PARFR
FINAL REPORT

DPE-0546-A-00-1003-00

July 1, 1986 - June 30, 1987

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

June 15, 1987

Jeffrey M. Spieler
Research Division
Office of Population
Agency for International Development
Washington, D.C. 20523

Dear Jeff:

In accordance with Cooperative Agreement DPE-0546-A-00-1003, enclosed please find 8 copies of PARFR's Final Report for the period July 1, 1986 through June 30, 1987. Volume I includes all administrative and financial information and final subagreement technical report summaries. Volumes II and III include complete copies of the final subagreement technical reports received during this reporting period.

Respectfully submitted,

Mary Nemeth

Mary Nemeth
Administrator

MN:rt
Enclosures

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

F I N A L R E P O R T

July 1, 1986 - June 30, 1987

Submitted to:

Research Division
Office of Population
Development Support Bureau
Agency for International Development
Washington, D.C. 20523

Submitted by:

Program for Applied Research on
Fertility Regulation
Northwestern University Medical School
Suite 1525
875 N. Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085

In compliance with
Cooperative Agreement DPE-0546-A-00-1003-00

Project Title and Contract Number:

Program for Applied Research on Fertility Regulation
DPE-0546-A-00-1003-00

Principal Investigator:

John J. Sciarra, M.D., Ph.D.
Professor and Chairman
Department of Obstetrics and Gynecology
Prentice Women's Hospital and Maternity Center
333 East Superior Street
Chicago, Illinois 60611

Contractor:

Northwestern University
c/o Sponsored Projects Administration
633 Clark Street, Room 1-203
Evanstor, Illinois 60201

Contract Period: July 1, 1981 - June 30, 1987

Reporting Period: July 1, 1986 - June 30, 1987

Total Expenditures Through June 30, 1987: $10,460,000.00

Total Expenditures July 1, 1986 Through June 30, 1987: $1,787,020.02

Total Expenditures Through June 30, 1987
Immunology Agreement: $700,000.00

Total Expenditures July 1, 1986 Through June 30, 1987
Immunology Agreement: $534,500.39

Total Award 7/1/81-6/30/87: $10,460,000

Total Award 9/1/85-6/30/87 Immunology Agreement: $700,000

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EXECUTIVE SUMMARY

This is the final report on the activities of PARFR for the period July 1, 1986 - June 30, 1987, the period of the one-year no-cost extension. When Northwestern University was informed that no further funds would be allocated, we immediately took the necessary actions to implement the termination procedures specified on pages 6 and 7, item 14 of Attachment 2 of the Standard Provisions in the Cooperative Agreement DPE-0545-A-00-1003-00.

After discussion with Northwestern University's Sponsored Project Administration staff and AID Technical Office, it was agreed that PARFR will not support any activities beyond March 31, 1987 in order to allow contributors sufficient time to submit final financial vouchers, PARFR to review the reports and complete all final vouchers for processing of payment to the contributing institutions. All contributors were informed in the last quarter of 1986 in order to allow them enough time to look for alternative sources of funding, if necessary, to complete the projects. The following is the list of projects that PARFR arranged for further funding and development by another AID-sponsored agency:

PARFR #	P.I.	Project	Status at Transfer	New Agency
385	Ralph Richart Columbia University	Toxicity Study of an Iodine Transcervical Sterilization Medium	IND completed.	FHI
376	C. Irving Meeker Maine Medical Ctr.	Pre IDE Studies - Tubal Clip	IDE amendment - authorization for Phase I obtained.	CONRAD
339	Lourens Zaneveld Rush-Presbyterian-St. Luke's Medical Center	Shug Device Study	IDE amendment - authorization for Phase I obtained.	CONRAD
339UI	Donald Waller The University of Illinois at Chicago	Toxicology of Silicone Implanted (SHUGS) in the Vas Deferens	IDE amendment - authorization for Phase I obtained.	CONRAD
338	Lourens Zaneveld Rush-Presbyterian-St. Luke's Medical Center	Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives	Pre-IND studies studies close to completion.	Proposal to be submitted to CONRAD.
338UI	Donald Waller The University of Illinois at Chicago	Toxicology Studies of Acrosin Inhibitors	Pre-IND studies studies close to completion.	Proposal to be submitted to CONRAD.

PARFR #	P.I.	Project	Status at Transfer	New Agency
315	Erwin Goldberg Northwestern Univ.	Immunologic Suppression of Fertility by Synthetic Antigenic Determinants of Lactate Dehydrogenase-C_4	Pre-clinical studies.	Proposal to be submitted to CONRAD.
375	Kenneth Tung University of New Mexico	Baboon Immunologic Evaluation with LDH-C_4 and *In Vitro* Fertilization Studies in Rodents	Pre-clinical studies.	Proposal to be submitted to CONRAD.
359	Bonnie Dunbar Baylor College of Medicine	Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins	Pre-clinical studies.	Proposal to be submitted to CONRAD.
379	Christopher Carron Duke University Medical Center	Idiotope Vaccine for Sperm-Targeted Immunocontraception	Pre-clinical studies.	Proposal to be submitted to CONRAD.
355	Nancy Alexander Oregon Regional Primate Research Center	Enhancement of the Secretory Immune Response to LDH-C_4	Pre-clinical studies.	Proposal to be submitted to CONRAD.
356a	Rajesh Naz The George Washington Univ.	Development of an Immunocontraceptive Vaccine: Role of 23-Kd Antigen in Immunoinfertility and Fertility Regulation	Pre-clinical studies.	Proposal to be submitted to CONRAD.
CD-RI series		Training and Collaboration in Immunocontraception	20 months completed.	All materials and files transferred to CONRAD.
309	Robert Chatterton Northwestern Univ. Medical School	Ovulation Inhibition by Anordrin	Pre-IND studies completed.	Unknown.

PARFR #	P.I.	Project	Status at Transfer	New Agency
386A	Howard Tatum Emory University	Phase II Poly NET 90 Injectable Study	Phase I completed. Phase II ongoing.	FHI
386C	Horacio Croxatto Instituto Chileno de Medicina Reproductiva	Phase II Poly NET 90 Injectable Study	Phase I completed. Phase II ongoing.	FHI
386F	Jerome Hoffman Mount Sinai Medical Center	Phase II Poly NET 90 Injectable Study	Phase I completed. Phase II ongoing.	FHI
386I	Giuseppe Benagiano Associazione per Studio della Riproduzione Umana	Phase II Poly NET 90 Injectable Study	Phase I completed. Phase II ongoing.	FHI
386J	Andrew Kaunitz University Hospital of Jacksonville	Phase II Poly NET 90 Injectable Study	Phase I completed. Phase II ongoing.	FHI
386M	Roberto Rivera Instituto de Investigacion Cientifica	Phase II Poly NET 90 Injectable Study	Phase I completed. Phase II ongoing.	FHI
387N	Brij Saxena Cornell University Medical College	Phase II Poly NET 90 Injectable Study - Pharmacokinetics	Phase I completed. Phase II ongoing.	FHI
387P	David Archer Magee-Women's Hosp.	Phase II Poly NET 90 Injectable Study - Pharmacokinetics	Phase I completed. Phase II ongoing.	FHI
387T	Jose P. Balmaceda The University of Texas Health Science Center	Phase I Poly NET 90 Injectable Study - Pharmacokinetics	Phase I completed.	
388M	Roberto Rivera Instituto de Investigacion Cientifica	Preparation and Characterization of Poly NET 30 Microspheres for Phase I Clinical Study	Phase I completed.	Unknown
391	Robert Rebar Northwestern University	Phase I Progesterone Microcapsule Clinical Trial	Phase I completed.	Unknown

PARFR #	P.I.	Project	Status at Transfer	New Agency
391C	Horacio Croxatto Instituto Chileno de Medicina Reproductiva	Progesterone Micro-capsules for Nursing Women	Phase I completed.	Unknown
	PARFR/Northwestern University	Poly Levonorgestrel	IND submitted.	Cross-reference to CONRAD granted.
	PARFR/Northwestern University	Poly NET + EE	IND submitted.	Cross-reference to CONRAD granted.
	Richard Sherins	Poly Testosterone	Phase I ongoing.	Cross-reference to CONRAD granted.

In relation to the clinical studies supported by PARFR, Northwestern's IRB (Attachment 1a and b) and each contributor (Attachment 2a-1) were informed about PARFR termination of support by March 31, 1987 in order to allow them, when appropriate, to look for another umbrella type of approval when needed. FDA was also informed, when appropriate, about each of our actions (Attachments 3a-d).

At the request of AID Technical Monitor, certain complete technical files were duplicated and forwarded to CONRAD (Attachment 4a and b). At the request of AID Technical Monitor, all remaining PARFR books and research monographs were sent to his attention. At no cost to AID, space was located in the Ward Building on the Chicago campus of Northwestern University to store all technical, financial, and other pertinent PARFR files and FDA files at a single location.

In relation to the final accounting and disposition of government property, an inventory schedule of property not consumed in the performance of this Cooperative Agreement was reviewed and all items accounted for. Copy of the inventory schedule was provided to the Technical Monitor on January 14, 1987 and Ms. Barbara Siegel of Sponsored Project Administration obtained permission from the AID Contracting Office to obtain title of the inventory for the Department of Obstetrics and Gynecology of the Medical School of Northwestern University.

Moving is scheduled for the week of June 22-27. Please note that the bulk of the moving costs will not be charged to AID. All equipment that was leased under the Cooperative Agreement will be returned to the lessors.

Sudler Marling, Inc., realty agent for the office rental space was informed in due time and no rent will be paid beyond June 30, 1987.

In order to maximize the resources allocated to direct research expenditures in this final no-cost extension year, the following administrative actions were taken:

1. Three staff members were terminated at varying times during this no-cost extension year representing a saving of $60,375.

2. Travel, domestic and international, was kept to a bare minimum, again saving these costs in order to apply them to the direct research expenditure line-item.

3. Office supplies, phone and communication expenditures were kept to a minimum. No new equipment was bought or leased since April, 1986.

4. For international meetings, alternate non-AID sources of funding were obtained in order to allow PARFR staff to participate in these efforts in relation to contraceptive development.

In December, 1986, a pre-audit survey requested by AID was performed by Mr. Buchanan of AID. It was decided by AID that a full audit of PARFR was not necessary.

Achievements by PARFR

Since 7/1/81, PARFR has received over $11,160,000 from AID through the present Cooperative Agreement (DPE-0546-A-00-1003-00; 7/1/81-6/30/86) and a No-Cost Extension through 6/30/87, to provide the following activities.

- Screen new contraceptive ideas for further development.
- Provide administrative, scientific, technical and financial support to domestic and foreign individuals and institutions for contraceptive research and development.
- Perform laboratory, pre-clinical studies and clinical trials of new or improved contraceptive modalities.
- Provide specialized scientific/technical training for developing country investigators.
- Obtain FDA approvals for Investigational New Drug Exemptions or Investigational Device Exemptions.
- Establish good working relationships with other agencies and industries to coordinate and collaborate research efforts without unnecessary duplication.
- Conduct symposia, workshops and seminars on issues related to the development of contraceptives.
- Widely disseminate research findings through its research monographs, workshop proceedings, publication of scientific papers and presentations at scientific meetings.

Working in partnership with the Office of Population of AID, the administrative framework of PARFR allowed it to seek and func promising research ideas in the field of fertility regulation, with minimal delays and maximum flexibility. About 150 research projects have been funded by PARFR that have addressed various aspects of contraceptive development.

Frequently, PARFR's involvement in research represented only the initial efforts in a development process that will not be completed for several years; for example, the development of an immunologic method of contraception against sperm antigens will take many years before a vaccine could become widely available. However, immunologic methods of contraception have the potential to

revolutionize contraception, as did the introduction of oral contraception in 1960. Based on the results from ongoing clinical trials, other projects supported by PARFR hold the promise of early introduction of new products; for example, the process for microencapsulating various steroids to be used as injectables that can provide continuous systemic contraception for periods of 30 to 180 days. The work of PARFR and the support provided to associated investigators have resulted in the submission of many applications for patents and initial approvals by the Food and Drug Administration.

PARFR has actively promoted and sought the participation of developing country institutions in its research activities. PARFR has provided funds and technical assistance to over 30 developing country institutions. The funds provided have been used for direct research expenditures, such as, personnel, patient care, laboratory supplies, animal purchase and maintenance, and travel and living expenses to attend PARFR workshops and symposia. Approximately 18% of PARFR funds have been expended for developing countries.

Part of the effect of providing support in developing countries is that even after PARFR projects terminate at these institutions, additional goal-oriented contraceptive development work is undertaken. This may be under other organizational funding or with funding from their own institutions.

The PARFR program has greatly contributed to the improving research competency in contraceptive development in developing countries. PARFR sponsored over 1,000 developing country researchers, investigators and clinicians to attend its workshops and symposia.

Approximately two-thirds of the PARFR workshop proceedings and research monographs were mailed to selected investigators and institutions in LDCs. Additionally, PARFR technical staff participated actively in LDC post-graduate activities, including lectures, practical demonstrations, and training.

PARFR/Northwestern University was selected by AID as the executing agency for a collaborative project with the Government of India in the specific field of immunocontraceptive development. The project agreement resulted, in part, from a PARFR initiative in 1982. The specific agreement became official in September, 1985. The project included: purchase of equipment and supplies for India's National Institute of Immunology; support of a research training programs in which Indian scientists come to the United States and vice versa; and sponsorship of research projects at institutions in both countries.

The purpose of this agreement was to provide scientific and technical instrumentation and training assistance in the field of reproductive immunology, specifically aimed at the development of a contraceptive vaccine. Since October 1985, $700,000 has been expended for the above purposes. It is noteworthy that the administrative indirect costs provided to Northwestern University have been only 5%. Importantly, the project has permitted the vital short-term training for Indian scientists in the laboratories of Harvard Medical School, Northwestern University, University of Wisconsin (Madison), Wayne State University, Baylor College of Medicine, George Washington University and the Salk Institute. All these trainees have returned or will return to the National Institute of Immunology, New Delhi for their continuing work in the field of immunocontraception.

It has been stated that there is an information gap among researchers in the field of fertility regulation, due to the many and varied disciplines involved, e.g., polymer and organic chemists, biologists, engineers, gynecologists, urologists. Each discipline has its own journals and meetings. Researchers from these various disciplines are rarely brought together to share and exchange information that could be instrumental in the promotion of new approaches to fertility regulation. PARFR set out to bridge this information gap through its scientific publications and by organizing meetings that include all of the various disciplines needed for the development of new and improved contraceptive methods.

As part of its objective to disseminate the findings of goal-oriented research in fertility regulation, PARFR had sponsored and organized a series of 14 workshops, each dealing with a specific aspect of contraceptive research. These workshops brought together many of the world's leading experts in the particular subject matter of the workshop, and included representatives from industry, private and public organizations funding contraceptive research, and government representatives. The format of the workshops included formal presentations of current material in the subject area and formal discussions of these presentations. The workshops were designed to allow the participants ample time for informal discussions. The result of these workshops had been to provide AID with current information on specific areas of contraceptive reproductive research. With this information AID has been in a better position to plan for future investigations, avoid unnecessary duplication of effort, and reduce the time for development of methods of contraception.

In addition to the annual workshops organized by PARFR, a series of regional symposia had been organized and sponsored by PARFR at the request of local governments, institutions and professional associations. In arranging these symposia, PARFR collaborated with the local AID missions to ensure that the appropriate persons were invited and the subject of the symposium met local needs. Over 4,000 individuals, including researchers, health care providers and government representatives are estimated to have participated in PARFR workshops, symposia and conferences.

PARFR staff and PARFR-sponsored investigators have participated in numerous national and international meetings in the area of contraceptive development. This participation included the presentation of current research data and the organization of special sessions. In addition, several PARFR scientific staff were actively involved in teaching clinical gynecology related to family planning at Northwestern University, and at postgraduate courses in the United States and abroad.

PARFR published the proceedings of its fourteen annual workshops and some of the regional symposia and conferences it has organized and sponsored. Usually, these workshop proceedings were published within months of the workshop and made available to all participants. Additional copies (1500) were provided to selected researchers, including the researchers affiliated with PARFR, medical libraries and other governmental and non-governmental organizations interested in contraceptive research. PARFR provided AID with 300 copies to distribute to its offices and missions around the world.

In 1980, PARFR initiated a research monograph called Research Frontiers in Fertility Regulation (RFFR). Each RFFR issue was concerned with a specific

tion of each topic by selected experts in the field. To date, 17 issues of RFFR have been published. Each issue of RFFR was peer-reviewed before being accepted for publication. About 6,000 copies of each issue were mailed from PARFR to researchers and other interested persons in developed and developing countries. About 65% of the copies were mailed to people in developing countries. The mailing list was periodically revised and continue to expand. At the time of the last revision, over 95% of the persons on the mailing list indicated they wished to continue to receive RFFR.

In preparation for the PARFR regional symposia, a syllabus was developed that includes scientific research papers and presentations. These were edited and printed by PARFR and distributed to participants at the meetings. Additional copies were mailed to key individuals in the geographic area, including AID missions. On occasion, PARFR has translated the English version into the local language.

PARFR staff and PARFR-sponsored researchers actively participated in the dissemination of information derived from the many research studies it has supported.

Recognizing that there are other private and public groups (both domestic and international) that support work on the development of new and improved methods of contraception, PARFR developed close ties with most of these groups. The extent of interaction of PARFR with CDB/NIH has been extensive. PARFR has had a special relationship with FHI and has delivered projects to FHI for Phase III clinical testing and development. WHO and The Population Council have actively participated in the organization, development and support of most PARFR workshops. Additionally, PARFR staff have served as resource persons to these organizations.

PARFR has recognized the need for interaction with private industry to provide the expertise and experience in the development of pharmaceutical or surgical instrument products. PARFR maintained particularly close relationships and undertook collaborative research with private sector organizations working in contraceptive development including the following:

BioNexus, Inc. (Raleigh, N.C.)
BioProducts, Inc. (Tucson, Arizona)
Bivona Surgical Instruments (Gary, Indiana)
Contraceptive Development Branch, National Institutes of Health
Family Health International (Research Triangle Park, N.C.)
G.D. Searle & Company (Skokie, Illinois)
Indian Council of Medical Research (New Delhi, India)
National Institute of Immunology (New Delhi, India)
Organon Pharmaceutical Corporation (Oss, Holland)
Ortho Pharmaceutical Corporation (Raritan, New Jersey)
Pavex, Inc. (San Juan Capistrano, California)
Reznik Instruments (Chicago, Illinois)
Stolle Research and Development Corporation (Lebanon, Ohio)
Storz GMBH (West Germany)
Syntex Corporation (Palo Alto, California)
3M Company (Minneapolis, Minnesota)
Upjohn Pharmaceutical Corporation (Kalamazoo, Michigan)
VLI Corporation (Irvine, California)
Wyeth Pharmacetical Corporation (Philadelphia, Pennsylvania)

Future Considerations

The Program for Applied Research on Fertility Regulation plans to continue its research and development activities within the Department of Obstetrics and Gynecology, Northwestern University. Several of the contraceptive leads developed by PARFR will be pursued with the support of the University and private funding. To this end, a regional office in Santiago, Chile has been established.

It is our firm intention to support in whatever ways possible the U.S. government's program in Family-Planning and Contraceptive Research and Development. Whenever called upon, PARFR will provide scientific and technical assistance to the government's CONRAD program and to other governmental and non-governmental agencies.

EXPENDITURES UNDER
COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
7/1/81 - 6/30/87

	7/1/81-6/30/82	7/1/82-6/30/83	7/1/83-6/30/84	7/1/84-6/30/85	7/1/85-6/30/86	7/1/86-6/30/87*	TOTAL
Research	$172,589.32	$ 932,128.91	$ 948,050.96	$1,312,972.21	$1,427,060.44	$1,282,320.78	$ 6,075,122.62
Workshops & Publications	44,738.69	131,185.49	90,921.16	124,655.87	59,953.32	- 0 -	451,454.53
Consultant Fees	12,100.05	24,895.62	26,463.68	33,370.54	48,446.00	13,266.27	158,542.16
Travel	48,284.32	75,312.44	142,505.35	100,021.43	98,655.37	19,380.18	484,159.09
Salaries & Fringe Benefits	211,795.99	234,928.90	259,571.68	277,957.44	296,665.99	240,911.45	1,521,831.43
Rent and Communication	69,508.51	133,538.86	123,188.71	146,728.25	152,126.53	104,802.45	729,893.31
Equipment	1,076.25	- 0 -	11,435.10	- 0 -	- 0 -	- 0 -	12,511.35
Indo-U.S. Agreement	- 0 -	- 0 -	- 0 -	- 0 -	165,499.61	534,500.39	700,000.00
Indirect Cost	89,835.09	185,063.84	202,979.05	233,318.52	188,950.12	126,338.89	1,026,485.51
	$649,928.22	$1,717,054.06	$1,805,115.69	$2,229,024.26	$2,437,357.38	$2,321,520.41	$11,160,000.00

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FERTILITY REGULATION

Northwestern University
Suite 1525
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(312) 664-0085
Cable: PARFR Chicago, Illinois

ATTACHMENT 1a

February 2, 1987

Joanne G. Richmond
Human Subject Specialist/Research
Services Administration
Northwestern University
Institutional Review Board
2-509 Crown
Evanston, IL 60201

Dear Ms. Richmond:

Due to the fact that PARFR does not have the authority and AID-funds to spend beyond June 30, 1987, we are in the process of closing down our clinical trials. From an administrative point of view, we have selected the date, March 31, 1987 to terminate projects in order to allow them to prepare final technical reports and obtain, when appropriate, IRB approval from other institutions. All of our contributors have been informed in writing of our plan of action.

Enclosed is the status of PARFR active projects. Please note that the projects marked with an asterisk are not going to be completed until October, 1987 and they will be covered from April 1st to completion by the Family Health International IRB.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
Enclosure
cc: William O. Park

STATUS OF PARFR ACTIVE PROJECTS, FEBRUARY, 1987

PRINCIPAL INVESTIGATOR	PROJECT TITLE	IRB APPROVAL DATE	STATUS
Howard J. Tatum, M.D. (Emory University)	Phase II Poly NET 90 Studies	2/21/86	Approval Needed to 3/31/87
*Horacio B. Croxatto, M.D. (Instituto Chileno de Medicina Reproductiva)	"	"	"
*Jerome J. Hoffman, M.D. (Mount Sinai Med. Ctr.)	"	"	"
*Giuseppe Benagiano, M.D. (Associazione per Studio della Reproduzione Umana)	"	"	"
*Andrew M. Kaunitz, M.D. (University Hospital of Jacksonville)	"	"	"
*Roberto Rivera, M.D. (Instituto de Investigacion Cientifica)	"	"	"
*Brij B. Saxena, Ph.D., D.Sc. (Cornell University Medical College)	"	"	"
*David Archer, M.D. (Magee-Women's Hospital)	"	"	"
Roberto Rivera, M.D. (Instituto de Investigacion Cientifica)	Phase I Poly NET 30 Study	"	Completed
Robert W. Rebar, M.D. (Nortnwestern Univ.)	Phase I Progesterone Microcapsule Clinical Trial	5/23/86	Active to 5/31/87
Horacio B. Croxatto, M.D. (Instituto Chileno de Medicina Reproductiva)	Progesterone Microcapsules for Nursing Women	3/3/86	Completed

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Northwestern University
Suite 1525
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Cable: PARFR Chicago, Illinois

September 18, 1986

Lourens J.D. Zaneveld, D.V.M., Ph.D.
Obstetrics-Gynecology Research
Rush-Presbyterian-St. Luke's Medical
Center
1753 West Congress Parkway
Chicago, IL 60612

RE: PARFR-338

Dear Dr. Zaneveld:

Due to administrative reasons, your project, "Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives," must be terminated no later than March 30, 1987. Final financial and technical reports must be received by PARFR no later than April 30, 1987. No exceptions will be made.

In addition, all future correspondence should be directed to my attention.

Sincerely,

Gerald L. Zatuchni, M.D., M.Sc.
Director

GIZ/aw
P.S.: Please sign and return the enclosed copy of this letter to assure me it has been received.

SIGNATURE: Lourens J.D. Zaneveld, D.V.M, Ph.D.

9/29/86
DATE

parfr

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
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(312) 664-0085
Cable: PARFR Chicago, Illinois

December 5, 1986

Lourens J.D. Zaneveld, D.V.M., Ph.D.
Obstetrics-Gynecology Research
Rush-Presbyterian-St. Luke's Medical Center
1753 West Congress Parkway
Chicago, IL 60612

RE: PARFR-338 and 339

Dear Dr. Zaneveld:

As indicated in my letter to you dated September 18, 1986, PARFR had decided to terminate all projects on March 31, 1987. Final financial and technical reports must be received no later than April 30, 1987.

Also the next quarterly financial report for the period of October 1 - December 31, 1986 should be received by PARFR no later than January 15, 1987.

If you have any questions regarding financial reports or procedures, please contact me.

Sincerely,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: Paul H. Matz

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FERTILITY REGULATION

Northwestern University
Suite 1525
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September 18, 1986

Donald P. Waller, Ph.D.
Assistant Professor
Department of Pharmacognosy and
Pharmacology
Room 353 - Pharmacy Building
University of Illinois at Chicago
P.O. Box 6998
Chicago, IL 60680

RE: PARFR-339UI

Dear Dr. Waller:

As stated in the enclosed letter to Dr. Zaneveld and due to administrative reasons, your project, "Toxicology of Silicone Implanted (SHUGS) in the Vas Deferens," must be terminated no later than March 30, 1987. Final financial and technical reports must be received by PARFR no later than April 30, 1987. No exceptions will be made.

In addition, all future correspondence should be directed to my attention.

Sincerely,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ/aw
cc: Lourens J.D. Zaneveld, D.V.M, Ph.D.
P.S.: Please sign and return the enclosed copy of this letter to assure me it has been received.

SIGNATURE: Donald P. Waller, Ph.D.

23 Sept 86
DATE

ATTACHMENT 2d

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

December 29, 1986

Howard J. Tatum, M.D.
Department of Gyn/Ob
Emory University School of Medicine
69 Butler Stret, S.e.
Atlanta, GA 30335

Dear Dr. Tatum:

As you are probably aware, PARFR did not obtain additional funding from AID to continue beyond June, 1987. For this reason, in order to comply with our own University's administrative regulations, we would like to inform you that on March 31, 1987, PARFR will no longer support your study, provide product liability insurance, and IRB umbrella approval. We do know that AID and FHI want to complete the study and we urge you to contact Dr. Albert Siemens at FHI in order to arrange funding with them for the period of April 1, 1987 to the completion of the study.

Your final financial voucher and final technical report must be received by PARFR no later than April 7, 1987. We regret to inform you that no exceptions will be made. Thank you for your cooperation in this study.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: J. Shelton
A. Siemens
John C. Richards

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

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875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

December 29, 1986

Horacio B. Croxatto, M.D.
J.V. Lastarria 29
Depto. 101
Santiago, CHILE

Dear Dr. Croxatto:

As you are probably aware, PARFR did not obtain additional funding from AID to continue beyond June, 1987. For this reason, in order to comply with our own University's administrative regulations, we would like to inform you that on March 31, 1987, PARFR will no longer support your study, provide product liability insurance, and IRB umbrella approval. We do know that AID and FHI want to complete the study and we urge you to contact Dr. Albert Siemens at FHI in order to arrange funding with them for the period of April 1, 1987 to the completion of the study.

Your final financial voucher and final technical report must be received by PARFR no later than April 7, 1987. We regret to inform you that no exceptions will be made. Thank you for your cooperation in this study.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: J. Shelton
A. Siemens
Emilia Lepe Poblete

ATTACHMENT 2f

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

December 29, 1986

Jerome J. Hoffman, M.D.
Director of Research in
Reproductive Physiology
Department of Research
Mount Sinai Medical Center
4300 Alton Road
Miami Beach, FL 33104

Dear Dr. Hoffman:

As you are probably aware, PARFR did not obtain additional funding from AID to continue beyond June, 1987. For this reason, in order to comply with our own University's administrative regulations, we would like to inform you that on March 31, 1987, PARFR will no longer support your study, provide product liability insurance, and IRB umbrella approval. We do know that AID and FHI want to complete the study and we urge you to contact Dr. Albert Siemens at FHI in order to arrange funding with them for the period of April 1, 1987 to the completion of the study.

Your final financial voucher and final technical report must be received by PARFR no later than April 7, 1987. We regret to inform you that no exceptions will be made. Thank you for your cooperation in this study.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: J. Shelton
A. Siemens
Andrew W. Behrman

ATTACHMENT 2g

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

December 29, 1986

Giuseppe Benagiano, M.D.
Professor, Director
1st Institute di Clinica
Obstetricia e Ginecologica
Associazione per Studio della
Riproduzione Umana
Via di Villa Patrizi, 22A
00161 Rome, ITALY

Dear Dr. Benagiano:

As you are probably aware, PARFR did not obtain additional funding from AID to continue beyond June, 1987. For this reason, in order to comply with our own University's administrative regulations, we would like to inform you that on March 31, 1987, PARFR will no longer support your study, provide product liability insurance, and IRB umbrella approval. We do know that AID and FHI want to complete the study and we urge you to contact Dr. Albert Siemens at FHI in order to arrange funding with them for the period of April 1, 1987 to the completion of the study.

Your final financial voucher and final technical report must be received by PARFR no later than April 7, 1987. We regret to inform you that no exceptions will be made. Thank you for your cooperation in this study.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: J. Shelton
A. Siemens
Michele Ermini, M.D.

parfr

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

December 29, 1986

Andrew M. Kaunitz, M.D.
Assistant Professor
Director, Division of Ambulatory Care
Department of Ob/Gyn
University Hospital of Jacksonville
655 West 8th Street
Jacksonville, FL 32209

Dear Dr. Kaunitz:

As you are probably aware, PARFR did not obtain additional funding from AID to continue beyond June, 1987. For this reason, in order to comply with our own University's administrative regulations, we would like to inform you that on March 31, 1987, PARFR will no longer support your study, provide product liability insurance, and IRB umbrella approval. We do know that AID and FHI want to complete the study and we urge you to contact Dr. Albert Siemens at FHI in order to arrange funding with them for the period of April 1, 1987 to the completion of the study.

Your final financial voucher and final technical report must be received by PARFR no later than April 7, 1987. We regret to inform you that no exceptions will be made. Thank you for your cooperation in this study.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: J. Shelton
A. Siemens
Daniel W. Smith

parfr

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

December 29, 1986

Roberto Rivera, M.D.
Director
Instituto de Investigacion Cientifica
Juarez University of Durango
Apartado Postal B-7
Durango, 34000 Durango, MEXICO

Dear Dr. Rivera:

As you are probably aware, PARFR did not obtain additional funding from AID to continue beyond June, 1987. For this reason, in order to comply with our own University's administrative regulations, we would like to inform you that on March 31, 1987, PARFR will no longer support your study, provide product liability insurance, and IRB umbrella approval. We do know that AID and FHI want to complete the study and we urge you to contact Dr. Albert Siemens at FHI in order to arrange funding with them for the period of April 1, 1987 to the completion of the study.

Your final financial voucher and final technical report must be received by PARFR no later than April 7, 1987. We regret to inform you that no exceptions will be made. Thank you for your cooperation in this study.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: J. Shelton
A. Siemens

parfr

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

December 22, 1986

Brij B. Saxena, Ph.D., D.Sc.
Professor of Biochemistry
and Endocrinology
Department of Ob/Gyn and Medicine
Division of Reproductive Endocrinology
The New York Hospital -
Cornell Medical Center
525 East 68th Street
New York, NY 10021

RE: Poly NET 90

Dear Dr. Saxena:

In relation to the Poly NET 90 Phase II endocrine study, please note that:

1. No additional endometrial biopsies will be accepted by Dr. Wilborn, effective immediately.

2. Effective immediately, order for RIA tracers will no longer be accepted by Serono and should be placed directly with Ms. Mary Nemeth, Administrator, PARFR.

3. Your contract terminates on March 31, 1987. We need to receive your final technical and financial vouchers by this date. No extension will be granted.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: Mary Nemeth

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

December 22, 1986

David Archer, M.D.
Associate Professor
Department of Ob/Gyn
University of Pittsburgh
Magee-Women's Hospital
Forbes Avenue and Halket Street
Pittsburgh, PA 15213

RE: Poly NET 90

Dear Dr. Archer:

In relation to the Poly NET 90 Phase II endocrine study, please note that:

1. No additional endometrial biopsies will be accepted by Dr. Wilborn, effective immediately.

2. Effective immediately, order for RIA tracers will no longer be accepted by Serono and should be placed directly with Ms. Mary Nemeth, Administrator, PARFR.

3. Your contract terminates on March 31, 1987. We need to receive your final technical and financial vouchers by this date. No extension will be granted.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: Mary Nemeth

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

ATTACHMENT 21

December 29, 1986

Robert W. Rebar, M.D.
Professor
Department of Ob/Gyn
Prentice Women's Hospital
333 E. Superior Street, 11th Floor
Chicago, IL 60611

RE: PARFR-391

Dear Dr. Rebar:

Following your phone conversation with Dr. Goldsmith on 12/22/86, we understand that you have completed the admission for this study. Since only 6 patients were admitted to the study, rather than the 8 women stated on page 3 of the subagreement, we are amending the budget to reflect the reduced number of volunteers. We would also like to call to your attention that PARFR cannot support your study beyond 3/31/87.

We must receive your final technical report and final financial vouchers by April 15, 1987. Your cooperation is greatly appreciated.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt

parfr

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

ATTACHMENT 3a

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

February 19, 1987

Solomon Sobel, M.D.
Director
Division of Metabolism and Endocrine
Drug Products HFN-810
Food and Drug Administration
Rockville, MD 20857

RE: Poly NET 90 Studies

Dear Dr. Sobel:

I regret that I was unable to attend the February 18th meeting but my associate, Dr. Goldsmith, reported to me about all the discussions, especially your favorable comments about this contraceptive modality which may become one day an approved FDA drug.

As has been previously indicated to you, PARFR will no longer be associated with further development and/or testing of the Poly NET 90 system beyond March 31, 1987. FHI has assumed all responsibilities for the completion of the Phase II trial, and for the initiation and monitoring of the Phase III trial.

If you need any additional information from PARFR, please do not hesitate to contact me.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
cc: Albert J. Siemens, Ph.D.
James D. Shelton, M.D.
Lee R. Beck, Ph.D.

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0065
Cable: PARFR Chicago, Illinois

February 17, 1987

Solomon Sobel, M.D.
Director
Division of Metabolism and Endocrine
Drug Products HFN-810
Food and Drug Administration
Rockville, MD 20857

RE: IND 27,826 -- "Testosterone Microspheres"
Amendment #2

Dear Dr. Sobel:

In relaton to your July 28, 1986 letter, please find enclosed the additional information for the twelve items on chemistry raised on pages 2 and 3 of the above-mentioned letter.

Also enclosed is preliminary information from Dr. Sherins, NIH, regarding the study on hypogonadal men conducted at NIH.

This study should probably be completed by May, 1987 and we will submit the information to you as soon as it is available.

We have followed your instructions and we have not initiated any further clinical studies before completing the hypogonadal men pharmacokinetic study (point 1 under Medical in your July 28, 1986 letter).

We would like to authorize cross-reference to this IND to Stolle Research and Development Corp. (Dr. Lee Beck), Agency for International Development (Dr. James Shelton), and CONRAD, Eastern Virginia Medical School (Dr. Gary Hodgen).

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
Enclosure
cc: Lee R. Beck, Ph.D.
James D. Shelton, M.D.
Gary D. Hodgen, Ph.D.
Richard J. Sherins, M.D.

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

February 2, 1987

Solomon Sobel, M.D.
Director
Division of Metabolism and Endocrine
Drug Products HFN-810
Food and Drug Administration
Rockville, MD 20857

RE: IND 28,263
Norethindrone/Ethinyl Estradiol
Microspheres

Dear Dr. Sobel:

In reference to the above-mentioned IND, please note that Northwestern University (PARFR) will not conduct and/or sponsor the Phase I clinical trial. We would like to authorize cross-reference to this IND to Stolle Research & Development Corporation laboratories (Dr. Lee Beck), Agency for International Development (Dr. James Shelton), and CONRAD - Eastern Virginia Medical School (Dr. Gary Hodgen).

If any of these institutions will conduct the Phase I clinical trial, they will be responsible to comply with your additional information request dated September 8, 1986.

It is our understanding that no further information or reporting is needed from us.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
Enclosure
cc: Lee R. Beck, Ph.D.
James D. Shelton, M.D.
Gary D. Hodgen, Ph.D.

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

ATTACHMENT 3d

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

February 2, 1987

Solomon Sobel, M.D.
Director
Division of Metabolism and Endocrine
Drug Products HFN-810
Food and Drug Administration
Rockville, MD 20857

RE: IND 28,102
Levonorgestrel Microspheres

Dear Dr. Sobel:

In reference to the above-mentioned IND, please note that Northwestern University (PARFR) will not conduct and/or sponsor the Phase I clinical trial. We would like to authorize cross-reference to this IND to Stolle Research & Development Corporation laboratories (Dr. Lee Beck), Agency for International Development (Dr. James Shelton), and CONRAD - Eastern Virginia Medical School (Dr. Gary Hodgen).

If any of these institutions will conduct the Phase I clinical trial, they will be responsible to comply with your additional information request dated September 8, 1986.

It is our understanding that no further information or reporting is needed from us.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
Enclosure
cc: Lee R. Beck, Ph.D.
James D. Shelton, M.D.
Gary D. Hodgen, Ph.D.

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

ATTACHMENT 4a
Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

February 20, 1987

Jeff Spieler
Research Division
Office of Population
Agency for International Development
Washington, D.C. 20523

Dear Jeff:

By separate mail, we are sending you the material requested in your January 27th letter. Please note that some technical reports (see enclosed list) are still not available to PARFR. As soon as they are received, copies will be forwarded to you.

If you need additional material, please advise me in writing.

Most cordially,

Gerald I. Zatuchni, M.D., M.Sc.
Director

GIZ:rt
Enclosures

ATTACHMENT 4b

P.I.	PARFR #	TITLE	REASON
Asch	347	"Studies on the Anovulatory Potency and Side Effects of an Inhibitory Analog of LH-RH in Cynomologous Monkeys"	See enclosed letter.
Asch	361	"Testosterone Microcapsule Formulation Study"	See enclosed letter.
Chatterton	309	"Ovulation Inhibition by Anordrin"	Final pharmacokinetic studies being analyzed.
Croxatto	391	"Progesterone Microcapsules for Nursing Women"	Study ongoing - final report expected in April.
Goldberg	315	"Immunologic Suppression of Fertility by Synthetic Antigenic Determinants of Lactate Dehydrogenase-C_4, Extension of PARFR-232"	See enclosed letter.
Lewis	362	"Combination Injectable Steroidal Microsphere - Continuation of PARFR-332"	See enclosed letter.
Rebar	391	"Phase I Progesterone Microcapsule Clinical Trial"	Project ongoing - final report expected in May.
Waller	338UI	"Toxicology Studies of Acrosin Inhibitors"	See enclosed letter.
Waller	339UI	"Toxicology of Silicone Implanted (SHUGS) in the Vas Deferens"	Project ongoing - final report expected in April - May.
Zaneveld	338	"Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives"	Project ongoing - final report expected in April - May.
Zaneveld	339	"Shug Device Studies"	Project ongoing - final report expected in April - May.

Scientific Travel

1. Research progress was monitored by review of technical reports and site visits. During this reporting period, the following visits were made by PARFR staff:

   a. 8/13-17/86 -- Alfredo Goldsmith, M.D., M.P.H.; Instituto Chileno de Medicine Reproductiva, Santiago, Chile (PARFR-386C -- Horacio B. Croxatto, M.D.).

   b. 8/23-26/86 -- Alfredo Goldsmith, M.D., M.P.H.; Associazione per Studio della Riproduzione Umana (PARFR-386I -- Giuseppe Benagiano, M.D.).

   c. 3/4-9/87 -- Alfredo Goldsmith, M.D., M.P.H.; Instituto Chileno de Medicine Reproductiva, Santiago, Chile (PARFR-386C -- Horacio B. Croxatto, M.D.).

2. Drs. Alfredo Goldsmith and John Sciarra attended a meeting with AID in Washington, D.C. on September 8, 1986, to discuss the transfer of projects to other AID-supported agencies.

3. Diane Krier-Morrow attended a meeting with AID in Washington, D.C. on September 8, 1986, to discuss the Indo-U.S. Immunology Agreement.

4. Dr. Alfredo Goldsmith attended a meeting with AID in Washington, D.C. on October 15, 1986, to present to CONRAD staff the PARFR portfolio of activities.

5. Drs. Alfredo Goldsmith, Gerald Zatuchni, John Sciarra, Ricardo Asch, Kamran Moghissi, and Joe Leigh Simpson attended the 12th IFFS Congress Meeting in Singapore on October 27-31, 1986. No cost was charged to AID.

6. Dr. Alfredo Goldsmith represented PARFR at a WHO meeting in Havana, Cuba on November 14-20, 1986. No cost was charged to AID.

7. Drs. Alfredo Goldsmith and John Sciarra attended a meeting with AID in Washington, D.C. on December 8, 1986, to discuss termination procedures and attend a debriefing regarding the Cooperative Agreement.

8. Dr. Alfredo Goldsmith attended a meeting with the FDA in Rockville, Maryland on February 17, 1987, in relation to Phase II and III of the Poly NET 90 study.

9. Drs. Alfredo Goldsmith and John Sciarra attended the Post-graduate Course in Seville, Spain on April 8-11, 1987, and delivered four lectures. No cost was charged to AID.

10. Drs. Gerald Zatuchni and John Sciarra attended the 9th Postgraduate Course on Infertility/Fertility in Florence, Italy on May 20-25, 1987. No cost was charged to AID.

11. Dr. Gerald Zatuchni was the external reviewer for two Ph.D. dissertations on the topic of the IUD at the University of Utrecht, The Netherlands in June, 1987. No cost was charged to AID.

LDC Involvement

During this reporting period, the following subagreements in LDCs were terminated:

1. PARFR-386C -- "Phase II Poly NET 90 Injectable Study"
   Horacio B. Croxatto, M.D., Instituto Chileno de Medicina Reproductiva,
   Santiago, Chile

2. PARFR-386M -- "Phase II Poly NET 90 Injectable Study"
   Roberto Rivera, M.D., Instituto de Investigacion Cientifica,
   Durango, Mexico

3. PARFR-388M -- "Phase I Poly NET 30 Injectable Study"
   Roberto Rivera, M.D., Instituto de Investigacion Cientifica,
   Durango, Mexico

4. PARFR-391C -- "Progesterone Microcapsules for Nursing Women"
   Horacio B. Croxatto, M.D., Instituto Chileno de Medicina Reproductiva,
   Santiago, Chile

LDC Research Funds

For the period 7/1/81-6/30/87, the following funds were expended for research in LDCs:

Country & PARFR #	Budget (Dollars)	Total Expenditures To Date
ARGENTINA		
PARFR-367	$ 9,900	$ 9,900.00
BRASIL		
PARFR-318B	14,982	14,701.49
PARFR-328B	14,122	10,792.00
PARFR-389	1,795	1,795.00
TOTAL BRASIL:	$ 30,899	$ 27,288.49
CHILE		
PARFR-301C	31,476	31,476.00
PARFR-310C	8,000	8,000.00
PARFR-311C	9,000	9,000.00
PARFR-316C	27,000	27,000.00
PARFR-327C	20,880	20,880.00
PARFR-341C	45,419	10,811.00
PARFR-386C	25,036	20,832.80
PARFR-391C	8,094	8,094.00
TOTAL CHILE:	$174,905	$136,093.80
EGYPT		
PARFR-300E	11,930	11,930.00
PARFR-314E	5,400	5,400.00
TOTAL EGYPT:	$ 17,330	$17,330.00
HONG KONG		
PARFR-366	5,302	2,564.20
MEXICO		
PARFR-300M	23,056	23,056.00
PARFR-330M	28,710	28,410.00
PARFR-341M	36,135	3,326.00
PARFR-386M	25,762	22,267.00
PARFR-388M	60,465	59,185.00
TOTAL MEXICO:	$174,128	$136,244.00
THAILAND		
PARFR-354	9,800	9,633.61
VENEZUELA		
PARFR-305V	24,049	21,512.10
PARFR-322V	3,600	3,600.00
PARFR-327V	19,535	17,043.23
TOTAL VENEZUELA:	$ 47,184	$ 42,155.33
TOTAL LDC:	$469,448	$381,209.43

Subagreements Terminated During the Period

7/1/86 - 6/30/87

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	INCLUSIVE PROJECT DATES	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
309	"Ovulation Inhibition by Anordrin" Robert T. Chatterton, Ph.D. Northwestern University Med. School Chicago, Illinois	1/1/82- 6/30/86	$ 83,531.83	$300,112.00
309a	"Toxicity Studies of Anordrin" Donald P. Waller, Ph.D. The University of Illinois at Chicago Chicago, Illinois	7/1/85- 9/30/86	42,966.79	93,313.00
315	"Immunologic Suppression of Fertility by Synthetic Antigenic Determinants of Lactate Dehydrogenase-C4, Extension of PARFR-232" Erwin Goldberg, Ph.D. Northwestern University Evanston, Illinois	3/1/82- 12/31/86	103,452.93	466,995.00
337T	"Intracervical Device Acceptability Study" Rochelle N. Shain, Ph.D. The University of Texas Health Science Center San Antonio, Texas	6/1/83- 6/30/86	2,411.51	12,420.68
338	"Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives" Lourens J.D. Zaneveld, D.V.M., Ph.D. Rush-Presbyterian-St. Luke's Medical Center Chicago, Illinois	7/1/83- 3/31/87	77,636.89	199,931.00
338UI	"Toxicology Studies of Acrosin Inhibitors" Donald P. Waller, Ph.D. The University of Illinois at Chicago Chicago, Illinois	7/1/84- 12/31/86	29,353.89	84,866.00
339	"SHUG Device Studies" Lourens J.D. Zaneveld, D.V.M., Ph.D. Rush-Presbyterian-St. Luke's Medical Center Chicago, Illinois	7/1/83- 3/31/87	24,448.83	70,453.83

Subagreements Terminated During the Period (cont'd)

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	INCLUSIVE PROJECT DATES	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
339UI	"Toxicology of Silicone Implanted (SHUG) in the Vas Deferens Donald P. Waller, Ph.D. The University of Illinois at Chicago Chicago, Illinois	7/1/84-3/31/87	$ 75,082.05	$147,740.00
341	"Phase I Poly NET 90 Injectable Study" Charles E. Flowers, Jr., M.D. The University of Alabama Birmingham, Alabama	12/1/83-12/31/85	32,153.90	76,126.23
355	"Enhancement of the Secretory Immune Response to LDH-C_4" Nancy J. Alexander, Ph.D. Medical Research Foundation of Oregon Portland, Oregon	4/15/84-12/31/86	11,976.01	98,820.71
356a	"Development of an Immunocontraceptive Vaccine: Role of 23-Kd Antigen in Immunoinfertility and Fertility Regulation" Rajesh K. Naz, Ph.D. The George Washington University Medical Center Washington, D.C.	12/1/84-6/30/86	11,032.88	98,190.16
359	"Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins" Bonnie S. Dunbar, Ph.D. Baylor College of Medicine Houston, Texas	6/1/84-9/30/86	20,287.10	117,835.66
360	"Inter- and Intra-Cycle Variation of Genital Peroxidases in Women" John C.M. Tsibris, Ph.D. The University of Illinois at Chicago Chicago, Illinois	7/1/84-9/30/86	31,775.70	99,817.35
363	"Laboratory Studies on an Antispermatogenic Agent - THP for Control of Male Fertility" John P. Wiebe, Ph.D. University of Western Ontario London, Ontario, Canada	10/1/84-12/31/86	5,250.67	93,968.97

Subagreements Terminated During the Period (cont'd)

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	INCLUSIVE PROJECT DATES	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
373	"90-Day Levonorgestrel Microspheres" Danny H. Lewis, Ph.D. Stolle Research & Development Corporation Decatur, Alabama	4/1/85-3/31/86	$ 11,234.00	$ 67,404.00
375	"Baboon Immunologic Evaluation with LDH-C_4 and In Vitro Fertilization Studies in Rodents" Kenneth S.K. Tung, M.D. University of New Mexico Albuquerque, New Mexico	5/1/85-9/30/86	40,935.41	62,576.00
378	"Preparation of Testosterone Microspheres" Danny H. Lewis, Ph.D. Stolle Research & Development Corp. Lebanon, Ohio	7/1/85-9/30/86	6,538.85	39,233.00
379	"Idiotope Vaccine for Sperm-Targeted Immunocontraception" Christopher P. Carron, Ph.D. Duke University Medical Center Durham, North Carolina	8/1/85-9/30/86	26,861.24	67,143.52
380	"Preliminary Evaluation of 3-M Impression Material as an Injectable Vas Occlusive Device" Lourens J.D. Zaneveld, D.V.M., Ph.D. Rush-Presbyterian-St. Luke's Medical Center Chicago, Illinois	8/1/85-4/30/86	1,474.56	10,832.00
382	"Postcoital Effectiveness of the Vaginal Spermicidal Barrier" Gerald S. Bernstein, Ph.D., M.D. Professional Staff Association, Los Angeles County, University of Southern California Medical Cent Los Angeles, California	10/1/85-9/30/86	2,798.81	3,651.68
384	"Effects of Immunization of Female Baboons with Zona Pellucida Antigens on Ovarian Functions" Vernon C. Stevens, Ph.D. The Ohio State University Research Foundation Columbus, Ohio	7/1/85-4/30/186	14,310.48	25,624.05

Subagreements Terminated During the Period (cont'd)

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	INCLUSIVE PROJECT DATES	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
385	"Toxicity Study of an Iodine Transcervical Sterilization Medium" Donald P. Waller, P.D. The Board of Trustess of The University of Illinois Chicago, Illinois	11/1/85-6/30/86	$ 47,555.00	$ 47,555.00
386A	"Phase II Poly NET 90 Injectable Study" Howard J. Tatum, M.D. Emory University Atlanta, Georgia	12/1/85-3/31/87	22,679.14	25,336.30
386C	"Phase II Poly NET 90 Injectable Study" Horacio B. Croxatto, M.D. Instituto Chileno de Medicina Reproductiva Santiago, Chile	12/1/85-3/31/87	16,802.80	20,832.80
386F	"Phase II Poly NET 90 Injectable Study" Jerome J. Hoffman, M.D. Mount Sinai Medical Center of Greater Miami Miami Beach, Florida	12/1/85-3/31/87	35,839.61	46,111.43
386I	"Phase II Poly NET 90 Injectable Study" Giuseppe Benagiano, M.D. Associazione per Studio della Riproduzione Umana Rome, Italy	12/1/85-3/31/87	14,914.00	18,249.00
386J	"Phase II Poly NET 90 Injectable Study" Andrew M. Kaunitz, M.D. University Hospital of Jacksonville Jacksonville, Florida	12/1/85-3/31/87	18,278.33	23,528.00
386M	"Phase II Poly NET 90 Injectable Study" Roberto Rivera, M.D. Instituto de Investigacion Cientifica Durango, Durango, Mexico	12/1/85-3/31/87	18,760.00	22,267.00

Subagreements Terminated During the Period (cont'd)

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	INCLUSIVE PROJECT DATES	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
387N	"Phase II Poly NET 90 Injectable Study - Pharmacokinetics" Brij B. Saxena, Ph.D., D.Sc. Cornell University Medical College New York, New York	12/1/85- 3/31/87	$ 85,953.46	$ 99,532.00
387P	"Phase II Poly NET 90 Injectable Study - Pharmacokinetics" David Archer, M.D. Magee Women's Hospital Pittsburgh, Pennsylvania	12/1/85- 3/31/87	82,195.37	95,951.00
387T	"Phase I Poly NET 90 Injectable Study - Pharmacokinetics" Jose P. Balmaceda, M.D. The University of Texas Health Science Center San Antonio, Texas	12/1/85- 11/30/86	68,192.79	68,192.79
388	"Preparation and Characterization of Poly NET 30 Microspheres for Phase I Clinical Study" Danny H. Lewis, Ph.D. Stolle Research & Development Corp. Decatur, Alabama	11/1/85- 3/31/86	11,724.00	29,307.00
388M	"Phase I Poly NET 30 Injectable Study" Roberto Rivera, M.D. Instituto de Investigacion Cientifica Durango, Durango, Mexico	3/1/86- 2/28/87	59,185.00	59,185.00
391	"Phase I Progesterone Microcapsule Clinical Trial" Robert W. Rebar, M.D. Northwestern University Chicago, Illinois	9/1/86- 3/31/87	35,164.27	35,164.27
391C	"Progesterone Microcapsules for Nursing Women" Horacio B. Croxatto, M.D. Instituto Chleno de Medicina Reproductiva Santiago, Chile	6/1/86- 3/31/87	8,094.00	8,094.00
CD-RI-001	"Training and Collaboration in Molecular Biology" William W. Chin, M.D. Joslin Diabetes Center Boston, Massachusetts	10/1/85- 6/30/86	6,382.00	33,350.08

Subagreements Terminated During the Period (cont'd)

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	INCLUSIVE PROJECT DATES	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
CD-RI-002	"Training and Collaboration in Bovine and Murine In Vitro Fertilization and Gamete Culture" Neal L. First, Ph.D. University of Wisconsin - Madison Madison, Wisconsin	10/21/85-3/31/86	$ 5,119.12	$ 7,249.12
CD-RI-005	"Training and Collaboration in Immunocontraception" Deborah Anderson, Ph.D. Brigham and Women's Hospital Boston, Massachusetts	4/27/86-6/30/86	7,835.39	7,835.39
CD-RI-006	"Training and Collaboration in Immunocontraception" Anthony G. Sacco, Ph.D. Wayne State University Detroit, Michigan	7/1/86-11/30/86	15,241.34	15,241.34
CD-RI-007	"Training and Collaboration in Immunocontraception" Erwin Goldberg, Ph.D. Northwestern University Evanston, Illinois	7/14/86-9/13/86	17,927.00	17,927.00
CD-RI-010	"Training and Cojllaboration in Peptide Synthesis and Purification" Jean Rivier, Ph.D. The Salk Institute La Jolla, California	1/1/87-6/30/87	23,280.00	23,280.00

Subagreements Terminated During the Period (cont'd)

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	INCLUSIVE PROJECT DATES	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
Direct Payments				
D-69	American Scientific Products Sarstedt, Inc. - Blood sampling tubes		$ 368.66	$ 1,738.94
D-70	Serono Diagnostics, Inc. Vira Chemicals, Inc. - RIA reagents		13,175.67	33,059.57
D-71	Scientific Supply Company - Pregnancy tests		1,023.75	3,439.40
D-74	Stolle Research & Development Corp. - NET RIAs		32,420.00	33,090.00
D-75	University of South Alabama - Histopathology studies		12,480.00	35,100.00
D-76	Cornell University Medical College - NET RIAs		1,000.00	1,000.00
D-77	Donald P. Waller, Ph.D. - Preparation of IND		3,600.00	3,600.00
D-78	Danny H. Lewis, Ph.D. - Preparation of documents for FDA		3,000.00	3,000.00

PERSONNEL

Effort and salary expenditures of PARFR personnel for this reporting period 7/1/86-6/30/87 are listed below:

Staff and Title	Effort in Man-Months	Salary
John J. Sciarra, M.D., Ph.D. Principal Investigator	1.2	$ 6,794.04
Gerald I. Zatuchni, M.D., M.Sc. Director	10.2	57,689.04
Alfredo Goldsmith, M.D., M.P.H. Head, Research Project Development	11.4	64,482.84
Diane Krier-Morrow, M.B.A.* Director of Administration	3.0	12,470.02
Mary Nemeth Administrator	12.0	20,700.83
Ruvenia Thomas Secretary II	12.0	23,700.86
Asenath Williamson** Secretary I	6.7	10,518.27
Josephine Harris*** Secretary I	3.2	5,189.62
Fringe Benefits	$39,365.93	

* Terminated 9/30/86.
** Terminated 1/9/87.
*** Terminated 10/5/86.

CONSULTANTS

For the period 7/1/86-6/30/87, the following is a list of Program Consultants, indicating their areas of expertise, contributions to the program, and payment thereof.

Consultant	Purpose	Effort	Fee
David A. Edelman, Ph.D. Biostatistician	Consultant Services 4/1/-6/30/86	16.5 days	$3,877.50
	Consultant Services 7/8-8/11/86	2.5 days	587.50
Forrest C. Greenslade, Ph.D. Regulatory Affairs Specialist	Consultant Services 4/15-5/3/86	1 day	251.27
Denise T. Resnik Regulatory Affairs Specialist	Consultant Services 4/15-5/3/86	4 days	900.00
Donald P. Waller, Ph.D. Reproductive Toxicologist	Consultant Services 7/14/86	1 day	50.00
		TOTAL	$5,666.27

BUDGET AND EXPENDITURES UNDER AID/DPE-0546-A-00-1003-00,
EFFECTIVE 7/1/81-6/30/87

TOTAL APPROPRIATION: $11,160,000
($10,460,000 PARFR and $700,000 add-on Indo-U.S. Immunology Agreement)

Category	Budget Appropriation	7/1/81 - 6/30/87 Total Expenditures
Research	$ 6,075,123	$ 6,075,122.62
Workshops and Publications	451,455	451,454.53
Consultants	158,542	158,542.16
Travel	484,159	484,159.09
Salaries and Fringe Benefits	1,521,831	1,521,831.43
Supplies, Communications and Rent	729,893	729,893.31
Equipment	12,511	12,511.35
Indirect Costs	1,026,486	1,026,485.51
Indo-U.S. Agreement	700,000	700,000.00
	$11,160,000	$11,160,000.00

EXPENDITURES UNDER AID/DPE-0546-A-00-1003-00
DURING THE PERIOD 7/1/86-6/30/87

Category	Expended 7/1/81-6/30/86	Expended 7/1/86-6/30/87	Total Expended 7/1/81-6/30/87
Research	$4,792,801.84	$1,282,320.78	$ 6,075,122.62
Workshops & Publications	451,454.53	- 0 -	451,454.53
Consultants	145,275.89	13,266.27	158,542.16
Travel	464,778.91	19,380.18	484,159.09
Salaries & Fringe Benefits	1,280,919.98	240,911.45	1,521,831.43
Supplies, Communications and Rent	625,090.86	104,802.45	729,893.31
Equipment	12,511.35	- 0 -	12,511.35
Indirect Costs	900,146.62	126,338.89	1,026,485.51
Indo-U.S. Agreement	165,499.61	534,500.39	700,000.00
TOTAL:	$8,838,479.59	$2,321,520.41	$11,160,000.00

BUDGET AND EXPENDITURES UNDER THE PARFR INDO-US IMMUNOLOGY AGREEMENT
AID/DPE-0546-A-00-1003-00
EFFECTIVE 9/1/85-6/30/87

TOTAL APPROPRIATION: $700,000

Category	Budget Appropriation	9/1/85 - 6/30/87 Total Expenditures
Indirect Costs	$ 49,147	$ 49,146.33
Supplies	58,424	58,424.09
Equipment	390,058	390,058.28
Travel	97,411	97,411.37
Subagreements	104,960	104,959.93
TOTAL:	$700,000	$700,000.00

EXPENDITURES UNDER THE PARFR INDO-US IMMUNOLOGY AGREEMENT
DURING THE PERIOD 7/1/86-6/30/87
AID/DPE-0546-A-00-1003-00

Category	Expended 9/1/85-6/30/86	Expended 7/1/86-6/30/87	Total Expended 9/1/85-6/30/87
Indirect Costs	$ 10,251.76	$ 38,894.57	$ 49,146.33
Supplies	15,792.27	42,631.82	58,424.09
Equipment	97,637.49	292,420.79	390,058.28
Travel	12,720.01	84,691.36	97,411.37
Subagreements	29,098.08	75,861.85	104,959.93
TOTAL:	$165,499.61	$534,500.39	$700,000.00

EQUIPMENT PURCHASED FOR THE NATIONAL INSTITUTE OF IMMUNOLOGY
UNDER THE PARFR INDO-U.S. IMMUNOLOGY AGREEMENT

Company	Brief Description	Cost
General Electric	Ultrasound equipment	$ 50,150.00
Precision Systems	Osmometer	4,083.00
Forma Scientific	CO_2 Incubators	8,151.00
Bachofer Gmbh	Micro-manipulator	10,595.00
GCA Corporation	Cell fusion system	16,573.22
Schueler and Company	Pipette puller	3,006.00
Medical System Corporation	Microforge	4,179.99
Fisher Scientific Products	Microcentrifuge	795.00
C. Reichert Optische Werks	Ultramicrotome	18,926.75
Jeol Ltd.	Microscope	72,868.99
Polysciences, Inc.	Microslicer and knife	9,520.00
Reichert Jung	Microtome	11,166.14
American Scientific Products	Pipette	2,175.43
Thomas Scientific	Powerpods	17,501.26
Cryo Resources	Semen analyzer	52,476.00
International Biotechnologies	DNA Sequencing Apparatus	2,960.00
Beckman Instruments	Computer software	3,845.00
Applied Biosystems	Protein sequencer	100,000.00
Pharmacia, Inc.	Power supply	1,103.50
		$390,058.28

TECHNICAL REPORT PROJECT SUMMARIES

The following final technical report summaries are included here; the complete final technical reports are included in Volumes II and III.

PARFR #	P.I.	Institution	Title	Page
309	Robert T. Chatterton, Ph.D.	Northwestern University	"Ovulation Inhibition by Anordrin"	52
309a	Donald P. Waller, Ph.D.	The University of Illinois at Chicago	"Toxicity Studies of Anordrin"	54
315	Erwin Goldberg, Ph.D.	Northwestern University	"Immunologic Suppression of Fertility by Synthetic Determinants of Lactate Dehydrogenase-C_4, Extension of PARFR-232"	55
337T	Rochelle N. Shain, Ph.D.	The University of Texas Health Science Center at San Antonio	"Intracervical Device Acceptability Study"	56
338	Lourens J.D. Zaneveld, D.V.M., Ph.D.	Rush-Presbyterian-St. Luke's Medical Center	"Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives"	57
338UI	Donald P. Waller, Ph.D.	The University of Illinois at Chicago	"Toxicology Studies of Acrosin Inhibitors"	58
339	Lourens J.D. Zaneveld, D.V.M., Ph.D.	Rush-Presbyterian-St. Luke's Medical Center	"Shug Device Studies"	59
339UI	Donald P. Waller, Ph.D.	The University of Illinois at Chicago	"Toxicology of Silicone Implanted (SHUG) in the Vas Deferens"	60
341	Charles E. Flowers, Jr., M.D.	The University of Alabama at Birmingham	"Phase I Poly NET 90 Injectable Study"	61
355	Nancy J. Alexander, Ph.D.	Oregon Regional Primate Research Center	"Enhancement of the Secretory Immune Response to LDH-C_4"	62
356a	Rajesh K. Naz, Ph.D.	The George Washington University	"Development of an Immunocontraceptive Vaccine: Role of 23-Kd Antigen in Immunoinfertility and Fertility Regulation"	63
359	Bonnie S. Dunbar, Ph.D.	Baylor College of Medicine	"Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins"	64

Technical Report Project Summaries (continued)

PARFR #	P.I.	Institution	Title	Page
360	John C.M. Tsibris, Ph.D.	The University of Illinois at Chicago	"Inter- and Intra-Cycle Variation of Genital Peroxidases in Women"	65
361	Ricardo H. Asch, M.D.	The University of Texas Health Science Center at San Antonio	"Testosterone Micro-capsule Formulation Study"	66
362	Danny H. Lewis, Ph.D.	Stolle Research and Development Corp.	"Combination Inject-able Steroidal Micro-sphere - Continuation of PARFR-332"	67
363	John P. Wiebe, Ph.D.	The University of Western Ontario	"Laboratory Studies on an Antispermatogenic Agent - THP for Con-trol of Male Fertiity"	68
364	Francisco J. Rojas, Ph.D.	The University of Texas Health Science Center at San Antonio	"Antifertility Effects of Microencapsulated LHRH Agonist"	69
373	Danny H. Lewis, Ph.D.	Stolle Research and Development Corp.	"90-Day Levonorgestrel Microsphere"	70
375	Kenneth S.K. Tung, M.D.	University of New Mexico	"Baboon Immunologic Evaluation with LDH-C_4 and *In Vitro* Fertilization Studies in Rodents"	71
378	Danny H. Lewis, Ph.D.	Stolle Research and Development Corp.	"Preparation of Testosterone Microspheres"	72
379	Christopher P. Carron, Ph.D.	Duke University Medical Center	"Idiotope Vaccine for Sperm-Targeted Immunocontraception"	73
380	Lourens J.D. Zaneveld, D.V.M., Ph.D.	Rush-Presbyterian-St. Luke's Medical Center	"Preliminary Evalua-tion of 3-M Impression Material as an Inject-able Vas Occlusive Device"	74
382	Gerald S. Bernstein, Ph.D., M.D.	Professional Staff Assn., Los Angeles County, University of Southern California Medical Ctr.	"Postcoital Effective-ness of the Vaginal Spermicidal Barrier"	75

Technical Report Project Summaries (continued)

PARFR #	P.I.	Institution	Title	Page
384	Vernon C. Stevens, Ph.D.	The Ohio State University Research Foundation	"Effects of Immunization of Female Baboons with Zona Pellucida Antigens on Ovarian Function"	76
385	Donald P. Waller, Ph.D.	The University of Illinois at Chicago	"Toxicity Study of an Iodine Transcervical Sterilization Medium"	78
386A	Howard J. Tatum, Ph.D.	Emory University	"Phase II Poly NET 90 Injectable Study"	79
386C	Horacio B. Croxatto, M.D.	Instituto Chileno de Medicina Reproductiva	"Phase II Poly NET 90 Injectable Study"	79
386F	Jerome J. Hoffman, M.D.	Mount Sinai Medical Center of Greater Miami	"Phase II Poly NET 90 Injectable Study"	79
386I	Giuseppe Benagiano, M.D.	Associazione per Studio della Riproduzione Umana	"Phase II Poly NET 90 Injectable Study"	79
386J	Andrew M. Kaunitz, M.D.	University Hospital of Jacksonville	"Phase II Poly NET 90 Injectable Study"	79
386M	Roberto Rivera, M.D.	Instituto de Investigacion Cientifica	"Phase II Poly NET 90 Injectable Study"	79
387N	Brij B. Saxena, Ph.D., D.Sc.	Cornell University Medical College	"Phase II Poly NET 90 Injectable Study - Pharmacokinetics"	80
387P	David Archer, M.D.	Magee-Women's Hospital	"Phase II Poly NET 90 Injectable Study - Pharmacokinetics"	80
387T	Jose P. Balmaceda, M.D.	The University of Texas Health Science Center at San Antonio	"Phase II Poly NET 90 Injectable Study - Pharmacokinetics"	81
388	Danny H. Lewis, Ph.D.	Stolle Research and Development Corp.	"Preparation and Characterization of Poly NET 30 Microspheres for Phase I Clinical Study"	82
388M	Roberto Rivera, M.D.	Instituto de Investigacion Cientifica	"Phase I Poly NET 30 Injectable Study"	83

PARFR #	P.I.	Institution	Title	Page
391	Robert W. Rebar, M.D.	Northwestern University Medical School	"Phase I Progesterone Microcapsule Clinical Trial"	84
391C	Horacio B. Croxatto, M.D.	Instituto Chileno de Medicina Reproductiva	"Progesterone Microcapsules for Nursing Women"	85
	Walter H. Wilborn, Ph.D.	University of South Alabama	"Response of Human Endometrium to 30 mg, 50 mg, 65 mg, and 100 mg of Norethindrone Microspheres: An Analysis by Correlative Light Microscopy, Histochemistry, Transmission Electron Microscopy, and Scanning Electron Microscopy"	86

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 309

TITLE: "Ovulation Inhibition by Anordrin"

INSTITUTION: Northwestern University Medical School

PRINCIPAL INVESTIGATOR: Robert T. Chatterton, Ph.D.

FUNDING PERIOD: 1/1/82-6/30/86 AMOUNT EXPENDED: $300,112

The objective of the studies was to complete preclinical testing of anordrin to comply with reccommendations of the FDA. Pharmacokinetic and metablism studies were conducted and hormones and cholesterol fractions were assayed in serum from animals in the toxicity study.

Three normal female cynomolgus monkeys received a 0.2 mg/kg body weight dose of ^{14}C-anordrin (9.0 mCi/mg) intravenously in a solution of propylene glycol (0.5 mg/ml) on the first day of menses. The duration of amenorrhea was noted and daily urine specimens were analyzed for pregnanediol. After return of menses, 3 monkeys then received the same dose of ^{14}C-anordrin by the intramuscular route in sesame oil (0.5 mg/ml). Another 3 monkeys received a 1.0 mg/kg body weight i.m. dose of ^{14}C-anordrin of the same specific activity. Sixteen blood samples were taken through an indwelling catheter on the day of administration of the dose, and daily blood samples were obtained thereafter until the radioactivity of the serum fell below 100 cpm/0.25 ml. Urine and feces were also collected daily until the level of radioactivity decreased to less than 100 cpm in 0.25 ml of urine. The average duration of amenorrhea after an intravenous dose of 0.2 mg/kg was 49 days; after the same dose i.m. to the same monkeys, the duration of amenorrhea was 60 days. After a 1.0 mg/kg dose i.m., two other monkeys had a period of amenorrhea of 74 days; one that received this dose had been amenorrheic for several months before treatment began and remained amenorrheic thereafter. Generally, menstruation occurred at about the time that the concentration of ^{14}C in serum reached 100 cpm/0.25 ml.

Kinetic analysis of anordrin itself was not possible with the standard procedure because drawing blood, allowing it to clot at room temperature, and temporary storage of the serum at 4°C prior to extraction of steroids was found to result in hydrolysis of one of the esters of anordrin. However, in an experiment in which blood was drawn into chilled, heparinized tubes, immediately centrifuged, and the plasma frozen, the half-life of anordrin was found to be 190 min. Using the standard procedure for sample collection in three monkeys, it was possible to determine the half-life of anordiol as 95.7 ± 6.6 min (S.D.). The steady state volume of distribution of anordiol was 3.0 ± 2.1 L (N=3), and its metabolic clearance rate was 0.035 ± 0.01 L/min. The concentration of anordiol in serum of monkeys receiving the 0.2 mg/kg dose of anordrin via an intramuscular route was so low that a good estimate of the relative availability is not possible. The peak concentration of

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL TECHNICAL REPORT SUMMARY

PARFR- 309 (CONTINUED)

TITLE:

INSTITUTION:

PRINCIPAL INVESTIGATOR: Robert T. Chatterton, Ph.D.

FUNDING PERIOD: AMOUNT EXPENDED:

anordiol in serum after 1.0 mg/kg dose of anordrin occurred as early as 1 day after injection and as late as 9 days, showing a large biological variation, but the concentration remained at a fairly level pleateau during this time in all monkeys.

Toxicity was evaluated by Dr. D. Waller at the University of Illinois. Monkeys, dogs, and rats received anordrin intramuscularly in sesame oil in schedules recommended by the FDA. Dr. Chatterton has measured cholesterol, HDL, prolactin, estradiol, testosterone, progesterone, cortisol, thryroxine, and calcitonin in the monkeys before treatment, and at monthly intervals just prior to each of the 3 administrations of anordrin and a month after the last injection. Cholesterol and HDL were also measured in rats before and after 90 days of anordrin treatment. In monkeys none of the measured hormones or lipoproteins were significantly altered by anordrin except for estradiol and cortisol. Reassay of estradiol with a more specific antiserum resulted in no significant change in the levels of this steroid hormone. Evidently, some metabolite(s) of anordrin other than anordiol, which was tested, cross-reacted with estradiol in the initial assay. The same possibility must be investigated with respect to cortisol.

Both cholesterol and HDL were significantly decreased in rats receiving anordrin. The concentration of LDL, calculated by difference, was not changed by the treatment.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL TECHNICAL REPORT SUMMARY

PARFR- 309a

TITLE: "Toxicity Studies of Anordrin"

INSTITUTION: The University of Illinois at Chicago

PRINCIPAL INVESTIGATOR: Donald P. Waller, Ph.D.

FUNDING PERIOD: 7/1/85-9/30/86 AMOUNT EXPENDED: $93,313

A 90-day subchronic toxicity study of anordrin administered intramuscularly was performed in three species. Sprague Dawley rats were dosed every fourth day (0, 0.8, 4 or 20 mg/kg), dogs (0, 0.4 and 1 mg/kg) and monkeys (0, 0.4, 4, 20 mg/kg) once every 30 days during the 90 day study period. Animals were weighed before each dosing or weekly. Blood samples were obtained at the end of the study for hormonal profiles. The animals were euthanized on the 91st day and major tissues were histopathologically examined. No major organ toxicity was observed, and histological changes were limited to the mammary tissue of all three species. These changes included mammary gland hyperplasia and lactiferous secretions. No major changes in normal profile were observed. These data support the nontoxic nature of anordrin.

SIGNATURE DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 315

TITLE: "Immunologic Suppression of Fertility by Synthetic Antigenic Determinants of Lactate Dehydrogenase-C_4, Extension of PARFR-23

INSTITUTION: Northwestern University

PRINCIPAL INVESTIGATOR: Erwin Goldberg, Ph.D.

FUNDING PERIOD: 3/1/82-12/31/86 AMOUNT EXPENDED: $466,995

The P.I. has prepared synthetic antigenic peptides of the sperm specific LDH-C_4 isozyme to elicit an immune response to the intact molecule that included a significant suppression of fertility. The P.I. has now solved the sequence of the human LDH-C_4 isozyme by cloning and sequencing cDNA from a human testes library. The peptide HC5-15 has been synthesized. In addition, two surface simulation peptides were designed, synthesized and tested for immunogenicity.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 337T

TITLE: "Intracervical Device Acceptability Study"

INSTITUTION: The University of Texas Health Science Center at San Antonio

PRINCIPAL INVESTIGATOR: Rochelle N. Shain, Ph.D.

FUNDING PERIOD: 6/1/83-6/30/86 AMOUNT EXPENDED: $12,420.68

To date a total of 820 questionnaires have been received from Finland. These include 295 initial inteviews (110-ICD, 100-LNG IUD, and 85-Nova T); 285 3-month follow-up interviews (106-ICD, 98-LNG IUD, and 81 Nova T); and 243 12-month interviews (92-ICD, 86-LNG IUD, and 65-Nova T). A total of 799 instruments have been coded and their data analyzed. Preliminary results from the 12-month follow-up data indicate that both the ICD and LNG IUD devices were associated with higher and shorter menses, more irregular cycles, more spotting, less cramping and some amenorrhea. The Nova T was associated with heavier and longer menses, more cramping, more spotting and more irregular cycling. On many 12-month follow-up measures (cannot feel the device, husbands cannot feel the device, change in desire for intercourse, have good or mostly good feelings about the device, dissatisfied with device, would recommend to friends), there were no significant differences between groups. However, a 12-months, 15% of ICD users, compared to only 6% of LNG IUD and 5% of Nova T users (p=.04), perceived especially bothersome side effects. Moreover, by the 12-month interval 24% of ICD users, compared to 8% of LNG IUD users and 9% of Nova T users (p= U.001), had discontinued their device and 8%, 1%, and [illegible] ICD, LNG and Nova T users, respectively, had expelled their device.

Although the ICD sample had experienced significantly m[illegible] discontinuations than the two control groups, ICD users in general were significantly more satisfied than the controls with their current device compared to their previously used IUDs. This seeming discrepancy is explained by group differences in prior contraceptive behavior patterns. Significantly more ICD users than members of the other two control groups volunteered for the present study because they disliked all other methods, were dissatisfied with the last method they used, were dissatisfied with their prior IUD, experienced unacceptable side effects from their prior IUD, and were dissatisfied with prior mechanical methods. Additionally, this group discontinued significantly more contraceptive methods because of dissatisfaction. As noted in previous reports, the ICD group as a whole appeared to be considerably more particular about contracetive methods in general and/or sensitive to side effects. This finding was confirmed by Tapani Luukkainen, M.D.

After all remaining interviews are received from Finland, multivariate regression analysis and analyses of covariance will be performed to determine the extent to which prior contraceptive behavior has influenced the higher discontinuation rates shown by ICD sample members and whether controlling for this behavior eliminates group differences in discontinuation.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 338

TITLE: "Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives

INSTITUTION: Rush-Presbyterian-St. Luke's Medical Center

PRINCIPAL INVESTIGATOR: Lourens J.D. Zaneveld, D.V.M., Ph.D.

FUNDING PERIOD: 7/1/83-3/31/87 AMOUNT EXPENDED: $199,931

The overall objective of this research is to develop a new contraceptive agent that is more potent and less toxic than nonoxynol-9, the most frequently used active ingredient of marketed vaginal contraceptives. It is well known that the sperm enzyme acrosin is essential for fertility and that acrosin inhibitors prevent fertilization. Therefore, acrosin inhibitors were synthesized, specifically aryl 4-guanidinobenzoates that possessed an already marketed phenol, and tested for their contraceptive and toxic properties. Our most recent efforts were aimed at obtaining all data necessary for submission of an IND to the FDA for a Phase I clinical trial with the optimal aryl 4-guanidinobenzoate. This has been accomplished and an IND is presently in preparation.

The vaginal contraceptive potency of AGB in the rabbit model is about 100 times higher than that of nonoxynol-9. The acute toxicity of AGB in mice and rats is about 5 times less than that of nonoxynol-9. AGB shows minimal or no vaginal irritation when tested in the rabbit model in contrast to nonoxynol-9. A mixture of AGB and nonoxynol-9 is no more irritating to the rabbit vagina than nonoxynol-9 alone. Thirty day or 50 day vaginal application of AGB to rats causes no histopathological changes in any of the organs and tissues examined, including the vagina. AGB is nonmutagenic as shown by the Ames test.

With the cooperation of the VLI, Santa Anna, CA, the Today sponge has been developed as a vaginal delivery system for AGB. The chemical and biochemical properties of AGB are not altered by the construction of the sponges whether or not nonoxynol-9 is also incorporated into the sponge. The release rate of AGB from the sponge is adequate for clinical use. A somewhat higher release rate of AGB is obtained when both nonxoynol-9 and AGB are incorporated into the sponge as compared to AGB alone.

From these preclinical studies, it can be concluded that AGB is potentially a highly potent vaginal contraceptive for women with minimal toxicity and vaginal irritation, and has a much higher therapeutic index than nonoxynol-9. Its chemical properties are such that it interacts rapidly with spermatozoa, that it remains bound to the sperm once interaction has taken place, and that it maintains stable in the vagina. For the Phase I clinical trial, AGB can be delivered in adequate concentrations to the vagina utilizing the Today sponge.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 338UI

TITLE: "Toxicology Studies of Acrosin Inhibitors"

INSTITUTION: The University of Illinois at Chicago

PRINCIPAL INVESTIGATOR: Donald P. Waller, Ph.D.

FUNDING PERIOD: 7/1/84-12/31/86 AMOUNT EXPENDED: $84,866

During the reporting period the difficulties in establishing an appropriate route of administration for the 30 day suchronic toxicity testing of AGB was resolved by discussion with the FDA.

A meeting at the FDA in Washington was arranged for May, 1986 to discuss the work previously performed on the AGB compound and carefully establish the requirements to complete preclinical toxicology testing for the AGB contraceptive.

The problem of the route to be utilized during the 30 day subchronic testing was addressed during the meeting. It was agreed that the gum acacia suspension could be used for the subchronic testing of AGB. It was also recommended that a group with nonoxynol in combination with AGB be included in the study. There were no major histopathological changes noted in any of the groups administered AGB vaginally.

All the groups receiving intraperitoneal injections of AGB or vehicle had peritonitis. This was a result of the daily injections into the peritoneal cavity and not considered drug related.

The most significant finding was an increase in dose-group associated dgenerative changes inthe kidneys for groups dosed with AGB. Similar changes were not noted in the vehicle control group of the group administered nonoxynol only. A no effect dose was not established for the renal changes caused by the AGB.

The subacute testing of the AGB demonstrates the relative safety of AGB administered vaginally. The renal damage observed after intraperitoneal administration raises some concern on long-term safety of this agent. More detailed studies will be required to characterize the risk associated with the administration of this drug.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 339

TITLE: "Shug Device Studies"

INSTITUTION: Presbyterian-St. Luke's Medical Center

PRINCIPAL INVESTIGATOR: Lourens J.D. Zaneveld, D.V.M., Ph.D.

FUNDING PERIOD: 7/1/83-3/31/87 AMOUNT EXPENDED: $70,453.83

The overall objective of this research is to develop a reversible vas deferens occlusion device. During previous contract periods such a device was developed and tested in primates. The device is called the Shug and it consists of two silicone plugs held together by a nylon suture. Primate experiments have shown that complete obstruction of sperm transport occurs when the Shug is in place and that all the animals ejaculate spermatozoa again at normal concentrations and motility after removal of the Shug. No side effects were observed.

Based on these results, an IDE was prepared and submitted to the FDA. The FDA had a number of questions, some of which were answered immediately, whereas others required more time. One of these was their requirement that life-time animal studies should be done with silicone in the vas, primarily to assure that no carcinogenic effects would be observed. For these reasons, the goals of our efforts during the past few years were primarily two fold: 1) to perform a long-term toxicity study of silicone in the vas; and 2) to continue responding to the questions by the FDA until the IDE was approved. Additionally, the Shug implantation technique was tested in men and optimized. At present, the toxicity study has almost been completed and FDA approval for the IDE has been obtained. An optimal implantation method for the human has been developed.

Implantation of the Shug was tested in 10 human volunteers at Propater, Sao Paulo, Brazil, under the direction of Dr. M. De Castro. This was an acute study employing men who were undergoing a vasectomy and the device was removed immediately after implantation. The technique which worked well in primates, was unsatisfactory for human use. Modifications were made and the implantation method that is now available is simple and requires no more time than a regular vasectomy. No pain was encountered by the men during implantation.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 339UI

TITLE: "Toxicology Studies of Acrosin Inhibitors"

INSTITUTION: The University of Illinois at Chicago

PRINCIPAL INVESTIGATOR: Donald P. Waller, Ph.D.

FUNDING PERIOD: 7/1/84-12/31/86 AMOUNT EXPENDED: $147,740

A new reversible male contraceptive device (SHUG) made primarily of silicone is being developed. Previous studies demonstrated its ability to block the flow of spermatozoa when placed in the monkey vas deferens. The silicone material must be tested for chronic target organ toxicity prior to clinical trials. Two hundred rats were divided into two groups: one group had sham operations and the other group had silicone noodles implanted in the vas deferens and secured by a suture. At six months and one year after implantation animals were sacrificed and the vas deferens removed, fixed, and histologically evaluated. Changes in the vas deferens attributed to the presence of the silicone were minimal-to-mild reactive hyperplasia in the proximal, surgical and distal segments of a few animals. An increased incidence of spermatoceles, aspermatic granulomas and chronic inflammatory changes within the inner walls of the vas deferens were also observed. These changes in the vas deferens are considered of minor toxicological importance.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 341

TITLE: "Phase I Poly NET 90 Injectable Study"

INSTITUTION: The University of Alabama at Birmingham

PRINCIPAL INVESTIGATOR: Charles E. Flowers, Jr., M.D.

FUNDING PERIOD: 12/1/83-12/30/85 AMOUNT EXPENDED: $76,126.23

The Stolle Research and Development Corporation (Stolle) undertook a series to improve the pharmacokinetics of the Phase II NET microsphere formulation in order to improve the release rates. The release rate was corrected by increasing the concentration of NET in the microspheres. Preclinical animal studies using baboons were conducted at UAB to confirm that the new formulation was suitable for use in the Phase II study. The UAB center agreed to undertake a Phase I dose-response study on the improved microsphere formulation.

Nine subjects were treated with one of three doses of Poly NET 90 to determine the optimal dose for a multicenter Phase II clinical trial using the same formulation. Following treatment with 50, 75, or 100 mg NET in polylactide-co-glycolide (PLGA) microspheres, blood samples were obtained from each subject at selected intervals to determine the NET release rate and effect on endogenous ovarian steroids. Pharmacokinetic data revealed consistent release of NET throughout the 90 day treatment interval, with successful ovulation inhibition in eight of the nine subjects. The ninth subject, who received the low dose (50 mg NET), had progesterone levels suggestive of ovulation in the first three weeks post-treatment. Eight of the nine subjects received a second injection 90 days after the first, and blood samples were obtained for further evaluation of the treatment pharmacokinetics and pharmacodynamics. All subjects remained on the study until they resumed normal menstrual patterns.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 355

TITLE: "Enhancement of the Secretory Immune Response to LDH-C_4"

INSTITUTION: Medical Research Foundation of Oregon

PRINCIPAL INVESTIGATOR: Nancy J. Alexander, Ph.D.

FUNDING PERIOD: 4/15/84-12/31/86 AMOUNT EXPENDED: $98,820.71

The P.I. has tested the ability of two adjuvants, cholera toxin B and muramyl dipeptide (MDP), to increase the secretory immune response to lactate dehydrogenase C_4 (LDH-C_4), a sperm-specific antigen. CD-1 female mice were primed with 100 ug LDH-C_4 in Complete Freund's Adjuvant (CFA) given intramuscularly (i.m.). Agarose plugs containing LDH-C_4 and/or the two adjuvants were surgically implanted into the right uterine horn one week following priming. The right uterine horn was ligated at only the cervical end. Serum, intestinal lavage, and intrauterine fluid samples from the left uterine horn were collected at weekly intervals. Analysis indicated that LDH-C_4 (100 ug) plug both adjuvants provided the highest intrauterine stimulation.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 356a

TITLE: "Development of an Immunocontraceptive Vaccine: Role of 23-Kd Antigen in Immunoinfertility and Fertility Regulation"

INSTITUTION: The George Washington University

PRINCIPAL INVESTIGATOR: Rajesh K. Naz, Ph.D.

FUNDING PERIOD: 12/1/84-6/30/86 AMOUNT EXPENDED: $98,190.16

The overall objective of this project is to investigate the use of FA-1, an antigen derived through the use of monoclonal antisperm antibody, MA-24, as an effective immunocontraceptive, and to investigate the role of FA-1 in involuntary immunoinfertility. It was proposed to characterize the FA-1, to test whether active immunization causes a cessation of fertility in female and male species, and to study the mechanisms of action of antibodies.

Achievements made

1. Development and characterization of the monoclonal antibody, MA-24.
2. Isolation and characterization of the fertilization antigen (FA-1).
3. Effects of active immunization with FA-1 on fertility of female animals.
4. Effects of FA-1 in involuntary immunoinfertility in humans.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 359

TITLE: "Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins"

INSTITUTION: Baylor College of Medicine

PRINCIPAL INVESTIGATOR: Bonnie S. Dunbar, Ph.D.

FUNDING PERIOD: 6/1/84-9/30/86 AMOUNT EXPENDED: $117,835.66

The P.I. has isolated and purified zona pellucida proteins and have sent samples to Dr. Shoba Seghal (India) and to Dr. Vernon Stevens (Ohio State University) for immunization of rhesus monkeys and baboons. The P.I. has immunized rabbits with a different form of a purified zona pellucida protein. The antibodies developed by these animals have been analyzed and the effects of immunization on ovarian function have been evaluated.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 360

TITLE: "Inter- and Intra-Cycle Variation of Genital Peroxidases in Women"

INSTITUTION: The University of Illinois at Chicago

PRINCIPAL INVESTIGATOR: John C.M. Tsibris, Ph.D.

FUNDING PERIOD: 7/1/84-9/30/86 AMOUNT EXPENDED: $99,817.35

The results show that guaiacol peroxidase (GP) in cervical mucus constitutes a useful parameter in defining the fertile period in women. GP could also serve an an initial diagnostic screening tool of some endocrinological abnormalities of the menstrual cycle. However, further analysis of the present hormone profiles and further experimentation will be required to document the use of GP as a simple diagnostic tool for insufficient progesterone production, prolonged follicular recruitment and possibly preovulatory hyperprolactinemia and endometriosis.

From the self-sampling and "clinical" (colorimetric) studies it becomes apparent that, 1) the women are capable of sampling suitable areas around their cervical os (dye study), 2) more effort is needed to optimize the chemical composition of the Q-tip, and 3) more work is needed to achieve the goal of a two-minute interval between extraction of the swab to full color development.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 361

TITLE: "Testosterone Microcapsule Formulation Study"

INSTITUTION: The University of Texas Health Science Center at San Antonio

PRINCIPAL INVESTIGATOR: Ricardo H. Asch, M.D.

FUNDING PERIOD: 7/1/84-6/30/86 AMOUNT EXPENDED: $106,839.85

FINAL TECHNICAL REPORT NOT RECEIVED.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 362

TITLE: "Combination Injectable Steroidal Microsphere - Continuation of PARFR-332"

INSTITUTION: Stolle Research and Development Corporation

PRINCIPAL INVESTIGATOR: Danny H. Lewis, Ph.D.

FUNDING PERIOD: 10/1/84-11/30/85 AMOUNT EXPENDED: $96,020

SEE IND #28,263. FINAL TECHNICAL REPORT NOT RECEIVED.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 363

TITLE: "Laboratory Studies on an Antispermatogenic Agent - THP for Control of Male Fertility"

INSTITUTION: The University of Western Ontario

PRINCIPAL INVESTIGATOR: John P. Wiebe, Ph.D.

FUNDING PERIOD: 10/1/84-12/31/86 AMOUNT EXPENDED: $93,968.97

The overall objective of the project was to determine the anti-fertility effects of intratesticular injections of 1,2,3-trihydroxypropane (THP; glycerol) solution in small mammals and subhuman primates. It was determined that THP exerts a dose-response on spermatogenic arrest and that the volume of THP injected must be adequate for dispersion of the substance throughout the testis. In adult rats 200 ul per testis of a 35-70% THP solution resulted in sutained infertility for at least 16 months. Histological evidence indicates that the treatment may be partially reversible in some instances and further work is required to establish the appropriate treatment regimens. Experiments with ^{14}C-THP have shown that the substance is 99.8% removed from the testes by 24 hours after an injection. An increase up to about 13% of the injected radioactivity is observed in the blood at 30 minutes, followed by a rapid decline. Some of the THP is metabolized to CO_2 and abut 10% of the radioactivity, appears in the urine and feces. Relatively small amounts ($<$ 2%) appear in the epididymides, prostate, seminal vesicles, kidney, liver, and body fat during the first 30 minutes after an injection and then rapdily decline to mere traces. Experiments to explore the possible mechanism of action of THP have shown that the tight junctions between Sertoli cells do not appear to be disrupted by the THP treatment and that the effect may be due to osmotic shock. Five separate experiments examined the effects of THP on gonadotropin receptors and serum gonadotropin levels from 1 to 120 days after the injection. The results indicate increases in serum LH and FSH by 4-8 days after the treatment and concomitant reduction in LH and FSH receptors in the testes; by 15-32 days after the treatment, the gonadotropins and the receptors have returned to control values and remain the same as the controls thereafter. Experiment with rabbits have shown that THP significantly reduces sperm formation and results in complete infertility in this species but that several injections appear to be necessary. Levels of androgen binding proteins in testicular tissues of rats and rabbits were not altered by the THP treatment. Experiments in progress with Squirrel monkeys show that a single injection abolishes sperm in ejaculates for at least 7 months (longest sampling to date) and that the serum hormone levels are not altered. Investigations to date have shown that THP has the potential of acting an an antifertility agent, not only in rats, but also in rabbits and monkeys and that the endocrine systems, libido, and accessory structures remain unaffected and no untoward side-effects are noted.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 364

TITLE: "Antifertility Effects of Microencapsulated LHRH Agonist"

INSTITUTION: The University of Texas Health Science Center at San Antonio

PRINCIPAL INVESTIGATOR: Francisco J. Rojas, Ph.D.

FUNDING PERIOD: 9/1/84-6/30/86 AMOUNT EXPENDED: $30,496.64

The present study was carried out with monkeys that underwent at least two consecutive normal, ovulatory menstrual cycles.

A single s.c. injection of D-Trp-6-LH-RH microcapsules every 30 days for 8 months resulted in inhibition of normal ovulation during treatment in all animals as determined by laparoscopy and hormone analysis.

Three of the five monkeys receiving D-Trp-6-LH-RH microcapsules only were amenorrheic. One monkey had uterine bleeding for two days on one occasion, another had one-day bleeding, twice, during the 8 months of treatment.

In the group receiving D-Trp-6-LH-RH microcapsules plus a progesterone challenge on day 25 of each month, four of the five monkeys had either cyclical or irregular uterine bleedings during treatment. One monkey had uterine bleeding only once.

All control animals presented monthly ovulatory cycles.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 373

TITLE: "90-Day Levonorgestrel Microspheres"

INSTITUTION: Stolle Research and Development Corporation

PRINCIPAL INVESTIGATOR: Danny H. Lewis, Ph.D.

FUNDING PERIOD: 4/1/85-3/31/86 AMOUNT EXPENDED: $67,404

The objective of this project is the development of a 90-day injectable microsphere system for the delivery of levonorgestrel. The availability of such a product would allow a direct comparison of levonorgestrel, norgestimate, and norethisterone to be made from basically the same controlled-release systems. The same general microencapsulation process and the same biodegradable polymeric excipient is involved in all three of the injectable contraceptive products. The microencapsulation process is the property of Ortho Pharmaceutical Corporation. Stolle R&D received permission from Ortho to produce a levonorgestrel formulation in order that a clinical comparison could be made of the three steroids. It is important to state that any further development of a levonorgestrel system by Stolle R&D would require authorization from Ortho.

The P.I. synthesized the appropriate 85:15 copolymer excipient, and developed a satisfactory microencapsulation procedure for levonorgestrel, characterized the prototype product in vitro, and conducted a pharmacokinetic study in the baboon model. The P.I. found that levonorgestrel can be formulated according to the Stolle/Ortho process to afford good controlled-release chaacteristics in vivo. The prototype formulation is quite similar to the 90-day norethisterone product in regard to microsphere size, distribution, visual appearance, and drug content. The baboon studies confirmed that the test material offers potential as a 90-day injectable contraceptive product.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 375

TITLE: "Baboon Immunologic Evaluation with LDH-C_4 and In Vitro Fertilization Studies in Rodens"

INSTITUTION: University of New Mexico

PRINCIPAL INVESTIGATOR: Kenneth S.K. Tung, M.D.

FUNDING PERIOD: 5/1/85-9/30/86 AMOUNT EXPENDED: $62,576

From detailed immunopathologic and clinical laboratory investigations on female baboons immunized with LDH-C_4 peptides 5-15, the P.I. did not detect any difference between LDH-X immunized baboons and untreated controls. All baboons had some evidence of hepatic dysfunction (hypergammaglobulinemia, anti-smooth muscle antibody, elevated LDH) and IgM deposits in the glomerular mesangia and Kupfer cells, suggestive of hepatic parasitic infection. Some of the anti-LDH-C_4 monoclonal antibodies from Dr. Goldberg were found to inhibit in vitro fertilization in mice. An experimental model for evaluating effects of antibody on fertility in vivo in mice was developed. Finally, injection of interleukin 2 (IL-2) was found to have profound adjuvant effect on anti-LDH-C_4 antibody response. Ongoing studies include detection of cell-mediated immune response to LDH-C_4, evaluation of in vivo effect of anti-LDH-C_4 antibody on mouse fertility, and ultrastructural localization of LDH-C_4 on mouse sperm.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 378

TITLE: "Preparation of Testosterone Microspheres"

INSTITUTION: Stolle Research and Development Corporation

PRINCIPAL INVESTIGATOR: Danny H. Lewis, Ph.D.

FUNDING PERIOD: 7/1/85-9/30/86 AMOUNT EXPENDED: $39,233

SEE IND #27,826. FINAL TECHNICAL REPORT NOT RECEIVED.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 379

TITLE: "Idiotope Vaccine for Sperm-Targeted Immunocontraception"

INSTITUTION: Duke University Medical Center

PRINCIPAL INVESTIGATOR: Christopher P. Carron, Ph.D.

FUNDING PERIOD: 8/1/85-9/30/86 AMOUNT EXPENDED: $67,143.52

The P.I. has examined the feasibility of inducing antigen-specific antisperm antibodies in mice by active immunization with rabbit antibodies prepared against the idiotype of murine monoclonal antisperm antibodies. While the P.I. has originally selected 4 different antibodies upon which to concentrate, the P.I. ultimately narrowed this to 2 mAbs, M29.6 and M42.15, because of practical considerations and because these 2 mAbs inhibit fertilization *in vivo* and *in vitro*. Sera from rabbits inoculated with purified mAbs were absorbed with normal mouse and isotype-specific immunoglobulin (Ig) and the anti-idiotype Ig fraction (Ab2) isolated by Protein A-chromatography. Binding specificity of Ab2 M42 for M42 was confirmed by measuring the reactivity of Ab2 with homologous and heterologous Mabs in an ELISA. Ab2 M42 competitively inhibited ^{125}I-labeled M42 binding to mouse sperm. Sperm extracts containing soluble M42 antigen inhibited binding of Ab2 to Abl by 70-75% suggesting that the Ab2 possessed subpopulations directed against sites similar or adjacent to the antigen-binding site of M42. Ab2 reactive with the antigen-combining site of M42 may cortain subpopulations which mimic the mouse sperm epitope recognized by M42. Immunization with Ab2 induced Ab3 which react with native antigen: mice immunized with Ab2 M42 develop antibodies (Ab3) which competitively inhibit binding of ^{125}I-labeled M42 to immobolized Ab2 M42. Similar results have been obtained with M29 Mab.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 380

TITLE: "Preliminary Evaluation of 3-M Impression Material as an Injectable Vas Occlusive Device"

INSTITUTION: Rush-Presbyterian-St. Luke's Medical Center

PRINCIPAL INVESTIGATOR: Lourens J.D. Zaneveld, D.V.M., Ph.D.

FUNDING PERIOD: 8/1/85-4/30/86 AMOUNT EXPENDED: $10,832

3-M Impression Material (vinyl polysiloxane impression material, light body-regular set, type 1, low viscosity; Dental Products, 3M, St. Paul, MN) was injected into the vasa of 4 dogs resulting in the obstruction of sperm transport. Five to six months after implantation, the dogs were euthanized. No gross pathological lesions were seen on autopsy. It can be concluded that 3-M Impression Material can prevent sperm transport through the vas without causing excessive pathology, at least for a 5-6 month period.

Before implantation, all the dogs produced semen samples that contained spermatozoa although ejaculation was somewhat erratic. After implantation of the 3-M Impression Material, several of the dogs were hesitant to ejaculate and one never ejaculated again no matter what was tried. Thus, postimplantation semen analyses are only available on three dogs.

After implantation, either no spermatozoa were found in the ejaculation, or only a few immotile, mostly decapitated sperm. On autopsy, all vasa appeared intact although some changes due to the implantation technique were occasionally noted. The material remained in place whether or not a suture had been passed through the vas and the material. No lesions were seen in the testis and epididymis, or in any of the abdominal and thoracic organs.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 382

TITLE: "Postcoital Effectiveness of the Vaginal Spermicidal Barrier"

INSTITUTION: Professional Staff Association, Los Angeles County - University of Southern California Medical Center

PRINCIPAL INVESTIGATOR: Gerald S. Bernstein, Ph.D., M.D.

FUNDING PERIOD: 10/1/85-9/30/86 AMOUNT EXPENDED: $3,651.68

The data demonstrate that the VSB had a marked inhibitory effect on sperm transport in the subjects studied. Every subject except one, was able to insert the device properly after a brief instruction session, and the device was in place over the cervix at the time of the post-coital test. These women who had used a diaphragm previously had less difficulty in learning the proper technique for inserting the VSB.

The results of this study suggest that further studies of the VSB are warranted in order to determine tolerance to clinical use and its efficacy in preventing pregnancy.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 384

TITLE: "Effects of Immunization of Female Baboons with Zona Pellucida Antigens on Ovarian Functions"

INSTITUTION: The Ohio State University Research Foundation

PRINCIPAL INVESTIGATOR: Vernon C. Stevens, Ph.D.

FUNDING PERIOD: 7/1/85-4/30/86 AMOUNT EXPENDED: $25,624.05

Studies of ZP-1 Animals

Five adult female baboons exhibiting ovulatory menstrual cycles were selected for study. Three control menstrual cycles were monitored for cycle length, sex skin changes and ovulation evidence by progesterone measurements. During the second of these three cycles, frequent serum samples were collected for estradiol and progesterone level assessment. During menstrual cycle days 1-5 of the fourth cycle, females were immunized with 0.5 mg of ZP-1, together with 0.25 mg of a synthetic adjuvant compound (CGP-11637), intramuscularly at two sites in a total volume of 1.0 ml. Two additional immunizations were given at 28 day intervals and a fourth injection was given at approximately six months after the initial immunization. Serum samples collected at frequent intervals were shipped to Houston for antibody level determinations. Frequent serum samples for estradiol and progesterone assessment were collected during the third cycle from the first immunization. Menstrual cycle parameters were monitored for nine cycles (or time equivalents in those with disrupted cycles) after "high" antibody levels were detected. At the end of this period (August 1985), ovaries from all the females were surgically removed, preserved according to instructions from Dr. Dunbar and shipped to her laboratory for histological evaluation.

Two of the five females exhibited few effects of treatment on cycle length or ovulation, however, the remaining three had at least one cycle of extended duration and/or anovulation. Two of these three became amenorrhic at the end of the study period with no evidence of ovulation.

Data showing levels of serum estradiol and progesterone levels in a control and a post-immunization cycle clearly demonstrate reduced estradiol levels in all animals following immunization while progesterone levels were little effected by this procedure. Progesterone levels were reduced in only one although the post-immunization cycle levels were compatible with normal luteal function. (This female became anovulatory in later cycles.)

Studies of ZP-3 Immunized Animals

The design of these studies was similar to those for ZP-1 antigen excepting a longer control period was used (antigen not available for immunization) and the immunization period extended due to a weak response to immunizations (low antibody levels). Hormone levels were assessed in control cycle 2 but post-immunization levels were not measured until cycles 7-8 from

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 384 (CONTINUED)

TITLE:

INSTITUTION:

PRINCIPAL INVESTIGATOR: Vernon C. Stevens, Ph.D.

FUNDING PERIOD: AMOUNT EXPENDED:

the first immunization. A total of four immunizations were given but these were spaced farther apart than those using ZP-1 due to unavailability of sufficient antigen at a given time.

Fewer effects on cycle length and ovulation were found in these females than those receiving ZP-1 immunizations. Two animals had two and one had one anovulatory cycle but no significant cycle length extensions were observed. While these alterations may have been related to immunizations, one or two anovulatory cycles during a period of 16-18 months is not uncommon in baboons receiving no treatment.

Quantities of ZP-3 antigen available for immunization were lower than for ZP-1 and perhaps for this reason, lower antibody levels were found following the initial immunizations. A final booster injection was made during cycles 6 or 7. Beginning with the onset of menses in the next cycle, frequent blood samples were collected to assess effects of immunization on serum estradiol and progesterone levles. The only observation of altered ovarian function made was an ovulatory in subsequent cycles.

It is apparent that immunizations with the ZP-1 antigen provided clearly interfered with ovarian function in the baboons. Effects found with ZP-3 immunizations were minor, if any, and a careful correlation of antibody levels and the few anovulatory cycles will need to be made before it can be determined whether this antigen holds promise for human antifertility vaccine development.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 385

TITLE: "Toxicity Study of an Iodine Transcervical Sterilization Medium"

INSTITUTION: The Board of Trustees of the University of Illinois"

PRINCIPAL INVESTIGATOR: Donald P. Waller, Ph.D.

FUNDING PERIOD: 11/1/85-6/30/86 AMOUNT EXPENDED: $47,555

A new ICF for the Femcept^R delivery system is being developed for transcervical sterilization of women. This study evaluated the effects of the ICF on the uterus and the peritoneal cavity. The ICF was prepared by BioNexus, Inc. (Raleigh, NC) and contained I_2, KI, glycerol, Vascoray^R and gum tragacanth. Fourteen cynomolgus monkeys were divided into four groups of at least three animals. One group was administered vehicle and another ICF transcervically until the uterine cavity and the fallopian tubes were filled with the dosing medium. Other groups were treated intraperitoneally with vehicle (0.5 ml) and ICF (0.5 ml). Blood was drawn from the animals before and during the experiment. All animals were necropsied 45 days after dosing and tissues examined histologically. No changes in the peritoneal cavity, the uterine tissues or blood parameters, including T_3 and T_4, were noted in any of the test groups compared to control. Histological changes were limited to fibrotic lesions in the lamina propria of the oviducts of monkeys administered ICF transcervically. These studies demonstrate the lack of toxicity of the ICF on the uterus and peritoneal cavity.

SIGNATURE DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 386A, C, F, I, J, M

TITLE: "Safety and Contraceptive Effectiveness of 65 mg and 100 mg of 90-Day Norethindrone Microspheres - Phase II Study"

INSTITUTION: Multi-center

PRINCIPAL INVESTIGATOR: PARFR

FUNDING PERIOD: 12/1/85-3/31/87 AMOUNT EXPENDED: $156,324.53

Ninety-four women were enrolled in a Phase II study to evaluate the safety and effectiveness of 65 mg and 100 mg doses of a 90-day microspheres.

Norethindrone levels were consistently lower for the 65 mg dose and this dose effectively prevented pregnancy for the period covered by this report.

Eleven women have discontinued the study, seven for reasons unrelated to the study product. Four women discontinued because of method-related problems, one because of skin rash (65 mg), one because of migraine headaches (100 mg), one because of amenorrhea (100 mg) and one because of headaches and insomnia (100 mg).

Seven adverse experiences have been reported -- three cases of skin rash (two in the 65 mg group and one in the 100 mg group), two cases of migraine headaches (one in each treatment group) and one each of ringing in the ears (65 mg) and a drop in hemoglobin (65 mg).

Women reported an increase in the number of days of spotting which had returned to baseline after a few months. The number of days of bleeding decreased from baseline.

In general, out-of-range laboratory values observed for women in the study occurred at scattered, irregular times post-injection and in most cases were clinically insignificant.

From an overall laboratory parameter standpoint, both dose levels were safe, with no obvious indication that the higher dose produced more abnormalities than the lower dose.

This study was conducted to determine the optimal Phase III dose of the NET microspheres. On the basis of this study, the 65 mg NET microsphere dose would be the choice.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 387N, P

TITLE: "A Endocrine of 65 mg and 100 mg of 90-Day Norethindrone Microspheres for Controlled Release Steroid Contraception - Phase II Study"

INSTITUTION: Multi-center

PRINCIPAL INVESTIGATOR: PARFR

FUNDING PERIOD: 12/1/85-3/31/87 AMOUNT EXPENDED: $195,483

Thirty-seven women were enrolled in a Phase II study to evaluate the effectiveness, safety and effect on ovarian function of 65 mg and 100 mg doses of 90-day NET microspheres.

Norethindrone levels were similar for the two doses and effectively prevented pregnancy for the period covered by this report.

Two women in the 65 mg treatment group and no wmen in the 100 mg treatment group had progesterone values suggestive of ovulation (> 3 ng/ml) during the 90-day period following the injection.

Four women have discontinued the study; one for mood changes and personal reasons, the second for severe leg pain, the third because she was moving from the area and the fourth because she was unable to return for scheduled testing.

Seven adverse experience considered serious or unanticipated were reported--two cases of skin irritation and one each of severe leg pain, functional cyst, daily headaches, a decrease in hemoglobin and a perforated uterus.

No disturbing side effects have been observed. Women reported an initial increase in the number of days of spotting after the first injection which return to baseline after the first 90 days.

Out-of-range laboratory values observed for women in the study occurred at scattered, irregular times post-injection and in most cases were clinically insignificant.

This study was conducted to determine the optimal Phase III dose of the NET microspheres. On the basis of this study, the 65 mg NET microsphere dose would be the choice.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 387T

TITLE: "Phase I Poly NET 90 Injectable Study - Pharmacokinetics"

INSTITUTION: The University of Texas Health Science Center at San Antonio

PRINCIPAL INVESTIGATOR: Jose P. Balmaceda, M.D.

FUNDING PERIOD: 12/1/85-11/30/86 AMOUNT EXPENDED: $68,192.79

Twenty women were enrolled to evaluate the safety and effect on ovarian function of 30 mg and 50 mg doses of 90-day NET microspheres.

There was an initial rise of NET values shortly after injection which decreased until the third week when values slowly rose and remained constant for at least 11 weeks.

Five women in the 30 mg treatment group and two women in the 50 mg treatment group had progesterone values suggestive of ovulation (> 3 ng/ml) during the 90-day post-injection period.

No disturbing side effects have been observed. Women reported an increase in the number of days of spotting during the 90 days of drug activity; spotting returned to baseline after the first 90 days.

Out-of-range laboratory values observed for women in the study occurred at scattered, irregular times post-injection and were clinically insiginificant.

This study was conducted to assess the safety and potential efficacy of two low doses of the NET microspheres. After the 30 mg dose, 50% of the women had serum progesterone levels suggestive of ovulation, whereas only 20% had ovulatory levels after the 50 mg dose.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL TECHNICAL REPORT SUMMARY

PARFR- 388

TITLE: "Preparation and Characterization of Poly NET 30 Microspheres for Phase I Clinical Study"

INSTITUTION: Stolle Research and Development Corporation

PRINCIPAL INVESTIGATOR: Danny H. Lewis, Ph.D.

FUNDING PERIOD: 11/1/85-3/31/86 AMOUNT EXPENDED: $29,307

The objective of this project was the preparation and preclinical characterization of Poly NET 30 microspheres for a Phase I clinical study. Clinical supplies were produced in the Stolle Parenteral Drug Facility in Cincinnati, Ohio and delivered to PARFR, Northwestern University on March 24, 1986 for distribution to clinical centers. The microspheres were manufactured by the patented Stolle microencapsulation process. The clinical materials were completely characterized in vitro and a short-term in vivo experiment was conducted in two baboons. A formal storage stability study was initiated as a task of this Subagreement at Stolle R&D on the Phase I supplies. The 30-day NET system is essentially developed. Future research and development efforts should be aimed at scale up and stability studies as the basic formulation is now in place.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 388M

TITLE: "Phase I Poly NET 30 Injectable Study"

INSTITUTION: Instituto de Investigacion Cientifica

PRINCIPAL INVESTIGATOR: Roberto Rivera, M.D.

FUNDING PERIOD: 3/1/86-2/28/87 AMOUNT EXPENDED: $59,185.00

Final results are provided in the complete technical report.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 391

TITLE: "Phase I Progesterone Microcapsule Clinical Trial"

INSTITUTION: Northwestern University

PRINCIPAL INVESTIGATOR: Robert W. Rebar, M.D.

FUNDING PERIOD: 9/1/86-3/31/87 AMOUNT EXPENDED: $35,164.27

We have now completed our PARFR funded study utilizing the progesterone microcapsules to determine their effects on the menstrual cycle in seven normal ovulating women. The data are now being analyzed and will be prepared for publication.

The progesterone microcapsules were injected into the seven women early in the follicular phase of their menstrual cycles. Progesterone levels definitely increased in five of the seven women and may have increased in the remaining two subjects. However, in one woman increased progesterone levels could be documented only one month after injection of the microcapsules, and in all progesterone levels increased only slightly, never achieving the expected value of 3 ng/ml.

Three of the women appeared to be anovulatory in the month following injection (two of these for two successive cycles each), and another subject had a cycle with a short luteal phase following administration of the progesterone microcapsules.

These data seem to suggest that progesterone microcapsules might well prove to be an effective form of contraception if appropriate release rates can be achieved. Certainly it would seem that additional research in this area is warranted.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 391C

TITLE: "Progesterone Microcapsules for Nursing Women"

INSTITUTION: Instituto Chileno de Medicina Reproductiva

PRINCIPAL INVESTIGATOR: Horacio B. Croxatto, M.D.

FUNDING PERIOD: 6/1/86-3/31/87 AMOUNT EXPENDED: $8,094

SEE FINAL TECHNICAL REPORT.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

Title: "Response of Human Endometrium to 30 mg, 50 mg, 65 mg, and 100 mg of Norethindrone Microspheres: An Analysis by Correlative Light MIcroscopy, Histochemistry, Transmission Electron Microscopy, and Scanning Electron Microscopy"

INSTITUTION: Multi-center

PRINCIPAL INVESTIGATOR: Walter H. Wilborn, Ph.D.

FUNDING PERIOD: 12/1/85-3/31/87 AMOUNT EXPENDED: $35,100

Biopsies were obtained at three centers. Eighty-nine biopsies were obtained from the 52 volunteers. A baseline, control, or pre-treatment biopsy was obtained from each potential subject. Treated biopsies were obtained from 27 of the 52 subjects.

All baseline and treated biopsies were evaluated by correlative light microscopy (LM), histochemistry, transmission electron microscopy (TEM), and scanning electron microscopy (SEM).

Evaluation of biopsies from 10 subjects at 2 months PI 30 mg NET showed that ovulation had probably been suppressed in all 10 subjects, although 2 biopsies contained inadequate endometrium for definitive evaluation, and 2 biopsies were in the proliferative phase undergoing repair. It appeared that the latter two subjects would have ovulated prior to 90 days PI, and that the remaining 8 subjects would not have ovulated before 90 days PI.

Evaluation of biopsies from 10 subjects at 2 months PI 50 mg NET showed that ovulation had probably been suppressed in all 10 subjects. However, adequate endometrium for definitive evaluation could not be obtained from one subject, and another subject had proliferative, but anovulatory, endometrium.

Bleeding occurred with both the 30 mg and the 50 mg injections, but the amount of bleeding and endometrial damage was far less with the 50 mg dose.

Biopsies were obtained from 6 subjects who had received 2, 65 mg NET injections separated by a 3 month interval. Proliferative endometrium was present in 3 of the 6 biopsies, and it seemed that the return of ovulatory cycles was eminent. Only 1 of the 3 remaining biopsies contained adequate endometrium for definitive evaluation. The endometrium was suppressed. Material available in the other 2 biopsies indicated that the 2 subjects would not have had ovulatory cycles for at least another month.

Biopsies were obtained from 8 subjects who had received 2, 100 mg NET injections separated by a 3 month interval. Two of 8 biopsies contained proliferative endometrium, and the remaining 6 had suppressed endometrium. It was not possible to conclude when the 8 subjects would have had ovulatory cycles.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR-

TITLE:

INSTITUTION:

PRINCIPAL INVESTIGATOR: Walter H. Wilborn, Ph.D. (continued)

FUNDING PERIOD: AMOUNT EXPENDED:

Based on the analyses of all biopsies and the correlation of data obtained by all investigative procedures, it was apparent that the 65 mg dose level of NET elicted a better endometrial response than did the 100 mg dose level. Furthermore, there was less shedding with the 65 mg dose level than with any other dose level tested. All dose levels resulted in microscopically recognizable bleeding. Fluctuations in ovarian activity occurred during continuous NET treatment, and bleeding and repair took place simultaneously.

SIGNATURE

DATE

PD-AAW-974

VOLUME II

PARFR
FINAL REPORT

DPE-0546-A-00-1003-00

July 1, 1986 - June 30, 1987

FINAL TECHNICAL REPORTS

The following final technical reports are included here in Volumes II and III.

PARFR #	P.I.	Institution	Title
VOLUME II			
309	Robert T. Chatterton, Ph.D.	Northwestern University	"Ovulation Inhibition by Anordrin"
309a	Donald P. Waller, Ph.D.	The University of Illinois at Chicago	"Toxicity Studies of Anordrin"
315	Erwin Goldberg, Ph.D.	Northwestern University	"Immunologic Suppression of Fertility by Synthetic Determinants of Lactate Dehydrogenase-C_4, Extension of PARFR-232"
337T	Rochelle N. Shain, Ph.D.	The University of Texas Health Science Center at San Antonio	"Intracervical Device Acceptability Study"
338	Lourens J.D. Zaneveld, D.V.M., Ph.D.	Rush-Presbyterian-St. Luke's Medical Center	"Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives"
338UI	Donald P. Waller, Ph.D.	The University of Illinois at Chicago	"Toxicology Studies of Acrosin Inhibitors"
339	Lourens J.D. Zaneveld, D.V.M., Ph.D.	Rush-Presbyterian-St. Luke's Medical Center	"Shug Device Studies"
339UI	Donald P. Waller, Ph.D.	The University of Illinois at Chicago	"Toxicology of Silicone Implanted (SHUG) in the Vas Deferens"
341	Charles E. Flowers, Jr., M.D.	The University of Alabama at Birmingham	"Phase I Poly NET 90 Injectable Study"
355	Nancy J. Alexander, Ph.D.	Oregon Regional Primate Research Center	"Enhancement of the Secretory Immune Response to LDH-C_4"
356a	Rajesh K. Naz, Ph.D.	The George Washington University	"Development of an Immunocontraceptive Vaccine: Role of 23-Kd Antigen in Immunoinfertility and Fertility Regulation"

Final Technical Reports (continued)

PARFR #	P.I.	Institution	Title
359	Bonnie S. Dunbar, Ph.D.	Baylor College of Medicine	"Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins"
VOLUME III			
360	John C.M. Tsibris, Ph.D.	The University of Illinois at Chicago	"Inter- and Intra-Cycle Variation of Genital Peroxidases in Women"
362	Danny H. Lewis, Ph.D.	Stolle Research and Development Corp.	"Combination Injectable Steroidal Microsphere - Continuation of PARFR-332"
363	John P. Wiebe, Ph.D.	The University of Western Ontario	"Laboratory Studies on an Antispermatogenic Agent - THP for Control of Male Fertiity"
364	Francisco J. Rojas, Ph.D.	The University of Texas Health Science Center at San Antonio	"Antifertility Effects of Microencapsulated LHRH Agonist"
373	Danny H. Lewis, Ph.D.	Stolle Research and Development Corp.	"90-Day Levonorgestrel Microsphere"
375	Kenneth S.K. Tung, M.D.	University of New Mexico	"Baboon Immunologic Evaluation with LDH-C_4 and *In Vitro* Fertilization Studies in Rodents"
378	Danny H. Lewis, Ph.D.	Stolle Research and Development Corp.	"Preparation of Testosterone Microspheres"
379	Christopher P. Carron, Ph.D.	Duke University Medical Center	"Idiotope Vaccine for Sperm-Targeted Immunocontraception"
380	Lourens J.D. Zaneveld, D.V.M., Ph.D.	Rush-Presbyterian-St. Luke's Medical Center	"Preliminary Evaluation of 3-M Impression Material as an Injectable Vas Occlusive Device"

Final Technical Reports (continued)

PARFR #	P.I.	Institution	Title
382	Gerald S. Bernstein, Ph.D., M.D.	Professional Staff Assn., Los Angeles County, University of Southern California Medical Ctr.	"Postcoital Effectiveness of the Vaginal Spermicidal Barrier"
384	Vernon C. Stevens, Ph.D.	The Ohio State University Research Foundation	"Effects of Immunization of Female Baboons with Zona Pellucida Antigens on Ovarian Function"
385	Donald P. Waller, Ph.D.	The University of Illinois at Chicago	"Toxicity Study of an Iodine Transcervical Sterilization Medium"
Poly Net 90 Studies (386 and 387 series)			
	Howard J. Tatum, Ph.D.	Emory University	"Phase II Poly NET 90 Injectable Study"
	Horacio B. Croxatto, M.D.	Instituto Chileno de Medicina Reproductiva	"Phase II Poly NET 90 Injectable Study"
	Jerome J. Hoffman, M.D.	Mount Sinai Medical Center of Greater Miami	"Phase II Poly NET 90 Injectable Study"
	Giuseppe Benagiano, M.D.	Associazione per Studio della Riproduzione Umana	"Phase II Poly NET 90 Injectable Study"
	Andrew M. Kaunitz, M.D.	University Hospital of Jacksonville	"Phase II Poly NET 90 Injectable Study"
	Roberto Rivera, M.D.	Instituto de Investigacion Cientifica	"Phase II Poly NET 90 Injectable Study"
	Brij B. Saxena, Ph.D., D.Sc.	Cornell University Medical College	"Phase II Poly NET 90 Injectable Study - Pharmacokinetics"
	David Archer, M.D.	Magee-Women's Hospital	"Phase II Poly NET 90 Injectable Study - Pharmacokinetics"
	Jose P. Balmaceda, M.D.	The University of Texas Health Science Center at San Antonio	"Phase II Poly NET 90 Injectable Study - Pharmacokinetics"

Final Technical Reports (continued)

PARFR #	P.I.	Institution	Title
388	Danny H. Lewis, Ph.D.	Stolle Research and Development Corp.	"Preparation and Characterization of Poly NET 30 Microspheres for Phase I Clinical Study"
388M	Roberto Rivera, M.D.	Instituto de Investigacion Cientifica	"Phase I Poly NET 30 Injectable Study"
391	Robert W. Rebar, M.D.	Northwestern University Medical School	"Phase I Progesterone Microcapsule Clinical Trial"
391C	Horacio B. Croxatto, M.D.	Instituto Chileno de Medicina Reproductiva	"Progesterone Microcapsules for Nursing Women"
	Walter H. Wilborn, Ph.D.	University of South Alabama	"Response of Human Endometrium to 30 mg, 50 mg, 65 mg, and 100 mg of Norethindrone Microspheres: An Analysis by Correlative Light Microscopy, Histochemistry, Transmission Electron Microscopy, and Scanning Electron Microscopy"

OVULATION INHIBITION BY ANORDRIN

PARFR 309

FINAL REPORT

Principal Investigator

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Ending Date of Current Contract: 6/30/86

Date of Last Technical Report: 11/85

Inclusive Dates of This Report: 7/85 - 6/86

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VII Hormone and lipoprotein concentrations in serum of monkeys and rats (tabular data) and rats

NARRATIVE SUMMARY

The objective of the studies conducted under this contract was to complete preclinical testing of anordrin to comply with recommendations of the FDA. Pharmacokinetic and metabolism studies were conducted at this institution, and hormones and cholesterol fractions were assayed in serum from animals in the toxicity study that was conducted at the College of Pharmacy, University of Illinois.

For pharmacokinetic and metabolism studies [2-^{14}C]anordrin was prepared by Amersham, Inc. This material was recrystallized with 20 mg of authentic anordrin that was synthesized by Dr G. Chettur of IITRI, Chicago. Identity of the labeled and unlabeled materials was confirmed by demonstrating that the specific activity of the mixture remained constant through 3 successive recrystallizations.

Four normal female cynomolgus monkeys received a 0.2 mg/kg body weight dose of ^{14}C-anordrin (9.0 mCi/mg) intravenously in a solution of propylene glycol (0.5 mg/ml) on the first day of menses. One monkey was eliminated because of difficulty with venipuncture. The duration of amenorrhea was noted and daily urine specimens were analyzed for pregnanediol. After return of menses, 3 monkeys then received the same dose of ^{14}C-anordrin by the intramuscular route in sesame oil (0.5 mg/ml). Another 3 monkeys received a 1.0 mg/kg body weight i.m. dose of ^{14}C-anordrin of the same specific activity. Sixteen blood samples

were taken through an indwelling catheter on the day of administration of the dose, and daily blood samples were obtained thereafter until the radioactivity of the serum fell below 100 cpm/0.25 ml. Urine and feces were also collected daily until the level of radioactivity decreased to less than 100 cpm in 0.25 ml of urine. Blood specimens were allowed to clot and were centrifuged. Serum was stored frozen at -20 C until it was analyzed. Urine was completely collected each day from trays that were mounted under the monkey cages. These trays were fitted with screens to filter out food and feces and were sprinkeled with sodium bisulfite each day. The urine was rinsed from the tray with water, and the entire solution was stored frozen in plastic jars.

The average duration of amenorrhea after an intravenous dose of 0.2 mg/kg was 49 days; after the same dose i.m. to the same monkeys, the duration of amenorrhea was 60 days. After a 1.0 mg/kg dose i.m., two other monkeys had a period of amenorrhea of 74 days; one that received this dose had been amenorrheic for several months before treatment began and remained amenorrheic thereafter. Generally, menstruation occurred at about the time that the concentration of ^{14}C in serum reached 100 cpm/0.25 ml.

The total radioactivity of the serum was determined in all samples. Unbound radioactive material was then extracted into diethyl ether and was chromatographed on silica gel thin-layer plates in a solvent system that clearly separated anordrin from the deesterified product 2a,17a-diethinyl-A-nor-androstane-

2B,17B-diol (anordiol) and more polar metabolites. Each ether extract having sufficient radioactivity was separated into compounds having the chromatographic mobility of anordrin, anordiol, a more polar metabolite III, and that which remained on the origin of the plate IV.

A large part of the radioactivity in serum remained unextracted. In samples obtained within a few hours after administration of the dose, most of the material could be extracted into acetone after saturation of the serum with NaCl. Application of the residue of this extract to chromatographic separation revealed that it contained the same compounds as had been separated from the ether extract. Evidently, these compounds were strongly bound in a form that is not readily released into diethyl ether.

Addition of ^{14}C-anordrin to serum <u>in vitro</u> proved that anordrin itself is strongly bound to a serum component that has a molecular weight of approximately 6000 daltons. The bound/free (B/F) ratio of ^{14}C-anordrin incubated with serum for 1 hr at 37°C was 10.4, similar to the ratio of 10.2 in serum after intravenous administration of ^{14}C-anordrin. The stronger binding of anordrin than anordiol to a plasma component probably accounts for its relatively longer half-life (see below).

All samples from monkeys to which ^{14}C-anordrin had been administered had radioactive metabolites that were not extractable into acetone unless the serum was first acidified to pH 3 such as is required for extraction of glucuronide or sulfate

conjugates of steroids. The presumed acid metabolites contained the majority of the ^{14}C after the i.m. administration of ^{14}C-anordrin. After extraction of neutral steroids from the serum, further extraction of acid metabolites resulted in complete removal of all radioactivity from the serum. The structure of the acid metabolites has not been determined.

Compound III has been purified in several chromatographic systems, but has not yet been identified structurally. It and compound IV have much longer serum half-lives <u>in vivo</u> than anordrin.

Kinetic analysis of anordrin itself was not possible with the standard procedure because drawing blood, allowing it to clot at room temperature, and temporary storage of the serum at 4°C prior to extraction of steroids was found to result in hydrolysis of one of the esters of anordrin. However, in an experiment in which blood was drawn into chilled, heparinized tubes, immediately centrifuged, and the plasma frozen, the half-life of anordrin was found to be 190 min. Using the standard procedure for sample collection in three monkeys, it was possible to determine the half-life of anordiol as 95.7 +/- 6.6 min (S.D.). A similar half-life was obtained for anordiol when pure ^{14}C-anordiol was injected in a fourth monkey (95 min). The steady state volume of distribution of anordiol was 3.0 +/- 2.1 L (N=3), and its metabolic clearance rate was 0.035 +/- 0.01 L/min. The concentration of anordiol in serum of monkeys receiving the 0.2 mg/kg dose of anordrin via an intramuscular route was so low that a

good estimate of the relative availability is not possible. The low concentration evidently is because of slow absorption from the injection site since the bioactivity of an intramuscular injection of the equivalent dose was greater than that of the i.v. dose. The peak concentration of anordiol in serum after the 1.0 mg/kg dose of anordrin occurred as early as 1 day after injection and as late as 9 days, showing a large biologic variation, but the concentration remained at a fairly level plateau during this time in all monkeys.

When the half-life of anordiol was compared to the duration of amenorrhea in the three monkeys that received the intravenous dose, a strong negative correlation was found, $r = -0.89$. This is evidence that in measuring the parameters of anordiol we are measuring the properties of administered anordrin that relate importantly to its biological activity. Nevertheless, a half-life of 95 min cannot explain the duration of effect. The effect is related more to the half-life of the total radioactivity in serum after 5 days from the time of injection, a time when the acid metabolites compose the majority of the radioactive compounds. The acid metabolites decrease in a linear first order manner with a half-life of 13.5 days. This is reflected by the eventual first order decrease in the concentration of metabolites in urine of 10 days or more. (The latter value could only be estimated in monkeys that received a higher dose of anordrin intramuscularly). Total excretion of metabolites of anordrin was 65% in feces and 35% in urine.

Toxicity was evaluated by Dr D Waller and his associates at the University of Illinois. Monkeys, dogs, and rats received anordrin intramuscularly in sesame oil in schedules recommended by the FDA. The pathology report will be submitted by Dr Waller. We have measured cholesterol, HDL, prolactin, estradiol, testosterone, progesterone, cortisol, thyroxine, and calcitonin in the monkeys before treatment, and at monthly intervals just prior to each of the 3 administrations of anordrin and a month after the last injection. Cholesterol and HDL were also measured in rats before and after 90 days of anordrin treatment. Rats were treated every fourth day during this time. In monkeys none of the measured hormones or lipoproteins were significantly altered by anordrin except for estradiol and cortisol. Reassay of estradiol with a more specific antiserum resulted in no significant change in the levels of this steroid hormone. Evidently, some metabolite(s) of anordrin other than anordiol, which was tested, cross-reacted with estradiol in the initial assay. The same possibility must be investigated with respect to cortisol.

Both cholesterol and HDL were significantly decreased in rats receiving anordrin. The concentration of LDL, calculated by difference, was not changed by the treatment.

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SUBSTANTIVE TECHNICAL RESEARCH REPORT

Statement of Objectives.

Complete preclinical testing under GLP huidelines to meet FDA requirements for a Phase I clinical trial.

Progress to Date.

A. Pharmacokinetics and Metabolism of Anordrin

I. Preparation of labeled and unlabeled anordrin.

Anordrin (2a,17a-diethynyl-A-nor-5a-androstane-2B,17B-diol dipropionate) was synthesized by Dr. G. Chettur of the Illinois Institute of Technology. The final product was crystallized from methanol. Mass spectra and elemental analysis of the crystalline material was in agreement with the proposed structure (M/e+ = 438; C,H calculated: Carbon 77.14, Hydrogen 8.74; C,H found: Carbon 77.17, Hydrogen 8.97). The melting point range was 149-151°C (lit. 152-152.5°C). The correspondence from Chettur is included in Appendix I; the procedure for the synthesis is in Appendix II. [2-^{14}C]Anordrin was prepared by Amersham, Inc. The radioactive compound (37,980 cpm) was added to 20 mg of the authentic anordrin provided by Dr. Chettur, and this mixture was crystallized to constant specific activity as shown in Table 1. The specific activity before crystallization was 1988 cpm/mg.

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TABLE 1

Recrystallization of 2-^{14}C-Anordrin

with Authentic Carrier Anordrin

1st crystals	1923 cpm/mg
1st M.L.	1900
2nd crystals	2241
2nd M.L.	1994
3rd crystals	2264
3rd M.L.	1988

This demonstrates the identity of the ^{14}C-labeled and unlabeled compounds.

II. Preparation of anordiol (2a,17a-diethynyl-A-nor 5a-androstane-2B,17B-diol) from anordrin and [2-^{14}C]anordrin.

Anordrin was dissolved in 25 times its weight of 1N NaOH in 90% aqueous methanol. After 1 hour at room temperature, the reaction was stopped by addition of an equivalent weight of 1 N HCl, and the product was extracted into diethyl ether after addition of 3 volumes of water to the reaction mixture. After evaporation of the ether, the product was recrystallized from methanol/water. It was found to be >95% pure by TLC in petroleum ether-acetone (3:1). A mixture of anordrin and its monoester and diol hydrolysis products was also prepared for TLC standards by treatment of anordrin in 1 N NaOH for 5 min at room temperature. Approximately 150 ug of the mixture was added to the samples before applying the samples to the plate. The compounds separate chromatographically as shown in Table 2. A scan of the radioactive final hydrolysis product in relation to the standards is shown in Fig 1. A marker of radioactive ink is put on the

103

Figure 1

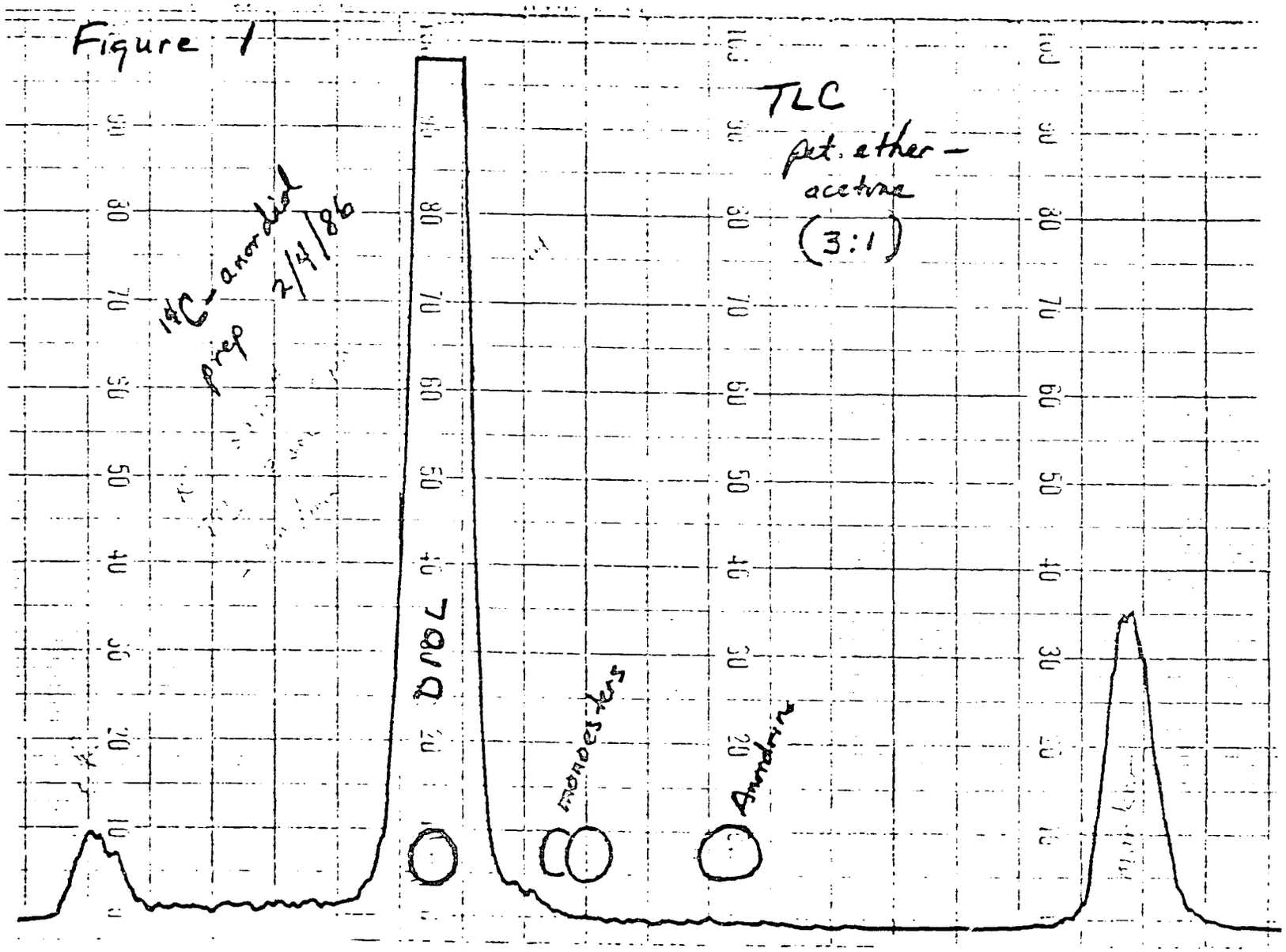

TLC
pet. ether –
acetone
(3:1)
14C-arachidic
prep
2/4/86
DIOL
monoesters
Arachidic

plate as a standard before scanning to align the chart produced by the scanner to the chromatogram. The standards were detected by spraying the plate with 5% H_2SO_4 in 80% ethanol and heating the plate for about 5 min at 100°C. The charred products appear as reddish spots and are marked on the chart. This chromatographic procedure was subsequently used to separate the metabolites of ^{14}C-anordrin in the pharmacokinetic study.

Table 2

Chromatographic Mobility of Anordrin and its Hydrolysis Products in the Petroleum Ether-Acetone (3:1) TLC System

	R_f
Anordrin	0.60
2-Monopropionate of anordrin	0.47
17-Monopropionate of anordrin	0.44
Anordiol	0.34

The hydrolysis products were also tested for purity by gas chromatography. The system used was a 5 ft x 2 mm i.d. column packed with 3% OV-1 on Supelcoport (Supelco, Inc.) operated isothermally at 230°C. The steroids were injected in CH_2Cl_2 solution and were quantified by a flame ionization detector. Anordrin appeared as a single peak with a retention time of 19 min. Anordiol had an R_T of 5.2 min., and cholesterol, used as an internal standard, had an R_T of 25.6 min.

III. <u>Extraction and purification of anordrin and its metabolites from serum and plasma.</u> The protocol for administration of ^{14}C-anordrin and blood sampling is included as

Appendix III. Serum or plasma samles (0.5 ml) were diluted with an equal volume of water and were extracted with diethyl ether. The ether was evaporated, and the residue was applied to TLC plates and chromatographed in the petroleum ether-acetone (3:1) system. The remaining aqueous phase was kept for further analysis.

Blood from monkeys CY99, CY101, and CY105 was allowed to stand at room temperature for 2 hr to allow clotting, and was then centrifuged to separate serum. The serum was then aspirated and put in screw-capped vials in the refrigerator for a few days until the extractions could be performed. This procedure, as will be described later, was found to result in hydrolysis of one of the propionate esters of anordrin. Therefore, in the initial studies anordrin had been largely converted to the monoester of anordiol before extraction. The extracts were found to contain very little radioactivity corresponding in mobility to the anordrin standard in the petroleum ether-acetone system, and the major product in the serum even as early as 3 min after an intravenous injection of ^{14}C-anordrin was ^{14}C-anordiol (Fig 2). At 8 and 10 min anordiol was still the major product (Fig 3). More polar products did not begin to appear until after 2 hr (Figs 4 and 5). A pool of serum extracts from days 11 through 18 was found to have almost exclusively very polar products that remained on the origin of the TLC plate (Fig 6). The material labeled "3" in Fig 5 (designated compound III) was pooled for further structural analysis. No definitive structure has been

Figure 2

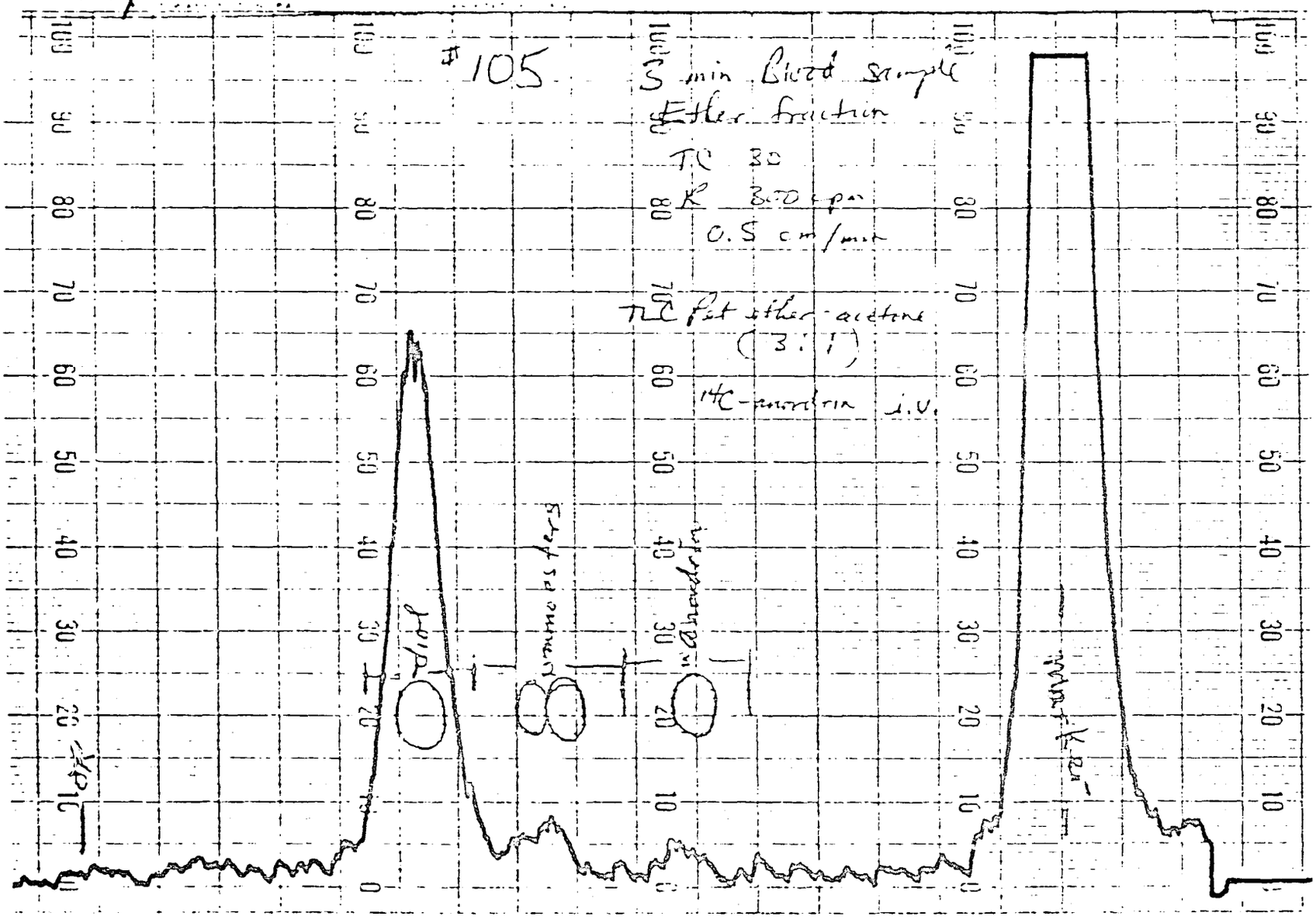

#105
5 min Blood sample
Ether fraction
TC 30
R 3.70 cpm
0.5 cm/min
TLC fat ether-acetone
(3:1)
14C-amorphin i.v.
Fatty acid
Cholesterol esters
Triglycerides
Amorphin
Rf
TC

Figure 3

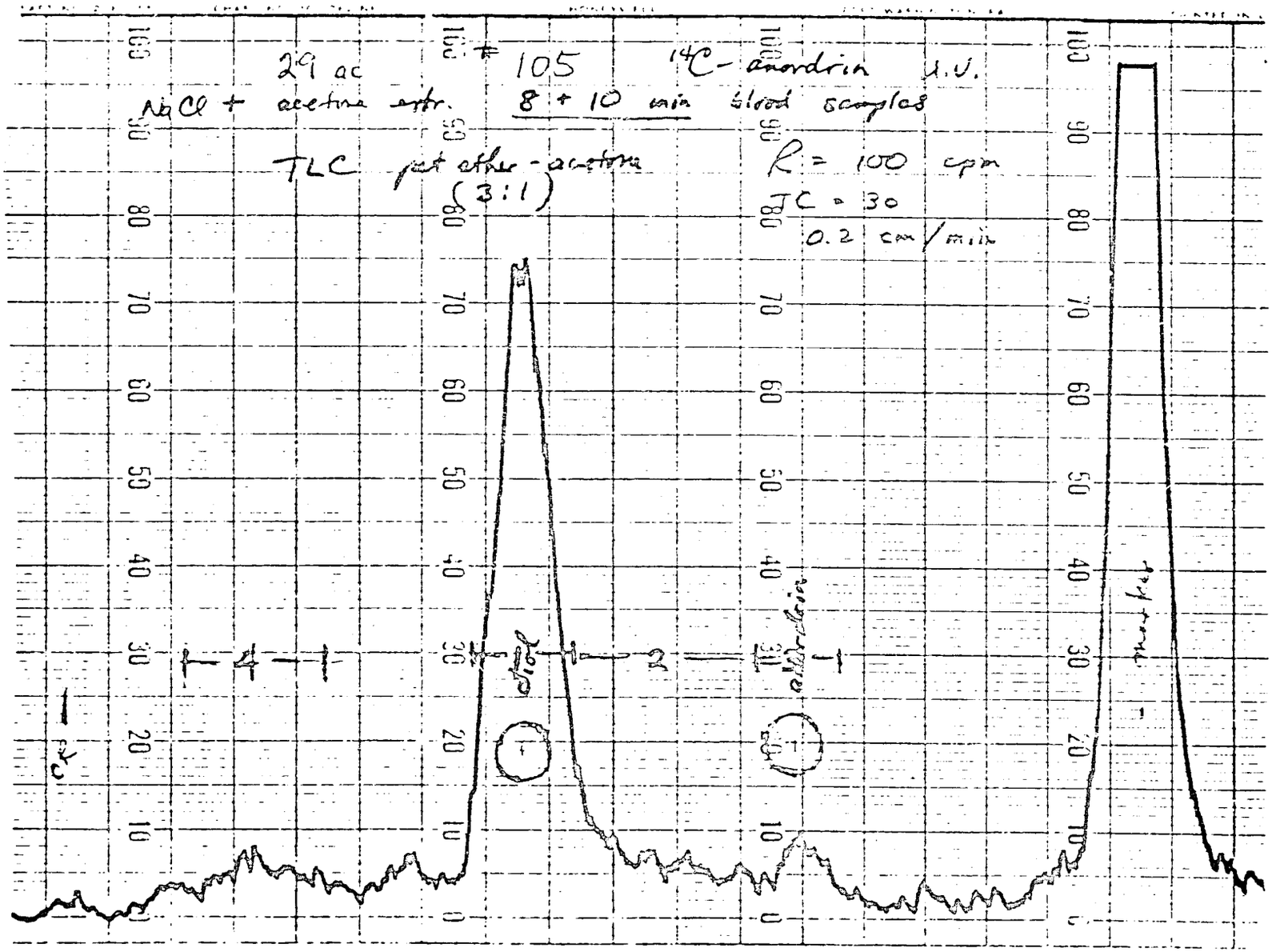

29 ac
NaCl + acetone extr.
TLC petr ether - acetone (3:1)
105
8 + 10 min
14C - anordrin
blood samples
R = 100 cpm
JC = 30
0.2 cm/min
blood
anordrin
metabolites
1
2
3
4

Figure 4

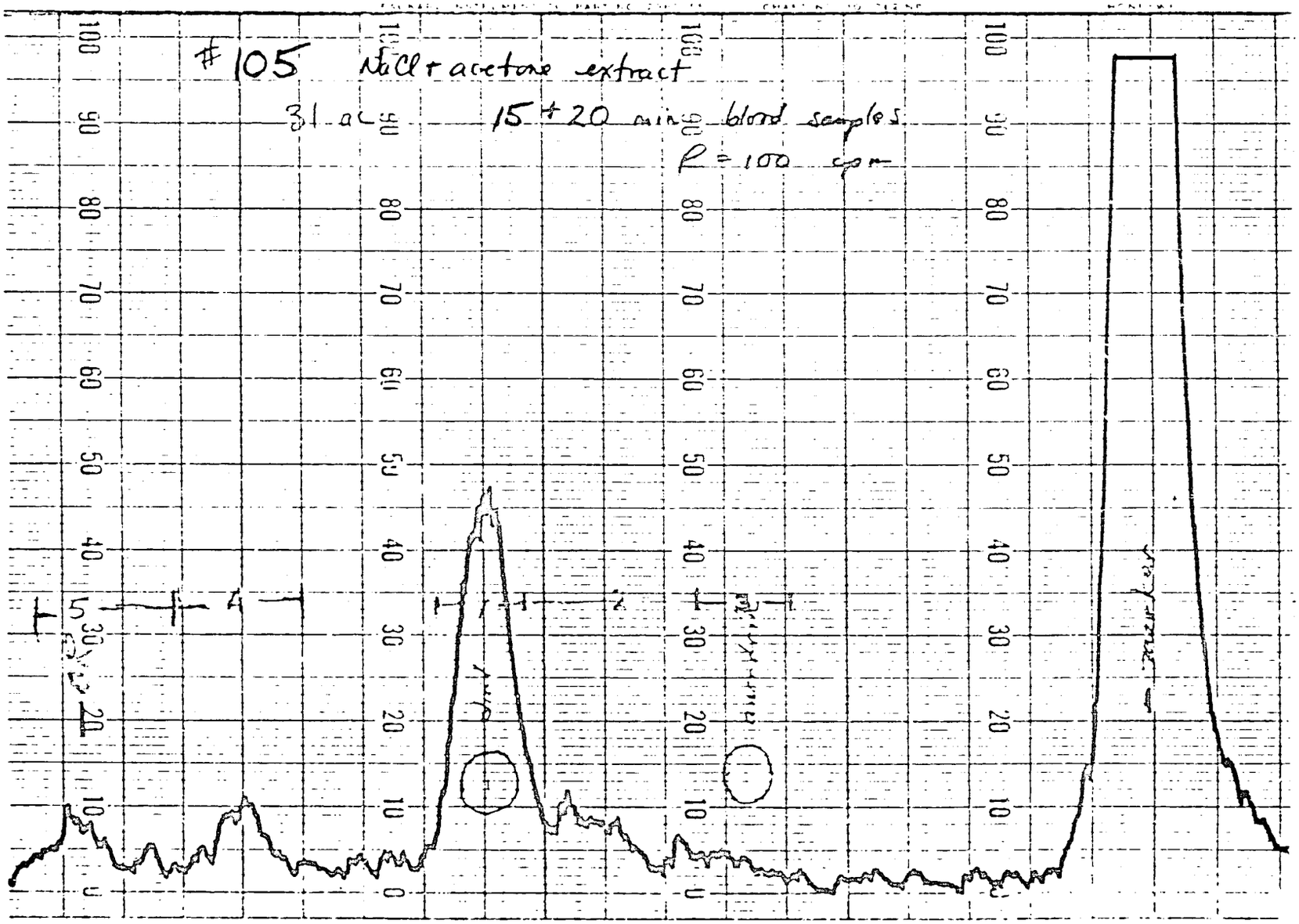

#105 NaCl + acetone extract
31 ac
15 + 20 min blood samples
R = 100 cpm

Figure 5

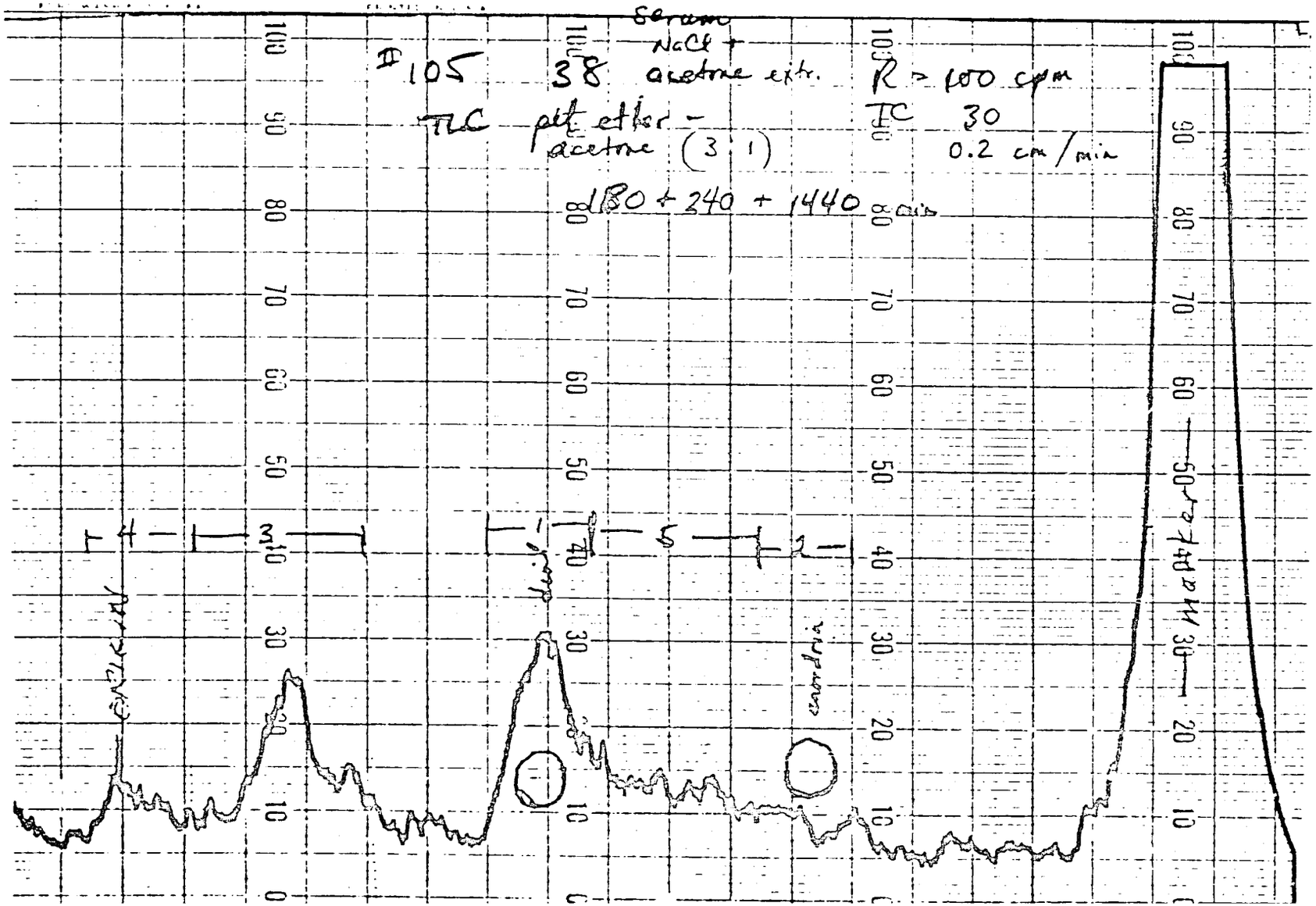
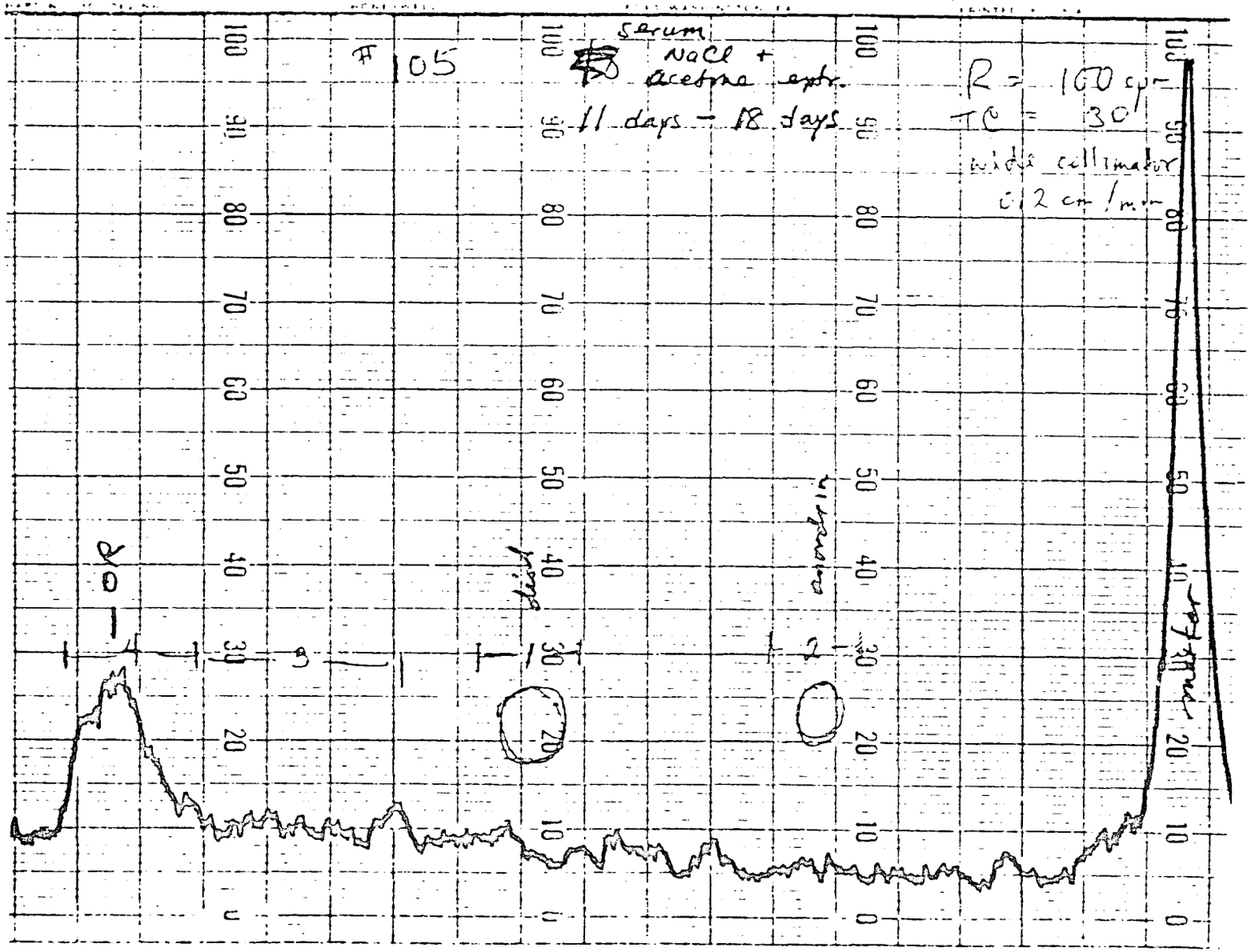

#105
38
Serum
NaCl +
acetone extr.
TLC pet ether -
acetone (3 : 1)
1180 + 240 + 1440
R = 100 cpm
FC 30
0.2 cm/min
Phytol
Squalene
Cholesterol
Vitamin A
Vitamin E
Vitamin K
Vitamin D

Figure 6


serum
NaCl +
acetone extr.
11 days – 18 days
R = 100 cpm
TC = 30'
wide collimator
0.2 cm/min
105
Rb – I
cpm
minutes

determined, but the compound apparently has an additional hydroxyl group.

Identity of anordiol purified from serum samples of monkey CY99 was demonstrated by addition of 13.6 mg of authentic anordiol to 4125 cpm of the compound eluted from TLC plates (SA of the mixture = 303 cpm/mg). Results of recrystallization of this mixture from methanol/water are shown in Table 3.

Table 3

Recrystallization of isolated 2-^{14}C-anordiol with authentic carrier anordiol

1st crystals	320 cpm/mg
1st M.L.	432
2nd crystals	319
2nd M.L.	298

The scans in Figs 2-6 are typical of patterns of metabolites obtained in extracts of serum from the other monkeys receiving an intravenous dose of ^{14}C-anordrin. Since there are basically 4 peaks of radioactivity that appear in these extracts, all serum samples were assayed for radioactive products that had the mobility of 1) anordrin, 2) anordiol, 3) compound III, and 4) products that remained on the origin of the chromatograms. Areas of the chromatograms for elution and counting were delimited by the standards of anordrin and anordiol as in the scans shown in Figs 2-6. The counts of compounds appearing in the ether-extractable fraction (free steroids) are given in Appendix IV, pp. 1-4.

IV. Binding of anordrin and its metabolites in serum. Serum was extensively washed with diethyl ether in an attempt to extract more of the ^{14}C-material, but a considerable proportion of the radioactivity remained unextractable. This material did not become ether-soluble after treatment at room temperature or at 37°C with 1M NaOH or 1M HCl, but it was extractable into acetone with the usual procedure for extracting acid conjugates of steroids, i.e., acidifying the aqueous solution to pH 3, saturating with NaCl, and extracting the conjugated steroid into acetone. Upon analysis of this acetone extract by TLC, it was discovered that the compounds extracted were similar to those originally extracted into ether. Compare Fig 3 (acetone extract) with Fig 2 (ether extract). Further, it was found that the efficiency of extracting the samples obtained within the first 20 min after an i.v. injection of ^{14}C-anordrin was the same with or without acidification of the aqueous samples.

In addition, it was found that when ^{14}C-anordrin was added to diluted serum *in vitro* it was largely unextractable into diethly ether, but was extractable into acetone after saturating the diluted serum with NaCl. After incubation with ^{14}C anordrin, ether-extracted serum was analyzed by gel filtration on Sephadex G-25 and by electrophoresis in 18% sucrose. By both procedures the bound ^{14}C-anordrin migrated with polypeptides having a molecular weight of less than 6000 daltons.

The method for extracting the bound fraction of anordrin and its metabolites was established as an acetone extraction of the

NaCl-saturated ether-extracted serum. This procedure will not allow extraction of steroid glucuronides of other acid conjugates into acetone. For example, metabolites present in serum after 3 days are not more than 60% extractable without acidification of the serum, but with acidification the remainder becomes acetone extractable (Appendix IV, p. 7.).

Serum collected from all monkeys during the first 20 min after an intravenous dose of ^{14}C-anordrin was extracted with ether and then with acetone to obtain both free (unbound) and bound steroid fractions (See Appendix IV, pp.1-4, for data). This procedure resulted in 77 +/- 6% recovery of labeled compounds from these samples without accounting for the acid metabolites. When the acid metabolites were accounted for after an i.m. injection in the same monkey, 95 +/- 18% of the serum was recovered in the sum of the fractions (Appendix IV, p. 7.).

The bound to free ratio (B/F) of anordrin in rapidly frozen serum samples obtained after an intravenous dose of ^{14}C-anordrin was 10.2 +/-1.0 (SE). This is similar to the ratio of 10.4 obtained after incubation of ^{14}C-anordrin with serum for 1 hr at 37°C, but different from the ratio of 8.6 obtained after incubation of ^{14}C-anordrin at room temperature. The B/F ratio of anordiol in serum of the monkeys that received ^{14}C-anordiol i.v. was 1.66 +/- 0.14 (SE). This is similar to the ratio of 1.7 obtained after incubation of ^{14}C-anordiol with serum at room temperature.

V. Conversion of ^{14}C-anordrin to ^{14}C-anordiol in serum.

Fresh blood was obtained from a monkey that had not received anordrin for over 5 months. Serum was separated and 3 ml was incubated with 2.0 ug of ^{14}C-anordrin (46,000 dpm) at 37°C for 48 hours. From 48 hr to 120 hr after the beginning of the incubation the serum was kept at 4°C. Aliquots of serum (0.3 ml) were taken at intervals for assay of bound and free ^{14}C-anordrin and its metabolite anordiol. The results are displayed in Table 4.

TABLE 4

Metabolism of 14C-Anordrin *in Vitro* (cpm/0.3 ml)

Time of	Unbound			Bound		
Incub	Anordrin	Monoest	Anordiol	Anordrin	Monoest	Anordiol
1 hr	689	97	53	7164	55	40
3 1/2	579	102	70	6264	132	52
6 1/2	526	168	77	6124	230	112
24	1830	384	168	3168	324	36
48	1086	543	159	2544	794	166
120	1570	3892	337	1576	424	8

[The abbreviation *Monoest* refers to the monoesters of anordiol, i.e. the partial hydrolysis products of anordrin.]

The half-life of conversion of anordrin to anordiol in serum *in vitro* at 37°C was 45 hours. Assuming a first order reaction, the half-time of conversion of anordrin to anordiol at 4°C was 77 hr. It is evident that partial hydrolysis of one of the esters of anordrin occurs at body temperature as well as at refrigeration temperature. The primary product is not, however, anordiol as shown by the accumulation of the monoesters at 120 hr.

The degree of binding observed in serum of monkeys that had

been given an intravenous injection of ^{14}C-anordrin is confirmed by these data. However, it is clear that the binding activity of the serum declined between 6 1/2 and 24 hr, and after further incubation at 4°C, binding had decreased to an even lower level.

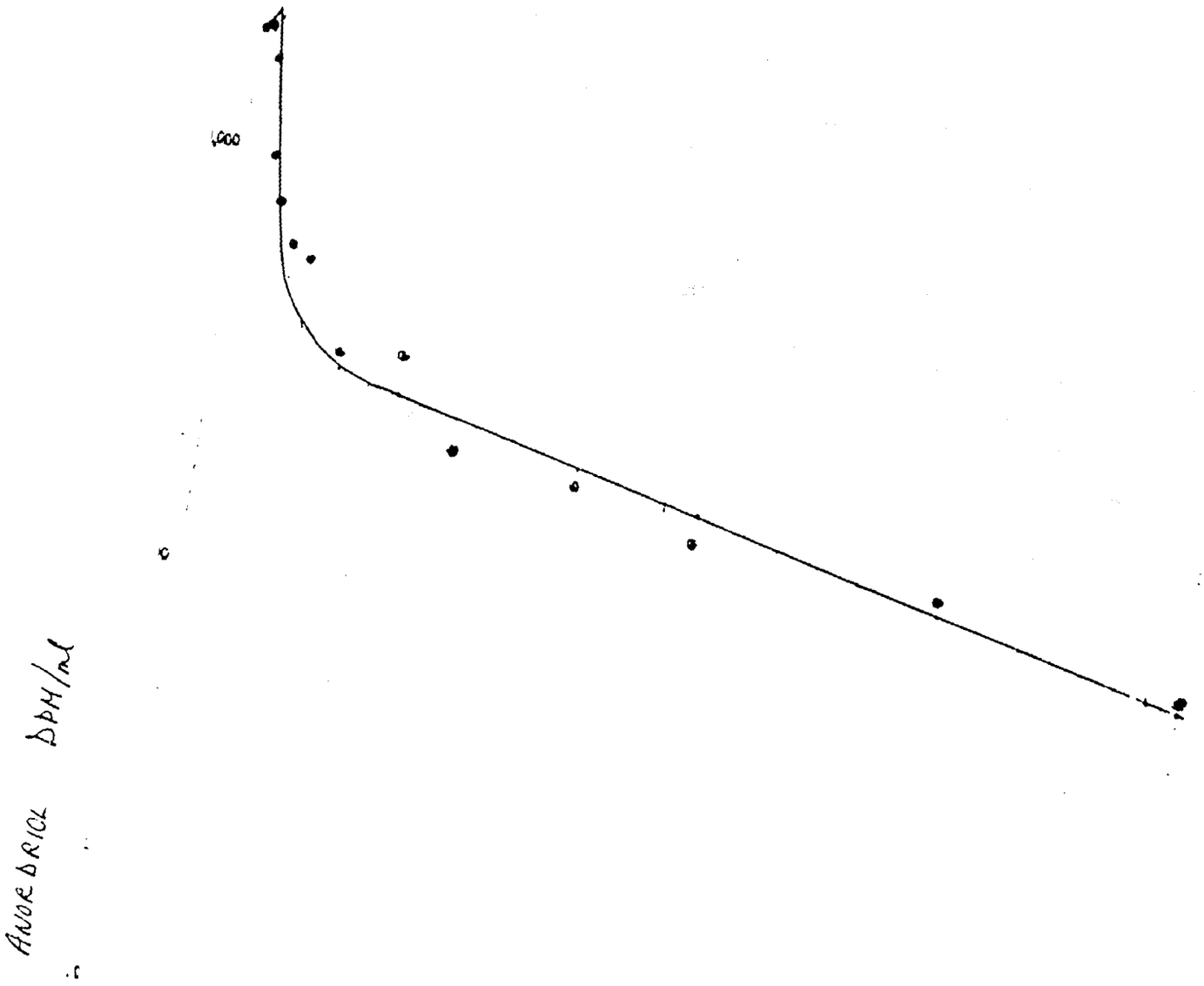
VI. Kinetics of anordrin and anordiol after intravenous administration.

Clearance from serum after an intravenous dose of ^{14}C-anordrin by standard procedures. Radioactive material was eluted from fractions separated by TLC as described above. Since a significant amount of the anordrin had been converted to the monoesters of anordiol in serum prepared by the standard procedure, only the data on anordiol was calculated in samples obtained from three monkeys after an intravenous injection of ^{14}C-anordrin. Figures 7-9 illustrate the disappearance of anordiol in serum of the three monkeys. For this purpose the total concentrations of the steroids were calculated by addition of the concentrations of bound and unbound anordiol in samples in which binding was determined. The mean half-life of clearance of anordiol was 95.7 +/- 6.6 (SD) min. The steady state volume of distribution was 3.0 +/- 2.1 (S.D.) L, and the metabolic clearance rate was 0.035 +/- 0.01 (S.D.) L/min.

Plasma clearance in rapidly frozen samples. Monkey CY104 was given the 0.2 mg/kg dose of ^{14}C-anordrin i.v. and blood was collected in heparinized tubes, rapidly chilled, centrifuged, and the cold plasma was immediately frozen in order to limit loss of

MONKEY 99

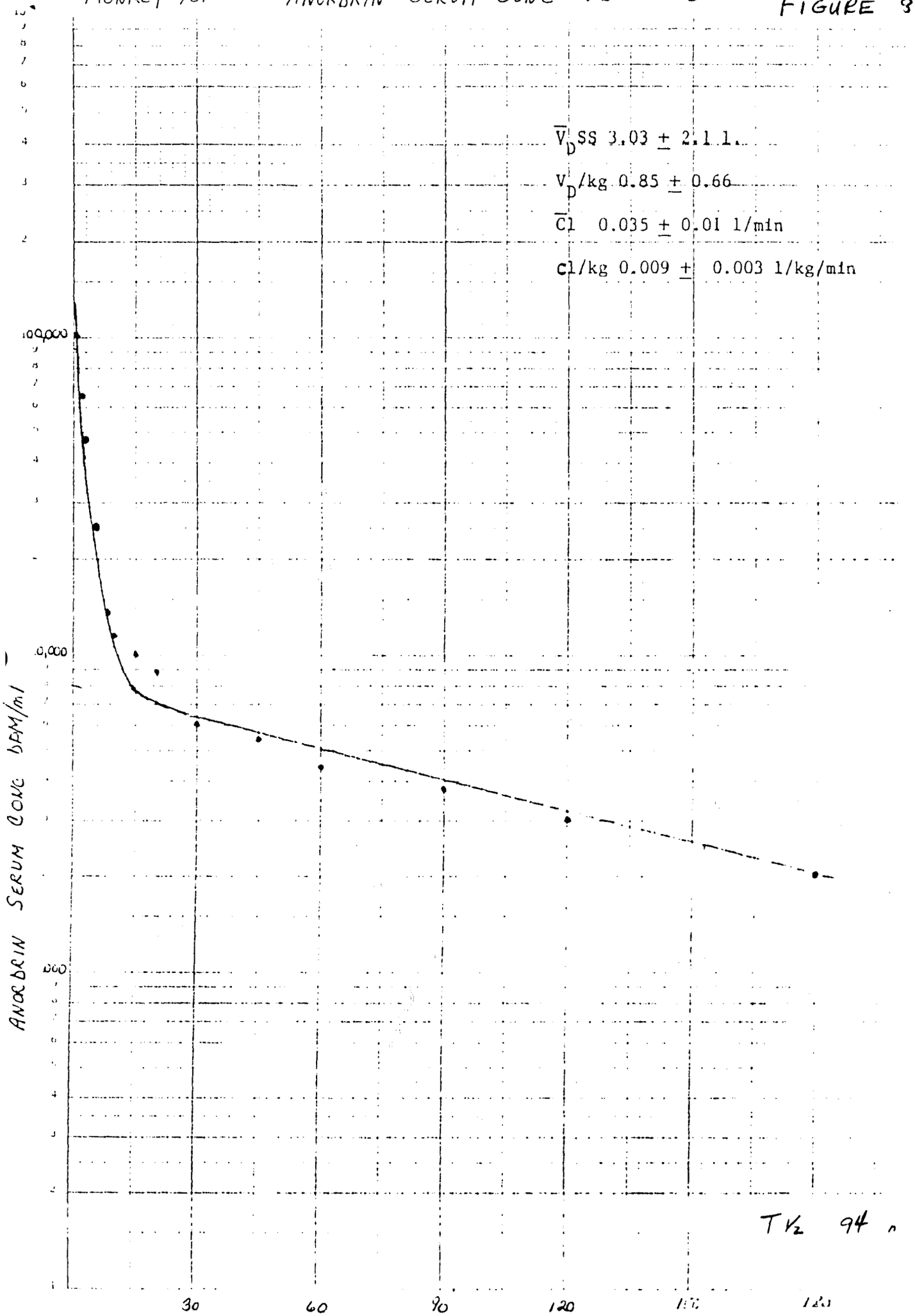
FIGURE 7



T½ 103 ± 15 SD m

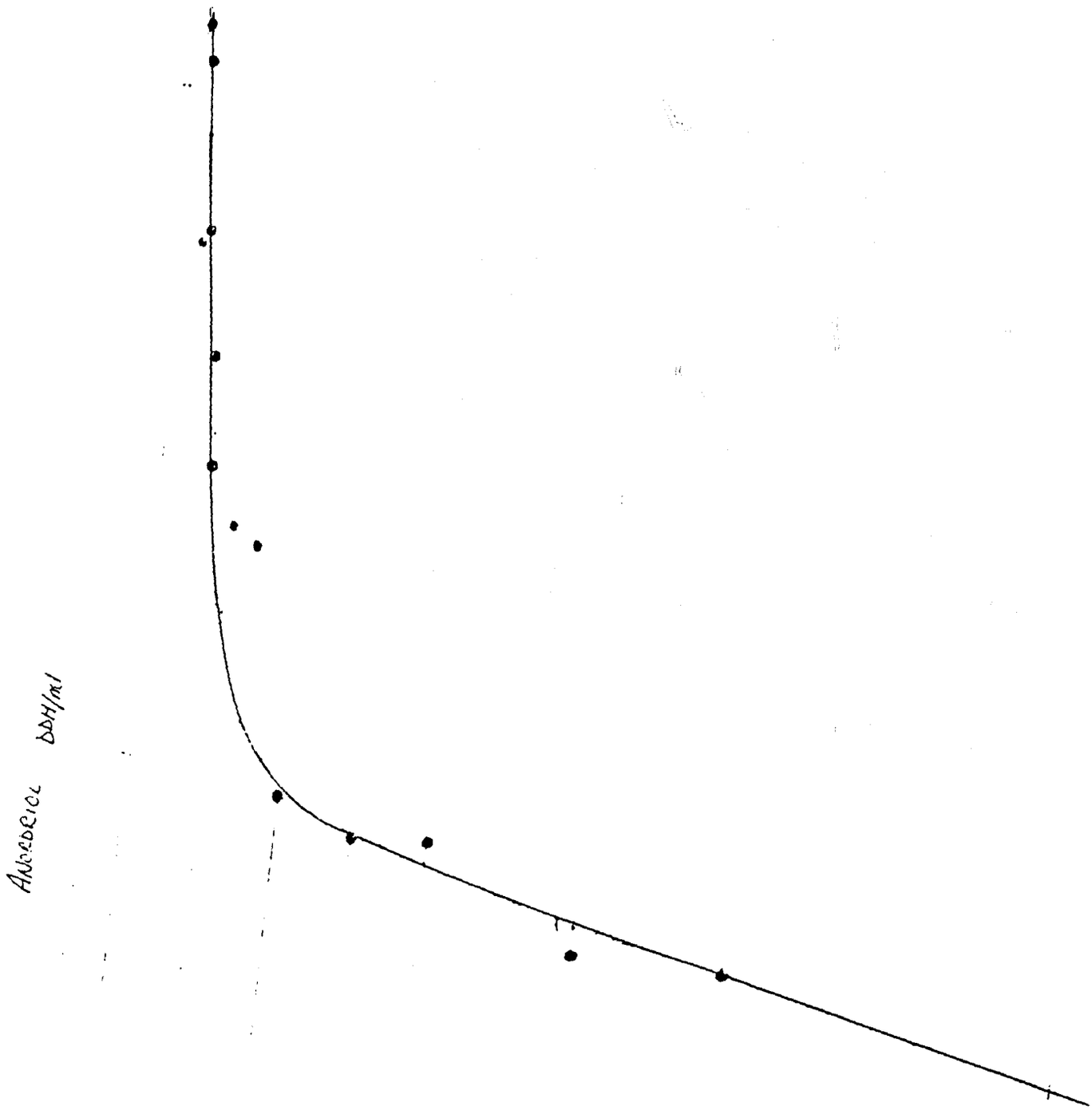
MONKEY 101 ANORDRIN SERUM CONC VS TIME

FIGURE 3



MONKEY 105

FIGURE 9



anordrin from the blood by hydrolysis after the blood was drawn from the animal. The data from this experiment is presented in Appendix IV, p. 5a-5d. Figure 10 shows the disappearance of ^{14}C-anordrin and the levels of ^{14}C-anordiol obtained in the first 60 min after administration of the dose. The half-life from the complete data shown in Figure 11 was 190 min.

VII Administration of the metabolite ^{14}C-anordiol. Because of the potential for conversion of some of the anordrin to anordiol in serum of monkeys given ^{14}C-anordrin during storage, we prepared ^{14}C-anordiol from ^{14}C-anordrin by hydrolysis of the esters as described above. The binding and kinetics of ^{14}C-anordiol after intravenous administration were compared with similar studies in which ^{14}C-anordrin was given. The data are summarized on p. 6 of Appendix IV. These data are illustrated . Figure 12. As can be seen the half-life of ^{14}C-anordiol determined in this way is essentially the same as that determined from studies in which ^{14}C-anordrin was administered. Thus, any conversion of anordrin to anordiol in serum did not significantly affect the results of the measured kinetics of anordiol. And, as is shown below, the clearance of anordiol may be the rate limiting factor in determining biological effect of anordrin.

VIII Relation between the half-life of anordiol and the duration of amenorrhea. Monkeys were examined daily for the presence of vaginal bleeding. The duration of amenorrhea is shown in Table 5.

FIGURE 10

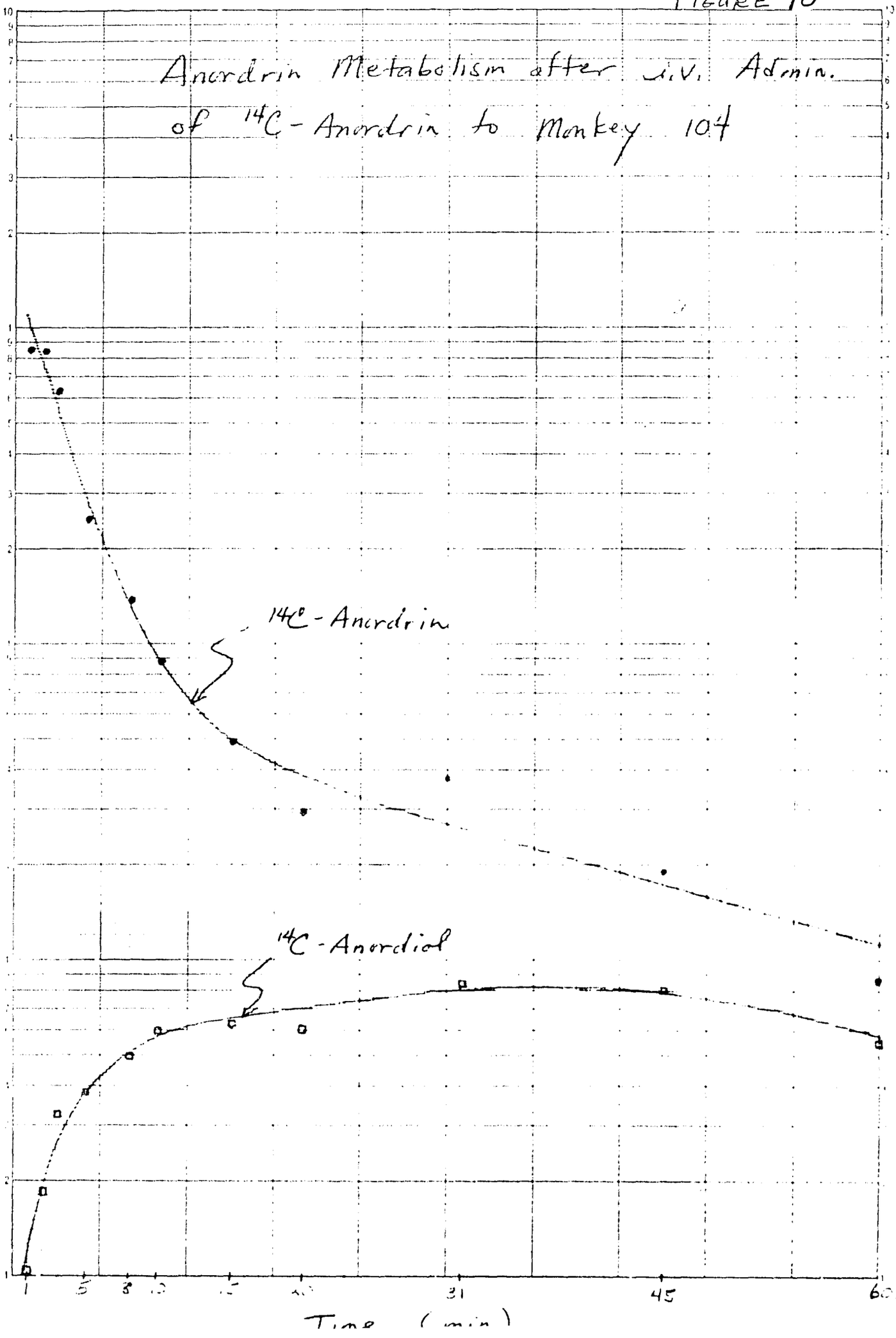

Anordrin Metabolism after i.v. Admin.
of 14C-Anordrin to Monkey 104
14C-Anordrin
14C-Anordiol
1
5
31
45
Time (min)

FIGURE 11

^{14}C - Anordrin i.v.
M # 104 Rapid
Freezing of Samples

$T_{1/2}$ = 190

FIG. 12

^{14}C-Anordiol Metabolism

M 102 i.V.

cpm/0.25 ml in serum

$T_{1/2}$ 95 min

TABLE 5

Duration of Amenorrhea after Anordrin (mg/kg) Treatment

Dose	Route	Monkey	Cycle Length	Remarks
0.2	i.v.	99	38	Amenorrheic >3 mo. before injection.
		101	44	Amenorrheic >3 mo. before injection.
		103	53	
		105	60	
		mean	48.8 days	
0.2	i.m.	99	58	
		101	57	
		103	65	
		105	62	
		mean	60.5 days	
1.0	i.m.	100	-	No menstruation. Amenorrheic >4 mo. before injection.
		102	71	
		104	77	
		mean	74 days	

Unexpectedly, two of the monkeys that had been amenorrheic for over 3 months menstruated between 30 and 40 days after administration of anordrin. The difference between the response to the 0.2 mg/kg dose i.v. and i.m. was analyzed by a paired t-test. The mean difference was 11.7 days which was statistically significant at the $P < 0.05$ level. The number of monkeys that received the two different doses intramuscularly is too small to draw conclusions from in this study, but there is ample evidence from previous studies for a dose response to intramuscularly administered anordrin.

The correlation between duration of amenorrhea and half-life of anordiol after the i.v. dose was calculated for monkeys CY99, CY101, and CY105. As stated earlier, the clearance of ^{14}C-

anordiol could not be determined in CY103 because of difficulty with intubation. The half-lives of anordiol in the three intubated monkeys was 103, 94, and 90 min, respectively, making the correlation coefficient = -0.89.

IX Calculated concentrations of anordiol in serum after i.m. administration of anordrin. Based on the specific activity of ^{14}C-anordrin of 9.0 mCi/mmol as supplied by Amersham Corp., the concentrations of anordiol in serum of monkeys that received the 1.0 mg/kg dose of ^{14}C-anordrin were calculated. Concentrations of anordiol are shown in Table 6. These concentrations are within the range of gas chromatographic methods if sufficient plasma is available, e.g., about 5 ml for gas chromatography with flame ionization detection. Alternatively, anordrin itself may be present in sufficient amounts to measure by gas chromatography or by radioimmunoassay, but we do not have sufficient data on the relationship between anordrin clearance and duration of effect to ascertain the value of anordrin measurements at the present time. However, antiserum to a conjugate of the 2-propionate monoester of anordiol has been prepared, and this may prove useful in measuring anordrin in serum of human subjects treated with anordrin.

TABLE 6

Concentration of Anordrin in Serum after Intramuscular Injection of 1.0 mg of Anordrin per Kg of Body Weight

	Anordiol (ng/ml)		
Time	CY100	CY102	CY104
45 min	1.6	1.6	0.4
60 min	2.4	2.4	0.8
120 min	2.8	5.2	2.0
240 min	4.4	6.0	3.6
360 min	7.2	13.6	4.8
24 hr	12.0	16.8	7.2
48 hr	11.2	20.8	7.2
72 hr	8.8	14.8	6.8
96 hr	6.8	10.4	10.0
120 hr	9.2	14.8	10.8
144 hr	9.2	10.8	13.2
168 hr	6.4	11.2	12.0
192 hr	6.0	12.4	14.4

* S.A. = 42.9 dpm/ng

X Relationship between metabolic clearance of anordrin metabolites and duration of effect. Although the half-life of anordiol appears to relate to the duration of effect of anordrin, the half-life is far too short to account for a duration of effect of over 30 days. If the active form of the drug must be present to continue to prevent follicular development, only the long-lived metabolites present in serum after 5 days can explain the biological activity. The decline in the total radioactivity per ml of serum forms a first order clearance rate after the first 7 days as shown in Figure 13. The metabolites in serum at this time consist primarily of acidic compounds which have not yet been identified.

FIGURE 13

#99 i.v. ^{14}C-Anordrin
Total cpm / 0.25 ml in serum

13½ day half-life

-p m-

XI Clearance of metabolites of ^{14}C-anordrin in urine.

Appearance of ^{14}C-labeled metabolites in urine occurs in a biphasic pattern. An initial first order decline of between 2.5 and 7.8 days with a slower clearance of 15 days or more characterizes the appearance of metabolites in urine. These data are included in Appendix VI. Graphic representations are on pp. 22-28. Steroid products of anordrin were extracted from urine initially with diethyl ether and then, after saturation with NaCl, with acetone. ^{14}C-Anordrin was not found in urine in detectable amounts, i.e., not more than 0.1% of excreted ^{14}C products. Approximately 40% could be extracted into ether from the urine excreted within the first 5 days after administration of the drug. None of the urinary products have been identified structurally.

Within the first 30 days approximately 35% of the ^{14}C dose could be accounted for in urinary products regardless of whether the dose had been given intravenously or intramuscularly.

B. Evaluation of Toxicity of Anordrin

I. Dosage and pathology reports.

These have been submitted separately by Dr. Donald Waller.

II. Hormone and lipoprotein changes in relation to dose in monkeys and rats.

Hormone concentrations were determined by standard radioimmunoassays. The Reproductive Endocrinology Laboratory is licensed by the State of Illinois Department of Public Health

(license no. 19901) and is certified by the College of American Pathologists. Cholesterol and HDL were assayed in serum of monkeys and rats by kits provided by Sigma Chemical Co. Prolactin, estradiol, testosterone, progesterone, and cortisol were assayed by procedures routinely used for human serum samples in the Reproductive Endocrinology Laboratory. These assays have been validated for use in the cynomolgus monkeys in previous studies conducted by our laboratory. Calcitonin was determined by Dr. Gerald Williams' laboratory at the West Side Veterans Administration Hospital, Chicago. This assay has been validated for both human and monkey studies previously by Dr. Williams. Thyroxine was determined by Dr. Charles Barsano in the Endocrine Laboratory at the North Chicago Veterans Administration Hospital.

Four monkeys were treated at each of four dose levels, 0, 1, 10, and 50 times the minimum effective dose. Blood from the test monkeys was drawn prior to each of the monthly doses of anordrin and one month after the third dose. Serum was separated and was stored frozen until the assays were performed. Blood was obtained from rats prior to treatment and again after 90 days of treatment with 0, 2, 10, or 50 times the minimal effective dose and serum was prepared and stored frozen.

Data was analyzed by Multiple Analysis of Variance (MANOVA) using the Statistical Package for the Social Sciences (SSPS PC+) on an IBM-XT personal computer. The procedure used was designed for analysis of data with repeated measures. The significance of differences due to dose and to time were determined by this program.

The data on the several hormone measures, cholesterol, and HDL in monkeys are summarized in Table 7. The values of Fisher's "F" and the probability level "P" are given. A significant dose response to anordrin was found only in estradiol and cortisol. There was a significant effect of time on the levels of most hormones and cholesterol fractions measured, but this was independent of the dose given to the monkeys, i.e., there was no dose x time interaction. Table 8 separates out the significance of the response to anordrin at the highest dose.

Since estradiol was found to increase significantly with dose of anordrin, the individual values with time and dose are shown in Table 9. Here the time effect becomes more apparent. The effect is greater at each dose as time progresses. However, the finding of increased serum levels of estradiol with anordrin treatment is contrary to all of the studies conducted in monkeys at lower doses in which it was shown that anordrin inhibits follicular development and estrogen excretion. Therefore, the serum was reassayed with a different, more specific antiserum to determine whether the measured increase was real or a result of binding of a metabolite of anordrin to the antiestradiol antiserum. As can be seen in Table 9A there was no significant association of measured estradiol with dose of anordrin when estradiol was measured with the more specific antiserum. Anordiol was tested for its ability to compete with ^{3}H-estradiol for binding to the first antiserum, but no binding could be

demonstrated.

Levels of serum cortisol were also significantly increased by the treatment, and are also shown in detail in Table 10. Here also the serum levels increase progressively with time. This response also must be investigated further in order to ascertain whether the increase is due to a metabolite of anordrin that increases in circulation with time and dose, or is due to a real increase in adrenal activity and elevation of serum cortisol.

The thyroxine response was not quite significant at the $P < 0.05$ level, but is presented in more detail for closer examination (Table 11). Here the increase appears to be only between the vehicle and the lowest dose without a further increase with doses greater than the minimal effective dose.

The data on cholesterol, HDL, and LDL is presented in Table 12. A significant decrease in both total cholesterol and HDL occurred with increasing dose levels of anordrin. As a result the levels of LDL, calculated by difference, did not change significantly (lower part of Table 12). This effect was different than that observed in monkeys in which neither cholesterol nor HDL were significantly altered by the treatment. This may be in part because the rats were treated every 4 days for a total of 90 days whereas the monkeys were treated only monthly. As can be seen from Table 12, cholesterol and HDL levels after treatment were not decreased in the 2x MED group, but only in the 10x and 50x groups despite the fact that the 2x group had lower initial levels of cholesterol.

Table 7

Summary of Anordrin Toxicity Study (Monkeys)

MANOVA

	Dose Response (D)		Effect of Time (T)		Interaction (DxT)	
	F	P	F	P	F	P
PRL	0.66	0.595	0.24	0.866	1.00	0.461
CHOL	0.35	0.793	15.0	<0.001**	0.69	0.710
HDL	2.36	0.128	8.55	<0.001**	1.16	0.351
E2	11.31	0.001**	13.82	<0.001**	5.82	<0.001**
T	2.05	0.161	14.79	<0.001**	1.14	0.359
P4	1.28	0.327	2.57	0.069	1.48	0.192
F	10.89	0.001*	13.86	<0.001**	1.74	0.115
Calc	1.12	0.378	6.55	0.001**	0.86	0.68
T4	3.00	0.073	9.92	<0.001**	1.53	0.174

Table 8

Effect of Anordrin (50 x MED) on Cholesterol and Hormone Levels in Monkeys

Analyte	Significance of Dose Reponse by MANOVA	
Cholesterol	0.79	N.S.
HDL	0.13	N.S.
Prolactin	0.60	N.S.
Cortisol	0.001	Highly Significant
Testosterone	0.16	N.S.
Progesterone	0.33	N.S.
Estradiol	0.001	Highly Significant
Thyroxine	0.07	N.S.
Calcitonin	0.38	N.S.

Table 9

Serum Estradiol pg/ml ± SE
Monkeys

Dose (MED)	Time (months) 0	1	2	3	mean
Vehicle	38 ± 8	18 ± 5	45 ± 17	54 ± 12	39 ± 8
x1	37 ± 4	22 ± 2	23 ± 3	24 ± 5	26 ± 4
x10	54 ± 26	72 ± 9	87 ± 15	90 ± 11	76 ± 5
x50	48 ± 3	80 ± 12	128 ± 22	147 ± 17	101 ± 22
	44 ± 4	48 ± 16	71 ± 23	79 ± 26	

Table 9A

Serum Estradiol in Monkeys, Second Assay with Specific Antiserum (pg/ml)

stive statistics based on 16 subjects processed

Dependent Variable	N	Mean	Standard Deviation	Minimum	Maximum
DOSE	16	2.500	1.155	1.000	4.000
E1	16	59.875	35.835	10.000	130.000
E2	16	20.563	15.362	10.000	68.000
E3	16	27.375	23.622	10.000	100.000
E4	16	52.938	43.189	23.000	190.000

Descriptive statistics for the subgroup: DOSE = 1 Vehicle

based on 4 subjects processed

Dependent Variable	N	Mean	Standard Deviation	Minimum	Maximum
E1	4	56.750	36.344	29.000	110.000
E2	4	30.000	26.382	10.000	68.000
E3	4	54.750	31.993	25.000	100.000
E4	4	106.750	62.946	37.000	190.000

Descriptive statistics for the subgroup: DOSE = 2 1 x MED

based on 4 subjects processed

Dependent Variable	N	Mean	Standard Deviation	Minimum	Maximum
E1	4	61.250	12.420	43.000	70.000
E2	4	23.500	11.030	13.000	34.000
E3	4	17.250	11.413	10.000	34.000
E4	4	30.000	4.690	25.000	34.000

Descriptive statistics for the subgroup: DOSE = 3 10 x MED

based on 4 subjects processed

Dependent Variable	N	Mean	Standard Deviation	Minimum	Maximum
E1	4	29.000	38.000	10.000	86.000
E2	4	15.250	9.179	10.000	29.000
E3	4	11.750	3.500	10.000	17.000
E4	4	34.500	8.737	23.000	44.000

Descriptive statistics for the subgroup: DOSE = 4 50 x MED

based on 4 subjects processed

Dependent Variable	N	Mean	Standard Deviation	Minimum	Maximum
	4	92.500	28.208	64.000	130.000
	4	13.500	4.123	10.000	18.000
	4	25.750	12.606	10.000	39.000
	4	40.500	6.608	34.000	48.000

Table 10

Serum Cortisol ng/ml ± SE
Monkeys

Dose (MED)	Time (months) 0	1	2	3	mean
Vehicle	234 ± 56	254 ± 31	233 ± 28	282 ± 62	251 ± 11
x1	179 ± 14	365 ± 43	325 ± 12	360 ± 36	307 ± 44
x10	350 ± 58	495 ± 10	495 ± 28	490 ± 64	458 ± 36
x50	280 ± 28	395 ± 29	385 ± 32	535 ± 66	399 ± 52
	260 ± 36	377 ± 50	360 ± 55	417 ± 58	

Table 11

Serum Thyroxine µg/dl ± SE
Monkeys

Dose (MED)	Time (months) 0	1	2	3	mean
Vehicle	7.2 ± 2.0	7.2 ± 0.8	7.8 ± 0.6	7.5 ± 0.5	7.4 ± 0.1
x1	8.2 ± 1.7	11.0 ± 1.6	13.8 ± 2.5	10.5 ± 0.6	10.9 ± 1.1
x10	7.8 ± 1.2	12.2 ± 0.8	11.2 ± 1.2	13.0 ± 0.9	11.0 ± 1.1
x50	7.2 ± 2.0	12.0 ± 0.8	14.0 ± 2.2	12.7 ± 1.6	11.5 ± 1.5
	7.6 ± 0.2	10.6 ± 1.2	11.7 ± 1.4	10.9 ± 1.3	

Table 12

Serum Cholesterol and HDL in Ra
mg/dl ± SE

Dose (MED)	Cholesterol Before	Cholesterol After	HDL Before	HDL After
Vehicle	94.6 ± 10.8	72.7 ± 4.7	42.3 ± 3.2	36.5 ± 4.6
x2	33.3 ± 3.3	73.8 ± 3.9	15.4 ± 1.8	49.7 ± 2.4
x10	70.1 ± 2.8	37.4 ± 3.4	42.1 ± 3.4	15.7 ± 1.5
x50	78.0 ± 4.0	38.4 ± 2.9	51.8 ± 4.0	13.2 ± 2.2

Dose Response
Cholesterol $P < 0.001$
HDL $P = 0.006$

	LDL mg/dl (Total Cholesterol - HDL) Before	After
V	52.3	36.2
2x	17.9	24.1
10x	28.0	21.7
50x	26.2	25.2
	31.1 ± 7.4 SE	26.8 ± 3.2 SE

Unanticipated Results

The only unanticipated results were the serum levels of estradiol in monkeys treated with anordrin in the toxicity testing aspect of the study, and the specificity of the assay is now being evaluated.

Research Design

No changes were made in the experimental design.

Operational Significance

The pharmacokinetics and metabolism studies show that anordrin has a relatively long half-life in the monkey, but that some of its metabolites have residence times in the body more in keeping with its long duration of effect on ovarian function.

The results of toxicity testing encourage us to proceed with Phase I clinical trials and eventual development of anordrin as a once-a-month contraceptive agent.

Dissemination and Utilization of Research Results

The studies on pharmacokinetics and metabolism have not yet been submitted for publication, but an abstract is being prepared for submission to the Endocrine Society. An abstract has been submitted to the Society of Toxicology for presentation at the 1987 annual meeting. The paper submitted is, "Toxicity Studies of Anordrin," by D.P. Waller, M. Szarley, J.C. Pena, C.D. Fakroddin, A. Martin, and R.T. Chatterton Jr.

Appendix I.

IIT Research Institute
10 West 35 Street, Chicago, Illinois 60616
312/567-4000

May 28, 1985
IITRI Project C06617

Dr. Robert T. Chatterton
Reproductive Endocrinology Laboratory
Department of Obstetrics & Gynecology
333 East Superior Street
Chicago, IL 60616

Dear Dr. Chatterton:

The first batch of α-Anordrin, synthesized as described in IITRI Proposal 84-234CC with the modifications suggested by you, yielded ~ 6g of crude material after reaction work up. Attempts to crystallize α-Anordrin from this crude yielded only a small amount of crystalline material. Therefore, the crude was chromatographed on silica gel column (30-60 mesh, Matheson, Coleman and Bell) and the α-Anordrin was eluted with heptane/acetone (10:1). The chromatographed material yielded a white crystalline material (~ 2.4g). TLC of this material showed a single spot, coincident with spot from the reference material, provided by you. IR, proton NMR and C-13 NMR of this material agreed with the proposed structure of α-Anordrin. However, melting point of this material was lower (softens at 140°C, melting range 145-150°C; literature: 152-152.5°C). The above material was crystallized from methanol. Mass spectra and elemental analysis of the crystallized material was in agreement with the proposed structure. (M/e^+ = 438; C, H analysis - calculated: Carbon 77.14; hydrogen 8.74; Found:~ Carbon 77.17; hydrogen 8.97). Once again, the melting point was lower (softens at 145°; melting range 148-150.5°C). The above material was recrystallized from methanol. However, the melting point of the recrystallized material remained the same as before. Therefore, the material was recrystallized from iso-octane. The melting point of this material is 149-151°C (softens at 145°C).

The amount of α-Anordrin with the above melting point is 1.97g. The intermediates, 500 mg to 1g quantities, are provided herewith. The estimate for additional 3g of α-Anordrin (Ref: Letter dated April 22, 1985) was based on crude yield of ~ 6g from reaction work up. It is seen that only about one third of this material can be recovered after purification steps. Therefore, the estimates may have to be revised somewhat to account for the additional labor. Hope this will not pose any problems.

Sincerely yours,

Govindan Chettur

Govindan Chettur
Research Chemist
Chemistry Research Section

cc :

MATERIALS SHIPPED TO DR. ROBERT T. CHATTERTON AT
NORTHWESTERN MEMORIAL HOSPITAL

		Wt (500mg - 1g range)
Androstanolone	(1)	✓
17-β Acetoxy-5 α Androstan-3-one	(2)	✓
17-β Acetoxy, 2,3 Seco-5 α Androstane-2,3-Dioic Acid	(3)	✓
17-β Acetoxy-Anor-5 α Androstan-2-one	(4)	✓
17-β Hydroxy-5 α Androstan-2-one	(5)	✓
Anor-5 α Androstan-2,17-Dione	(6)	✓
2α,17 α Diethynyl-Anor-5 α Androstan-2β, 17β-Diol	(7)	✓
2α,17 α Diethynyl-Anor-5 α Androstan-2β, 17β-Diol Dipropionate (α-Anordrin)	(8)	1.97g

IIT Research Institute
10 West 35 Street, Chicago, Illinois 60616
312/567-4000

April 22, 1985

Dr. Robert T. Chatterton
Reproductive Endocrinology Laboratory
Department of Obstetrics & Gynecology
333 East Superior Street
Chicago, IL 60616

Dear Dr. Chatterton,

To answer your letter of April 16th, inquiring the feasibility of making more α-anordrin, I have drawn up the following time and cost estimates.

Since I am synthesizing α-anordrin, as per the present contract, I estimate the cost of supplying an additional 3g of α-anordrin to be $10,000. I could supply an additional 7g of α-anordrin for $20,000.

I estimate the time for supplying an additional 3g to be by the end of May '85 and if you are asking for an additional 7g, I could supply it by the end of June '85.

I am keeping aside 500 mg to 1g of the major intermediates of the synthesis. I will be saving approximately the same amounts from the remaining synthetic steps.

Hope this answers the points raised in your letter.

Thanking you

Sincerely,

Govindan Chettur
Research Chemist
Chemistry Research Section

Approved:

D. J. Moschandreas, Ph.D.
Research Director
Chemistry and Chemical Engineering
Research Department

The following materials were delivered on September 3, 1985 as part of the requirements of the IITRI-Project C06617.

Compound	Amount
1. Androstanolone (1) (starting material for batches 2 and 3 Aldrich; Lot No. 2708PJ)	4.62 g
2. 17-β Acetoxy-5α-Androstan-3-one (2)	1.50 g
3. 17β-Acetoxy, 2,3 Seco-5α Androstan 2,3 Dioic Acid (3)	0.49 g
4. 2α, 17α Diethynyl-Anor-5α Androstan (8) 2β, 17β-Diol Dipropionate (α-Anordrin)	2.16 g
5. 2α, 17α Diethynyl-Anor-5α Androstan (8) 2β, 17β Dioldiropionate (α-Anordrin)	1.84 g

Received 9/3/85

John M. Ince

Procedure for the Synthesis of α-Anordrin:

The procedure currently employed in our laboratory for synthesis of α-Anordrin is described below:

Step 1: 17β-hydroxy-5α-androstan-3-one (1) to 17β-acetoxy-5α-androstan-3-one (2)

Excess acetic anhydride is added to a solution of 17β-hydroxy-5α-androstan-3-one (Aldrich) in dry pyridine. The reaction mixture is allowed to stir overnight at room temperature. Following neutralization with ice-cold 10% HCl, the mixture is extracted with $CHCl_3$. The organic layer is washed with 10% HCl, 5% $NaHCO_3$, saturated NaCl and dried over anhydrous Na_2SO_4. Evaporation of the solvent yields 2 in greater than 95% yield.

TLC is performed in petroleumether/acetone (3/1). 2 may be recrystallized from the same solvents. NMR: 4.63 (t, 17 H), 2.03 (s, $COCH_3$), 1.02 and 0.81 (2s, 18 and 19-Me). m.p. 152-156°C.

Step 2: 2 to 17β-acetoxy-2,3-seco-5α-androstane-2,3-dioic acid (3)

This transformation is carried out according to procedures described by Scribner[1]. 11.12 g 2 are dissolved in 75 ml of acetic acid, 2.5 ml H_2O, and 0.75 ml methanol, and heated to 60°C. with stirring. A solution of 13.5 g CrO_3 in 30 ml acetic acid and 25 ml H_2O is added slowly so that the temperature remains between 60-65°C. The reaction mixture is then maintained at this temperature for 2 1/2 hours. 75 ml of H_2O, preheated to 60°C., are added and the reaction is allowed to stand at room temperature overnight. Filtration and washing of the filter cake with water gives, after drying, 4.54 g (36%) of white crystalline 3, m.p. 226-228°C. NMR: 4.63 (t, 17 H), 2.03 (s, $COCH_3$), 0.82 and 0.78 (2s, 18 and 19-Me).

Step 3: 3 to 17β-acetoxy-Anor-5α-androstan-2-one (4)

The A-ring of the steroid is reclosed to a five-membered ring according to Scribner's procedure[1]. A sandbath and short-path (5 cm) right-angle tube are used instead of a Woods metal bath and Claissen adapter, respectively. 7.28 g 3 are dissolved in 60 ml acetic anhydride and refluxed for 2 hr. The reflux condenser is replaced by a distillation head, and 60 ml of liquid are collected at atmospheric pressure. The distillation head is replaced by the short-path tube and a collecting flask, and the dark green distillation residue is slowly heated to 260°C. with 10-15 mm vacuum applied from the side-arm of the receiving flask. The temperature is maintained between 260-270°C. for 1 1/2 hr. The vacuum is then increased, and heat is applied to the side-arm through use of a heating tape to force the distillation into the receiving flask. The crude product, a yellow solid, is dissolved in $CHCl_3$ and washed with 5% $NaHCO_3$ and saturated NaCl. After drying over Na_2SO_4 and stripping of the solvent, 4.32 g 4 are obtained (71%). NMR: 4.63 (t, 17 H), 2.01 (s, $COCH_3$), 0.85 and 0.81 (2s, 18 and 19-Me). m.p. 112-113.5[1].

Procedure for Synthesis of α-Anordrin (cont.)

Step 4: 4 to 17β-hydroxy-Anor-5α-androstan-2-one (5)

4 is dissolved in 1N NaOH in 80% ethanol and stirred, at room temperature, for two hours or longer. The reaction mixture is neutralized with ice-cold 1N HCl and extracted with ethyl acetate. Following drying over Na_2SO_4 and
stripping of the solvent, 5 is obtained in approximately 95% yield. m.p. 189-192°C. (lit. m.p. 191-194°C.[2]). NMR: 0.85 and 0.76 (2s, 18 and 19-Me).

Step 5: 5 to Anor-5α-androstan-2,17-dione (6)

Jones reagent (13.36 g CrO_3 in 11.5 ml conc. H_2SO_4, diluted to 50 ml with H_2O) is added dropwise, with stirring, to a solution of 5 in acetone at 0-5°C. until a reddish color remains. After approximately 30 minutes, isopropanol is added to remove excess Jones reagent. Following addition of ice cold water, the reaction mixture is extracted with ether. The ethereal layer is washed with 5% $NaHCO_3$, dried over Na_2SO_4, and stripped. The white solid obtained is recrystallized from ether to give 6. m.p. 170-171°C (lit. m.p. 171-173°C.[2]). NMR: 0.89 (s, overlapping 18 and 19-Me).

Step 6: 6 to 2α,17α-diethynyl-Anor-5α-androstan-2β,17β-diol (7)

Ethynylation of 6, carried out according to Crabbe's procedure[3], yields a mixture of epimers at the C-2 position, as shown by NMR. Although repeated recrystallization from CH_2Cl_2/petroleum ether can result in pure 7, chromatography of the esterified Anor steroids separates the epimers more efficiently. Dry acetylene gas is bubbled through a solution of 9.3 g lithium acetylide-ethylene diamine complex in 16 ml anhydrous DMSO for 20 min. with stirring. The reaction mixture is cooled to 0-5°C. and 3.26 g 6 in 96 ml anhydrous DMSO is added. After bubbling through acetylene gas for an additional 30 min., the reaction is quenched with 10% aqueous ammonium chloride, filtered, and extracted with dichloromethane. The organic layer is washed with water, dried, filtered, and stripped to yield 2.54g (66%) of a pale yellow oil. In order to separate out any unreacted material, a Girard's separation is performed. 10 ml of propionic acid and 2 g Girard "T" reagent is added per gram of crude reaction product. This is heated at 100°C with reflux for 30 min. The mixture is then cooled in an ice bucket. A solution of 17 g anhydrous Na_2CO_3 in 150 ml H_2O is added per gram of crude material. The pH of the reaction mixture is checked to be sure it is alkaline. The mixture is extracted 3 times with 100 ml of 1:1 ethyl ether:petroleum ether. The organic layer is washed 3 times with 100 ml distilled water. The organic layer is dried over Na_2SO_4 and then evaporated. NMR: 2.56 (with shoulder) and 2.49 (-CCH), 0.93 (with shoulder) and 0.85 (18 and 19-Me). m.p. 169-170°C.

Step 7: 7 to 2α,17α-diethynyl-Anor-5α-androstane-2β,17β-diol dipropionate (8)

Crabbe's procedure[3] is used to esterify 7. Excess propionic anhydride (15 ml) is added to a solution of 1.6g 7 in 20 ml dry pyridine. The reaction mixture is refluxed for 15-17 hr at 112-117°C., yielding a dark brown solution. Methanol is added to the cooled reaction mixture to

Procedure for Synthesis of α-Anordrin (cont.)

Step 7 (cont.):

decompose excess propionic anhydride. After addition of ice water and neutralization with 10% HCl, the solution is extracted with dichloromethane. The organic layer is washed with 10% HCl and water, dried, filtered, and stripped, yielding 1.46 g of a light brown oil. Recrystallization from methanol at this point to yield pure α-Anordrin has not been successful. However, chromatography over silica gel with petroleum ether/acetone (3/1) as the eluting solvent does separate the C-2 epimers. Fractions containing α-Anordrin are combined, the solvent stripped, and the resulting solid recrystallized from methanol. m.p. 152.0-152.5°C. NMR: 2.58 and 2.54 (2s, -CCH), 1.13 (t, 2 $-CH_2CH_3$, 8Hz), 0.86 and 0.84 (2s, 18 and 19-Me).

(Note: The amounts of reactants described in the synthesis of α-Anordrin are from actual syntheses run in the laboratory. Amounts of various components of the reactions vary proportionally to the amount of steroid present.)

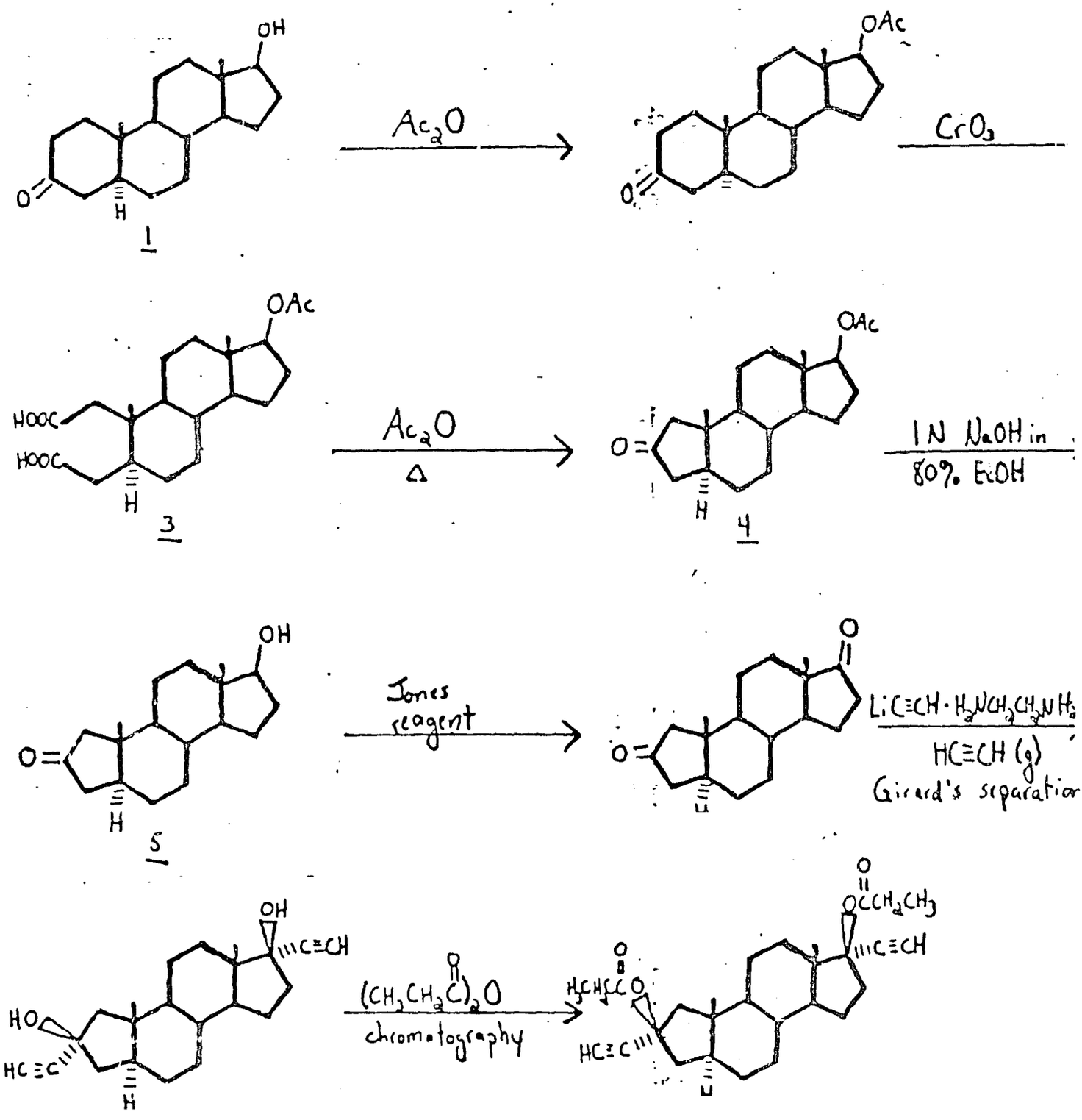
REFERENCES

1. Scribner, R.N. in "Organic Reactions in Steroid Chemistry, v.2," ed. J. Fried and J.A. Edwards, Van Nostrand Reinhold, N.Y., 1972, p.409.

2. Minson, M. and J. Jacques, Bull Soc Chim Fr, 1965, p.71.

3. Crabbe, P. et al, Steroids, 33:85, 1979.

Synthesis of anordrin



Appendix III

DETAILED DESCRIPTION OF THE STUDIES

Pharmacokinetics and Metabolism of Anordrin.

Anordrin synthesized under GLP guidelines will be utilized for the study. It will be mixed in proportions with ^{14}C-anordrin to give 25 Ci doses in 0.2 mg/kg b.w. and 1.0 mg/kg b.w. dose. An aliquot of the ^{14}C-anordrin obtained from New England Nuclear Corp. will be recrystallized with authentic anordrin to establish purity of the labeled compound before use. The ^{14}C-anordrin dose will be dissolved in sesame oil for i.m. administration to the test animals.

Six monkeys will be quarantined for two months. During the second month, urine will be collected daily to determine the occurence of normal ovarian hormone production and ovulation from assays of urinary pregnanediol glucuronide and estrone glucuronide. Only monkeys with normal menstrual cycles and normal ovarian function will be used in the study. They will also be examined by a veterinarian to exclude any animals with any detectable disease. They will also undergo the usual testing procedures for tuberculosis.

The specific details of the intubation and sampling procedure is as follows:

Fast monkeys for 12 hr prior to study.
Provide water ad lib.
Anesthetize monkeys with ketamine intramuscularly. Anesthesia will be maintained for 4-6 hours only.
Place angiocath by cut down in a jugular vein with a heparinized stopcock attached for blood drawing.
Insert a peripheral line for fluid replacement during anesthesia.
Insert a catheter for urine collection.
Draw blood for protein-binding studies with ^{3}H-anordrin, and for RBC/plasma partition of ^{3}H-anordrin.
Give ^{14}C-anordrin (25 μCi) intramuscularly.
Draw 1.5 ml blood samples at 0, 5, 15, 20, 30, and 45 min and 3.0 ml blood samples at 60, 90, 120, 180, 240, 360, 420, 480, and 1440 min.
Replace fluid with Ringer's solution.
Draw urine from the catheter at hourly intervals, emptying the bladder each time, for the first 8 hours.
Continue 12 hr urine collections for 4 days.
Assay plasma for total ^{14}C-steroids, for conjugated and unconjugated metabolites, and for unmetabolized ^{14}C-anordrin.
Three monkeys will be studied at each dose level.

Plasma samples will be analyzed by addition of an aliquot to liquid scintillation fluid such as Bray's for direct count of ^{14}C in a liquid scintillation spectrometer. Another aliquot will be extracted with diethyl ether to extract unmetabolized anordrin and any unconjugated metabolites into the organic solvent. A count of part of the ether and remaining water soluble fractions will be taken to determine the extent of conjugation to glucuronides and other water-soluble forms that occurs as clearance proceeds. The larger part of the ether-soluble material from every 4th sample in the sequence will be analyzed by thin-layer chromatography to determine the extent of unconjugated metabolites in the ^{14}C material in this fraction. If 90% or more of ^{14}C in the

ether fraction is unmetabolized ^{14}C-anordrin, this analysis will not have to be done on all samples in order to estimate the disappearance characteristics if the drug. The total accumulation of ^{14}C-steroids in urine during the course of the experiment will be measured as well as the occurrence of conjugates that can be hydrolyzed by β-glucuronidase and sulfatase.

Plasma samples will also be analyzed for anordrin by radioimmunoassay. Data will be analyzed according to the method described by Berman and Weiss (SAAM Manual. Public Health Service Publication No. 1703, U.S. Government Printing Office, Washington, D.C., 1967) with the Saam 23 computer program on the Control Data Corp Cyber 170/130 computer.

To analyze the oral bioavailability of anordrin, the three monkeys studied at 1 mg/kg dose, will be studied two weeks after the initial study by giving 1.0 mg/kg anordrin orally and drawing blood for anordrin RIA at time 0, 15, 30, 45, 60, 120, 180, 240, 360, 480, and 1440 minutes after dosing. For these studies no anesthesia will be used.

Time Schedule

The date for beginning evaluation of monkeys for entry into the study will be July 1, 1985. The animals therefore must be

Appendix IV

Anacardin Metabolism #99 i.v. net cpm / 0.25 ml serum

Time	Free anacard	diol	polar	orig	Bound anacard	diol	polar	orig
0								
1	66	2506	43	36	321	1246	63	119
2	96	2504	45	40	351	1286	73	119
3	46	4855	75	52	136	1429	66	109
5	27	2510	92	71	87	739	56	101
8	17	1492	110	98	64	420	46	103
10	–	1148	129	64	–	331	47	98
15	–	946	169	82	–	245	46	101
20	–	913	178	93	–	183	41	73
30					–	674	174	167
45					–	711	154	224
60					–	442	141	187
90					–	400	135	199
120					–	327	117	187
180					–	285	169	165
240					–	197	157	99
11/1						66	125	174

Combined Free and Bound

Anordrin Metabolism #101 i.v. net cpm / 0.25 ml serum

Time		Free anord	Free diol	Free polar	Free orig	Bound anord	Bound diol	Bound polar	
0	33								
1		228	18,187	210	93	430	1305	84	157
2		55	11,455	154	73	285	1268	78	144
3		73	7,991	195	50	349	1084	88	140
5		10	4,246	218	61	96	666	74	136
8		4	2,371	264	77	69	272	67	128
10		–	2,004	327	118	—	242	60	134
15		–	1,764	372	90	—	174	64	148
20		–	1,578	429	106		138	60	153
30							1172	281	283
45							1056	315	288
60							847	267	289
90							733	235	299
120							593	296	283
180							392	161	259
240							272	161	
11/15							138	121	

Combined Free and Bound

Anordrin Metabolism #103 iv. net cpm/0.25 ml serum

Time		Free				Bound			
		guard	dial	polar	orig	guard	dial	polar	orig
0	<2								
1	0.14	89	6,236	96	46	671	864	18	78
2	*	53	8,220	133	44	62	387	57	84
3		100	6,815	139	69	—	129	45	127
5		102	5,540	189	66	53	173	51	120
8		—	3,020	214	60	48	130	50	124
10		—	2,119	257	96		121	63	125
15			1,010	473	229		279	46	97
20			1,012	254	69		95	48	105
30	.25ml						202	162	250
10/8	70ml						121	93	214
10/9	no serum								
10/11			Combined Free and Bound				40	37	288
10/14							11	39	212
10/16							9	26	116
10/18							3	9	93
10/21							0	7	56

* 2 min 0.45 ml second assay

137

Anordrin Metabolism #105 (i.v.) cpm / 0.25 ml

Time		Free anord	Free diol	Free polar	Free ori	Bound anord	Bound diol	Bound polar	Bound ori
0	10/14/86								
1	0.09 ml	622	21,594	327	66	1783	6922	350	—
2	0.15	—	18,560	300	67	1170	4287	243	—
3	0.5	12	6,551	94	55	135	1429	68	109
5	0.25	8	7,062	210	188	174	1478	51	202
8	0.18	—	2889	167	177	—	1166	36	288
10	0.3	3	1647	153	141	—	552	59	163
15	0.4	2	1239	205	147	—	306	91	126
20	0.3	0	1078	195	167	—	303	[illegible]	121
30	0.5					—	335	110	55
45							277	119	52
60							282	128	62
90							160	99	42
120							154	116	47
180									
240									
10/15							19	83	36
10/16							25	60	28
10/17							6	35	25
10/18							6	31	21
10/19							7	28	27
10/20							11	36	27
10/21							5	29	[illegible]

Combined Free and Bound

Acid from the labelling
Serum sampling with rapid freezing (-70°C)

m 104 i.v.

Time (min)	Total cpm	in 0.25 ml	fraction	
	around	dial	pster	acid
2/5/80				
1	8335	101	132	142
2	8397	196	162	164
3	6217	326	242	103
5	2424	386	370	84
8	1370	501	448	126
10	904	600	224	49
15	410	652	146	[illegible]
20	291	600	210	60
31	317	616	406	112
40	246	632	436	124
60	166	542	304	116
90	146	412	214	160
120	140	316	[illegible]	204
180	116	402	[illegible]	240
240	81	312	204	270
2/6	18	100	135	120
2/7	11	48	142	116

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ANALYSIS OF METABOLITES AFTER 14C-ANORDRIN WITH RAPID FREEZING OF SERUM.

ANORDRIN

Time (min)	Ether Extract Total	Ether Extract Net	Acetone Extr. Total	Acetone Extr. Net	Ratio A/E
0	104	24.00	93	83.00	0.35
2	1623	1,603.00	14757	14,727.00	9.19
5	1110	1,080.00	10025	10,025.00	9.28
10	590	570.00	8089	8,059.00	14.14
15	368	338.00	3440	3,410.00	10.09
20	227	207.00	1784	1,754.00	8.47
		4,022.00 +		38,038.00	

ANALYSIS OF METABOLITES AFTER 14C-ANORDRIN WITH RAPID FREEZING OF SERUM.

ANORDIOL

Time (min)	Ether Extract Total	Ether Extract Net	Acetone Extr. Total	Acetone Extr. Net	Ratio A/E
0	51	21.00	93	63.00	3.00
2	45	15.00	112	82.00	5.47
5	78	43.00	167	132.00	3.07
10	101	71.00	324	324.00	4.70
15	244	214.00	440	410.00	1.92
20	298	268.00	586	556.00	2.07
		637.00		1,582.00	

ANALYSIS OF METABOLITES AFTER 14C-ANORDRIN WITH RAPID FREEZING OF SERUM.

COMPOUND III

Time (min)	Ether Extract Total	Ether Extract Net	Acetone Extr. Total	Acetone Extr. Net	Ratio m/E
0	33	7.00	41	11.00	7.67
2	56	26.00	152	132.00	5.08
5	53	23.00	163	133.00	[illegible]
10	52	22.00	215	185.00	8.45
15	109	79.00	133	153.00	1.7[illegible]
20	134	104.00	[illegible]	197.00	[illegible]
		257.00		812.00	

ANALYSIS OF METABOLITES AFTER 14C-ANORDRIN WITH RAPID FREEZING OF SERUM.

COMPOUND IV

Time (min)	Ether Extract Total	Ether Extract Net	Acetone Extr. Total	Acetone Extr. Net	Ratio N/E
0	37	7.00	41	11.00	1.57
2	36	6.00	90	60.00	10.00
5	35	5.00	119	89.00	17.80
10	37	7.00	109	79.00	11.29
15	32	2.00	105	75.00	37.50
20	33	3.00	121	91.00	30.33
		30.00		405.00	

Anaerobic Metabolism in M 102 2/27

	Time	Free (cpm/0.25 ml sample)				Bound (cpm/0.25 ml)				
		acid	dial	polar	orig	acid	dial	polar	orig	Total dial
2/27	0									
	1	5	1150	127	30	34	2939	452	530	4092
	2	1	631	77	10	19	1746	348	225	2407
	3	0	651	147	10	17	1442	345	224	2293
	5	3	754	172	14	4	1282	343	222	2040
	8	–	671	202	13	11	1261	271	185	2102
	10	4	700	248	6	7	1080	260	159	1830
	15	0	625	312	5	10	1084	345	196	1609
	20	3	700	259	8	5	691	265	177	1654
	30	4	411	205	6	0	651	312	209	1270
	44	2	449	341	4	8	700	292	218	1149
	60	0	400	291	4	1	439	220	155	859
	90	2	351	420	17	0	423	227	126	774
	120	4	261	423	21	0	296	245	126	563
	180	2	220	492	23	0	193	165	119	423
	240	2	197	279	15	6	240	262	211	393
2/28		2	91	223	16	0	56	203	62	
3/1		0	6	101	10	1	28	143	59	
3/2		0	5	91	13	3	10	82	50	
3/3		0	2	26	9	4	4	69	42	
3/4		1	1	15	10	0	7	59	51	
3/5		2	2	10	2	0	6	25	12	
3/6		2	5	6	4	0	3	26	11	
3/7		2	2	1	2	3	3	19	20	

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Aneurin Metabolism #99 i.m. net cpm / 0.25 ml

Time		ansrd	dial	polar	urine	acid metab	aq residue
12/11/65	0.2 ml	0	0	0	0	Bg = 32	
12/12	0.5	—	6	10	12	78	0
12/13	0.5	—	82	21	23	133	0
12/14	0.15		—	—	—	—	—
12/15	0.5		11	23	19	197	5
12/16	"		11	30	32	249	0
12/17	"		14	32	40	292	0
12/18			20	20	41	274	0
12/19			21	37	25	246	20
12/20			14	44	23	294	4
12/21			7	30	14	575	0
12/22			2	26	23	277	4
12/23			4	29	21	259	2
12/24			1	22	22	249	0
12/25			8	21	19	254	0
12/26			3	12	25	254	0
12/28							
12/29							
12/30							
12/31							

Anordrin Metabolism #101 cim. net cpm / 0.25 ml

Time	anord	diol	polar	orig
0 12/30/65	0	0	0	0
12/31	—	6	2	9
1/1	—	—	—	—
1/2	—	7	9	21
1/3	—	—	—	—
1/4	—	7	13	26
1/5	—	6	10	23
1/6		13	14	19
1/7		10	12	20
1/8		—	—	—
1/9		11	43	70
1/10		12	39	40
1/11		18	38	42
1/12		6	46	40
1/13		4	35	39
1/14		1	25	43
1/15		0	53	40
1/16		0	29	40
1/17		0	14	20

B_{ij} = .32

Anordrin Metabolism #103 d.m., net cpm / 0.25 ml

Time	ansrd	diol	polar	orig
12/2/85				
12/3	–	5	5	14
12/4		7	8	7
12/5	–	7	4	17
12/6	–	8	5	13
12/7	–	13	20	33
12/8		13	20	55
12/9		7	10	25
12/10		10	17	47
12/11		9	36	24
12/12		10	32	27
12/14		4	29	24
12/15		7	25	20
12/16		9	50	36
12/17		—	—	—
12/18		5	26	19
12/19		5	26	19
12/20		7	27	13

Anesdrin Metabolism #105 dim net cpm / 0.25 ml

Time	anred	diol	polar	acid	
12/16/85					
12/17	—	1	9	6	
12/18	—	51	18	9	
12/19	—	11	23	13	
12/20	—	12	37	15	
12/21	-	11	30	17	
12/22		11	21	19	replaced
12/23		4	21	20	
12/24		6	17	11	
12/25					
12/26		5	30	27	
12/27		11	29	60	
12/28		2	39	59	
12/29		2	30	30	replaced
12/30		3	28	20	
12/31		7	39	27	
1/1		—	—	—	
1/2		3	20	26	
1/3					
1/4		2	21	29	

Appendix IV

Amoebic Metabolism 100 cim. net cpm / 0.25 ml serum

Time		anerd	diel	polar	orig	
11/6/65	0	0	0	0	0	B_0 32
15		0	0	2	12	
30	0.35 ml	0	6	7	34	
45		0	14	8	19	
60		0	22	10	20	
120		0	25	9	15	
240	0.4 ml	0	41	12	36	6/3/66 spike
360		0	65	33	22	
11/7		—	108	83	110	
11/8		—	101	126	178	
11/9			82	86	201	
11/10			62	80	196	
11/11			85	116	211	
11/12			83	114	217	
11/13			60	92	231	
11/14			56	94	218	
11/15			68	121	200	
11/16						
11/17						
11/18						
11/19						

circled values are averages of two assays

Anacetin Metabolism 102 i.m. net cpm / 0.25 ml

Time		anacetin	diol	polar	ori.
10/25/85		0	0	0	0
5	0.4 ml	0	0	4	25
15	0.13 ml	—	—	—	—
30		0	14	11	40
45		0	14	10	29
60		0	22	9	30
120		0	48	22	26
240		0	55	25	16
360		0	124	60	34
10/26		—	154	148	132
10/27		—	188	250	282
10/28		—	137	221	162
10/29			94	188	204 *
10/30			126	200	274
10/31			100	104	304
11/1			104	163	230
11/2			113	182	255
11/3					
11/4					
11/5					
11/6					
11/7					

* circled values are average of duplicate assays

Anordrin Metabolism 104 dim. net cpm / 0.25 ml

Time		anord	diol	polar	orig
10/18/85		0	0	0	0
5	0.17 ml	—	—	—	—
15	0.16 ml	—	—	—	—
30		0	2	4	29
45		0	2	5	20
60		0	7	7	19
120		0	19	13	13
240		0	33	18	16
300		0	45	32	29
10/19		—	64	92	80
10/20		—	64	127	103
10/21			62	56	116 —
10/22			92	143	320
10/23			100	134	333
10/24			120	157	346
10/25			111	142	351
10/26			130	211	432
10/27					
10/28					
10/29					
10/30					
10/31					
11/1					
11/2					

Amiodrin metabolism 104 (dim.) cpm / 0.25 ml serum

Time		Free anord	Free diol	Free polar	Free orig	Bound anord	Bound diol	Bound polar	
0	10/18/85					4	0	1	6
5						0	0	1	5
15						1	3	2	17
30						2	6	3	9
45							5	2	11
60							12	14	12
120							18	9	16
240							24	15	24
360							42	50	44
10/19							57	75	79
10/20							50	51	61
10/21		38	648	806	318		56	94	81
10/22							73	100	117
10/23							94	111	208
10/24							102	111	
10/25							106	129	
10/26							116	116	466
10/27							104	96	460
10/28							76	110	446
10/29							70	90	408
10/30		3	103	685	133		70	68	352
10/31							36	70	266
11/1							34	44	354
11/2							34	42	244
11/3									

Appendix V

Aurin Pharmacokinetics (i.v.)

Serum (conc. to in 0.25 ml)

#99 10/31/65	total cpm
0 min	20
1	5922
2	6177
3	7741
5	4219
8	2935
10	2362
15	2181
20	2087
30	1702
45	1611
60	1376
90	1293
120	1254
180	1072
240	931
11/1	916
11/2	675
11/3	562
11/4	476
11/5	320
11/6	290
11/7	299
11/8	279
11/9	260
11/11	219
11/13	193
11/15	162
11/18	126
11/20	106
11/22	102
11/25	91
11/27	72
11/29	66

#101 11/14/65	total cpm
0 min	18
1 min	23,855
2	14,625
3	9,613
5	5760
8	3874
10	3465
15	3040
20	2786
30	2606
45	2483
60	2002
90	1661
120	1601
180	1226
240	1154
11/15	840
11/16	924
11/17	555
11/18	467
11/19	421
11/20	333
11/21	289
11/22	229
11/25	199
11/27	163
11/29	123
12/2	116
12/4	104
12/5	101
12/6	89
12/9	99
12/11	76
12/13	70

#103 10/7/65	total cpm
0	
1	5903
2	9785
3	6554
5	5335
7	3608
10	2715
15	2009
20	1800
30	1036
10/8	1040
10/9	1027
10/11	1104
10/14	664
10/16	526
10/18	425
10/21	407
10/23	375
10/25	270
10/26	232
10/29	225
11/1	193
11/4	169
11/8	131
11/11	109
11/15	96
11/18	47

Anordrin Pharmacokinetics

Serum (counts in 0.25 ml)

#105 (i.v.) 10/14/85	total cpm	#99 (i.m.) 12/11/85		#101 (i.m.) 12/30/85	
0		0	89?	0	47
1	20,386	3½ hr	123	21 hr	116
2	15,271	20½ hr	194	47 hr	166
3	9988	12/12 47½ hr	254	95 hr	208
5	5842	12/14	245	1/5	211
8	3945	12/15	294	1/6	218
10	3375	12/16	379	1/7	267
15	2855	12/17	403	1/8	484
20	2564	12/18	405	1/9	393
30	2198	12/19	310	1/10	292
45	1956	12/20	419	1/11	288
60	1871	12/21	318	1/12	300
90	1544	12/22	313	1/13	298
120	1441	12/23	349	1/14	282
180	1397	12/24	302	1/15	299
240	1284	12/26	299	1/16	271
10/15	1049	12/27	305	1/17	234
10/16	833	12/28	311	1/18	272
10/17	630	12/29	330	1/19	221
10/18	620	12/30	240	1/20	225
10/19	600	12/31	229	1/21	190
10/20	566	1/2	242	1/22	200
10/21	641	1/4	210	1/23	172
10/23	581	1/5	246	1/24	187
10/25	519	1/6	227	1/25	155
10/28	445	1/8		1/27	118
10/30	429	1/10	167	1/29	125
11/1	429	1/13	158	1/31	112
11/5	256	1/15	150	2/3	98
11/8	245	1/17	144	2/5	81
11/11	226	1/20	137	2/7	77
11/15	150	1/22	123	2/10	74
11/18	142	1/24	123	2/12	
		1/27	108		

Anordrin Pharmacokinetics

Serum (counts in 0.25 ml)

#103 (i.m.)

12/2/25	
0	42
2 hr	61
17 hr	85
44 hr	114
12/5	125
12/6	142
12/7	207
12/8	218
12/9	200
12/10	267
12/11	239
12/12	204
12/13	241
12/14	223
12/15	216
12/16	228
12/17	237
12/18	210
12/19	208
12/20	185
12/21	188
12/22	186
12/23	215
12/24	174
12/25	162
12/26	171
12/29	168
12/30	152
12/31	151
1/2	149
1/4	121
1/6	140
1/8	
1/13	105
1/15	109
1/17	110
1/20	105
1/22	89
1/24	91
1/27	73

#105 (i.m.)

12/16/25	
0	156 ?
24 hr	177
12/18	232
12/19	86
12/20	261
12/21	257
12/22	273
12/23	300
12/24	287
12/26	303
12/27	338
12/28	344
12/29	295
12/30	294
12/31	328
1/2	216
1/4	277
1/5	278
1/6	271
1/8	276
1/10	227
1/13	228
1/15	214
1/17	178
1/18	—
1/19	—
1/20	182
1/22	160
1/24	161
1/27	157
1/29	140
[illegible]	[illegible]
2/5	10
2/7	13
2/10	1
2/12	

Anerdrin Pharmacokinetics

Serum (conc. in 0.25 ml)

#100

(i.m. 10 mg)

11/6/85	
0	—
5 min	67
15	—
30	132
45	115
60	144
120	148
240	199
360	263
24 hr.	666
11/6 —	1216
11/9	1050
11/10	1096
11/11	1140
11/12	1127
11/13	1422
11/14 —	1426
11/15	1201
11/16	1040
11/17	906
11/18	992
11/19	895
11/20	764
11/21	772
11/22	769
11/23	805
11/24	713
11/25	767
11/26	697
11/27	678
11/29	6[illegible]1
11/30	714
12/1	700
[illegible]	[illegible]

12/3	584
12/4	579
12/5	600
12/6	585
12/7	545
12/8	563
12/9 —	569
12/10	527
12/11	469
12/12	450
12/13	428
12/14	403
12/15	456
12/16	497
12/18	398
12/20	316
12/22	209
12/24	269
12/26	258
12/28 —	252
12/31	228
1/2	229
1/4	196
1/6 —	216
1/8 —	226
1/10	163
1/13	168
1/15	150
1/17	142
1/20	133
1/22	123
1/24	115
1/27	106

Anordrin Pharmacokinetics

Serum (counts in 0.25 ml)

#102

(Anordrin, 1.0 mg)

10/25/85	
0	20
5 min	68
15	96
30	119
45	119
60	124
120	167
240	202
360	355
26½ hr	846
10/27	1236
10/28	1169
10/29	1195
10/30	1585
10/31	1302
11/1	1376
11/2	1390
11/3	1248
11/4	1659
11/5	1272
11/6	1138
11/7	1012
11/8	1020
11/9	1180
11/10	1125
11/11	911
11/12	807
11/13	792
11/14	885
11/15	649
[illegible]	651
11/17	565
11/18	558
11/19	529
11/20	473
11/21	407
11/22	392
11/23	385
11/24	397
11/25	355
11/26	308
11/27	299
11/29	251
11/30	252
12/1	239
12/2	220
12/3	234
12/4	244
12/6	208
12/9	195
12/11	165
12/13	159
12/16	165
12/18	153
12/20	105
12/22	104
12/24	92
12/26	83
12/28	85
12/31	78
1/2	80

Anordrin Pharmacokinetics

Serum (counts in 0.25 ml)

#104

(i.m., 1.0 mg)

10/18/85	
0	24
5 min	55
15	80
30	97
45	108
60	103
120	136
240	180
360	217
22½ hr	574
10/20	894
10/21	719
10/22	1021
10/23	1328
10/24	1597
10/25	1649
10/26	2060
10/27	2014
10/28	2375
10/29	2266
10/30	2338
10/31	2222
11/1	2025
11/2	1925
11/3	1790
11/4	1846
11/5	1428
11/6	1270
11/7	1115
11/8	1051
11/9	910
11/10	1004
11/11	996
[illegible]	951
11/13	1007
11/14	965
11/15	804
11/16	757
11/17	684
11/18	670
11/19	595
11/20	560
11/21	550
11/22	502
11/23	512
11/24	529
11/25	462
11/26	
11/27	346
11/29	315
12/2	307
12/4	299
12/6	268
12/9	269
12/11	257
12/13	211
12/16	223
12/18	201
12/20	149
12/22	134
12/24	118
12/26	116
12/28	116
12/31	98
1/2	93

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Appendix VI

* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY99 * I.V. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPM/ml	VOL g/day
1	K	1 CY99	11/01/85		25800		01	1	9545	270
2	K	2 CY99	11/02/85		14500		01	2	5917	245
3	K	3 CY99	11/03/85		13300		01	3	6064	220
4	K	4 CY99	11/04/85		8100		01	4	4238	190
5	K	5 CY99	11/05/85		5900		01	5	2173	270
6	K	6 CY99	11/06/85		4100		01	6	1834	225
7	K	7 CY99	11/07/85		3400		01	7	1563	220
8	K	8 CY99	11/08/85		2200		01	8	952	235
9	K	9 CY99	11/09/85		1100		01	9	510	220
10	K	10 CY99	11/10/85		1100		01	10	540	205
11	K	11 CY99	11/11/85		700		01	11	411	170
12	K	12 CY99	11/12/85		540		01	12	231	235
13	K	13 CY99	11/13/85		400		01	13	184	215
14	K	14 CY99	11/14/85		410		01	14	195	210
15	K	15 CY99	11/15/85		450		01	15	228	195
16	K	16 CY99	11/16/85		290		01	16	140	205
[illegible]	K	17 CY99	11/17/85		260		01	17	100	255
18	K	18 CY99	11/18/85		250		01	18	120	205
19	K	19 CY99	11/19/85		220		01	19	115	195
20	K	20 CY99	11/20/85		220		01	20	92	240
21	K	21 CY99	11/21/85		180		01	21	76	240
22	K	22 CY99	11/22/85	3	200	0.8	01	22	74	265
23	K	23 CY99	11/23/85	4	170	1.0	01	23	65	260
24	K	24 CY99	11/24/85	7	180	1.7	01	24	73	245
25	K	25 CY99	11/25/85	14	250	2.9	01	25	118	210
26	K	26 CY99	11/26/85	12	170	2.7	01	26	84	225
27	K	27 CY99	11/27/85	16	200	3.8	01	27	86	235
28	K	28 CY99	11/28/85	35	165	4.7	01	28 Not collected	121	135
29	K	29 CY99	11/29/85	35	165	4.7	01	29 - 2 day collected	121	135
30	K	30 CY99	11/30/85	30	140	5.7	01	30	72	190
31	K	31 CY99	12/01/85	30	250	6.0	01	31	124	200
32	K	32 CY99	12/02/85	93	180	21.9	01	32	78	235
33	K	33 CY99	12/03/85	25	200	5.9	01	33	85	235
34	K	34 CY99	12/04/85	75	140	17.2	01	34	60	230
35	K	35 CY99	12/05/85	37	140	9.4	01	35	54	255
36	K	36 CY99	12/06/85	30	150	7.7	01	36	68	215
37	K	37 CY99	12/07/85	15	160	3.7	01	37	64	245
38	K	38 CY99	12/08/85	19	120	3.4	01	38	68	180
39	K	39 CY99	12/09/85	8	90	2.0	01	39 Menses	36	250
40	K	40 CY99	12/10/85	14	160	2.5	01	40 Menses	90	175
41	K	41 CY99	12/11/85	9	170	2.2	01	41 Menses	69	250

****** 07:30:14 *******

* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY99 * I.M. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Pdrol mg/ml	T_CPM /100	T_Pd	SERIES day		REMARKS	NET_C CPMml	VOL g/day
42	K	42 Cr99	12/12/85		2500		02	01		955	265
43	K	43 Cr99	12/13/85		4200		02	02		1712	245
44	K	44 Cr99	12/14/85		3800		02	03		1869	200
45	K	45 Cr99	12/15/85		4100		02	04		2166	190
46	K	46 Cr99	12/16/85		5400		02	05		2715	200
47	K	47 Cr99	12/17/85		6200		02	06		2219	280
48	K	48 Cr99	12/18/85		4000		02	07		1786	255
49	K	49 Cr99	12/19/85		4100		02	08		1540	265
50	K	50 Cr99	12/20/85		4900		02	09		1330	355
51	K	51 Cr99	12/21/85		4900		02	10		1639	300
52	K	52 Cr99	12/22/85		4300		02	11		1577	270
53	K	53 Cr99	12/23/85		4300		02	12		2134	200
54	K	54 Cr99	12/24/85		3500		02	13		1433	245
55	K	55 Cr99	12/25/85		3300		02	14	Not collected	2200	150
56	K	56 Cr99	12/26/85		3300		02	15	- 2 days collected	2200	150
57	K	57 CY99	12/27/85		3300		02	16		1240	270
58	K	58 Cr99	12/28/85		3100		02	17		1073	290
59	K	59 Cr99	12/29/85		3100		02	18		1227	250
60	K	60 Cr99	12/30/85		3100		02	19		1200	260
61	K	61 Cr99	12/31/86		3100		02	20		1120	280
62	K	62 Cr99	01/01/86		2100		02	21	Not collected	1319	160
63	K	63 Cr99	01/02/86	2	2100	0.6	02	22	- 2 days collected	1319	320
64	K	64 Cr99	01/03/86		1300		02	23	Not collected	975	133
65	K	65 CY99	01/04/86	2	1300	0.5	02	24	- 2 days collected	975	133
66	K	66 Cr99	01/05/86	2	1200	0.5	02	25		464	255
67	K	67 Cr99	01/06/86				02	26	Urine collections	0	0
68	K	68 Cr99	01/07/86				02	27	discontinued until	0	0
69	K	69 CY99	01/08/86				02	28	1/23/86	0	0
70	K	70 Cr99	01/09/86				02	29		0	0
71	K	71 Cr99	01/10/86				02	30		0	0
72	K	72 Cr99	01/11/86				02	31		0	0
73	K	73 Cr99	01/12/86				02	32		0	0
74	K	74 Cr99	01/13/86				02	33		0	0
75	K	75 CY99	01/14/86				02	34		0	0
76	K	76 Cr99	01/15/86				02	35		0	0
77	K	77 Cr99	01/16/86				02	36		0	0
78	K	78 Cr99	01/17/86				02	37		0	0
79	K	79 CY99	01/18/86				02	38		0	0
80	K	80 Cr99	01/19/86				02	39		0	0
81	K	81 Cr99	01/20/86				02	40		0	0
82	K	82 Cr99	01/21/86				02	41		0	0
83	K	83 Cr99	01/22/86				02	42		0	0
84	K	84 Cr99	01/23/86	10	370	2.4	02	43		153	240
85	K	85 Cr99	01/24/86	5	560	1.5	02	44		187	300
86	K	86 Cr99	01/25/86	8	630	2.0	02	45		246	250
87	K	87 Cr99	01/26/86		480	2.2	02	46	Not collected	411	123
88	K	88 Cr99	01/27/86	18	480	2.2	02	47	- 2 days collected	411	123
89	K	89 Cr99	01/28/86	6		1.8	02	48		0	295
90	K	90 Cr99	01/29/86	7		2.6	02	49		0	370
91	K	91 Cr99	01/30/86	7		2.4	02	50		0	340

* Endosis * Moxidrin Pharmacokinetics Studies - Monkey *

PK_URINE* CY99 * I.M. 0.2 mg/kg

* print out from PRINT OUT * 12/07/00 * JKM *

Record	Labnum	Subject	DATE	Total ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPM/ml	VOL g/day
92	R	92 CY99	01/31/00	15	550	3.9	02	51	203	260
93	R	93 CY99	02/01/00	10	480	3.8	02	52	125	360
94	R	94 CY99	02/02/00	7	400	2.7	02	53	161	285
95	R	95 CY99	02/03/00	5	380	1.2	02	54	155	245
96	R	96 CY99	02/04/00	3	520	1.0	02	55	154	335
97	R	97 CY99	02/05/00	1	440	0.3	02	56 Menses	136	325
98	R	98 CY99	02/06/00	2	360	0.6	02	57 Menses	112	320

****** 07:27:11 *******

* ENDOSTS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY100 * I.M. 1.0 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPM/ml	VOL g/day
99	K 99	CY100	11/07/85		6700		03 1		3327	200
100	K 100	CY100	11/08/85		9400		03 2		5356	175
101	K 101	CY100	11/09/85		20200		03 3		7783	260
102	K 102	CY100	11/10/85		15800		03 3		7914	200
103	K 103	CY100	11/11/85		17100		03 5		11774	145
104	K 104	CY100	11/12/85		25300		03 6		12050	210
105	K 105	CY100	11/13/85		25400		03 7		12386	205
106	K 106	CY100	11/14/85		28300		03 8		15322	185
107	K 107	CY100	11/15/85		21200		03 9		14114	150
108	K 108	CY100	11/16/85		23600		03 10		8577	275
109	K 109	CY100	11/17/85		18400		03 11		6799	270
110	K 110	CY100	11/18/85		15700		03 12		8511	185
111	K 111	CY100	11/19/85		10400		03 13		5467	190
112	K 112	CY100	11/20/85		11700		03 14		5568	210
113	K 113	CY100	11/21/85		6800		03 15		3388	200
114	K 114	CY100	11/22/85		7700		03 16		4269	180
115	K 115	CY100	11/23/85		5600		03 17		3003	185
116	K 116	CY100	11/24/85		6500		03 18		2949	220
117	K 117	CY100	11/25/85		8000		03 19		4119	195
118	K 118	CY100	11/26/85		7400		03 20		3708	200
119	K 119	CY100	11/27/85		5300		03 21		2216	240
120	K 120	CY100	11.28/85		4200		03 22	Not collected	3507	120
121	K 121	CY100	11/29/85		4200		03 23	- 2 days collected	3507	120
122	K 122	CY100	11/30/85		3600		03 24		2051	185
123	K 123	CY100	12/01/85		3800		03 25		1727	220
124	K 124	CY100	12/02/85		7200		03 26		3410	210
125	K 125	CY100	12/03/85		4200		03 27		2137	195
126	K 126	CY100	12/04/85		4600		03 28		2230	205
127	K 127	CY100	12/05/85		5100		03 29		2015	255
128	K 128	CY100	12/06/85		4300		03 30		1652	260
129	K 129	CY100	12/07/35		3500		03 31		1281	270
130	K 130	CY100	12/08/85		4400		03 32		1985	220
131	K 131	CY100	12/09/85		5000		03 33		2128	235
132	K 132	CY100	12/10/85		3100		03 34		1595	195
133	K 133	CY100	12/11/85		3600		03 35		1300	280
134	K 134	CY100	12/12/85		3500		03 36		1600	220
135	K 135	CY100	12/13/85		3000		03 37		1258	240
136	K 136	CY100	12/14/85		2400		03 38		1005	235
137	K 137	CY100	12/15/85		2700		03 39		1643	165
138	K 138	CY100	12/16/85		2800		03 40		1394	200
139	K 139	CY100	12/17/85		3200		03 41		1048	305
140	K 140	CY100	12/18/85		2800		03 42		1093	255
141	K 141	CY100	12/19/85		1500		03 43		673	230
142	K 142	CY100	12/20/85		2100		03 44		680	305
143	K 143	CY100	12/21/85		1900		03 45		564	330
144	K 144	CY100	12/22/85		2100		03 46		895	240
145	K 145	CY100	12/23/85		1700		03 47		828	200
146	K 146	CY100	12/24/85		2300		03 48		833	275
147	K 147	CY100	12/25/85		1600		03 49	Not collected	922	173
148	K 148	CY100	12/26/85		1600		03 50	- 2 days collected	922	173

ENDOSIS # Anordrin Pharmacokinetics Studies - Monkey

PK_URINE* CY100 * I.M. 1.0 mg/kg

print out from PRINT OUT # 12/09/86 # JRH

		SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPM/ml	VOL g/day
149	K	149 CY100	12/27/85		1700		03 51		682	245
150	K	150 CY100	12/28/85		1400		03 52		516	275
151	K	151 CY100	12/29/85		1600		03 53		580	270
152	K	152 CY100	12/30/85		1600		03 54		781	205
153	K	153 CY100	12/31/85		1100		03 55		449	250
154	K	154 CY100	01/01/86		950		03 56	Not collected	525	180
155	K	155 CY100	01/02/86		950		03 57	- 2 days collected	525	180
156	K	156 CY100	01/03/86		800		03 58	Not collected	407	200
157	K	157 CY100	01/04/86		800		03 59	- 2 days collected	407	200
158	K	158 CY100	01/05/86		730		03 60		242	300
159	K	159 CY100	01/06/86		1100		03 61		491	220
160	K	160 CY100	01/07/86		900		03 62		301	300
161	K	161 CY100	01/08/86		630		03 63		295	215
162	K	162 CY100	01/09/86		830		03 64		249	335
163	K	163 CY100	01/10/86		780		03 65		332	235
164	K	164 CY100	01/11/86		420		03 66		227	185
165	K	165 CY100	01/12/86		570		03 67		238	240
166	K	166 CY100	01/13/86	< 1	800	0.3	03 68		262	305
167	K	167 CY100	01/14/86	< 1	710	0.3	03 69		279	255
168	K	168 CY100	01/15/86	2	530	0.5	03 70		207	255
169	K	169 CY100	01/16/86	2	530	0.7	03 71		157	340
170	K	170 CY100	01/17/86	2	460	0.6	03 72		173	275
[illegible]	K	171 CY100	01/18/86	2	350	0.5	03 73		154	230
[illegible]	K	172 CY100	01/19/86	2	330	0.5	03 74		143	230
173	K	173 CY100	01/20/86	2	860	0.6	03 75		277	310
174	K	174 CY100	01/21/86	2	620	0.8	03 76		149	415
175	K	175 CY100	01/22/86	3	430	1.1	03 77		117	370
176	K	176 CY100	01/23/86	2	440	0.6	03 78		141	315
177	K	177 CY100	01/24/86	2	380	0.6	03 79	Blood in tray	126	305
178	K	178 CY100	01/25/86	2	310	0.4	03 80		146	215
179	K	179 CY100	01/26/86		400	0.2	03 81	Not collected	204	195
180	K	180 CY100	01/27/86	< 1	400	0.2	03 82	- 2 days collected	204	195
181	K	181 CY100	01/28/86	< 1	380	0.3	03 83		117	325
182	K	182 CY100	01/29/86	1	360	0.3	03 84		115	310
183	K	183 CY100	01/30/86	2	340	0.6	03 85		119	285
184	K	184 CY100	01/31/86	2	410	0.7	03 86		113	365
185	K	185 CY100	02/01/86	1	330	0.4	03 87		82	400
186	K	186 CY100	02/02/86	< 1	390	0.5	03 88		82	475
187	K	187 CY100	02/03/86	1	280	0.3	03 89		107	265
188	K	188 CY100	02/04/86	1	340	0.3	03 90		98	350
189	K	189 CY100	02/05/86	< 1	300	0.4	03 91		72	420
190	K	190 CY100	02/06/86	< 1	200	0.3	03 92		60	340
191	K	191 CY100	02/07/86	1	230	0.3	03 93		67	345
192	K	192 CY100	02/08/86	1	190	0.3	03 94		69	275
193	K	193 CY100	02/09/86		200	0.3	03 95	Not collected	68	295
194	K	194 CY100	02/10/86	1	200	0.3	03 96	- 2 days collected	68	295
195	K	195 CY100	02/11/86	1	170	0.2	03 97		73	235
	K	196 CY100	02/12/86	1	250	0.3	03 98		78	325
	K	197 CY100	02/13/86	1	200	0.3	03 99		64	315
	K	198 CY100	02/14/86	< 1	200	0.3	03 100		59	340

* ENDOSTS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY100 * I.M. 1.0 mg/kg

* print out from PRINT OUT * 12/09/86 * JWM *

recno	LABNUM	SUBJECT	DATE	Pdrol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPM/ml	VOL g/day
199	K 199	CY100	02/15/86	< 1		0.3	03 101		0	300
200	K 200	CY100	02/16/86		150	0.2	03 102	Not collected	59	253
201	K 201	CY100	02/17/86	< 1	150	0.2	03 103	- 2 days collected	59	253
202	K 202	CY100	02/18/86	< 1	250	0.5	03 104		53	475
203	K 203	CY100	02/19/86	< 1	240	0.5	03 105		49	490
204	K 204	CY100	02/20/86	< 1	170	0.3	03 106		50	340
205	K 205	CY100	02/21/86	< 1	190	0.3	03 107		57	325
206	K 206	CY100	02/22/86	< 1	240	0.5	03 108		48	495
207	K 207	CY100	02/23/86				03 109		0	0
208	K 208	CY100	02/24/86	1	210	0.3	03 110		63	335
209	K 209	CY100	02/25/86	1.94			03 111		43	425
210	K 210	CY100	02/26/86	3.23			03 112		50	315
211	K 211	CY100	02/27/86				03 113	Missing	0	0
212	K 212	CY100	02/28/86				03 114	Urine collect. Ended	0	0

****** 07:44:47 *******

* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY101 * I.V. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LabNum	SUBJECT	DATE	Pdrol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPM/ml	VOL g/day
213	K 213	Cy101	11/15/85		24600		01	1	13308	185
214	K 214	Cy101	11/16/85		7400		01	2	4324	170
215	K 215	Cy101	11/17/85		4200		01	3	2255	185
216	K 216	Cy101	11/18/85		3000		01	4	1589	190
217	K 217	Cy101	11/19/85		1700		01	5	1044	165
218	K 218	Cy101	11/20/85		1900		01	6	720	260
219	K 219	Cy101	11/21/85		1000		01	7	587	170
220	K 220	Cy101	11/22/85		910		01	8	431	210
221	K 221	Cy101	11/23/85		620		01	9	325	190
222	K 222	Cy101	11/24/85		490		01	10	239	205
223	K 223	Cy101	11/25/85		540		01	11	278	195
224	K 224	Cy101	11/26/85		330		01	12	167	195
225	K 225	Cy101	11/27/85		310		01	13	152	205
226	K 226	Cy101	11/28/85		215		01	14 Not collected	177	120
227	K 227	Cy101	11/29/85		215		01	15 - 2 days collected	177	120
228	K 228	Cy101	11/30/85		150		01	16	81	190
229	K 229	Cy101	12/01/85		190		01	17	81	235
230	K 230	Cy101	12/02/85		130		01	18	62	210
231	K 231	Cy101	12/03/85		100		01	19	46	220
232	K 232	Cy101	12/04/85		140		01	20	72	190
233	K 233	Cy101	12/05/85		120		01	21	51	240
234	K 234	Cy101	12/06/85		110		01	22	44	240
[illegible]	K 235	Cy101	12/07/85		130		01	23	54	235
236	K 236	Cy101	12/08/85		110		01	24	48	235
237	K 237	Cy101	12/09/85		120		01	25	57	215
238	K 238	Cy101	12/10/85	1	80	0.2	01	26	41	195
239	K 239	Cy101	12/11/85	2	110	0.5	01	27	44	240
240	K 240	Cy101	12/12/85	4	110	0.9	01	28	51	215
241	K 241	Cy101	12/13/85	4	94	0.9	01	29	41	230
242	K 242	Cy101	12/14/85	7	75	1.5	01	30	34	220
243	K 243	Cy101	12/15/85	10	79	2.4	01	31	33	240
244	K 244	Cy101	12/16/85	13	70	2.6	01	32	35	200
245	K 245	Cy101	12/17/85	10	110	3.3	01	33	34	330
246	K 246	Cy101	12/18/85	10	56	2.4	01	34	24	235
247	K 247	Cy101	12/19/85	12	98	2.4	01	35	49	200
248	K 248	Cy101	12/20/85	11	120	3.2	01	36	40	290
249	K 249	Cy101	12/21/85	11	68	3.1	01	37	24	285
250	K 250	Cy101	12/22/85	10	50	2.6	01	38	19	265
251	K 251	Cy101	12/23/85	12	47	2.8	01	39	20	235
252	K 252	Cy101	12/24/85	14	65	4.5	01	40	20	325
253	K 253	Cy101	12/25/85		38	3.1	01	41 Not collected	23	163
254	K 254	Cy101	12/26/85	19	38	3.1	01	42 - 2 days collected	23	163
255	K 255	Cy101	12/27/85	13	55	3.2	01	43	22	250
256	K 256	Cy101	12/28/85		100		01	44 Menses	41	245
257	K 257	Cy101	12/29/85		130		01	45 Menses	45	285
258	K 258	Cy101	12/30/85	5	100	1.2	01	46 Menses	44	235

****** 07:47:06 *******

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* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY101 * I.M. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day		REMARKS	NET_C CPM/ml	VOL g/day
259	K	259 CY101	12/31/85		2100		02	1		872	240
260	K	260 CY101	01/01/86		2600		02	2	Not collected	1750	148
261	K	261 CY101	01/02/86		2600		02	3	- 2 days collected	1750	148
262	K	262 CY101	01/03/86		3250		02	4	Not collected	2248	145
263	K	263 CY101	01/04/86		3250		02	5	- 2 days collected	2248	145
264	K	264 CY101	01/05/86		3100		02	6		807	380
265	K	265 CY101	01/06/86		6400		02	7		2740	235
266	K	266 CY101	01/07/86		3800		02	8		2592	145
267	K	267 CY101	01/08/86		5300		02	9		2164	245
268	K	268 CY101	01/09/86		6400		02	10		1983	325
269	K	269 CY101	01/10/86		2600		02	11		1279	215
270	K	270 CY101	01/11/86		3900		02	12		1890	205
271	K	271 CY101	01/12/86		4000		02	13		1705	235
272	K	272 CY101	01/13/86		4000		02	14		1024	390
273	K	273 CY101	01/14/86		6700		02	15		2385	280
274	K	274 CY101	01/15/86		2500		02	16		833	300
275	K	275 CY101	01/16/86		3500		02	17		1247	280
276	K	276 CY101	01/17/86		2200		02	18		897	240
277	K	277 CY101	01/18/86		2900		02	19		1244	235
278	K	278 CY101	01/19/86		1800		02	20		647	285
279	K	279 CY101	01/20/86	1	1900	0.3	02	21		677	285
280	K	280 CY101	01/21/86	1	2500	0.3	02	22		866	285
281	K	281 CY101	01/22/86	1	1500	0.3	02	23		540	270
282	K	282 CY101	01/23/86	1	2500	0.3	02	24		890	285
283	K	283 CY101	01/24/86	1	1500	0.3	02	25		464	330
284	K	284 CY101	01/25/86	1	1500	0.3	02	26		543	280
285	K	285 CY101	01/26/86		1000	0.1	02	27	Not collected	710	140
286	K	286 CY101	01/27/86	1	1000	0.1	02	28	- 2 days collected	710	140
287	K	287 CY101	01/28/86	1	590	0.3	02	29		173	340
288	K	288 CY101	01/29/86	2	700	0.6	02	30		221	315
289	K	289 CY101	01/30/86	1	590	0.3	02	31		198	300
290	K	290 CY101	01/31/86	1	510	0.3	02	32		171	300
291	K	291 CY101	02/01/86	1	520	0.3	02	33		161	325
292	K	292 CY101	02/02/86	2	470	0.5	02	34		187	250
293	K	293 CY101	02/03/86	2	330	0.5	02	35		122	270
294	K	294 CY101	02/04/86	2	530	0.7	02	36		143	370
295	K	295 CY101	02/05/86	1	360	0.4	02	37		102	355
296	K	296 CY101	02/06/86	1	270	0.3	02	38		105	255
297	K	297 CY101	02/07/86	2	303		02	39		101	300
298	K	298 CY101	02/08/86	4	345		02	40		103	335
299	K	299 CY101	02/09/86		238	0.0	02	41	Not collected	159	150
300	K	300 CY101	02/10/86	9	238	1.4	02	42	- 2 days collected	159	150
301	K	301 CY101	02/11/86	7	262	1.9	02	43		97	270
302	K	302 CY101	02/12/86	8	635	2.3	02	44		219	290
303	K	303 CY101	02/13/86	6	272	1.5	02	45		111	245
304	K	304 CY101	02/14/86	4	325	1.3	02	46		97	335
305	K	305 CY101	02/15/86	4	0	1.1	02	47		0	285
306	K	306 CY101	02/16/86		176	0.8	02	48	Not collected	96	183
307	K	307 CY101	02/17/86	10	175	0.8	02	49	- 2 days collected	96	182
308	K	308 CY101	02/18/86	8	202	2.6	02	50		62	325

* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY101 * I.M. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JMH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPMml	VOL g/day
309	K 309	CY101	02/19/86	8	297	3.6	02 51		66	450
310	K 310	CY101	02/20/86	8	174	2.4	02 52		58	300
311	K 311	CY101	02/21/86	8	215	2.9	02 53		59	365
312	K 312	CY101	02/22/86	9	162	2.9	02 54		50	325
313	K 313	CY101	02/23/86		0		02 55	Missing	0	0
314	K 314	CY101	02/24/86	8	201		02 56	Menses	53	380
315	K 315	CY101	02/25/86	2.98	166		02 57	Menses ends	35	475

****** 07:50:03 *******

* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY102 * I.M. 1.0 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day		REMARKS	NET_C CPMml	VOL g/day
316	K 316	CY102	10/26/85		0		03	1		0	255
317	K 317	CY102	10/27/85		20020		03	2		7280	275
318	K 318	CY102	10/28/85		37467		03	3		16290	230
319	K 319	CY102	10/29/85		23564		03	4		11782	200
320	K 320	CY102	10/30/85		40114		03	5		20057	200
321	K 321	CY102	10/31/85		15522		03	6		6209	250
322	K 322	CY102	11/01/85		40001		03	7		16327	245
323	K 323	CY102	11/02/85		19934		03	8		10775	185
324	K 324	CY102	11/03/85		25038		03	9		9819	255
325	K 325	CY102	11/04/85		25652		03	10		13501	190
326	K 326	CY102	11/05/85		17933		03	11		7472	240
327	K 327	CY102	11/06/85		17751		03	12		8453	210
328	K 328	CY102	11/07/85		11506		03	13		5114	225
329	K 329	CY102	11/08/85		16744		03	14		7788	215
330	K 330	CY102	11/09/85		13068		03	15		5227	250
331	K 331	CY102	11/10/85		13709		03	16		6528	210
332	K 332	CY102	11/11/85		15095		03	17		7275	180
333	K 333	CY102	11/12/85		10558		03	18		4223	250
334	K 334	CY102	11/13/85		8689		03	19		3342	260
335	K 335	CY102	11/14/85		30267		03	20		13452	225
336	K 336	CY102	11/15/85		6314		03	21		3323	190
337	K 337	CY102	11/16/85		5927		03	22		2577	230
338	K 338	CY102	11/17/85		4308		03	23		1657	260
339	K 339	CY102	11/18/85		3942		03	24		1792	220
340	K 340	CY102	11/19/85		3326		03	25		1584	210
341	K 341	CY102	11/20/85		3526		03	26		1469	240
342	K 342	CY102	11/21/85		2220		03	27		965	230
343	K 343	CY102	11/22/85		2832		03	28		1156	245
344	K 344	CY102	11/23/85		2467		03	29		1028	240
345	K 345	CY102	11/24/85		2045		03	30		909	225
346	K 346	CY102	11/25/85		2394		03	31		1088	220
347	K 347	CY102	11/26/85		1955		03	32		869	225
348	K 348	CY102	11/27/85		1905		03	33		762	250
349	K 349	CY102	11/28/85		1027		03	34	Not collected	694	148
350	K 350	CY102	11/29/85		1020		03	35	- 2 days collected	694	147
351	K 351	CY102	11/30/85		942		03	36		471	200
352	K 352	CY102	12/01/85		1058		03	37		504	210
353	K 353	CY102	12/02/85		1605		03	38		479	335
354	K 354	CY102	12/03/85		685		03	39		326	210
355	K 355	CY102	12/04/85		804		03	40		335	240
356	K 356	CY102	12/05/85		1053		03	41		376	280
357	K 357	CY102	12/06/85		778		03	42		278	280
358	K 358	CY102	12/07/85		667		03	43		342	195
359	K 359	CY102	12/08/85		799		03	44		296	270
360	K 360	CY102	12/09/85		737		03	45		301	245
361	K 361	CY102	12/10/85		572		03	46		254	225
362	K 362	CY102	12/11/85		611		03	47		235	260
363	K 363	CY102	12/12/85		593		03	48		228	260
364	K 364	CY102	12/13/85		495		03	49		194	255
365	K 365	CY102	12/14/85		432		03	50		192	225

* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY102 * I.M. 1.0 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPMml	VOL g/day
366	K 366	CY102	12/15/85		412		03 51		217	190
367	K 367	CY102	12/16/85		504		03 52		229	220
368	K 368	CY102	12/17/85	4	470		03 53		165	285
369	K 369	CY102	12/18/85	10	500		03 54		185	270
370	K 370	CY102	12/19/85	7	281		03 55		134	210
371	K 371	CY102	12/20/85	8	314	2.3	03 56		110	285
372	K 372	CY102	12/21/85	12	291	3.4	03 57		102	285
373	K 373	CY102	12/22/85	15	208	3.9	03 58		80	260
374	K 374	CY102	12/23/85	17	246	5.4	03 59		77	320
375	K 375	CY102	12/24/85	27	237	7.7	03 60		83	285
376	K 376	CY102	12/25/85		157		03 61	Not collected	101	155
377	K 377	CY102	12/26/85	42	157	6.5	03 62	- 2 days collected	101	155
378	K 378	CY102	12/27/85	41	192	9.8	03 63		80	240
379	K 379	CY102	12/28/85	20	168	5.6	03 64		60	280
380	K 380	CY102	12/29/85	23	202	7.2	03 65		64	315
381	K 381	CY102	12/30/85	32	189	8.6	03 66		70	270
382	K 382	CY102	12/31/85	28	159	7.4	03 67		60	265
383	K 383	CY102	01/01/86		131	0.0	03 68	Not collected	77	170
384	K 384	CY102	01/02/86	34	131	0.0	03 69	- 2 days collected	77	170
385	K 385	CY102	01/03/86		125		03 70	Not collected	64	195
386	K 386	CY102	01/04/86		125		03 71	- 2 days collected	64	195
387	K 387	CY102	01/05/86		180		03 72	Meases- 1/4	57	315
[illegible]	K 388	CY102	01/06/86		220		03 73		63	350
389	K 389	CY102	01/07/86		146		03 74		41	355
390	K 390	CY102	01/08/86		117		03 75		45	260
391	K 391	CY102	01/09/86		136		03 76		40	340
392	K 392	CY102	01/10/86		125		03 77		41	305
393	K 393	CY102	01/11/86		103		03 78		39	265
394	K 394	CY102	01/12/86		125		03 79		64	195

****** 07:53:56 ******

* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY103 * I.V. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day		REMARKS	NET_C CPM/ml	VOL g/day
395	K 395	CY103	10/08/85		0		01	01	Missing	0	0
396	K 396	CY103	10/09/85		39900		01	2		21555	185
397	K 397	CY103	10/10/85		10990		01	3		5435	200
398	K 398	CY103	10/11/85		22500		01	4		9168	245
399	K 399	CY103	10/12/85		19600		01	5		8913	220
400	K 400	CY103	10/13/85				01	6	Missing	0	0
401	K 401	CY103	10/14/85				01	7	"	0	0
402	K 402	CY103	10/15/85				01	8	"	0	0
403	K 403	CY103	10/16/85				01	9	"	0	0
404	K 404	CY103	10/17/85				01	10	"	0	0
405	K 405	CY103	10/18/85				01	11	"	0	0
406	K 406	CY103	10/19/85				01	12	"	0	0
407	K 407	CY103	10/20/85				01	13	"	0	0
408	K 408	CY103	10/21/85				01	14	"	0	0
409	K 409	CY103	10/22/85		12400		01	15		3188	390
410	K 410	CY103	10/23/85		7000		01	16		2403	290
411	K 411	CY103	10/24/85		1500		01	17		889	165
412	K 412	CY103	10/25/85		1200		01	18		377	320
413	K 413	CY103	10/26/85		1200		01	19		298	400
414	K 414	CY103	10/27/85		360		01	20		254	140
415	K 415	CY103	10/28/85		760		01	21		355	215
416	K 416	CY103	10/29/85		600		01	22		305	195
417	K 417	CY103	10/30/85		540		01	23		249	215
418	K 418	CY103	10/31/85		800		01	24		185	430
419	K 419	CY103	11/01/85		1100		01	25		369	290
420	K 420	CY103	11/02/85		3000		01	26		564	530
421	K 421	CY103	11/03/85		580		01	27		235	245
422	K 422	CY103	11/04/85		330		01	28		178	185
423	K 423	CY103	11/05/85		390		01	29		220	175
424	K 424	CY103	11/06/85		330		01	30		159	205
425	K 425	CY103	11/07/85		280		01	31		99	280
426	K 426	CY103	11/08/85		360		01	32		147	245
427	K 427	CY103	11/09/85		460		01	33		139	330
428	K 428	CY103	11/10/85		190		01	34		97	200
429	K 429	CY103	11/11/85		280		01	35		85	330
430	K 430	CY103	11/12/85	11	420	5.9	01	36		78	535
431	K 431	CY103	11/13/85	19	260	4.1	01	37		120	215
432	K 432	CY103	11/14/85	34	390	13.1	01	38		100	385
433	K 433	CY103	11/15/85	26	410	12.5	01	39		86	480
434	K 434	CY103	11/16/85	46	160	9.2	01	40		92	200
435	K 435	CY103	11/17/85	34	360	12.9	01	41		94	380
436	K 436	CY103	11/18/85	58	210	13.6	01	42		88	235
437	K 437	CY103	11/19/85	68	220	19.7	01	43		76	290
438	K 438	CY103	11/20/85	55	230	15.9	01	44		80	290
439	K 439	CY103	11/21/85	83	220	19.9	01	45		91	240
440	K 440	CY103	11/22/85	80	280	26.8	01	46		83	335
441	K 441	CY103	11/23/85	68	200	22.4	01	47		61	330
442	K 442	CY103	11/24/85	52	200	14.6	01	48		70	280
443	K 443	CY103	11/25/85	63	170	12.6	01	49		86	200
444	K 444	CY103	11/26/85	43	570	20.9	01	50		118	485

* ENDOSTS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY103 * I.V. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JWH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPM/ml	VOL g/day
445	K 445	CY103	11/27/85	41	230	16.6	01 51		57	405
446	K 446	CY103	11/28/85		155	0.0	01 52	Not collected	69	228
447	K 447	CY103	11/29/85		155	0.0	01 53	- 2 days collected	69	228
448	K 448	CY103	11/30/85	27	120	7.0	01 54		45	260
449	K 449	CY103	12/01/85	18	130	5.5	01 55	Menses- 11/29 - 12/1	43	305

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* ENDO575 * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY103 * I.M. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPM/ml	VOL g/day
450	K 450	CY103	12/03/85		740		02 1		361	205
451	K 451	CY103	12/04/85		2200		02 2		1147	190
452	K 452	CY103	12/05/85		3000		02 3		1202	250
453	K 453	CY103	12/06/85		2300		02 4		1146	205
454	K 454	CY103	12/07/85		3000		02 5		1321	230
455	K 455	CY103	12/08/85		4300		02 6		2255	190
456	K 456	CY103	12/09/85		4200		02 7		1752	240
457	K 457	CY103	12/10/85		4700		02 8		2198	215
458	K 458	CY103	12/11/85		5200		02 9		2185	240
459	K 459	CY103	12/12/85		4000		02 10		1703	235
460	K 460	CY103	12/13/85		3900		02 11		1535	255
461	K 461	CY103	12/14/85		3500		02 12		1484	235
462	K 462	CY103	12/15/85		3000		02 13		1254	285
463	K 463	CY103	12/16/85		4100		02 14		1800	225
464	K 464	CY103	12/17/85		5700		02 15		1597	360
465	K 465	CY103	12/18/85		3100		02 16		1257	250
466	K 466	CY103	12/19/85		2800		02 17		2066	135
467	K 467	CY103	12/20/85		3400		02 18		1167	290
468	K 468	CY103	12/21/85		3100		02 19		1177	265
469	K 469	CY103	12/22/85		3200		02 20		1297	250
470	K 470	CY103	12/23/85		3600		02 21		1858	205
471	K 471	CY103	12/24/85		3200		02 22		1134	280
472	K 472	CY103	12/25/85		2250		02 23	Not collected	1491	153
473	K 473	CY103	12/26/85		2250		02 24	- 2 days collected	1491	153
474	K 474	CY103	12/27/85		2700		02 25		1244	215
475	K 475	CY103	12/28/85		2700		02 26		1244	215
476	K 476	CY103	12/29/85		2000		02 27		884	225
477	K 477	CY103	12/30/85		2600		02 28		1160	225
478	K 478	CY103	12/31/85		2300		02 29		840	270
479	K 479	CY103	01/01/86		1450		02 30	Not collected	918	158
480	K 480	CY103	01/02/86		1450		02 31	- 2 days collected	918	158
481	K 481	CY103	01/03/86		1550		02 32	Not collected	967	163
482	K 482	CY103	01/04/86		1550		02 33	- 2 days collected	967	163
483	K 483	CY103	01/05/86		1200		02 34		340	345
484	K 484	CY103	01/06/86		1800		02 35		693	260
485	K 485	CY103	01/07/86		1500		02 36		304	495
486	K 486	CY103	01/08/86		890		02 37		355	250
487	K 487	CY103	01/09/86		1000		02 38		279	365
488	K 488	CY103	01/10/86		1400		02 39		502	275
489	K 489	CY103	01/11/86		1200		02 40		420	275
490	K 490	CY103	01/12/86		1000		02 41		433	240
491	K 491	CY103	01/13/86	3	1200	0.9	02 42		403	300
492	K 492	CY103	01/14/86	4	1000	1.1	02 43		391	265
493	K 493	CY103	01/15/86	10	1000	2.9	02 44		359	285
494	K 494	CY103	01/16/86	8	810	2.9	02 45		224	360
495	K 495	CY103	01/17/86	12	470	2.4	02 46		237	200
496	K 496	CY103	01/18/86	27	980	6.9	02 47		385	255
497	K 497	CY103	01/19/86	23	850	6.7	02 48		294	290
498	K 498	CY103	01/20/86	30	980	10.8	02 49		272	360
499	K 499	CY103	01/21/86	31	770	8.5	02 50		281	275

* ENDOSYS * Anororin Pharmacokinetics Studies - Monkey *

PK_URINE* CY103 * I.M. 0.2 mg/kg

* print out from Pdiol UPGRADE * 12/17/86 * RNW *

recno	LmoNum	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPMml	VOL g/day
500	K	500 CY103	01/22/86	31	620	8.7	02 51		221	280
501	K	501 CY103	01/23/86	43	680	12.5	02 52		235	290
502	K	502 CY103	01/24/86	67	740	19.8	02 53		249	295
503	K	503 CY103	01/25/86	71	700	17.8	02 54		278	250
504	K	504 CY103	01/26/86		700	15.0	02 55	Not collected	273	250
505	K	505 CY103	01/27/86	60	700	15.0	02 56	- 2 days collected	273	250
506	K	506 CY103	01/28/86 ns				02 57		111	111
507	K	507 CY103	01/29/86	18	620		02 58		111	111
508	K	508 CY103	01/30/86	5	510		02 59		129	395
509	K	509 CY103	01/31/86	33	550		02 60		199	275
510	K	510 CY103	02/01/86	15	550		02 61		165	335
511	K	511 CY103	02/02/86	6	470		02 62	Menses	146	320
512	K	512 CY103	02/03/86	2	330		02 63	Menses ends	133	250

****** 14:15:35 *******

* ENDOSTS * Androdrin Pharmokinetics Studies - Monkey *

PK_URINE* CY104 * I.M. 1.0 mg/kg

* print out from Pdiol UPGRADE * 12/17/86 * NNW *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day		REMARKS	NET_C CPM/ml	VOL g/day
513	K 513	CY104	10/19/85				03	01	Missing	0	0
514	K 514	CY104	10/20/85				03	02	"	0	0
515	K 515	CY104	10/21/85				03	3	"	0	0
516	K 516	CY104	10/22/85		7200		03	4		3200	225
517	K 517	CY104	10/23/85		5500		03	5		4200	130
518	K 518	CY104	10/24/85		5100		03	6		3300	155
519	K 519	CY104	10/25/85		14800		03	7		7207	205
520	K 520	CY104	10/26/85		30800		03	8		12337	250
521	K 521	CY104	10/27/85		24000		03	9		9992	240
522	K 522	CY104	10/28/85		30100		03	10		17721	170
523	K 523	CY104	10/29/85		24800		03	11		12399	200
524	K 524	CY104	10/30/85		22100		03	12		11650	190
525	K 525	CY104	10/31/85		34500		03	13		14375	240
526	K 526	CY104	11/01/85		30700		03	14		13345	230
527	K 527	CY104	11/02/85		21200		03	15		12825	165
528	K 528	CY104	11/03/85		26800		03	16		13400	215
529	K 529	CY104	11/04/85		23900		03	17		12558	190
530	K 530	CY104	11/05/85		19500		03	18		11797	165
531	K 531	CY104	11/06/85		18100		03	19		10368	175
532	K 532	CY104	11/07/85		13800		03	20		6414	215
533	K 533	CY104	11/08/85		10400		03	21		4174	250
534	K 534	CY104	11/09/85		7900		03	22		2642	300
535	K 535	CY104	11/10/85		6300		03	23		2757	230
536	K 536	CY104	11/11/85		6700		03	24		3362	200
537	K 537	CY104	11/12/85				03	25		0	335
538	K 538	CY104	11/13/85		5000		03	26		2471	225
539	K 539	CY104	11/14/85		8100		03	27		3225	250
540	K 540	CY104	11/15/85		5600		03	28		2231	250
541	K 541	CY104	11/16/85		5100		03	29		1871	275
542	K 542	CY104	11/17/85		4100		03	30		1445	285
543	K 543	CY104	11/18/85		3300		03	31		1482	220
544	K 544	CY104	11/19/85		2600		03	32		721	365
545	K 545	CY104	11/20/85		2800		03	33		589	470
546	K 546	CY104	11/21/85		2100		03	34		838	250
547	K 547	CY104	11/22/85		3000		03	35		659	450
548	K 548	CY104	11/23/85		2400		03	36		582	415
549	K 549	CY104	11/24/85		1600		03	37		610	270
550	K 550	CY104	11/25/85		1400		03	38		587	245
551	K 551	CY104	11/26/85		1600		03	39		418	380
552	K 552	CY104	11/27/85		1600		03	40		440	360
553	K 553	CY104	11/28/85		1150		03	41	Not collected	303	385
554	K 554	CY104	11/29/85		1150		03	42	- 2 days collected	303	385
555	K 555	CY104	11/30/85		1400		03	43		315	445
556	K 556	CY104	12/01/85		1100		03	44		258	430
557	K 557	CY104	12/02/85		1100		03	45		233	465
558	K 558	CY104	12/03/85		560		03	46		255	220
559	K 559	CY104	12/04/85		590		03	47		288	205
560	K 560	CY104	12/05/85		780		03	48		209	375
561	K 561	CY104	12/06/85		510		03	49		208	245
562	K 562	CY104	12/07/85		840		03	50		166	505

* ENDOSIS * Amoxicillin Pharmacokinetics Studies - Monkey *

PK_URINE* CY104 * I.M. 1.0 mg/kg

* print out from Patol OrbRMDE * 12/17/88 * NRM *

Techo	Chemon	Subject	DATE	Patol ng/ml	T_CPM /100	T_Pd day	SERIES	REMARKS	NET_C CPMal	VOL g/day
563	K	563 CY104	12/08/85		550	03	51		234	235
564	K	564 CY104	12/09/85		520	03	52		195	265
565	K	565 CY104	12/10/85		540	03	53		260	270
566	K	566 CY104	12/11/85		530	03	54		188	280
567	K	567 CY104	12/12/85		560	03	55		181	320
568	K	568 CY104	12/13/85		320	03	56		91	350
569	K	569 CY104	12/14/85		270	03	57		76	350
570	K	570 CY104	12/15/85		250	03	58		139	180
571	K	571 CY104	12/16/85		290	03	59		111	260
572	K	572 CY104	12/17/85		350	03	60		115	305
573	K	573 CY104	12/18/85	< 1	270	03	61		94	290
574	K	574 CY104	12/19/85	1	230	03	62		68	340
575	K	575 CY104	12/20/85	2	280	03	63		84	335
576	K	576 CY104	12/21/85	2	270	03	64		70	385
577	K	577 CY104	12/22/85	2	260	03	65		74	345
578	K	578 CY104	12/23/85	6	260	03	66		82	320
579	K	579 CY104	12/24/85	5	200	03	67	Blood in tray	77	265
580	K	580 CY104	12/25/85	ns	160	03	68	Not collected	69	235
581	K	581 CY104	12/26/85	5	160	03	69	- 2 days collected	69	235
582	K	582 CY104	12/27/85	7	200	03	70		70	285
583	K	583 CY104	12/28/85	14	170	03	71		68	245
584	K	584 CY104	12/29/85	7	240	03	72		70	340
585	K	585 CY104	12/30/85	11	210	03	73		65	325
[illegible]	K	586 CY104	12/31/85	14	240	03	74		77	305
587	K	587 CY104	01/01/86	ns	160	03	75	Not collected	60	263
588	K	588 CY104	01/02/86	9	160	03	76	- 2 days collected	60	263
589	K	589 CY104	01/03/86	ns	170	03	77	Not collected	73	233
590	K	590 CY104	01/04/86	5	170	03	78	- 2 days collected	73	233
591	K	591 CY104	01/05/86		150	03	79		42	355
592	K	592 CY104	01/06/86		240	03	80		83	290
593	K	593 CY104	01/07/86		430	03	81		137	315
594	K	594 CY104	01/08/86		160	03	82		54	295
595	K	595 CY104	01/09/86		170	03	83		33	500
596	K	596 CY104	01/10/86		180	03	84		57	320
597	K	597 CY104	01/11/86		120	03	85		39	310
598	K	598 CY104	01/12/86		120	03	86		39	310

****** 14:19:51 *******

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* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY105 * I.V. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day		REMARKS	NET_C CPMml	VOL g/day
599	K 599	CY105	10/15/85				01	01	Missing	0	0
600	K 600	CY105	10/16/85				01	2	"	0	0
601	K 601	CY105	10/17/85				01	3	"	0	0
602	K 602	CY105	10/18/85				01	4	"	0	0
603	K 603	CY105	10/19/85				01	5	"	0	0
604	K 604	CY105	10/20/85				01	6	"	0	0
605	K 605	CY105	10/21/85				01	7	"	0	0
606	K 606	CY105	10/22/85		4700		01	8		2560	185
607	K 607	CY105	10/23/85		2700		01	9		2105	130
608	K 608	CY105	10/24/85		2000		01	10		942	210
609	K 609	CY105	10/25/85		2000		01	11		1048	195
610	K 610	CY105	10/26/85		1900		01	12		1283	150
611	K 611	CY105	10/27/85		1400		01	13		569	250
612	K 612	CY105	10/28/85		1500		01	14		758	200
613	K 613	CY105	10/29/85		1200		01	15		611	200
614	K 614	CY105	10/30/85		1700		01	16		927	185
615	K 615	CY105	10/31/85		1800		01	17		1300	135
616	K 616	CY105	11/01/85		2400		01	18		911	265
617	K 617	CY105	11/02/85		1000		01	19		589	170
618	K 618	CY105	11/03/85		1300		01	20		672	195
619	K 619	CY105	11/04/85		1200		01	21		716	170
620	K 620	CY105	11/05/85		960		01	22		457	210
621	K 621	CY105	11/06/85		1600		01	23		402	400
622	K 622	CY105	11/07/85		1400		01	24		401	355
623	K 623	CY105	11/08/85		1000		01	25		246	410
624	K 624	CY105	11/09/85		610		01	26		277	220
625	K 625	CY105	11/10/85		550		01	27		191	290
626	K 626	CY105	11/11/85		470		01	28		279	170
627	K 627	CY105	11/12/85		650		01	29		269	240
628	K 628	CY105	11/13/85		500		01	30		200	250
629	K 629	CY105	11/14/85		420		01	31		199	210
630	K 630	CY105	11/15/85		200		01	32		119	165
631	K 631	CY105	11/16/85		300		01	33		120	250
632	K 632	CY105	11/17/85		200		01	34		92	220
633	K 633	CY105	11/18/85		250		01	35		75	335
634	K 634	CY105	11/19/85		150		01	36		90	165
635	K 635	CY105	11/20/85		140		01	37		75	185
636	K 636	CY105	11/21/85		130		01	38		62	215
637	K 637	CY105	11/22/85		170		01	39		72	230
638	K 638	CY105	11/23/85		150		01	40		63	230
639	K 639	CY105	11/24/85		99		01	41		52	190
640	K 640	CY105	11/25/85		150		01	42		69	215
641	K 641	CY105	11/26/85	3	120	0.7	01	43		52	225
642	K 642	CY105	11/27/85	3	150	0.7	01	44		59	245
643	K 643	CY105	11/28/85		90	2.9	01	45	Not collected	61	145
644	K 644	CY105	11/29/85	20	90	2.9	01	46	- 2 days collected	61	145
645	K 645	CY105	11/30/85	20	140	3.9	01	47		71	195
646	K 646	CY105	12/01/85	12	110	2.6	01	48		52	215
647	K 647	CY105	12/02/85	15	140	3.5	01	49		62	230
648	K 648	CY105	12/03/85	44	83	8.1	01	50		45	185

* ENDOSTS * Anordrin Pharmacokinetics Studies - Monkey *

'K_URINE* CY105 * I.V. 0.2 mg/kg

print out from PRINT OUT * 12/09/86 * JWH *

recno	LabNUM	SUBJECT	DATE	Pdrol ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPM/ml	VOL g/day
049	K 049	CY105	12/04/85	10	140	2.2	01 51		64	220
050	K 050	CY105	12/05/85	22	120	5.3	01 52		48	240
051	K 051	CY105	12/06/85	25	110	6.5	01 53		41	260
052	K 052	CY105	12/07/85	26	150	6.8	01 54		56	260
053	K 053	CY105	12/08/85	16	100	4.0	01 55		40	250
054	K 054	CY105	12/09/85	16	76	3.2	01 56		38	200
055	K 055	CY105	12/10/85	6	89	1.4	01 57		38	235
056	K 056	CY105	12/11/85	24	92	6.5	01 58		34	270
057	K 057	CY105	12/12/85	10	68	2.1	01 59		42	210
058	K 058	CY105	12/13/85	4	110	0.9	01 60	Menses	45	235
059	K 059	CY105	12/14/85	5	90	1.2	01 61	Menses	36	250
060	K 060	CY105	12/15/85	4	85	0.9	01 62	Menses	36	235
061	K 061	CY105	12/16/85	3	89	0.7	01 63	Menses	37	240

****** 08:07:21 *******

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* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

PK_URINE* CY105 * I.M. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JNH *

recno	LABNUM	SUBJECT	DATE	Paral ng/ml	T_CPM /100	T_Pd	SERIES day	REMARKS	NET_C CPMml	VOL g/day
002	K 002	CY105	12/17/85		2600		02 01		739	350
003	K 003	CY105	12/18/85		2100		02 2		757	280
004	K 004	CY105	12/19/85		2000		02 3		952	210
005	K 005	CY105	12/20/85		3800		02 4		1509	250
006	K 006	CY105	12/21/85		6000		02 5		1967	305
007	K 007	CY105	12/22/85		5400		02 6		2211	245
008	K 008	CY105	12/23/85		5500		02 7		2574	215
009	K 009	CY105	12/24/85		5300		02 8	Blood in tray	1798	295
070	K 070	CY105	12/25/85		4250		02 9	Not collected	3338	128
071	K 071	CY105	12/26/85		4250		02 10	- 2 days collected	3338	128
072	K 072	CY105	12/27/85		5000		02 11		2127	235
073	K 073	CY105	12/28/85		4400		02 12		1721	255
074	K 074	CY105	12/29/85		5000		02 13		2027	245
075	K 075	CY105	12/30/85		4900		02 14		1948	250
076	K 076	CY105	12/31/85		4800		02 15		2089	230
077	K 077	CY105	01/01/86		2900		02 16	Not collected	2270	128
078	K 078	CY105	01/02/86		2900		02 17	- 2 days collected	2270	128
079	K 079	CY105	01/03/86		2500		02 18	Not collected	1707	145
080	K 080	CY105	01/04/86		2500		02 19	- 2 days collected	1707	145
081	K 081	CY105	01/05/86		1700		02 20		454	380
082	K 082	CY105	01/06/86		2100		02 21		793	270
083	K 083	CY105	01/07/86		2400		02 22		979	250
084	K 084	CY105	01/08/86		1800		02 23		515	350
085	K 085	CY105	01/09/86		1700		02 24		443	380
086	K 086	CY105	01/10/86		1600		02 25		635	255
087	K 087	CY105	01/11/86		970		02 26		423	230
088	K 088	CY105	01/12/86		930		02 27		365	255
089	K 089	CY105	01/13/86	< 1	1100	0.5	02 28		218	500
090	K 090	CY105	01/14/86	< 1	1100	0.3	02 29		426	260
091	K 091	CY105	01/15/86	1	1200	0.3	02 30		468	260
092	K 092	CY105	01/16/86	< 1	1100	0.3	02 31		328	350
093	K 093	CY105	01/17/86	3	1100	0.8	02 32		419	255
094	K 094	CY105	01/18/86	< 1	960	0.3	02 33	Blood in tray	326	295
095	K 095	CY105	01/19/86	< 1	790	0.4	02 34		222	355
096	K 096	CY105	01/20/86	< 1	890	0.3	02 35		281	315
097	K 097	CY105	01/21/86	< 1	700	0.3	02 36		207	340
098	K 098	CY105	01/22/86	1	650	0.3	02 37		240	270
099	K 099	CY105	01/23/86		490		02 38		183	270
700	K 700	CY105	01/24/86	2	650	0.6	02 39		203	320
701	K 701	CY105	01/25/86	1	570	0.2	02 40		243	235
702	K 702	CY105	01/26/86		600	0.6	02 41	Not collected	205	300
703	K 703	CY105	01/27/86	2	600	0.6	02 42	- 2 days collected	205	300
704	K 704	CY105	01/28/86	2	530	0.6	02 43		166	320
705	K 705	CY105	01/29/86	3	670	0.8	02 44		242	275
706	K 706	CY105	01/30/86	2	370	0.6	02 45		129	300
707	K 707	CY105	01/31/86	2	310	0.6	02 46		112	280
708	K 708	CY105	02/01/86	2	420	0.6	02 47		143	290
709	K 709	CY105	02/02/86	3	570	0.8	02 48		212	270
710	K 710	CY105	02/03/86	5	400	1.2	02 49	Blood in tray	160	250
711	K 711	CY105	02/04/86	6	610	2.1	02 50		171	355

* ENDOSYS * Anordrin Pharmokinetics Studies - Monkey *

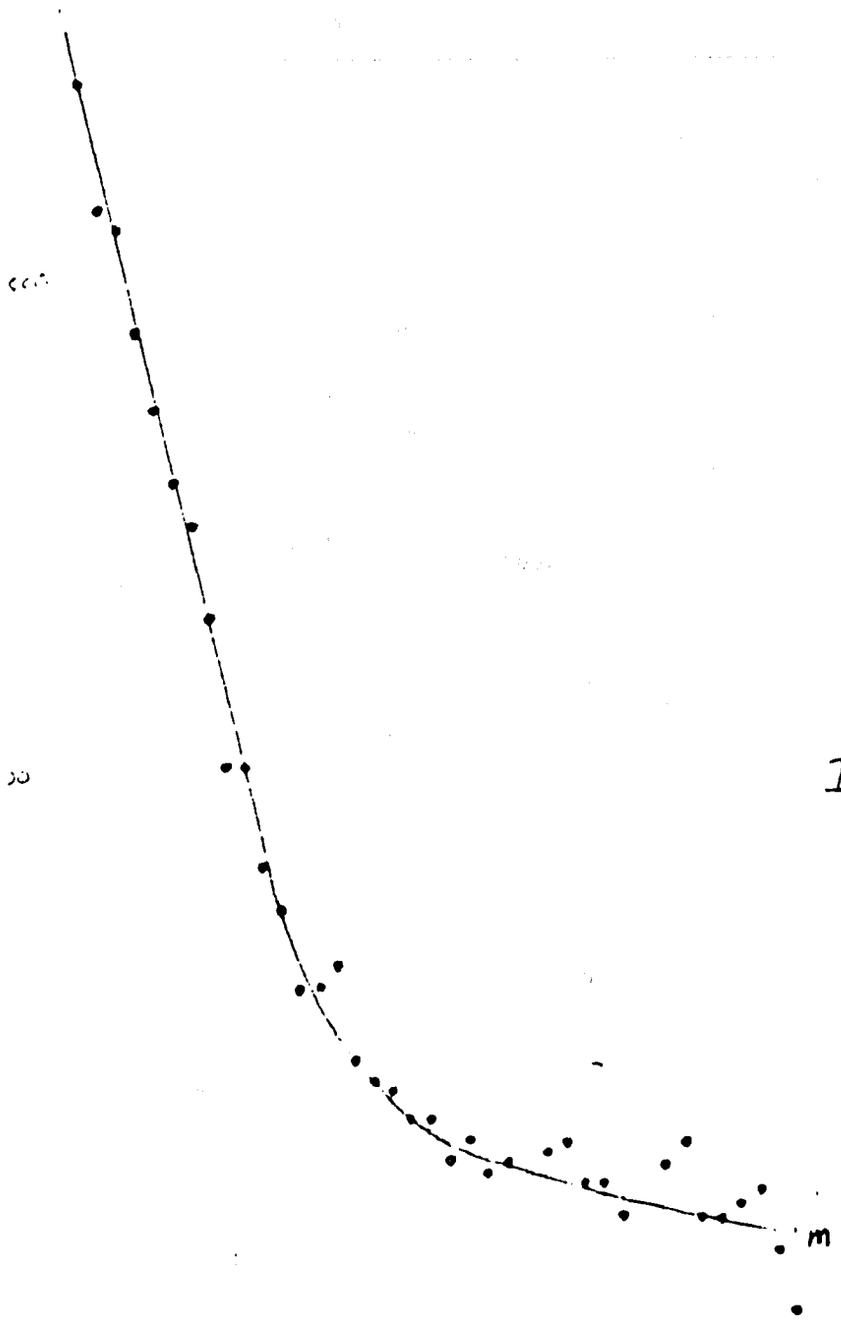
PK_URINE* CY105 * I.M. 0.2 mg/kg

* print out from PRINT OUT * 12/09/86 * JKM *

Recno	LABNUM	SUBJECT	DATE	Pdiol ng/ml	T_CPM /100	T_Pd	SERIES day		REMARKS	NET_C CPM/ml	VOL g/day
712	K	712 CY105	02/05/86	6	470	1.6	02	51		169	275
713	K	713 CY105	02/06/86	3	360	0.9	02	52		125	285
714	K	714 CY105	02/07/86	3	350	0.8	02	53		129	270
715	K	715 CY105	02/08/86	3	370	0.8	02	54		138	270
716	K	716 CY105	02/09/86		230	0.0	02	55	Not collected	147	158
717	K	717 CY105	02/10/86	3.96	230	0.0	02	56	- 2 days collected	147	158
718	K	718 CY105	02/11/86	2	290	0.6	02	57		99	295
719	K	719 CY105	02/12/86	2	260	0.6	02	58	Menses	93	275
720	K	720 CY105	02/13/86	2	250	0.6	02	59	Menses	89	275

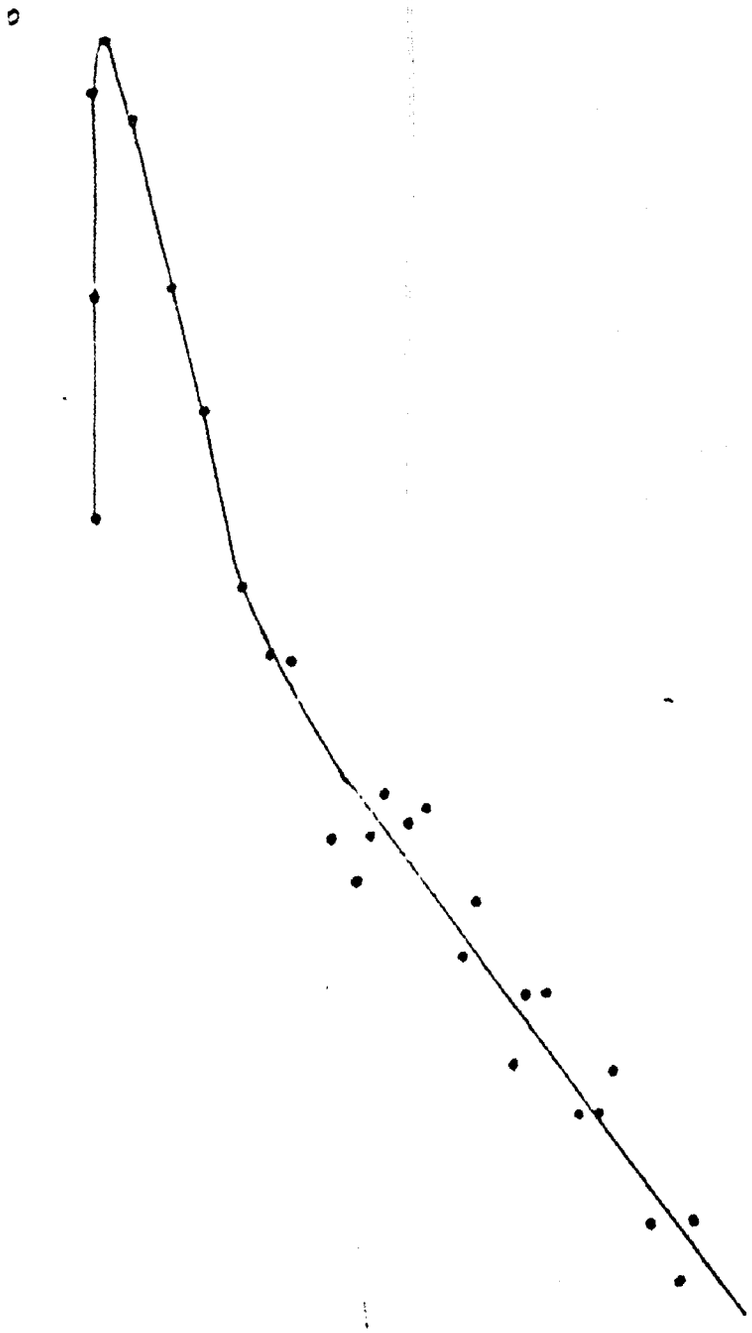
****** 08:10:24 *******

m #99 i.v.
^{14}C - Amoxicillin
Total cpm/day
in Urine

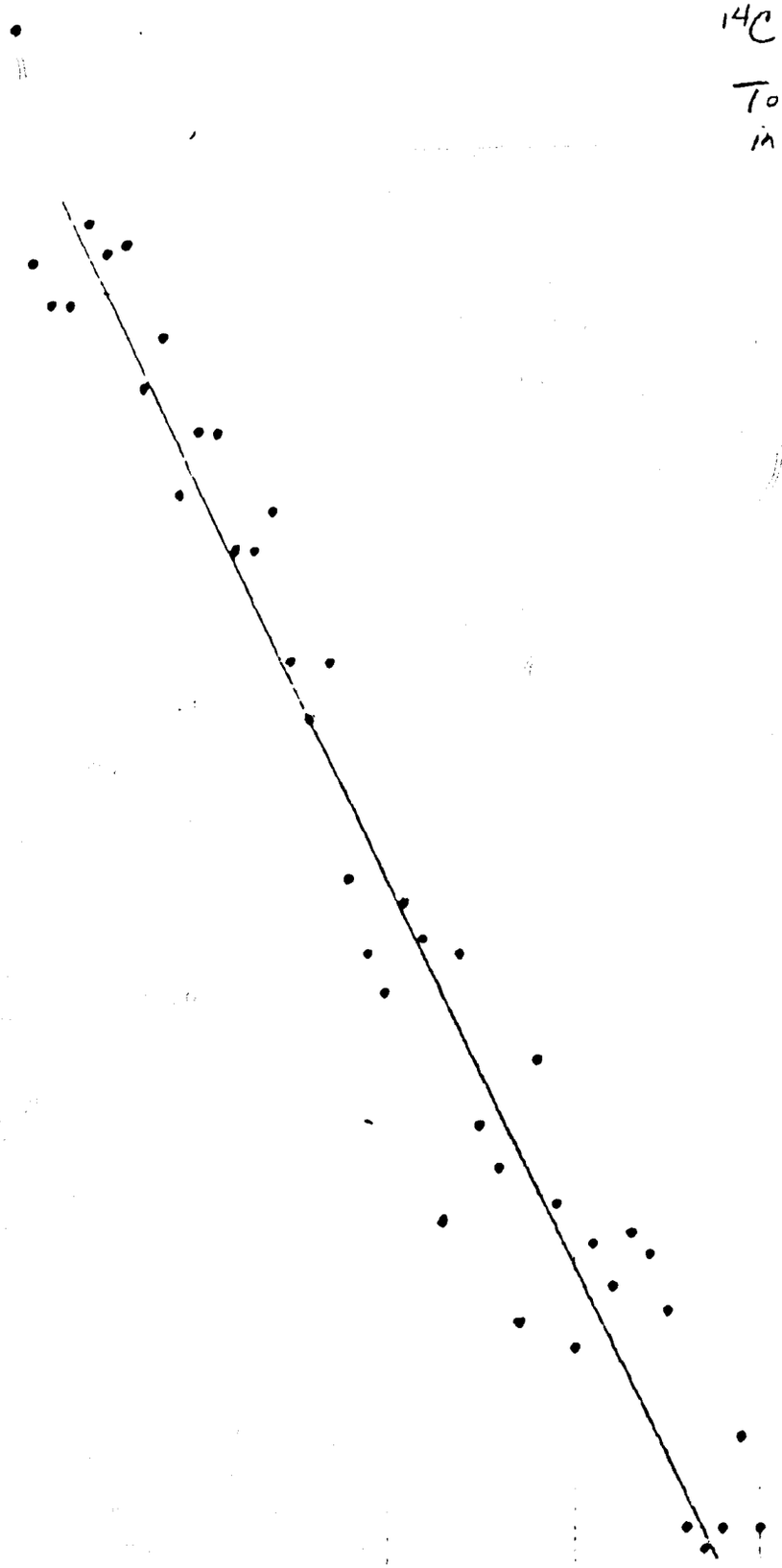


Initial $T_{1/2}$ = 5.4 days

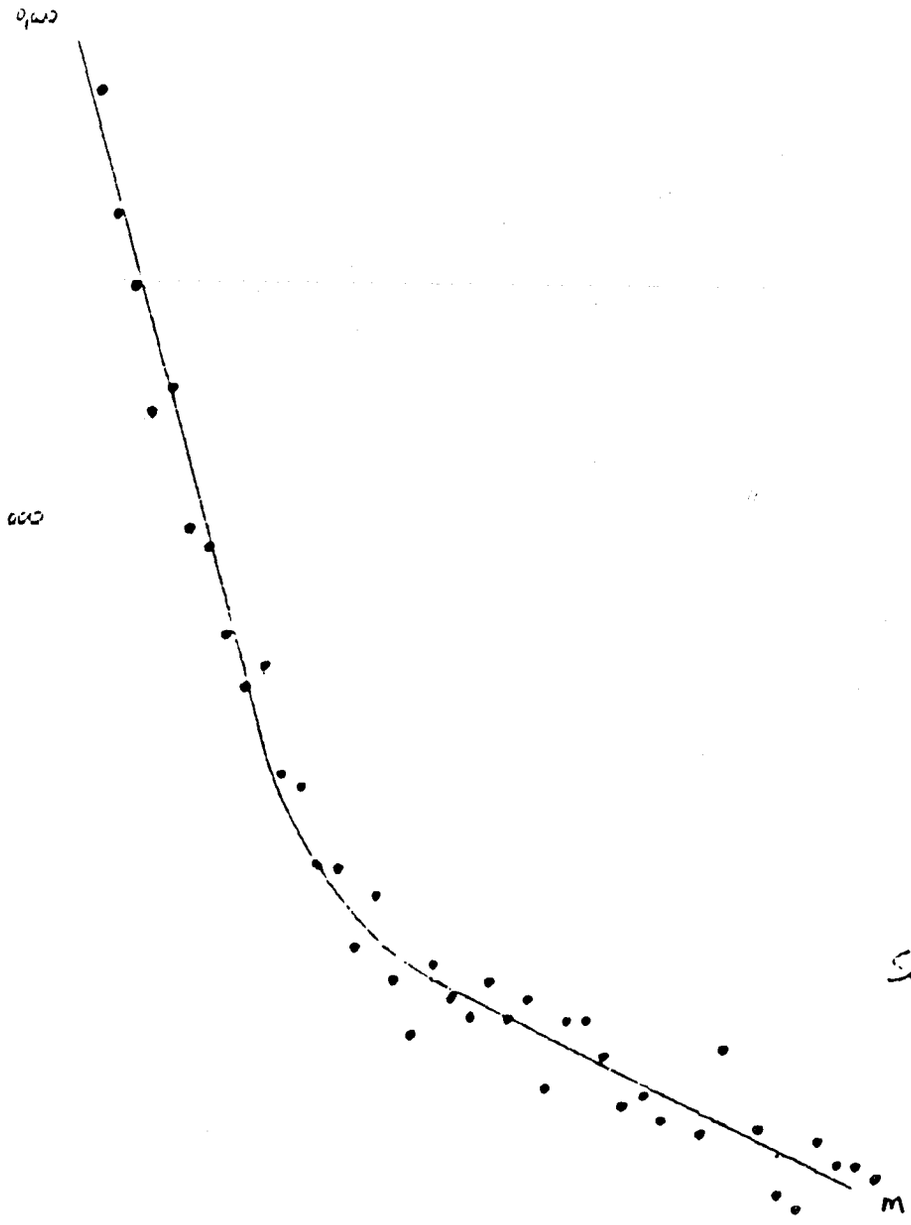
M #100 dim.
14C - Anordrin
Total cpm /day
in Urine



M #100 i.m.
^{14}C - Anordrin
Total cpm / day
in Urine



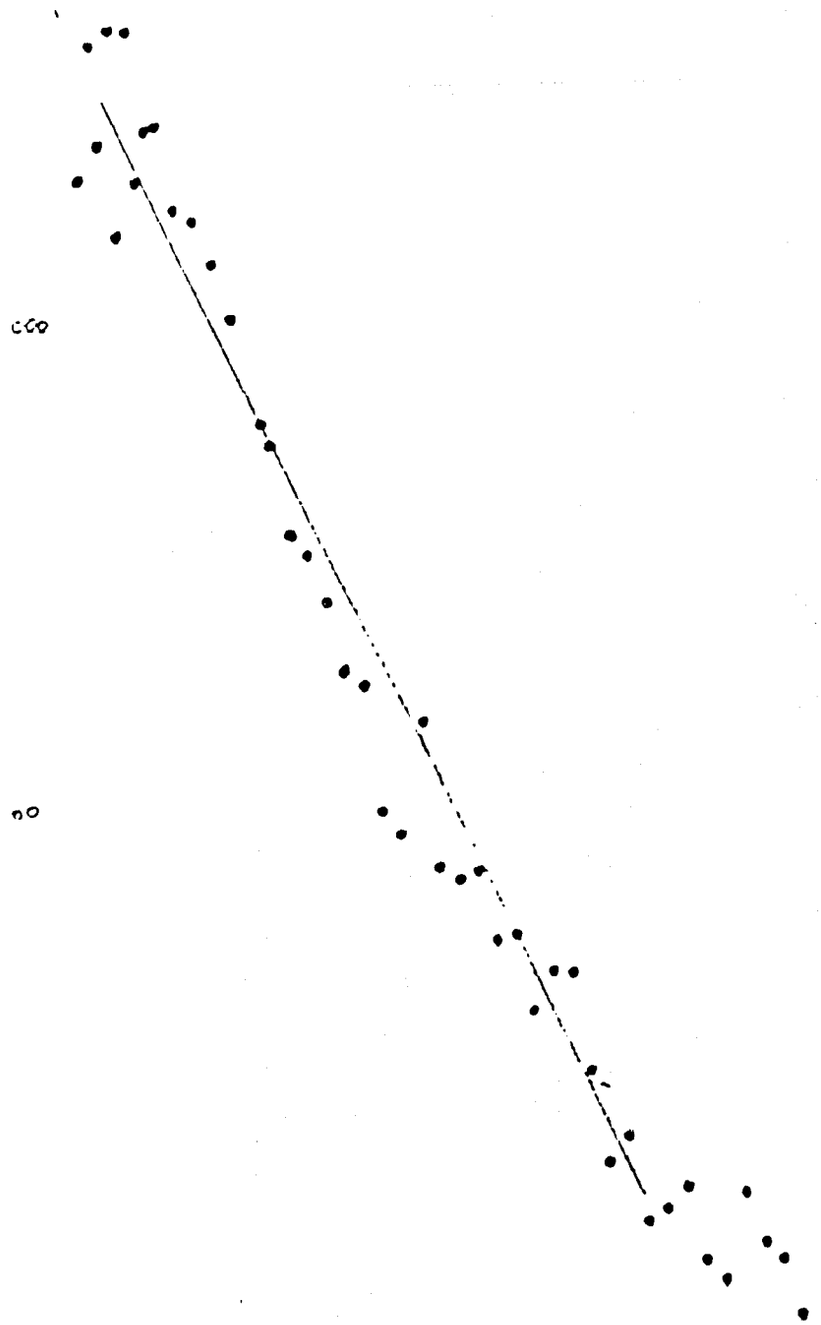
T ½ = 15 days



M # 101 i.v.
^{14}C - Anordrin
Total cpm / day
in Urine

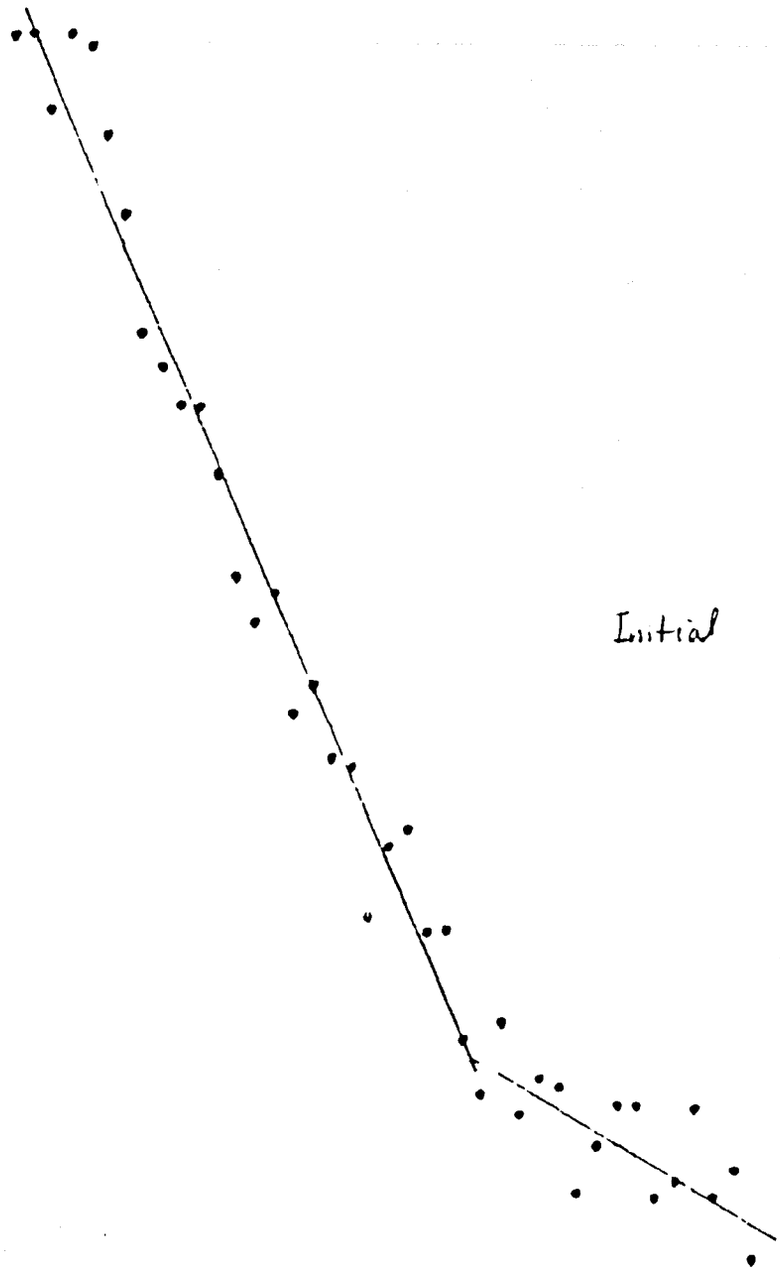
Initial $T_{1/2}$ = 2.5 days
Secondary $T_{1/2}$ = 11.0 days

M # 102 i.m.
^{14}C - Anordrin
Total cpm/day in
Urine



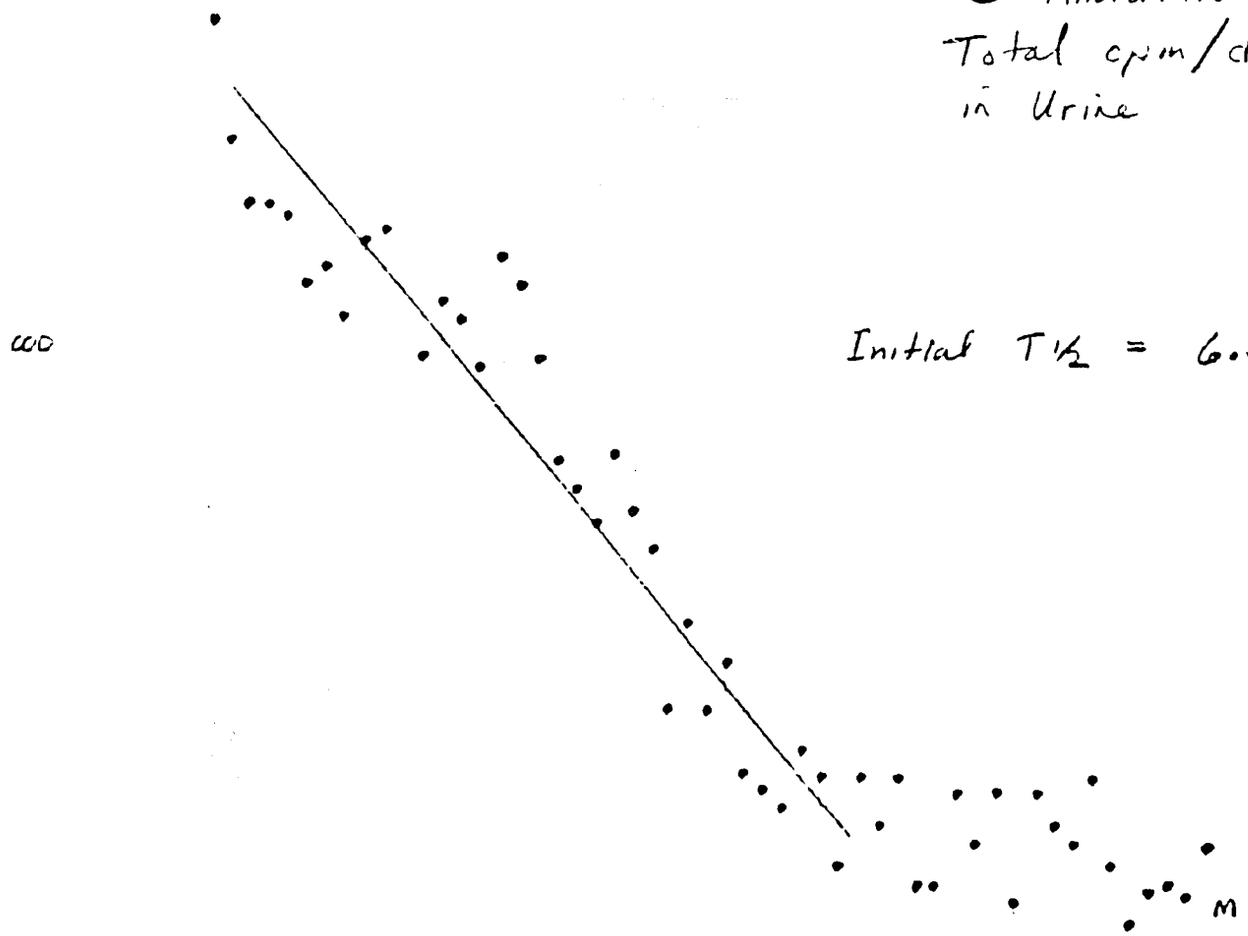
$T_{1/2}$ = 7.8 days

M #104 i.m.
^{14}C - Anordrin
Total cpm / day
in Urine



Initial $T_{1/2}$ = 6.9 days

M # 105 i.v.
^{14}C - Anordrin
Total cpm/day
in Urine



cpm / day x 10^{-2}

Appendix VII
Dose 1

* ENDOSYS * Anordrin Toxicology Studies *

TOXANORD* * VehCon

* print out from PRINT OUT * 12/05/86 * jnh *

SUBJECT	DATE	Chol mg/dL	FSH mIUml	PRL ng/ml	HDL mg/dl	E2 pg/ml	T pg/ml	P4 pg/ml	F ng/ml	T4 ug/dl	Calc pg/ml
Monkey - 193	10/23/85	160	< 4	< 2	71	52	220	3	156	4	6.8
Monkey - 193	11/22/85	170	< 4	4	67	22	110	< 1	174	8	6.8
Monkey - 193	12/21/85	150	< 4	21	65	92	120	< 1	152	9	7.5
Monkey - 193	01/20/86	180	< 4	22	88	88	170	< 1	160	5	8.0
Monkey - 195	10/23/85	140	< 4	2	59	36	92	5	120	5	6.3
Monkey - 195	11/22/85	110	< 4	< 2	57	29	140	4	240	5	5.9
Monkey - 195	12/21/85	150	< 4	11	71	47	110	5	240	4	8.2
Monkey - 195	01/20/86	170	< 4	10	72	44	59	7	190	4	8.1
Monkey - 211	10/23/85	120	< 4	< 2	110	26	36	< 1	340	13	6.5
Monkey - 211	11/22/85	110	< 4	< 2	83	< 10	13	< 1	280	13	6.9
Monkey - 211	12/21/85	160	4	9	94	22	11	< 1	260	9	6.5
Monkey - 211	01/20/86	150	< 4	< 2	87	30	2	7	380	7	6.5
Monkey - 215	10/23/85	110	< 4	17	89	29	39	< 1	320	7	4.8
Monkey - 215	11/22/85	81	< 4	2	76	< 10	42	< 1	320	7	4.8
Monkey - 215	12/21/85	86	< 4	< 2	78	18	41	< 1	280	9	5.6
Monkey - 215	01/20/86	130	< 4	< 2	72	56 0		< 1	400	13	6.1

****** 15:33:20 *******

Dose 2

* ENDOSYS * Anordrin Toxicology Studies *

TOXANORD* * 1×ETD

* print out from PRINT OUT * 12/05/86 * jnh *

SUBJECT	DATE	Chol mg/dL	FSH mIUml	PRL ng/ml	HDL mg/dl	E2 pg/ml	T pg/ml	P4 pg/ml	F ng/ml	T4 ug/dl	Calc pg/ml
Monkey - 199	10/23/85	120 <	4 <	2	63	40	34	< 1	180	9	7.9
Monkey - 199	11/22/85	190 <	4 <	2	72	26	24	1	440	10	7.8
Monkey - 199	12/21/85	160 <	4 <	2	64	28	0 <	1	320	13	8.0
Monkey - 199	01/20/86	230 <	4 <	2	83	16	1	2	320	12	8.0
Monkey - 201	10/23/85	110 <	4	27	90	44	110	4	200	5	5.6
Monkey - 201	11/22/85	110 <	4	7	63	17	42	< 1	280	7	6.4
Monkey - 201	12/21/85	130 <	4	4	70	25	30	< 1	300	14	7.1
Monkey - 201	01/20/86	130 <	4	18	72	20	15	< 1	280	10	7.6
Monkey - 204	10/23/85	140 <	4	3	82	40	290	< 1	196	8	5.7
Monkey - 204	11/22/85	100 <	4	29	72	26	56	1	300	14	5.6
Monkey - 204	12/21/85	150 <	4	22	65	26	21	2	320	11	7.4
Monkey - 204	01/20/86	230 <	4 <	2	NS	40	54	2	400	12	6.9
Monkey - 212	10/23/85	89 <	4	13	52	25	13	< 1	140	7	6.3
Monkey - 212	11/22/85	65 <	4 <	2	51	19	21	< 1	440	13	6.8
Monkey - 212	12/21/85	76	4 <	2	49	13	0 <	1	360	11	8.2
Monkey - 212	01/20/86	140 <	4 <	2	56	20	4	< 1	440	14	8.8

****** 15:35:03 *******

Dose 3

* ENDOSYS * Anordrin Toxicology Studies *

TOXANORD* * 10xETD

* print out from PRINT OUT * 12/05/86 * jnh *

SUBJECT	DATE	Chol mg/dL	FSH mIUml	PRL ng/ml	HDL mg/dl	E2 pg/ml	T pg/ml	P4 pg/ml	F ng/ml	T4 ug/dl	Calc pg/ml
Monkey - 194	10/23/85	110	45	2	64	23	150	< 1	260	6	7.2
Monkey - 194	11/22/85	130	< 4	< 2	51	56	25	1	480	13	7.4
Monkey - 194	12/21/85	150	< 4	2	58	73	35	1	460	12	8.9
Monkey - 194	01/20/86	140	< 4	< 2	50	72	33	< 1	420	16	12.2
Monkey - 205	10/23/85	160	< 4	< 2	110	132	200	< 1	320	8	6.1
Monkey - 205	11/22/85	110	< 4	< 2	66	94	39	1	480	21	7.3
Monkey - 205	12/21/85	130	< 4	< 2	68	132	7	2	520	14	6.9
Monkey - 205	01/20/86	170	4	< 2	65	96	12	2	460	19	8.1
Monkey - 213	10/23/85	110	< 4	15	62	38	49	< 1	300	8	6.0
Monkey - 213	11/22/85	160	< 4	< 2	41	80	6	< 1	500	11	7.6
Monkey - 213	12/21/85	110	4	< 2	44	67	1	< 1	560	8	5.4
Monkey - 213	01/20/86	170	< 4	< 2	44	120	2	1	400	12	6.0
Monkey - 214	10/23/85	130	< 4	2	91	24	160	1	520	9	10.0
Monkey - 214	11/22/85	190	< 4	20	57	59	23	< 1	520	10	11.0
Monkey - 214	12/21/85	110	< 4	3	63	78 0		< 1	440	11	9.2
Monkey - 214	01/20/86	150	< 4	6	79	74	14	< 1	680	9	13.0

****** 15:24:12 *******

Dose 4

* ENDOSYS * Anordrin Toxicology Studies *

TOXANORD* * 50xETD

* print out from PRINT OUT * 12/05/86 * jnh *

SUBJECT	DATE	Chol mg/dL	FSH mIUml	PRL ng/ml	HDL mg/dl	E2 pg/ml	T pg/ml	P4 pg/ml	F ng/ml	T4 ug/dl	Calc pg/ml
Monkey - 191	10/23/85	110	< 4	2	59	38	110	2	200	8	5.8
Monkey - 191	11/22/85	120	< 4	38	54	76	55	< 1	380	10	6.8
Monkey - 191	12/21/85	120	< 4	15	53	118	95	< 1	480	15	6.0
Monkey - 191	01/20/86	120	< 4	9	66	156	46	< 1	360	11	11.6
Monkey - 197	10/23/85	100	< 4	< 2	65	50	200	3	280	8	7.0
Monkey - 197	11/22/85	76	< 4	< 2	46	65	29	1	360	11	8.6
Monkey - 197	12/21/85	60	< 4	< 2	36	168	77	2	340	11	10.4
Monkey - 197	01/20/86	150	< 4	< 2	53	168	180	1	680	17	9.1
Monkey - 200	10/23/85	150	< 4	< 2	74	54	150	6	320	8	6.0
Monkey - 200	11/22/85	120	< 4	< 2	64	116	84	2	360	12	5.2
Monkey - 200	12/21/85	120	< 4	< 2	54	156	60	3	360	14	5.8
Monkey - 200	01/20/86	170	< 4	9	49	168	150	3	360	13	6.5
Monkey - 203	10/23/85	100	< 4	3	70	49	250	< 1	320	6	7.2
Monkey - 203	11/22/85	110	4	17	43	65	130	< 1	480	9	6.9
Monkey - 203	12/21/85	120	< 4	5	60	71	60	< 1	360	12	4.7
Monkey - 203	01/20/86	170	< 4	27	66	96	79	< 1	540	10	5.7

****** 15:32:28 *******

* ENDOSYS * Anordrin Toxicology Studies *

FOXANORD* * CONTRL

* print out from PRINT OUT * 12/08/86 * JNH *

SUBJECT	DATE	Chol mg/dL	HDL mg/dl
RAT - 001	10/24/85	100	27
RAT - 002	10/24/85	61	32
RAT - 003	10/24/85	78	18
RAT - 008	10/24/85	52	36
RAT - 010	10/24/85	78	32
RAT - 015	10/24/85	210	NS
RAT - 020	10/24/85	87	54
RAT - 022	10/24/85	110	56
RAT - 026	10/24/85	82	44
RAT - 035	10/24/85	73	50
RAT - 037	10/24/85	73	44
RAT - 041	10/24/85	86	48
RAT - 045	10/24/85	91	68
RAT - 054	10/24/85	82	32
RAT - 056	10/24/85	77	36
RAT - 058	10/24/85	82	42
RAT - 063	10/24/85	77	32
RAT - 064	10/24/85	230	NS
RAT - 066	10/24/85	45	40
RAT - 078	10/24/85	100	64
RAT - 001	01/21/86	91	79
RAT - 002	01/21/86	87	42
RAT - 003	01/21/86	61	28
RAT - 008	01/21/86	74	40
RAT - 010	01/21/86	87	46
RAT - 015	01/21/86	96	50
RAT - 020	01/21/86	57	34
RAT - 022	01/21/86	100	40
RAT - 026	01/21/86	74	18
RAT - 035	01/21/86	83	54
RAT - 037	01/21/86	70	9
RAT - 041	01/21/86	78	46
RAT - 045	01/21/86	52	18
RAT - 054	01/21/86	22	28
RAT - 056	01/21/86	NS	NS
RAT - 058	01/21/86	70	30
RAT - 063	01/21/86	65	59
RAT - 064	01/21/86	83	46
RAT - 066	01/21/86	35	1
RAT - 078	01/21/86	96	48

****** 10:51:06 *******

* ENDOSYS * Anordrin Toxicology Studies *
TOXANORD* * *2x*

* print out from PRINT OUT * 12/08/86 * JNH *

SUBJECT	DATE	Chol mg/dL	HDL mg/dl
RAT - 005	10/24/86	53	44
RAT - 007	10/24/86	71	62
RAT - 013	10/24/86	62	40
RAT - 027	10/24/86	76	58
RAT - 028	10/24/86	67	64
RAT - 046	10/24/86	71	48
RAT - 047	10/24/86	98	68
RAT - 050	10/24/86	67	68
RAT - 052	10/24/86	53	39
RAT - 057	10/24/86	62	34
RAT - 060	10/24/86	96	39
RAT - 061	10/24/86	70	42
RAT - 062	10/24/86	70	46
RAT - 068	10/24/86	70	40
RAT - 069	10/24/86	100	60
RAT - 070	10/24/86	61	58
RAT - 075	10/24/86	100	58
RAT - 079	10/24/86	91	52
RAT - 080	10/24/86	70	48
RAT - 082	10/24/86	48	52
RAT - 005	01/21/86	18	14
RAT - 007	01/21/86	36	9
RAT - 013	01/21/86	27	22
RAT - 027	01/21/86	55	24
RAT - 028	01/21/86	55	23
RAT - 046	01/21/86	36	14
RAT - 047	01/21/86	41	1
RAT - 050	01/21/86	NS	NS
RAT - 052	01/21/86	38	22
RAT - 057	01/21/86	13	11
RAT - 060	01/21/86	25	7
RAT - 061	01/21/86	17	32
RAT - 062	01/21/86	50	9
RAT - 068	01/21/86	21	18
RAT - 069	01/21/86	17	9
RAT - 070	01/21/86	NS	NS
RAT - 075	01/21/86	42	7
RAT - 079	01/21/86	42	22
RAT - 080	01/21/86	50	18
RAT - 082	01/21/86	17	16

****** 10:47:20 *******

* ENDOSYS * Anordrin Toxicology Studies *

TOXANORD* * *10x*

* print out from PRINT OUT * 12/08/86 * JNH *

	DATE	Chol mg/dL	HDL mg/dl
RAT - 004	10/24/85	70	46
RAT - 006	10/24/85	70	22
RAT - 009	10/24/85	83	44
RAT - 016	10/24/85	61	15
RAT - 018	10/24/85	78	46
RAT - 019	10/24/85	70	46
RAT - 023	10/24/85	52	44
RAT - 024	10/24/85	91	68
RAT - 025	10/24/85	52	39
RAT - 029	10/24/85	74	26
RAT - 031	10/24/85	65	58
RAT - 032	10/24/85	61	34
RAT - 033	10/24/85	61	24
RAT - 034	10/24/85	78	48
RAT - 042	10/24/85	70	54
RAT - 043	10/24/85	65	54
RAT - 051	10/24/85	65	48
RAT - 071	10/24/85	78	64
RAT - 072	10/24/85	96	72
RAT - 076	10/24/85	61	34
RAT - 004	01/21/86	17	16
RAT - 006	01/21/86	29	5
RAT - 009	01/21/86	29	24
RAT - 016	01/21/86	46	19
RAT - 018	01/21/86	25	7
RAT - 019	01/21/86	13	9
RAT - 023	01/21/86	46	14
RAT - 024	01/21/86	46	28
RAT - 025	01/21/86	29	16
RAT - 029	01/21/86	38	19
RAT - 031	01/21/86	42	22
RAT - 032	01/21/86	NS	NS
RAT - 034	01/21/86	33	16
RAT - 033	01/21/86	33	12
RAT - 042	01/21/86	57	14
RAT - 043	01/21/86	43	19
RAT - 051	01/21/86	67	9
RAT - 071	01/21/86	NS	NS
RAT - 072	01/21/86	57	NS
RAT - 076	01/21/86	24	18

****** 10:48:35 *******

* ENDOSYS * Anordrin Toxicology Studies *

TOXANORD* * *50x*

* print out from PRINT OUT * 12/08/86 * JNH *

SUBJECT	DATE	Chol mg/dL	HDL mg/dl
RAT - 011	10/24/85	96	64
RAT - 012	10/24/85	87	64
RAT - 014	10/24/85	61	27
RAT - 017	10/24/85	140	100
RAT - 021	10/24/85	87	48
RAT - 030	10/24/85	70	36
RAT - 038	10/24/85	70	52
RAT - 039	10/24/85	57	30
RAT - 040	10/24/85	61	40
RAT - 048	10/24/85	74	40
RAT - 049	10/24/85	74	46
RAT - 053	10/24/85	100	74
RAT - 055	10/24/85	74	42
RAT - 059	10/24/85	70	36
RAT - 065	10/24/85	70	50
RAT - 067	10/24/85	78	50
RAT - 073	10/24/85	70	54
RAT - 074	10/24/85	57	40
RAT - 077	10/24/85	78	72
RAT - 081	10/24/85	78	56
RAT - 011	01/21/86	24	9
RAT - 012	01/21/86	38	22
RAT - 014	01/21/86	57	14
RAT - 017	01/21/86	48	9
RAT - 021	01/21/86	29	< 1
RAT - 030	01/21/86	NS	NS
RAT - 038	01/21/86	48	< 1
RAT - 039	01/21/86	29	14
RAT - 040	01/21/86	29	19
RAT - 048	01/21/86	38	19
RAT - 049	01/21/86	62	38
RAT - 053	01/21/86	14	7
RAT - 055	01/21/86	62	32
RAT - 059	01/21/86	38	14
RAT - 065	01/21/86	43	7
RAT - 067	01/21/86	22	9
RAT - 073	01/21/86	43	14
RAT - 074	01/21/86	35	9
RAT - 077	01/21/86	35	9
RAT - 081	01/21/86	35	5

****** 10:49:51 *******

FINAL REPORT

TOXICITY STUDIES OF ANORDRIN

Program for Applied Research on Fertility Regulation (PARFR)
Northwestern University Subcontract 309A

Principal Investigator: D.P. Waller Ph.D.
Associate Professor
Department of Pharmacodynamics
College of Pharmacy

Institution: University of Illinois at Chicago
Health Sciences Center
P.O. Box 6998
Chicago, Illinois 60680

Subagreement Period:

Starting Date: 1 July 1985
Ending Date: 30 September 1986

A. SUMMARY

Anordrin , an A-nor steroid, will inhibit ovulation when administered intramuscularly to monkeys at a dose of 0.4 mg/kg. Preclinical studies of anordrin need to be performed prior to the initiation of Phase I clinical trials. Preclinical studies for this compound were recommended by Dr. Chatterton in cooperation with contacts at FDA. They include 90 day subchronic studies in three species.

The current experiment evaluated the toxicity of Anordrin in three species, rats, dogs and monkeys. Rats were dosed every fourth day, dogs and monkeys once every 30 days during the 90 day study period. The animals were sacrificed on the 91st day and histopathological examination of the tissues performed.

No major organ toxicity was observed. However, some changes in the mammary tissue occurred. In all three species, mammary gland hyperplasia and lactiferous secretions were observed in treated groups of animals. These were considered of minor toxicological importance.

B. BACKGROUND

Anordrin is a steroid used in China as an injectable contraceptive agent. An attempt is being made to begin clinical trials of this agent in the United States. Since anordrin has not undergone toxicity evaluation in the U.S., it must be adequately tested in preclinical toxicity testing. Acute doses of 800 mg/kg body weight of anordrin have been administered to mice without lethality. This supports the general lack of acute toxicity of steroids. Thus the acute LD_{50} of anordrin will not be required.

However the standard 90 day subacute toxicity testing will be required in three species. The effective dose for inhibition of ovulation in the monkey(0.4 mg/kg) was used to the determine the doses to be tested. Doses were established according to recommendations for toxicity testing of anordrin supplied by Dr. Chatterton of Northwestern University (Appendix 1).

C. OBJECTIVES

The primary objective of this study was to perform the 90-day subchronic toxicity testing of anordrin in three species, the rat, dog and monkey.

D. PROGRESS TO DATE

The test compound was obtained from Dr. Chatterton. All doses were administered intramuscularly as a solution of anordrin in Sesame Oil(NF). Blood was collected from all animals just prior to the first dosing for a complete hemogram with differential and a sample also sent to Northwestern University for lipid analysis.

Part I: Rats

Eighty mature female Sprague Dawley rats were divided into four groups and administered, vehicle, 0.08, 0.40, or 2.00 mg/kg of anordrin every four days for a total of ninety days.

All animals were weighed once every four days just prior to injection and clinically observed immediately and four hours after the injection. They were also observed each day for general mortality and morbidity.

On day ninety one, blood was obtained for analysis and the animals were autopsied. Samples of all major tissues were obtained for histopathological examination.

Histological examination of the animals revealed treatment related changes were confined to the mammary tissues of test animals in the the two highest treatment groups(4 and 20 mg/kg). The changes consisted of: "a dose-related incidence of lactation(secretory phase), galactocyst formation and lobular hyperplasia of glandular tissue. The galactocyst formation consisted of a cystic dilatation of mammary ducts with a lactiferous secretion. The glandular hyperplasia was confined to six Group III animals and was graded as minimal to moderate in severity." A complete histopathology report is attached as Appendix 2.

Part II: Dogs

Twelve dogs(pure bred female Beagles) were divided into three groups and administered vehicle, 0.40 or 1.00 mg/kg of anordrin every thirty days for a total of ninety days.

The animals were weighed and clinically observed weekly during the 90 day test period and just prior to dosing(days 1,31 and 61).

On day ninety one blood was obtained for clinical analysis and the animals were autopsied. Samples of all major organs were obtained for histopathological examination.

Histological examination revealed treatment related tissue changes were confined to the mammary tissue of animals in both groups treated with anordrin. The changes consisted of "a dose related incidence of lactiferous secretions within mammary ducts. This change was focal in distribution and graded as mild to moderate in relative severity.". A complete histopathology report is attached as Appendix 3.

Part III: Monkeys

Sixteen female Cynomolgus monkeys were obtained and divided into four groups and administered vehicle, 0.04, 0.40 or 2.00 mg/kg every thirty days for a total of 90 days.

All animals were weighed and clinically observed weekly and immediately prior to the administration of the test material. The test material was administered on days 1, 31 and 61. Urine was collected on a twenty-four hour basis from the monkeys on days 88,89 and 90. The samples were sent to Northwestern University for analysis.

On day ninety one, blood was obtained for analysis and the animals were autopsied. Samples from all major organs were obtained for histopathological examination.

Histopathological examination revealed treatment related changes confined to the mammary tissue of test animals in all three groups treated with anordrin. The changes consisted of "a dose-related incidence of diffuse lobular hyperplasia of mammary gland tissue and lactiferous secretions. These changes were graded as minimal to mild in relative severity". A complete histopathology report is attached as Appendix 4.

Summary

The histopathological changes observed in the three species tested were consistent. Each exhibited similar changes in mammary tissue. These effects may be due to the partial estrogenic activity of anordrin. A no effect dose level for mammary effects was 0.8 mg/kg in the rat. A no effect dose level was not observed in the dog or the monkey. The lowest dose tested in the monkey was the dose at which ovulation is inhibited. Even at this dose three out of the four treated animals had observable mammary tissue changes.

This evaluation supports the general nontoxic nature of anordrin when administered once a month for a 90 day period of time. Although the mammary gland changes are of minor toxicological significance, they should be carefully considered. It should be established whether or

not similar changes in mammary tissue were observed for other steroid agents previously tested. This is particularly important since the monkey exhibited changes in mammary tissue even at the lowest dose tested.

E. UNANTICIPATED RESULTS

None

F. RESEARCH DESIGN

The original proposal was written with a dosing schedule for rats to be injected three times at four day intervals. Discussions just prior to submission of the proposed study changed the dosing of the rats to once every four days for ninety days. This change in dosing schedule resulted in the need for a much greater amount of anordrin. The initial request for the amount of anordrin needed for the study was based upon the early dosing schedule of rats. Upon initiation of the study it was recognized the calculation for the amount of anordrin needed for the study was inadequate and additional quantities of anordrin were requested and obtained from Dr. Chatterton, Northwestern University.

G. OPERATIONAL SIGNIFICANCE

These studies support the general non-toxic nature of anordrin in animals when administered by monthly injections for a period of ninety days.

H. BUDGET REPORT

See Appendix 5.

The final costs of this project exceeded the amount of the award by $1,447. This was a result of greater than anticipated personnel costs. Additional costs were caused by a short delay in the onset of the experiment due to problems with delivery of acceptable animals and additional personnel needed during the days of necropsy of the study animals.

RECOMMENDATIONS FOR TOXICITY TESTING OF ANORDRIN
4/27/83

Since anordrin has not undergone toxicity evaluation in the U.S., the standard toxicity requirements for steroids that must precede phase I clinical trials have to be done. Since steroids are recognized as not being acutely lethal and we have already administered doses of 800 mg/kg body weight of anordrin to mice, establishment of the LD_{50} will not be required. The effective dose upon which toxicity testing will be based is the effective dose/kg body weight in the monkey. Therefore, determination of the effective dose for inhibition of ovulation in rats and dogs will not be required. Tests for mutagenicity are also not required. The following treatments will be required for evaluation of toxicity:

Rat: 3 injections at 4-day intervals. Doses of 2, 10, and 50 times the minimal effective dose (20 rats/dose).

Dog: 3 monthly injections at 10 and 25 times the minimal effective dose (4 dogs/dose).

Monkey: 3 monthly injections at 1, 10, and 50 times the minimal effective dose (4 monkeys/dose).

The minimal effective dose is defined as that dose/kg body weight that results in at least a 31-day delay in ovulation in all monkeys. During treatment, the animals will be weighed periodically, food consumption determined, and general health assessed as prescribed by guidelines provided by the FDA. Blood will be drawn from monkeys for assessment of cortisol, prolactin, estradiol, LH and FSH, thyroid hormones, antithrombin III, and fibrinolysins before administration and at 1 and 25 days after each dose of anordrin. Blood clotting time and blood pressure will also be assessed once before and twice during the study. Liver and kidney enzymes in serum

will be measured as part of the SMA 20 on duplicate samples from each monkey before treatment and during the last month, as will the several classes of serum lipoproteins.

At termination of the toxicity study each animal will be autopsied and specimens will be taken from all organs for histology and pathological evaluation.

APPENDIX 2

DONOVAN E. GORDON, D.V.M., PH.D., LTD.
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HIGHLAND PARK, ILLINOIS 60035

312/432-2565

June 26, 1986

Ms. Jill C. Penna
Toxicology Research Laboratory
University of Illinois
835 S. Wolcott Street
Chicago, IL 60612

Dear Ms. Penna:

I am submitting herewith my histopathology report prepared in connection with Project No. 0004, Part I, which pertains to the 90-Day Toxicity Study of Anordrin in Female Sprague-Dawley Rats.

Sincerely,

Donovan E. Gordon, D.V.M.

DEG:pw
Enc.

Testing Facility & Address	Sponsor
Donovan E. Gordon, D.V.M., Ph.D., Ltd. 833 Kimball Rd., Highland Park, IL 60035	Toxicology Research Laboratory University of IL, Chicago, IL 60612

Study Title

90-Day Intramuscular Toxicity Study of Anordrin In Female Sprague-Dawley Rats

Study Number	Summary Date
Project No. 0004, Part I	June 25, 1986

A histopathologic examination was conducted on Hematoxylin-eosin stained sections of selected tissues from a total of 77 Sprague-Dawley female rats that were sacrificed at the conclusion of the above study. The tissues submitted for examination, by light microscopy, consisted of brain, spinal cord (2 segments), eye, pituitary, mammary gland, esophagus, lung, trachea, liner, stomach, small and large intestines, spleen, kidneys, thymus, thyroid, parathyroid, adrenals, pancreas, urinary bladder, uterus and cervix, ovaries, lymph node, sternum with skeletal muscle, heart, aorta and gross lesion(s)/masses.

A total of 941 slides containing stained tissue sections were received in two separate shipments on April 1 and 16, 1986 from Propath, Inc., 1080 Nerge Road, Elk Grove Village, Illinois; where they were prepared.

The necropsy of animals, collection and fixation of tissues were conducted by the Toxicology Research Laboratory at the University of Illinois in Chicago, Illinois.

The experimental group descriptions are as follows:

Experimental Group	Treatment	Number of Animals
I	Anordrin, 0.8 mg/Kg	20
II	Anordrin, 4 mg/Kg	20
III	Anordrin, 20 mg/Kg	20
IV	Vehicle Control	17

All histologic changes observed in the tissues examined were recorded in the accompanying "Histopathology Incidence Tables" which are identified by experimental group and animal number. These tables also constitute an accountability of all tissues submitted for examination. The grading system and abbreviations used in the pathology tables are as follows:

Grade I = Minimal severity	X = Within normal limits
Grade 2 = Mild severity	L = Microscopic lesion
Grade 3 = Moderate severity	F = Focal
Grade 4 = Marked severity	M = Multifocal
A = Tissue absent	D = Diffuse

The incidences of microscopic lesions are summarized by group and sex in the Pathology Appendices as follows:

Pathology Appendix A: Incidence of Lesions Considered to be Treatment Related

Pathology Appendix B: Lesion Incidence Comparison Table

Pathology Appendix C: Histopathology Lesion Severity and Incidence Table

Pathology Appendix D: Correlation of Gross and Microscopic Findings

Results

Treatment-related changes were confined to the mammary tissue of test animals in Groups II and III which is summarized in Appendix A. The changes consist of a dose-related incidence of lactation (secretory phase), galactocyst formation and lobular hyperplasia of glandular tissue. The galactocyst formation consists of a cystic dilatation of mammary ducts with a lactiferous secretion. The glandular hyperplasia was confined to six Group III animals and was graded as minimal to moderate in severity. The above changes were not evident among test animals in Group I or the vehicle control animals.

The remainder of the tissue changes observed among the vehicle control and test animals were regarded as lesions of naturally occurring diseases or agonal lesions related to the sacrifice procedure. The overall incidence and relative severity of these changes were similar, in most instances, among the vehicle control and test groups.

Discussion

Treatment-related changes ascribed to the systemic effects of Anordrin over a 90-day period were confined to the mammary gland tissue of 6/18 (33%) and 17/18 (94%) female rats at the 4 and 20 mg/Kg dosage levels, respectively, which consisted of lactation, galactocyst formation and/or glandular hyperplasia. These findings were of minor toxicological significance. The 0.8 mg/Kg level of Anordrin was regarded as a no-effect level for these changes.

Donovan E. Gordon, D.V.M.
Diplomate, American College of
Veterinary Patholgists

PATHOLOGY APPENDIX A

INCIDENCE OF LESIONS CONSIDERED TO BE TREATMENT-RELATED

INCIDENCE OF LESIONS CONSIDERED TO BE TREATMENT RELATED

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

TISSUE LESION(S)	GROUP I 0.8 mg/Kg	GROUP II 4 mg/Kg	GROUP III 20 mg/Kg	GROUP IV 0 mg/Kg	
MAMMARY GLAND	(20)	(18)	(18)	(17)	
Secretory phase	0/20	6/18	17/18	0/17	
Galacocyst formation	0/20	1/18	6/18	0/17	
Glandular hyperplasia	0/20	0/18	6/18	0/17	

( ) = number of animals for which tissue was represented

PATHOLOGY APPENDIX B

LESION INCIDENCE COMPARISON TABLE

LESION INCIDENCE COMPARISON TABLE

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

TISSUE LESION(S)	GROUP I 0.8 mg/Kg	GROUP II 4 mg/Kg	GROUP III 20 mg/Kg	GROUP IV 0 mg/Kg	
BRAIN	(20)	(20)	(20)	(17)	
SPINAL CORD-1	(20)	(20)	(19)	(17)	
SPINAL CORD-2	(13)	(12)	(12)	(8)	
EYE	(19)	(20)	(20)	(17)	
PITUITARY	(9)	(4)	(9)	(7)	
MAMARY GLAND	(20)	(18)	(18)	(17)	
Secretory phase	0/20	6/18	17/18	0/17	
Galactocyst formation	0/20	1/18	6/18	0/17	
Glandular hyperplasia	0/20	0/18	6/18	0/17	
ESOPHAGUS	(20)	(20)	(20)	(17)	
LUNG	(20)	(20)	(20)	(17)	
Lymphoid infiltrate,peribronchia/iolar	1/20	1/20	4/20	6/17	
Lymphoid infiltrate, perivascular	6/20	8/20	5/20	6/17	
Congestion with edema	3/20	3/20	7/20	2/17	
Alveolar macrophages	2/20	2/20	0/20	3/17	
Hemorrhage, recent, agonal	0/20	0/20	0/20	2/17	
TRACHEA	(20)	(20)	(19)	(16)	
Lymphoid infiltrate, submucosal	9/20	5/20	12/19	6/16	
Inflammatory exudate in lumen	0/20	0/20	2/19	1/16	

Code: ( ) = number of animals for which tissue was represented

LESION INCIDENCE COMPARISON TABLE

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

TISSUE LESION(S)	GROUP I 0.8 mg/Kg	GROUP II 4 mg/Kg	GROUP III 20 mg/Kg	GROUP IV 0 mg/Kg	
LIVER	(20)	(20)	(20)	(17)	
Bile duct proliferation	0/20	1/20	0/20	0/17	
STOMACH	(20)	(20)	(20)	(17)	
SMALL INTESTINE (DUODENUM)	(20)	(20)	(20)	(17)	
LARGE INTESTINE (COLON)	(20)	(20)	(20)	(17)	
Nematode parasite in lumen	2/20	1/20	1/20	0/17	
PARATHYROID(S)	(6)	(4)	(3)	(7)	
SPLEEN	(20)	(20)	(19)	(17)	
Increased pigment	1/20	4/20	4/19	0/17	
KIDNEYS	(20)	(20)	(20)	(17)	
Microconcretions, unilateral	2/20	2/20	1/20	2/17	
Microconcretions, bilateral	3/20	0/20	2/20	1/17	
Lymphoid infiltrate, unilateral	0/20	0/20	0/20	1/17	
THYMUS	(20)	(19)	(15)	(15)	
THYROID(S)	(11)	(9)	(15)	(12)	
ADRENAL(S)	(19)	(20)	(19)	(16)	
PANCREAS	(20)	(20)	(20)	(17)	
URINARY BLADDER	(20)	(20)	(20)	(16)	
OVARIE(S)	(19)	(20)	(19)	(17)	

Code: ( ) = number of animals for which tissue was represented

LESION INCIDENCE COMPARISON TABLE

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

TISSUE LESION(S)	GROUP I 0.8 mg/Kg	GROUP II 4 mg/Kg	GROUP III 20 mg/Kg	GROUP IV 0 mg/Kg	
UTERUS	(20)	(17)	(17)	(17)	
Lumenal dilatation-hydrometria	2/20	7/17	4/17	2/17	
Metritis, chronic	2/20	1/17	3/17	0/17	
CERVIX	(20)	(20)	(19)	(17)	
Cervicitis, chronic	2/20	0/20	0/19	0/17	
LYMPH NODE	(19)	(20)	(17)	(15)	
STERNUM	(15)	(20)	(19)	(15)	
Inflammation, soft tissue	0/15	0/20	1/19	0/15	
SKELETAL MUSCLE	(20)	(20)	(20)	(17)	
HEART	(20)	(20)	(20)	(16)	
Myocardial degeneration & fibrosis	1/20	0/20	1/20	0/16	
AORTA	(20)	(20)	(17)	(16)	

Code: ( ) = number of animals for which tissue was represented

PATHOLOGY APPENDIX C

HISTOPATHOLOGY LESION SEVERITY AND INCIDENCE TABLE

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP I - 0.8 mg/Kg												
		ANIMAL NO.	1	5	7	13	27	28	46	47	52	57	58	60	
1	BRAIN		X	X	X	X	X	X	X	X	X	X	X	X	1
2	SPINAL CORD-1		X	X	X	X	X	X	X	X	X	X	X	X	2
3	SPINAL CORD-2		A	X	X	A	A	A	X	X	X	A	X	X	3
4	EYE		X	X	X	X	X	X	X	X	X	X	X	A	4
5	PITUITARY		X	X	A	X	A	A	X	X	X	A	A	A	5
6	MAMMARY GLAND		X	X	X	X	X	X	X	X	X	X	X	X	6
7	ESOPHAGUS		X	X	X	X	X	X	X	X	X	X	X	X	7
8	LUNG		L	L	X	X	L	X	X	L	X	X	X	L	8
9	Lymphoid infiltrate, periobronchial/iolar						1/F								9
10	Lymphoid infiltrate, perivascular						2/F								10
11	Congestion with edema		3/D	2/M						2/M					11
12	Alveolar macrophages													1/F	12
13	TRACHEA		X	X	L	L	L	L	X	L	X	L	L	X	13
14	Lymphoid infiltrate, submucosal				2/F	3/M	1/F	3/F		2/F		3/M	3/M		14
15	LIVER		X	X	X	X	X	X	X	X	X	X	X	X	15
16	STOMACH		X	X	X	X	X	X	X	X	X	X	X	X	16
17	SMALL INTESTINE (DUODENUM)		X	X	X	X	X	X	X	X	X	X	X	X	17
18	LARGE INTESTINE (COLON)		L	X	X	L	X	X	X	X	X	X	X	X	18
19	Nematode parasite in lumen		P			P									19
20	PARATHYROID(S)		X	A	X	A	A	A	A	A	A	X	A	A	20

Legend: L = Microscopic Lesion(s) Not Remarkable
P = Present, no grade
A = Tissue Absent

1 = Minimal
2 = Mild
3 = Moderate
4 = Marked Severity

S = Solitary Focus
F = Focal
M = Multifocal
D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	I - 0.8 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	61	62	68	69	75	79	80	82				
1	BRAIN		X	X	X	X	X	X	X	X				1
2	SPINAL CORD-1		X	X	X	X	X	X	X	X				2
3	SPINAL CORD-2		X	A	A	X	X	X	X	X				3
4	EYE		X	X	X	X	X	X	X	X				4
5	PITUITARY		X	X	X	A	A	A	A	A				5
6	MAMMARY GLAND		X	X	X	X	X	X	X	X				6
7	ESOPHAGUS		X	X	X	X	X	X	X	X				7
8	LUNG		L	L	L	X	L	L	X	X				8
9	Lymphoid infiltrate, peribronchial/iolar												1/20	9
10	Lymphoid infiltrate, perivascular		2/M	2/F	1/M		2/F	1/F					6/20	10
11	Congestion with edema												3/20	11
12	Alveolar macrophages			1/F									2/20	12
13	TRACHEA		L	X	X	X	X	X	X	L				13
14	Lymphoid infiltrate, submucosal		1/M							2/F			9/20	14
15	LIVER		X	X	X	X	X	X	X	X				15
16	STOMACH		X	X	X	X	X	X	X	X				16
17	SMALL INTESTINE (DUODENUM)		X	X	X	X	X	X	X	X				17
18	LARGE INTESTINE (COLON)		X	X	X	X	X	X	X	X				18
19	Nematode parasite in lumen												2/20	19
20	PARATHYROID(S)		X	A	A	A	A	A	X	X				20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary Focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP I - 0.8 mg/Kg, continued											
		ANIMAL NO.	1	5	7	13	27	28	46	47	52	57	58	60
	SPLEEN		X	X	X	X	X	L	X	X	X	X	X	X
	Increased pigment							1/D						
	KIDNEYS		XX	XX	XX	XL	XX	XL	XX	XX	XX	XX	XX	LL
	Tubules-microconcretions unilateral					1/F		1/F						
	Tubules-microconcretions, bilateral													2/M
	THYMUS		X	X	X	X	X	X	X	X	X	X	X	X
	THYROID(S)		X	X	A	X	A	X	A	X	A	X	A	X
	ADRENALS		XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
	PANCREAS		X	X	X	X	X	X	X	X	X	X	X	X
	URINARY BLADDER		X	X	X	X	X	X	X	X	X	X	X	X
	UTERUS		X	L	X	X	X	L	L	X	X	X	X	X
	Lumenal dilatation-hydrometria							1						
	Metritis, chronic			3					2					
	CERVIX		X	L	X	X	X	X	L	X	X	X	X	X
	Cervicitis, chronic			2					1					
	LYMPH NODE		X	X	X	X	X	X	X	X	X	X	X	X
	STERNUM		A	X	A	X	A	X	X	A	X	X	A	X
	SKELETAL MUSCLE		X	X	X	X	X	X	X	X	X	X	X	X
	HEART		X	X	X	X	X	X	X	X	X	X	X	X

Legend. L = Microscopic Lesion(s)
X = Not Remarkable
P = Present, no grade
A = Not Present, Absent

1 = Minimal
2 = Mild
3 = Moderate
4 = Marked Congestion

f = Solitary Focus
F = Focal
M = Multifocal
D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	I - 0.8 mg/Kg, continued										GROUP INCIDENCE	
		ANIMAL NO.	61	62	68	69	75	79	80	82				
1	SPLEEN		X	X	X	X	X	X	X	X				1
2	Increased pigment												1/20	2
3	KIDNEYS		XX	LL	XX	XX	LL	XX	XX	XX				3
4	Tubules-microconcretions, unilateral												2/20	4
5	Tubules-microconcretions, bilateral			1/F			1/F						3/20	5
6	THYMUS		X	X	X	X	X	X	X	X				6
7	THYROID(S)		X	A	A	A	A	X	X	X				7
8	ADRENALS		XX	XA	XX	XX	XX	XX	AA	XA				8
9	PANCREAS		X	X	X	X	X	X	X	X				9
10	URINARY BLADDER		X	X	X	X	X	X	X	X				10
11	UTERUS		X	X	X	X	L	X	X	X				11
12	Lumenal dilatation-hydrometria						2						2/20	12
13	Metritis, chronic												2/20	13
14	CERVIX		X	X	X	X	X	X	X	X				14
15	Cervicitis, chronic												2/20	15
16	LYMPH NODE		A	X	X	X	X	X	X	X				16
17	STERNUM		X	X	X	X	X	X	X	X				17
18	SKELETAL MUSCLE		X	X	X	X	X	X	X	X				18
19	HEART		X	X	X	X	X	X	L	X				19
20	Myocardial degeneration & fibrosis								2/F				1/20	20

Legend: L = Microscopic Lesion(s) 1 = Minimal F = Solitary Focus
X = Not Remarkable 2 = Mild f = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP I - 0.8 mg/Kg, continued												
		ANIMAL NO.	1	5	7	13	27	28	46	47	52	57	58	60	
1	AORTA		X	X	X	X	X	X	X	X	X	X	X	X	1
2	OVARIES		XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	2
3															3
4															4
5															5
6															6
7															7
8															8
9															9
10															10
11															11
12															12
13															13
14															14
15															15
16															16
17															17
18															18
19															19
20															20

Legend: L = Microscopic Lesion(s) — Not Remarkable; Present, no grade; A = Tissue Absent
1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Marked Severity
f = Solitary focus; f = Focal; M = Multifocal; - = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	I - 0.8 mg/Kg, continued										GROUP INCIDENCE	
		ANIMAL NO.	61	62	68	69	75	79	80	82				
1	AORTA		X	X	X	X	X	X	X	X				1
2	OVARIES		AA	XX	XX	XX	XA	XX	XX	XX				2
3														3
4														4
5														5
6														6
7														7
8														8
9														9
10														10
11														11
12														12
13														13
14														14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary Focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP II - 4 mg/Kg												
		ANIMAL NO.	4	6	9	16	18	19	23	24	25	29	31	32	
1	BRAIN		X	X	X	X	X	X	X	X	X	X	X	X	1
2	SPINAL CORD-1		X	X	X	X	X	X	X	X	X	X	X	X	2
3	SPINAL CORD-2		X	X	A	A	A	X	A	A	X	X	X	X	3
4	EYE		X	X	X	X	X	X	X	X	X	X	X	X	4
5	PITUITARY		A	A	A	X	A	A	A	A	A	A	A	A	5
6	MAMMARY GLAND		X	X	X	L	X	X	X	L	X	X	L	L	6
7	Secretory phase					P				P			P	P	7
8	ESOPHAGUS		X	X	X	X	X	X	X	X	X	X	X	X	8
9	LUNG		L	L	X	L	L	L	X	L	X	X	X	L	9
10	Congestion with edema		2/M												10
11	Lymphoid infiltrate, perivascular			1/F		1/F	2/F	1/F		1/F				1/F	11
12	Alveolar macrophages							1/F							12
13	TRACHEA		L	X	X	X	X	X	X	X	X	X	X	L	13
14	Lymphoid infiltrate, submuscosal		1/F											1/F	14
15	LIVER		X	X	X	X	X	L	X	X	X	X	X	X	15
16	Bile duct proliferation							1/F							16
17	STOMACH		X	X	X	X	X	X	X	X	X	X	X	X	17
18															18
19															19
20															20

Legend: L = Microscopic Lesion(s)
X = Not Remarkable
P = Present, no grade
A = Tissue Absent

1 = Minimal
2 = Mild
3 = Moderate
4 = Marked Severity

f = Solitary focus
F = Focal
M = Multifocal
D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	II - 4 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	33	34	42	43	51	71	72	76				
1	BRAIN		X	X	X	X	X	X	X	X				1
2	SPINAL CORD-1		X	X	X	X	X	X	X	X				2
3	SPINAL CORD-2		A	X	A	X	X	X	X	A				3
4	EYE		X	X	X	X	X	X	X	X				4
5	PITUITARY		X	A	A	X	X	A	A	A				5
6	MAMMARY GLAND		L	X	A	A	L	X	X	X				6
7	Secretory phase		P				P						6/20	7
8	Galactocyst formation						P						1/20	8
9	ESOPHAGUS		X	X	X	X	X	X	X	X				9
10	LUNG		L	L	X	X	L	L	L	L				10
11	Congestion with edema							2/M	2/M				3/20	11
12	Lymphoid infiltrate, perivascular			1/F						1/F			8/20	12
13	Alveolar macrophages		1/F										2/20	13
14	Lymphoid infiltrate, peribronchial/iolar						1/F						1/20	14
15	TRACHEA		L	X	X	X	X	L	L	X				15
16	Lymphoid infiltrate, submuscosal		3/M					2/M	3/M				5/20	16
17	LIVER		X	X	X	X	X	X	X	X				17
18	Bile duct proliferation												1/20	18
19	STOMACH		X	X	X	X	X	X	X	X				19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal S = Solitary Focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

TISSUE LESION(S)	GROUP II - 4 mg/KG, continued													
	ANIMAL NO.	4	6	9	16	18	19	23	24	25	29	31	32	
1 SMALL INTESTINE (DUODENUM)		X	X	X	X	X	X	X	X	X	X	X	X	1
2 LARGE INTESTINE (COLON)		X	X	X	X	X	L	X	X	X	X	X	X	2
3 Nematode parasite in lumen							P							3
4 SPLEEN		X	X	L	L	X	X	X	X	L	X	X	X	4
5 Increased pigment				1/D	1/D					2/D				5
6 KIDNEYS		XX	XX	XX	XX	XX	XL	XX	XX	XL	XX	XX	XX	6
7 Tubules-microconcretions, unilateral							2/M			1/F				7
8 THYMUS		X	X	X	X	X	X	X	X	X	X	X	X	8
9 THYROID(S)		A	X	A	A	X	A	X	X	A	A	X	X	9
10 PARATHYROID(S)		A	A	A	A	X	A	X	X	A	A	A	A	10
11 ADRENALS		XX	XX	XX	XX	XX	XX	XX	XX	XA	XX	XX	XX	11
12 PANCREAS		X	X	X	X	X	X	X	X	X	X	X	X	12
13 URINARY BLADDER		X	X	X	X	X	X	X	X	X	X	X	X	13
14 UTERUS		X	X	X	L	L	L	X	X	A	L	X	L	14
15 Lumenal dilatation-hydrometria					1	1	2						2	15
16 Metritis, chronic											1			16
17 CERVIX		X	X	X	X	X	X	X	X	X	X	X	X	17
18 LYMPH NODE		X	X	X	X	X	X	X	X	X	X	X	X	18
19 STERNUM		X	X	X	X	X	X	X	X	X	X	X	X	19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary Focus
X = Not Remarkable 2 = Mild f = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	II - 4 mg/Kg, continued										GROUP INCIDENCE	
		ANIMAL NO.	33	34	42	43	51	71	72	76				
1	SMALL INTESTINE (DUODENUM)		X	X	X	X	X	X	X	X				1
2	LARGE INTESTINE (COLON)		X	X	X	X	X	X	X	X				2
3	Nematode parasite in lumen												1/20	3
4	SPLEEN		X	X	L	X	X	X	X	X				4
5	Increased pigment				1/D								4/20	5
6	KIDNEYS		XX	XX	XX	XX	XX	XX	XX	XX				6
7	Tubules-microconcretions, unilateral												2/20	7
8	THYMUS		X	X	A	X	X	X	X	X				8
9	THYROID(S)		A	X	A	A	A	X	X	A				9
10	PARATHYROID(S)		A	A	A	A	A	A	X	A				10
11	ADRENALS		XX	XX	XX	XX	XX	XA	XX	XX				11
12	PANCREAS		X	X	X	X	X	X	X	X				12
13	URINARY BLADDER		X	X	X	X	X	X	X	X				13
14	UTERUS		A	X	L	A	X	L	L	X				14
15	Lumenal dilatation-hyrometria				2			1	2				7/17	15
16	Metritis, chronic												1/17	16
17	CERVIX		X	X	X	X	X	X	X	X				17
18	LYMPH NODE		X	X	X	X	X	X	X	X				18
19	STERNUM		X	X	X	X	X	X	X	X				19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary Focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP II - 4 mg/Kg, continued												
		ANIMAL NO. 4	6	9	16	18	19	23	24	25	29	31	32	
1	SKELETAL MUSCLE	X	X	X	X	X	X	X	X	X	X	X	X	1
2	HEART	X	X	X	X	X	X	X	X	X	X	X	X	2
3	AORTA	X	X	X	X	X	X	X	X	X	X	X	X	3
4	OVARIES	XX	XA	XX	XX	XX	XX	XX	XX	XX	XA	XX	XX	4
5														5
6														6
7														7
8														8
9														9
10														10
11														11
12														12
13														13
14														14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) Not Remarkable
X = Present, no grade
A = Tissue Absent

1 = Minimal
2 = Mild
3 = Moderate
4 = Marked Severity

S = Solitary Focus
F = Focal
M = Multifocal
D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	II - 4 mg/Kg, continued										GROUP INCIDENCE	
		ANIMAL NO.	33	34	42	43	51	71	72	76				
1	SKELETAL MUSCLE		X	X	X	X	X	X	X	X				1
2	HEART		X	X	X	X	X	X	X	X				2
3	AORTA		X	X	X	X	X	X	X	X				3
4	OVARIES		XX	XA	XX	XX	XX	XX	XX	XX				4
5														5
6														6
7														7
8														8
9														9
10														10
11														11
12														12
13														13
14														14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal S = Solitary Focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP III - 20 mg/Kg												
		ANIMAL NO. 11	12	14	17	21	30	38	39	40	48	49	53	
1	BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	1
2	SPINAL CORD-1	X	X	X	X	X	X	X	X	X	X	X	X	2
3	SPINAL CORD-2	A	X	X	A	A	X	X	X	X	X	A	X	3
4	EYE	X	X	X	X	X	X	X	X	X	X	X	X	4
5	PITUITARY	X	X	A	A	A	A	A	X	X	A	A	X	5
6	MAMMARY GLAND	L	L	L	L	L	X	L	L	L	A	A	L	6
7	Secretory phase	P	P	P	P	P		P	P	P			P	7
8	Galactocyst formation		P		P					P				8
9	Glandular hyperplasia							3					3	9
10	ESOPHAGUS	X	X	X	X	X	X	X	X	X	X	X	X	10
11	LUNG	L	L	L	L	X	X	L	X	X	L	L	L	11
12	Lymphoid infiltrate, perivascular	1/F		1/F								1/F	2/F	12
13	Congestion with edema		3/M	2/D	2/M			2/D						13
14	Lymphoid infiltrate, peribronchial/iolar										1/F	2/F		14
15	TRACHEA	L	L	L	X	X	A	X	X	L	L	L	L	15
16	Lymphoid infiltrate, submucosal	2/F	2/F	3/M						1/F	3/M	2/M	3/M	16
17	Inflammatory exudate in lumen											P		17
18	LIVER	X	X	X	X	X	X	X	X	X	X	X	X	18
19														19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal S = Solitary focus
[illegible] Remarkable 2 = Mild f = Focal
[illegible] esent, no grade 3 = Moderate M = Multifocal
A [illegible] ssue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	III - 20 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	55	59	65	67	73	74	77	81				
1	BRAIN		X	X	X	X	X	X	X	X				1
2	SPINAL CORD-1		X	X	X	X	X	A	X	X				2
3	SPINAL CORD-2		X	A	A	X	X	A	X	A				3
4	EYE		X	X	X	X	X	X	X	X				4
5	PITUITARY		X	X	A	X	A	X	A	A				5
6	MAMMARY GLAND		L	L	L	L	L	L	L	L				6
7	Secretory phase		P	P	P	P	P	P	P	P			17/18	7
8	Galactocyst formation			P				P		P			6/18	8
9	Glandular hyperplasia			1				1	2	2			6/18	9
10	ESOPHAGUS		X	X	X	X	X	X	X	X				10
11	LUNG		X	L	L	L	L	X	X	X				11
12	Lymphoid infiltrate, perivascular					2/F							5/20	12
13	Congestion with edema				2/D	2/D	2/M						7/20	13
14	Lymphoid infiltrate, peribronchial/iolar			2/F	1/F								4/20	14
15	TRACHEA		L	L	L	L	X	L	X	X				15
16	Lymphoid infiltrate, submucsal		3/M	2/F	3/M	3/M		2/F					12/19	16
17	Inflammatory exudite in lumen				P								2/19	17
18	LIVER		X	X	X	X	X	X	X	X				18
19														19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary Focus
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HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

TISSUE LESION(S)	GROUP	III - 20 mg/Kg, continued												
	ANIMAL NO.	11	12	14	17	21	30	38	39	40	48	49	53	
1 STOMACH		X	X	X	X	X	X	X	X	X	X	X	X	1
2 SMALL INTESTINE (DUODENUM)		X	X	X	X	X	X	X	X	X	X	X	X	2
3 LARGE INTESTINE (COLON)		X	X	X	X	X	X	X	X	X	X	X	X	3
4 SPLEEN		L	X	X	X	X	X	X	L	X	X	X	X	4
5 Increased pigment		1/D							1/D					5
6 KIDNEYS		XX	XX	XX	XL	LL	XX	LL	XX	XX	XX	XX	XX	6
7 Tubules - microconcretions, unilateral					1/F									7
8 Tubules - microconcretions, bilateral						2/M		2/F						8
9 THYMUS		A	A	X	X	X	X	X	X	X	X	A	X	9
10 THYROID(S)		X	A	A	X	X	A	A	X	X	A	X	X	10
11 PARATHYROID(S)		A	A	A	A	X	A	A	A	X	A	A	A	11
12 ADRENALS		XX	XX	XX	XX	XX	XX	XX	AA	XX	XA	XX	XX	12
13 PANCREAS		X	X	X	X	X	X	X	X	X	X	X	X	13
14 URINARY BLADDER		X	X	X	X	X	X	X	X	X	X	X	X	14
15 UTERUS		A	X	L	L	X	A	X	L	X	L	A	X	15
16 Lumen dilatation-hydrometria				2	3				2		2			16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s)
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HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	III - 20 mg/Kg, continued										GROUP INCIDENCE	
		ANIMAL NO.	55	59	65	67	73	74	77	81				
1	STOMACH		X	X	X	X	X	X	X	X				1
2	SMALL INTESTINE (DUODENUM)		X	X	X	X	X	X	X	X				2
3	LARGE INTESTINE (COLON)		X	X	X	X	X	X	L	X				3
4	Nematode parasite in lumen								P				1/20	4
5	SPLEEN		X	A	L	L	X	X	X	X				5
6	Increased pigment				1/D	1/D							4/19	6
7	KIDNEYS		XX	XX	XX	XX	XX	XX	XX	XX				7
8	Tubules-microconcretions, unilateral												1/20	8
9	Tubules-microconcretions, bilateral												2/20	9
10	THYMUS		X	X	X	X	A	A	X	X				10
11	THYROID(S)		X	X	X	X	X	X	X	X				11
12	PARATHYROID(S)		A	X	A	A	A	A	A	A				12
13	ADRENALS		XX	XX	XX	XX	XA	XX	XX	XX				13
14	PANCREAS		X	X	X	X	X	X	X	X				14
15	URINARY BLADDER		X	X	X	X	X	X	X	X				15
16	UTERUS		X	X	L	L	L	X	X	X				16
17	Lumenal dilatation-hydrometria												4/17	17
18	Metritis, chronic				2	2	2						3/17	18
19														19
20														20

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HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP III - 20 mg/Kg, continued												
		ANIMAL NO. 11	12	14	17	21	30	38	39	40	48	49	53	
1	CERVIX	A	X	X	X	X	X	X	X	X	X	X	X	1
2	LYMPH NODE	X	X	X	X	X	X	X	X	X	X	A	A	2
3	STERNUM	X	X	X	X	A	X	X	X	X	X	X	X	3
4	SKELETAL MUSCLE	X	X	X	X	X	X	X	X	X	X	X	X	4
5	HEART	X	X	X	L	X	X	X	X	X	X	X	X	5
6	Myocardial degeneration & fibrosis				1/F									6
7	AORTA	A	X	X	X	A	X	X	A	X	X	X	X	7
8	OVARIES	XX	XA	XX	XX	XX	XA	XX	XX	XX	XX	XX	XX	8
9														9
10														10
11														11
12														12
13														13
14														14
15														15
16														16
17														17
18														18
19														19
20														20

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HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	III - 20 mg/Kg, continued										GROUP INCIDENCE	
		ANIMAL NO.	55	59	65	67	73	74	77	81				
1	CERVIX		X	X	X	X	X	X	X	X				1
2	LYMPH NODE		X	X	X	X	X	X	A	X				2
3	STERNUM		X	X	X	X	X	L	X	X				3
4	Inflammation, chronic, soft tissue							2/F					1/19	4
5	SKELETAL MUSCLE		X	X	X	X	X	X	X	X				5
6	HEART		X	X	X	X	X	X	X	X				6
7	Myocardial degeneration & fibrosis												1/20	7
8	AORTA		X	X	X	X	X	X	X	X				8
9	OVARIES		XX	XX	AA	XX	XX	XX	XX	XX				9
10														10
11														11
12														12
13														13
14														14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary Focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

TISSUE LESION(S)	GROUP IV - Vehicle Control													
	ANIMAL NO.	2	3	8	10	15	20	22	26	35	37	41	45	
1	BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	1
2	SPINAL CORD-1	X	X	X	X	X	X	X	X	X	X	X	X	2
3	SPINAL CORD-2	X	A	X	A	A	X	A	X	A	A	A	X	3
4	EYE	X	X	X	X	X	X	X	X	X	X	X	X	4
5	PITUITARY	A	X	A	A	A	X	A	X	A	A	X	X	5
6	MAMMARY GLAND	X	X	X	X	X	X	X	X	X	X	X	X	6
7	ESOPHAGUS	X	X	X	X	X	X	X	X	X	X	X	X	7
8	LUNG	L	X	X	L	L	X	L	X	L	L	X	X	8
9	Lymphoid infiltrate, peribronchial/iolar	1/F						1/F			1/F			9
10	Lymphoid infiltrate, perivascular	2/F						2/F		2/F				10
11	Alveolar macrophages	1/F												11
12	Congestion with edema				2/D									12
13	Hemorrhage, recent, agonal					2/F				1/F				13
14	TRACHEA	X	X	X	X	L	X	L	X	L	A	X	X	14
15	Lymphoid infiltrate, submucosal					1/F		1/F		1/F				15
16	LIVER	X	X	X	X	X	X	X	X	X	X	X	X	16
17	STOMACH	X	X	X	X	X	X	X	X	X	X	X	X	17
18	SMALL INTESTINE (DUODENUM)	X	X	X	X	X	X	X	X	X	X	X	X	18
19	LARGE INTESTINE (COLON)	X	X	X	X	X	X	X	X	X	X	X	X	19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	IV - Vehicle Control										GROUP INCIDENCE	
		ANIMAL NO.	54	63	64	66	78							
1	BRAIN		X	X	X	X	X							1
2	SPINAL CORD-1		X	X	X	X	X							2
3	SPINAL CORD-2		X	X	A	A	X							3
4	EYE		X	X	X	A	X							4
5	PITUITARY		A	X	A	A	A							5
6	MAMMARY GLAND		X	X	X	X	X							6
7	ESOPHAGUS		X	X	X	X	X							7
8	LUNG		L	L	X	L	L							8
9	Lynphoid infiltrate, peribronchial/iolar		1/F			2/M	2/M						6/17	9
10	Lymphoid infiltrate, perivascular		2/M			1/M	1/F						6/17	10
11	Alveolar macrophages		1/F			2/F							3/17	11
12	Congestion with edema			2/F									2/17	12
13	Hemorrhage, recent,agonal												2/17	13
14	TRACHEA		X	L	X	L	L							14
15	Lymphoid infiltrate, submucosal			1/F		3/F	4/M						6/16	15
16	Inflammatory exudate in lumen					P							1/16	16
17	LIVER		X	X	X	X	X							17
18	STOMACH		X	X	X	X	X							18
19	SMALL INTESTINE (DUODENUM)		X	X	X	X	X							19
20	LARGE INTESTINE (COLON)		X	X	X	X	X							20

Legend: L = Microscopic Lesion(s) 1 = Minimal S = Solitary Focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

TISSUE LESION(S)	GROUP IV - Vehicle Control, continued													
	ANIMAL NO.	2	3	8	10	15	20	22	26	35	37	41	45	
1 SPLEEN		X	X	X	X	X	X	X	X	X	X	X	X	1
2 KIDNEYS		XL	XX	XX	LL	XX	XX	XX	XX	XX	XL	XX	XL	2
3 Lymphoid infiltrate, unilateral		2/f												3
4 Tubules-microconcretions, bilateral					1/F									4
5 Tubules-microconcretions, unilateral											2/F		1/F	5
6 THYMUS		X	X	X	A	X	X	X	X	X	X	X	X	6
7 THYROID(S)		X	A	X	X	X	X	A	A	A	A	X	X	7
8 PARATHYROID(S)		X	A	X	A	A	A	A	A	A	A	X	A	8
9 ADRENALS		AA	XX	XA	XA	XA	XX	XX	XX	XA	XX	XX	XX	9
10 PANCREAS		X	X	X	X	X	X	X	X	X	X	X	X	10
11 URINARY BLADDER		X	X	X	X	X	X	X	X	X	X	X	X	11
12 UTERUS		X	L	X	X	X	X	X	X	X	X	X	X	12
13 Lumenal dilatation-hydrometria			2											13
14 CERVIX		X	X	X	X	X	X	X	X	X	X	X	X	14
15 LYMPH NODE		X	X	X	X	X	A	X	X	X	X	X	X	15
16 STERNUM		X	X	X	X	A	X	X	X	A	X	X	X	16
17 SKELETAL MUSCLE		X	X	X	X	X	X	X	X	X	X	X	X	17
18 HEART		X	X	X	X	X	X	X	X	X	X	X	X	18
19 AORTA		X	X	X	X	X	X	X	X	X	X	X	X	19
20														20

Legend: L = Microscopic Lesion(s) — Not Remarkable; X = Present, no grade; A = Tissue Absent
1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Marked Severity
f = Solitary Focus; F = Focal; M = Multifocal; D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	IV - Vehicle Control, continued										GROUP INCIDENCE	
		ANIMAL NO.	54	63	64	66	78							
1	SPLEEN		X	X	X	X	X							1
2	KIDNEYS		XX	XX	XX	XX	XX							2
3	Lymphoid infiltrate, unilateral												1/17	3
4	Tubules-microconcretions, bilateral												1/17	4
5	Tubules-microconcretions, unilateral												2/17	5
6	THYMUS		X	A	X	X	X							6
7	THYROID(S)		X	X	X	X	X							7
8	PARATHTROIDS		X	X	X	A	X							8
9	ADRENALS		XX	XX	XX	XA	XX							9
10	PANCREAS		X	X	X	X	X							10
11	URINARY BLADDER		A	X	X	X	X							11
12	UTERUS		X	X	X	L	X							12
13	Lumen dilatation-hydrometria					1							2/17	13
14	CERVIX		X	X	X	X	X							14
15	LYMPH NODE		X	X	A	X	X							15
16	STERNUM		X	X	X	X	X							16
17	SKELETAL MUSCLE		X	X	X	X	X							17
18	HEART		X	X	X	X	X							18
19	AORTA		X	X	A	X	X							19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal S = Solitary Focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

TISSUE LESION(S)	GROUP	IV - Vehcile Control, continued												
	ANIMAL NO.	2	3	8	10	15	20	22	26	35	37	41	45	
OVARIES		XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XA	1
														2
														3
														4
														5
														6
														7
														8
														9
														10
														11
														12
														13
														14
														15
														16
														17
														18
														19
														20

Legend: L = Microscopic Lesion(s) Not Remarkable
Absent, no grade
Tissue Absent

1 = Minimal
2 = Mild
3 = Moderate
4 = Marked Severity

S = Solitary Focus
F = Focal
M = Multifocal
D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE SPRAGUE-DAWLEY RATS

	TISSUE LESION(S)	GROUP	IV - Vehicle Control, continued										GROUP INCIDENCE	
		ANIMAL NO.	54	63	64	65	73							
1	OVARIES		XX	XX	XX	XX	XX							1
2														2
3														3
4														4
5														5
6														6
7														7
8														8
9														9
10														10
11														11
12														12
13														13
14														14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal S = Solitary Focus
N = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

PATHOLOGY APPENDIX D

CORRELATION OF GROSS & MICROSCOPIC FINDINGS

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
90-DAY INTRAMUSCULAR TOXICITY STUDY OF ANORDRIN IN
FEMALE SPRAGUE-DAWLEY RATS

Species Rats Sex Female Group Number I Dosage Level 0.8 mg/Kg

Animal Number	Tissue	Gross Observations	Microscopic Observations
028	Uterine Horns	Enlarged, filled with greenish fluid	Unremarkable*
046	Uterine Horns	Enlarged, filled with greenish fluid	Cervicitis & Metritis
061	Left Rear Leg	Vehicle Blisters	Tissue Not Examined
069	Uterine Horns	Enlarged, filled with clear fluid	Unremarkable*
079	Uterus	Swollen slightly	Unremarkable*

*Macroscopic observation unconfirmed

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
90-DAY INTRAMUSCULAR TOXICITY STUDY OF ANORDRIN IN
FEMALE SPRAGUE-DAWLEY RATS

Species Rats Sex Female Group Number II Dosage Level 4.0 mg/Kg

Animal Number	Tissue	Gross Observations	Microscopic Observations
006	Uterine Horns	Enlarged, filled with green fluid	Unremarkable*
	Rt. Hindleg & Mammary Gld.	Vehicle Blisters	Hindleg Tissue Not Examined Mammary Tissue Unremarkable*
016	Uterine Horns	Enlarged, filled with clear fluid	Hydrometria
019	Thoracic Lymph Nodes	Enlarged	Unremarkable*
	Uterus	Enlarged	Hydrometria
024	Right Rear Leg	Vehicle Blisters	Tissue Not Examined
025	Uterus	Distended	Tissue Absent
032	Uterus	Distended	Hydrometria
033	Uterus	Enlarged, greenish color	Tissue Absent
034	Uterus	Filled with fluid	Unremarkable*
051	Lungs	Small (1 mm) nodules present	No Nodular Lesions Noted*
071	Uterus	Distended, greenish color	Hydrometria
072	Uterus	Distended slightly, greenish color	Hydrometria
076	Uterus	Enlarged, filled with fluid	Unremarkable*

*Macroscopic observation unconfirmed.

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
90-DAY INTRAMUSCULAR TOXICITY STUDY OF ANORDRIN IN
FEMALE SPRAGUE-DAWLEY RATS

Species Rats Sex Female Group Number III Dosage Level 20 mg/Kg

Animal Number	Tissue	Gross Observations	Microscopic Observations
014	Uterus	Slight thickening	Hydrometria
	Muscle - Injection Site	Vehicle Blisters	Tissue Not Examined
017	Ileum	Lesion	Tissue Not Examined
	Lf. Uterine Horn	Thickened Area (4 mm)	Hydrometria
030	Uterus	Enlarged, distended with green fluid	Tissue Not Examined
	Rt. Ovary	Atrophied	Tissue Absent
040	Pelvic Cavity	Vehicle Blisters	Tissue Not Examined
	Thigh Muscle	Oil Blisters	Tissue Not Examined
048	Uterus	Inflammed, Vehicle Blisters	Hydrometria
	Thigh Muscles	Blisters filled with creamy material	Tissue Not Examined
053	Pelvic Region	Vehicle Blisters	Tissue Not Examined
059	Uterus	Enlarged, filled with fluid	Unremarkable*
065	Pelvic Region	Oil Blisters	Tissue Not Examined
	Uterine Horn	Inflammed, greenish in color	Metritis
	Muscle-Hindleg	Contains Vehicle	Tissue Not Examined

*Macroscopic observation unconfirmed

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
90-DAY INTRAMUSCULAR TOXICITY STUDY OF ANORDRIN IN
FEMALE SPRAGUE-DAWLEY RATS

Species Rats Sex Female Group Number III-continued Dosage Level 20 mg/Kg

Animal Number	Tissue	Gross Observations	Microscopic Observations
067	Uterine Horns	Blister filled with oil near horn Swollen, greenish color	Tissue Not Examined Metritis
073	Uterus	Enlarged and discolored	Metritis
074	Uterus	Filled with fluid	Unremarkable*
077	Uterus	Enlarged	Unremarkable*
081	Uterus	Filled with fluid	Unremarkable*

*Macroscopic observation unconfirmed

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
90-DAY INTRAMUSCULAR TOXICITY STUDY OF ANORDRIN IN
FEMALE SPRAGUE-DAWLEY RATS

Species Rats Sex Female Group Number IV Dosage Level 0 mg/Kg

Animal Number	Tissue	Gross Observations	Microscopic Observations
002	Uterus	Distended	Unremarkable*
008	Rt. Hindleg	Vehicle Blisters	Tissue Not Examined
020	Rt. Hindleg	Vehicle Blisters	Tissue Not Examined

*Macroscopic observation unconfirmed

APPENDIX 3

DONOVAN E. GORDON, D.V.M., PH.D., LTD.
Veterinary Pathology Consultant
833 KIMBALL ROAD
HIGHLAND PARK, ILLINOIS 60035

312/432-2565

August 5, 1986

Ms. Jill C. Pena
Toxicology Research Laboratory
University of Illinois
835 S. Wolcott Street
Chicago, IL 60612

Dear Ms. Pena:

I am submitting herewith my histopathology report prepared in connection with Project No. 0004, Part I, which pertains to the 90-Day Toxicity Study of Anordrin in Female Beagle Dogs.

Sincerely,

D. E. Gordon

Donovan E. Gordon, D.V.M.

DEG/mp

Enc.

Testing Facility Address	Sponsor
Donovan E. Gordon, D.V.M., Ph.D., Ltd. 833 Kimball Rd., Highland Park, IL 60035	Toxicology Research Laboratory University of IL. Chicago, IL 60612

Study Title

90-Day Intramuscular Toxicity Study of Anordrin in Female Beagle Dogs

Study Number	Summary Date
Project No. 0004, Part I	August 4, 1986

A histopathologic examination was conducted on Hematoxylin-eosin stained sections of selected tissues from a total of 12 female Beagle dogs that were sacrificed at the conclusion of the above study. The tissues submitted for examination, by light microscopy, consisted of brain, spinal cord (2 segments), eye, pituitary, mammary gland, esophagus, lung, trachea, liver, stomach, small and large intestines, spleen, kidneys, thymus, thyroid, parathyroid, adrenals, pancreas, urinary bladder, uterus and cervix, ovaries, lymph node, sternum with skeletal muscle, heart and aorta.

A total of 309 slides containing stained tissue sections were received in two separate shipments on July 1 and 31, 1986 from Propath, Inc., 1080 Nerge Road, Elk Grove Village, Illinois; where they were prepared.

The necropsy of animals, collection and fixation of tissues were conducted by the Toxicology Research Laboratory at the University of Illinois in Chicago, Illinois.

The experimental group descriptions are as follows:

Experimental Group	Treatment	Number Of Animals
I	Anordrin, 4 mg/Kg	4
II	Anordrin, 10 mg/Kg	4
III	Vehicle Control	4

All histologic changes observed in the tissues examined were recorded in the accompanying "Histopathology Incidence Tables" which are identified by experimental group and animal number. These tables also constitute an accountability of all tissues submitted for examination. The grading system and abbreviations used in the pathology tables are as follows:

Grade I = Minimal severity	X = Within normal limits
Grade 2 = Mild severity	L = Microscopic lesion
Grade 3 = Moderate severity	F = Focal
Grade 4 = Marked severity	M = Multifocal
A = Tissue absent	D = Diffuse

The incidences of microscopic lesions are summarized by group and sex in the Pathology Appendices as follows:

Pathology Appendix A: Incidence of Lesions Considered to be Treatment Related

Pathology Appendix B: Lesion Incidence Comparison Table

Pathology Appendix C: Histopathology Lesion Severity and Incidence Table

Results

Treatment-related changes were confined to the mammary tissue of test animals in Groups I and II which is summarized in Appendix A. The changes consist of a dose-related incidence of lactiferous secretions within mammary ducts. This change was focal in distribution and graded as mild to moderate in relative severity. This change was not evident among the vehicle control animals.

The remainder of the tissue changes observed among the vehicle control and test animals were regarded as lesions of naturally occurring diseases and they were confined to the lung and thymus gland. The incidence and relative severity of these changes were similar among the vehicle control and test groups.

Discussion

Treatment-related changes ascribed to the systemic effects of Anordrin over a 90-day period were confined to the mammary gland tissue of 2/4 and 3/4 female dogs at the 4 and 10 mg/Kg dosage levels, respectively, which consisted of the presence of lactiferous secretions with ducts. These findings were of minor toxicological significance. The no-effect level of Anordrin that induces this change was not established in this study.

Donovan E. Gordon, D.V.M.
Diplomate, American College of
Veterinary Pathologists

PATHOLOGY APPENDIX A

INCIDENCE OF LESIONS CONSIDERED TO BE TREATMENT-RELATED

INCIDENCE OF LESIONS CONSIDERED TO BE TREATMENT RELATED

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE BEAGLE DOGS

TISSUE LESION(S)	GROUP I 4.0 mg/Kg	GROUP II 10 mg/Kg	GROUP III 0 mg/Kg		
MAMMARY GLAND	(4)	(4)	(4)		
Lactiferous secretion in ducts	2/4	3/4	0/4		

Code: ( ) = number of animals for which tissue was represented

PATHOLOGY APPENDIX B

LESION INCIDENCE COMPARISON TABLE

LESION INCIDENCE COMPARISON TABLE

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE BEAGLE DOGS

TISSUE LESION(S)	GROUP I 4.0 mg/Kg	GROUP II 10 mg/Kg	GROUP III 0 mg/Kg		
BRAIN	(4)	(4)	(4)		
SPINAL CORD - 1	(4)	(4)	(4)		
SPINAL CORD - 2	(1)	(1)	(0)		
EYE	(4)	(4)	(4)		
PITUITARY	(3)	(4)	(3)		
Cyst	1/3	0/4	0/3		
MAMMARY GLAND	(4)	(4)	(4)		
Lactiferous secretion in ducts	2/4	3/4	0/4		
ESOPHAGUS	(4)	(4)	(4)		
LUNG	(4)	(4)	(4)		
Bronchopneumonia, chronic	1/4	1/4	0/4		
TRACHEA	(4)	(4)	(4)		
LIVER	(4)	(4)	(4)		
STOMACH	(4)	(4)	(4)		
SMALL INTESTINE - DUODENUM	(4)	(4)	(4)		
LARGE INTESTINE - COLON	(4)	(4)	(4)		
SPLEEN	(4)	(4)	(4)		
KIDNEYS	(4)	(4)	(4)		
THYMUS	(4)	(4)	(4)		
Cysts	1/4	0/4	2/4		

Code: ( ) = number of animals for which tissue was represented

LESION INCIDENCE COMPARISON TABLE

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE BEAGLE DOGS

TISSUE LESION(S)	GROUP I 4.0 mg/Kg	GROUP II 10 mg/Kg	GROUP III 0 mg/Kg		
THYROID(S)	(2)	(2)	(0)		
PARATHYROID(S)	(1)	(1)	(0)		
PANCREAS	(4)	(4)	(4)		
URINARY BLADDER	(3)	(4)	(4)		
UTERUS	(3)	(4)	(4)		
OVARIE(S)	(4)	(4)	(4)		
CERVIX	(3)	(4)	(4)		
LYMPH NODE	(3)	(1)	(2)		
STERNUM	(4)	(4)	(4)		
SKELETAL MUSCLE	(4)	(4)	(4)		
HEART	(4)	(4)	(4)		
AORTA	(4)	(4)	(4)		
ADRENALS	(4)	(4)	(4)		

Cod[illegible] ( ) = number of animals for which tissue [illegible]as represented

PATHOLOGY APPENDIX C

HISTOPATHOLOGY LESION SEVERITY AND INCIDENCE TABLE

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY
OF ANORDRIN IN FEMALE BEAGLE DOGS

	TISSUE LESION(S)	GROUP	I - 4.0 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	3775	3781	3782	3786								
1	BRAIN		X	X	X	X								1
2	SPINAL CORD - 1		X	X	X	X								2
3	SPINAL CORD - 2		X	A	A	A								3
4	EYE		X	X	X	X								4
5	PITUITARY		X	A	X	L								5
6	Cyst					2/F							1/3	6
7	MAMMARY GLAND		L	X	L	X								7
8	Lactiferous secretion in ducts		2/F		3/F								2/4	8
9	ESOPHAGUS		X	X	X	X								9
10	LUNG		L	X	X	X								10
11	Bronchopneumonia, chronic		2/F										1/4	11
12	TRACHEA		X	X	X	X								12
13	LIVER		X	X	X	X								13
14	STOMACH		X	X	X	X								14
15	SMALL INTESTINE - DUODENUM		X	X	X	X								15
16	LARGE INTESTINE - COLON		X	X	X	X								16
17	SPLEEN		X	X	X	X								17
18	KIDNEYS		X	X	X	X								18
19	THYMUS		X	L	X	X								19
20	Cysts			2/F									1/4	20

Legend: L = Microscopic Lesion(s) 1 = Minimal S = Solitary focus
X = Present Remarkable 2 = Mild F = Focal
A = Present, no grade 3 = Moderate M = Multifocal
4 = Marked/Severe D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY
OF ANORDRIN IN FEMALE BEAGLE DOGS

	TISSUE LESION(S)	GROUP	I - 4.0 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	3775	3781	3782	3786								
1	THYROID(S)		X	X	A	A								1
2	PARATHYROID(S)		A	X	A	A								2
3	PANCREAS		X	X	X	X								3
4	URINARY BLADDER		X	X	X	A								4
5	UTERUS		X	X	X	A								5
6	OVARIE(S)		X	X	X	X								6
7	CERVIX		X	X	X	A								7
8	LYMPH NODE		X	A	X	X								8
9	STERNUM		X	X	X	X								9
10	SKELETAL MUSCLE		X	X	X	X								10
11	HEART		X	X	X	X								11
12	AORTA		X	X	X	X								12
13	ADRENALS		X	X	X	X								13
14														14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal S = Solitary Focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked [illegible]

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY
OF ANORDRIN IN FEMALE BEAGLE DOGS

	TISSUE LESION(S)	GROUP	II - 10.0 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	3776	3779	3784	3785								
1	BRAIN		X	X	X	X								1
2	SPINAL CORD - 1		X	X	X	X								2
3	SPINAL CORD - 2		A	A	X	A								3
4	EYE		X	X	X	X								4
5	PITUITARY		X	X	X	X								5
6	MAMMARY GLAND		L	L	L	X								6
7	Lactiferous secretions in ducts		2/F	2/F	2/F								3/4	7
8	ESOPHAGUS		X	X	X	X								8
9	LUNG		X	L	X	X								9
10	Bronchopneumonia, chronic			2/F									1/4	10
11	TRACHEA		X	X	X	X								11
12	LIVER		X	X	X	X								12
13	STOMACH		X	X	X	X								13
14	SMALL INTESTINE - DUODENUM		X	X	X	X								14
15	LARGE INTESTINE - COLON		X	X	X	X								15
16	SPLEEN		X	X	X	X								16
17	KIDNEYS		X	X	X	X								17
18	THYMUS		X	X	X	X								18
19	THYROID(S)		A	X	X	A								19
20	PARATHYROID(S)		A	A	X	A								20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary focus
[illegible]nt Remarkable 2 = Mild F = Focal
[illegible]resent, no grade 3 = Moderate M = Multifocal
[illegible] 4 = Marked [illegible]

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY
OF ANORDRIN IN FEMALE BEAGLE DOGS

	TISSUE LESION(S)	GROUP	II - 10.0 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	3776	3779	3784	3785								
1	PANCREAS		X	X	X	X								1
2	URINARY BLADDER		X	X	X	X								2
3	UTERUS		X	X	X	X								3
4	OVARIE(S)		X	X	X	X								4
5	CERVIX		X	X	X	X								5
6	LYMPH NODE		A	X	A	A								6
7	STERNUM		X	X	X	X								7
8	SKELETAL MUSCLE		X	X	X	X								8
9	HEART		X	X	X	X								9
10	AORTA		X	X	X	X								10
11	ADRENALS		X	X	X	X								11
12														12
13														13
14														14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary Focus
N = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY
OF ANORDRIN IN FEMALE BEAGLE DOGS

	TISSUE LESION(S)	GROUP	III - Vehicle Control										GROUP INCIDENCE	
		ANIMAL NO.	3777	3778	3780	3783								
1	BRAIN		X	X	X	X								1
2	SPINAL CORD - 1		X	X	X	X								2
3	SPINAL CORD - 2		A	A	A	A								3
4	EYE		X	X	X	X								4
5	PITUITARY		A	X	X	X								5
6	MAMMARY GLAND		X	X	X	X								6
7	ESOPHAGUS		X	X	X	X								7
8	LUNG		X	X	X	X								8
9	TRACHEA		X	X	X	X								9
10	LIVER		X	X	X	X								10
11	STOMACH		X	X	X	X								11
12	SMALL INTESTINE - DUODENUM		X	X	X	X								12
13	LARGE INTESTINE - COLON		X	X	X	X								13
14	SPLEEN		X	X	X	X								14
15	KIDNEYS		X	X	X	X								15
16	THYMUS		L	X	L	X								16
17	Cysts		2/F		1/F								2/4	17
18	THYROID(S)		A	A	A	A								18
19	PARATHYROID(S)		A	A	A	A								19
20	PANCREAS		X	X	X	X								20

Legend: L = Microscopic Lesion(s) X = Not Remarkable P = Present, no grade A = Tissue Absent
1 = Minimal 2 = Mild 3 = Moderate 4 = Marked Severity
S = Solitary Focus F = Focal M = Multifocal D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY
OF ANORDRIN IN FEMALE BEAGLE DOGS

	TISSUE LESION(S)	GROUP	III - Vehicle Control										GROUP INCIDENCE	
		ANIMAL NO.	3777	3778	3780	3783								
1	URINARY BLADDER		X	X	X	X								1
2	UTERUS		X	X	X	X								2
3	OVARIE(S)		X	X	X	X								3
4	CERVIX		X	X	X	X								4
5	LYMPH NODE		A	A	X	X								5
6	STERNUM		X	X	X	X								6
7	SKELETAL MUSCLE		X	X	X	X								7
8	HEART		X	X	X	X								8
9	AORTA		X	X	X	X								9
10	ADRENALS		X	X	X	X								10
11														11
12														12
13														13
14														14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) X = Not Remarkable P = Present, no grade
1 = Minimal 2 = Mild 3 = Moderate
f = Solitary Focus F = Focal M = Multifocal

DONOVAN E. GORDON, D.V.M., PH.D., LTD.
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312/432-2566

August 12, 1986

Ms. Jill C. Pena
Toxicology Research Laboratory
Department of Pharmacology
University of Illinois
835 S. Wolcott Street
Chicago, IL 60612

Dear Ms. Pena:

I am submitting herewith my histopathology report prepared in connection with Project No. 0004, Part I, which pertains to the 90-Day Toxicity Study of Anordrin in Female Cynomolgus Monkeys.

Sincerely,

Donovan E. Gordon, D.V.M.

DEG:pw
Enclosure

Testing Facility & Address	Sponsor
Donovan E. Gordon, D.V.M., Ph.D., Ltd. 833 Kimball, Highland Park, IL 60035	Toxicology Research Laboratory University of IL, Chicago, IL 60612

Study Title

90-Day Intramuscular Toxicity Study of Anordrin In Female Cynomolgus Monkeys

Study Number	Summary Date
Project No. 0004, Part I	August 9, 1986

A histopathologic examination was conducted on Hematoxylin-eosin stained sections of selected tissues from a total of 16 female cynomolgus monkeys that were sacrificed at the conclusion of the above study. The tissues submitted for examination, by light microscopy, consisted of brain, spinal cord (2 segments) eye, pituitary, mammary gland, esophagus, lung, trachea, liver, stomach, small and large intestine, spleen, kidneys, thymus, thyroid, parathyroid, adrenals, pancreas, urinary bladder, uterus and cervix, ovaries, lymph node, sternum with skeletal muscle, heart, aorta and gross lesions.

A total of 388 slides containing stained tissue sections were received in two separate shipments on July 1 and 31, 1986 from Propath, Inc., 1080 Nerge Road, Elk Grove Village, Illinois; where they were prepared.

The necropsy of animals, collection and fixation of tissues were conducted by the Toxicology Research Laboratory at the University of Illinois in Chicago, Illinois.

The experimental group descriptions are as follows:

Experimental Group	Treatment	Number of Animals
I	Anordrin, 0.4 mg/Kg	4
II	Anordrin, 4 mg/Kg	4
III	Anordrin, 20 mg/Kg	4
IV	Vehicle Control	4

All histologic changes observed in the tissues examined were recorded in the accompanying "Histopathology Incidence Tables" which are identified by experimental group and animal number. These tables also constitute an accountability of all tissues submitted by examination. The grading system and abbreviations used in the pathology tables are as follows:

Grade 1 = Minimal severity	X = Within normal limits
Grade 2 = Mild severity	L = Microscopic lesion
Grade 3 = Moderate severity	F = Focal
Grade 4 = Marked severity	M = Multifocal
A = Tissue absent	D - Diffuse

The incidences of microscopic lesions are summarized by group and sex in the Pathology Appendices as follows:

Pathology Appendix A: Incidence of Lesions Considered to be Treatment Related

Pathology Appendix B: Lesion Incidence Comparison Table

Pathology Appendix C: Histopathology Lesion Severity and Incidence Table

Pathology Appendix D: Correlation of Gross and Microscopic Findings

Results

Treatment-related changes were confined to the mamary tissue of test animals in Groups I, II and III which are summarized in Appendix A. The changes consist of a dose-related incidence of diffuse lobular hyperplasia of mammary gland tissue and lactiferous secretions. These changes were graded as minimal to mild in relative severity. The above changes were not evident among the vehicle control animals.

The remainder of the tissue changes observed among the vehicle control and test animals were regarded as lesions of naturally occurring diseases unrelated to the test material. The overall incidence and relative severity of these changes were similar, in most instances, among the vehicle control and test groups.

Discussion

Treatment-related changes ascribed to the systemic effects of Anordrin over a 90-day period were confined to the mammary gland tissue of 3/4, 4/4 and 4/4 female monkeys at the 0.4, 4 and 20 mg/Kg dosage levels, respectively, which consisted of mammary gland hyperplasia with lactiferous secretions. These findings were of minor toxicological significance. The no-effect level of Anordrin with respect to these changes was not established in this study.

Donovan E. Gordon

Donovan E. Gordon, D.V.M.
Diplomate, American College of
Veterinary Pathologists

PATHOLOGY APPENDIX A

INCIDENCE OF LESIONS CONSIDERED TO BE TREATMENT-RELATED

INCIDENCE OF LESIONS CONSIDERED TO BE TREATMENT RELATED

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

TISSUE LESION(S)	GROUP I 0.4 mg/Kg	GROUP II 4 mg/Kg	GROUP III 20 mg/Kg	GROUP IV 0 mg/Kg
MAMMARY GLAND	(4)	(4)	(4)	(4)
Hyperplasia, lobular	3/4	4/4	4/4	0/4
Lactiferous secretions	2/4	4/4	3/4	0/4

Code: ( ) = number of animals for which tissue was represented

PATHOLOGY APPENDIX B

LESION INCIDENCE COMPARISON TABLE

LESION INCIDENCE COMPARISON TABLE

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

TISSUE LESION(S)	GROUP I 0.4 mg/Kg	GROUP II 4 mg/Kg	GROUP III 20 mg/Kg	GROUP IV 0 mg/Kg
BRAIN-CEREBRUM & CEREBELLUM	(4)	(4)	(4)	(4)
SPINAL CORD-1	(1)	(0)	(2)	(3)
SPINAL CORD-2	(0)	(0)	(0)	(0)
EYE	(4)	(4)	(4)	(4)
PITUITARY	(4)	(4)	(4)	(4)
MAMMARY GLAND	(4)	(4)	(4)	(4)
Hyperplasia, lobular	3/4	4/4	4/4	0/4
Lactiferous secretions	2/4	4/4	3/4	0/4
ESOPHAGUS	(4)	(4)	(4)	(4)
LUNG	(4)	(4)	(4)	(4)
Lung mite pigment	3/4	2/4	0/4	0/4
Bronchiolitis, chronic, parasitic	1/4	0/4	0/4	0/4
TRACHEA	(4)	(4)	(4)	(4)
LIVER	(4)	(4)	(4)	(4)
Mononuclear cell infiltrate, portal	0/4	2/4	1/4	2/4
STOMACH	(4)	(4)	(4)	(4)
SMALL INTESTINE-DUODENUM	(4)	(4)	(4)	(3)
LARGE INTESTINE-COLON	(4)	(4)	(4)	(4)
Colitis, parasitic (Oesophagostomum spp.)	0/4	0/4	0/4	1/4
SPLEEN	(4)	(4)	(4)	(4)

Code: ( ) = number of animals for which tissue was represented

LESION INCIDENCE COMPARISON TABLE

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

TISSUE LESION(S)	GROUP I 0.4 mg/Kg	GROUP II 4 mg/Kg	GROUP III 20 mg/Kg	GROUP IV 0 mg/Kg
PANCREAS	(3)	(4)	(4)	(4)
THYROID(S)	(3)	(4)	(4)	(4)
PARATHYROID(S)	(1)	(4)	(2)	(2)
ADRENAL(S)	(3)	(4)	(4)	(4)
KIDNEYS	(4)	(4)	(4)	(4)
Nephritis, chronic, bilateral	1/4	0/4	0/4	0/4
Nephritis, chronic, unilateral	1/4	0/4	0/4	0/4
Fibrosis, cortex, unilateral	0/4	0/4	1/4	0/4
URINARY BLADDER	(4)	(4)	(4)	(4)
Cystitis, chronic	1/4	0/4	0/4	0/4
THYMUS	(4)	(2)	(2)	(2)
Cysts	0/2	0/2	1/2	1/2
UTERUS	(4)	(4)	(4)	(4)
CERVIX	(4)	(4)	(4)	(4)
Inflammation, chronic	1/4	0/4	0/4	0/4
OVARIES	(4)	(4)	(4)	(4)
Cysts, follicular, unilateral	0/4	0/4	0/4	1/4
LYMPH NODE	(4)	(4)	(4)	(4)
STERNUM	(4)	(4)	(4)	(4)

( ) = number of animals for which tissue was represented

LESION INCIDENCE COMPARISON TABLE

90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

TISSUE LESION(S)	GROUP I 0.4 mg/Kg	GROUP II 4 mg/Kg	GROUP III 20 mg/Kg	GROUP IV 0 mg/Kg	
SKELETAL MUSCLE	(4)	(4)	(4)	(4)	
Parasitic cysts, Sacrosporidia spp.	3/4	0/4	0/4	0/4	
HEART	(4)	(4)	(4)	(4)	
Fibrosis, myocardial	1/4	0/4	0/4	0/4	
Myocarditis, chronic	0/4	0/4	0/4	1/4	
AORTA	(4)	(4)	(4)	(4)	

Code: ( ) = number of animals for which tissue was represented

PATHOLOGY APPENDIX C

HISTOPATHOLOGY LESION SEVERITY AND INCIDENCE TABLE

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

	TISSUE LESION(S)	GROUP	I - 0.4 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	5199	5201	5204	5212								
1	BRAIN-CEREBRUM & CEREBELLUM		X	X	X	X								
2	SPINAL CORD-1		X	A	A	A								
3	SPINAL CORD-2		A	A	A	A								
4	EYE		X	X	X	X								
5	PITUITARY		X	X	X	X								
6	MAMMARY GLAND		L	L	L	X								
7	Hyperplasia, lobular		1/D	1/D	1/D								3/4	
8	Lactiferous secretions		1/D		1/D								2/4	
9	ESOPHAGUS		X	X	X	X								
10	LUNG		L	L	L	X								
11	Lung mite pigment		2/F	2/M	2/M								3/4	
12	Bronchiolitis, chronic, parasitic			3/F									1/4	
13	TRACHEA		X	X	X	X								
14	LIVER		X	X	X	X								
15	STOMACH		X	X	X	X								
16	SMALL INTESTINE-DUODENUM		X	X	X	X								
17	LARGE INTESTINE-COLON		X	X	X	X								
18	SPLEEN		X	X	X	X								
19	PANCREAS		X	X	A	X								1
20	THYROIDS		XX	XX	XX	AA								2

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary focus
X = Not Remarkable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

	TISSUE LESION(S)	GROUP	I - 0.4 mg/Kg										GROUP INCIDENCE
		ANIMAL NO.	5199	5201	5204	5212							
	PARATHYROIDS		AA	XA	AA	AA							
	ADRENALS		XA	XX	XX	AA							
	KIDNEYS		XX	LL	XL	XX							
	Nephritis, chronic, bilateral			3/F									1/4
	Nephritis, chronic, unilateral				2/F								1/4
	URINARY BLADDER		X	X	L	X							
	Cystitis, chronic				2/D								1/4
	THYMUS		X	X	X	X							
	UTERUS		X	X	X	X							
10	CERVIX		X	L	X	X							
11	Inflammation, chronic			2/D									1/4
12	OVARIES		XX	XA	XX	AA							
13	LYMPH NODE		X	X	X	X							
14	STERUM		X	X	X	X							
15	SKELETAL MUSCLE		X	L	L	L							
16	Parasitic cyst(s), Sarcosporidia spp.			P	P	P							3/4
17	HEART		L	X	X	L							
18	Fibrosis, myocardial		2/F										1/4
19	Myocarditis, chronic					1/F							1/4
20	AORTA		X	X	X	X							

Legend: Microscopic Lesion(s)
Not Recorded
Present, no grade
Lesion Absent
1 = Minimal
2 = Mild
3 = Moderate
4 = Marked/Severe
F = Solitary Focus
f = Focal
M = Multifocal
D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

	TISSUE LESION(S)	GROUP	II - 4.0 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	5194	5205	5213	5214								
1	BRAIN-CEREBRUM & CEREBELLUM		X	X	X	X								1
2	SPINAL CORD-1		A	A	A	A								2
3	SPINAL CORD-2		A	A	A	A								3
4	EYE		X	X	X	X								4
5	PITUITARY		X	X	X	X								5
6	MAMMARY GLAND		L	L	L	L								6
7	Hyperplasia, lobular		2/D	2/D	1/D	2/D							4/4	7
8	Lactiferous secretions		1/D	1/D	1/D	1/D							4/4	8
9	ESOPHAGUS		X	X	X	X								9
10	LUNG		L	L	X	X								10
11	Lung mite pigment		2/F	1/F									2/4	11
12	TRACHEA		X	X	X	X								12
13	LIVER		L	X	X	L								13
14	Mononuclear cell infiltrate, portal		1/F			1/M							2/4	14
15	STOMACH		X	X	X	X								15
16	SMALL INTESTINE-DUODENUM		X	X	X	X								16
17	LARGE INTESTINE-COLON		X	X	X	X								17
18	SPLEEN		X	X	X	X								18
19	PANCREAS		X	X	X	X								19
20	THYROIDS		XX	XX	XX	XX								20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary focus
A = Not Accessible 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
X = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

	TISSUE LESION(S)	GROUP	II - 4.0 mg/Kg										GROUP INCIDENCE
		ANIMAL NO.	5194	5205	5213	5214							
1	PARATHYROIDS		XA	XX	XX	XA							1
2	ADRENALS		XX	XX	XX	XX							2
3	KIDNEYS		XX	XX	XX	XX							3
4	URINARY BLADDER		X	X	X	X							4
5	THYMUS		A	X	A	X							5
6	UTERUS		X	X	X	X							6
7	CERVIX		X	X	X	X							7
8	OVARIES		AX	XX	XX	XX							8
9	LYMPH NODE		X	X	X	X							9
10	STERUM		X	X	X	X							10
11	SKELETAL MUSCLE		X	X	X	X							11
12	HEART		X	X	X	X							12
13	AORTA		X	X	X	X							13
14													14
15													15
16													16
17													17
18													18
19													19
20													20

Legend: X = Microscopic Lesion(s) Not Remarkable
Present, no grade
A = Tissue Absent
1 = Minimal
2 = Mild
3 = Moderate
4 = Marked Severity
f = Solitary Focus
f = Focal
m = Multifocal
d = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

	TISSUE LESION(S)	GROUP	III - 20.0 mg/Kg										GROUP INCIDENCE
		ANIMAL NO.	5191	5197	5200	5203							
1	BRAIN-CEREBRUM & CEREBELLUM		X	X	X	X							
2	SPINAL CORD-1		X	A	A	X							
3	SPINAL CORD-2		A	A	A	A							
4	EYE		X	X	X	X							
5	PITUITARY		X	X	X	X							
6	MAMMARY GLAND		L	L	L	L							
7	Hyperplasia, lobular		2/D	2/D	2/D	2/D							4/4
8	Lactiferous secretions		1/D	1/D		1/D							3/4
9	ESOPHAGUS		X	X	X	X							
10	LUNG		X	X	X	X							
11	TRACHEA		X	X	X	X							
12	LIVER		X	X	L	X							
13	Mononuclear cell infiltrate, portal				1/F								1/4
14	STOMACH		X	X	X	X							
15	SMALL INTESTINE-DUODENUM		X	X	X	X							
16	LARGE INTESTINE-COLON		X	X	X	X							
17	SPLEEN		X	X	X	X							
18	PANCREAS		X	X	X	X							
19	THYROIDS		XX	XA	XX	XX							
20	PARATHYROIDS		XA	XA	AA	AA							

Legend: L = Microscopic Lesion(s) 1 = Minimal F = Solitary Focus
X = Not Remarkable 2 = Mild f = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

	TISSUE LESION(S)	GROUP	III - 20.0 mg/Kg										GROUP INCIDENCE	
		ANIMAL NO.	5191	5197	5200	5203								
1	ADRENALS		XX	XA	XX	XX								1
2	KIDNEYS		XX	XX	XX	XL								2
3	Fibrosis, cortex, unilateral					2/F							1/4	3
4	URINARY BLADDER		X	X	X	X								4
5	THYMUS		A	L	A	X								5
6	Cysts			2/F									1/2	6
7	UTERUS		X	X	X	X								7
8	CERVIX		X	X	X	X								8
9	OVARIES		XX	XX	XX	XX								9
10	LYMPH NODE		X	X	X	X								10
11	STERNUM		X	X	X	X								11
12	SKELETAL MUSCLE		X	X	X	X								12
13	HEART		X	X	X	X								13
14	AORTA		X	X	X	X								14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary Focus
[illegible] Not Remarkable 2 = Mild F = Focal
[illegible] Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

	TISSUE LESION(S)	GROUP	IV - Vehicle Control										GROUP INCIDENCE	
		ANIMAL NO.	5193	5195	5211	5215								
1	BRAIN-CEREBRUM & CEREBELLUM		X	X	X	X								1
2	SPINAL CORD-1		A	X	X	X								2
3	SPINAL CORD-2		A	A	A	A								3
4	EYE		X	X	X	X								4
5	PITUITARY		X	X	X	X								5
6	MAMMARY GLAND		X	X	X	X								6
7	ESOPHAGUS		X	X	X	X								7
8	LUNG		X	X	X	X								8
9	TRACHEA		X	X	X	X								9
10	LIVER		X	X	L	L								10
11	Mononuclear cell infiltrate, portal				2/F	1/M							2/4	1
12	STOMACH		X	X	X	X								1
13	SMALL INTESTINE-DUODENUM		X	X	A	X								1
14	LARGE INTESTINE-COLON		L	X	X	X								1
15	Colitis, parasitic (Oesophagostomum spp.)		3/F										1/4	1
16	SPLEEN		X	X	X	X								1
17	PANCREAS		X	X	X	X								1
18	THYROIDS		XX	XX	XX	XX								1
19	PARATHYROIDS		XA	AA	XX	AA								1
20	ADRENALS		XX	XX	XX	XX								2

Legend: L = Microscopic Lesion(s) 1 = Minimal f = Solitary Focus
X = Not Examinable 2 = Mild F = Focal
P = Present, no grade 3 = Moderate M = Multifocal
A = Tissue Absent 4 = Marked Severity D = Diffuse

HISTOPATHOLOGY INCIDENCE TABLE
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

	TISSUE LESION(S)	GROUP	IV - Vehicle Control										GROUP INCIDENCE	
		ANIMAL NO.	5193	5195	5211	5215								
1	KIDNEYS		XX	XX	XX	XX								1
2	URINARY BLADDER		X	X	X	X								2
3	THYMUS		L	A	X	A								3
4	Cysts		2/F										1/2	4
5	UTERUS		X	X	X	X								5
6	CERVIX		X	X	X	X								6
7	OVARIES		XL	XX	XX	XX								7
8	Cysts, follicular, unilateral		P/F										1/4	8
9	LYMPH NODE		X	X	X	X								9
10	STERUM		X	X	X	X								10
11	SKELETAL MUSCLE		X	X	X	X								11
12	HEART		X	X	L	X								12
13	Myocarditis, chronic				1/F								1/4	13
14	AORTA		X	X	X	X								14
15														15
16														16
17														17
18														18
19														19
20														20

Legend: L = Microscopic Lesion(s) Not Remarkable; P = Present, no grade; A = Tissue Absent
1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Marked Severity
F = Solitary Focus; f = Focal; M = Multifocal; D = Diffuse

PATHOLOGY APPENDIX D

CORRELATION OF GROSS & MICROSCOPIC FINDINGS

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

Species Primate Sex Female Group Number I Dosage Level 0.4 mg/Kg

Animal Number	Tissue	Gross Observations	Microscopic Observations
5212	Left Adrenal	Smaller than normal	Tissue not examined

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

Species Primate **Sex** Female Group Number II **Dosage Level** 4.0 mg/Kg

Animal Number	Tissue	Gross Observations	Microscopic Observations
5213	Heart	Discolored Area	None*
5214	Heart	Less Firm	None*
	Skin-base of tail	Swollen	Tissue not examined
	Skin-perivulvar	Swollen	Tissue not examined

*macroscopic observation unconfirmed

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

Species Primate **Sex** Female Group Number III Dosage Level 20.0 mg/Kg

Animal Number	Tissue	Gross Observations	Microscopic Observations
5191	Urinary Bladder	Stone in lumen	None*
5197	Ovaries	Cysts, bilateral	None*
	Inguinal Lymph Node	Enlarged	Tissue not examined

*macroscopic observation unconfirmed

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS
90-DAY INTRAMUSCULAR TOXICITY STUDY OF
ANORDRIN IN FEMALE CYNOMOLGUS MONKEYS

Species Primate Sex Female Group Number IV Dosage Level 0 mg/Kg

Animal Number	Tissue	Gross Observations	Microscopic Observations
5193	Lung	Adhesions, bilateral Black specks, bilateral	None*
	Urinary Bladder	Adhesions to abdominal wall	None*

*macroscopic observation unconfirmed

FINAL TECHNICAL REPORT

I. Project Report Summary

A. General Information

1. Immunologic Suppression of Fertility by Synthetic Antigenic Determinants of LDH-C_4. PARFR-315, Extension of PARFR-232.

2. Erwin Goldberg, Principal Investigator, Department of Biochemistry, Molecular Biology and Cell Biology.

3. Northwestern University

4. Subcontract Period
   a. March 1, 1982
   b. December 31, 1986

5. Last Technical Report Submitted: June 26, 1986

6. Reporting Period: June 27, 1986-December 31, 1986

B. Narrative Summary

We have prepared synthetic antigenic peptides of the sperm specific LDH-C_4 isozyme to elicit an immune response to the intact molecule that included a significant suppression of fertility. We have now solved the sequence of the human LDH-C_4 isozyme by cloning and sequencing cDNA from a human testes library. The peptide HC5 15 has been synthesized. In addition, two surface simulation peptides were designed, synthesized and tested for immunogenicity.

II. Substantive Technical Research Report

A. Statement of Objectives

The objective of this project is to develop a contraceptive vaccine consisting of a synthesizable antigenic peptide that will provoke antibodies against the LDH-C_4 enzyme of spermatozoa.

B. Accomplishments of the Project

This work has been focused on the immunologic suppression of fertility by synthetic antigenic determinants of the sperm-specific isozyme of lactate dehydrogenase. This isozyme is known as LDH-C_4 (sometimes LDH-X). The general objective is to develop a contraceptive consisting of an antigenic peptide or peptides that provoke antibodies against LDH-C_4. The rationale for utilizing this antigen is based on previous work that demonstrated a significant inhibition of fertility in female mice, rabbits and baboons immunized with LDH-C_4. It appears that circulating antibodies access the female reproductive tract as a transudate of serum and bind to LDH-C_4 on the surface of spermatozoa thereby impeding their transport to the site of fertilization. LDH-C_4 was the first sperm antigen that could be purified to

homogeneity and chemically characterized. Therefore it seemed likely that this isozyme could form the basis of a practical contraceptive. However, it is unlikely that the natural product could be isolated in sufficient quantity for wide usage, particularly if LDH-C_4 from human testis or semen is employed. In addition it would be most difficult to control the purity of the material in a mass isolation scheme. Our strategy then was to attempt to find an antigen amenable to chemical synthesis to provide the greatest flexibility and insure specificity of the immune response. A small peptide bearing an antigenic determinant of LDH-C_4 could be substituted for the natural product if it induced an immune response to the native protein. Such peptides were identified by isolating fragments of the native protein that bound rabbit anti-mouse LDH-C_4 and in turn provoked an immune response to the native protein. The general feasibility of this approach has now been established.

We identified 9 peptides that contained antigenic determinants to which antibodies to LDH-C_4 could bind. We selected several of these for further testing on the basis of their accessibility on the surface of the intact tetramer and their amino acid sequence differences from similar regions of the somatic lactate dehydrogenases. In general, we found that we could stimulate an immune response to the native protein when we immunized animal models with synthetic peptides conjugated to diphtheria toxoid as carrier. Most importantly, one of these peptides representing that part of the sequence designated 5 to 15 of the LDH-C_4 molecule was used as antigen in a fertility trial. We demonstrated a 71% inhibition of pregnancy in 14 female baboons that had been immunized with the peptide diphtheria toxoid conjugate. The effect appeared to be reversible. Simultaneously, we refined the molecular structure by computer graphics modeling of LDH-C_4 from x-ray crystallographic data. We developed a molecular model that reveals that some of our ppptides are unlikely to be accessible as antibody binding sites, while other determinants involve a combination of residues from peptides that are distant in terms of sequence but spatially close in terms of position on the molecule. Thus we have been able to propose two surface simulation peptides and these have been synthesized.

Another and exciting aspect of our recent work is the isolation of the human *ldh-c* gene. This accomplishment represents a breakthrough permitting us to deduce the amino acid sequence of human LDH-C_4. On this basis we have compared the 5 to 15 peptide from the mouse with 5 to 15 peptide from the human sequence and find that there are some significant amino acid substitutions. Since these differences are sufficient to affect immunogenicity we have synthesized the human 5 to 15 peptide for further analysis. This peptide determinant should provoke antibodies of greater specificity and higher affinity for human LDH-C_4 and therefore may prove more effective than the mouse 5 to 15 peptide as an immunocontraceptive. We plan to test this hypothesis.

Other studies in progress involve determining the optimal ratio of peptide to carrier and the dose response properties of each of the peptides. As noted above, the peptide 5-15 is effective as an immunogen in blocking fertility reversibly in female baboons. Therefore, we are attempting to "fast track" our studies with this peptide. Although we have immunized rabbits with HC 5-15 and produced artibodies to this peptide, we are unable to assess the reaction of these antibodies with human LDH-C_4. It will first be necessary to obtain this isozyme by genetic engineering technology. Now that we have been successful in isolating and cloning the human *ldh-c* gene, we will be able to

incorporate this gene into an expression vector and thereby obtain relatively large quantities of the enzyme itself. Of course, this has many implications, not the least of which is an abundant supply of antigen that may serve ultimatley as the best vaccine. In addition we plan to use recombinant DNA technology to incorporate the *ldh-c* gene or fragment thereof into a viral vector that can then be used for immunization. This strategy may permit immunization to stimulate a long term response requiring infrequent booster injections. Also, we should be able to test a system such as adenoma or vaccinia that is already acceptable for human use. This would obviate the need for the adjuvant which has so far been required in studies with virtually all sperm antigens. The comparison of sequence differences of antigenic peptides from mouse and human LDH-C is presented in Table 1.

Two surface simulation peptides have been synthesized. These consist of a combination of residues from peptide 5-15 and peptide 211-226 appropriately spaced to simulate their relative positions on the surface of LDH-C_4. These sequences are:

```
     1        5   6  211      219      222 223 224
I.   S - G*- E - Q - A - G*- T - G*- D - K - E

    216      219      222 223 224          1       5   6
II.  A - G*- T - G*- D - K - E - G*- G*- S - G*- E - Q
```

* spacer glycine

These peptides have been injected into rabbits, however it is too soon to determine the nature and extent of the antibody response.

C. Unanticipated Results

None

D. Research Design

No changes

E. Operational Significance

Not applicable

F. Dissemination and Utilization of Research Results

Not applicable

G. Involvement of Minority Personnel and Women

Not applicable

H. Work Plan for Coming Reporting Period

Not applicable

TABLE 1

COMPARATIVE SEQUENCES OF HUMAN AND MOUSE LDH-C_4 ANTIGENIC DETERMINANTS

Antigenic Site[1]	Species[2]	Sequence
5-15	MC	EELIQ**NL**V**PE**D**K**
	HC	.Q..E**K**.I**E**D.**E**
41-55	MC	GLADELALVD**ADT**DK
	HC	D.....**VAL**..
58-74	MC	GEALDLLHGSLFLSTPK
	HC	..MM..Q.....F..S.
97-110	MC	RM**VS**GQTRL**D**LLQR
	HC	.Q**QE**.E...**A**.V..
176-210	MC	LGVNPTSCHGWVLGEHGDSSVPIWSGVNVAGVTLK
	HC	...H.....II.....L.....A..
211-226	MC	SLNPA**I**GTDSDKEHWK
	HC	T.D.K**L**.....
231-243	MC	QVV**E**GGYEV**L**NMK
	HC	..I**Q**SA..I**I**KL.
283-303	MC	EEVFLSIPCVLGQSGITDFVK
	HC	..L.....RN.VS.V..
304-316	MC	VNMT**A**EEE**G**LLKK
	HC	I.LN**S**...**A**.F..

[1] Numbers refer to chemical sequence

[2] MC = murine LDH-C_4; HC = human LDH-C_4

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February 2, 1987

Gerald I. Zatuchni, M.D., M.Sc.
Director
Program for Applied Research on
Fertility Regulation
Northwestern University - Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611

RE: PARFR-337T

Dear Jerry:

I received your letter of January 21, 1987, requesting my final report. I am sending you a copy of the letter and report sent to PARFR on July 24, 1986. This letter confirms that I had received permission from PARFR to submit my Six Month Technical Report Summary as my final report until the time that publications from this work were ready. Publications have been delayed because of missing interviews. We compiled a list of all missing questionnaires and sent it to Finland; we recently received a response with some of the missing material. However, a few questionnaires still cannot be found. We are in the process of deciding whether to exclude these cases or use a statistical technique that would compensate for the missing data points. I do not anticipate that we will have a draft publication before this summer. As soon as a manuscript is ready I will send it to you.

With best regards,

Rochelle

Rochelle N. Shain, Ph.D.
Associate Professor

dv
Enclosures

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7703 Floyd Curl Drive
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Medical School
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July 24, 1986

Diane Krier-Morrow, M.B.A.
Director of Administration
Program for Applied Research
on Fertility Regulation
Northwestern University - Ste. 1525
875 N. Michigan Ave.
Chicago, IL 60611

RE: PARFR 337T
Intracervical Device
Acceptability Study

Dear Diane:

Enclosed you will find my 6-month Technical Report Summary. As per our telephone discussion, this summary will serve as my final report until the time that publications from this work are sent to PARFR.

Sincerely yours,

Rochelle Shain

Rochelle N. Shain, Ph.D.
Associate Professor

dv
Enclosure

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 337T

TITLE: "Intracervical Device Acceptability Study"

INSTITUTION: The University of Texas Health Science Center at San Antonio

PRINCIPAL INVESTIGATOR: Rochelle N. Shain, Ph.D.

FUNDING PERIOD: 6/1/83-6/30/86 AMOUNT FUNDED: $12,502

Below is a project summary for the January 1, 1986 - June 30, 1986 period.

To date a total of 820 questionnaires have been received from Finland. These include 295 initial interviews (110-ICD, 100-LNG IUD, and 85-Nova T); 285 3-month follow-up interviews (106-ICD, 98-LNG IUD, and 81 Nova T); and 243 12-month interviews (92-ICD, 86-LNG IUD, and 65-Nova T). A total of 799 instruments have been coded and their data analyzed. Preliminary results from the 12-month follow-up data indicate that both the ICD and LNG IUD devices were associated with lighter and shorter menses, more irregular cycles, more spotting, less cramping and some amenorrhea. The Nova T was associated with heavier and longer menses, more cramping, more spotting and more irregular cycling. On many 12-month follow-up measures (cannot feel the device, husbands cannot feel the device, change in desire for intercourse, have good or mostly good feelings about the device, dissatisfied with device, would recommend to friends), there were no significant differences between groups. However, at 12-months, 15% of ICD users, compared to only 6% of LNG IUD and 5% of Nova T users (p=.04), perceived especially bothersome side effects. Moreover, by the 12-month interval 24% of ICD users, compared to 8% of LNG IUD users and 9% of Nova T users (p=<0.001), had discontinued their device and 8%, 1%, and 1% of ICD, LNG and Nova T users, respectively, had expelled their device.

Although the ICD sample had experienced significantly more discontinuations than the two control groups, ICD users in general were significantly more satisfied than the controls with their current device compared to their previously used IUDs. This seeming discrepancy is explained by group differences in prior contraceptive behavior patterns. Significantly more ICD users than members of the other two control groups volunteered for the present study because they disliked all other methods, were dissatisfied with the last method they used, were dissatisfied with their prior IUD, experienced unacceptable side effects from their prior IUD, and were dissatisfied with prior mechanical methods. Additionally, this group discontinued significantly more contraceptive methods because of dissatisfaction. As noted in previous reports, the ICD group as a whole appeared to be considerably more particular about contraceptive methods in general and/or sensitive to side effects. This finding was confirmed by Tapani Luukkainen, M.D.

After all remaining interviews are received from Finland, multivariate regression analysis and analyses of covariance will be performed to determine the extent to which prior contraceptive behavior has influenced the higher discontinuation rates shown by ICD sample members and whether controlling for this behavior eliminates group differences in discontinuation.

Rochelle Shain

FINAL REPORT

PREPARATION OF ACROSIN INHIBITORS (ARYL GUANIDINOBENZOATES) FOR PHASE I CLINICAL TRIAL

Program for Applied Research and Fertility Regulation - PARFR 338

Principal Investigator: L.J.D. Zaneveld, D.V.M., Ph.D.
Professor
Department of Obstetrics and Gynecology, and
Department of Biochemistry
Rush University

Institution: Rush-Presbyterian-St. Luke's Medical Center
1763 West Congress Parkway
Chicago, IL 60612

Period Covered: Until March 31, 1987

1. PROJECT REPORT SUMMARY

a. Narrative Summary

The overall objective of this research is to develop a new contraceptive agent that is more potent and less toxic than nonoxynol-9, the most frequently used active ingredient of marketed vaginal contraceptives. It is well known that the sperm enzyme acrosin is essential for fertility and that acrosin inhibitors prevent fertilization. Therefore, acrosin inhibitors were synthesized, specificially aryl 4-guanidinobenzoates that possessed an already marketed phenol, and tested for their contraceptive and toxic properties. Our most recent efforts were aimed at obtaining all data necessary for submission of an IND to the FDA for a Phase I clinical trial with the optimal aryl 4-guanidinobenzoate. This has been accomplished and an IND is presently in preparation.

A large number of studies were performed during the present and previous contract periods that have led to the identification of 4'-acetamidophenyl 4-guanidinobenzoate (AGB) as the preferred compound for the Phase I clinical trial. AGB is a highly potent pseudoirreversible inhibitor of isolated human acrosin. Once bound to acrosin, extensive dialysis at pH 3.0 over a 2 day period is required before partial dissociation occurs. No dissociation should occur at neutral pH. Since spermatozoa remain in the acidic vagina for about 6 hours maximally before entry into the cervix and uterus whose fluids have a neutral pH, no dissociation of the enzyme and inhibitor should occur while the spermatozoa are transported and stored in the female genital tract. AGB inhibits the acrosin of whole human spermatozoa when mixed for 2 minutes with semen, indicating that AGB interacts very rapidly with the sperm. The compound

cannot be removed from the spermatozoa by washing which provides further evidence that AGB will not be removed from the spermatozoa during their transport through the female genital tract.

AGB is stable at the vaginal pH of about 4.0 for at least 3 months, indicating that it can be placed vaginally without losing its properties. AGB also inhibits the motility of human spermatozoa but this antimotility effect is about 10-15 times less than that of nonoxynol-9. Thus, even though the dose of AGB that will be used in the clinical trial is high enough to cause sperm immobilization, its primary mechanism of action will remain its acrosin inhibitory property.

The vaginal contraceptive potency of AGB in the rabbit model is about 100 times higher than that of nonoxynol-9. The acute toxicity of AGB in mice and rats is about 5 times less than that of nonoxynol-9. AGB shows minimal or no vaginal irritation when tested in the rabbit model in contrast to nonoxynol-9. A mixture of AGB and nonoxynol-9 is no more irritating to the rabbit vagina than nonoxynol-9 alone. Thirty day or 50 day vaginal application of AGB to rats causes no histopathological changes in any of the organs and tissues examined, including the vagina. AGB is nonmutagenic as shown by the Ames test.

With the cooperation of the VLI, Santa Anna, CA, the Today sponge has been developed as a vaginal delivery system for AGB. The chemical and biochemical properties of AGB are not altered by the construction of the sponges whether or not nonoxynol-9 is also incorporated into the sponge. The release rate of AGB from the sponge is adequate for clinical use. A somewhat higher release rate of AGB is obtained when both nonoxynol-9 and AGB are incorporated into the

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sponge as compared to AGB alone.

A simple assay has been developed that measures the acrosin content of human spermatozoa. This assay together with sperm motility can be utilized in the Phase I clinical trial to monitor if the functional activity of the spermatozoa collected from the vagina and cervix has been impaired. An HPLC technique has been developed to determine AGB levels in fluids and should be useful to measure the AGB levels in the genital tract fluids and blood during the clinical trial.

From these preclinical studies, it can be concluded that AGB is potentially a highly potent vaginal contraceptive for women with minimal toxicity and vaginal irritation, and has a much higher therapeutic index than nonoxynol-9. Its chemical properties are such that it interacts rapidly with spermatozoa, that it remains bound to the sperm once interaction has taken place, and that it maintains stable in the vagina. For the Phase I clinical trial, AGB can be delivered in adequate concentrations to the vagina utilizing the Today sponge.

II. SUBSTANTATIVE TECHNICAL RESEARCH REPORT

A. Statement of Objectives

The overall objective of this research is to develop a new vaginal contraceptive agent that is more potent and less toxic than nonoxynol-9. More recently, the goals were to obtain all the preclinical data necessary for submission of an IND to the FDA utilizing the agent 4'-acetamidophenyl 4-guanidinobenzoate (AG) that was found to possess the most desirable

properties as a vaginal contraceptive based on preclinical studies.

B. Progress to Date

The following is a brief summary of all the progress that has been made in the synthesis and evaluation of the aryl 4-guanidinobenzoates, followed by more specific information gained with 4'-acetamidophenyl 4-guanidinobenzoate (AGB) which was selected for the Phase I clinical trial.

General Overview of Results Obtained with the Synthesized Aryl 4-Guanidinobenzoates

A significant amount of evidence supports the use of sperm enzyme inhibitors as contraceptive agents (1,2). Of particular interest are inhibitors of the acrosomal enzyme acrosin. Numerous animal investigations have shown that acrosin is essential for fertilization (2). The same is true for the human. Infertile men are known to possess two to three times lower acrosin levels than asymptomatic men (3,4). Procedures known to reduce the fertilizing capacity of spermatozoa such as sperm banking (cryopreservation), also reduce sperm acrosin levels, even in cases where sperm motility is retained (5,6). Finally, sperm acrosomal abnormalities are known to be associated with male infertility (7). Thus, inhibition of sperm acrosin should lead to infertility. Indeed, a large number of animal studies have shown that acrosin inhibitors prevent fertilization whether they are administered vaginally, into the uterus or intraperitoneally (1,2). Several different types of acrosin inhibitors have been evaluated for their vaginal contraceptive use (1,2,8-12) but the aryl 4-guanidinobenzoates are by far the most potent acrosin inhibitors and

antifertility agents with the lowest toxicity. The following summarizes the data obtained with these aryl 4-guanidinobenzoates by our laboratories (2,13-31, and unpublished results).

1. Twenty-seven aryl 4-guanidinobenzoates were synthesized, including nine that possess phenols currently in clinical use. Such phenols include, among others, acetaminophen, methyl- and ethylparaben and methylsalicylate. Guanidinobenzoic acid is also relatively nontoxic. Thus, it was felt that the combination of the guanidinobenzoic acid and a clinically used phenol should lead to a relatively nontoxic and clinically acceptable agent. This, indeed, appears to be the case (see further). The following experiments primarily relate to the studies done with the guanidinobenzoic acid derivatives containing the clinically utilized phenols.

2. The nine synthesized aryl 4-guanidinobenzoates containing clinically used phenols and a tenth that was purchased (the methylumbelliferone derivative) were found to be potent inhibitors of human acrosin with I_{50} values ranging from 3×10^{-7} to 7×10^{-10} M. The compounds form pseudoirreversible complexes with human acrosin. The K_{app} of selected aryl guanidinobenzoates ranged from 10^{-8} to 10^{-9} M and the k_2 (acylation constant) was about 10^{-2} sec. The acrosin-inhibitor complex dissociates only after extensive dialysis at pH 3.0 over a 2 day period with frequent changes (1000 fold the volume of the dialyzed material) in dialysate. Therefore, no dissociation of acrosin and inhibitor will occur during the period that the sperm is in the vagina (approximately 6 hours). Once the sperm enter cervical mucus and the uterus, the pH of the surrounding medium changes to about 7 which prevents dissociation. These data imply that acrosin will remain tightly bound to inhibitor while the sperm are

in the female genital tract, even if the sperm remain stored for 4 days or longer in the cervical mucus or uterus. The contraceptive studies in animal models support this.

3. Treatment of whole human semen with 10^{-4} M aryl 4-guanidinobenzoates (including AGB) for less than 2 min, completely inhibited sperm acrosin in a dose dependent manner. With 10^{-5} M and 10^{-6} M aryl 4-guanidinobenzoate, 80% anu 50% inhibition were, respectively, observed. Thus, the aryl guanidinobenzoates react extremely quickly with human spermatozoa at very low concentrations. This is essential, because at midcycle, spermatozoa may rapidly enter cervical mucus. The amount of AGB that will be used in the clinical trial (approximately 100 mg/day) is orders of magnitude higher than 10^{-4} M. Thus, at the dose placed vaginally, extremely rapid and complete inhibition of the acrosin of all the ejaculated spermatozoa should occur.

Experiments were also performed to determine how tightly the aryl 4-guanidinobenzoates (including AGB) bind to human spermatozoa in order to ascertain that the inhibitors cannot not be washed away from the sperm during sperm entry into cervical mucus. For this purpose, whole human semen was treated with inhibitor (at the concentrations described above) for up to 2 min and immediately washed by centrifugation over Ficoll. Free aryl guanidinobenzoate will not pass through the Ficoll. The acrosin on the sperm remained inhibited, showing that the inhibitor cannot be washed away from the spermatozoa.

Recent studies further indicate that AGB not only inhibits acrosin (both present as the active form on the sperm or as an inactive form called

proacrosin) but also the activation of proacrosin to acrosin.

4. Selected aryl 4-guanidinobenzoates were tested for their ability to prevent in vitro fertilization using: 1) mouse gametes; and 2) the human sperm-zona free hamster oocyte penetration assay ("humster test"). At very low inhibitor concentrations (10^{-4} to 10^{-6} M), penetration was prevented in the humster test. At even lower concentrations, fertilization was prevented in the mouse in vitro fertilization system (the I_{50} was approximately 10^{-8} M). The antifertility activity of the inhibitors was shown to parallel their acrosin inhibition activity. The lower amounts required for antifertility activity in the mouse system is not surprising, since the mouse eggs possessed a zona pellucida, whereas this layer was removed from the hamster eggs. The latter test primariliy indicates the ability of human spermatozoa to undergo capacitatio the acrosome reaction, and to fuse with investment-free (denuded) oocytes. Acrosin appears to be particularly important for the penetration of spermatozoa through the zona pellucida of the egg, although this enzyme is also involved in the acrosome reaction. Thus, it can be expected that even lower concentrations of the inhibitor than those observed for the humster test will inhibit fertilization of zona-intact human eggs by human spermatozoa.

In order to verify that aryl guanidinobenzoates can prevent the acrosome reaction, the effect of an aryl 4-guanidinobenzoate on the guinea pig acrosome reaction was studied. The guinea pig was used because the acrosome reaction is readily visible in this species and because a synchronous acrosome reaction is available, making it possible to separate capacitation from the acrosome reaction. At 10^{-5} M, complete inhibition of the acrosome reaction occurred. No effect on capacitation was noted. Preliminary studies with human

spermatozoa indicate that AGB also prevents the acrosome reaction of human spermatozoa.

5. Nonoxynol-9 immobilized all human sperm at 257 μg/ml in the Sander-Cramer test (30 sec. test). No antimotility effect was observed at nonoxynol-9 concentrations below 5.1 μg/ml. For the methylsalicylate-, ethylparaben-, and acetaminophen-derivatives, these values were 213 and 71 μg/ml, 228 and 30 μg/ml, 1460 and 146 μg/ml, respectively. These results show that the aryl guanidinobenzoates also have antimotility properties. Therefore, at the levels that will be used in women (100 mg/day), AGB will have a dual mechanism of action: it will inhibit both acrosin and spermatozoal motility. However, the primary mechanism of contraception will remain its acrosin inhibitory activity.

6. Comparative rabbit vaginal contraceptive studies were performed with all nine derivatives containing clinically utilized phenols, as well as with nonoxynol-9. Low concentrations (100 μg/ml) of the aryl 4-guanidinobenzoates were used to determine contraceptive efficacy due to acrosin inhibition and not by any antimotility action. Two ml (200 μg/rabbit) were applied. Seven of the nine aryl guanidinobenzoates greatly depressed the fertility rate (the fertility rate of these agents varied from 0.5 to 33%, as compared to 85 to 90% in the controls). The most potent inhibitors (the acetaminophen-, ethylparaben- and methylsalicylate-derivatives) resulted in fertility rates of 6%, 0.5% and 7%, respectively. By contrast, much higher concentrations of nonoxynol-9 (2,000 μg and 20,000 μg per rabbit) were required to depress the fertility rates to 25% and 17%, respectively. Thus, even at these high doses of nonoxynol-9, the decrease in fertilization did not reach that of the much lower doses of these three aryl 4-guanidinobenzoates. The results with

nonoxynol-9 confirm previous data which showed its relatively poor in vivo efficacy (32-36). Higher concentrations of the aryl 4-guanidinobenzoates should reduce the fertility rate of the inhibitors to zero, but such concentrations were not employed since no differentiation between the acrosin inhibitory activity of the compounds and their antimotility effect can then be made. These results show that certain aryl 4-guanidinobenzoates, including AGB, are at least 100 times more potent as vaginal contraceptives than nonoxynol-9. It should further be noted that the concentration of aryl 4-guanidinobenzoate used in the rabbit was orders of magnitude lower than that which will be applied to women.

7. Primate vaginal contraceptive experiments with an aryl 4-guanidinobenzoate confirmed the rabbit experiments, i.e., that very low doses of aryl 4-guanidinobenzoate are contraceptive. Comparative studies with Delfen Vaginal Cream (Ortho) showed that the aryl 4-guanidinobenzoate, even at 100 times lower concentrations than the nonoxynol-9 content in Delfen Vaginal Cream, had the same or slightly higher contraceptive potency than the Cream.

8. Acute toxicity (LD_{50}) studies were performed in mice with all the aryl 4-guanidinobenzoates containing clinically used phenols and nonoxynol-9. The material was injected I.P. Nonoxynol-9 (LD_{50} = 180 mg/kg under the same conditions) was more toxic than any of the aryl guanidinobenzoates. Several aryl guanidinobenzoates, including AGB, had LD_{50}'s of about 1000 mg/kg with some having an LD_{50} > 2000 mg/kg.

9. Based on the above contraceptive and acute toxicity studies, four aryl guanidinobenzoates were selected for further study: the guanidinobenzoic acid

derivatives of acetaminophen (AGB), methylsalicylate (MSGB), ethylparaben (EGB) and methylumbelliferone (MUGB).

10. The acute toxicity studies were repeated with the first three of these compounds in mice and rats. The results were similar in the mouse and rat, with respective LD_{50}'s of 829 and 1000 mg/kg for AGB, of 261 and 250 mg/kg for EGB, and 1000 mg/kg for MSGB. MUGB has an LD_{50} greater than 2000 mg/kg. Based on these acute toxicity evaluations and the rabbit vaginal contraceptive studies, it can be calculated that the therapeutic index (toxic dose/contraceptive dose) for these selected aryl guanidinobenzoates ranges from approximately 5,000 to more than 40,000. By contrast, the therapeutic index of nonoxynol-9 is about 200 under these same conditions.

11. The Ames test was performed with four aryl guanidinobenzoates (MUGB, MSGB, EGB and AGB) and none showed mutagenicity. A mixture of nonoxynol-9 and one of the aryl guanidinobenzoates was also tested and shown to be nonmutagenic.

12. Two of the aryl 4-guanidinobenzoates (MSGB and MUGB) were tested for mutagenicity by the micronucleus assay. No significant induction of micronuclei was found even at dose levels 125-250 times that will be employed in the Phase I clinical trial.

13. Preliminary chronic vaginal toxicity studies were performed with the four aryl 4-guanidinobenzoates and nonoxynol-9 by applying them for 50 days to the vagina of rats at 100 mg/rat (the highest amount that could be used due to the small vagina of the rat and viscosity of the suspension). GLP conditions were not employed. None of the animals died in any of the treatment groups. None

of the compounds caused a significant increase in organ or tissue pathology, including that of the vagina, as compared to the controls. The dose of aryl guanidinobenzoate employed on a body weight basis was more than 100 times higher than that which will be used in the clinical trial.

14. Vaginal irritation studies were subsequently performed under GLP conditions using the rabbit as animal model. Dose levels were employed that were about 20 times higher than those that will be used (or are being used in case of nonoxynol-9) in the human. The compounds (in gum acacia) were applied daily for 10 days. During the first series of tests, MUGB, AGB, MSGB and EGB were compared to nonoxynol-9. AGB did not cause any vaginal irritation, i.e., was no different than the control (vehicle-treated) animals. The other aryl 4-guanidinobenzoates did cause some vaginal irritation but none were as severe as nonoxynol-9 which is well known to be quite irritating to the rabbit vagina EGB was the only inhibitor which approached the irritation observed after nonoxynol-9 administration. Although all 4 compounds appear to be safe for clinical use, it was decided to focus on AGB for a clinical trial since it is essentially non-irritating to the vagina, it has low acute and chronic toxicity, and it possesses high vaginal contraceptive potency.

Preclinical Results Obtained with 4-Acetamidophenyl 4-Guanidinobenzoate Hydrochloride (AGB)

1. AGB Inhibition of Purified Human Acrosin

Highly purified acrosin was prepared from human spermatozoa and the interaction with AGB was tested. The ED_{50} of AGB was 8×10^{-9} M. The K_m was 5.65×10^{-9}

M, the k_2 was 2.9×10^{-3} sec^{-1} and the k_2/K_m was 51.3 M^{-1} sec^{-1}. These values indicate that the AGB interacts extremely rapidly with acrosin and are consistent with pseudoirreversible binding.

Dialysis experiments showed that at least 2 days of dialysis at pH 3.0 are required before some of the AGB is removed from human sperm acrosin. No dissociation will occur at all when dialysis is performed at pH 7.0 to 8.0. Since the sperm are at a pH of 4.0 only while in the vagina for maximally 6 hours after which sperm entry into the cervix and uterus is completed (the fluids of these organs have a pH of 7.0 to 8.0), these results indicate that no dissociation of AGB from acrosin will occur while the spermatozoa are transported through the female genital tract.

2. AGB Treatment of Whole Human Spermatozoa: Inhibition of Acrosin

AGB was mixed with human semen (pooled ejaculates) for 2 min, after which the supernatant fluid (seminal plasma and inhibitor that did not bind to the sperm) was removed from the spermatozoa by Ficoll centrifugation. The acrosin activity of the spermatozoa was tested. The ED_{50} was 0.013 mg/ml semen and the ED_{84} was 0.074 mg/ml semen. Additionally, the results show that AGB cannot be washed away from human spermatozoa. This provides further evidence that AGB will not be removed from the spermatozoa while the gametes are transported through the female genital tract.

3. Stability of AGB

Stability studies with AGB were performed at pH 4.0, approximating the vaginal pH, and compared to pH 6.0 and 8.0. At the vaginal pH, AGB was stable over a 3 month period. It was slightly less stable at pH 6.0 and somewhat more unstable at pH 8.0. Since the sponges will only be in the vagina for a 2.5 hour period during the clinical trial, it can be concluded that AGB will remain stable.

4. Antimotility Activity of AGB

The Sander-Cramer test was performed. Different concentrations of AGB were mixed with human semen and the motility of the spermatozoa was observed after 30 sec. The ED_{50} of AGB was 0.57 mg/ml and the ED_{84} was 1.52 mg/ml. Under exactly the same conditions, the ED_{50} of nonoxynol-9 was 0.04 mg/ml and the ED_{84} was 0.08 mg/ml. Thus, although the primary mechanism of action of AGB remains its acrosin inhibitory properties, at the dose that will be used for the clinical trial (100 mg/day) AGB will also inhibit the motility of the spermatozoa. It was further found that the antimotility effect of AGB and nonoxynol-9 were additive when these drugs were mixed.

5. Vaginal Contraceptive Potency of AGB in the Rabbit Model

AGB (0.1 mg/ml) in placebo gel (2 ml) was placed inside the vagina of rabbits (n=4) and the rabbits mated. Thirty-six hours later, the genital tracts were excised, the embryos collected and the percent fertilization calculated. Mean fertilization of the AGB was 8%. Under exactly the same conditions, the placebo gel gave a mean fertilization of 82%. Nonoxynol-9 at 10 (1 mg/ml) and

100 (10 mg/ml) fold higher concentrations than AGB gave mean fertilizations of 32% and 16%, respectively. Thus, AGB is orders of magnitude more potent as a vaginal contraceptive than nonoxynol-9 in the rabbit model.

6. Development of the Today Sponge for Release of AGB

Dr. Saxena at VLI Co. prepared AGB-releasing Today sponges. The AGB was pulverized to a very small particle size, mixed with the other components of the Today sponge to get uniform distribution and the sponges were constructed by the usual VLI process. One group of the sponges also contained nonoxynol-9 (N-9). The highest amount of AGB that could be incorporated and uniformly distributed in the sponge was 350 mg.

In vitro testing of the AGB released from the sponge showed that chemical and biochemical properties of AGB were not altered by the sponge construction. HPLC analysis of the released AGB showed that it was not hydrolyzed into its subunits. Also, the enzyme inhibitory properties of AGB were not altered. The released AGB had the same spermicidal properties as it had before incorporation.

To measure the release rate and to mimic "infinite sink" conditions in order to simulate the fact that AGB is probably rapidly removed from the vagina (primarily due to leakage), the sponges were placed in a beaker containing 900 ml of formic acid buffer at pH 4.0 (the approximate pH of the vaginal fluid) (37). The buffer was replaced after 12 hrs, 24 hrs, 36 hrs, 48 hrs and once daily thereafter for 7 days. The amount of AGB released at each time point was determined by analyzing a sample by high performance liquid chromatography

(HPLC).

The amount of AGB released over a 1 day period from the sponge containing 350 mg AGB was 75 mg if the sponge did not contain nonoxynol-9 and 120 mg if the sponge did contain nonoxynol-9. These are the approximate dose levels that should achieved for the Phase I clinical trial (100 mg/day).

7. Acute Toxicity Studies (Not GLP)

Acute toxicity was evaluated in two species usign the intraperitoneal route of administration of a gum acacia suspension. The LD_{50} values obtained were as follows:

Mice	829 mg/kg
Rats	1000 mg/kg

Under exactly the same conditions, the LD_{50} for nonoxynol-9 in mice was 180 mg/kg. Thus, the acute toxicity of AGB is approximately 5 times less than that of nonoxynol-9.

8. Preliminary Subchronic Evaluation (Not GLP)

Rats were administered AGB in a gum acacia suspension for 50 days by vaginal application (10 mg/animal or approximately 40 mg/kg). There were no deaths in the group administered AGB vaginally. No pathology of the vagina, liver, kidney and lung was observed by either gross observations or by histopathology.

AGB was also injected intraperitoneally in rats. The mortality for the intraperitoneal injection of AGB was as follows:

Dose (mg/kg)	no. dead/no. treated
20	0/6
100	3/6
500	6/6

Nonoxynol-9 was also evaluated in parallel to AGB in rats. Mortality was as follows:

25	1/6
100	4/6

9. Mutagenicity

Mutagenicity of AGB was tested in a Forward Mutation Assay employing Salmonella Typhernurium Strain TM677 with both activated and non-activated systems. AGB demonstrated no mutagenicity activity in this assay.

10. Vaginal Irritation Studies (GLP)

Two vaginal irritation studies were performed. Both studies exposed rabbits to gum acacia suspensions as daily doses for ten days. Nonoxynol-9 was also tested as positive control.

Study 1 evaluated several different aryl 4-guanidinobenzoates. The results for AGB and nonoxynol-9 are given below.

	Dose (mg/day)	Dose (mg/kg)	Synopsis of vaginal changes observed
AGB	150	50	No observable effects
N-9	500	167	Moderate acute inflammation of the proximal vagina: moderate acute inflammation of the distal vagina.

Study 2 evaluated AGB alone and in combination with nonoxynol-9.

	Dose (mg/day)	Dose (mg/kg)	Synopsis of vaginal changes observed
AGB	75	25	None except for some mild focal mucosal ulceration in the proximal vaginal only.
AGB with N-9	75 250	25 50	Mucosal ulceration and moderate submucosal necrosis in the proximal and middle vagina.
N-9	250	50	Mucosal ulceration and moderate submucosal necrosis in the proximal and middle vagina.

Attempts were also made to determine the vaginal irritation of the AGB-releasing Today sponge. However, such studies were unsuccessful. When the sponges were placed near the vagina orifice, the sponges were spontaneously pushed out of the vagina, usually within 12 hours. Rabbits with sponges placed deep into the vagina near the cervix retained the sponges for 24 hours. However, these sponges could not be removed and replaced each day since the inserted sponges could not be grabbed with forceps or otherwise due to the very long vagina of the rabbit and the bending of the vagina over the pubic bone. Subsequently, sponges with suture material tied around them were placed deep in the vagina near the cervix with the string exiting the vaginal orifice to facilitate removal of sponges. However, these sponges were usually not retained for more than 12 hours. It appears that the rabbits pull on the string. Finally, a stricture was made in the vagina utilizing umbilical tape. This required abdominal surgery. Although the sponges were retained in the vagina, they could not be replaced on a regular basis.

It is concluded that preclinical vaginal irritation studies with the sponge cannot be performed. However, the Today sponge has been applied now for a number of years to women without complaint of vaginal irritation. AGB was shown to be essentially non-irritating to the rabbit vagina even at doses 25 to 50 times higher than that which will be used in the clinical trial. Also, AGB was applied for a 10 day period in contrast to the 2-4 hour period that will be used the trial. Finally, AGB did not enhance the vaginal irritation of nonoxynol-9. Thus, it can be expected that the Today sponge releasing AGB should be non-irritating to the human vagina.

11. 30 Day Subchronic Studies (GLP)

Vaginal Application

Nine week old Sprague Dawley rats were treated vaginally for 30 days with one of the following: 1) vehicle only (5% gum acacia); 2) AGB (10 mg/ml); 3) AGB (10 mg/ml) and nonoxynol-9 (15%); and 4) nonoxynol-9 (15%). Twenty to twenty-five rats were used in each group. On day 31, the rats were sacrificed, tissues collected, fixed and sent to Pathology Associates, Inc. for histopathological study. It was concluded that neither AGB nor nonoxynol-9 either separately or together resulted in any significant histopathologic changes in any organ including the vagina

Intraperitoneal Application

Nine week old Sprague Dawley rats were treated intraperitoneally for 30 days with one of the following: 1) vehicle only (5% gum acacia); 2) AGB (7 mg/ml); 3) AGB (14 mg/ml); 4) AGB (28 mg/ml); 5) AGB (7 mg/ml) and nonoxynol-9 (10%); and 6) nonoxynol-9 (10%). Twenty to thirty rats were used in each treatment group. On day 31, the rats were sacrificed, tissues collected, fixed and sent to Pathology Associates, Inc., for histopathologic study. It was concluded that: 1) intraperitoneal AGB administration results in degenerative kidney changes; 2) gum acacia both with and without AGB and/or nonoxynol-9 resulted in hepatocellular cytoplasmic vacuolization; and 3) virtually all rats showed peritonitis but the incidence, distribution and severity was greater in those groups given nonoxynol-9 and/or AGB.

12. Preliminary Pharmacokinetic Studies

Blood samples were obtained on the day of sacrifice from rabbits treated in the vaginal irritation studies (see Section 10). These samples were assayed for acetaminophen to determine if blood levels of acetaminophen during treatment were increased. No acetaminophen was detected (sensitivity of assay 0.4 μg/ml).

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PROJECT TITLE: "Toxicology Studies of Acrosin Inhibitors"

PARFR Subagreement Number: PARFR-338UI

Principal Investigator: Donald P. Waller
Associate Professor of Pharmacology
Department of Pharmacodynamics
College of Pharmacy

312-996-5385

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Subagreement Period:
Starting date: July 1, 1984
Ending date: December 31, 1986

Date of Last Technical Report Submitted to PARFR: January 30, 1986

Inclusive Dates of this Technical Report: January 1-December 31, 1986.

Narrative Summary

During the reporting period the difficulties in establishing an appropriate route of administration for the 30 day subchronic toxicity testing of AGB was resolved by discussions with the FDA. Following FDA recommendations the 30 day subchronic toxicity testing was performed.

A. Statement of Objectives:

To provide toxicology information to support the submission of an IND for a Phase I clinical trial of an arylguanidinobenzoate as a new and effective vaginal contraceptive.

B. Progress to Date:

Radiolabelled AGB was obtained to determine pharmacokinetic parameters of vaginally absorbed AGB. The original proposal was written for tests utilizing the ^{14}C labelled compound. No source was identified for producing the ^{14}C labelled compound. Only the tritiated compound could be obtained. The radiolabelled compound was not in large quantity and at minimal specific activity.

Initial tests with the compound indicated that the radiolabel was not adequate in either specific activity or amount to perform a meaningful evaluation of pharmacokinetic parameters.

Further attempts to obtain adequate radiolabelled compound were not successful.

A letter was written to the FDA during September 1985 to establish the requirements for submission of an IND for an AGB containing vaginal contraceptive (Attachment 1). The letter was forwarded to the appropriate section of the FDA and a response was received during April, 1986 (See attachment 2). A meeting at the FDA in Washington was arranged for May 1986 to discuss the work previously performed on the AGB compound and carefully establish the requirements to complete preclinical toxicology testing for the AGB contraceptive (Attachment 3). The meeting was attended by Dr. L.J.D. Zaneveld (Rush-Presbyterian-St. Lukes Medical Center, Dr. A Goldsmith (PARFR), J. M Spieler (AID) and myself. A summary of the meeting written by the FDA is attached (Attachment 4).

The problem of the route to be utilized during the 30 day subchronic testing was addressed during the meeting. It was agreed that the gum acacia suspension could be used for the subchronic testing of AGB. It was also recommended that a group with nonoxynol in combination with AGB be included in the study. The addition of these test groups resulted in

ten groups of rats included in the study rather than the eight groups specified in the original proposal to PARFR.

A new protocol was written to include the additional groups and the study was performed (See Attachment 5). Details of the experiment are included in the GLP study protocol.

A draft report of the histopathological results has been received (Attachment 6). Only the summary of the draft report is included in this report. The complete document is more than 350 pages and will be forwarded to PARFR when the final report is obtained.

There were no major histopathological changes noted in any of the groups administered AGB vaginally.

All the groups receiving intraperitoneal injections of AGB or vehicle had peritonitis. This was a result of the daily injections into the peritoneal cavity and not considered drug related. More extensive changes characterized by extramedullary hematopoiesis (EMH) was observed in the higher dose groups of AGB and nonoxynol treated groups. This was a result of more severe peritonitis in these groups. The increased peritonitis was probably a result of foreign body reaction to the presence of either the AGB and/or the nonoxynol.

The most significant finding was an increase in dose-group associated degenerative changes in the kidneys for groups dosed with AGB. Similar changes were not noted in the vehicle control group of the group administered nonoxynol only. A no effect dose was not established for the renal changes caused by the AGB.

The effect of AGB on the kidney should be further studied to determine the no-effect dose level.

C. Unanticipated Results

The degenerative changes in the kidney are of some concern for clinical testing of AGB. Studies on the kidney changes should be performed to determine a no-effect dose level and the nature of the renal damage. In addition, a second species should be studied to determine if the effect may be specific for the rat.

D. Research Design

Additional groups of animals were added to the 30 day subchronic toxicity test. These groups were added at the recommendation of the FDA. The groups added and numbers of animals in each of the groups are found in Attachment 5.

E. Operational Significance

The subacute testing of the AGB demonstrates the relative safety of AGB administered vaginally. The renal damage observed after intraperitoneal administration raises some concern on long term safety of this agent. More detailed studies will be required to characterize the risk associated with the administration of this drug.

F. Dissemination and Utilization of Research Results

The 30 day subacute toxicity study will be the subject of abstracts and a manuscript to be submitted to a toxicology journal.

G. Work Plan for Coming Reporting Period N/A

H. Budget Forecast

The costs for the studies performed exceeded the projected budget(Attachment 6). The FDA recommended addition of two groups of animals in the subchronic testing required; additional technician time (to perform dosing and to autopsy the animals), increased numbers of animals to be purchased and housed; and increased numbers of slides to be processed and interpreted.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administr
8757 Georgia Avenue
Silver Spring MD 20910

OCT 3 1985

Donald P. Waller, Ph.D.
The University of Illinois at Chicago
Department of Pharmacodynamics
833 South Wood Street
Box 6998
Chicago, Illinois 60680

Dear Dr. Waller:

This is in response to your letter dated September 5, 1985, concerning the new vaginal contraceptive sponge with acrosin inhibitor. This contraceptive sponge is a drug product, therefore, your letter is being forwarded to Dr. Solomon Sobel, Director, Division of Metalbolism and Endocrine Drug Products, Center for Drugs and Biologics, Food and Drug Administration, 5600 Fishers Lane, Rockville, Maryland 20857.

Dr. Sobel will respond to your letter.

Sincerely yours,

Lillian Yin, Ph.D.
Director, Division of OB-GYN,
ENT, and Dental Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

cc: Dr. Solomon Sobel

THE UNIVERSITY OF ILLINOIS AT CHICAGO

College of Pharmacy
Department of Pharmacodynamics
833 South Wood Street
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5 September 1985

Lillian Yin, Ph.D.
Director
Division of OB-GYN, ENT, and Dental Devices
Office of Device Evaluation
Center for Devices and Radiological Health
Food and Drug Administration
8757 Georgia Avenue (HFZ-470)
Silver Spring, Maryland 20910

Dear Dr. Yin,

We are beginning to prepare an IND for a new vaginal contraceptive device which uses the Today^R sponge as the vehicle for the administration of an acrosin inhibitor(aryl 4-guanidinobenzoate). This device will be identical to the current Today^R sponge containing nonoxynol, except that it will either contain the aryl 4-guanidinobenzoate only or both nonoxynol-9 and the aryl 4-guanidinobenzoate. The test devices will be manufactured by VLI Corporation which currently markets the Today^R sponge.

We have conducted rabbit vaginal irritation tests using suspensions of the aryl 4-guanidinobenzoate. In contrast to nonoxynol-9, it appears to have little or no effect on the vaginal mucosa at concentrations up to about 10 times the expected clinical dose.

We are planning the next series of toxicology testing which we hope will lead to approval for Phase I clinical testing. We currently plan to perform a second rabbit vaginal irritation test using the sponge device. This irritation test will utilize a sponge which contains the greatest amount of aryl 4-guanidinobenzoate which the manufacturer can incorporate into the sponge. Unfortunately this dose is not a large multiple of the expected clinical dose. Release studies in vitro have shown that about 20% of the total material is released from the sponge over a 24 hour period. We plan to place the sponge into the vagina of the rabbit and replace it after 24 hours for ten consecutive days. This will result in continuous exposure of the rabbit vagina to the acrosin inhibitor for ten days.

In addition to the rabbit vaginal irritation test, we will conduct a 30 day subchronic toxicity test using rats. Both the intraperitoneal and vaginal routes of administration will be used. The intraperitoneal injection will be at the maximum dose possible for a reasonable amount of inhibitor in a gum acacia suspension. From previous studies we expect this dose will be approximately fifty times the expected exposure assuming 100% of the clinical dose is absorbed from the vagina. For the vaginal route of administration, we anticipate placing a sponges without the inhibitor, sponges containing inhibitor and sponge containing inhibitor as well as nonxynol-9 in three groups of animals. There will be 50 animals in each group. The sponge will be replaced each day with a new piece of sponge. We will perform a whole body histological examination of the animals upon completion of the 30 day dosing period .

The Phase I clinical trial will consist of a small number of women(20 subjects who will have undergone tubal ligation to insure no pregnancy will occur). The sponge will be inserted in the vagina and remain for a short period of time(less than 3 hours). During the time of exposure it is anticipated that intercourse will occur. Upon completion of intercourse, vaginal fluid will be collected, the sponge will be removed and the female will immediately douche and the solutions collected. The vaginal washings will be used to cleanse the vagina and remove the released test material. The vaginal fluid and washings will be evaluated for the presence and condition of sperm and the amount of released inhibitor.

We would like to know if the above testing will be adequate for initiating a Phase I clinical trial as described. We would be happy to travelto Washington on an informal basis to discuss in more detail the requirements for such a device.

Please let us know a convenient date when we may visit with you or someone in your section to discuss the above future IND submission.

It was a pleasure to talk with you in Geneva. I hope we can continue our discussions in the near future.

Sincerely,

Donald P. Waller, Ph.D.
Associate Professor

cc:L.J.D. Zaneveld

Attachment 2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Rockville MD 20857

APR 18

Donald P. Waller, Ph.D.
Department of Pharmacodynamics
College of Pharmacy
The University of Illinois at Chicago
833 South Wood Street, Box 6998
Chicago, IL 60680

Dear Dr. Waller:

This is in response to your letter dated September 5, 1985, to Dr. Lillian Yin of our Office of Device Evaluation regarding a vaginal contraceptive using the Today sponge plus aryl 4-guanidinobenzoate (an acrosin inhibitor). We apologize for the delay in responding to your inquiry.

The preclinical studies as outlined in your letter should be adequate to allow initiation of the proposed Phase I clinical trials. We enclose the forms you will need to request a Notice of Claimed Investigational Exemption for a New Drug (IND) before beginning that clinical trial. Also enclosed are several pamphlets addressing various aspects of the IND submission. If you have additional questions or would like to come in for a meeting with us, please contact Ms. Lee Ripper at 301-443-3490.

In addition, we enclose a copy of the original pharmacology review of the new drug application for the Today sponge for your information. However, the studies needed for your proposed product would not be identical to those required for the Today sponge since we do not have the marketing experience with 4-guanidinobenzoate that we had with nonoxynol-9.

We hope this is helpful to you.

Sincerely yours,

Solomon Sobel

Solomon Sobel, M.D.
Director
Division of Metabolism and
Endocrine Drug Products, HFN-810
Office of Biologics Research and Review
Center for Drugs and Biologics

Enclosures

NDA 18-683

4 November 1981

VLI Corporation
Costa Mesa, California

Submission: 22 September 1981

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

Secure Contraceptive Sponge (Previously called: Collatex Contraceptive Sponge)

OTC Vaginal Contraceptive Sponge
containing the spermicide Nonoxynol-9

Related: IND 16,222

Supplier: Major components.
Urethane Prepolymer ([redacted]
[redacted]
Nonoxynol-9 ([redacted]) - [redacted]
Final Product.
Manufactured by VLI Corporation

Formulation: Final dry sponge composition.

Urethane Foam	[redacted] gms	[redacted]	
Nonoxynol-9	1.0 gm	+ [redacted]	(ca. [redacted] dry wt.)
Citric Acid	[redacted] mg	+ [redacted]	
Sodium Citrate (dihydrogen)	[redacted] mg	+ [redacted]	
Benzoic Acid	[redacted] mg	+ [redacted]	
Sorbic Acid	[redacted] mg	+ [redacted]	
Sodium Metabisulfite	[redacted] mg	+ [redacted]	
Polyester (100%) woven tape	0.47 x 15.24 cm		
Polyester (100%) Scrim	2.5 x 4 cm		

The cup-shaped contraceptive sponge is about 55 mm in diameter by 25 mm overal (ca. 15 mm at the depression) with a polyester loop for removal from the vagina.

Indication: Vaginal Contraceptive

Dose:
The SECURE Contraceptive Sponge is placed in the vagina, by the user, prior to intercourse. It can be inserted anytime from immediately prior to up to two days before intercourse. Coitus can occur multiple times during the recommended 48-hour wearing.

NOV 6 1981

Preclinical Studies:
(Due to the nature of this product and that of the preclinical studies this Pharmacology Review varies from the form of the usual summary.)

In-Vitro - Spermicide Elution Studies. (VLI Corporation?)

A series of sponges containing various nonoxynol-9 (N-9) spermicide levels (0, 10, 20, 30%) by dry sponge weight, with and without preservatives and with and without buffered citric acid were tested for pH and spermicidal effectiveness of saline rinses from the sponges (up to 40 individual 5 min. soak/squeeze cycles with 20 ml physiological saline.)

The pH of the eluate rinses averaged ca. 5.3 (in range of that of the normal vagina - 4.5 to 5.5).

Preservatives alone and citric acid alone had no detectable spermicidal activity. Neither the preservatives nor the citric acid enhanced the spermicidal effect of the wash eluates on spermatozoa.

Spermicidal effectiveness of the 30% N-9 sponge was lost by the 20th physiological saline rinse; by the 5th rinse for a 20% N-9 sponge and by the 3rd rinse for a 10% N-9 sponge.

The 30% N-9 sponges released spermicide more readily than did 10 and 20% sponges as well as a greater percentage of total spermicide content with 20 consecutive rinses.

After five soak/squeeze saline cycles, about 50% spermicide is washed from the 30% spermicide sponge. The incremental amount per rinse drops to less than 5 mg after the 5th rinse, however spermicidal effectiveness is maintained until rinse 20.

After the first 20 rinses, 680 mg (ca. 53%) of N-9 was removed from sponge Lot 041278 (containing 1290 mg N-9) and 110 mg by the second 20 rinses for a total of 61%. The dilution (of eluates) beyond which spermicidal effects were lost was 1:16 by the first 20 rinses and 1:1 by the second 20 rinses.

Both the amount of spermicide available and the percent released increase as the level of N-9 is raised above 30%, but in lesser increments than that seen between 20 and 30% sponges.

The dilution beyond which spermicidal effectiveness is lost for eluates of the 30% (manufactured) sponge after soaking/agitate cycles at 37°C is as follows:

One Day	1:128 pre-squeeze	1:512 post-squeeze
Five Days	1:128 pre-squeeze	1:256 post-squeeze

The amount of spermicide released from a 30% spermicidal (3.45 g N-9) sponge with soaking/agitation in 37°C saline pre and post squeeze is:

One Day		2 Days		3 Days	
Pre - squeeze	Post	Pre - squeeze	Post	Pre - squeeze	Post
1280 (37%)	520 (15%)	1730 (50%)	470 (14%)	970 (28%)	280 (8%)

4 Days		5 Days	
Pre - squeeze	Post	Pre - squeeze	Post
1390 (40%)	280 (8%)	1230 (36%)	480 (14%)

Laboratory Studies of the Release of Nonoxynol-9 from Polymeric Intravaginal Contraceptive Sponges. [redacted] M.D., [redacted] Ph. D., M.D., [redacted] Ph. D.

This DRAFT Article included the following in addition to information not covered elsewhere:

UV Analysis (223 nm) proved superior to bioassay and gravimetric analysis.

Washing Experiments. Following equilibration in 20 ml 0.9% NaCl for 5 min., sponges were squeezed and fluid tested.

Total recovery of N-9 from saline rinses and extraction of sponges with chloroform averaged 94.2% ± 5.5.

Incubation Experiments. Incubation at 37°C in 0.9% NaCl with slow agitation up to 5 days.

The greatest amount of N-9 was released on the first day with relatively small amounts appearing on subsequent days - the average daily release was an exponential function of the initial quantity of N-9 in the sponge. Total recovery of N-9 includir chloroform extractable material was 96.3% ± 1.7% for one day incubation and 95.5% ± 2.1% for the 5 days incubation.

Titers of spermicidal activity were equivalent for 30% N-9 sponges up to ca. 15 mos. old.

USP Toxicity Tests.

[redacted] Signed [redacted] 9/16/77; [redacted] 8/5/77. Four standard toxicity tests (USP) were performed for the urethane sponge containing nonoxynol-9.

The eluates were prepared using 4 gms of dry sponge with 20 ml of saline and with 20 ml of cottonseed oil, soaked at 70°C for 24 hrs.

1. Acute systemic injection test with both saline (i.v.) and cottonseed oil (i.p.) extracting media in mice.
   The nonoxynol-9 eluate resulted in deaths of 5/5 mice tested.

2. Intracutaneous injection test with both saline and cottonseed oil extracting media in rabbits.
   The nonoxynol-9 eluate intracutaneaneous reactivity was marked in all rabbit injection sites.

3. 7 Day - Acute intramuscular implantion of sponges in rabbits.
   A moderate tissue reaction was reported.

4. Tissue culture with inhibition of cell growth.
   The cytotoxicity score for the eluates was 4 - toxic to WI-38 cells under the prescribed test parameters.

USP toxicity tests for the urethane foam without nonoxynol-9 showed no significant differences from controls.

Toxicity of Scrim - [redacted]
Report dtd. 29 June 1981; Signed: [redacted]

Polyester woven mesh scrim impregnated in the sponge during molding to add stren

USP Tests.

1. Acute systemic toxicity in mice. 120.0 cm^2/20.0 ml
   In sodium chloride - i.v.; ethanol in sodium chloride - i.v.;
   PEG 400 - i.p.; cottonseed oil - i.p.
   No significantly greater systemic reaction than controls.

2. Intracutaneous toxicity in 2 rabbits per extract (same eluates as acute tox.)
   No significantly greater tissue reaction than control.

3. 7-Day Implantation test in 2 rabbits.
   No significant macroscopic tissue reaction was reported compared to contr

Dermal Toxicity - [redacted].

Primary Skin Irritation Test on Rabbits. Report dtd. 21 Feb 78. Signed: [redacted] Ph.D.

Sponges containing 30% by dry weight nonoxynol-9 were cut into one inch square blocks to which 0.5 ml of saline was added to moisten. Blocks were placed under gauze on abraded and nonabraded skin on backs of six albino rabbits. Patches were removed at 24 hrs. with observations at 24 and 72 hrs.

Erythema was observed in the intact and abraded skin of 1 animal. - Rated as nominally irritating - and according to the Draize average not a primary irritant to rabbit skin.

Acute Dermal Toxicity Test in Rabbits. Report dtd. 21 Feb 78. Signed [redacted] Ph.D.

1 3/4" dia. sponges were soaked in 40 cc deionized water for 18 hrs. at ambient temperatures and liquid squeezed out by hand. 20 ml/kg was applied to intact skin of 2M and 3F New Zealand White rabbits and to the abraded skin of 3M and 2F. Occlusive coverings were removed after 24 hrs. with observations for 14 days.

There was some decreased activity, but no deaths.

Repeat Insult Patch Test (10-RIPT) on 52 Human Subjects. Report dtd 10 Mar 78.
Signed: [redacted] B.S., [redacted] M.D.

Test material was prepared by soaking a sponge in 40 cc distilled water for 15 min. The water was hand squeezed from the sponge and ca. 0.2 ml of this nonoxynol-9 extract was dropped onto a 2 cm sq. of Webril non-woven cotton fabric affixed to Dermicil cloth tape which was applied to the inner aspect of the arm or back and covered with Blenderm hypo-allergenic tape. Patches were removed after 24 hrs. 24 or 48 (weekends) hr. rest periods followed. The procedure was repeated for 10 applications. 10-14 days after the last patch a challange patch was applied.

Results: 12 subjects showed slight and transient reactions
1 subject reacted repeatedly during the induction phase - but no reaction at challange
1 subject reacted at challange - minimal and transient.

Mutagenicity:

Ames Test. [redacted] 23 Aug 77. Signed: [redacted] B.S.; [redacted] Ph.D. [redacted]

Eluate of Urethane Foam Sponges containing nonoxynol-9.

Eluates - 5 gm of sponges were diced and agitated with 60 ml saline for 7 at 37°C.

The eluate prepared from the test sponges containing nonoxynol-9 was non mu for all 5 test strains of Salmonella typhimurium (TA-1535, TA-1537, TA-1538, TA TA-100) - activated and non-activated.

[redacted] Report 3 April 1979. Signed: [redacted] B.S.; [redacted] Ph.D.

Collatex Contraceptive Sponge Lot 120878 with 30% by dry weight of nonoxyn using Fischer's Media for Leukemic cells of Mice with 5 days agitation.

The eluate of the Collatex Contraceptive Sponge was non-mutagenic for the 5 test strains utilized in this study.

Mouse Lymphoma Assay. [redacted] dtd 6 Apr 1979 Signed: [redacted] B.S.; [redacted] Ph.D.

Collatex Contraceptive Sponge Lot 120878 with 30% by dry weight of nonoxyn

Eluate - One Collatex Contraceptive Sponge was placed in 60 ml Fisher's Med for leukemic cells of mice and agitated for 5 days. Following the agitation per the sponge was squeezed and the fluid filtered before test.

It is reported that none of the dilutions of the eluate yielded an Induced Mutation Frequency greater than 2 and that therefore, the eluate of Collatex Contraceptive Sponge was non-mutagenic for all dilutions in which it was not cytotoxic to the Mouse Lymphoma cells.

Ames - Salmonella/Microsome Plate Test. [redacted] Report date Oct 1980. Signed: [redacted] Ph.D.; [redacted] Ph.D.

The test material (Lot 072380) was cut into small pieces; 60 ml physiologic saline was added to 5 g of the material pieces and shaken at 37°C for 5 days(?). The saline solution was then squeezed out of the foam and filtered.

The test material extract did not exhibit toxicity with any of the indicato strains used. The results both in the absence and in the presence of a rat live activation system were negative.

Mouse Lymphoma Forward Mutation Assay. [redacted] dtd. July, 1979. Signed: [redacted], Ph.D.; [redacted] Ph.D.

Collatex Contraceptive Sponge - Nonoxynol-9 at 30% of Dry Sponge Weight (1.15 gm/sponge) Lot 120878. Test Material 10806-27-1. [redacted]

One sponge was agitated with Fischer's growth medium for 5 days at 37°C. The sponge was then squeezed ; the medium collected and filtered before use.

The test material was reported as not detectably mutagenic over a wide toxicity range under non-activation conditions. It was not activated by S9 microsomes to mutagenic products detectable in the assay. Concentrations up to a highly toxic level of 7.5 µl/ml were assayed - 10 µl/ml was lethal.

Mouse Lymphoma Forward Mutation Assay. [redacted] Report dtd. Oct. 1980. Test Material Lot 072380.
Signed: [redacted] Ph.D.; [redacted] Ph.D.

Extraction - 5 gm of white solid foam polymer with attached "cloth" ribbon per 60 ml of Fisher's medium shaken 5 days at 37°C. Medium was removed by squeezing and then filtered.

Concentrations greater than 6.25 µl/ml were completely toxic. The extract was considered to be inactive in the Mouse Lymphoma Forward Mutation Assay. Significant changes in the mutant frequency at the TK locus were not induced in L5178Y mouse lymphoma cells. 10 µl/ml was highly toxic in the presence and absence of rat liver S9 metabolic activation.

Microbiological Studies.
[redacted] Jan. 1981. Drs. [redacted].

Expt. 1 - Staph aureus and Staph epidermis showed no growth in undiluted solutions of 100% nonoxynol-9 and a water/spermicide/preservative mixture (25% nonoxynol-9) used to manufacture contraceptive sponges.

Expt. 2. - Undiluted solutions as above did not support growth of E. coli, Staph aureus, Candida albicans, Pseudomonas aeruginosa, Aspergillus niger and Toxic Shock Staph aureus at test periods of 24 hrs. 7 days and 14 days.

Expt. 3 - The second expt. was repeated following the USP XX Microbial Tests "51" protocol (modified). The water/spermicide/preservative mixture did not support the growth of any of the test organisms.

Expt. 4. - The contraceptive sponge innoculated with each of the test organisms of expt. 3 did not support the growth of any of them. Aspergillus niger was reduced from ca. 2×10^5 at Day 0 to about 70 colonies at day 21, to 5 colonies at day 28.

Expt. 5. - Cultures from swabs of 3 vaginal sites in 5 women prior to and immediately after wearing the Collatex Contraceptive Sponge for 2 days (also from a sample of the sponge) indicated that the sponge did not significantly alter the microbial environment of the vagina.

Animal Intravaginal Studies (Summaries Only)
Dr. [redacted]

Rats - 28 Day Retention. No controls and no. animals studied was not given. Small tampons were fashioned from sponges containing nonoxynol-9. Stainless steel sutures were used to close the vaginal opening, delaying (but not totally preventing) expulsion of the test material. Rats gnawed the sutures in many cases.

Rats retained the sponge for weekly periods up to 28 days. No vaginal irritation was evident, but flattening of the vaginal mucosal surface rugae was typical. Occasionally, small amounts of white or yellow exudate was noted.

Monkey - (one female rhesus)

No attempt was made to avoid menstrual cycles. The sponge remained in place through one menstrual cycle. The monkey retained the sponge for up to 32 days without removal. No unusual morphology was found upon biopsy of the vagina.

Note - For the rat and monkey the pathologist reported that the slides were very difficult to interpret because of the manner in which specimens and sections were taken.

Monomeric Residues.

Any polymeric product in contact with the body raises the concern of release of monomers which are residual products from polymerization or prepolymers.

It is reported that theoretically the following may be found in the finished contraceptive sponge: 2,4- and 2,6-toluene diisocyanates (TDI) and their hydrolysis products, 2,4- and 2,6-toluene diamines (TDA); trimethylolpropane (TMOP); polyethylene glycol 1000 (PEG); 4,4'-methylenebis (cyclohexylisocyanate) and its hydrolysis product, 4,4'-methylenebis (cyclohexylamine).

2,6-TDA has been reported by the National Cancer Institute to be not carcinogenic. Of concern is 2,4-TDA which was analyzed as a component of hair dyes by Ames and Burnett in 1975 and found to be a potential mutagen. Further reports indicate that topical application to mice for 18 months at levels formerly used in hair dyes caused no adverse effects. NIOSH has listed 2,4-TDA as a suspected carcinogen.

According to [redacted]. (vol. 1.3 p 249), improvements in the manufacturing process and in the formulation of [redacted] prepolymers have lowered the typical TDA content of [redacted] foams to less than one part-per-million and further that [redacted] grades [redacted] (used in the manufacture of the current Secure Vaginal Contraceptive Sponge) have been modified to generate no TDA on foaming.

They state that a newly-developed analytical method (based on analyses by High Pressure Liquid Chromatography) which allows the detection of as little as 50 parts-per-billion of TDA so far has been unable to detect TDA in [redacted] foams prepared from [redacted] under recommended conditions.

Foreign Data: No foreign preclinical studies are present in this application.

Quality Assurance - Statements are present for recent studies.

Comments and Evaluation:

The Secure Contraceptive Sponge, measuring ca. 2.5 x 5.5 cm, is composed primarily of a polyurethane foam . in which ca. 30% (1 gm ± 15%) of the nonionic surfactant nonoxynol-9 (N-9) by dry weight is incorporated during the manufacturing process. The amount of nonoxynol-9 is not as excessive as it might seem since N-9 plays a dual role i.e. not only as a spermicide but also due to its surfactant property, as an agent needed for foaming of the urethane material. Other minor components and the polyester scrim and woven polyester tape molded into the edge of the sponge to serve as a removal strip would not be expected to cause any toxicity problems. Possible problems of the originally proposed wash and reuse product would appear to be avoided with the current single use recommendation. (Coitus, however, can occur multiple times during the recommended two day wearing period.)

Due to the nature of the product, the preclinical studies varied somewhat from the usual pharmacology and toxicology studies.

In-vitro spermicide elution studies showed spermicidal effectiveness of the 30% Nonoxynol-9 sponge to be maintained up to the 20th physiological saline rinse. Although about 50% of the spermicide is washed from the sponge after five soak/squeeze cycles only about 53% is removed after 20 rinses. The release rate appears to be governed by an interaction between the urethane polymer and nonoxynol-9 during manufacture. Total recovery of N-9 including chloroform extractable material was in the range of 96%.

The standard USP toxicity tests for the urethane sponge with and without nonoxynol-9 gave results about as expected. Eluates of the sponge killed all mice in the acute systemic injection test and intracutaneous reactivity was marked at injection sites in rabbits. Moderate tissue reaction followed 7 days i.m. implantation in rabbits. Eluates were toxic to WI-38 cells in tissue cultur The urethane foam without nonoxynol-9 showed no toxicity or significant differences from controls in these tests.

The polyester woven mesh scrim (same compound as the retrival loop), impregnated into the sponge to add strength, when studied by the first 3 tests above gave results comparable to that of controls.

The skin irritation tests in rabbits and human subjects in general gave responses of a low order of irritation. The acute dermal toxicity test in rabbits showed a decrease in activity but no deaths.

In-vitro microbiological studies carried out on sponges fresh from the package and on those worn for 2 days as well as solutions of the water/spermicide mixture used in the manufacture of the Secure sponges showed no support for growth of various test organisms. Cultures from vaginal swabs of users of the sponge indicated that the sponge did not significantly alter the vaginal microbial environment.

Originally there was concern over the fact that urethane foams were reported to contain fraction components of low molecular weight polymeric diamines which might possess a carcinogenic potential. The 2,4 toluene diamine has been reported by NCI as not carcinogenic and the 2,6 toluene diamine reported by NIOSH as a suspected carcinogen had not shown a carcinogenic potential following topical application to mice for 18 mos. at levels used in hair dyes. In addition improvements in the formation of prepolymers have further decreased the probability of toxicity in the final product and newer analytical methodology (High Pressure Liquid Chromatography), sensitive to 50 ppb of TDA, has not shown any TDA in the foam.

The current [redacted] prepolymer is reported to differ from the previous [redacted] in that it has a lower order of magnitude level of isocyanate, thereby substantially decreasing any TDI (toluene diisocyanate) or their hydrolysis products, TDA monomer residuals. Therefore, the newer [redacted] prepolymer would not be expected to cause any problem of carcinogenicity.

Due to concerns over any potential problems of mutagenicity caused by toluene diisocyanate or toluene diamine residues or to any other substances, Ames Salmonella, Microsome Mutagenesis and Mouse Lymphoma Assays were carried out. These tests were performed on three lots of VLI Corporation production and according to the sponsor ranged in age from 3 years to recent production.

The eluates of the nonoxynol-9 containing sponges were non-mutagenic in the Ames test when tested by 2 different firms.

Originally a Mouse Lymphoma Assay by [redacted] (Pharm. Rev. of IND 16,222 dtd. 4/10/79 by Dr. V. Berliner) utilizing a foam extract had shown a slight dose-related increase in mutation frequency in the activation series (evaluated by [redacted] as not inducing significant forward mutation under the conditions of the assay). This study was not included in the NDA, however, the material used ([redacted]) had a slightly different chemical composition than the [redacted] used in the present sponge. Two additional Mouse Lymphoma Assays by [redacted] and one by [redacted] did not show extracts of the sponge to be mutagenic.

As realized by the sponsor, the vaginal irritation studies carried out in only one rhesus monkey which carried a tampon made from the sponge for 32 days and the study in rats (sponge had to be retained by sutures) for up to 28 days are of relatively little value in comparison to the clinical usage. No unusual morphology was reported, however, in the opinion of the pathologist slides were difficult to interpret due to their poor quality.

Labeling - From the standpoint of Pharmacology, labeling appears satisfactory.

Conclusions:

Nonoxynol-9 has been used as a spermicide for a number of years and its safety for use in vaginal contraceptives has been established. The Federal Register, Vol. 45, No. 241, December 12, 1980 re Vaginal Contraceptive Drug Products for Over-The-Counter Human Use regards the nonionic surfactant Nonoxynol-9 as a Category I vaginal contraceptive safe and effective for OTC use.

The primary role of the sponge is as a carrier of nonoxynol-9 whose role is unique in that as a surfactant it contributes to the structure of the foam sponge as well as being a spermicide whose potency is influenced by its reactivity with the sponge.

The extensive investigations of the functional and toxicological properties of the Secure Contraceptive Sponge did not indicate any toxicological problems which would preclude its use as a vaginal contraceptive.

Recommendation: From the standpoint of Pharmacology, approval of this application can be recommended.

cc:
Original NDA 18-683; HFD-180
Trip NDA 18-683; HFD-130 NDA 18-683
HFD-130 Pharmacology; HFD-130 DHertig

D. Hertig
David H. Hertig
Pharmacologist

V Berliner

4 November 1981

Pharmacology Input to Institutional Summary Basis of Approval for Secure Contraceptive Sponge NDA 18-683

Prepared by: D. Hertig
Approved by: V. R. Berliner, Ph.D.
Supervisory Pharmacologist

IV. Pharmacology

A. Pharmacological Profile

Secure Contraceptive Sponge for use as a vaginal contraceptive is composed primarily of a polyurethane foam in which 30% (ca. 1 gm) of the nonionic surfactant nonoxynol-9 by dry weight is incorporated during the manufacturing process.

The pharmacological profile of nonoxynol-9 is well known, its having been classified as a Category I vaginal contraceptive safe and effect for OTC use (The Federal Register, Vol. 45, No. 241, December 12, 198(re Vaginal Contraceptive Drug Products for Over-The-Counter-Human Use)

B. Toxicity Tests

Due to the nature of the product, the preclinical studies varied somewhat from the usual type pharmacology and toxicology studies.

1. In-vitro spermicide elution studies.
   Spermicidal effectiveness of the 30% nonoxynol-9 sponge was maintained up to the 20th physiological saline rinse.

2. Standard USP toxicity tests for plastic materials.
   Eluates of the sponge
   a. Acute systemic i.v. injection test - killed all mice.
   b. Intracutaneous reactivity - was marked at injection sites in rabbits.
   c. I.M. Implantation in rabbits - after 7 days, marked tissue reaction.
   d. Toxicity to WI-38 cells - toxic in tissue culture.

The urethane foam without nonoxynol-9 showed no toxicity or significant differences from controls in these tests.

The polyester scrim (same substance as the retrival loop) impregnated into the sponge to add strength, gave results comparable to controls in tests a,b,c above.

3. Skin Irritation Tests - in rabbits and human subjects in general gave responses of a low order of irritation.

4. Acute Dermal Toxicity - (application of 20 ml/kg eluate produced by soaking sponge in water for 18 hrs. - occlusive coverings removed after 24 hrs. with 14 days observation) tests in rabbits showed a decrease in activity but no deaths.

5. In-vitro microbiological studies. Tests carried out on sponges fresh from the package and on those worn for 2 days as well as solutions of the water/spermicide mixture used in the sponge manufacture showed no support for growth of various test organisms.

   Cultures from vaginal swabs of users of the sponge indicated that the sponge did not significantly alter the vaginal microbial environment.

6. Mutagenicity Tests

   a. Ames Test

      The eluates of nonoxynol-9 containing sponges were non-mutagenic when tested by 2 different firms.

   b. Mouse Lymphoma Tests.

      Three assays by two different firms did not show extracts of the sponge to be mutagenic.

7. Vaginal Irritation Studies.

   Monkey - One rhesus monkey carried a tampon made from the sponge for 32 days.

   Rats - Sponges were retained up to 28 days.

No unusual morphology was reported for either species.

THE UNIVERSITY OF ILLINOIS AT CHICAGO

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(312) 996-0888

2 May 1986

Ms. Lee Ripper
Consumer Safety Officer
Office of Biologics Research and Review
Center for Drugs and Biologics
Food And Drug Administration
HFN-310, Rm 14B03
5600 Fishers Lane
Rockville, MD 20857

Dear Ms. Ripper,

Thank you for arranging a meeting on 12 May 1986 at the FDA to discuss the toxicology testing requirements for initiating clinical trials for an acrosin inhibitor incorporated into a Today sponge. The meeting will be attended by myself, Dr. L.J.D. Zaneveld (the investigator who is developing the acrosin inhibitors as a vaginal contraceptive and co-investigator), Dr. Alfredo Goldsmith (Program for Applied Research on Fertility Regulation which funds the acrosin project) and Jeffrey M. Spieler (AID).

Our letter of 5 September, 1985 outlined proposed preclinical toxicology testing for our new vaginal contraceptive product. These tests included a 30 day subchronic toxicity test using rats and a ten day vaginal irritation test using rabbits. We now find it difficult to perform the above test using simple dosing techniques for the vaginal route of exposure for both of the above tests.

The vagina of both the rabbit and the rat expell the sponge device within 24 hours. This results in an indefinite exposure period. We are aware of the reported procedures for retention of the sponge device in the vagina which include stainless steel sutures to close the vagina of the rat and umbilical tape around the vagina of the rabbit to create a stricture. We have tried these procedures and found them to be less than satisfactory. We would like to propose the following changes from the studies in our previous letter.

30 DAY SUBCHRONIC TOXICITY TEST IN RATS

Two routes of administration were proposed. The intraperitoneal route presents no problems and can be performed using normal testing procedures. The vaginal administration of the sponge on a daily basis will present problems in determining the exact exposure of the animal to the sponge containing an acrosin inhibitor. The sponge in a rat is retained for several hours, but is consistently expelled prior to the usual 24 hour dosing period. The suturing of the vaginal opening prevents daily administration of the sponge material but causes stress and discomfort to the animal.

We are proposing to administer the acrosin material on a daily basis as a gum acacia suspension to allow administration of accurate doses at multiples of the expected exposure level. This will insure maximal doses of the acrosin inhibitor and provide a good indication of potential problems related to systemic exposure after vaginal administration and direct contact of the vaginal tissue with the acrosin inhibitor.

VAGINAL IRRITATION IN THE RABBIT

The rabbit vagina also expels the sponge. We have tried different sizes and shapes of sponges without success. Making a stricture in the vagina using umbilical tape has been successfully performed. However, this procedure will not permit replacement of the sponge at daily intervals and requires abdominal surgery.

We would like to perform the vaginal irritation study using rabbits with sponges placed directly in the vagina without any special procedures to insure retention of the sponge. The rabbits will be observed at two hour intervals for the first twelve hours to insure retention of the sponges during this period. Sponges will be replaced each morning. All sponges which remain in the vagina will be removed prior to the insertion of new sponges. A short piece of suture material will be attached to the sponge to facilitate removal of any remaining sponges. The daily replacement of the sponges will continue for 10 days. Three groups will be tested. One group will be administered sponge material only and the remaining groups will be administered sponges containing one of two concentrations of the acrosin inhibitor. It is anticipated that no more than twice the concentration of the acrosin inhibitor to be used in the clinical trials can be incorporated into the sponges due to manufacturing limitations.

These dosing schedules represent testing to provide maximal information on potential toxic effects of the new acrosin inhibitors used as vaginal contraceptives in a Today sponge.

We look forward to discussions with you next week.

Sincerely,

Donald P. Waller, Ph.D., D.A.B.T.
Associate Professor of Pharmacology

Pre-IND
4'-acetamidophenyl 4-guanidinobenzoate (AGB)

May 12, 1986

MEMORANDUM OF MEETING

Representing the Sponsor:

Donald P. Waller, Ph.D., Associate Professor, Section of OB/GYN, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois
Lourens J.D. Zaneveld, D.V.M., Ph.D., Professor and Director, Section of OB/GYN, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois
Alfredo Goldsmith, M.D., PARFR, Northwestern University
Jeffrey M. Spieler, AID

Representing the Food and Drug Administration:

Solomon Sobel, M.D., Director, DMEDP
Alex Jordan, Ph.D., Supervisory Pharmacologist
David Hertig, Pharmacologist
Al Weiner, M.D., Medical Officer
Lee Ripper, Consumer Safety Officer

Background: See submission dated May 2, 1986.

Discussion: The visitors explained that AGB is less spermicidal than nonoxynol-9 instead acting by inhibition of the sperm enzyme acrosin which is needed for fertilization. AGB binds to acrosin and is carried up into the uterus/tubes. With its acrosin inactivated, the sperm cannot penetrate the zona pellucida. AGB is to be incorporated into the Today sponge. Although the N-9 can be omitted from the sponge, the resultant product is not as "spongy." Also the N-9 seems to somehow aid release of AGB, perhaps because the AGB is solubilized by N-9 or because the conformation of the sponge is different. In response to an FDA question about possible chemical reaction between N-9 and AGB, the sponsors replied that neither seems to affect the activity of the other. FDA noted that they should confirm that there is no chemical reaction between the two.

Currently they are experiencing difficulties in doing vaginal irritation studies. If they place small stringless sponges in rabbits without strictures, a high percentage will retain the sponges for 12 hours before spontaneously expelling them. But when they add a removal string the animal pulls it out. If strictures are used, the sponges cannot be changed daily; if not used, retention time cannot be guaranteed. Therefore, FDA agreed that the firm could conduct their vaginal administration studies using a gum acacia suspension of AGB; it will also be used for the IP studies. Using gum acacia suspension, the drug can be administrationed at about a hundred-fold dose of the sponge.

The Phase I post-coital clinical trial on sterilized women will compare the Today sponge versus the sponge with AGB only versus the Today sponge plus AGB. The sponge will be in the vagina about 4 hours with a maximum of 6-12 hours. Measurements will include AGB both in vaginal fluid and remaining in the sponge, and number of sperm in cervical mucus. If a sufficient number of live sperm are found, a hamster egg penetration test will be done.

Conclusions: For Phase I FDA will accept the 30-day subchronic study in rats [3 groups (N-9 alone, AGB + N-9 combination, AGB alone) X 2 routes of administration (IP and intravaginally)] plus what rabbit data they already have. However, to completely meet the two species by two routes preclinical guidelines, a rabbit IP study must be completed, and the results submitted and reviewed before Phase II may begin. FDA noted that because of our combination policy, sponsors must show that all drugs in a combination contribute to the product's activity. Also, FDA is interested in how the sponge without AGB will perform compared to the N-9 sponge + AGB because of our desire for a vaginal contraceptive product that causes less irritation than does N-9.

Lee Ripper

Lee Ripper
June 17, 1986

TOXICOLOGY RESEARCH LABORATORY
UNIVERSITY OF ILLINOIS AT CHICAGO
HEALTH SCIENCES CENTER
CHICAGO, IL 60680

A. Study Identification

1. Title — 30-Day Subchronic Toxicity Test of 4'-Acetamidophenyl 4-guanidinobenzoate hydrochloride(AGB) in rats.

2. Objective — Evaluate potential toxicity of AGB in rats after vaginal and intraperitoneal administration for 30 days .

3. Study Number — 0023

B. Study Management

1. Testing Laboratory — Toxicology Research Laboratory
University of Illinois at Chicago
1940 W. Taylor
Chicago, IL 60612

Liaison — Edmund G. Anderson, Ph.D.
Director of TRL & Head,
Department of Pharmacology
University of Illinois at Chicago
College of Medicine
835 S. Wolcott St.
Chicago, IL 60612

Study Director — Donald P. Waller, Ph.D.
Department of Pharmacodynamics
College of Pharmacy
University of Illinois at Chicago
833 South Wood St.
Chicago, IL 60612

Pathology — Pathology Associates, Inc.
10075 Tyler Place
Hyatt Park II
Ijamsville, Maryland 21754

Histology — Pathology Associates, Inc.
10075 Tyler Place
Hyatt Park II
Ijamsville, Maryland 21754

2. Sponsor — Program for Applied Research in Fertility Regulation(PARFR)
Northwestern University
Diane Krier-Morrow
Tel (312) 644-0085

C. Proposed Schedule

1.	Arrival date	21 September 1986
2.	Starting date	28 September 1986
3.	Completion	28 October 1986
4.	Final Report	7 November 1986

D-1. Test Article Identification

1. Name: 4'-Acetamidophenyl 4-Guanidinobenzoate Hydrochloride(See attachment I)

D-2. Test System Identification

1.	Species	Rat
2.	Strain/substrain	Sprague Dawley
3.	Sex	Female
4.	Number	200
5.	Source	Charles River
6.	Age	9 weeks
7.	Body weight range	175-200 g
8.	Identification per SOP	:ANM008
9.	Randomization per SOP:	TXG072
10.	Housing	BRL Room:
11.	Quarantine period per SOP No.:	ANM003
12.	Husbandry per SOP No.:	BRL Standard Protocol
13.	Food and Water	ad libitum

E. Experimental Design

1. Study design

AGB is a potential vaginal contraceptive. This study is to assess potential toxicity of systemically absorbed AGB after short term repeated exposure. Rats will be administered AGB suspended in gum acacia for 30 consecutive days using both the intraperitoneal and vaginal routes of administration. On day 31 the animals will be sacrificed, organs removed and fixed for histopathological examination.

2. Method of Administration and Justification

Both the intraperitoneal and vaginal routes of administration will be utilized. The peritoneal route will insure absorption and systemic exposure of the rats to AGB. The vaginal route of administration is the route of administration to be used in humans.

3. Dosing

All dosing will be performed between the hours of 0800 and 1100 hours for 30 consecutive days.

Intraperitoneal Administration(TXG014): Each rat will be administered a volume of 0.5 ml/100 g body weight of one of four dosing preparations to achieve a dose of 35, 70 and 140 mg/kg of AGB/kg body weight.

Vaginal Administration: An eye dropper will be filled with 0.2 ml of the substace to be administered. The rat will be picked up and turned over on its back, the eye dropper inserted gently into the vaginal orifice and its contents expelled. The rat will be held in the supine position for 30 seconds following the administration and then returned to its cage. A clean eye dropper will be used for each animal.

4. Test Substance: The test substance will be provided by Dr. L.J.D. Zaneveld. Receipt for the material will be in accordance with SOP MAT001. Storage and labeling will be according to MAT002 and MAT003. The material will be dispensed per SOP MAT004. A test/Control Article Fact Sheet will be completed according to SOP MAT005.

5. Animals

a. The care, maintenance, and housing of animals will be per SOP ANM002.

b. Animals will be group-caged for this test and the number of animals per cage will not exceed three.

c. Animals will be randomized into groups per CMP003.

6. Experimental design

Two hundred mature female rats will be allowed three days after arrival for acclimatization. Each animal will then be weighed(TXG022) and identified (ANM005) prior to quarantine(ANM003). On the eighth day the animals will be weighed and randomly divided into six groups per SOP TXG072. The number of animals in each group, the route of administration and the preparation to be dosed are as follows:

Group 1--(30 animals) Administered vaginally 0.2 ml of a 5% solution of gum acacia.

Group 2--(30 animals) Administered vaginally 0.2 ml of a 5% gum acacia suspension containing 10 mg/ml of AGB.

Group 3--(30 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension.

Group 4--(30 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 7 mg/ml of AGB.

Group 5--(40 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 14 mg/ml of AGB.

Group 6--(40 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 28mg/ml of AGB.

All animals will be weighed per SOP TXG022 and observed daily per SOP TXG028. On day 31 after the first dose of AGB or vehicle control the animls will be sacrificed by carbon dioxide asphyxiation(TXG024).

a. Gross Necropsy-- All test animals will be subjected to complete gross necropsy per SOP PTH001. Tissues will be processed per SOP PTH002, fixed in 10% buffered formalin and sent to the histologist, per SOP PTH003.

b. Histopathology---All gross lesions will be examined microscopically. In addition, for animals in the control and dosing groups and all animals which die during the study: Brain(at least three levels), spinal cord (at least 2 levels), eye, pituitary gland, mammary gland, esophagus, lung (with mainstream bronchi), trachea, liver, stomach, small and large intestine, spleen, kidneys, thymus, thyroid, parathyroid, adrenals, pancreas, urinary bladder, heart, aorta, ovaries, complete reproductive tract including fallopian tubes, uterine horns and vagina, a representative lymph node, sternum, and sciatic nerve with skeletal muscle will be observed.

F. Experimental Evaluation

Evaluation of the slides obtained from the procedures described above will be made Pathology Associates, Inc.(PAI). PAI will submit the report to TRL within 14 days from receipt of tissues.

G. Data Retention (inclusion in the Archives)

1. Records

a. Protocol and any addendums attached to it.

b. Standard Operating Procedures; ADM011, ADM013, ADM017, ANM002, ANM003, ANM005 ANM008, CMP003, MAT001, MAT002, MAT003, MAT004, MAT005, PTH001, PTH002, PTH003, QAU002, QAU003, QAU004, QAU005, TXG006, TXG014, TXG022, TXG024, TXG028 and any addendums or changes made to them during the course of the study.

c. Laboratory notebook

A separate laboratory notebook will be maintained for the study. The laboratory notebook for this study will be divided into three parts.

I. Initial Information

This part of the notebook will contain data which deals with the initiation of the study.

a) Animal ID and History Record (TRL012-2.84): This form will contain general information about the animal which will be listed in identification number order. Included will be all the information required by the appropriate SOP as specified in section E-3.

b.) Animal Randomization Sheet (TRL053-09.86): This form documents the randomization of the animals as described in D-2, 9. It will list the animals by group, ID#, body weight (g), the mean weight of each group, the standard deviation within the group and the analysis of variance for the groups.

II. Daily Information

Daily information will include the observations made of the animals on a day to day basis.

a) General Observation Sheet (TRL007-12.83): Per SOP No.: XG006. This form will be filled out twice daily, once at approximately 0700 hours and at approximately 1900 hours. The animals will be observed for general condition. All the animals in the study will be placed on one General Observation Sheet.

III. Weekly Information

Clinical Observation: Clinical evaluations will be performed once each week according to SOP TXG028 and recorded on the clinical observation form(TRL 007).

IV. Concluding Information

The Concluding Information section will contain the information on the clinical observations of the animals and the necropsy forms.

a) Necropsy Forms (TRL010-12.83 & TRL011-12.83): These forms will be completed for each animal as they are necropsied. Specifically, they concern the general observations at necropsy, the formation of any masses or lesions and the tissues collected for histopathology. The latter form is submitted to the pathologists with a copy kept in the raw data files until such time that the form is completed by the pathologist.

d. Other Raw Data

Raw data other than the laboratory notebooks will be maintained in the archives. This will include tissue specimens not used in histology (both wet specimens and tissue blocks), correspondence directly relating to the study and any other data the administration of TRL deems relevant to the study.

e. Equipment Records

Equipment records will be kept detailing the calibration and use of the balance used in this study.

2. Archive storage

a. All records pertaining to this study will be kept in the archives located in Room 409 of the MSA building, University of Illinois. Archive records will be maintained per SOP No.: ADM013.

3. Final Report

The final report will follow guidelines in SOP No.: ADM017.

H. Conditions and Monitoring

1. QAU
The QAU will monitor the study as instructed in SOP No.: QAU004. The records will be maintained in accordance with SOP Nos.: QAU002, QAU003 and QAU005.

2. Amendments to the study will be fully documented in accordance with SOP No.: ADM011.

3. Sponsor audits will be permitted at the request of PARFR with at least one day notification.

I. Protocol Approval

Prepared By: _____ 22 Sep 86
D.P. Waller, Study Director DATE

Reviewed By: _____ Sept 22, 1986
J.C. Pena, Assistant Director, TRL DATE

Reviewed By: _____ Sep 24, 1986
B.T. Bennett, Director of BRL DATE

Reviewed By: _____
R.C. Schoenbeck, QAU Officer DATE

Approved: _____
E.G. Anderson, Program Director, TRL DATE

Toxicology Research Laboratory
University of Illinois at Chicago

DEVIATION FROM PROTOCOL/SOP

==========

Study Number: 0023 Date Requested: 14 October 1986
Title: 30-Day Subchronic Toxicity Test of 4'-Acetamidophenyl 4-guanidinobenzoate hydrochloride(AGB in rats.

Study Director: Donald P. Waller

Request By: Donald P. Waller

Prepared By: Donald P. Waller (Name) Study Director (Title)

Nature of Deviation: The current protocol divides two hundred animals into six groups:

Group 1--(30 animals) Administered vaginally 0.2 ml of a 5% solution of gum acacia.

Group 2--(30 animals) Administered vaginally 0.2 ml of a 5% gum acacia suspension containing 10 mg/ml of AGB.

Group 3--(30 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension.

Group 4--(30 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 7 mg/ml of AGB.

Group 5--(40 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 14 mg/ml of AGB.

Group 6--(40 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 28mg/ml of AGB.

The number of animals will be increased to two hundred and fifty and the dosing groups will be increased to ten dosing groups as follows:

Group 1--(20 animals) Administered vaginally 0.2 ml of a 5% solution of gum acacia.

Group 2--(25 animals) Administered vaginally 0.2 ml of a 5% gum acacia suspension containing 10 mg/ml of AGB.

Group 3--(25 animals) Administered vaginally 0.2 ml of a 5% gum acacia suspension containing 10 mg/ml of AGB and 15% nonoxynol-9.

Group 4--(20 animals) Administered vaginally 0.2 ml of a 5% gum acacia suspension containing 15% nonoxynol-9.

Group 5--(20 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension.

Group 6--(30 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 7 mg/ml of AGB.

Group 7--(30 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 14 mg/ml of AGB.

Group 8--(35 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 28mg/ml of AGB.

Group 9--(25 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 7 mg/ml of AGB and 10% nonoxynol-9.

Group 10--(20 animals) Administered intraperitoneally 1 ml/200 grams of body weight a 5% gum acacia suspension containing 10% nonoxynol-9.

Attachment: NO

Reason for Request: The six dosing groups in the protocol were determined according to the original proposal to the sponsoring agency. However, at a subsequent meeting with the FDA attended by myself, Dr. L.J.D. Zaneveld, Dr. Alfredo Goldsmith(PARFR) and Jeffrey Spieler(AID) it was suggested by the FDA that additional groups be added to examine the effects of AGB in combination with nonoxynol-9. The revised groups of animals are to comply with the above suggestions.

Attachment: NO

==========

ACTION:

Approved: X Date in Effect: 14 Oct 1986

Denied: _____

STUDY DIRECTOR: _____

==========

Reason for Action (if different from above): _____

_____

_____

Attachment: ____yes X no

---

cc:
Program Director date sent: 10/14/86
Sponsor date sent: 10/14/86

TRL019-3.84

PAI Pathology Associates, Inc.

10075 Tyler Plac
Hyatt Park II
Ijamsville, Maryland .
(301) 663-1644

PATHOLOGY REPORT
FOR

30-DAY SUBCHRONIC TOXICITY TEST OF 4'-ACETAMIDOPHENYL 4-GUANIDINOBENZOATE HYDROCHLORIDE (AGB) IN RATS

STUDY NO. 0023

DRAFT

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I. PATHOLOGY NARRATIVE

PATHOLOGY REPORT

30-DAY SUBCHRONIC TOXICITY TEST OF 4^1-ACETAMIDOPHENYL 4-GUANIDINOBENZOATE HYDROCHLORIDE (AGB) IN RATS

INTRODUCTION

This is the final pathology report by Pathology Associates, Inc. (PAI) to the Toxicology Research Laboratory, University of Illinois at Chicago, representing the support required to conclude the study designated, "30-Day Subchronic Toxicity Test of 4^1-Acetamidophenyl 4-Guanidinobenzoate Hydrochloride (AGB) in Rats," as described in the protocol for Study No. 0023.

EXPERIMENTAL DESIGN AND METHODS

Two hundred fifty 9-week old female Sprague Dawley rats were divided into 10 dose groups and administered AGB and/or nonoxynol-9 suspended in 5% gum acacia (vehicle) for 30 consecutive days using the intraperitoneal (IP) or vaginal routes of administration, according to the following protocol:

GROUP	NO. OF RATS	ROUTE	TREATMENT
1	20	Vaginal [1]	Vehicle Only
2	25	Vaginal [1]	AGB, 10mg/ml
3	25	Vaginal [1]	AGB(10mg/ml) and nonoxynol-9(15%)
4	20	Vaginal [1]	nonoxynol-9 (15%)
5	20	IP [2]	Vehicle Only
6	30	IP [2]	AGB (7mg/ml)
7	30	IP [2]	AGB (14mg/ml)
8	35	IP [2]	AGB (28mg/ml)
9	25	IP [2]	AGB(7mg/ml) and nonoxynol-9(10%)
10	20 [3]	IP [2]	nonoxynol-9 (10%)

1. 0.2 ml administered vaginally
2. 1 ml/200 grams of body weight administered intraperitoneally
3. Only 19 animals were received.

On day 31 after the first treatment, the rats were sacrificed by carbon dioxide asphyxiation and necropsied. Tissues were fixed in 10% neutral buffered formalin, processed, and evaluated microscopically.

Microscopic findings are presented by dose/sex group with all diagnoses for individual animals in the Tabulated Animal Data Tables (Section III). The codes used as entries in these tables are explained in the Reports Code Table (Appendix 1), while topographic/morphologic abbreviations are explained in the Abbreviations List (Appendix 2). Microscopic findings for all groups are summarized in the Project Summary Tables (Section II). Tabulated data in Section II are divided by route of administration, with groups 1-4 (vaginal) presented separately from groups 5-10 (intraperitoneal). Microscopic findings are correlated with gross lesions, if possible, in Section IV (Correlation of Gross and Microscopic Findings).

RESULTS

No dose group related lesions were noted microscopically in the vaginal treatment groups. In group 2 a slight increase in the number of kidneys showing mineralization was noted, but this is not considered to be biologically significant.

Numerous microscopic changes were noted in all of the groups given intraperitoneal injections. In group 5 (vehicle only) treatment-related changes were consistently noted in the liver, with 20/20 rats having hepatocellular cytoplasmic vacuolization and 16/20 rats having foci of subacute inflammation in the livers. Peritonitis (inflammation of the capsular or serosal surface of abdominal organs) was noted in eight rats in group 5. In each case, the inflammation was localized to one or two organs or segments of the intestine.

In groups 6-10 peritonitis was noted in all but one animal (Gp 8 - #39). In most instances numerous organs, particularly the liver and spleen, were affected. In only a few rats was the inflammation limited to small foci in the mesentery, around the pancreas, or on the serosa of one or two abdominal organs. Hepatocellular cytoplasmic vacuolization was seen in nearly all of the animals in groups 6-10.

In the kidneys, dose-group associated changes were noted in groups 6, 7, 8, and 9, and consisted of changes in the renal tubules and/or interstitium. Chronic nephropathy was diagnosed in 14/30, 21/30, 20/35, and 11/25 rats in groups 6, 7, 8, and 9, respectively. In addition, renal tubular regeneration was diagnosed in 8/30, 3/30, 4/25, and 7/25 rats in groups 6, 7, 8, and 9, respectively. No similar changes were present in groups 5 or 10.

Extramedullary hematopoiesis (EMH) was diagnosed in the spleens of some rats in groups 7 through 10, but not 5 and 6. This was attributed to the more severe peritonitis with associated hemorrhage in some rats in these groups.

Interstitial inflammation was present in the lungs of 80-100% of the rats in each of the ten groups and represents infection by adventitious agents, probably viral.

DISCUSSION

The most numerous and striking changes noted at necropsy were lesions on the surface of abdominal organs and within the mesentery. These consisted primarily of thickened, white membranes over some or all surfaces, particularly the liver and spleen, adhesions between liver lobes or between organs, and masses in the mesentery or attached to abdominal organs. These changes correlated well with the peritonitis in groups 5-10, and particularly in groups 6-10. In addition to the chronic inflammation and fibrosis on the serosal surfaces, inflammation was also seen in deeper portions of some tissues, particularly segments of the bowel. In several cases, inflammation in the submucosa or mucosa indicated possible perforation of the bowel during intraperitoneal administration. Abscesses, multifocal chronic inflammation and hemorrhage, particularly within the mesentery, and inflammatory or hyperplastic changes in the mesenteric lymph nodes in response to the peritonitis accounted for the masses noted at necropsy. As a part of the chronic inflammation, numerous granulomas or multinucleated giant cells were present. Many contained refractile material. It is obvious that while some peritonitis resulted from intraperitoneal administration of 5% gum acacia alone, the peritonitis was potentiated by either AGB alone, nonoxynol-9 alone, or the two chemicals together.

Liver changes were noted in most rats from all groups treated intraperitoneally but not vaginally. The most striking microscopic change consisted of hepatocellular vacuolization. In most cases, the affected hepatocytes were swollen by numerous tightly-packed, small, round vacuoles. In some livers, the cytoplasm of affected hepatocytes contained no distinct vacuoles, but instead, the cytoplasm was relatively clear with a stippling of organelles. In the more severely affected livers, the diffuse cytoplasmic swelling caused marked distortion of the liver lobes. While all of the livers in group 5 had vacuolar change, few liver lesions were noted at necropsy. In groups 6 through 10, however, most rats were noted to have swollen livers with blunt edges or pale livers. In these groups, the thickened capsules, adhered lobes, and swollen hepatocytes distorted many livers markedly.

Because rats from all groups treated intraperitoneally had hepatocellular vacuolar change, it can be inferred that the change was due in part to either the procedure or to the 5% gum acacia vehicle. It is possible that the vacuolization represents toxic change resulting from metabolism of the intraperitoneally administered material and that the material administered vaginally was not absorbed to the same degree. The subacute inflammation diagnosed in 16/20, 7/30, 1/30, and

2/25 rat livers in groups 5, 6, 7, and 9, respectively, consisted of tiny foci of mixed inflammatory cells, usually in groups of ten cells or less. The significance is not known.

The chronic nephropathy diagnosed in rats in groups 6, 7, 8, and 9 consisted of renal tubular regeneration, interstitial inflammation, and fibrosis primarily within the cortex or near the cortico-medullary junction. These changes were not present in rats in other groups. In addition, less severe changes, probably of similar origin, were present in these same four groups given AGB in 5% gum acacia intraperitoneally. These consisted of renal tubular regeneration alone or interstitial inflammation alone. Tubular regeneration was present only in rats from groups 6, 7, 8, and 9 with the exception of one rat in group 3. The presence of these renal changes in rats given AGB intraperitoneally but not vaginally indicates that AGB causes renal toxicity, but is not absorbed in substantial amounts through the vagina. No renal toxicity resulted from gum acacia alone or nonoxynol-9 in gum acacia either intraperitoneally or vaginally administered.

The white capsular membrane observed at necropsy on many of the kidneys of rats having peritonitis was not seen microscopically because the renal capsule tends to separate from the outer cortical surface during processing.

Other changes are considered incidental or insignificant and will not be discussed further.

CONCLUSION

Under the conditions of this experiment neither AGB nor nonoxynol-9 administered vaginally either separately or together resulted in any significant histopathologic changes. 4^1-Acetamidophenyl 4-Guanidinobenzoate Hydrochloride (AGB), when administered intraperitoneally resulted in degenerative kidney changes. Gum acacia (5%) administered intraperitoneally both with and without AGB and/or nonoxynol-9 resulted in hepatocellular cytoplasmic vacuolization. Peritonitis was seen in virtually all rats treated intraperitoneally, but the incidence, distribution and severity was greater in those groups given AGB and/or nonoxynol-9.

Lucas H. Brennecke, D.V.M.
Diplomate, A.C.V.P.
January 29, 1987

PAI Pathology Associates, Inc.

10075 Tyler Place
Hyatt Park II
Ijamsville, Maryland 217[illegible]
(301) 663-1644

August 15, 1986

Dr. Donald Waller
University of Illinois at Chicago
Toxicology Research Laboratory
835 South Wolcott
Chicago, IL 60612

INVOICE NO. 3181-018-1

For professional and technical services provided for Dr. Donald Waller for the AGB study.

600 slides prepared @ $6.13	$3,678.00
TOTAL INVOICE AMOUNT	$3,678.00

TERMS: Net 30

Deborah M. Fowles
Manager of Accounting and Administration

PAI Pathology Associates, Inc.

10075 Tyler Place
Hyatt Park II
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August 30, 1986

Dr. Donald Waller
University of Illinois at Chicago
Toxicology Research Laboratory
835 South Wolcott
Chicago, IL 60612

INVOICE NO. 3181-018-2

For professional and technical services provided for Dr. Donald Waller for the AGB study.

700 slides prepared @ $6.13	$4,291.00
TOTAL INVOICE AMOUNT	$4,291.00

TERMS: Net 30

Deborah M. Fowles
Manager of Accounting and Administration

PAI **Pathology Associates, Inc.**

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September 15, 1986

Dr. Donald Waller
University of Illinois at Chicago
Toxicology Research Laboratory
835 South Wolcott
Chicago, IL 60612

INVOICE NO. 3181-018-3

For professional and technical services provided for Dr. Donald Waller for the AGB study.

750 slides prepared @ $6.13	$4,597.50
TOTAL INVOICE AMOUNT	$4,597.50

TERMS: Net 30

Deborah M. Fowles
Manager of Accounting and Administration

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September 30, 1986

Dr. Donald Waller
University of Illinois at Chicago
Toxicology Research Laboratory
835 South Wolcott
Chicago, IL 60612

INVOICE NO. 3181-018-4

For professional and technical services provided for Dr. Donald Waller for the AGB study.

800 slides prepared @ $6.13	$4,904.00
TOTAL INVOICE AMOUNT	$4,904.00

TERMS: Net 30

Deborah M. Fowles
Manager of Accounting and Administration

PAI Pathology Associates, Inc.

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October 15, 1986

Dr. Donald Waller
University of Illinois at Chicago
Toxicology Research Laboratory
835 South Wolcott
Chicago, IL 60612

INVOICE NO. 3181-018-5

For professional and technical services provided for Dr. Donald Waller for the AGB study.

150 slides prepared @ $6.13	$919.50
1900 tissues evaluated @ $2.10	3,990.00
TOTAL INVOICE AMOUNT	$4,909.50

TERMS: Net 30

Deborah M. Fowles
Manager of Accounting and Administration

376

PAI **Pathology Associates, Inc.**

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Hyatt Park II
Ijamsville, Maryland 2
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October 31, 1986

Dr. Donald Waller
University of Illinois at Chicago
Toxicology Research Laboratory
835 South Wolcott
Chicago, IL 60612

INVOICE NO. 3181-018-6

For professional and technical services provided for Dr. Donald Waller for the A study.

2375 tissues evaluated @ $2.10	$4,987.50
TOTAL INVOICE AMOUNT	$4,987.50

TERMS: Net 30

Deborah M. Fowles
Manager of Accounting and Administration

PAI Pathology Associates, Inc.

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Ijamsville, Maryland 21[illegible]
(301) 663-1644

November 15, 1986

Dr. Donald Waller
University of Illinois at Chicago
Toxicology Research Laboratory
835 South Wolcott
Chicago, IL 60612

INVOICE NO. 3181-018-7

For professional and technical services provided for Dr. Donald Waller for the AGB study.

2375 tissues evaluated @ $2.10	$4,987.50
TOTAL INVOICE AMOUNT	$4,987.50

TERMS: Net 30

Deborah M. Fowles
Manager of Accounting and Administration

FINAL REPORT

SHUG DEVICE STUDIES

Program for Applied Research and Fertility Regulation - PARFR 339

Principal Investigator: L.J.D. Zaneveld, D.V.M., Ph.D.
Professor
Department of Obstetrics and Gynecology,
and
Department of Biochemistry
Rush University

Institution: Rush-Presbyterian-St. Luke's Medical Center
1763 West Congress Parkway
Chicago, IL 60612

Period Covered: Until March 31, 1987

1. PROJECT REPORT SUMMARY

A. Narrative Summary

The overall objective of this research is to develop a reversible vas deferens occlusion device. During previous contract periods such a device was developed and tested in primates. The device is called the Shug and it consists of two silicone plugs held together by a nylon suture. Primate experiments have shown that complete obstruction of sperm transport occurs when the Shug is in place and that all the animals ejaculate spermatozoa again at normal concentrations and motility after removal of the Shug. No side effects were observed.

Based on these results, an IDE was prepared and submitted to the FDA. The FDA had a number of questions, some of which were answered immediately, whereas others required more time. One of these was their requirement that life-time animals studies should be done with silicone in the vas, primarily to assure that no carcinogenic effects would be observed. For these reasons, the goals of our efforts during the past few years were primarily two fold: 1) to perform a long term toxicity study of silicone in the vas; and 2) to continue responding to the questions by the FDA until the IDE was approved. Additionally, the Shug implantation technique was tested in men and optimized. At present, the toxicity study has almost been completed and FDA approval for the IDE has been obtained. An optimal implantation method for the human has been developed.

The toxicity study was performed in rats. After modifying the Shug and the implantation technique so that the silicone could readily be placed into the

animals, the study was initiated. Sham control implants were also performed. To date, rats have been euthanized after 6 months and 1 year. No vas toxicity has been identified on histophathologic study. The two year observation period will be completed within the next 5 months which will end the toxicological study. This work is under the direction of Dr. Don P. Waller, Associate Professor, Department of Pharmacodymanics, University of Illinois at Chicago.

Other deficiencies identified by the FDA included: 1) the lack of detailed information on the chemical composition of the Shug components; and 2) additional information regarding the biocompatibility of the device materials besides the rat toxicity study. A visit was made to the FDA, the questions were discussed and clarified, and a new amendment was submitted. Some deficiencies still remained and were responded to in subsequent letters. One of these required that the effect of the Shug on human spermatozoa was evaluated. Also, the protocol for the clinical trial as well as the consent form needed to be revised. This was done. Finally, some minor questions remained dealing with the clinical trial itself due to the change from PARFR to CONRAD. Satisfactory answers were provided and the IDE was approved pending identification of the clinical sites where the studies are to take place and IRB approvals at these sites.

Implantation of the Shug was tested in 10 human volunteers at Propater, Sao Paolo, Brasil, under the direction of Dr. M. DeCastro. This was an acute study employing men who were undergoing a vasectomy and the device was removed immediately after implantation. The technique which worked well in primates, was unsatisfactory for human use. Modifications were made and the implantation method that is now available is simple and requires no more time than a regular

vasectomy. No pain was encountered by the men during implantation.

The project has been transferred to CONRAD, the clinical sites have been identified, modifications in the protocol and consent forms were made in response to the Human Subjects committees at these sites, and final IRB approvals are being sought. It is expected that the clinical trial will be initiated within the next 4 months.

SUBSTANTATIVE TECHNICAL RESEARCH REPORT

Details of the toxicity studies in the rat can be found in the Final Report from Dr. D.P. Waller, Associate Professor, Department of Pharmacodynamics, University of Illinois at Chicago, and will not be presented here.

The work done to provide the additional information that was required by the FDA is detailed in the correspondence with the FDA (see following pages). Finally, the results of the acute implantation study in men is described in a letter to Dr. Lillian Yin, Director, Division of Ob/Gyn, ENT and Dental Services at the FDA and is also attached.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

FINAL **TECHNICAL REPORT SUMMARY**

PARFR- 339UI

TITLE: "Toxicology Studies of Acrosin Inhibitors"

INSTITUTION: The University of Illinois at Chicago

PRINCIPAL INVESTIGATOR: Donald P. Waller, Ph.D.

FUNDING PERIOD: 7/1/84-12/31/86 AMOUNT EXPENDED: $147,740

A new reversible male contraceptive device (SHUG) made primarily of silicone is being developed. Previous studies demonstrated its ability to block the flow of spermatozoa when placed in the monkey vas deferens. The silicone material must be tested for chronic target organ toxicity prior to clinical trials. Two hundred rats were divided into two groups: one group had sham operations and the other group had silicone noodles implanted in the vas deferens and secured by a suture. At six months and one year after implantation animals were sacrificed and the vas deferens removed, fixed, and histologically evaluated. Changes in the vas deferens attributed to the presence of the silicone were minimal-to-mild reactive hyperplasia in the proximal, surgical and distal segments of a few animals. An increased incidence of spermatoceles, aspermatic granulomas and chronic inflammatory changes within the inner walls of the vas deferens were also observed. These changes in the vas deferens are considered of minor toxicological importance.

SIGNATURE

DATE

341

APR 23 1999

PHASE I POLY NET 90 - 90 DAY INJECTABLE CONTRACEPTIVE STUDY

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Subagreement Period: 12/01/83-11/30/85

Date of Last Report: 10/31/85

FINAL REPORT: 12/01/83 to 11/30/85

FINAL TECHNICAL REPORT

I. PROJECT REPORT SUMMARY

A. General Information

1. PARFR 341 - "Phase I Poly NET 90 Injectable Study

2. Principal Investigators:

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3. Institution:

University of Alabama at Birmingham
Department of Obstetrics and Gynecology
University Station
Birmingham, AL 35294

4. Subagreement Period: December 1, 1983-December 30, 1985

5. Date Last Technical Report: October 31, 1985

6. Progress Report Inclusive Dates: December 1, 1983-December 30, 1985

B. Narrative Summary Statement

The purpose of the original subcontract was to perform a Phase II clinical study on the Poly NET 90 Day Injectable Microsphere Formulation. The study was to be conducted at five centers. The center at the University of Alabama at Birmingham (UAB) was to be responsible for the collection and reporting of the results from the other cooperating centers which include San Antonio, Rome, Chile, and Atlanta. The UAB Center was also responsible for manufacturing the microsphere doses and conducting a preclinical test in baboons on the master batch of Norethisterone (NET) microspheres to be used for the Phase II studies.

The results of the preclinical baboon pharmacokinetic studies (reference June 25 technical progress report) revealed a problem with the test formulation. The initial NET release rate was slower than expected. Based on these pharmacokinetic data the decision was made not to use this microsphere formulation for the Phase II clinical study. The clinical investigators were instructed to discontinue the study except at two centers (San Antonio and Chile) where some subjects had already been treated with the first injection. The investigators at these centers were instructed not to treat any additional subjects or to give second injections to any of the subjects that had already been treated. They were further instructed to provide alternate methods of contraception to the subjects that had been treated and to continue to follow these subjects until the serum levels of NET could no longer be detected. Blood samples obtained at periodic intervals from these subjects were sent to the University of Alabama at Birmingham for NET analysis. In addition to this, the investigators monitored the serum levels of estrogen and progesterone and kept records on vaginal bleeding patterns. There were 14 subjects treated at the San Antonio center and three subjects at the Chile center. Initiation of the Phase II study at the other three centers was delayed pending the availability of an acceptable NET microsphere formulation. One subject at the San Antonio center became pregnant during the first cycle posttreatment, thus confirming the problem with the release rate.

The Stolle Research and Development Corporation (Stolle), under a research agreement with PARFR (PARFR 357), undertook a series of studies to improve the pharmacokinetics of the Phase II NET microsphere formulation in order to improve the release rates. The results of this effort were presented in the Stolle technical progress report (PARFR subagreement 357). In summary, this study determined the cause of the slower than expected release rates and discovered the means to correct the release rate. The release rate was corrected by increasing the concentration of NET in the microspheres. Preclinical animal studies using baboons were conducted at UAB to confirm that the new formulation was suitable for use in the Phase II study. In order to determine the dose of NET microspheres to be used for the resumption of the Phase II study, it was decided to modify the clinical protocol at the UAB center. The UAB center agreed to undertake a Phase I dose-response study on the improved microsphere formulation in lieu of the Phase II study as previously described. The clinical protocol was changed to include infertile or nonsexually active subjects instead of fertile sexually active subjects. In addition, three different doses of the microsphere formulation were tested.

Nine subjects were treated with one of three doses of Poly NET 90 to determine the optimal dose for a multicenter Phase II clinical trial using the same formulation. Following treatment with 50, 75, or 100 mg NET in poly-lactide-co-glycolide (PLGA) microspheres, blood samples were obtained from each subject at selected intervals to determine the NET release rate and effect on endogenous ovarian steroids. Pharmacokinetic data revealed consistent release of NET throughout the 90 day treatment interval, with successful ovulation inhibition in eight of the nine subjects. The ninth subject, who received the low dose (50 mg NET), had progesterone levels suggestive of ovulation in the first three weeks posttreatment. Eight of the nine subjects received a second injection 90 days after the first, and blood samples were obtained for further evaluation of the treatment pharmacokinetics and pharmacodynamics. All subjects remained on the study until they resumed normal menstrual patterns, the last subject to complete the study in early January, 1986.

II. Substantive Technical Research Report

A. Statement of Objectives

The original objective of this project was to undertake a Phase II clinical trial of a long-acting injectable NET microsphere formulation. During the course of the project the objective was modified to include a Phase I study at the UAB center prior to initiation of the Phase II studies at the other cooperating centers. The purpose of this change was to test performance of the microsphere delivery system in a small group of human subjects prior to releasing the master batch of microspheres for expanded Phase II studies. This change was necessitated by the failure of the original batch of microspheres produced for the Phase II study to provide adequate release rates of NET during the first month posttreatment.

B. Progress to Date

The technical and administrative tasks performed by the UAB staff on this project included the following:

1. Preparation of the original master batch of NET microspheres intended for use in expanded Phase II studies at UAB and the other cooperating centers.

2. Preclinical evaluation of the original microsphere batch in baboons.

3. Recruitment of patients and initiation of the Phase II study at the UAB center.

4. Evaluation of the serum levels of NET in 19 subjects treated with the original microsphere batch at two of the cooperating centers.

5. Analysis of preliminary baboon and human pharmacokinetic data obtained from the original master batch of NET microspheres which led to the decision to terminate the Phase II study at the UAB and other cooperating centers. This decision was based on pharmacokinetic data that showed that the NET release rate during the first month posttreatment was not sufficient to ensure contraceptive efficacy.

6. Initiation of the collaborative research effort with Stolle to improve the release kinetics of the NET microsphere formulation. The objective of this research was to adjust the NET release rates during the first month posttreatment without compromising the quality of the microsphere formulation.

7. Preclinical pharmacokinetic studies in baboons to test various modified microsphere formulations produced by Stolle. The purpose of these baboon studies was to select the most promising improved formulation to reinitiate the Phase II studies.

8. Pharmacokinetic studies in human subjects (Phase I at UAB) to test the most promising improved microsphere formulation based on the baboon pharmacokinetic studies prior to releasing this formulation for use in expanded Phase II studies. The purpose of this Phase I study was to confirm the results observed in baboons in a small group of human subjects prior to releasing the microspheres for use in the Phase II study. The details of these various tasks have been described in previous progress reports. The decision was made to terminate the original Phase II studies on the basis of baboon pharmacokinetic studies and pharmacokinetic studies in the first 19 patients who were treated with the original master batch of microspheres. Finally, it was determined, based on the results of Task 5, that the new microsphere formulation was satisfactory for use in expanded Phase II studies.

Baboon Pharmacokinetic Studies

The results of the baboon pharmacokinetic studies on the master batch of NET microspheres that were made at the UAB and Iowa for the Phase II clinical studies are shown in Figure I. The baboons were given a 75 mg dose of microencapsulated NET, which is the same dose that was intended for use in the clinical study. The human dose is approximately three times higher than what is necessary for ovulation inhibition in the baboon.

The serum levels of NET gradually increased until about day 20 posttreatment (PT), at which time serum NET reached high enough levels to suppress ovulation. The serum NET levels remained relatively constant until about day 50 PT. After day 50, there was an abrupt decrease due to biodegradation of the microspheres. The early burst effect seen in previous pharmacokinetic studies was absent. The reduction of the burst effect was due to less free NET on the finished microspheres, indicating improved encapsulation. It is important to note that the pattern of NET release from this formulation during the first 2 months posttreatment was different from what occurred in the previous Phase I studies on 90-day NET microsphere formulations. Instead of a gradual decrease in the serum NET levels during the first month posttreatment there was a gradual increase. This represents a problem with regard to the efficacy of the formulation for providing contraceptive protection, because the serum levels of NET may not be high enough during the first month posttreatment to suppress ovulation. This could result in unwanted pregnancies. Unfortunately, the Phase II study was started at two of the cooperating centers before this baboon data was available. Shortly after the initiation of the Phase II study at the San Antonio Center, one of the 14 subjects treated with the injectable microsphere formulation became pregnant during the first month posttreatment. We immediately obtained blood samples from the subjects at the San Antonio center and from several subjects who were treated at the Chile Center and evaluated the serum levels of NET by radioimmuno-assay. The results of these pharmacokinetic studies are shown in

Figure 2. These results confirm the findings in the baboons that the serum levels during the first month posttreatment are much lower than expected based on previous studies using NET microsphere formulations. There were no adverse effects observed in any of the subjects treated with this formulation.

According to the protocol, a single pregnancy did not justify the discontinuation of the study. However, we decided to discontinue the study because the pharmacokinetic data clearly suggested the possibility that other subjects might become pregnant during the first month posttreatment because of the low serum NET levels. We therefore decided to discontinue the use of this batch of microcapsules in favor of making a new batch which would provide higher serum NET levels during the first month posttreatment. At the time this decision was made, ten subjects had been recruited at the UAB center for the Phase II clinical trial. These subjects were at various stages of evaluation during the pretreatment phase when the decision was made to discontinue this study. Accordingly, these subjects were released from this study and the clinical study at UAB was deferred until the problems with the microsphere formulation could be satisfactorily resolved.

Modification of Microsphere Formulations

The next step in the program was carried out in collaboration with Stolle. At this point in the program, Stolle assumed responsibility for the manufacture of the microsphere formulations. A series of experiments was run by Stolle to change the microencapsulation process in order to achieve faster release rates during the first month posttreatment without compromising the quality of the microspheres. This was achieved by increasing the concentration of NET in the microspheres. The UAB worked in close collaboration with Stolle, transferring the information we had gained from the microencapsulation work done at UAB and at Southern Research Institute. UAB then carried out the pharmacokinetic studies in baboons using improved microsphere formulations produced by Stolle. Figure 3 shows the serum NET levels in four baboons treated with two different microsphere formulations.

Formulation A is a composite of five batches of NET microspheres and Formulation B was a composite of six batches. The size range and percent drug loading of both formulations were identical. Formulation B had a greater proportion of microspheres in the smaller size range, i.e. 25 - 45 microns. Formulations A and B both provided satisfactory serum levels of NET during the first month post-treatment. The explanation for the increased release rate as compared to the earlier batch, is that these microspheres contained a higher concentration of NET (i.e. approximately 45%). It was determined as part of the Stolle research program that increasing the concentration of NET in the microspheres causes a corresponding increase in the in vivo release rate. Formulation B was expected to produce higher serum NET levels during the first few weeks than Formulation A. This was attributed to the greater proportion of smaller microspheres in Formulation B, as the size of the microspheres is a second parameter that controls the rate of release. The serum levels of NET were relatively constant for Formulation A, without erratic fluctuations and there was little or no burst from either formulation.

Based on these findings, a decision was made in collaboration with the PARFR and AID staff to procede with the Phase I clinical trial using three different doses of Formulation A. At this point, a decision was made not to use this microsphere batch for Phase II studies until the baboon data could be verified in a small group of human subjects. It is for this reason that the continuation of the program at UAB was a Phase I and not a Phase II study. This required making two changes in the original clinical protocol. All subjects recruited for the Phase I study must be infertile to avoid the risk of pregnancy. In addition, the protocol required collection of a greater number of blood samples, especially during the first month posttreatment, in order to obtain sufficient pharmacokinetic data to judge the acceptability of this microsphere formulation for use in expanded Phase II studies. This action was taken in order to prevent a repeat of the problem that occurred earlier, when a a microsphere formulation that did not have adequate release rates to supress ovulation was used in unprotected women.

Phase I Clincial Trial at UAB

The microsphere formulation selected for use in the Phase I clinical trial at UAB comprised microspheres 25-90 microns in diameter containing 45% by weight NET in a PLGA excipient (Batch # SR016). The preclinical evaluation of this formulation in baboons indicated that the rate and duration of NET release were appropriate for further evaluation in human subjects. Following a pretreatment control cycle during which blood samples were obtained to verify the occurrence of ovulation, three groups of three subjects each were injected intramuscularly with microspheres containing either 50, 75, or 100 mg of NET suspended in 1.5 ml of vehicle comprising 2% carboxymethycellulose and 1% Tween-20 in water. The subjects were instructed to keep a menstrual diary and to return to the clinic for blood sampling at selected intervals after treatment. The NET pharmacokinetic data for these subjects and the endogenous hormone data are shown in Figure 4. The subjects treated with the 100 mg dose of NET had no evidence of ovulation throughout the initial 90-day treatment interval, and the serum NET levels were greater than 1 ng/ml in 95% of the samples acquired from these subjects. We knew from previous experience that serum NET levels in excess of 1 ng/ml effectively inhibit ovulation in both baboons and women. The 75 mg dose also inhibited ovulation during the first 90 days posttreatment and serum NET levels exceeded 1 ng/ml in 75% of the samples evaluated. One subject treated with the 50 mg NET dose had serum progesterone levels suggestive of ovulation during the first 21 days posttreatment. In the low dose group, 58% of the samples contained over 1 ng/ml of NET. Eight of the nine subjects were given a second injection 3 months after the first injection, and blood samples were obtained for analysis of steroid profiles. The serum NET levels following the second injection did not differ significantly from those observed following the first injection.

The purpose of this Phase I study was to test the performance of the master batch of NET microspheres intended for use in expanded Phase II studies. This test of performance was based solely on the pharmacokinetic profiles observed in the subjects following treatment with this formulation. The primary objective was to

test whether or not the deficiencies in the earlier microsphere formulation with regard to the slow release rate during the first month posttreatment had been corrected. The results from this study clearly show that the improved microsphere formulation provides satisfactory release rates during the entire 90-day treatment interval. Moreover, the pharmacokinetic data following the second injection clearly showed that there is not a build up of serum NET with additional treatment. It was decided, based on these results, that this improved NET microsphere formulation was acceptable for use in the expanded Phase II studies. The decision was made at this point to discontinue any further treatment of the subjects on the Phase I study at UAB, and to reinitiate the Phase II study at the other cooperating centers.

D. Research Design Changes.

There were several major changes during the course of this program of research. The project initially began as a Phase II study that was terminated because of an unexpected pregnancy. There was a significant delay prior to reinitiating the clinical study, which was changed to a Phase I study. During this period of delay several additional necessary tests were added to further evaluate improved microsphere formulations prior to the reinitiation of the Phase II studies.

E. Operational Significance (N/A)

F. Dissemination/Utilization of Research Results

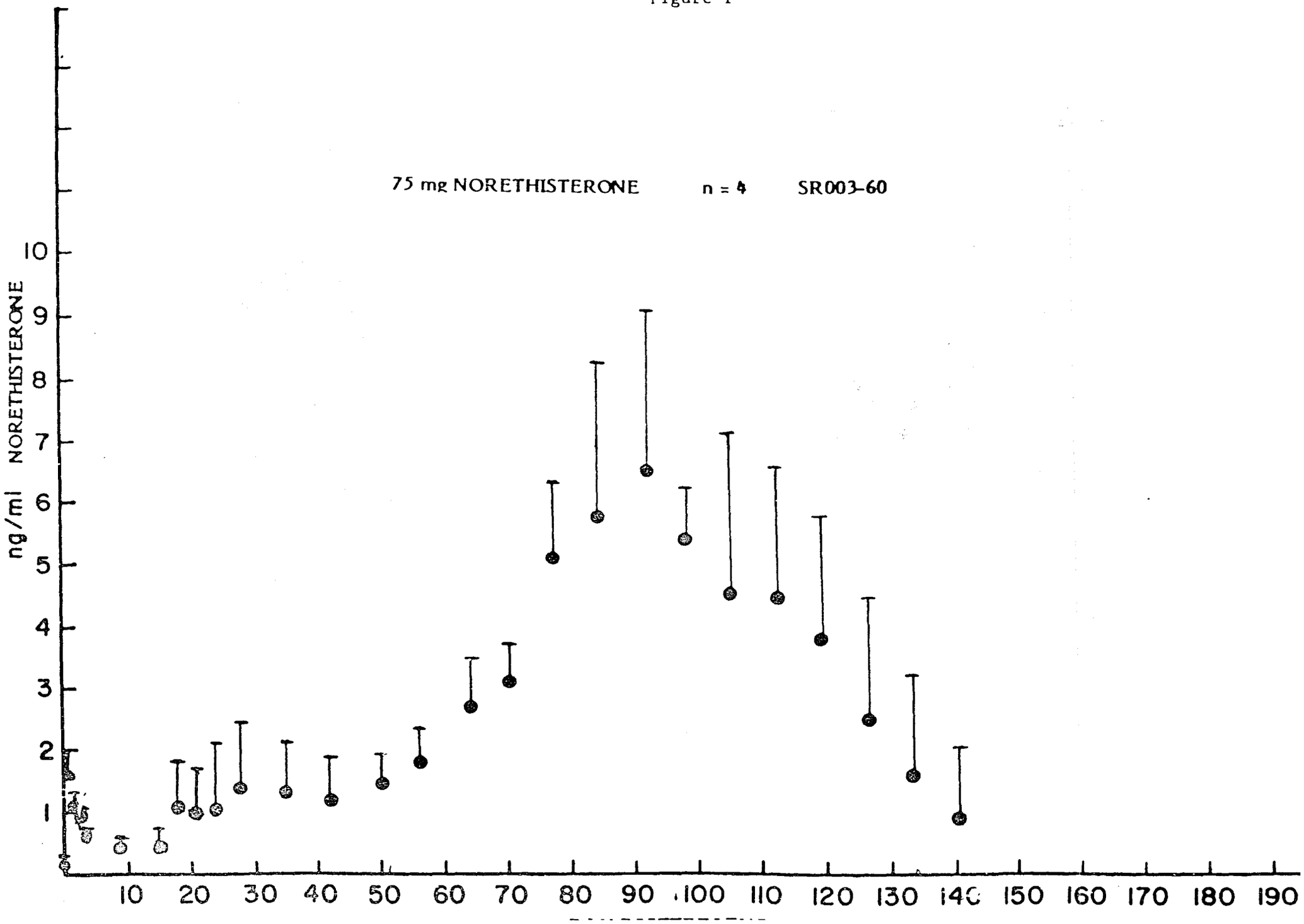
The results from these studies have been discussed at planning sessions to enable final dose selection for the Phase II clinical trials. There has been no scientific publication of these results.

G. Work Plan for the Coming Period (N/A)

H. Budget

The attached table lists the original budget as approved 12/01/83 for this program of research and the actual expenditures on the project. The justification for the difference between the estimated and actual budget is that significant changes were made in the work plan of the original project that resulted in long delays and expenditures in the catagories not otherwise anticipated.

Figure 1


75 mg NORETHISTERONE
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SR003-60
ng/ml NORETHISTERONE
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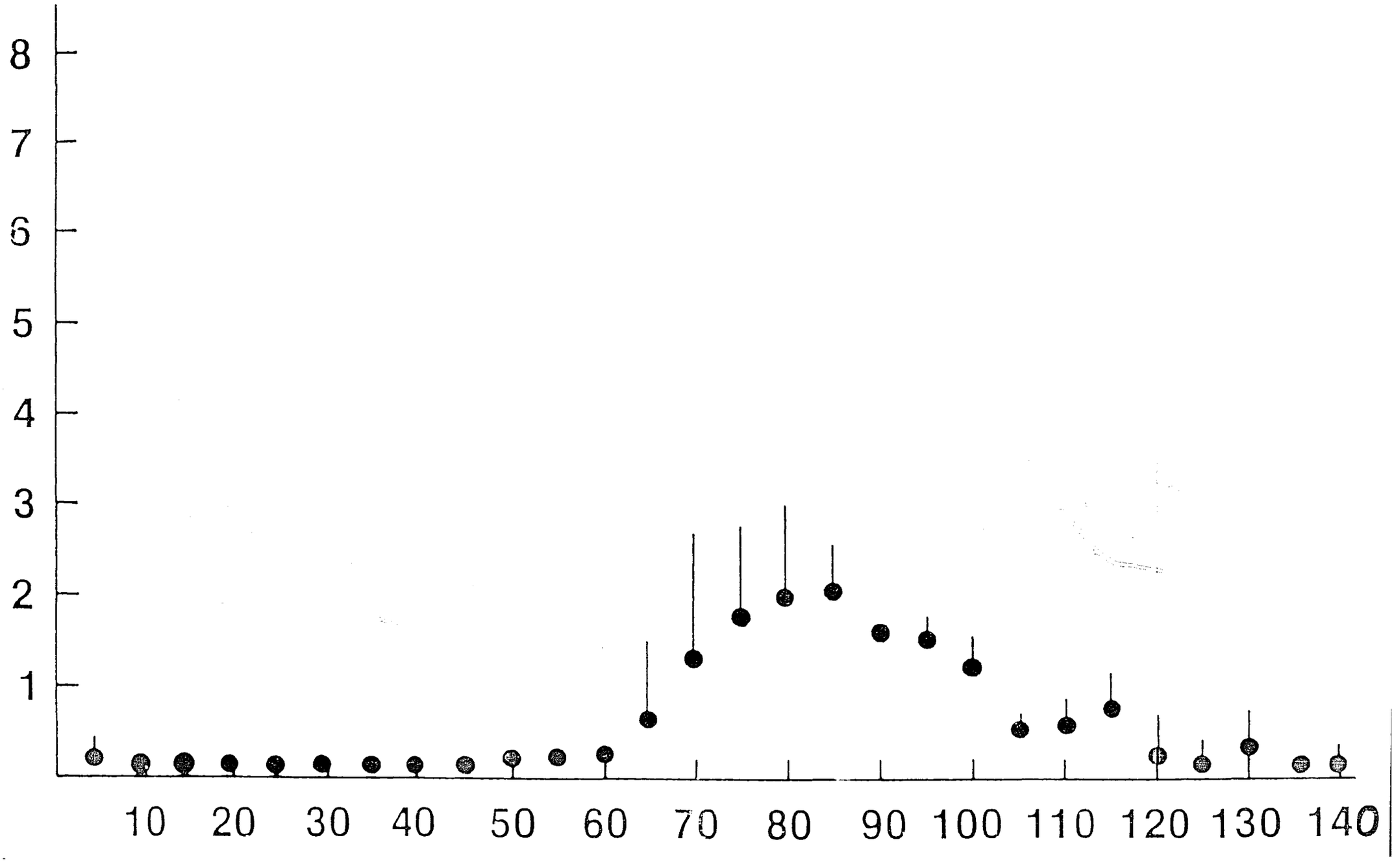
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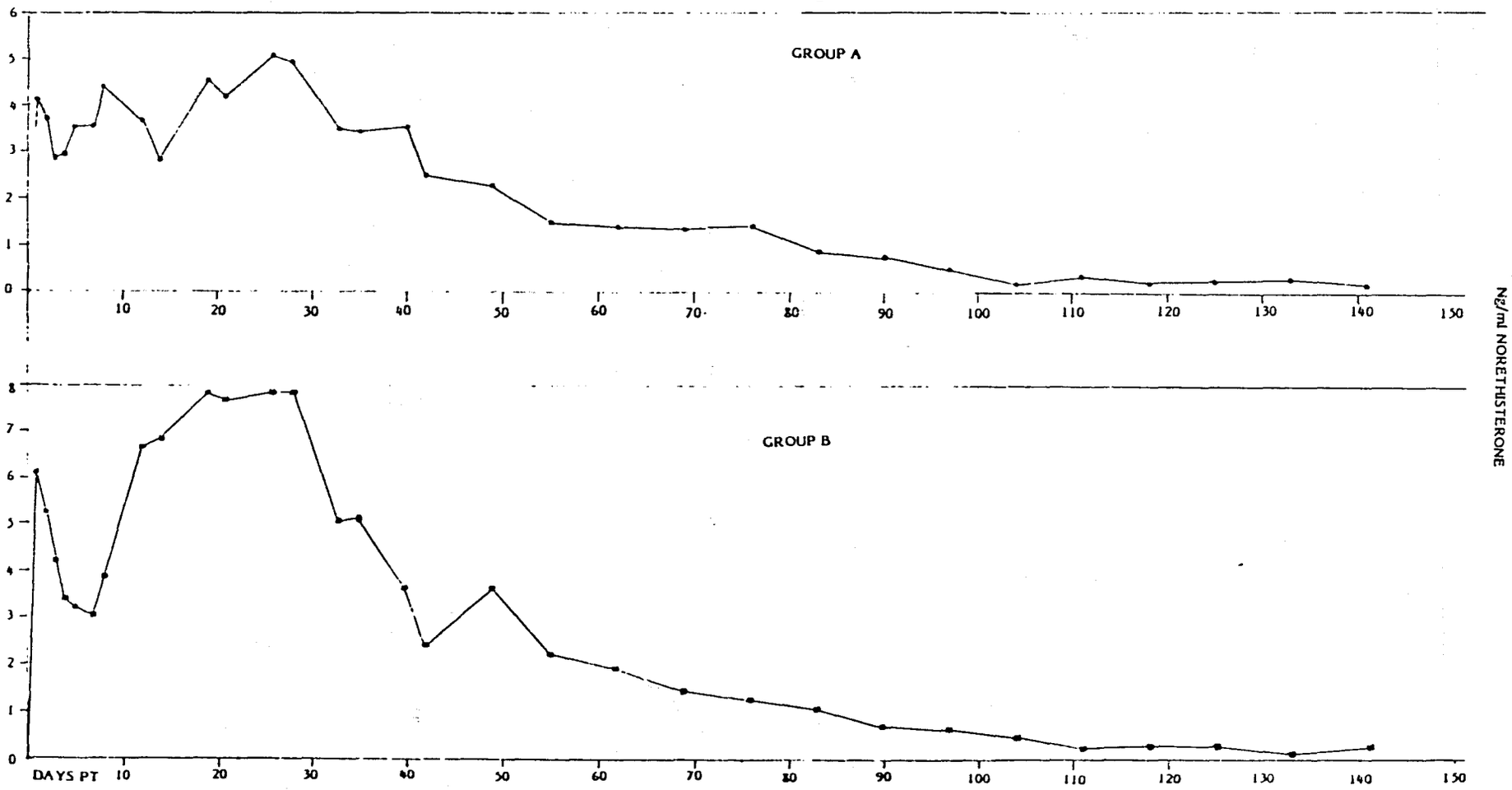
Figure 3


GROUP A
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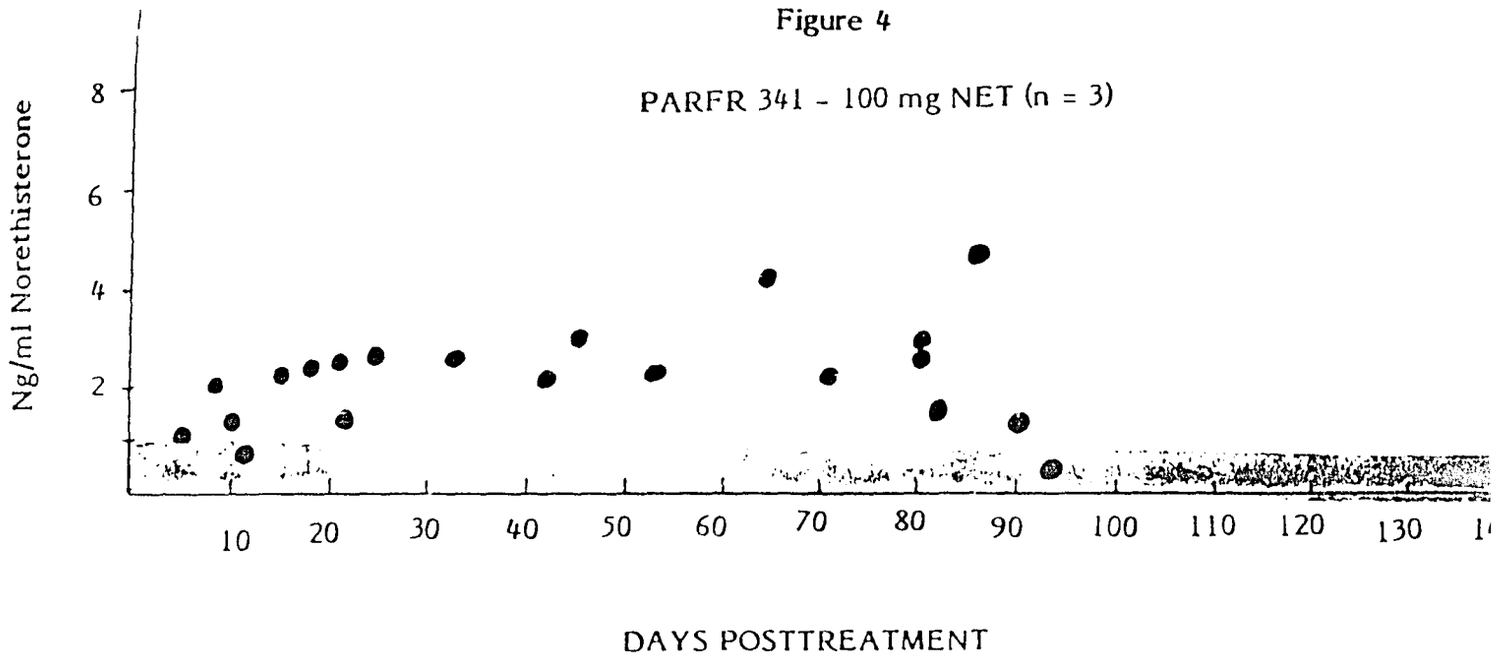
ng/ml NORETHISTERONE

Figure 4


PARFR 341 - 100 mg NET (n = 3)
Ng/ml Norethisterone
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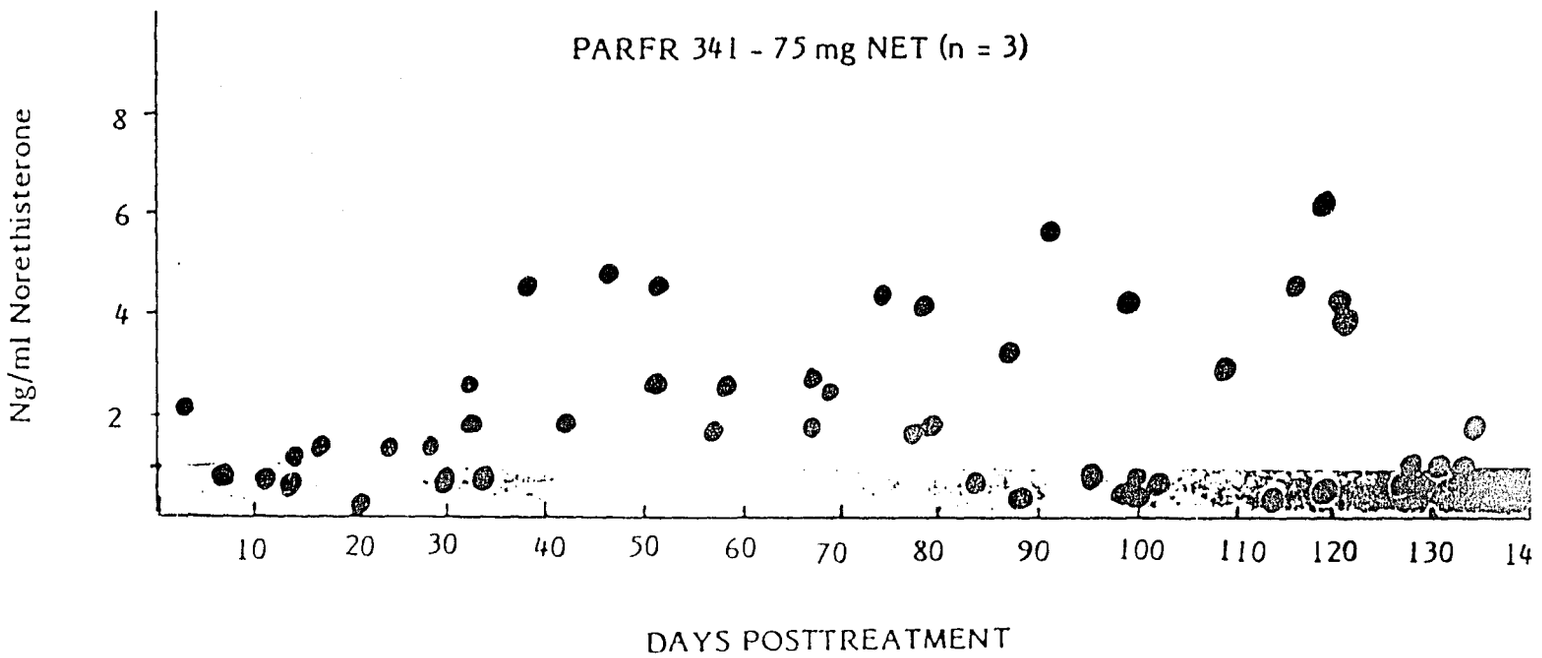
DAYS POSTTREATMENT


PARFR 341 - 75 mg NET (n = 3)
Ng/ml Norethisterone
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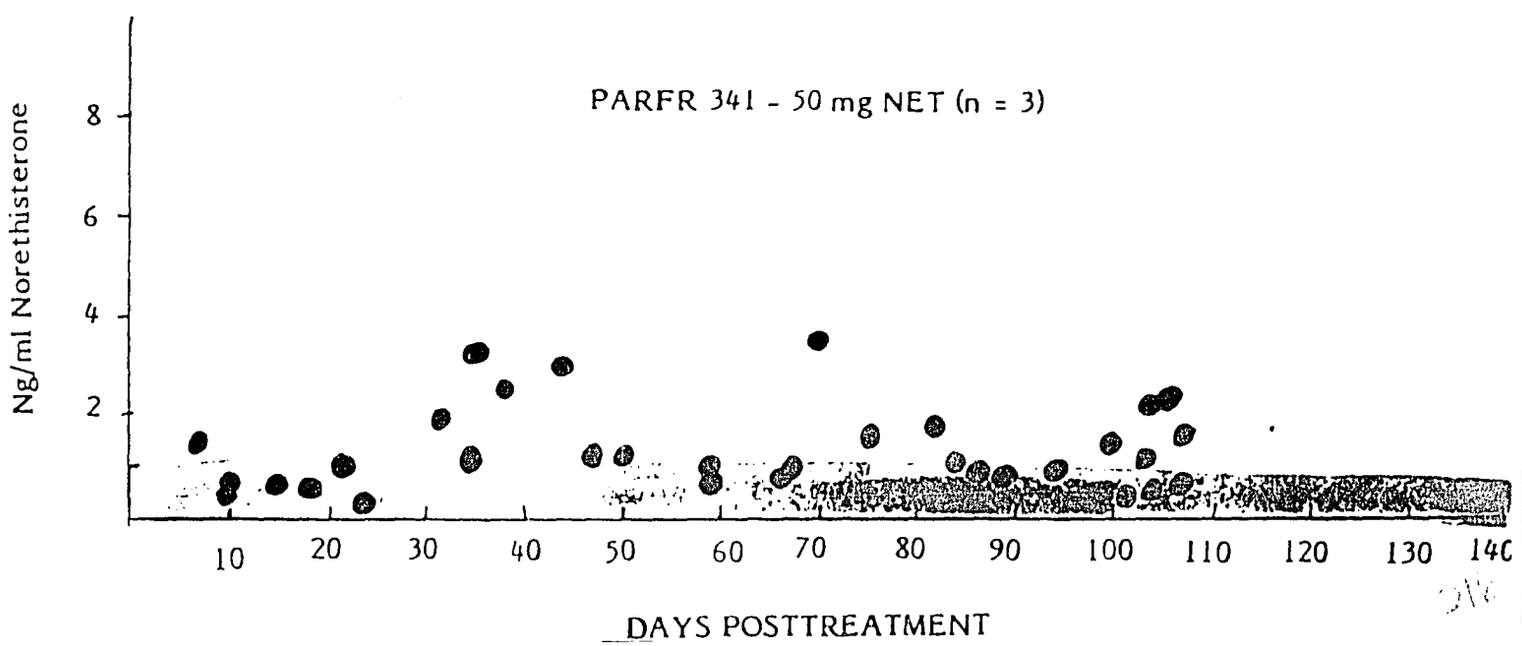
DAYS POSTTREATMENT


PARFR 341 - 50 mg NET (n = 3)
Ng/ml Norethisterone
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120
130
DAYS POSTTREATMENT

PROJECT TITLE: "Phase I Poly NET 90 Injectable Study"

PRINCIPLE INVESTIGATOR: Charles E. Flowers, Jr., M.D.

PARFR-341 Financial Report for the Period (Dates) From 12/1/83 to 11/30/85.

CATEGORY	12/1/83-11/30/85 BUDGET
Salaries	
Lee R. Beck, Ph.D. Co-Investigator	$12,000
Nurse Practitioner	1,021
Lab Technician	12,473
RIA Supplies and Materials	11,621
Clinical Costs	
Patient Recruitment	8,520
Telephone, Xerox, Etc.	40.05
Laboratory Costs	
Pap Smear	200
Pregnancy Tests	1,502
Modified SMAC	500
Urinalysis	98
Histological Services	
Collection, processing and evaluation of endometrial biopsies	8,680
Subtotal	$56,705
Overhead (39.5%)	22,444
TOTAL PARFR-341	$79.149

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

Technical Report

I. PROJECT REPORT SUMMARY

A. General Information

1. Enhancement of the Secretory Immune Response to LDH-C_4 (PARFR-355)

2. Nancy J. Alexander, Ph.D.
Oregon Regional Primate Research Center
505 N.W. 185th Avenue
Beaverton, Oregon 97006
(503) 645-1141, ext. 262

3. Institution: Oregon Regional Primate Research Center

4. Subagreement Period: 4/15/84 - 12/31/86

5. Last Technical Report Submitted: 2/26/86

6. Inclusive Dates of This Technical Report: 2/27/86 - 8/31/86

B. Narrative Summary

We have tested the ability of two adjuvants, cholera toxin B and muramyl dipeptide (MDP), to increase the secretory immune response to lactate dehydrogenase C_4 (LDH-C_4), a sperm-specific antigen. CD-1 female mice were primed with 100 ug LDH-C_4 in Complete Freund's Adjuvant (CFA) given intramuscularly (i.m.). Agarose plugs containing LDH-C_4 and/or the two adjuvants were surgically implanted into the right uterine horn one week following priming. The right uterine horn was ligated at only the cervical end. Serum, intestinal lavage, and intrauterine fluid samples from the left uterine horn were collected at weekly intervals. Analysis indicated that LDH-C_4 (100 ug) plus both adjuvants provided the highest intrauterine stimulation.

II. TECHNICAL RESEARCH REPORT

A. Statement of Objectives

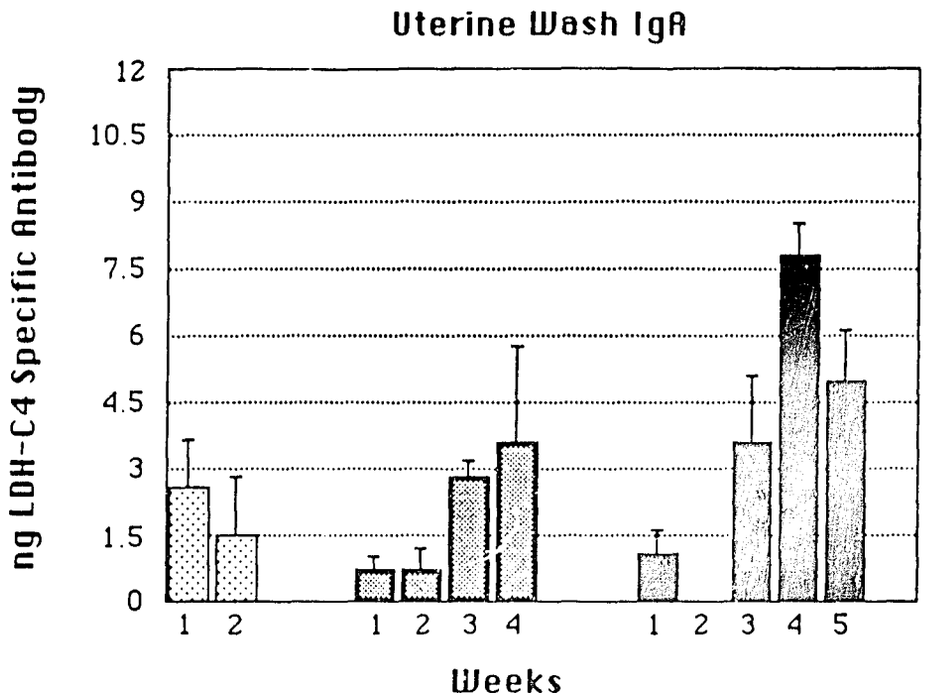
The objective of this project is to develop an immunization procedure that will make possible a strong secretory immune response to lactate dehydrogenase C_4 (LDH-C_4), a sperm-specific antigen. The results will aid in the development of a contraceptive vaccine.

B. Progress to Date

At the beginning of this study, CD-1 mice were bled via the retro-orbital plexus and divided into groups of five. Animals were then injected intramuscularly with a solution containing 100 ug LDH-C_4 in CFA, thereby priming systemically to insure a high response. Uterine agarose plugs were then implanted. These plugs contained phosphate-buffered saline (PBS); LDH-C_4 (100 ug); cholera toxin B (CTB) (10 ug); muramyl dipeptide (MDP) (10 ug); LDH-C_4 (100 ug) + MDP (100 ug); LDH-C_4 (100 ug) + CTB; or LDH-C_4 (100 ug) + MDP (100 ug) + CDP (100 ug). After insertion of the plug into the right uterine horn, both ends were ligated. Care was taken to avoid damaging the uterine artery. The left horn was ligated only at the cervical end.

At weekly intervals, mice were first bled to obtain a serum sample. Collection of an intestinal lavage sample followed. The procedure for this collection included intragastric administration of a lavage solution followed by an intraperitoneal injection of 0.1 mg of pilocarpine. Intestinal discharges were obtained in a solution containing soybean trypsin protease inhibitor and EDTA soon after. Centrifugation was used to clarify the samples and phenylmethylsufonylfluoride (PMSF). The mice were then killed by cervical dislocation. After a ventral transverse incision, the left uterine horn was isolated. One end was cut and 200 ul of saline were flushed through by means of a Hamilton syringe. Samples of all three types were stored at -20°C until sent to Dr. Goldberg. In his laboratory, enzyme-linked immunosorption assays (ELISAs) were performed to determine LDH-C_4-specific IgA and IgG levels.

Our results (Figures 1 and 2) indicate that mice in Group 7 that received LDH-C_4 (100 ug) + MDP (10 ug) + CTB (10 ug) received uterine IgA/IgG stimulation superior to any of the other groups ($p < 0.05$). Uterine and serum IgG levels were not significantly different between the groups (Figures 3-6); lavage immunoglobulin levels were negligible for all of the groups (data not shown). These findings indicate that the presence of both adjuvants is important in inducing a maximal IgA intrauterine response to LDH-C_4. Therefore, this immunization protocol will be used in future studies.

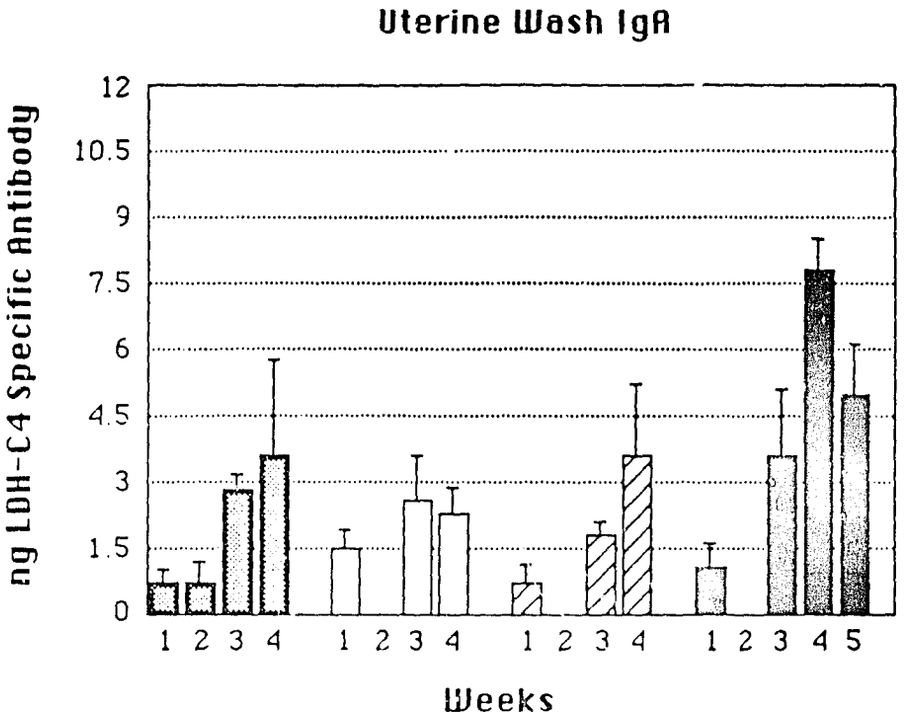


Group 1: agarose plug only

Group 4: LDH-C_4 (100 μg)

Group 7: LDH-C_4 (100 μg) + MDP (10 μg) + CTB (10 μg)

FIGURE 1



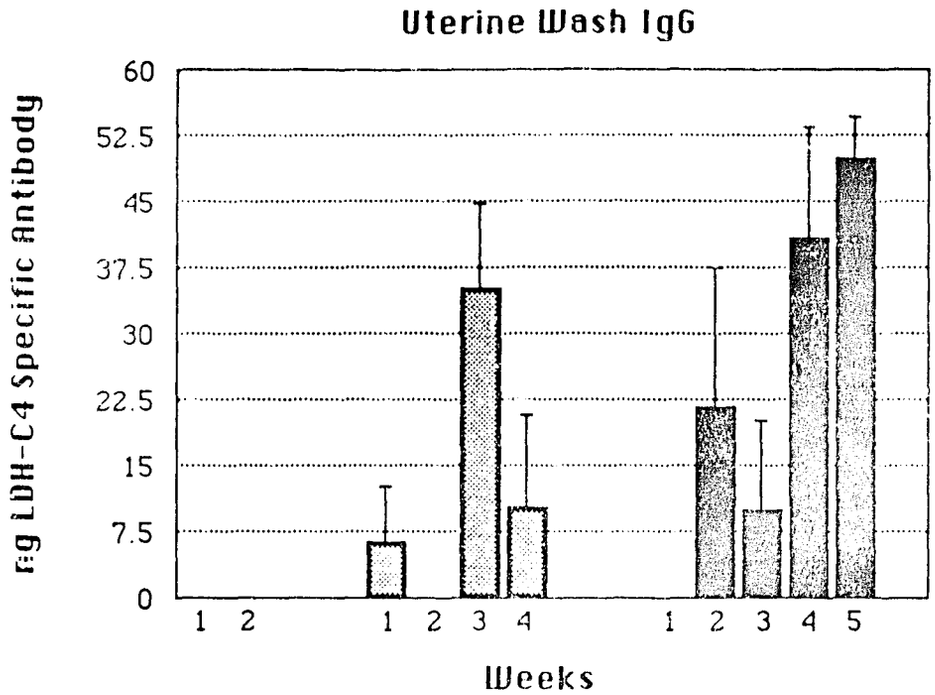
Group 4: LDH-C_4 (100 μg)

Group 5: LDH-C_4 (100 μg) + MDP (10 μg)

Group 6: LDH-C_4 (100 μg) + CTB (10 μg)

Group 7: LDH-C_4 (100 μg) + MDP (10 μg) + CTB (10 μg)

FIGURE 2

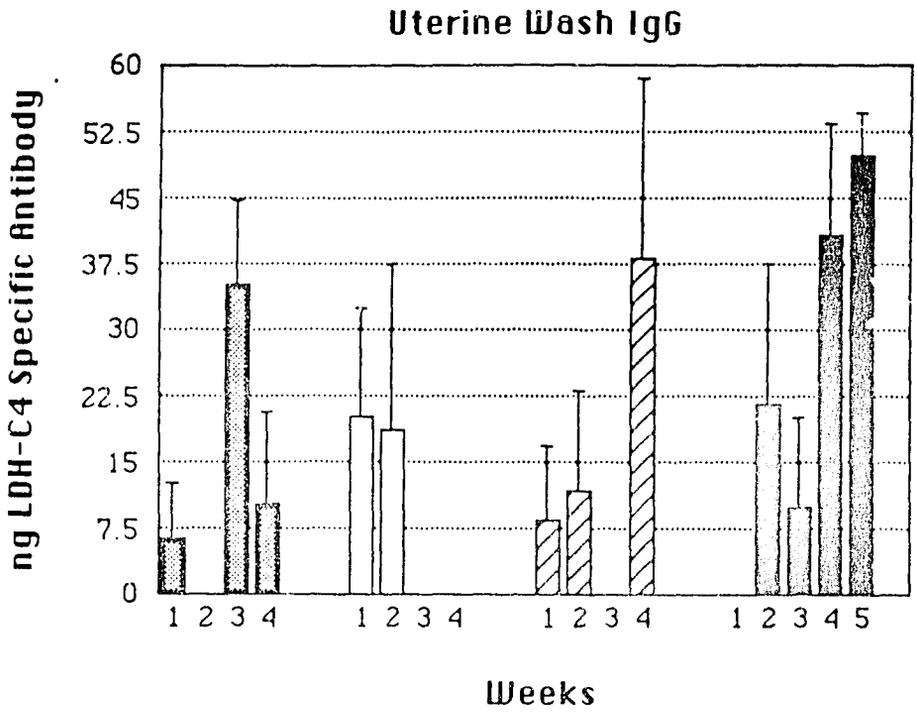


Group 1: agarose plug only

Group 4: LDH-C_4 (100 μg)

Group 7: LDH-C_4 (100 μg) + MDP (10 μg) + CTB (10 μg)

FIGURE 3



Group 4: LDH-C_4 (100 μg)

Group 5: LDH-C_4 (100 μg) + MDP (10 μg)

Group 6: LDH-C_4 (100 μg) + CTB (10 μg)

Group 7: LDH-C_4 (100 μg) + MDP (10 μg) + CTB (10 μg)

FIGURE 4

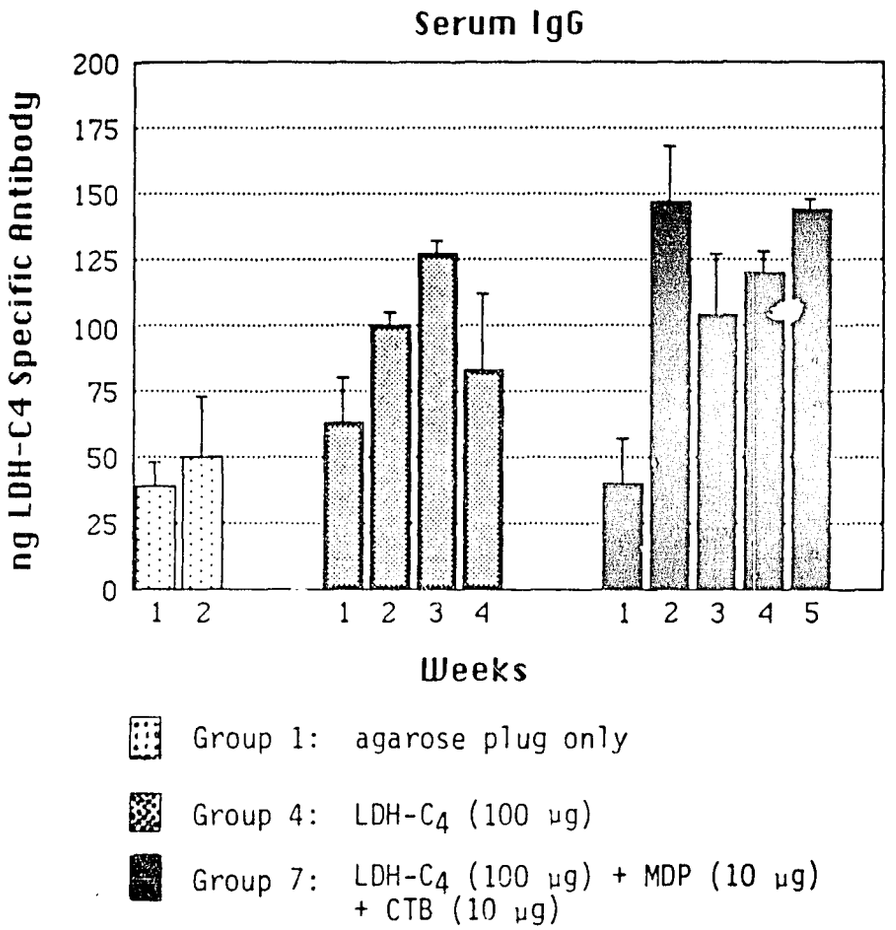
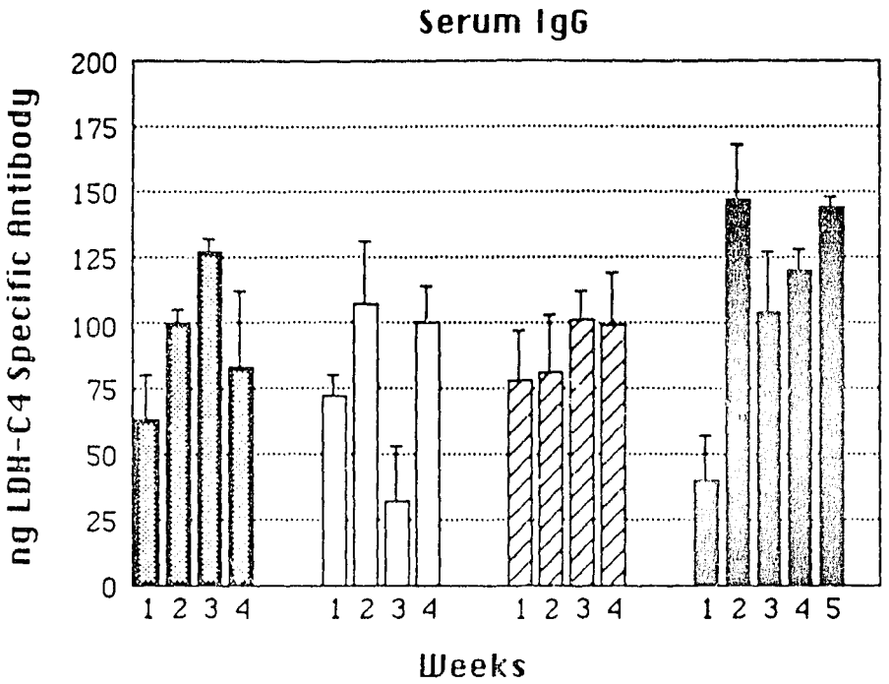

Serum IgG
ng LDH-C4 Specific Antibody
200
175
150
125
100
75
50
25
0
1 2
1 2 3 4
1 2 3 4 5
Weeks
Group 1: agarose plug only
Group 4: LDH-C4 (100 µg)
Group 7: LDH-C4 (100 µg) + MDP (10 µg) + CTB (10 µg)

FIGURE 5



Group 4: LDH-C_4 (100 μg)

Group 5: LDH-C_4 (100 μg) + MDP (10 μg)

Group 6: LDH-C_4 (100 μg) + CTB (10 μg)

Group 7: LDH-C_4 (100 μg) + MDP (10 μg) + CTB (10 μg)

FIGURE 6

Using the protocol found to provide maximal IgA intrauterine stimulation, our next goal was to establish the most stimulating immunization route. This was done, as before, using groups of five CD-1 females. After initial systemic priming (i.m.) with LDH-C_4 (100 µg) in saline, each group received the antigen 1 week later using intramuscular + CFA on day 7 (Group 1); intramuscular + CFA on day 0 and 7 (Group 2); intramuscular + CFA on day 0, intragastric on day 7 (Group 3); intramuscular + CFA on day 0, intragastric on day 7, intrauterine on day 14 (Group 4); intramuscular + CFA on day 0 and 7, intrauterine on day 14 (Group 5); intramuscular + CFA on day 0, intrauterine on day 7 (Group 6). Serum, lavage, and uterine wash samples were taken at weekly intervals and LDH-C_4-specific antibody levels were measured as previously described. We have received the serum data from Dr. Goldberg (Table 1) and await the lavage data. With this information, two important considerations for a contraceptive vaccine will be known: (1) route of immunization, and (2) selection of adjuvants able to enhance the intrauterine IgA response without increasing the serum IgA response.

Table 1: Serum values (ng/ml of IgG anti-LDH-C_4) from mice immunized by various routes.

	Days post immunization			
Group	21	28	35	42
I	668 (3/5)*		89 (5/5)	
II	708 (5/5)		4247 (5/5)	
III	4 (1/5)		21 (1/5)	
IV	303 (5/5)	486 (5/5)	629 (5/5)	1257 (5/5)
V	166 (5/5)	947 (5/5)	1835 (5/5)	1075 (4/5)
VI	396 (5/5)	1102 (5/5)	4019 (5/5)	268 (3/4)

*Number of mice responding.

C. Unanticipated Results

Mouse mortality was 3% owing to surgical complications.

D. Research Design

Priming of the mice with 100 μg of LDH-C_4 in CFA was done to insure a response.

E. Operational Significance

Not applicable.

F. Dissemination and Utilization of Research Results

Not applicable.

G. Work Plan for Coming Reporting Period

Not applicable.

H. Budget Forecast

-0-

FINAL TECHNICAL REPORT

I. Project Report Summary

A. General Information

1. Project Title and Subagreement Number: Development of an Immunocontraceptive Vaccine: Role of 23-kD Antigen in Immunoinfertility and Fertility Regulation. PARFR - 356a (originally PARFR-356).

2. Principal Investigator
Rajesh K. Naz, Ph.D.
Assistant Professor and Director,
In Vitro Fertilization Laboratory
Department of Obstetrics and Gynecology
The George Washington University Medical Center
2150 Pennsylvania Avenue, N.W., Washington, D.C. 20037
(Telephone: (202) 676-4614, 5086, 5087)
Signature _____

3. Institution
The George Washington University Medical Center

4. Subagreement Period
December 1, 1984 to June 30, 1986

B. Progress to Date

(a) General objective

The overall objective of this project is to investigate the use of FA-1, an antigen derived through the use of monoclonal antisperm antibody, MA-24, as an effective immunocontraceptive, and to investigate the role of FA-1 in involuntary immunoinfertility. It was proposed to characterize the FA-1, to test whether active immunization causes a cessation of fertility in female and male species, and to study the mechanisms of action of antibodies.

(b) Achievements made

(1) Development and characterization of the monoclonal antibody, MA-24

BALB/c mice were immunized intraperitoneally with 10^6 sperm (washed ejaculated or epididymal human sperm) in Freund's complete adjuvant. Hybridomas were prepared by fusion of the mouse myeloma line P3-NS1/1-Ag4-1 with spleen cells from mice with high antibody titers to sperm. The hybridomas secreting these antibodies were selected and cloned. The preliminary screening was performed by an indirect enzyme-linked immunosorbent assay (ELISA) with whole washed human sperm or a membrane preparation

solubilized with LIS (0.3M lithium diiodosalicylate). Positive clones (reading higher than 2 standard deviations above the mean for controls) were selected and recloned, and 10^7 hybrid cells were injected into BALB/c mice previously treated with pristane to generate ascites fluid.

Of 40 putative clones secreting antibodies to sperm, one (MA-24) was selected for detailed analysis. (Naz et al., Science, 342-344, 1984) The MA-24 clone determined was to be of the 2a subclass of immunoglobulin G (IgG_{2a}) bythe agar-gel double diffusion technique. This clone was negative in the agglutination and immobilization assays, two methods that we use to test for antibodies to sperm in serum of humans with involuntary infertility. MA-24 was germ cell-specific and did not bind to lymphocytes, erythrocytes, fibroblasts, lymphoblastoid cells, K562 leukemia cells, or other somatic cells by indirect immunofluorescence technique (IFT). The results of IFT were further checked in the absorption assay and in a test of residual activity in the ELISA after the antibodies had been incubated with various somatic tissues. In all of these and subsequent studies, both the supernatant from myeloma cell cultures and ascites fluids obtained from mice injected with other hybridoma clones were used as controls.

The MA-24 clone showed binding to the postacrosomes, midpieces, and tails of viable and methanol-fixed human spermatozoa by IFT. It showed the same binding pattern with capacitated, uncapacitated, and reduced (swollen-head) sperm treated with dithiothreitol and Triton X-100. It also reacted with murine, rabbit, bull and rhesus monkey sperm. It did not react with seminal plasma for vasectomized humans. In the Western blot enzyme immunobinding assay, the MA-24 clone reacted with a single 23-kD protein band when a detergent-solubilized membrane preparation of human tests was used. Thus, MA-24 was specifically directed to an intrinsic membrane protein of sperm and not to one absorbed onto the sperm surface from accessory gland fluids.

The tissue-specific but not species-specific MA-24 antibody to the 23-kD protein completely blocked binding and penetration of zona-free hamster ova by human sperm. MA-24 also significantly inhibited fertilization in vitro of marine oocytes by murine sperm. (Naz et al., Science, 342-344, 1984)

(2) <u>Isolation and characterization of the fertilization antigen (FA-1)</u>

The antigen (FA-1) to which the MA-24 antibody is directed was isolated from both human ejaculated sperm and testis by immunoaffinity chromatography. As the availability of human germ cells is limited for use in the isolation of FA-1, we purified and characterized it from murine germ cells. (Naz et al., Proc. Natl. Acad. Sci. USA, 83, 5713-5717, 1986)

FA-1 was purified from either deoxycholate- or lithium diiodosalicylate-solubilized murine testes by immunoaffinity chromatography using a monoclonal antibody, MA-24, which inhibited fertilization **in vitro.** The FA-1 was recovered at high (11.4) or low (2.8) pH using stepwise elution procedures of the deoxycholate or lithium diiodosalicylate extracts, respectively. Both of these fractions showed a single band of 47 kD when analyzed by $NaDodSO_4$/PAGE and silver staining. Following removal of the detergent and extensive dialysis at pH 5.8 or treatment with 0.15 M NaCl, even in the presence of detergent, a monomer of 23 kD was detected. Two-dimensional PAGE of FA-1 showed, 4 or 5 polypeptides in the 47-kD or 23-kDa range. The dialyzed FA-1 contained a major 23-kD and a minor 48-kD band when separated on both sucrose and cesium chloride gradients. High performance size-exclusion chromatography showed a major peak at 23 kD and a minor peak at 50 kD. Further analysis of the 23kD peak by reverse-phase chromatography resolved the antigen into 3 peaks, which gave similar two-dimensional gel patterns as the native FA-1. Lectin affinity chromatography on a lens culinaris column demonstrated that a part of the antigen was bound to the lectin while the rest was not. The FA-1 revealed a positive reaction with periodic-Schiff reagent and contained glucose and mannose, which together constituted 18.8% of the total antigen mass. Amino acid analysis showed a high percentage of aspartic acid, glumatic acid, serine, and glycine.

(3) <u>Effects of active immunization with FA-1 on fertility of female animals</u>

Female rabbits were actively immunized against the fertilization antigen (FA-1) isolated from lithium diiodosalicylate (LIS)-solubilized murine testis (Naz, submitted for publication). Three trials were performed in order to check the effect of immunization on fertility. In all of these trials, there was a significant ($P<.001$) reduction of fertility as determined by the percentage of 8-day implants/corpora lutea ratio (FA-1, 0-26.3%; adjuvant control, 79.4-100%). A complete block was observed in animals which received intravenous immunization with the antigen. Antisera collected from FA-1-immunized rabbits were negative in the agglutination and the immobilization techniques, and demonstrated modal titers of $\geq$ 1:2560 in the enzyme linked immunosorbent assay (ELISA) using FA-1. Antisera were tissue-specific and showed binding to the specific protein bands of 47,000 and 23,000 Mr, dimeric and monomeric forms of FA-1, respectively, in the Western blot procedure. Ova collected from rabbits inseminated with sperm which had been treated with antiserum for immunized rabbits showed reduced fertilization rates (anti-FA-1, 3.9-27.7%; control rabbit serum, 87.8%). There was again a reduction in percentage of the 9-day implants/corpora lutea ratio in the rabbits inseminated with treated sperm (anti-FA-1, 10.7%; control rabbit serum, 72.7%). It is concluded that active immunization with FA-1 resulted in a tissue-specific immune response which caused a reduction of fertility in rabbits, by a mechanism(s) involving an inhibition of the fertilization process.

(4) Role of FA-1 in involuntary immunoinfertility in humans

FA-1 isolated from either human or murine testes cross-react with sera from immunoinfertile patients (Naz et al., Science, 342-344, 1986; Naz, 1986 in Immunological Approaches to Contraception and Promotion of Fertility, ed. Talwar, G.P. (Plenum, New York, pp. 183-190). Sera from infertile patients, which had a block of **in vitro** fertilization (IVF), demonstrated a strong binding with FA-1 in ELISA. These results indicate that antibodies to FA-1 may be capable of blocking fertilization and fertility in humans. (Naz, 1986, in Advances in Vaccinology, ed. Talwar, G.P. (Springer-Verlag, New York, in press)

(c) Recommendations regarding unfinished work

Initially we isolated FA-1 using MA-24 immunoaffinity chromatography, which gave us enough antigen to perform preliminary characterization. For a large scale analysis, we need a large quantity of the antigen. FA-1 should be isolated using c-DNA recombinant technology and peptide synthesis approach for testing in sub-human primates. This will also provide homogeneous FA-1 required for the development of contraceptive vaccine.

PROJECT SUMMARY REPORT

A. General Information

1. Title and PARFR Subagreement Number - Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins. No. PARFR 359.

2. Dr. Bonnie S. Dunbar
Department of Cell Biology
Baylor College of Medicine
Houston, TX 77030
(713) 799-6255

3. Baylor College of Medicine

4. Subagreement Period - 06/01/84-10/30/86

5. Date of Last Technical Report Submitted - 06/30/86

6. Inclusive Dates of This Technical Report - 07/01-10/30/86

B. Narrative

During this funding period, we have isolated and purified zona pellucida proteins and have sent samples to Dr. Shoba Seghal (India) and to Dr. Vern Stevens (Ohio State University) for immunization of rhesus monkeys and baboons. We have immunized rabbits with a different form of a purified zona pellucida protein. The antibodies developed by these animals have been analyzed and the effects of immunization on ovarian function have been evaluated. These accomplishments are the first step in evaluating the efficacy of utilizing ZP proteins as immunocontraceptives in humans.

I. TECHNICAL RESEARCH REPORT

A. Objectives

It has been clearly established that active heteroimmunization of a variety of mammalian species with zona pellucida (ZP) will cause the production of antibodies which inhibit fertilization by preventing the spermatozoa from binding to the surface of the zona pellucida.

These studies have led to hopes that an effective immunological contraceptive method could be developed utilizing the ZP as an immunogen. More recently, studies have been carried out which demonstrate that porcine ZP preparation will result in altered ovarian functions in the rabbit and bonnet monkeys. The rabbit studies by Dunbar and colleagues first demonstrated that small primordial and primary ovarian follicles are damaged at the stage at which the ZP is forming. Therefore, subsequent differentiation of steroid-producing granulosa cells does not occur in these animals. Studies by Sacco use the major porcine ZP antigen purified by chromatographic methods demonstrated that this form of the ZP antigen also resulted in alteration of bonnet monkeys' ovarian function.

The present studies were designed to use the denatured and deglycosylated forms of ZP proteins for the following reasons: (a) to determine if the deglycosylated proteins are immunogenic; and (b) to determine if purified proteins cause alteration in ovarian function as do the native proteins.

The objectives of the proposed work were: a) determine whether primates develop antibodies against porcine zona pellucida (ZP) proteins which cross-react with their own zona pellucida; b) to characterize these antigens using established radioimmunoassay, enzyme-linked immunoassay, and 2D-PAGE Western blotting methods; and c) to determine whether these antibodies interfere with ovarian function as well as fertility.

B. Results of Project

All immunized animals were analyzed for antibody levels and ovarian function over a period of one year. The characterization of antibodies is summarized in Table 1. Examples of immunoblot analysis of these animals is shown in Figure 1.

DISCUSSION OF RESULTS

Characterization of Antibodies

The antibody titers obtained with the purified proteins were lower than those obtained with heat-solubilized porcine zonae pellucidae in this study as well as in previous animal studies. This could be attributed to three factors: (a) less protein was used per immunization; (b) protein purification methods require solubilized proteins to be used, therefore, some conformational determinants which may be involved in immunogenicity as well as antigenicity; or (c) a synthetic adjuvant was used instead of complete Freund's adjuvant.

Immunoblot analysis of rhesus monkeys immunized with HSPZ demonstrates that all three proteins are antigenic. The antibodies from baboons injected with the ZPI immunogen recognize antigens or both proteins I and III even though the purified deglycosylated protein III detected protein I, but not III by immunoblot analysis. These results strongly suggest that proteins I and III share some antigenic determinants. Because the chemical process which is used to deglycosylate and purify the ZP proteins is different from that used to separate the proteins for immunoblot analysis, it is probable that not all antigenic determinants are recognized by this procedure. This would explain why antibodies from animals immunized with protein III recognize only protein I on immunoblot analysis.

Although it is possible that either purified protein I and III could contaminate each others preparations, the fact that no antibodies are developed against protein II in either Group I or Group II antigen would argue against this. Because protein II is more abundant than protein III and is closer in both molecular weight and charge to either, it might be expected to involve equal or greater antibody titers than protein III because it would provide a greater extent of contamination.

In summary, these studies demonstrate that non-human primates immunized with total purified and deglycosylated proteins will develop antibodies to ZP antigens which alter ovarian structure and function. Group I baboons had higher titers of antibodies, reduced estrogen levels as well as reduced numbers of antral follicles. Group II baboons had lower antibody titers and did not exhibit dramatic changes in hormonal levels over the immunization although the ovarian histology suggests that the early development of primary follicles are being altered which could later effect ovarian granulosa cell differentiation. The antibodies which appear to be responsible are directed against ZP protein I and the extent of this effect is related to the antibody titer.

Future Studies Necessary Based on Present Study

Because antibodies to porcine ZP protein I appear to be responsible for the alteration in ovarian physiology, it is apparent that attention should be focused on ZP proteins II and III. These proteins appear to be important molecules in the fertilization process since they are susceptible to proteolysis by the sperm acrosomal enzyme, acrosin. Because these proteins are far less abundant than protein I and because they can easily be contaminated with this protein, it is apparent that future studies need to be

directed towards the use of recombinant DNA technology for the generation of sufficient quantities of purified protein for use as immunogen.

Summary of Rabbit ZP Studies

Four (4) rabbits immunized with deglycosylated protein I and complete Freund's adjuvant developed only low titers of antibodies (<1000 titer by ELISA). Immunoblot analysis gave patterns identical to those shown in Figure 1. These studies demonstrate that the immunogenicity of ZP glycoproteins is greatly reduced following denaturation and deglycosylation. These demonstrate that future studies will require methods for immunogenicity enhancement if purified proteins or peptides are to be used to determine efficacy in eliciting antibodies which may effectively inhibit fertilization.

Table 1

Characterization of Antibodies From Primates

Immunized with Porcine ZP Antigen

Animal	Antigen Used	Adjuvant Used	Highest Antibody Titer During Study[a]	ZP Antigens Recognized by 2D-PAGE
Group I - Baboons (immunized by Dr. Vern Stevens)				
	Purified ZPI[b]			
Zerna	"	CGP-11637	124,000	ZPI & III
Winnie	"	"	62,000	I & III
Ariel	"	"	62,000	I, II, & III
Xandra	"	"	124,000	I & III
Dona	"	"	62,000	I & III
Group II - Baboons (immunized by Dr. Vern Stevens)				
	Purified Deglycosylated ZPIII[c]			
Laurel	"	CGP-11637	8,000	I
Ukena	"	"	8,000	I
Effy	"	"	4,000	I
Whitney	"	"	4,000	I
Ina	"	"	4,000	I
Rhesus Monkeys (immunized by Dr. Shoba Seghal)				
#1	HSPZ + CFA		> 250,000	I, II & III
#2	"		"	"
#3	"		"	"
#4	"		"	"
#5	"		"	"

[a]Antibody titer determined by enzyme-linked immunoassay: value is 50% serum giving 50% maximum binding.

[b]ZPI purified by electroelution of the most glycoprotein (I) from preparative isoelectric focusing gels.

[c]Heat-solubilized porcine ZP were treated with trifluoromethane sulfonic acid to remove majority of carbohydrate. Preparative SDS gels were run and stained with Coomassie blue ZP protein III (molecular weight of 75K) was isolated and electroeluted from the PAGE gel.

Table 2

Summary of Effects of Immunization
with ZP Immunizations on Ovarian Function

	Serum[a] Estrogen Levels	Summary of Cycle[b] and Ovulation at Experiment End	Ovarian[c] Histology (avg. follicles/xsect.)
Group I - Baboons			
Zerna	slight reduction	ovulation	old corpora lutea 1 antral follicle
Winnie	significant reduction	ovulation	10 antral follicles
Ariel	significant reduction	short follicular phase	1 antral follicle
Xandra	reduction	no ovulation	1 old corpora lutea 1 antral follicle
Dona	reduction	no ovulation	no large follicles and corpora lutea
Group II - Baboons			
Laurel	normal	ovulation	no antral follicles[d]
Ukena	normal	ovulation	4 antral follicles[d]
Effy	normal	ovulation	2 antral follicles
Whitney	normal	ovulation	6 antral follicles
Ina	normal	ovulation	5 antral follicles
Control Baboons			
	-	-	>40 antral follicles
	-	-	>40 antral follicles
	-	-	>30 antral follicles
Rhesus Monkeys			
1	-	No	-
2	-	No	-
3	-	No	-
4	-	No	-
5	-	No	-

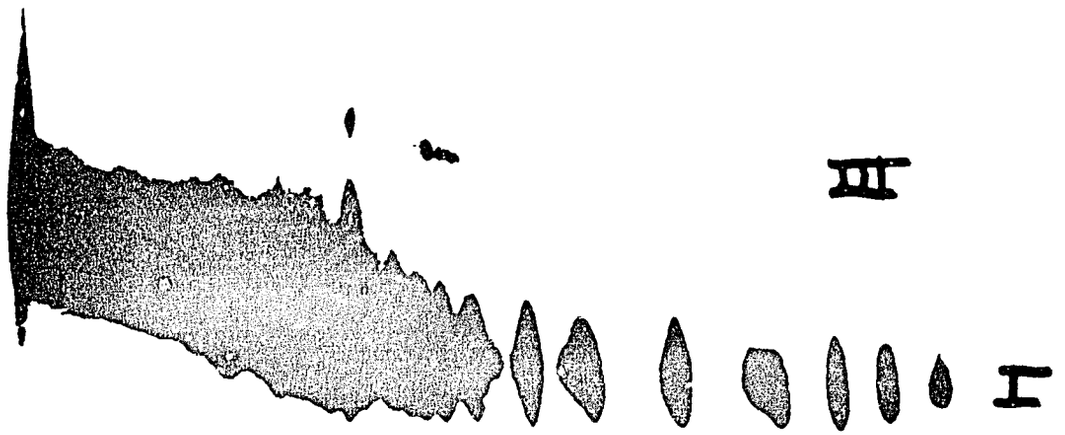
[a]Levels measured by Dr. Stevens Gros before and after immunization (from final technical report to PARFR by D. V. Stevens)

[b]Ovulation determined by progesterone levels and menses in baboons and by color of sex skin in rhesus monkeys.

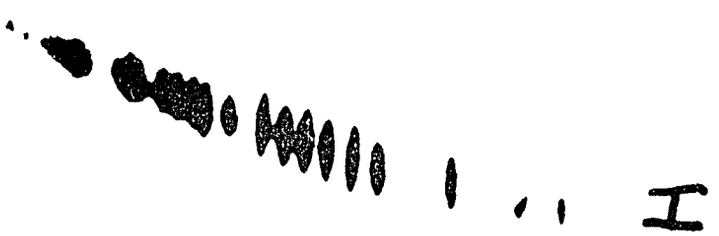
[c]Ovaries from group one were frozen during shipment from Ohio to Texas. Therefore, only late stages of follicular development could be determined (see examples in Figure 2).

[d]Granulosa cells in majority of antral follicles had pycnotic nuclei which suggested atresia in follicles as compared to control animals.

Figure 1: Examples of two-dimensional PAGE immunoblot patterns obtained by antibodies from immunized monkeys (A) Pattern obtained by all baboons from Group I. (B) Pattern obtained by all baboons from Group I.

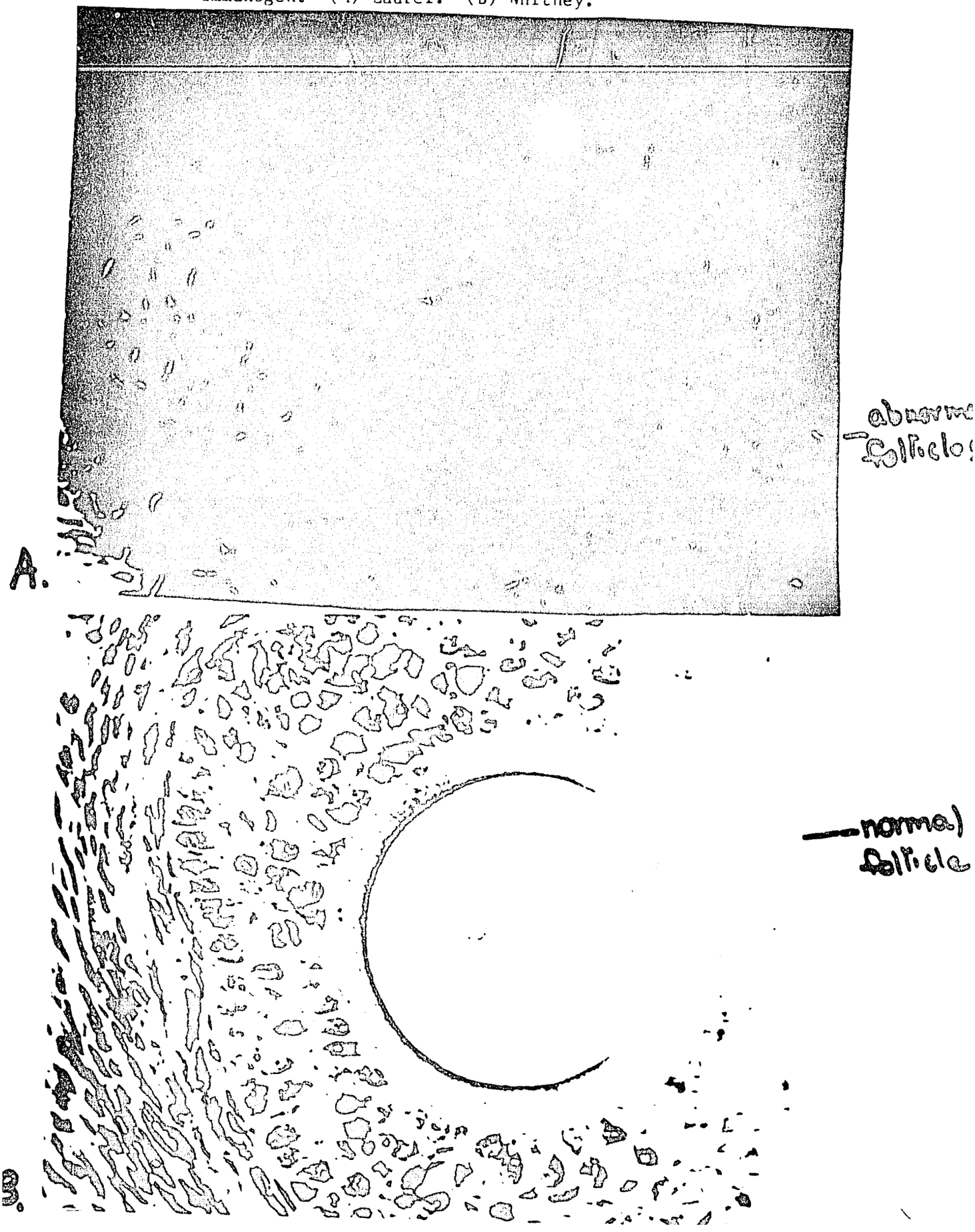


A.



B.

Figure 2: Examples of ovarian histology from animals immunized with ZP immunogen. (A) Laurel. (B) Whitney.



PD-AAW-474

VOLUME III

PARFR
FINAL REPORT

DPE-0546-A-00-1003-00

July 1, 1986 - June 30, 1987

FINAL PROGRESS REPORT OF THE PARFR-360 PROJECT
(July 1, 1984 - September 30, 1985)

The objective of this project was to test the reproductibility of the cervical mucus guaiacol peroxidase (GP) patterns in ten (10) volunteers, each tested for six (6) menstrual cycles. Daily samples of cervical mucus were collected with a fertility cannula at the external os, following the insertion of a speculum. GP activity was measured spectrophoretically after extraction of the specimen in 0.5 M $CaCl_2$-10 mM Tris-Cl pH 7.2 buffer and brief homogenization in a glass-tefflon mechanical homogenizer; the homogenates were spun at 2000 g for 10 min before analysis for GP activity. To define and "verify" the time of ovulation, daily first-morning urine samples were collected for determination of LH and pregnanediol levels. The hormone assays were done by RIA in Dr. R.T. Chatterton's laboratory.

One-sided cusum analysis of the data was first done by Dr. Ronald H. Gray at Johns Hopkins University and finally, on the 58 cycles, by Dr. Patricia Langenberg at the University of Illinois at Chicago. The cusum analysis was done according to J.P. Royston, Ovulation: Methods for its prediction and detection, S.L. Jeffcoate, ed., John Wiley and Sons, Ltd., 1983 p. 19-32. Reference level was defined as one standard deviation below the mean of the first five observations of the log-transformed data. In order to qualify as a "drop", 1) the data values had to stay below the reference level for 3 consecutive days, and 2) the cumulative difference from the reference level dropped below 2 standard deviations. The first day of the sequence (below the reference level) was the day of the drop. Correspondingly, in the postovulatory day of the "rise" the GP values have to be "above" the two criteria (reference level and cumulative difference).

The results of the cusum analysis are shown on Table 1. For a variety of reasons only 5 volunteers completed all six cycles, 2 finished 5 cycles and the remaining 18 cycles were done with 6 volunteers. One volunteer (JP) became pregnant during her test cycle, although she had assured us that she would not have unprotected intercourse during the study months. The three cycles of volunteer KS and the cycle of SW were anovulatory, as indicated by their hormone levels (see complete data in Appendix 1). The first two cycles of MK showed abnormally low urine LH levels.

Some of the cycles shown in Table I and Appendix 1 did not "trigger" a drop or a rise signal according to the cusum test. We are now analyzing the hormone and BBT levels of these cycles to determine if these parameters would provide a clue for the no-trigger observation.

Table 2 shows the distribution of the ovulation prediction ("drop"-days before the LH + 1 day) in 43 cycles; the data were obtained from Table 1. Dr. Ronald H. Gray had kindly performed a cusum analysis of our earlier GP data and discussed with Dr. Patricia Langenberg, our statistician, how to perform the cusum test. In his August 29, 1985 letter to Dr. A. Goldsmith, Dr. Gray outlined some criteria in evaluating the usefulness of the GP method in natural family planning:

a) A <u>late</u> signal, 2 days before ovulation, is considered equivalent to a method failure.
b) An <u>optimal</u> signal is 3-6 days before ovulation.
c) A <u>premature</u> signal would be 7 days before ovulation.

According to these criteria, the data in Table 2 show 9.3% late signal, 67.6% optimal signal and 23.2% premature signal. We agree with Dr. Gray that it is impossible to translate these percentages to expected pregnancy rates. Although a 9.3% "failure" rate may seem acceptable for NFP, we believe that the cycles with 2 day prediction show some underlying abnormality which would preclude that they could be potentially conception cycles. Further analysis of these data (Appendix 1) and of the pre-PARFR-360 cycles, where daily serum LH, P4 and E2 levels were determined, would shed some light into the "normality" of the GP profiles. Such analysis is now underway as we prepare manuscripts for publication. We will send these manuscripts to PARFR before they are submitted for publication.

Table 1 also gives an outline of the mean fertile period, namely, 6.78±0.33 SE days as determined from the GP profiles of 37 cycles.

Cusum analysis of GP profiles was also done in 22 menstrual cycles which were studied in 1982-83. In these cycles we found a mean 3.14±0.36 SE days trigger of the pre-ovulatory drop in GP values and a mean 2.27±0.24 SE days trigger of the post-ovulatory rise in GP. In these cycles the day of ovulation is designated as the serum LH+1 day. In the fifth cycle of volunteer SS (Table 3) we collected daily early morning serum and first morning urine samples thus establishing (Table 3) that, at least in this cycle, the urine LH peak occurred 24 hours later than the serum LH peak. Therefore, the mean drop-rise values of -5.11±0.31 to +1.68±0.18 obtained from Table 1 would seem compatible with the corresponding -3.14±0.36 to + 2.27±0.24 values obtained from a different group of volunteers, since there is one day difference in the estimation of day 0 between the two groups of women.

Further analysis of the urine pregnanediol, BBT and GP values of the cycles in Table 1 and Appendix 1 will be performed and compared with the serum progesterone and BBT and GP values of the earlier 22 cycles. This analysis will help determine the "dependability" of the GP profiles. We expect that the GP profiles will provide a reliable parameter in predicting the time of ovulation and in defining the fertile period in women.

FINAL PROGRESS REPORT OF THE PARFR-360 PROJECT
FROM JULY 1, 1985 TO SEPTEMBER 30, 1986.

The objectives and procedures of this project were, in chronological order as follows: 1) to evaluate the ability of twenty (20) women to sample their cervicovaginal secretions using a prototype sampling device containing a retractable Calgiswab (calcium alginate swab). After self-sampling, cervical mucus would be sampled at the external os by a technician following the insertion of a speculum. Both samples would be extracted with the calcium buffer and the GP content measured spectrophotometrically.

Thirty (30) volunteers would be recruited to participate in a "dye-study" to help optimize the self-sampling procedure. Specifically these volunteers would be asked to insert the sampling device until they think it reached the posterior fornix. Then the nurse or physician would insert through the hollow sampling device a Calgiswab which had been slightly immersed in one of three dye solutions, namely, methylene blue, gentian violet or indigo. The dye mark, visualized after inserting a speculum, would show the position reached by the swab.

2) Test if vaginal infections interfered with the GP test. Twenty (20) patients diagnosed with monilia and trichomonas or non-specific vaginitis would be studied for six (6) days following their initial visit and initiation of treatment. A technician would insert a speculum and collect cervical mucus at the external os using a fertility canula and "vaginal secretions" at the posterior fornix using a Calgiswab.

3) Characterize biochemically cervical mucus GP during the cycle. Both normal and infected cervical mucus would be studied. Additionally, semen samples from 15 volunteers would be collected to be used in in vitro studies with GP preparations and assess how sperm and seminal fluids inhibit the GP assay of cervical mucus samples obtained by the users of the method.

4) Develop a colorimetric test for GP. A separate group of ten (10) volunteers would be studied for one menstrual cycle each. The technician would collect two samples after the insertion of a speculum. First, cervical mucus at the external os using a canula and second cervicovaginal secretions at the posterior fornix using a Calgiswab. The first sample would serve as "control" to show the GP "profile" and would be assayed spectrophotometrically after the usual extraction/homogenization procedure. The swab would be dropped in a solution containing the 0.5 M $CaCl_2$ extracting buffer, guaiacol and H_2O_2 and the color developed would be compared in the next 5 min with an appropriate color chart. The GP "profiles" of the two assays would be compared and the reagent concentrations adjusted to optimize the GP reaction as a home test-kit method.

RESULTS

DYE-STUDY TO TEST SELF-SAMPLING TECHNIQUE (APPENDIX 2)

To optimize the cervical mucus sampling procedure 29 women were instructed on how to insert the prototype sampling device. They marked with the swab dipped in methylene blue, gential violet or indigo carmine the position reached by the device; the dye mark was visualized after inserting a speculum. We tried different methods with the first 15 volunteers with a 60-80% success rate, and

tested the optimal procedure with the last 14 volunteers with a 93% success rate only one woman was unsuccessful in reaching her posterior fornix or os, due to the position of her cervix. The supine squatting positions were equally good and comfortable for inserting the device as the standing position was preferred only in 16-20% of the cases. The squatting position was used in the self-sampling study.

Sampling Procedure:

The volunteer is instructed to insert the device, with swab inside it, until she reaches her posterior fornix. As the device is inserted the curved tip of it should be pointed towards her right. When she reaches the fornix she pushes the swab forward to expose the tip and turns the device 360°. Then she retracts the swab inside the device and pulls the device out.

SELF-SAMPLING STUDY (APPENDIX 3)

The summary page of Appendix 3 contains our comparison of the technician-sampled and volunteer-sampled GP profiles of twenty (20) menstrual cycles. There are three inherent difficulties in attempting to compare the two GP profiles. 1) The volunteers obtained cervicovaginal secretions at a location of the posterior of the vagina which is not exactly known and may vary from day to day, whereas the technician could always visualize the external os. In the latter case no attempt was made to collect either a constant amount of mucus or all of the mucus present. 2) Even at midcycle the cervical mucus is not a homogeneous suspension of aqueous and gel-like materials and even repeated samplings at the external os could give widely different GP contents (units/g wet mucus). As demonstrated in the "clinical-colorimetric" study of 10 cycles (see below) the Calgiswab seems to retain some GP even in the 0.5 M $CaCl_2$-Tris solutions whereas the extraction/homogenization of the canula sample is more efficient. Also, in the "infections" study of 20 women (see below) the swab sampling was done by the technician at the posterior fornix and with the use of a speculum, and yet the swab GP values were consistently lower than the canula GP values. This suggests that either the swab retains a lot of GP or that the concentration of GP at the posterior fornix might be lower (for a variety of reasons) than that at the external os. Even if the concentration is lower this does not pose a problem since there is adequate GP activity to give us a strong color, as was found in the "colorimetric" study of 10 cycles (see below). Nevertheless, this self-sampling study is very useful since it identified problems which must be solved before the method is suitable as a home self-test.

Only in 7 out of 20 cycles (35%) was the self-sampled GP profile judged as similar to the canula-sampled GP profile and of apparent equal value as an indicator of impending ovulation. Moreover, in 4 of the 7 "good" cycles we noticed very low levels of follicular phase GP (u/g); one explanation would be that low GP values indicate the presence of a form (isoenzyme, monomer) of GP which does not "stick" to the Calgiswab as tightly as another form, for example, the luteal phase GP. Strengthening that explanation is the observation that in 75% of the cycles tested (last column of summary page, Appendix 3) the Calgiswab samples showed luteal phase GP significantly lower than that of canula samples. The radioiodinated cervical mucus samples (see below) could be used to test if the Calgiswab preferentially retains certain proteins in cervical mucus. Alternatively, the Calgiswab may retain some small peptides or non-protein molecules which may influence the observed GP activity.

In terms of the net weight of canula vs swab samples we do not believe that there are significant, consistent differences between them. Two possible explanations for the consistently very low GP content of the Calgiswab samples from volunteers SS, CV and GG are, a) all three consistently failed to reach far enough into the posterior vaginal area, and b) all three had in their mucus some factor(s) which caused the apparent lowering of the GP content. That factor cannot be excessively acidic vaginal pH since one of them (MC) was shown to have normal pH. Moreover, MC had participated in the dye-study and is an experienced gynecologist's assistant; however, at present she has been unable to conceive.

STUDY OF GP IN WOMEN WITH VAGINAL INFECTIONS (APPENDIX 4)

Twenty women were recruited from our Clinic or from advertising within the University of Illinois; the women were selected randomly, in terms of the day of their cycle. A physician documented, but did not quantitate, their infection and prescribed treatment. Four women refused treatment but we decided to include them in the study to test if the untreated infection had an impact on the observed GP levels. In the attached graphs (Appendix 4) the arrows on the X-axis indicate the days of treatment.

It seems that none of these infection were serious enough to cause a noticeable invasion of the cervical mucous with WBC peroxidases (as was seen previously in one patient with severe yeast infection). Also, the medications did not seem to affect, positively or negatively, the GP present (not even daily vinegar douches). The drugs could, in principle, affect both the production of GP as well as the in vitro GP assay. Unfortunately, under our protocol we could not quantitate the degree of infection or have a more extensive follow up of the patients.

We believe that mild vaginal infections would not exert an appreciable effect on the observed GP levels.

COLORIMETRIC (CLINICAL) STUDY (APPENDIX 5)

We have studied the 10 cycles outlined in the summary page of Appendix 5. Our objective was to begin testing conditions for the development of a kit. We developed the five standard colors shown in the color photograph to represent appropriate ranges of absorbancy. Then decided that 2 ml of the 0.5 M $CaCl_2$-Tris buffer, 26 mM guaiacol and 0.6 mM H_2O_2 would be a good starting point in extracting the GP activity from the Calgiswab within 2 min or so.

The first cycle tested (J.J.) showed good promise (the legend of Figure 1 outlines our procedure and the tabular data are shown in Appendix 5). In that cycle we saw too much color which gave a 3-day instead of the 4-5 day prediction as expected from the cervical mucus GP data shown in column 2 of the Table and the graph. Nevertheless, we decided not to dilute the reagents since in another concomitant cycle (D.S.) we were observing very low cervical and swab GP values. In some cycles we took a second swab and also dipped the first swab in a fresh solution of guaiacol and H_2O_2 (referred to as Phase 2). To our surprise almost all of the GP activity must have remained bound to the Calgiswab as shown by the intense colors of Phase 2. Thus, in the limited time of 2-5 min extraction only a small amount, if any, of GP was extracted into the solution. The intra-iodination method of radiolabelling cervical mucus proteins (including the autoradioiodination of GP and its subunits) is described in the next section of

this report and will prove most useful in, a) clarifying the efficiency recovery from the swab of the putative subunits of cervical mucus GP, under different times and conditions of extraction, and b) screening the suitability of other materials, besides calcium alginate, to coat the "swab". Additional studies are clearly needed to solve the extraction/color development problem. The system may be more complex than is currently assumed, especially if the observed midcycle drop in the specific activity of GP (Units/g wet mucus) is a combination of a) dilution (due to the increased amounts of secretion, as we have discussed in two of our recent publications), and b) the presence of endogenous GP inhibitors, e.g. estrogens. What is not known yet, but could have a bearing on the clinical trials of the method, is the possibility that the swab binds one compound (e.g. inhibitor of GP) more tightly than others (e.g. proteins and GP itself). We had observed earlier that cotton Q-tips would bind GP so tightly than no GP activity could be demonstrated.

Notice that in the self sampling studies, we observed lower concentration of GP in the swab samples than in canula samples, possibly because the time of extraction (which varied from 30 min onwards) was not long enough in all cases.

Since the GP method to predict ovulation is based on a timed measurement of specific activity it is important that the net sample weight does not fluctuate widely from day to day. The summary Table and the tabular data of Appendix 5 show the mean sample weights which do not seem to vary appreciably within a given cycle and person.

Although all 10 cycles showed significant midcycle variations in the spectrophotometrically measured GP (U/g) of cervical mucus, only two cycles (J.J, and M.P., Figures 2 and 3) gave promising results with the colorimetric test. We are convinced that further research along the outlined lines will eliminate this initial difficulty.

INTRA-IODINATION OF NATIVE HUMAN CERVICAL MUCUS (APPENDIX 6)

In addition to colorimetric reactions, such as guaiacol oxidation, peroxidases catalyze the iodination of protein tyrosine residues in the presence of iodide and hydrogen peroxide. The radioiodination of proteins is being used to label proteins in intact cells, cell homogenates or to label purified proteins containing tyrosine residues and to study metabolism, and the structure and function of biological systems.

We decided to request from PARFR assistance in initiating biochemical studies of cervical mucus GP in order to accelerate our progress in the adaptation of the method to home use. The use of endogenous cervical mucus GP to radiolabel proteins located near GP and interacting with GP had three major advantages: 1) It could be accomplished in the native state (without the need to denature the mucin structure, as is the case in all biochemical studies reported so far), 2) It could be done with a few milligrams of mucus, which made the study feasible throughout the menstrual cycle, and 3) The reagents, iodide-125 and H_2O_2, were small enough to penetrate the rather compact mucin structure during the early follicular and luteal phases.

GP requires high salt concentrations to be extracted from cervical mucus. We could take advantage of the "particulate" nature of PG, iodinate the mucus, then perform a fractional extraction of the various components (depicted in Schema 1) and finally use electrophoresis which can resolve proteins according to their size and charge. However, the required high salts (0.5 M $CaCl_2$) needed for GP extraction would interfere with all electrophoretic procedures and in the absence of high salt GP would precipitate. Therefore, we had to resort to non-ionic "solubilizers" of particulate proteins such as the detergent Triton X-100. This, is how we found that millimolar concentrations of Ca^{++} are required for GP activity because in the presence of detergent GP remained soluble but had lost all its activity in guaiacol oxidation.

Schema 1 shows the steps in our analysis of cervical mucus and a hypothetical diagram of the mucin structure. The supporting framework consists of mucin chains cross-linked via covalent tyrosine-tyrosine bonds. The cross-linking is presumably catalysed by GP in the presence of H_2O_2. Following the iodination reaction, soluble proteins and hormones (e.g. relaxin, LH, other serum proteins, sex hormones, prostaglandins, etc) would be removed with the buffer extraction (only 5 mM Ca^{++} was present to preserve GP in its active form). The 0.5 M $CaCl_2$ extraction would remove GP and other non-covalently bound proteins in cervical mucus; 0.5 M $CaCl_2$ or 1 M NaCl work as chaotropic agents. Reduction of -S-S- bonds with mercaptoethanol (2-ME) and sodium dodecyl sulfate (SDS) would extract from the "scaffold" all proteins which are "neighbors" of GP (since they are iodinated by GP) and are held together via cysteine-cysteine bonds. Such drastic treatment could liberate any "cross-linking" proteins, the putative elements holding in place the three-dimensional structure of mucins. It has been shown that the mucin structure becomes less-crosslinked at midcycle thus allowing the penetration of spermatozoa. Until recently, we would not have attributed -S-S- bond formation to a peroxidase (although GP participates in an oxidative process) but Turner et al. (J. biol. Chem. 261:13056, 1986) reported that ovoperoxidase interacts with a thiolhistidine compound. Therefore, it is our working hypothesis that GP either catalyzes the cross-linking of mucins or GP serves as a bridge or linker in the mucin structure.

The treatment of the remaining mucin structure with N-glycanase, in the presence of 2-ME and SDS, should remove all the N-linked carbohydrates from the mucin structure and potentially liberate other iodinated proteins. Finally, complete hydrolysis of the last pellets (not with 6 N HCl but enzymatically, since HCl could de-iodinate the iodotyrosine residues) should liberate the radiolabelled and non-labelled tyrosine-tyrosine dimer. The dimer can be easily separated by chromatography and identified by its characteristic fluorescence spectrum.

RESULTS AND DISCUSSION

Figures 4, 5 and 7 show the results of our first intra-iodination experiments with human cervical mucus. Following iodination with endogenous GP we have extracted/treated the mucus according to the general steps shown in Schema 1; the details of the methods and procedures are given in the next section. One- and two-dimensional electrophoretic separation of the extracts is followed by gel autoradiography.

Figure 4 shows the results obtained with mucus samples obtained during the cycle of volunteer L.A. Two mucus samples had been obtained during her participation in the colorimetric trial (Figure 4A); the days were numbered from

the onset of menses. In this series of iodinations we skipped step 1 and did only step 2 (calcium extraction of mucus). Following iodination we treated an aliquot of the extract with 0.3 M disuccinimidyl suberate, a bifunctional reagent which cross-links protein through N-terminal or lysine amino groups. In the top row of the two dimensional gels there is a significant change in the patterns from day 11 to 28. This is a demonstration of the architecture of the Ca-extractable proteins of human cervical mucus from the vantage point of the guaiacol peroxidase. From the extent of protein iodination, it became apparent that the level of GP activity in the mucus did not correlate with iodination levels, as if the iodination was independent of GP concentration! However, this has been observed in numerous other peroxidase-catalysed protein iodinations (cf. Huwiler and Kohler, Eur. J. Biochem. 147:469-476, 1985) where the kinetics of iodination depend mainly on the reactivity and concentration of the tyrosyl residues. It is interesting that the bifunctional reagent (DSS) does not cause an indiscriminate cross-linking of proteins to give aggregates with very large molecular weight. Instead it appears that DSS forms an 80-90 kDa complex which is apparently more acidic (pI ca. 6) than its components and somewhat independent of the day of the cycle.

Figure 5 compares the iodination patterns of mucus from two women, one having a vaginal yeast infection. There appears to be a groups of proteins in the range of 70 kDa and pI=4.7-5.2 which is only present in the infected mucus. At present we do not know whether the differences are due to individual variations among the two women, are due to the different days of the cycle or if the new bands represent white blood cell or yeast proteins which were in the vicinity of GP and became labelled.

Samples from volunteer L.A. (Figure 6) were used to obtain the data shown on Figure 7. These samples were treated according to the four steps shown in Schema 1. With buffer extraction (5 mM calcium-Tris buffer) two main bonds are seen in the range of 50-66 kDa. Calcium (0.5 M) extraction, which removes most of the GP activity, gave two similar bands and a smaller bond around 10 kDa. Myeloperoxidase which was shown also to be calcium dependent (Shiro et al. J. Biol. Chem. 261:9382, 1986) consists of a 57 and a 10 kDa subunits. Thus, it appears likely that cervical mucus GP has also two subunits and that both become autoiodinated. GP staining of the gels should clarify some of these assumptions. High-performance liquid chromatography could also be used to separate these proteins. Reconstitution experiments could then be performed to determine the function of each subunit.

The reduction of the remaining materials with 2-ME showed the presence of some very large iodinated proteins which must have been released from the mucin structure by reducing -S-S- bonds. Also some small proteins were released and were more prominent in the Day 17 sample.

After N-glycanase treatment some intensely labelled, small proteins were extracted. It is not unreasonable to suggest that they represent "linker" proteins rather than being mechanically "trapped" in carbohydrate-rich sections of mucus.

We believe that this method will prove invaluable in 1) understanding the mucus structure and the hormone influence on such structure during the menstrual cycle, and 2) evaluating the suitability of various materials for sampling human cervical mucus. Because this portion of the work demanded more time than had been anticipated we were unable to test the effect of the ejaculate on GP activity. A

more meaningful study with semen components and GP will be feasible once the intra-iodination data have been better analyzed and some of the radiolabelled proteins been identified.

INTRA-IODINATION OF NATIVE CERVICAL MUCUS

PROCEDURE

Human cervical mucus was removed from the external os with a fertility cannula. The sample (20-400 mg) was gently dispersed into 0.3 ml of 0.3 M Tris-Cl/5 mM $CaCl_2$ for 30 min at room temperature. Sodium iodide-125 (5 uL, 0.5 mCi) was added and the sample was stirred gently with a glass rod for 10 min. The iodination reaction was initiated with 5 uL of 0.3% hydrogen peroxide. After 10 min the sample was transferred to a dialysis bag (mol. weight cut-off 1,000) and dialyzed at room temperature for 3 x 1 hr in 1 L of 10 mM Tris-Cl pH 7.2 buffer. The dialysate was centrifuged overnight at 70,000g and 10°C.

The supernatant solution, containing all soluble mucous proteins, is designated as Solution A.

The 70,000g pellets were incubated overnight at room temperature in 10 mM Tris-Cl pH 7.2/0.5 M $CaCl_2$ to extract guaiacol peroxidase and other "particulate" proteins. The suspensions were spun for 2 hours at 70,000g and 10°C. The supernatant solutions were dialyzed overnight at 4°C against a 10 mM $CaCl_2$, 10 mM HEPES pH 7.2, 0.05% Triton X-100 solution to remove excess calcium as dictated for further electrophoresis experiments. The dialysate is designated as Solution B.

To the last pellets 0.17% sodium dodecyl sulfate (SDS) and 34 mM 2-mercaptoethanol were added and the suspensions heated to 95°C for 30 min. The heated suspensions were spun at 70,000g and 10°C for 12 hours. The supernatant solution was designated as Solution C.

To the last pellets were added 0.17% SDS, 34 mM 2-mercaptoethanol and the suspensions were heated at 95°C for 5 min. After cooling to room temperature the following compounds were added at the indicated final concentrations: 0.2 M sodium phosphate pH 8.6, 10 mM EDTA, 1.25% NP-40 detergent and 5 units/ml N-glycanase (peptide: N-glycosidase F, Genzyme Corp., Boston, MA). These suspensions were incubated at 37°C in a shaker for 48 hours, and then centrifuged at 70,000g for 4 hours at 10°C. The supernatant solution was designated as Solution D. The pellets are stored at -20°C and will be completely hydrolyzed enzymatically (Neutralase, ICN Biochemicals) and analyzed by HPLC (fluorescence detection) to determine the presence of the putative tyrosine-tyrosine dimers formed via ether or C-C bonds.

PROCEDURES FOR POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) AND ISOELECTRIC FOCUSING (IEF) OF ^{125}I-LABELLED SOLUTIONS A-D

1st dimension (IEF and NEPHGE)[1]: 9.2 M urea, 2% NP-40, 5.64% acrylamide/DATD (from a stock of 31.96% acrylamide and 5.64% DATD) and 2.04% ampholines (1% pH 3.5-10, 0.72% pH 5-7, 0.32% pH 9-11). Polymerize IEF tube gels with 15 ul of 0.28 g/ml ammonium persulfate per 10 ml gel mixture and NEPHGE gels with 20 ul per 10 ml mixture.

2nd dimension (SDS-PAGE)[2] - Separating gel: 5-20% acrylamide (0.08-032%

Bisacrylamide as crosslinker), 0,1% SDS, 0.056% ammonium persulfate, 0.08% TEMED (in 1.5 M Tris-Cl pH 8.8)

- Stacking gel: 4.5% acrylamide, 0.12% Bisacrylamide, 0.1% SDS, 0.058% TEMED (0.5 M Tris-Cl p 6.8), 0.053% amm. persulfate.

Molecular Weight Protein Standards (Bio-Rad Laboratories, Richmond, CA)

Proteins	Molecular Weight
lysozyme	14,400
soybean trypsin inhibitor	21,500
carbonic anhydrase	31,000
ovalbumin	45,000
bovine serum albumin (BSA)	66,000
phosphorylase B	92,000
beta-galactosidase	116,000
myosin	200,000

The molecular weight markers are prepared for use by making a 1:20 dilution into the sample buffer for SDS-PAGE. The sample buffer consists of 5% SDS, 8% glycerol, 0.71 M 2-mercaptoethanol and 0.004% bromophenol blue. The proteins are then denatured by incubation at 95°C for 5 minutes. A 20 ul sample volume is sufficient to give well stained bands each containing 2 ug of protein.

References

Groppi, Jr., V.E. and Coffino, P. G1 and S phase mammalian cells synthesize histones at equivalent rates. Cell 21:195-204 (1980).

O'Farrell, P.Z., Goodman, H.M. and O'Farrell, P.H. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12:1133-1142 (1977).

Silver Staining of Proteins Following Polyacrylamide Gel Electrophoresis

Materials:

All solutions are prepared from analytical grade reagents. Deionized, glass-distilled water is used in the preparation of all solutions. The volumes of these solutions are sufficient to stain a gel measuring approximately 15 x 15 cm., and can be adjusted accordingly for larger or smaller gels.

1) Solution A. 2000 ml of 50% (V/V) methanol.
2) Solution B. Ammoniacal silver nitrate solution is prepared as follows: mix 63 ml of 0.36% NaOH solution and 4.2 ml of concentrated ammonium hydroxide. Then 12 ml of 20% (W/V) silver nitrate solution is added dropwise while the solution is vigorously mixed. After mixing, 220 ml of water is added.
3) Solution C. 500 ml of 0.005% (W/V) citric acid, 0.019% (V/V) formaldehyde (0.25 ml of 38% (V/V) formaldehyde per 500 ml).
4) Solution D. 500 ml of 50% (V/V) methanol, 3% (V/V) glycerol, 10% (V/V) acetic acid.

All of the above solutions are prepared fresh as required and used immediately.

Methods:

Approximately 500 ml of each solution is used for each wash (with the exception of Solution B) and the gel is continually agitated at 20-50 rpm on a shaker table.

1) Proteins are separated by polyacrylamide gel electrophoresis and the gel is then placed in a tray and washed 16-24 hours in Solution A, with 2-3 changes (Several changes and an overnight soak are recommended for high % gels, thick gels, or gradient gels).
2) Prepare Solution B which is should be used within 5-10 minutes of preparation. Rinse the gel with 3-4 changes of distilled water. Stain the gel in Solution B with gentle agitation for 15 (with thin gels) - 30 (thick gels) minutes.
3) Rinse the gel with agitation in distilled water for 5 minutes, with 3-4 changes.
4) Visualize the proteins by the addition of Solution C and gently rocking the gel dish. When the image has developed to the desired intensity (usually within 5 minutes), remove the gel and place in a tray containing Solution D to stop stain development. Wash in this solution for at least 3 hours; the gel can, however, be stored for several days without a noticeable change in color or background staining, prior to drying it preparatively for autoradiography.

Autoradiography:

After drying the electrophoresed, silver-stained polyacrylamide gel onto a piece of absorbent filter paper (using a Bio-Rad Model 483 slab dryer and Precision Model D25 vacuum pump, with 2 cold traps in the vacuum line) for 2-3 hours at 80°C, the gel is now ready for autoradiography. Using direct exposure, place the gel in contact with a piece of Kodak X-OMAT AR Film and store in a Wolf Deluxe cassette containing 2 Wolf hi-plus intensifying screens. Exposure is done at -70°C for a variable length of time determined by the amount of radioactivity on the gel.

Pertinent References:

1) Gradient Gels
   a) Esposito, J. and Obijeski, J. High Resolution Polyacrylamide Gradient Gel Electrophoresis. Preparative Biochemistry 6:431-42 (1976).

2) Silver Staining
   a) Merrill, C., Goldman, D. and VanKeuren, M. Simplified Silver Protein Detection and Image Enhancement Methods in Polyacrylamide Gels. Electrophoresis 3:17-23 (1982).
   b) Morrissey, J. Silver Stain for Proteins in Polyacrylamide Gels: A Modified Procedure with Enhanced Uniform Sensitivity. Analytical Biochemistry 117:307-10 (1981).
   c) Poehling, H.M. and Neuhoff, V. Visualization of Proteins with a Silver "Stain": A Critical Analysis. Electrophoresis 2:141-7 (1981).

3) Autoradiography
   a) Laskey, R. and Mills, A. Enhanced Autoradiographic Detection of P-32 and I-125 Using Intensifying Screens and Hypersensitized Film. FEBS Letters 82:314-16 (1977).
   b) Swanstrom, R. and Shank, P. X-ray Intensifying Screens Greatly Enhance the Detection by Autoradiography of the Radioisotopes P-32 and I-125. Analytical Biochemistry 86:184-92 (1978).
   c) Eastman Kodak Publication M3-508 (1986). Autoradiography of Macroscopic Specimens.

APPENDIX 1

November 15, 1986

PARFR-360

PARFR CYCLES 1984-85

Name	C_1	C_2	C_3	C_4	C_5	C_6
LC	9/17/84-10/17	10/21/84-11/20	1/25/85-2/18	2/22/85-3/20	3/24/85-4/18	4/22/-5/17
MC	9/9/84-10/4	10/8/84-11/5	1/8/85-2/4	2/8/85-3/4	3/8/85-3/31	4/4/85-4/27
MK	9/20/84-10/17a	10/22/84-11/14a	11/19/84-12/9	1/8/85-1/29	2/2/85-2/25	3/1/85-3/21
SS	7/11/84-8/2	8/9/84-8/30	9/5/84-9/27	10/2/84-10/24	10/29-11/19	11/23/84-12/
KS	9/22/84-10/19*	10/22/84-11/9*	1/26/85-2/14*			
VE	10/2/84-10/24	10/29/84-11/21	11/26/84-12/13			
SO	11/7/84-12/1	1/2/85-1/23	1/28/85-2/18	2/23/85-3/17	3/21/85-4/12	4/17/85-5/9
JJ	1/14/85-2/8	2/13/85-3/8	3/12/85-4/10	4/15/85-5/8		
PC	2/5/85-3/3	3/7/85-4/1	4/5/85-4/30	5/4/85-5/28	6/2/85-6/23	
GH	2/12/85-3/4	3/9/85-3/31	4/3/85-4/27	5/2/85-5/23	5/27-6/17	
CM	3/21/85-4/13	4/17/85-5/9	5/14/85-6/5			
SF	2/25/85-3/16					
JP	9/14/84-10/19					
AT	3/25/85-4/28	5/2/85-5/29				
SW	6/29/84-7/20*					

* = Anovulatory cycles

a = Too low urine LH levels.

APPENDIX 1

TABLE 1

One-sided cusum analysis of the trigger day before (drop) and after (rise) ovulation in 5 menstrual cycles from 13 volunteers (day of ovulation is designated as day zero and is th peak urine LH +1day (see Appendix 1)

Volunteer	Cycle	Drop (days before ovulation)	Rise (days after ovulation)	Drop-to-rise Interval (days)
SS	1	6	2	8
SS	2	4	1	5
SS	3	4	1	5
SS	4	4	2	6
SS	5	5	2	7
SS	6	3	-	-
LC	1	9	1	10
LC	2	5	-	-
LC	3	7	2	9
LC	4	10	1	11
LC	5	5	4	9
LC	6	2	1	3
VE	1	4	1	5
VE	2	3	2	5
VE	3	4	2	6
SO	1	7	1	8
SO	2	-	1	-
SO	3	4	1	5
SO	4	-	3	-
SO	5	5	3	8
SO	6	5	2	7
MC	1	7	2	9
MC	2	9	-	-
MC	3	6	1	7
MC	4	7	1	8
MC	5	6	1	7
MC	6	5	2	7
PC	1	-	3	-
PC	2	-	5	-
PC	3	7	3	10
PC	4	3	2	5
PC	5	4	1	5

(continued)

APPENDIX 1

TABLE 1 (continued)

Volunteer	Cycle	Drop (days before ovulation)	Rise (days after ovulation)	Drop-to-rise Interval (days)
GH	1	3	0	3
GH	2	4	5	9
GH	3	2	4	6
GH	4	-	-	-
GH	5	3	1	4
JJ	1	5	2	7
JJ	2	5	1	6
JJ	3	8	1	9
CM	1	-	3	-
CM	2	4	3	7
CM	3	2	-	-
SF	1	6	0	6
AT	1	3	1	5
AT	2	8	1	9
JP (conceived)	1	5	1	6
MK	3	2	-	-
MK	4	-	0	-
MK	5	-	-	-
MK	6	6	-	-

Cusum Analysis
No-trigger, drop: 8 cycles
No-trigger, rise: 8 cycles
Mean days of drop: 5.11 ± 0.31 SEM, n=37, excluding non-trigger pairs
Mean days of rise: 1.68 ± 0.18 SEM, n=27, excluding non-trigger pairs
Mean drop-to-rise interval: 6.78 ± 0.33 SEM days, n=37

Note: Six other cycles of volunteers MK (2), KS (3), SW (1) were not included in the analysis (see text).

APPENDIX 1

TABLE 2

DISTRIBUTION OF OVULATION PREDICTION

Prediction (days before the urine LH + 1 day)	2	3	4	5	6	7	8	9	10
Percent of 43 cycles (from Table 1)	9.3	14.0	21.0	21.0	11.6	11.7	4.6	4.6	2.3

TABLE 3

Serum and urine LH peaks in one cycle

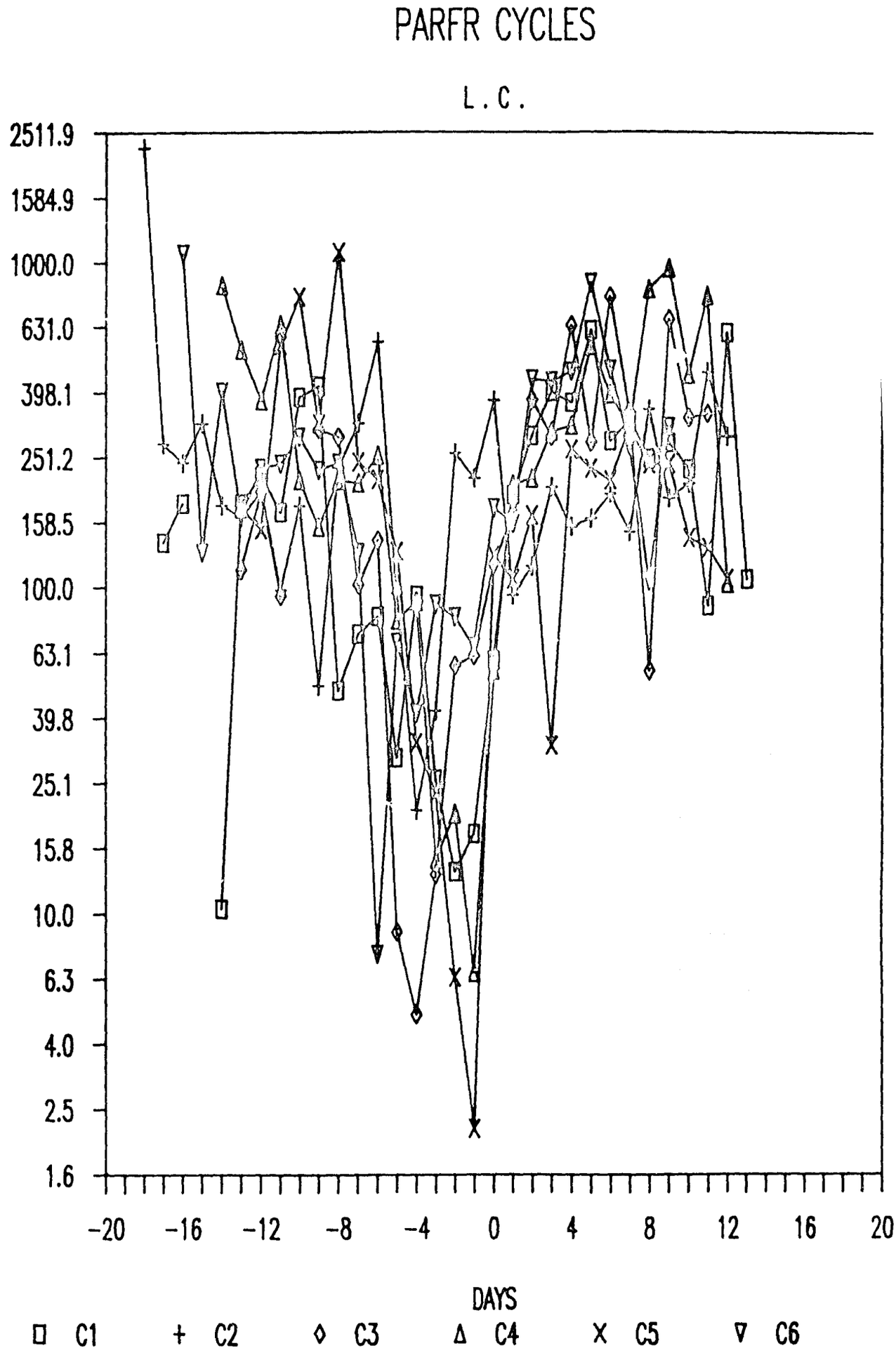
S.S.

SERUM

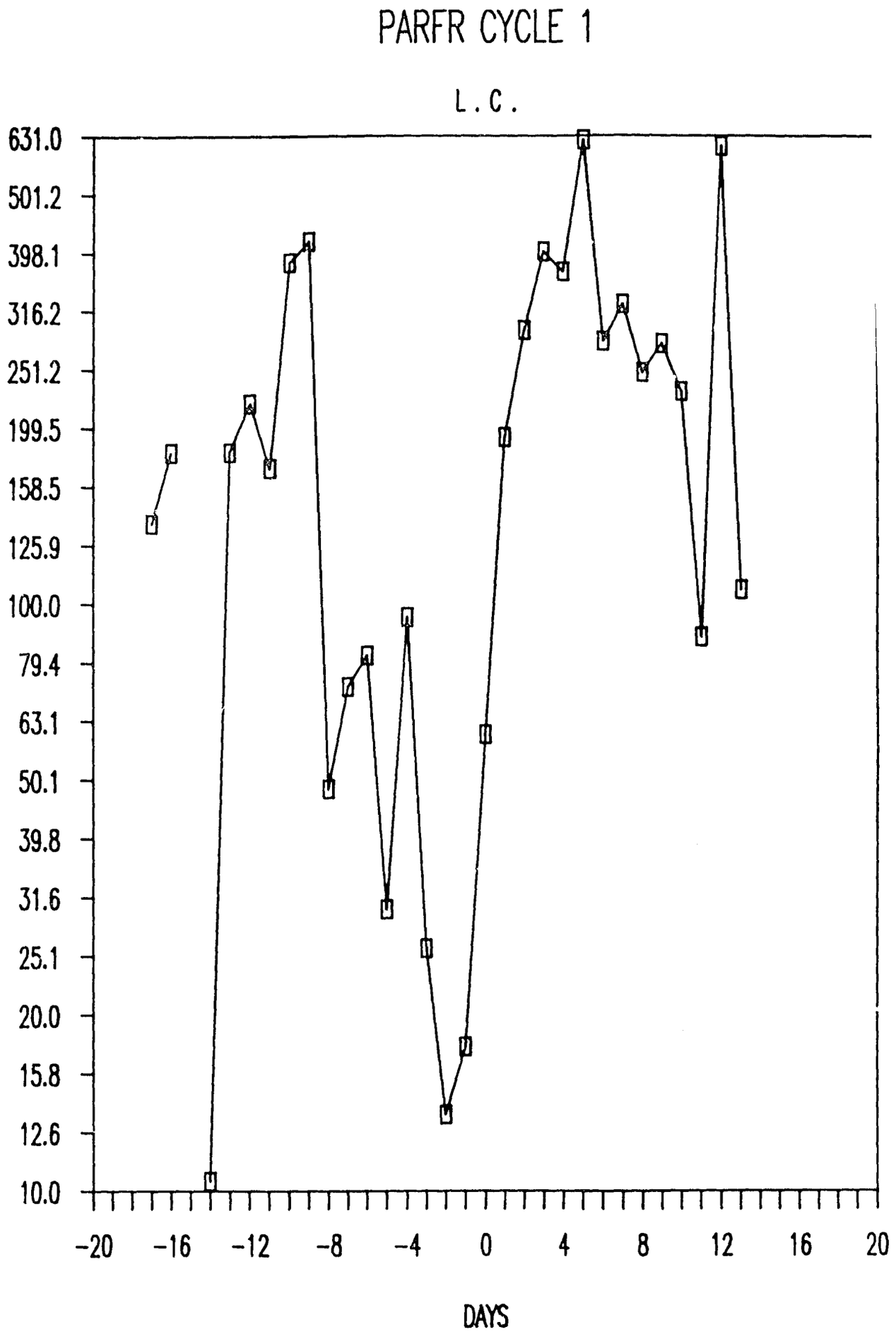
DATE	DFOM	OVD	GPC	GPV	LH	P4	E2
10/29/84	5	-8	82.6	---			19.1
10/30/84	6	-7	235.5	175.2			40.8
10/31/84	7	-6	213.0	37.4			57.1
11/1/84	8	-5	191.5	---			73.0
11/2/84	9	-4	25.1	18.1	9.4	0.4	101.7
11/3/84	10	-3	---	19.9	13.1	0.4	122.2
11/4/84	11	-2	2.5	10.3	14.4	0.4	196.7
11/5/84	12	-1	---	2.7	61.2	0.8	207.4
11/6/84	13	0	---	---	39.3	1.5	103.5
11/7/84	14	1	0.0	---	14.5	3.8	35.2
11/8/84	15	2	124.4	33.0	8.1	9.6	38.2
11/9/84	16	3	102.5	252.1	6.3	14.8	56.0
11/10/84	17	4	388.6	57.0	5.0	21.9	68.6
11/11/84	18	5	347.4	135.1	3.3	22.8	87.4
11/12/84	19	6	37.9	---	3.1	21.0	63.9
11/13/84	20	7	67.8	56.1	5.9	15.6	86.5
11/14/84	21	8	72.5	81.1	3.9	11.3	70.6
11/15/84	22	9	107.5	78.7	2.9	9.4	58.4
11/16/84	23	10	25.4	43.9		10.3	79.9
11/17/84	24	11	308.8	---		3.8	27.2
11/18/84	25	12	112.6	238.0		1.4	7.4
11/19/84	26	13	104.1	278.0		0.8	7.4

URINE

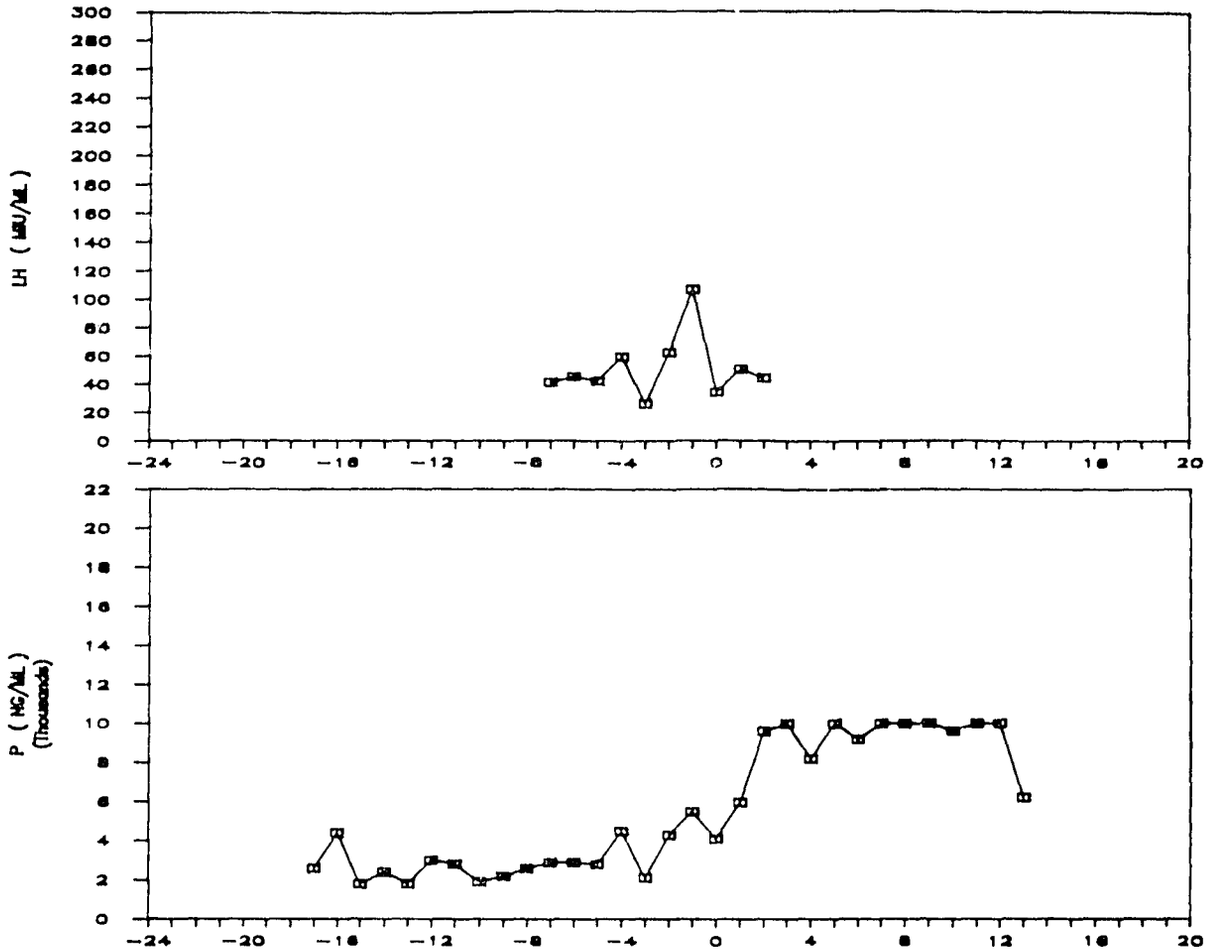
DATE	DFOM	OVD	GPC	GPV	LH	PREG	E2
10/29/84	5	-9	82.6			700.0	
10/30/84	6	-8	235.5			1100.0	
10/31/84	7	-7	213.0			1300.0	
11/1/84	8	-6	191.5			700.0	
11/2/84	9	-5	25.1			1000.0	
11/3/84	10	-4	---			1300.0	
11/4/84	11	-3	2.5		24.6	1300.0	
11/5/84	12	-2	---		18.8	500.0	
11/6/84	13	-1	---		142.3	1600.0	
11/7/84	14	0	0.0		12.1	1300.0	
11/8/84	15	1	124.4		30.1	1600.0	
11/9/84	16	2	102.5		71.5	3200.0	
11/10/84	17	3	388.6		50.9	5400.0	
11/11/84	18	4	347.4			7200.0	
11/12/84	19	5	37.9			10000.0	
11/13/84	20	6	67.8			6300.0	
11/14/84	21	7	72.5			6600.0	
11/15/84	22	8	107.5			7600.0	
11/16/84	23	9	25.4			5900.0	
11/17/84	24	10	308.8			4200.0	
11/18/84	25	11	112.6			2400.0	
11/19/84	26	12	104.1			2200.0	

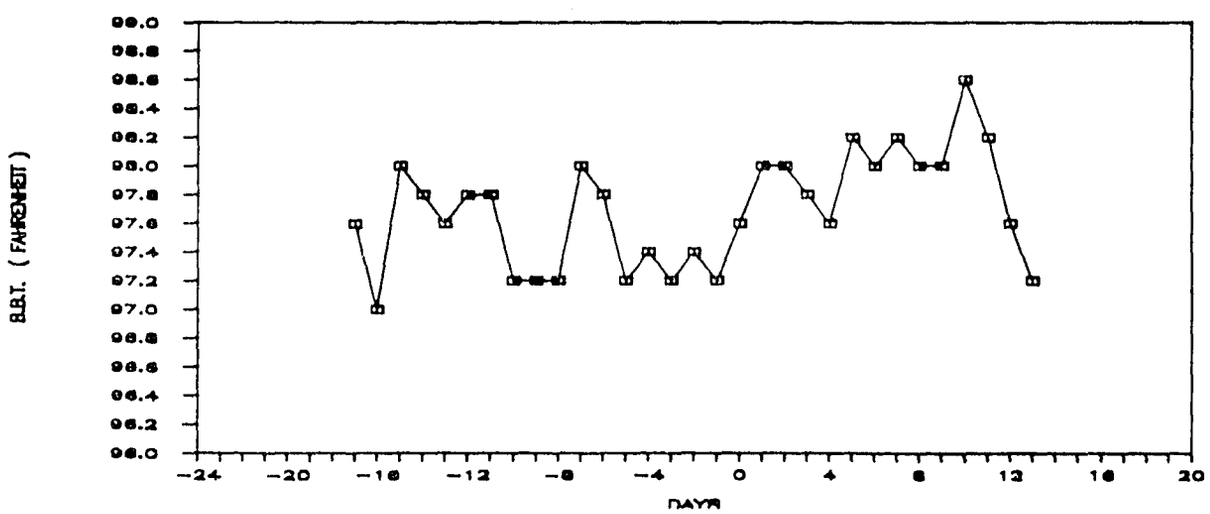

PARFR CYCLES
L.C.
2511.9
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS
C1
C2
C3
C4
C5
C6

NAME	DATE	DFOM	OVD	GPC	LH	P	BBT	REMARKS
L.C.	9/17/84	4	-17	137.3		2600	97.6	PARFR CYCLE 1
	9/18/84	5	-16	181.2		4400	97.0	
	9/19/84	6	-15	---		1800	98.0	
	9/20/84	7	-14	10.4		2400	97.8	
	9/21/84	8	-13	181.6		1800	97.6	
	9/22/84	9	-12	219.8		3000	97.8	
	9/23/84	10	-11	170.4		2800	97.8	
	9/24/84	11	-10	384.5		1900	97.2	
	9/25/84	12	-9	417.4		2200	97.2	
	9/26/84	13	-8	48.5		2600	97.2	
	9/27/84	14	-7	72.4	41	2900	98.0	
	9/28/84	15	-6	81.8	45	2900	97.8	
	9/29/84	16	-5	30.2	42	2800	97.2	
	9/30/84	17	-4	95.2	59	4500	97.4	
	10/1/84	18	-3	25.9	26	2100	97.2	
	10/2/84	19	-2	13.5	62	4300	97.4	
	10/3/84	20	-1	17.6	106	5500	97.2	
	10/4/84	21	0	60.0	34	4100	97.6	
	10/5/84	22	1	193.4	50	6000	98.0	
	10/6/84	23	2	294.6	44	9600	98.0	
	10/7/84	24	3	401.5		10000	97.8	
	10/8/84	25	4	371.2		8200	97.6	
	10/9/84	26	5	621.2		10000	98.2	
	10/10/84	27	6	282.2		9200	98.0	
	10/11/84	28	7	326.3		10000	98.2	
	10/12/84	29	8	248.9		10000	98.0	
	10/13/84	30	9	279.0		10000	98.0	
	10/14/84	31	10	231.4		9600	98.6	
	10/15/84	32	11	88.0		10000	98.2	
	10/16/84	33	12	606.8		10000	97.6	
	10/17/84	34	13	105.8		6200	97.2	

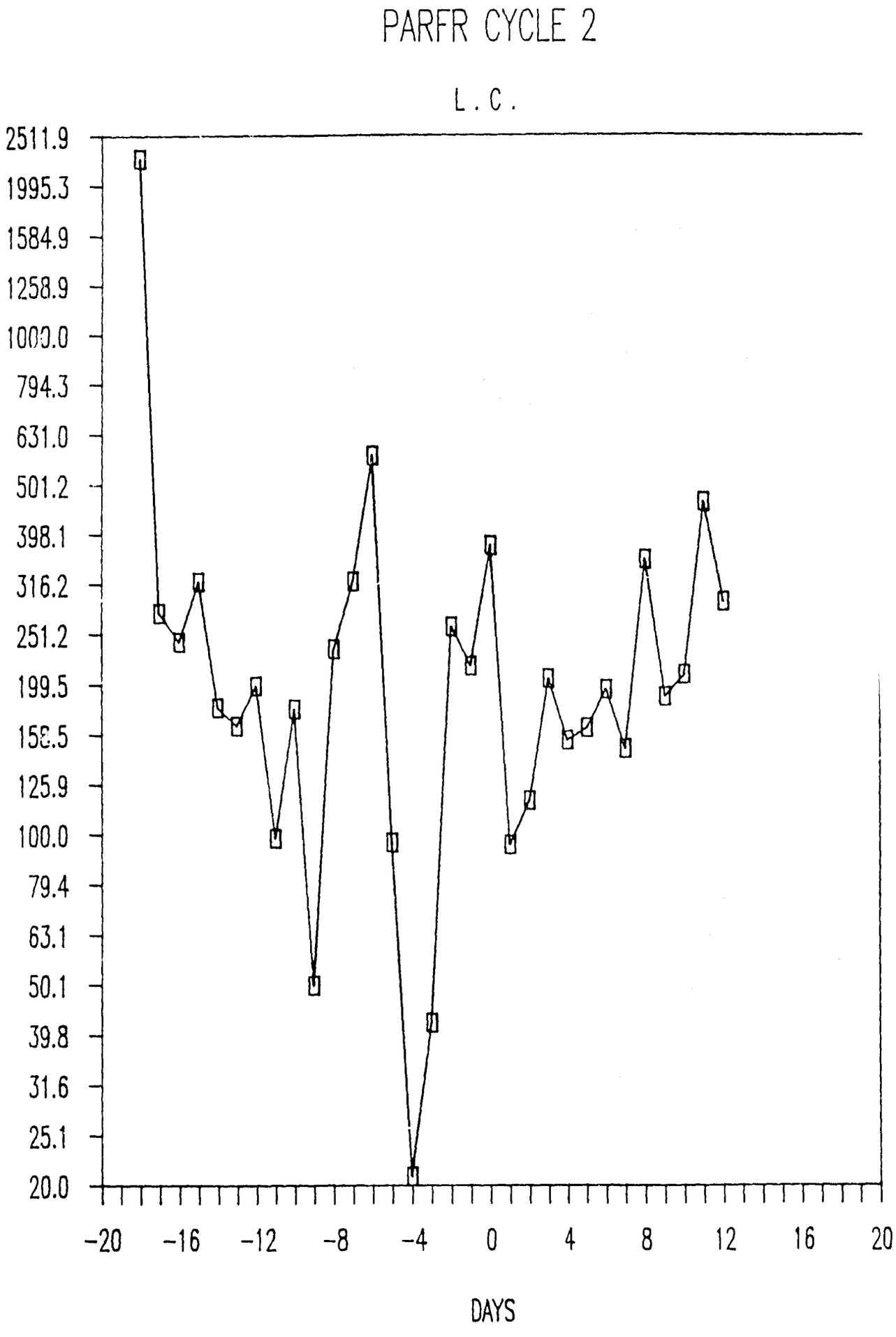

PARFR CYCLE 1
L.C.
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

PARFR CYCLE 1



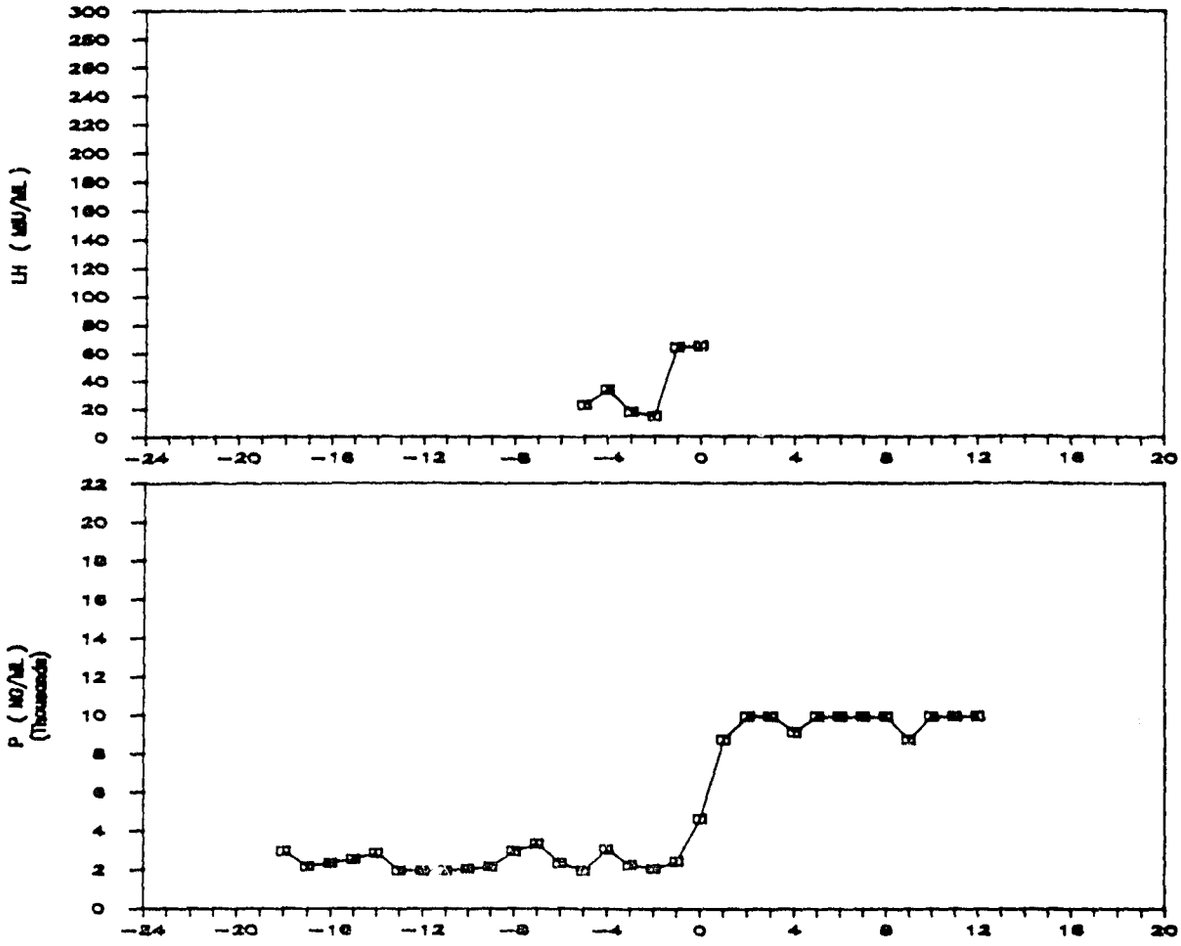


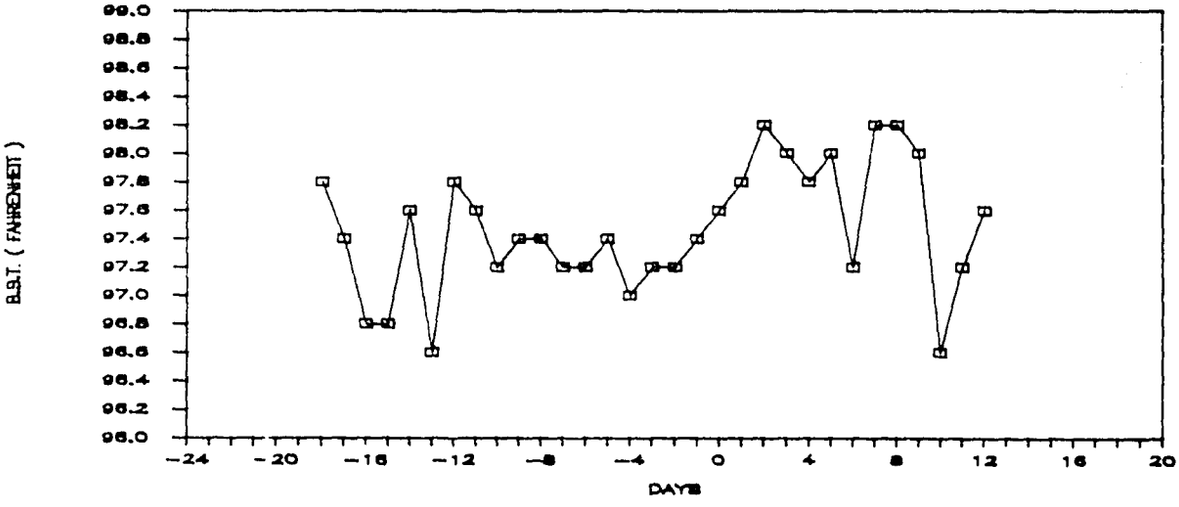
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
L.C.	10/21/84	4	-18	2257.7			3000.0		97.8	PARFR CYCLE 2
	10/22/84	5	-17	276.4			2200.0		97.4	
	10/23/84	6	-16	242.3			2400.0		96.8	
	10/24/84	7	-15	319.6			2600.0		96.8	
	10/25/84	8	-14	179.2			2900.0		97.6	
	10/26/84	9	-13	164.7			2000.0		96.6	
	10/27/84	10	-12	197.9			2000.0		97.8	
	10/28/84	11	-11	98.4			2000.0		97.6	
	10/29/84	12	-10	178.2			2100.0		97.2	
	10/30/84	13	-9	50.0			2200.0		97.4	
	10/31/84	14	-8	234.4			3000.0		97.4	
	11/1/84	15	-7	320.7			3400.0		97.2	
	11/2/84	16	-6	571.3			2400.0		97.2	
	11/3/84	17	-5	96.5		22.0	2000.0		97.4	
	11/4/84	18	-4	20.8		33.0	3100.0		97.0	
	11/5/84	19	-3	42.1		17.0	2300.0		97.2	
	11/6/84	20	-2	260.0		14.0	2100.0		97.2	
	11/7/84	21	-1	217.3		63.0	2500.0		97.4	
	11/8/84	22	0	377.7		64.0	4700.0		97.6	
	11/9/84	23	1	95.3			8800.0		97.8	
	11/10/84	24	2	116.9			10000.0		98.2	
	11/11/84	25	3	204.5			10000.0		98.0	
	11/12/84	26	4	154.1			9200.0		97.8	
	11/13/84	27	5	163.5			10000.0		98.0	
	11/14/84	28	6	194.7			10000.0		97.2	
	11/15/84	29	7	148.2			10000.0		96.2	
	11/16/84	30	8	353.2			10000.0		98.2	
	11/17/84	31	9	188.1			9800.0		98.0	
	11/18/84	32	10	208.3			10000.0		96.6	
	11/19/84	33	11	460.3			10000.0		97.2	
	11/20/84	34	12	290.4			10000.0		97.6	


PARFR CYCLE 2
L.C.
2511.9
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

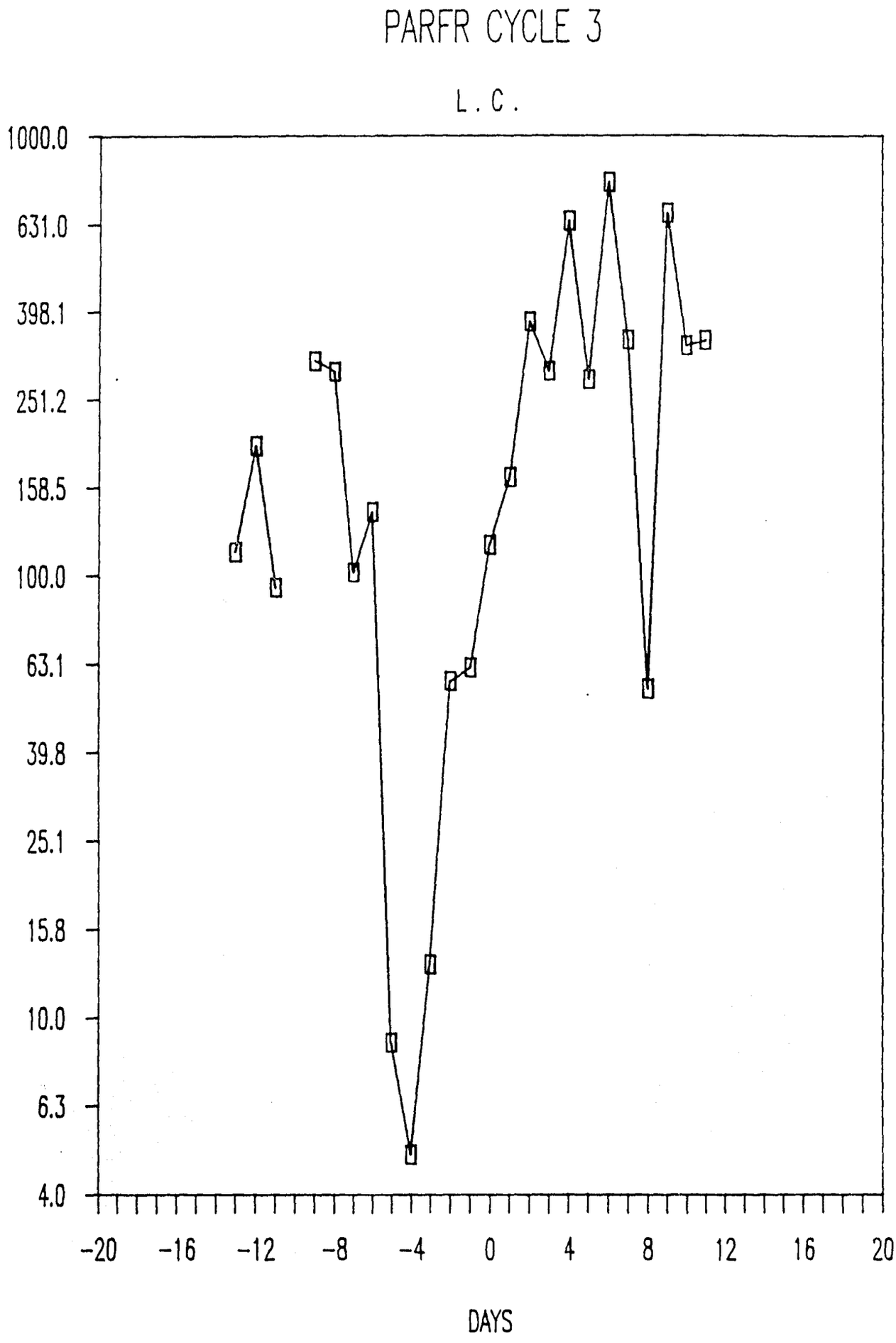
L.C

PARFR CYCLE 2



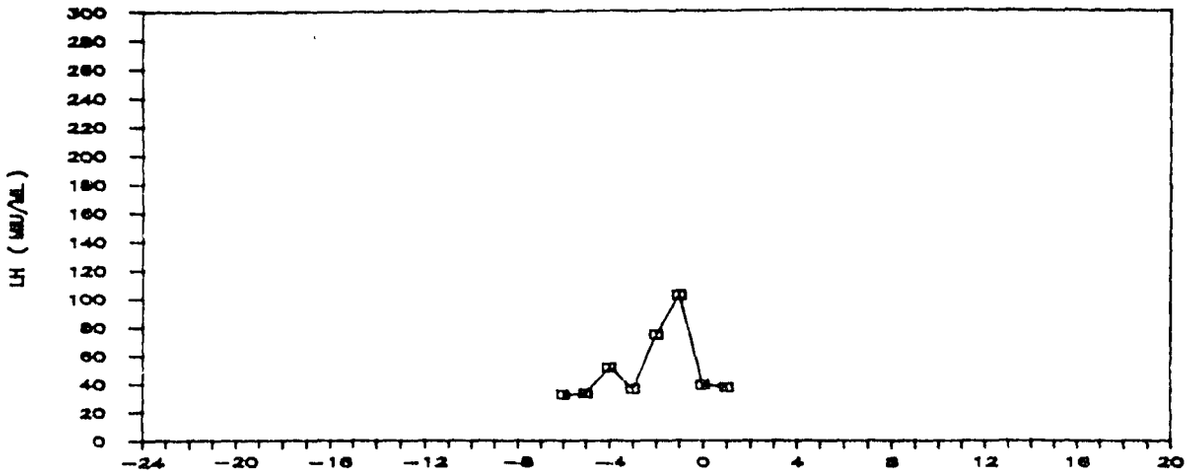


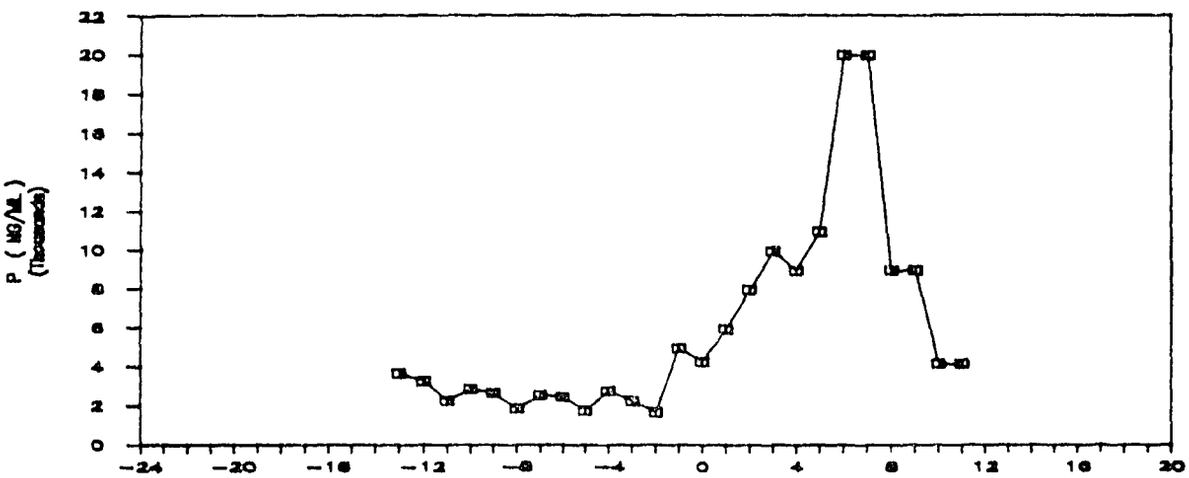
NAME	DATE	DFOM	DVD	GPC	GPV	LH	P	E2	BBT	REMARKS
L.C.	1/25/85	4	-13	113.6			3700.0		97.2	PAPER CYCLE 3
	1/26/85	5	-12	196.9			3200.0		98.0	
	1/27/85	6	-11	94.3			2300.0		97.4	
	1/28/85	7	-10	---			2900.0		97.2	
	1/29/85	8	-9	307.2			2700.0		97.2	
	1/30/85	9	-8	290.2			1900.0		97.2	
	1/31/85	10	-7	102.2			2600.0		97.4	
	2/1/85	11	-6	140.0		32.0	2500.0		97.2	
	2/2/85	12	-5	8.8		33.0	1800.0		97.6	
	2/3/85	13	-4	4.9		51.0	2800.0		97.8	
	2/4/85	14	-3	13.2		36.0	2300.0		96.8	
	2/5/85	15	-2	57.9		74.0	1700.0		97.2	
	2/6/85	16	-1	62.1		102.0	5000.0		97.8	
	2/7/85	17	0	118.2		39.0	4300.0		98.2	
	2/8/85	18	1	168.4		37.0	6000.0		97.4	
	2/9/85	19	2	378.8			8000.0		98.2	
	2/10/85	20	3	292.5			10000.0		98.2	
	2/11/85	21	4	641.1			9000.0		98.2	
	2/12/85	22	5	280.2			11000.0		98.2	
	2/13/85	23	6	785.6			20000.0		97.8	
	2/14/85	24	7	345.2			20000.0		98.0	
	2/15/85	25	8	55.6			9000.0		98.0	
	2/16/85	26	9	667.5			9000.0		98.2	
	2/17/85	27	10	333.5			4200.0		98.4	
	2/18/85	28	11	342.2			4200.0		97.8	

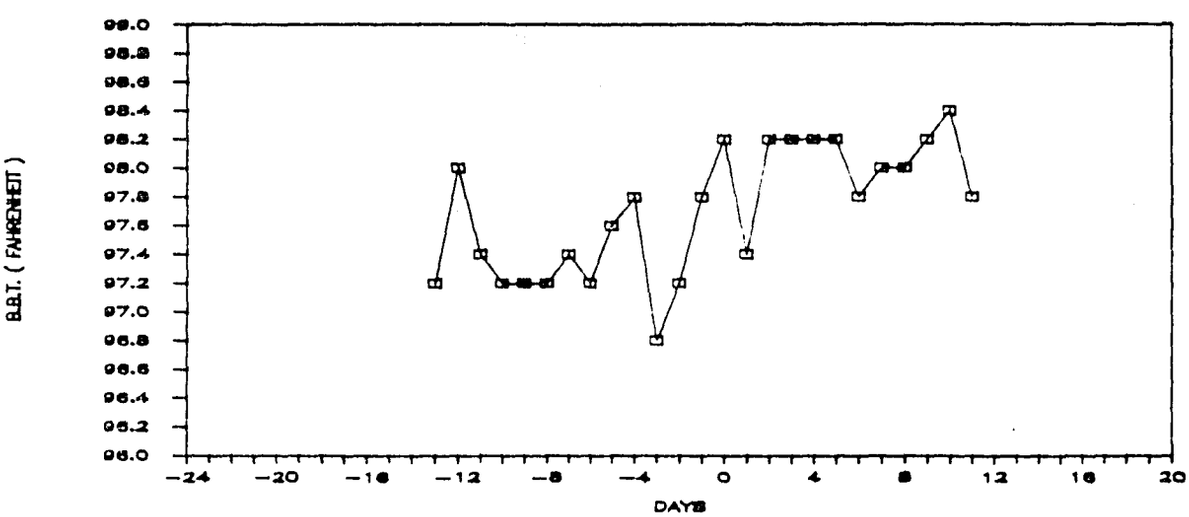

PARFR CYCLE 3
L.C.
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

L.C.

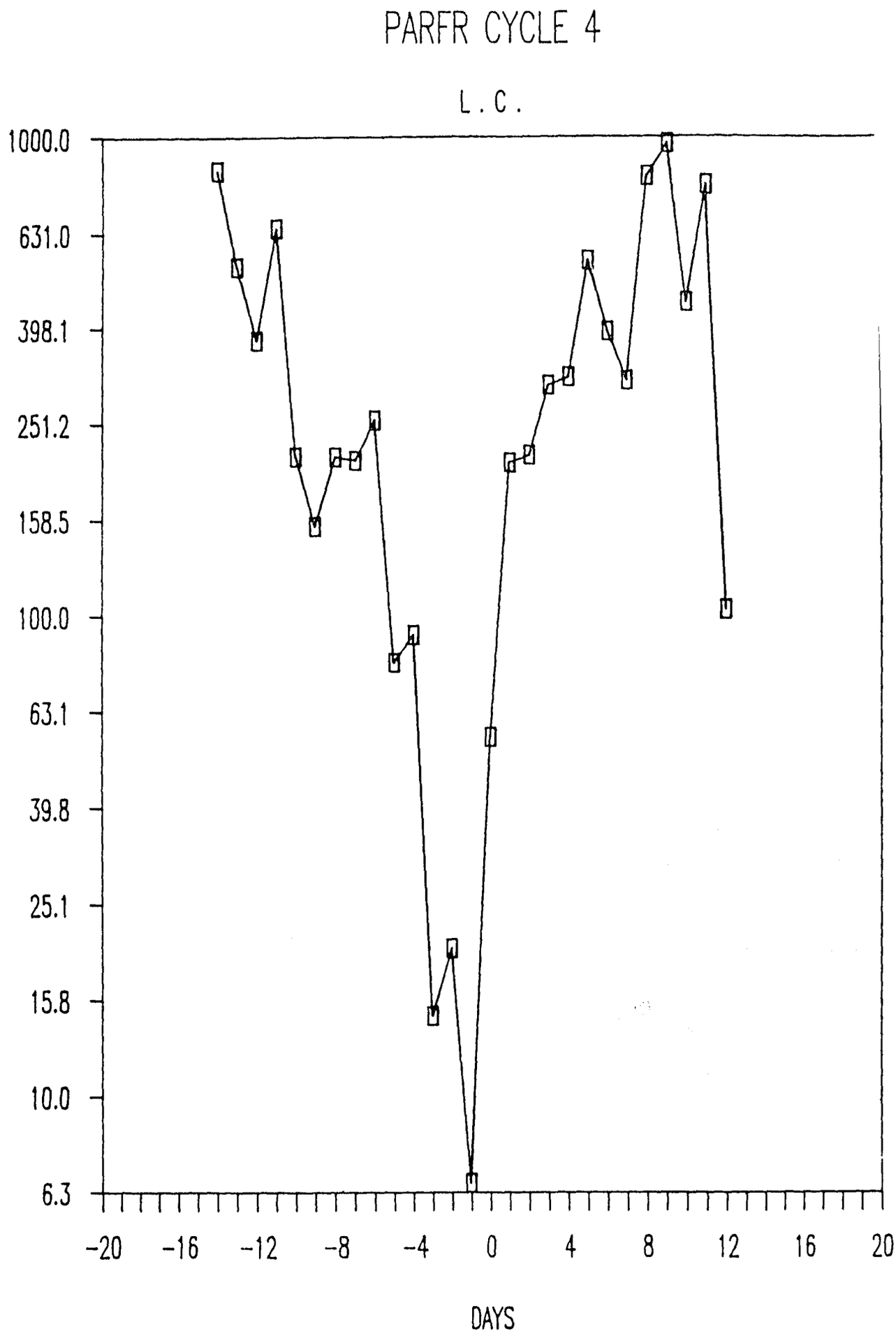
PARFR CYCLE 3



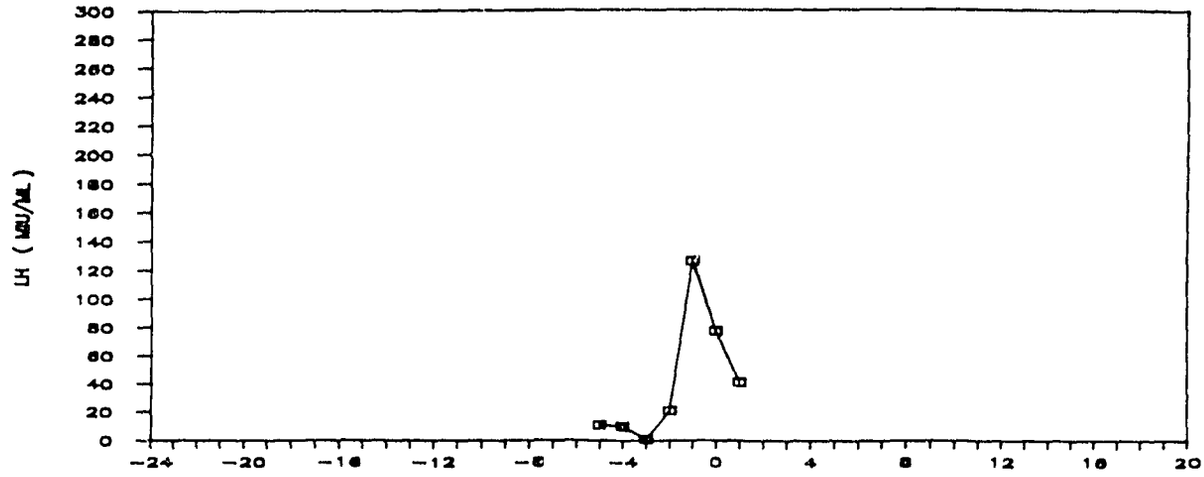


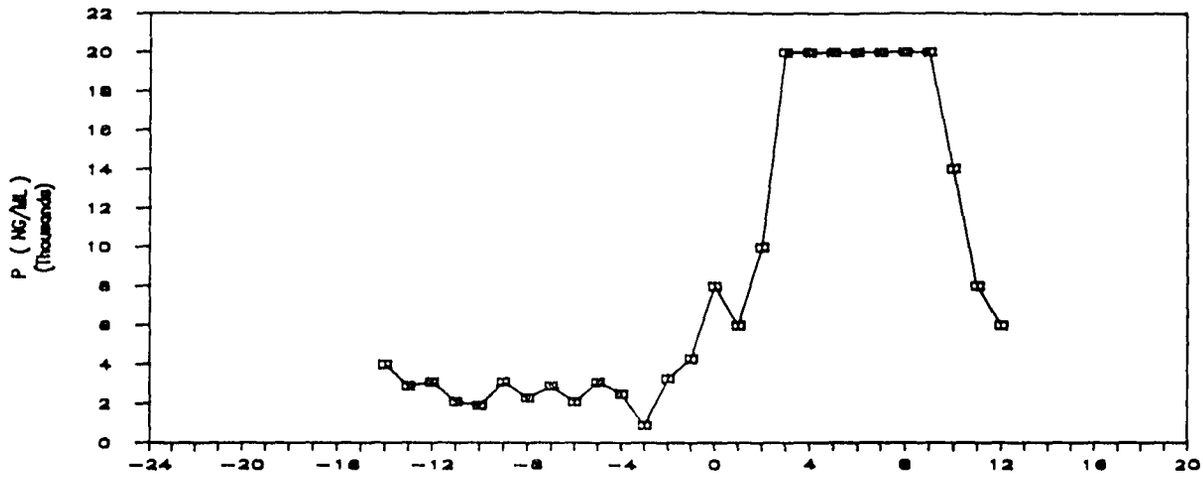


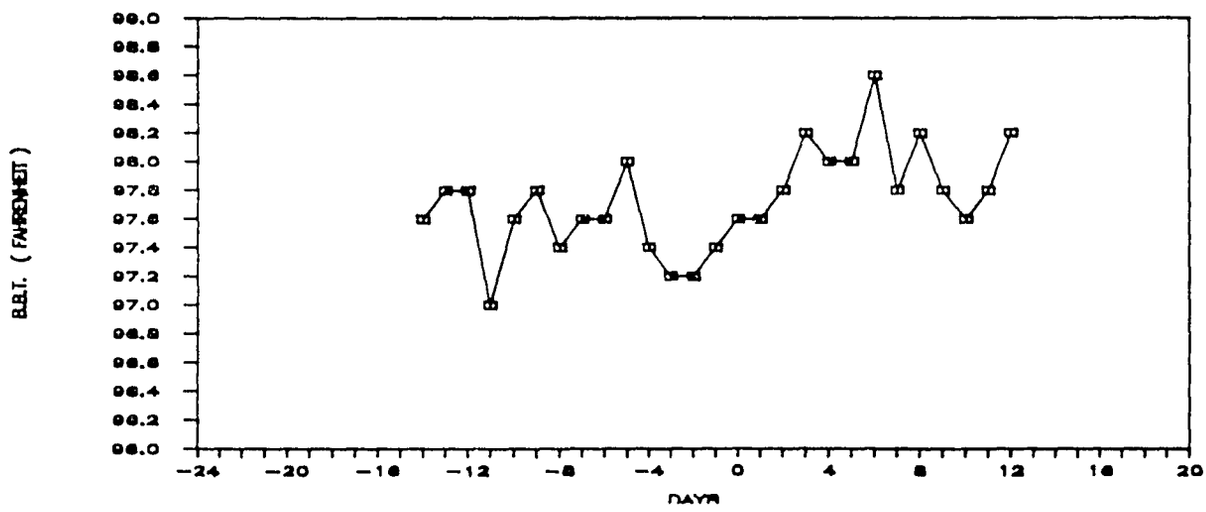
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
L.C.	2/22/85	4	-14	853.3			4000.0		97.6	PARFR CYCLE 4
	2/23/85	5	-13	538.2			2900.0		97.8	
	2/24/85	6	-12	376.1			3100.0		97.8	
	2/25/85	7	-11	546.9			2100.0		97.0	
	2/26/85	8	-10	214.4			1900.0		97.6	
	2/27/85	9	-9	153.6			3100.0		97.8	
	2/28/85	10	-8	214.2			2300.0		97.4	
	3/1/85	11	-7	210.7			2900.0		97.6	
	3/2/85	12	-6	255.5			2100.0		97.6	
	3/3/85	13	-5	79.9		11.0	3100.0		98.0	
	3/4/85	14	-4	91.3		10.0	2500.0		97.4	
	3/5/85	15	-3	14.7		1.0	900.0		97.2	
	3/6/85	16	-2	20.3		21.0	3200.0		97.2	
	3/7/85	17	-1	6.6		126.0	4300.0		97.4	
	3/8/85	18	0	55.9		77.0	8000.0		97.6	
	3/9/85	19	1	209.1		41.0	6000.0		97.6	
	3/10/85	20	2	217.2			10000.0		97.8	
	3/11/85	21	3	303.4			20000.0		98.2	
	3/12/85	22	4	315.8			20000.0		98.0	
	3/13/85	23	5	553.3			20000.0		98.0	
	3/14/85	24	6	393.3			20000.0		98.6	
	3/15/85	25	7	310.9			20000.0		97.8	
	3/16/85	26	8	827.5			20000.0		98.2	
	3/17/85	27	9	963.2			20000.0		97.8	
	3/18/85	28	10	452.4			14000.0		97.6	
	3/19/85	29	11	791.4			8000.0		97.8	
	3/20/85	30	12	102.9			6000.0		98.2	


PARFR CYCLE 4
L.C.
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

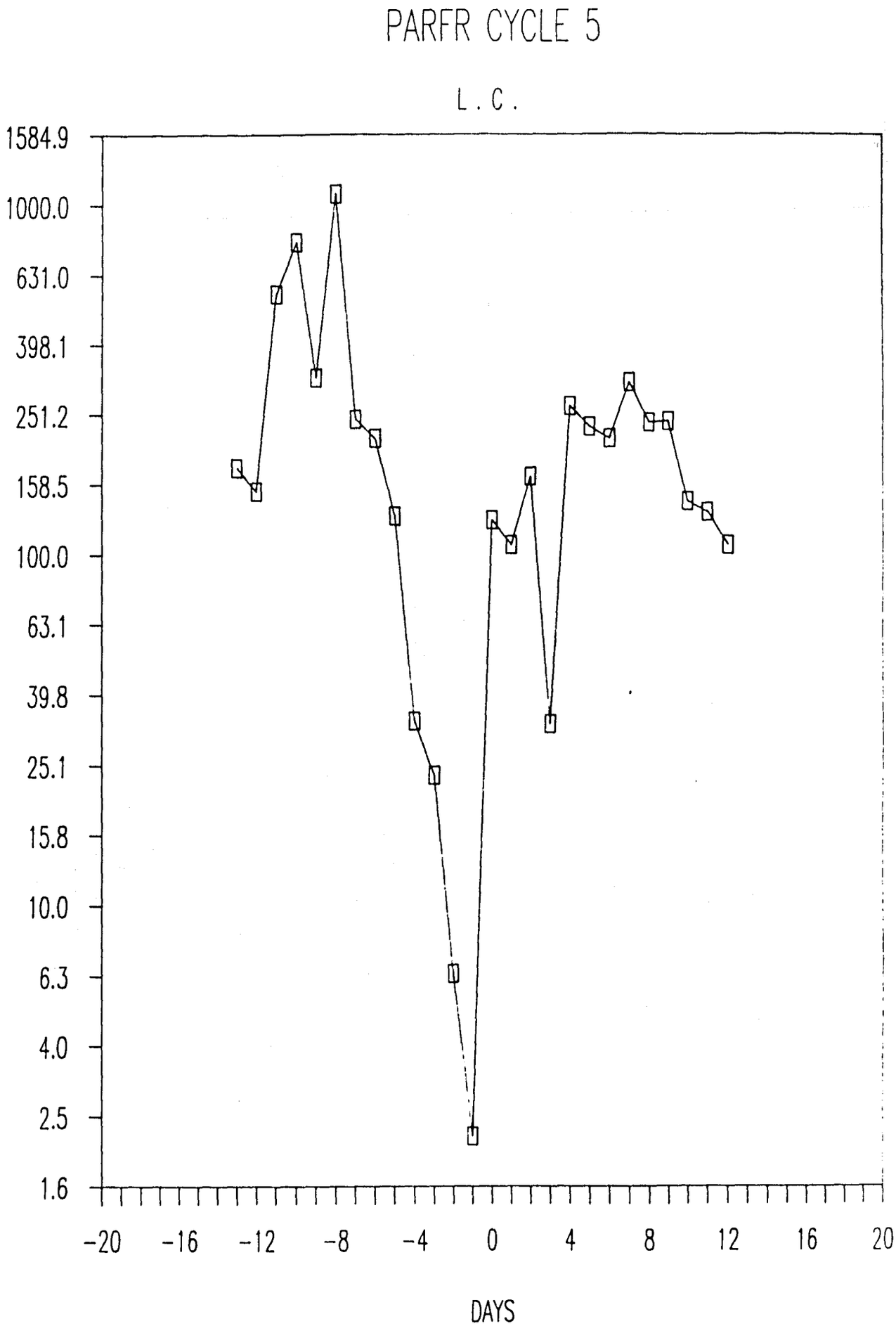
PARFR CYCLE 4



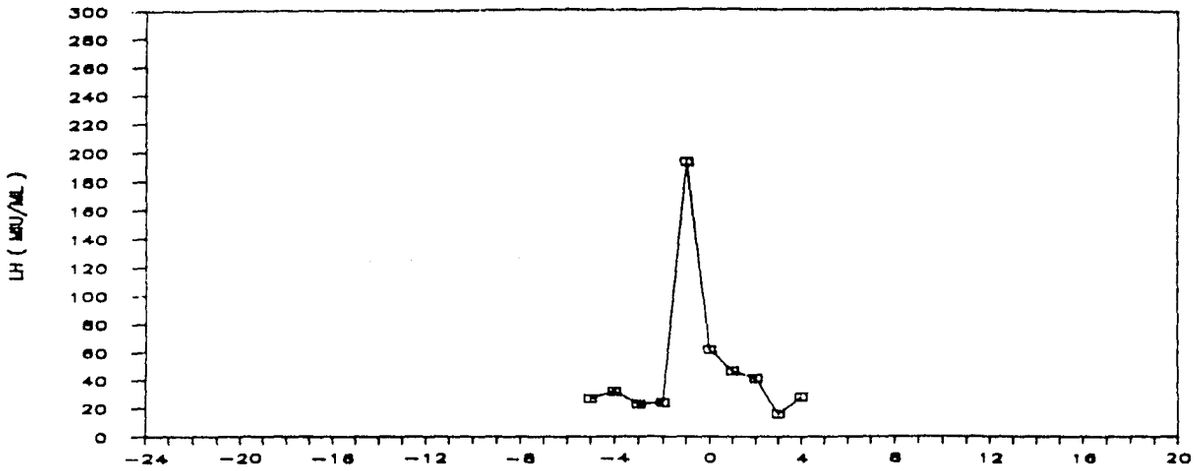


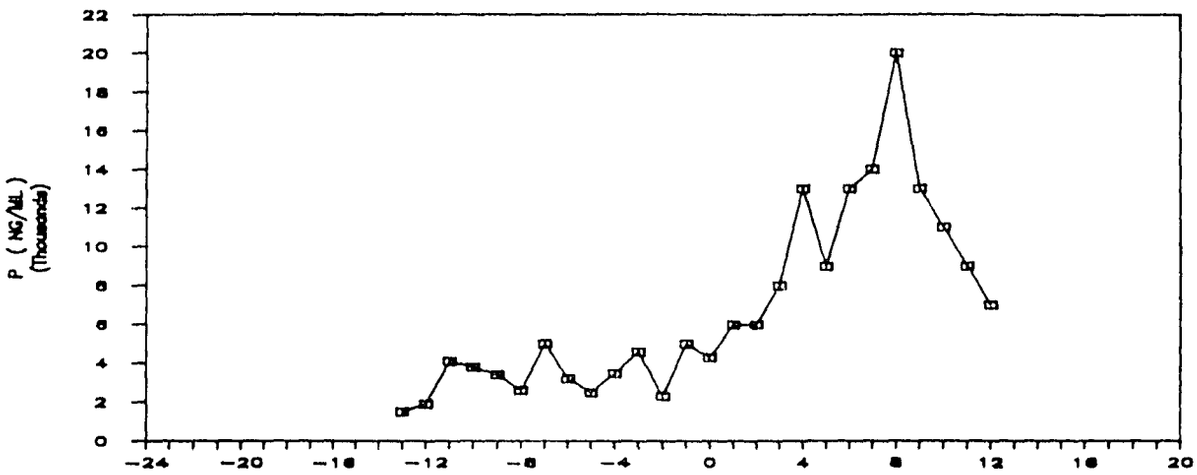


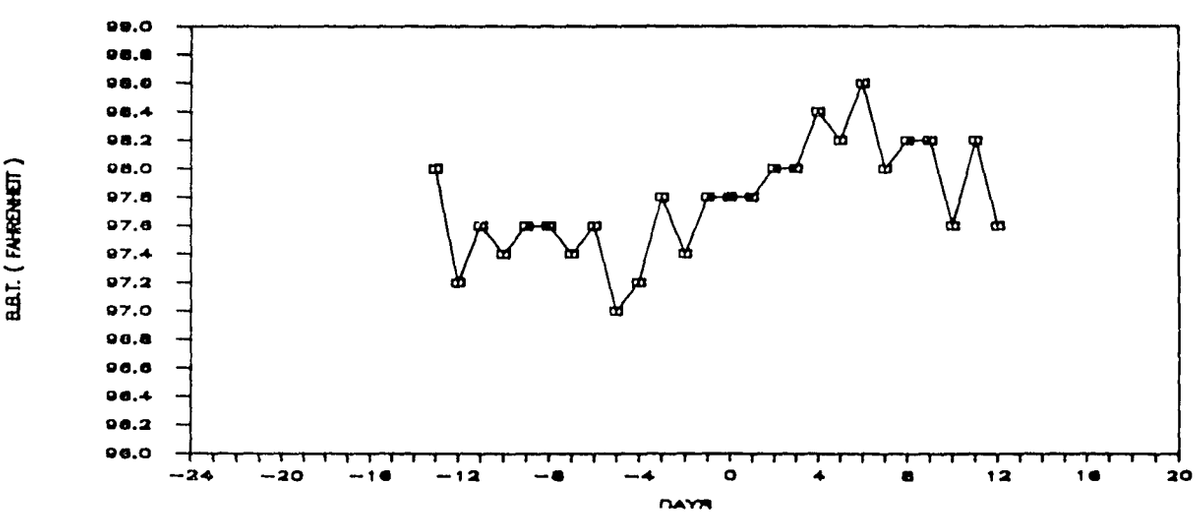
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
L.C.	3/24/85	4	-13	177.1			1500.0		98.0	PARFR CYCLE 5
	3/25/85	5	-12	151.4			1900.0		97.2	
	3/26/85	6	-11	556.2			4100.0		97.6	
	3/27/85	7	-10	785.7			3800.0		97.4	
	3/28/85	8	-9	320.0			3400.0		97.6	
	3/29/85	9	-8	1075.5			2600.0		97.6	
	3/30/85	10	-7	243.8			5000.0		97.4	
	3/31/85	11	-6	215.6			3200.0		97.6	
	4/1/85	12	-5	129.4		27.0	2500.0		97.0	
	4/2/85	13	-4	33.6		32.0	3500.0		97.2	
	4/3/85	14	-3	23.5		23.0	4600.0		97.8	
	4/4/85	15	-2	6.4		24.0	2300.0		97.4	
	4/5/85	16	-1	2.2		193.0	5000.0		97.8	
	4/6/85	17	0	125.7		61.0	4700.0		97.8	
	4/7/85	18	1	106.9		46.0	6000.0		97.8	
	4/8/85	19	2	167.6		41.0	6000.0		98.0	
	4/9/85	20	3	33.0		16.0	8000.0		98.0	
	4/10/85	21	4	266.2		28.0	12000.0		98.4	
	4/11/85	22	5	232.2			9000.0		98.2	
	4/12/85	23	6	214.4			13000.0		98.6	
	4/13/85	24	7	310.4			14000.0		98.0	
	4/14/85	25	8	238.8			20000.0		98.2	
	4/15/85	26	9	240.0			13000.0		98.2	
	4/16/85	27	10	141.8			11000.0		97.6	
	4/17/85	28	11	132.3			9000.0		98.2	
	4/18/85	29	12	106.3			7000.0		97.6	


PARFR CYCLE 5
L.C.
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

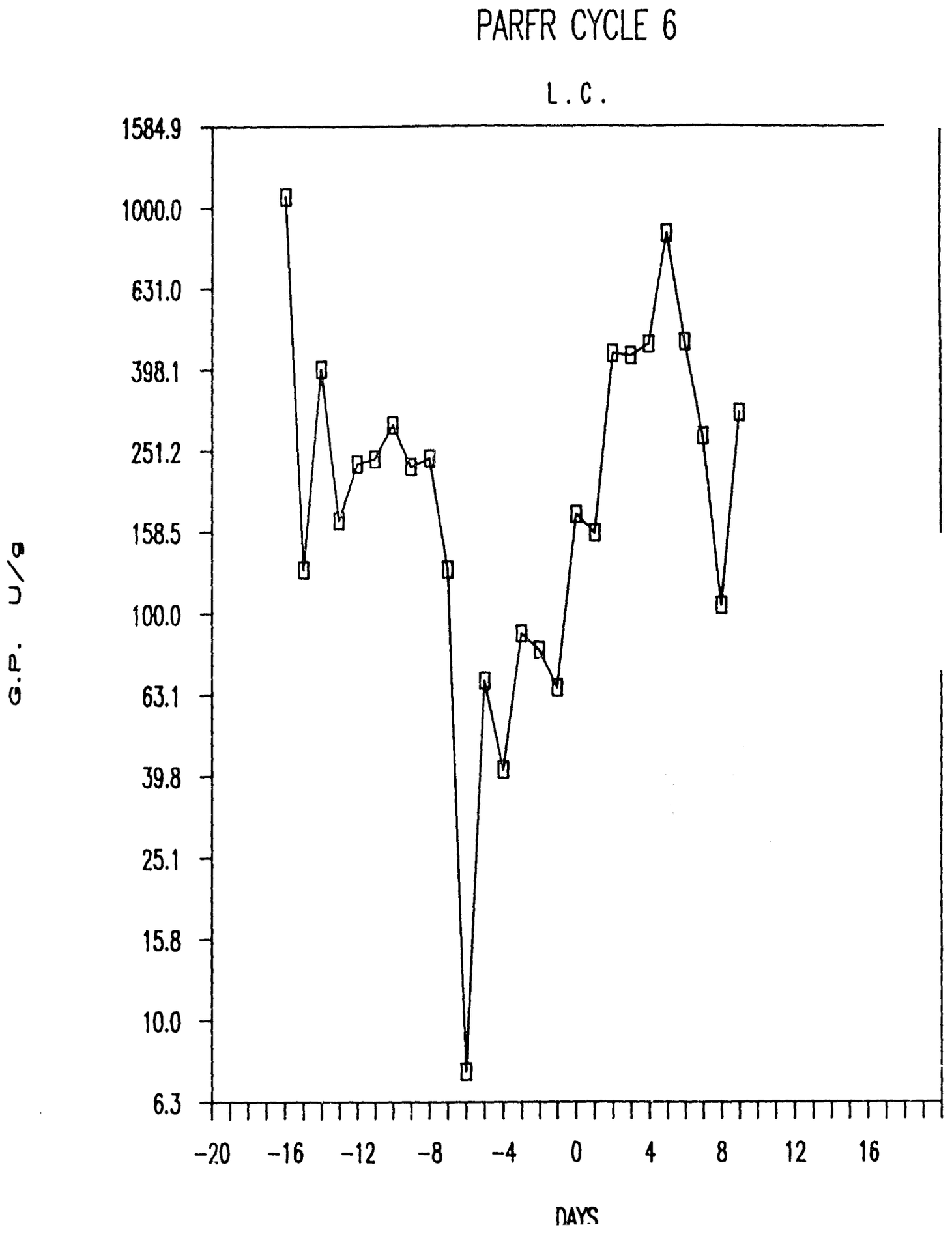
PARFR CYCLE 5



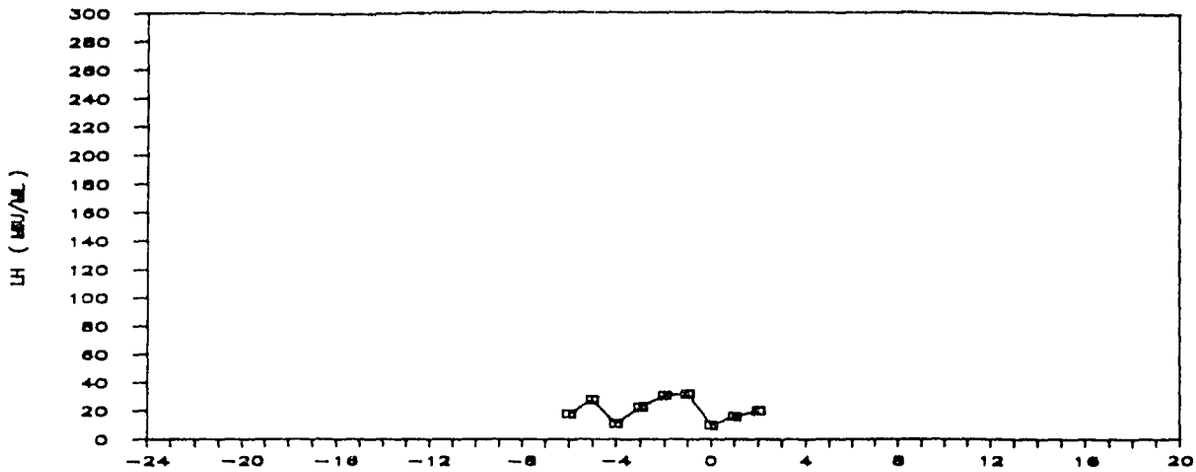


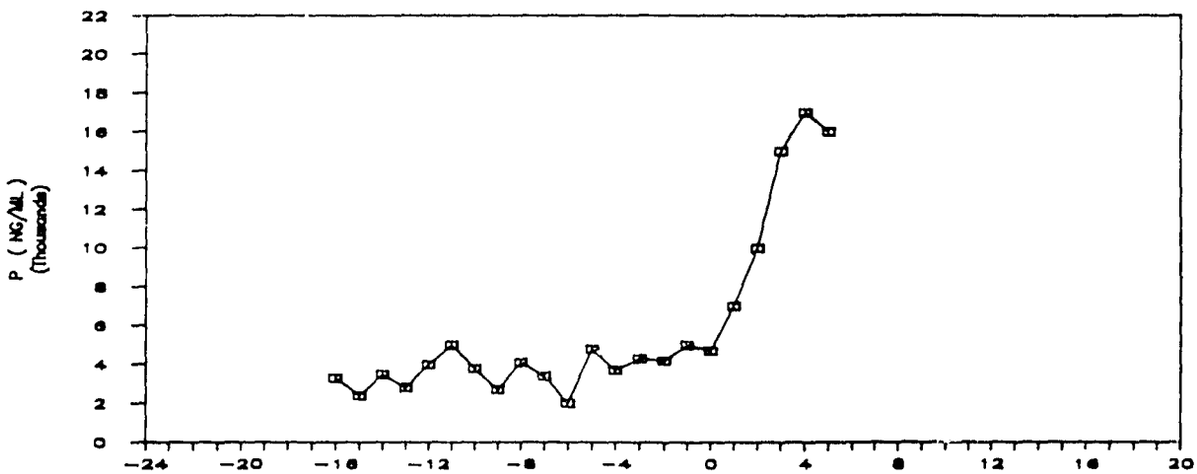


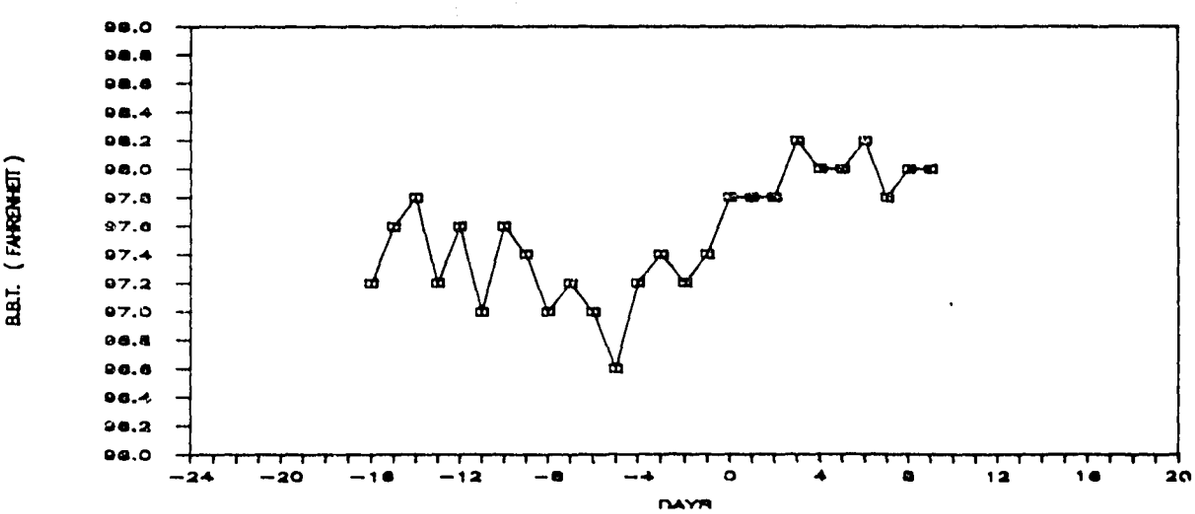
NAME	DATE	DFOM	OVD	GPC	LH	P	BBT	REMARKS
L.C.	4/22/85	4	-16	1067.9		3300.0	97.2	PARFR CYCLE
	4/23/85	5	-15	128.3		2400.0	97.6	
	4/24/85	6	-14	399.1		3500.0	97.8	
	4/25/85	7	-13	168.9		2800.0	97.2	
	4/26/85	8	-12	233.5		4000.0	97.6	
	4/27/85	9	-11	239.9		5000.0	97.0	
	4/28/85	10	-10	290.5		3800.0	97.6	
	4/29/85	11	-9	229.6		2700.0	97.4	
	4/30/85	12	-8	241.8		4100.0	97.0	
	5/1/85	13	-7	128.7		3400.0	97.2	
	5/2/85	14	-6	7.5	18.0	2000.0	97.0	
	5/3/85	15	-5	68.4	28.0	4800.0	96.6	
	5/4/85	16	-4	41.4	11.0	3700.0	97.2	
	5/5/85	17	-3	89.5	23.0	4300.0	97.4	
	5/6/85	18	-2	81.4	31.0	4200.0	97.2	
	5/7/85	19	-1	65.9	32.0	5000.0	97.4	
	5/8/85	20	0	175.9	10.0	4700.0	97.8	
	5/9/85	21	1	158.4	16.0	7000.0	97.8	
	5/10/85	22	2	438.3	20.0	10000.0	97.8	
	5/11/85	23	3	433.3		15000.0	98.2	
	5/12/85	24	4	462.2		17000.0	98.0	
	5/13/85	25	5	868.3		16000.0	98.0	
	5/14/85	26	6	468.0			98.2	
	5/15/85	27	7	273.3			97.8	
	5/16/85	28	8	104.7			98.0	
	5/17/85	29	9	312.2			98.0	

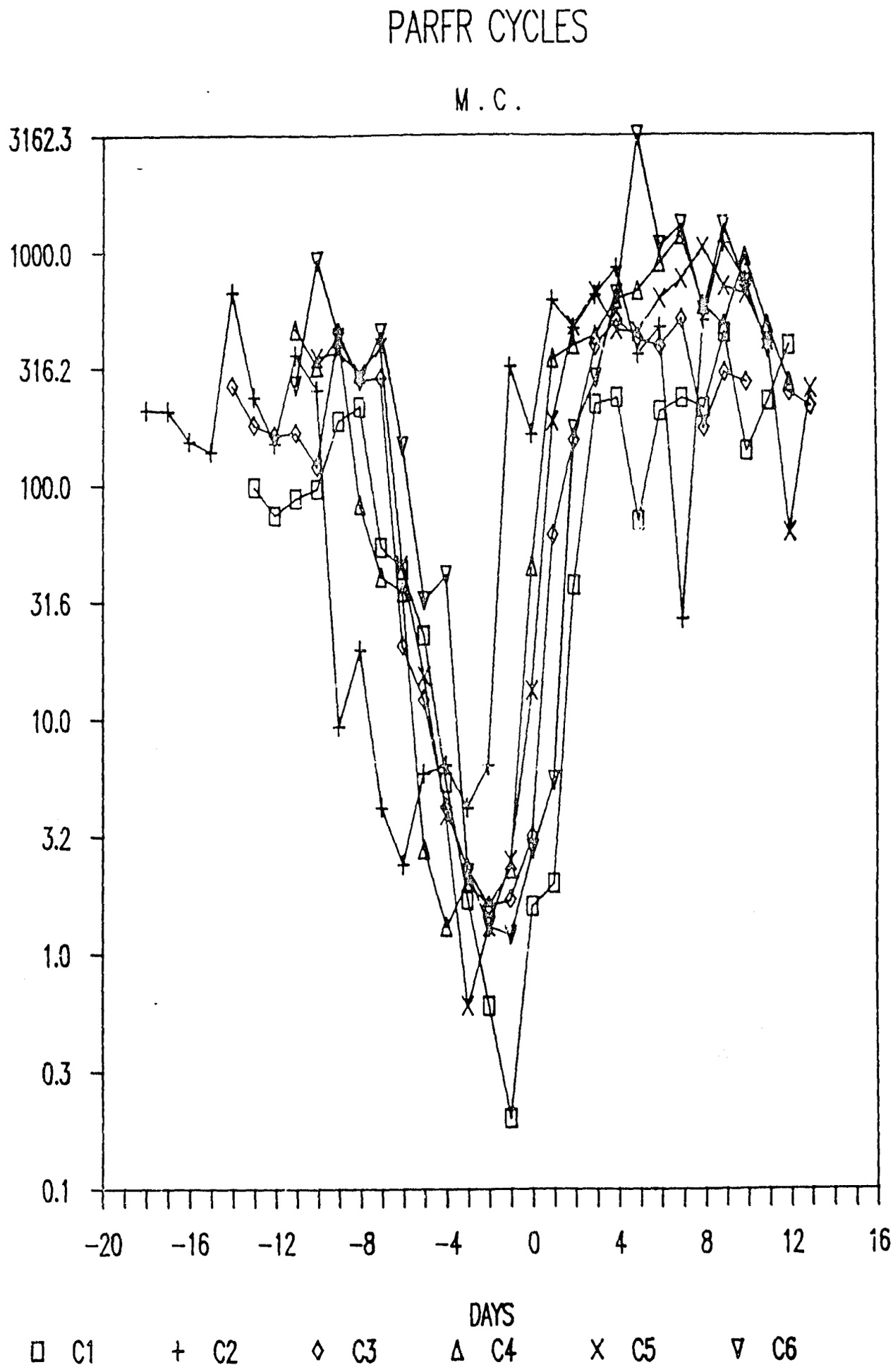

PARFR CYCLE 6
L.C.
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
G.P. U/g
-20
-16
-12
-8
-4
0
4
8
12
16
DAYS

PARFR CYCLE 6

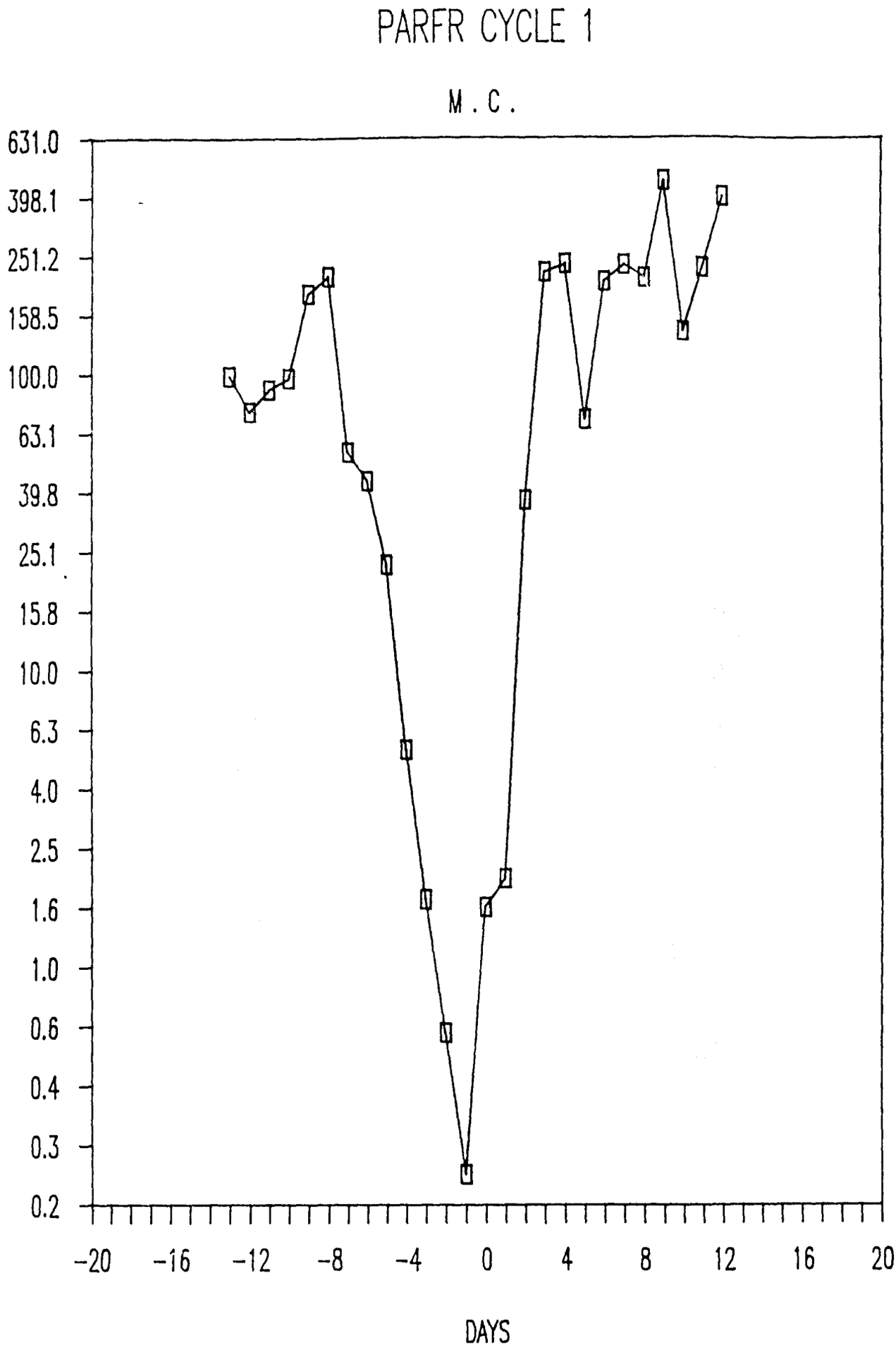






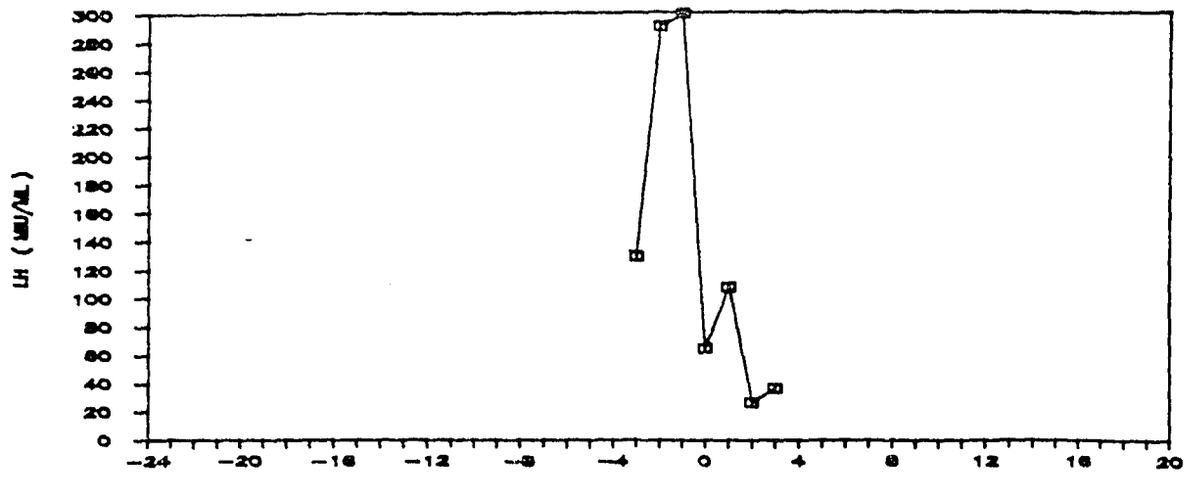

PARFR CYCLES
M.C.
3162.3
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-20
-16
-12
-8
-4
0
4
8
12
16
DAYS
C1
C2
C3
C4
C5
C6

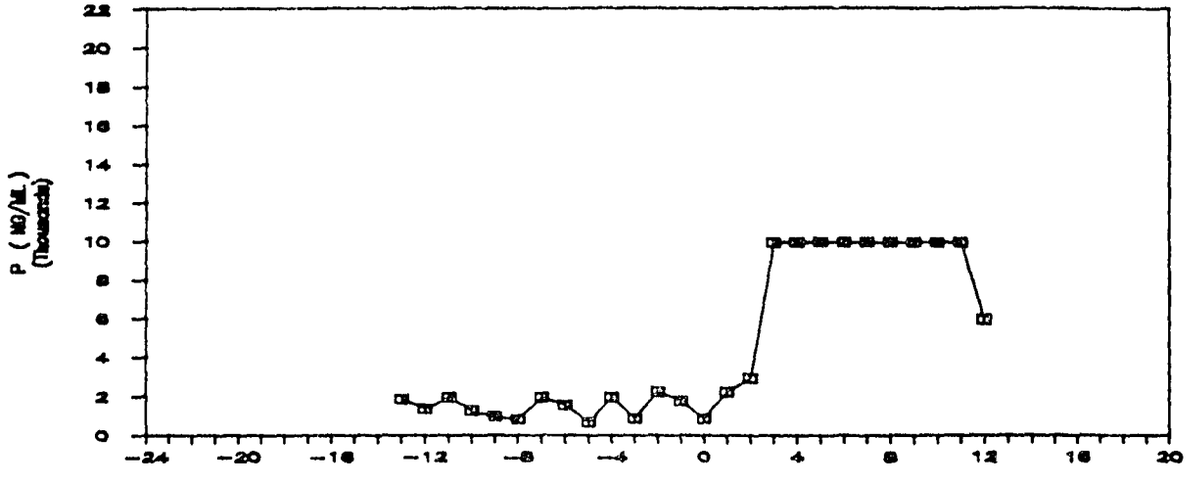
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
M.C.	9/9/84	3	-13	98.5			1900.0		---	PARFR CYCLE 1
	9/10/84	4	-12	74.3			1400.0		---	
	9/11/84	5	-11	88.3			2000.0		---	
	9/12/84	6	-10	96.5			1300.0		97.8	
	9/13/84	7	-9	188.4			1000.0		97.4	
	9/14/84	8	-8	216.4			870.0		97.8	
	9/15/84	9	-7	54.5			2000.0		97.6	
	9/16/84	10	-6	43.5			1600.0		98.0	
	9/17/84	11	-5	22.8			700.0		97.6	
	9/18/84	12	-4	5.4			2000.0		97.2	
	9/19/84	13	-3	1.7		130.0	900.0		97.6	
	9/20/84	14	-2	0.6		291.0	2300.0		97.6	
	9/21/84	15	-1	0.2		307.0	1800.0		97.8	
	9/22/84	16	0	1.6		65.0	900.0		98.0	
	9/23/84	17	1	2.0		108.0	2300.0		97.8	
	9/24/84	18	2	37.7		27.0	3000.0		97.8	
	9/25/84	19	3	223.2		37.0	10000.0		97.8	
	9/26/84	20	4	238.4			10000.0		97.8	
	9/27/84	21	5	70.7			10000.0		98.0	
	9/28/84	22	6	207.2			10000.0		98.4	
	9/29/84	23	7	236.1			10000.0		98.2	
	9/30/84	24	8	213.3			10000.0		98.2	
	10/1/84	25	9	450.7			10000.0		98.0	
	10/2/84	26	10	139.9			10000.0		98.2	
	10/3/84	27	11	229.6			10000.0		98.0	
	10/4/84	28	12	398.1			6000.0		97.4	

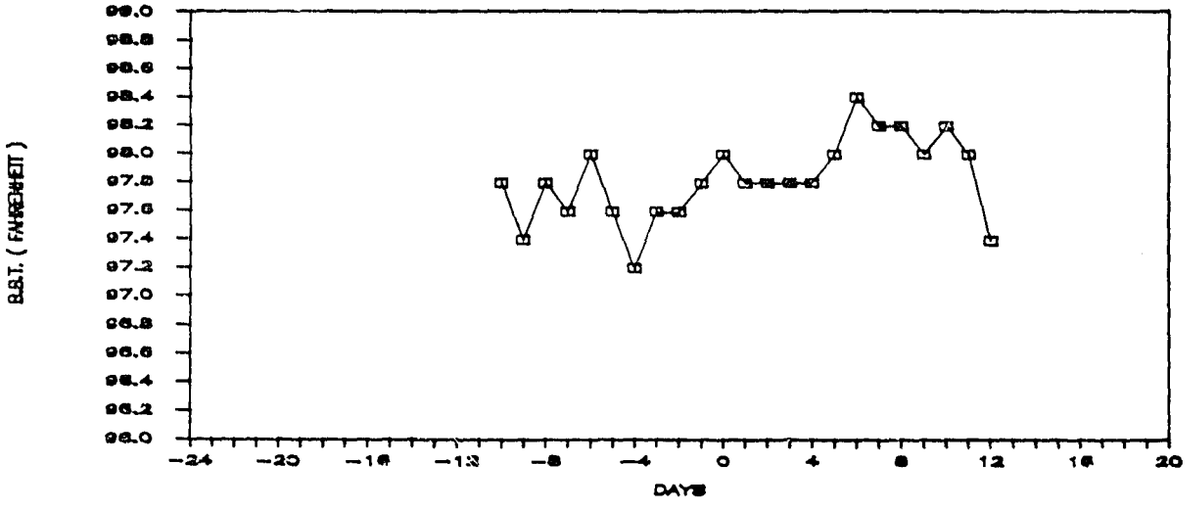

PARFR CYCLE 1
M . C .
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
0.6
0.4
0.3
0.2
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.C.

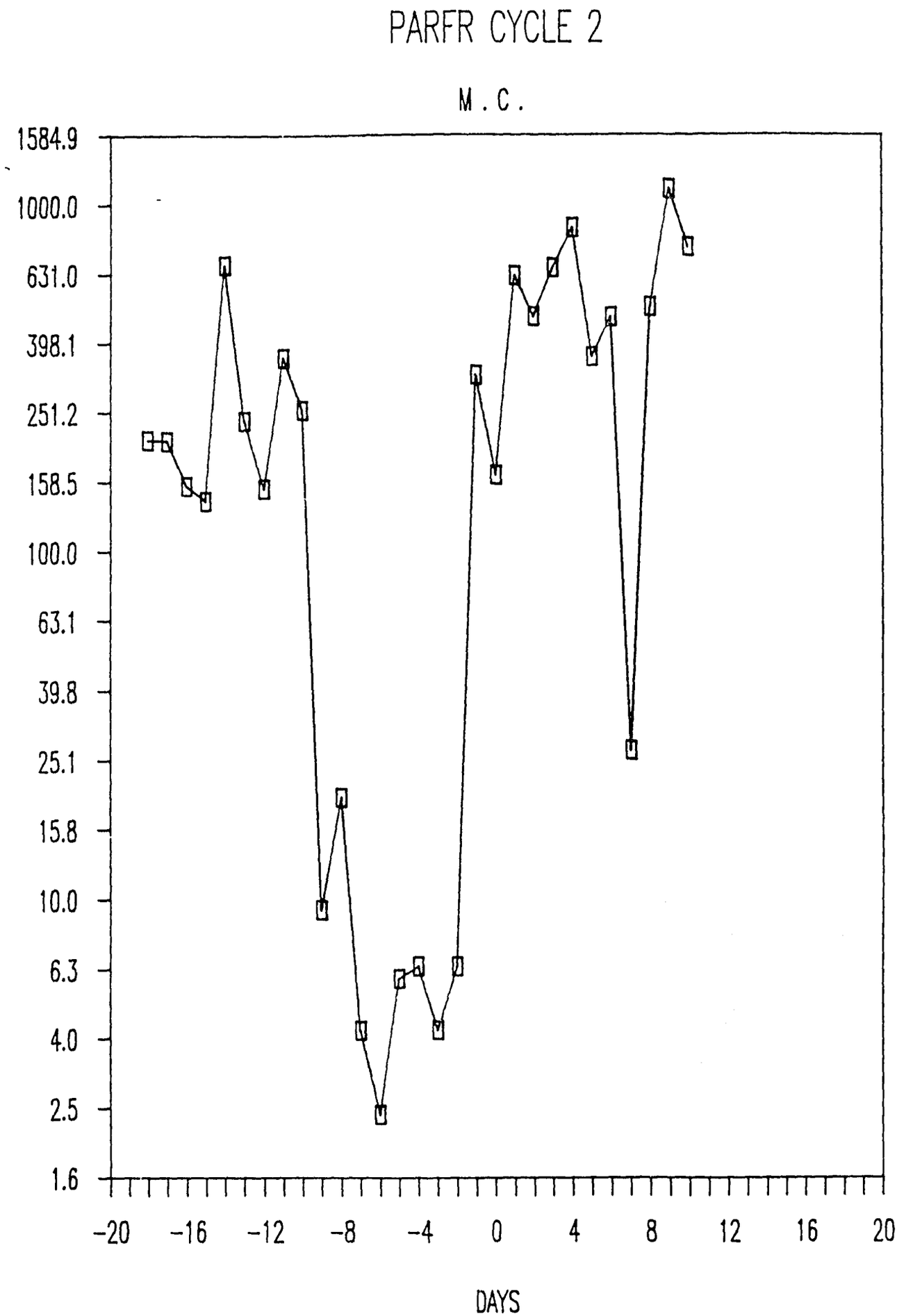
PARFR CYCLE 1





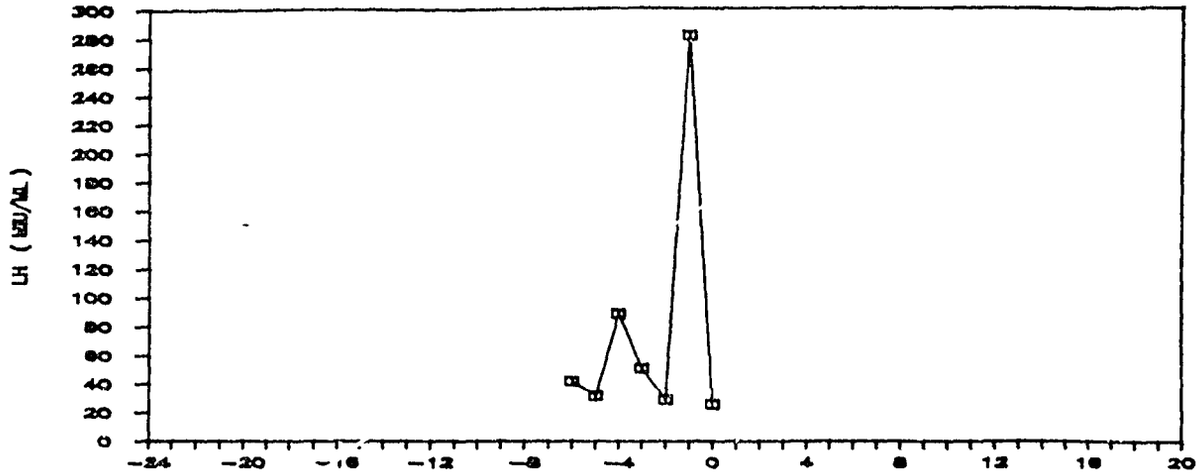


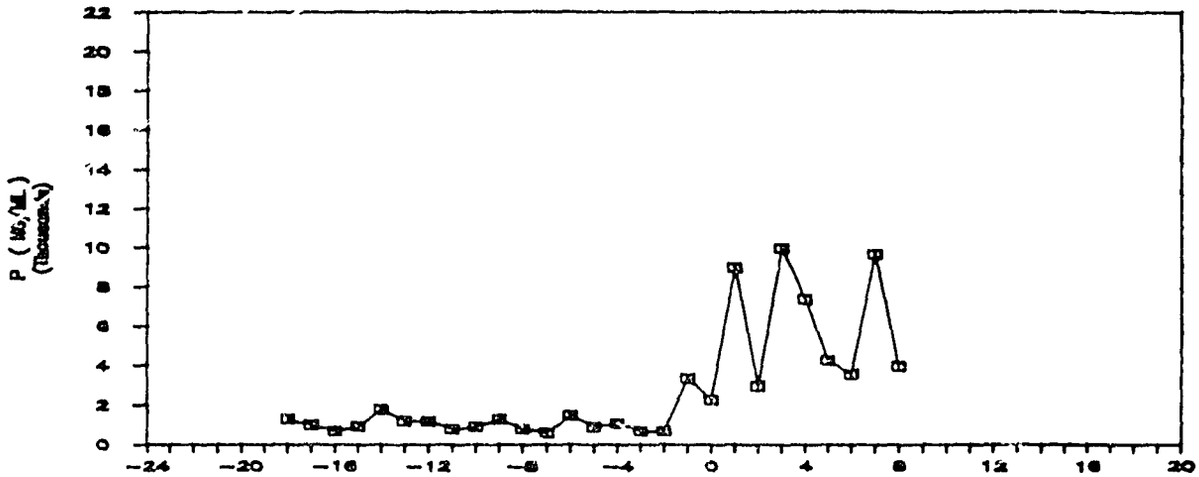
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
M.C.	10/8/84	4	-18	209.4			1300.0		98.0	PARFR CYCLE 2
	10/9/84	5	-17	207.6			1000.0		98.0	
	10/10/84	6	-16	153.7			700.0		97.8	
	10/11/84	7	-15	139.2			930.0		97.6	
	10/12/84	8	-14	670.0			1800.0		97.6	
	10/13/84	9	-13	237.1			1200.0		97.8	
	10/14/84	10	-12	150.8			1200.0		97.6	
	10/15/84	11	-11	357.9			800.0		97.8	
	10/16/84	12	-10	253.6			900.0		97.6	
	10/17/84	13	-9	9.3			1300.0		97.5	
	10/18/84	14	-8	19.6			800.0		97.8	
	10/19/84	15	-7	4.2			600.0		97.8	
	10/20/84	16	-6	2.4		42.0	1500.0		97.6	
	10/21/84	17	-5	5.9		32.0	900.0		97.8	
	10/22/84	18	-4	6.4		89.0	1100.0		97.6	
	10/23/84	19	-3	4.2		51.0	700.0		98.0	
	10/24/84	20	-2	6.4		29.0	700.0		97.8	
	10/25/84	21	-1	322.2		282.0	3400.0		97.8	
	10/26/84	22	0	165.8		26.0	2300.0		98.2	
	10/27/84	23	1	621.8			9000.0		98.2	
	10/28/84	24	2	474.2			3000.0		98.2	
	10/29/84	25	3	655.7			10000.0		98.0	
	10/30/84	26	4	859.6			7400.0		98.2	
	10/31/84	27	5	364.1			4300.0		98.0	
	11/1/84	28	6	473.5			3600.0		98.0	
	11/2/84	29	7	26.7			9700.0		98.0	
	11/3/84	30	8	505.5			4000.0		98.2	
	11/4/84	31	9	1105.1					98.6	
	11/5/84	32	10	752.9					97.6	

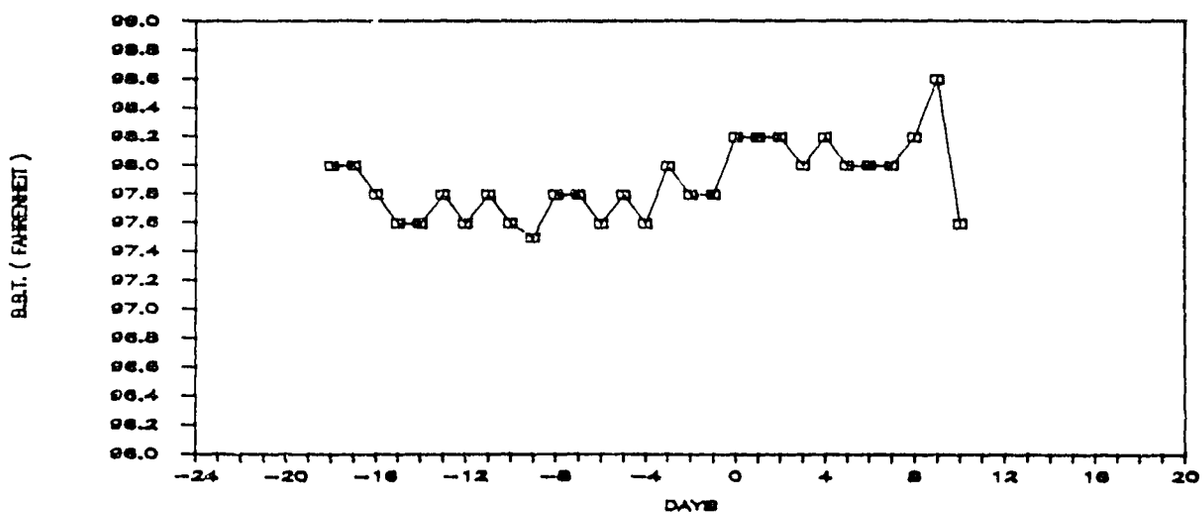

PARFR CYCLE 2
M.C.
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.C

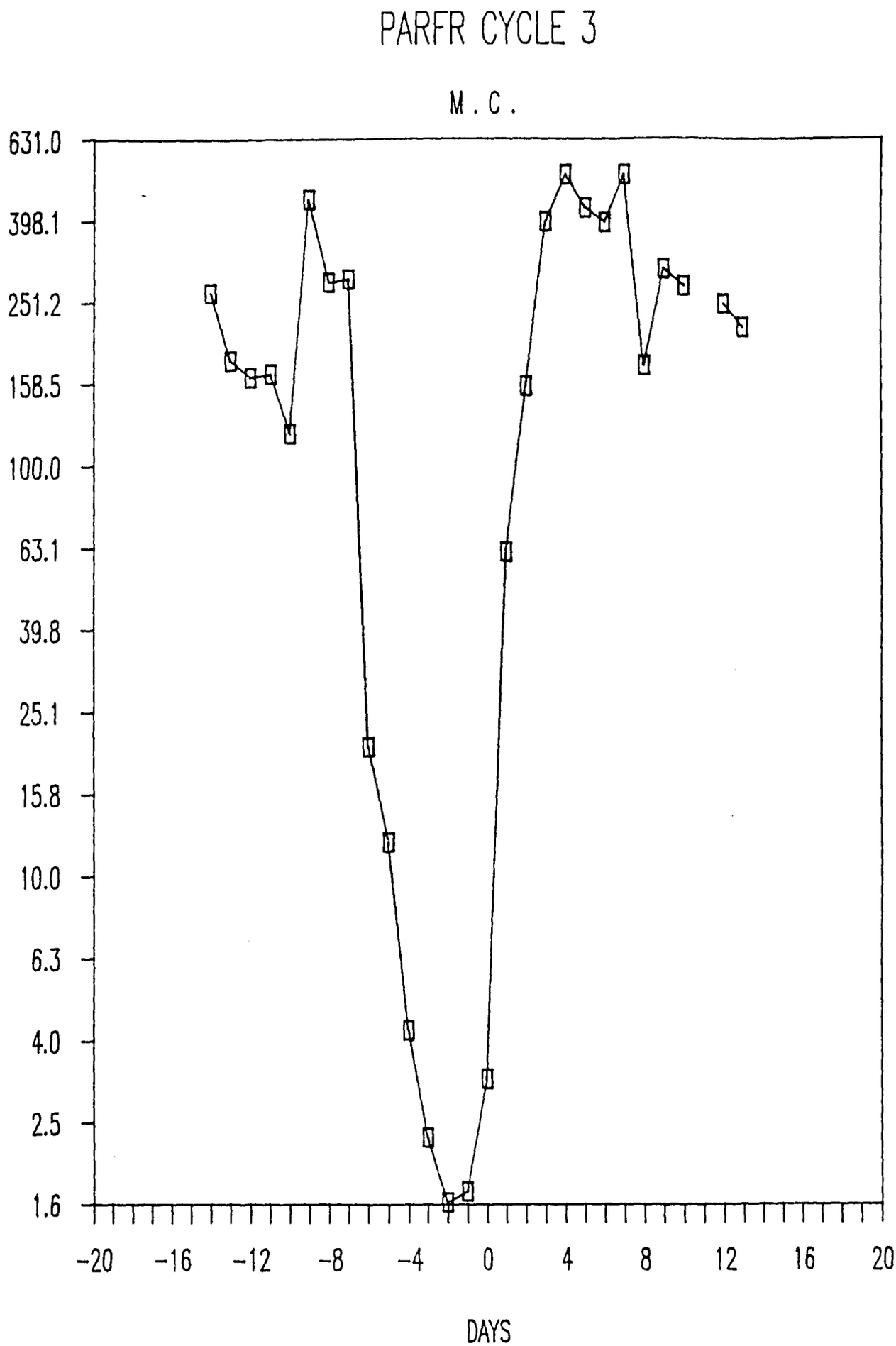
PARFR CYCLE 2





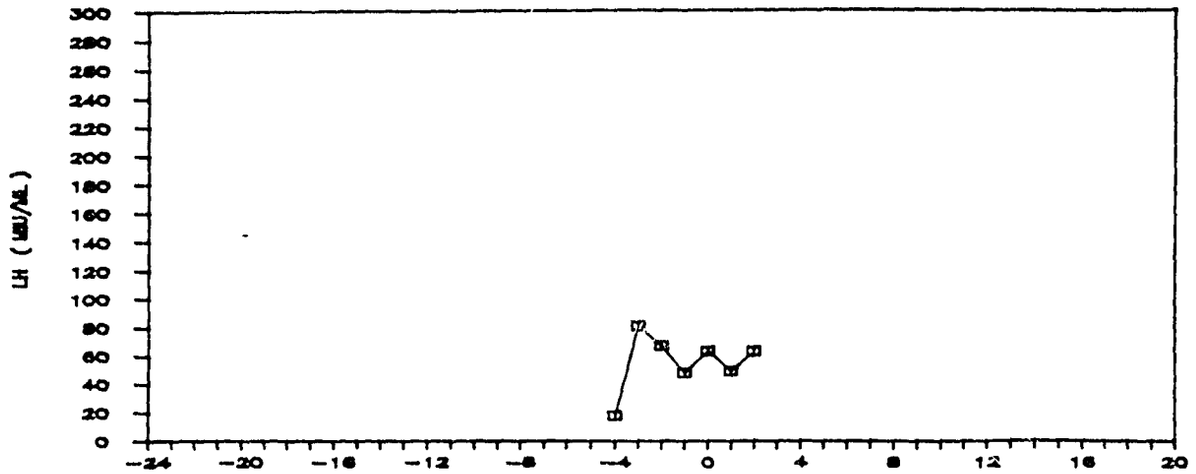


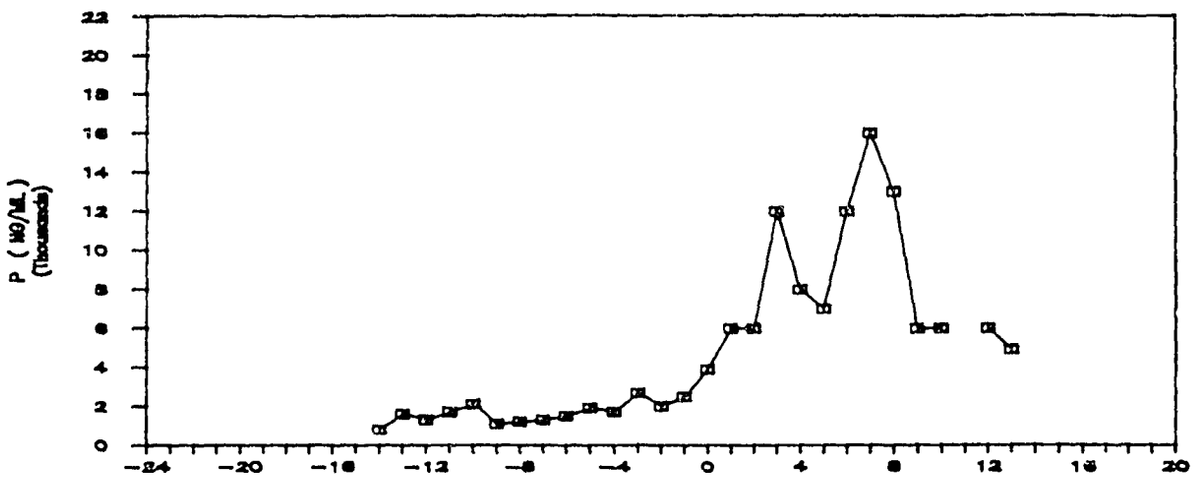
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
M.C.	1/8/85	4	-14	265.3			800.0		97.8	PARFR CYCLE 3
	1/9/85	5	-13	180.0			1600.0		97.8	
	1/10/85	6	-12	163.7			1300.0		98.2	
	1/11/85	7	-11	167.0			1700.0		97.4	
	1/12/85	8	-10	119.8			2100.0		97.8	
	1/13/85	9	-9	448.9			1100.0		98.4	
	1/14/85	10	-8	281.5			1200.0		97.8	
	1/15/85	11	-7	286.3			1300.0		97.4	
	1/16/85	12	-6	20.6			1500.0		97.6	
	1/17/85	13	-5	12.1			1900.0		97.6	
	1/18/85	14	-4	4.2		18.0	1700.0		97.8	
	1/19/85	15	-3	2.3		81.0	2700.0		97.8	
	1/20/85	16	-2	1.6		67.0	2000.0		98.0	
	1/21/85	17	-1	1.7		48.0	2500.0		97.6	
	1/22/85	18	0	3.2		63.0	3900.0		98.2	
	1/23/85	19	1	61.8		49.0	6000.0		98.0	
	1/24/85	20	2	156.8		63.0	6000.0		98.0	
	1/25/85	21	3	395.3			12000.0		98.0	
	1/26/85	22	4	514.0			8000.0		98.0	
	1/27/85	23	5	427.8			7000.0		98.0	
	1/28/85	24	6	393.8			12000.0		98.0	
	1/29/85	25	7	514.1			16000.0		98.0	
	1/30/85	26	8	175.5			13000.0		98.0	
	1/31/85	27	9	302.0			6000.0		98.0	
	2/1/85	28	10	273.8			6000.0		98.0	
	2/2/85	29	11	---			---		---	
	2/3/85	30	12	248.1			6000.0		98.2	
	2/4/85	31	13	217.3			4900.0		---	

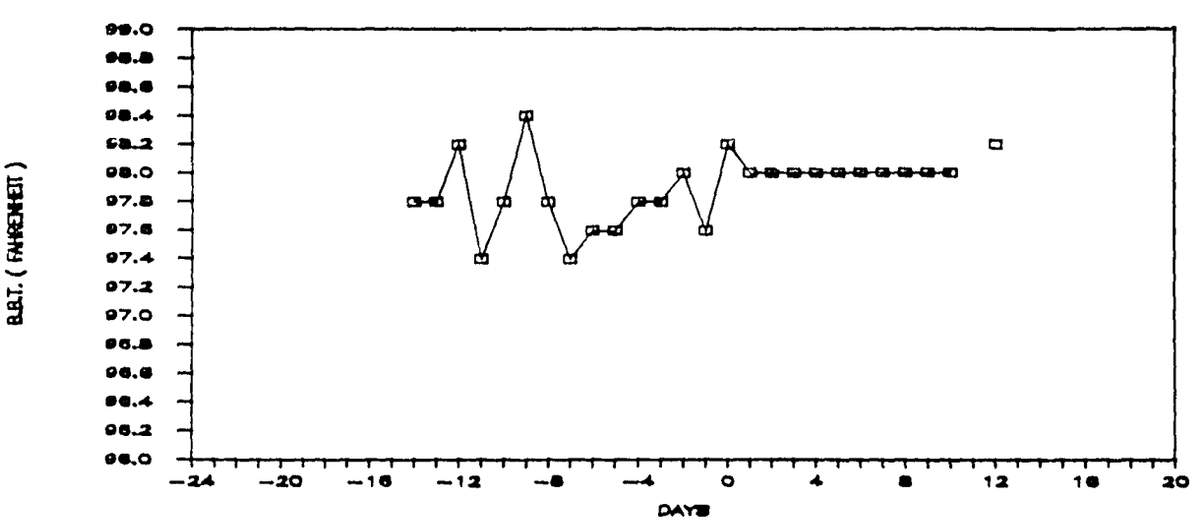

PARFR CYCLE 3
M.C.
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.C.

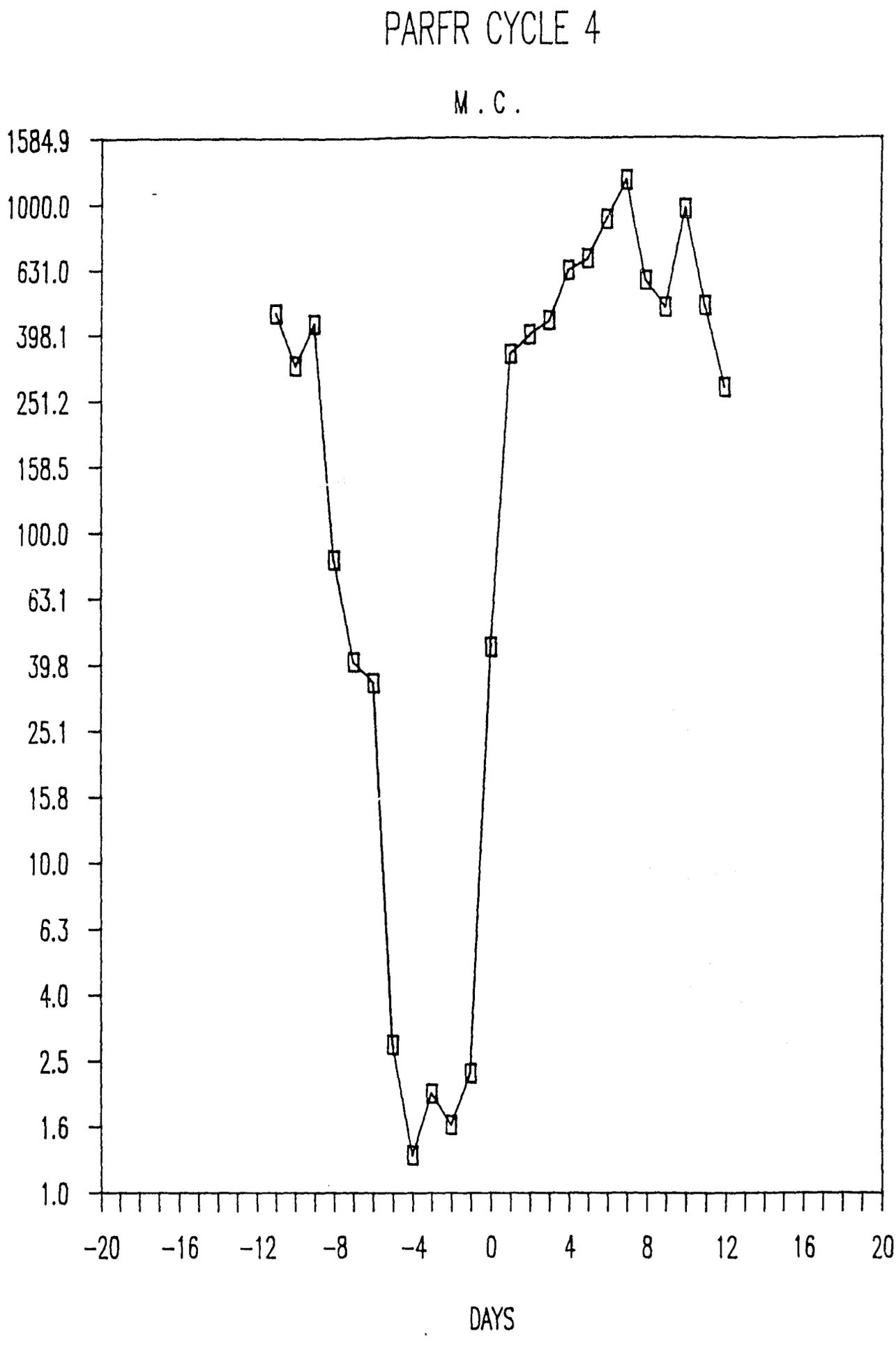
PARFR CYCLE 3





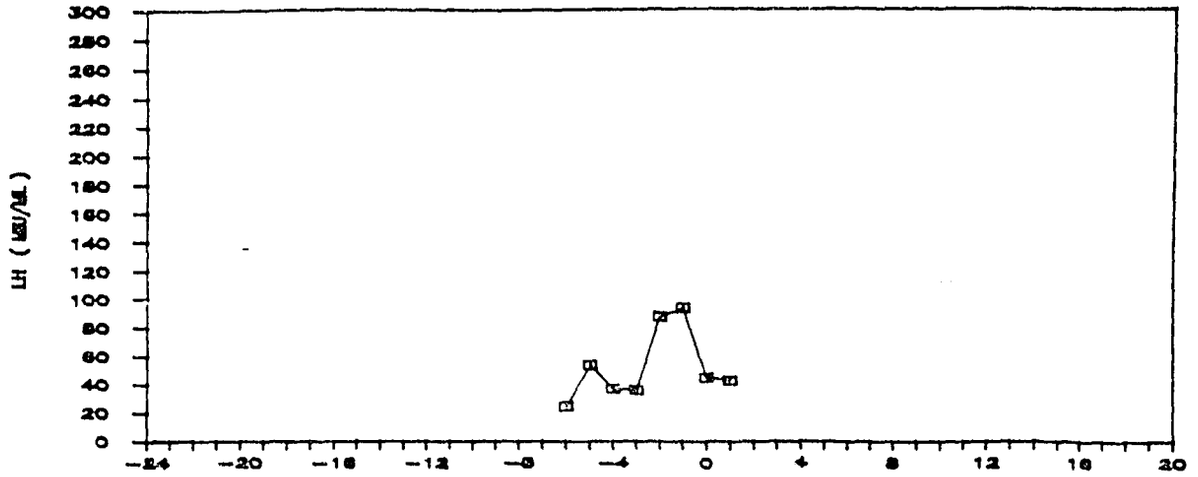


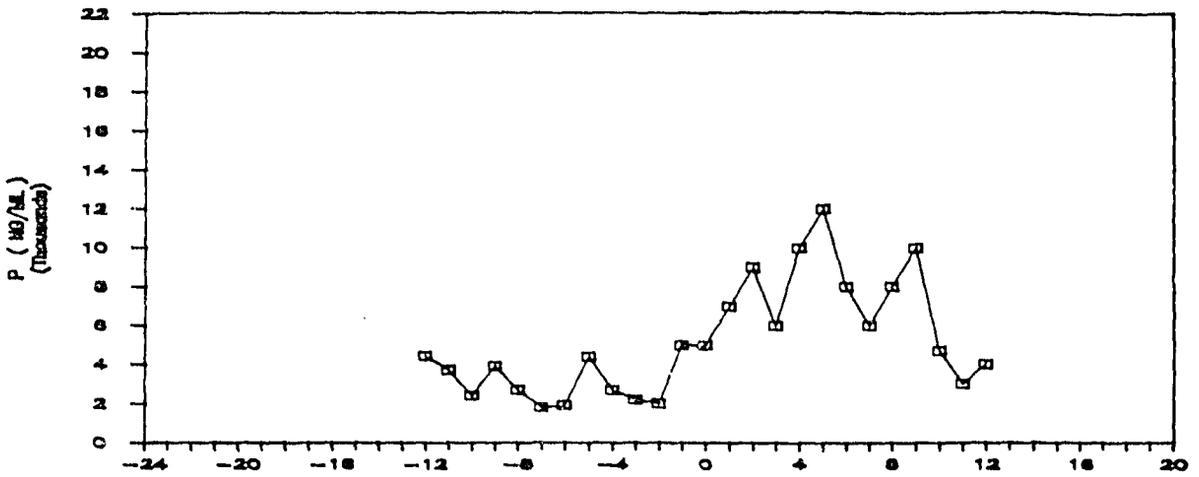
DATE	DATE	DFOM	DVD	GPC	GPV	LH	P	E2	BBT	REMARKS
M.C.	2/8.85	4	-12				4400.0		99.0	PARFR CYCLE 4
	2/9/85	5	-11	463.3			3700.0		98.4	
	2/10/85	6	-10	320.5			2400.0		97.8	
	2/11.85	7	-9	430.3			3900.0		98.0	
	2/12/85	8	-8	32.7			2700.0		97.4	
	2/13.85	9	-7	40.5			1800.0		97.6	
	2/14.85	10	-6	35.0		25.0	1900.0		98.0	
	2/15.85	11	-5	2.8		54.0	4400.0		97.8	
	2/16.85	12	-4	1.3		37.0	2700.0		97.8	
	2/17.85	13	-3	2.0		36.0	2200.0		97.8	
	2/18.85	14	-2	1.6		88.0	2000.0		97.8	
	2/19/85	15	-1	2.3		94.0	5000.0		97.3	
	2/20.85	16	0	45.2		45.0	5000.0		98.0	
	2/21.85	17	1	350.5		43.0	7000.0		98.2	
	2/22/85	18	2	401.1			9000.0		98.0	
	2/23.85	19	3	443.3			6000.0		98.0	
	2/24.85	20	4	629.3			10000.0		98.4	
	2/25.85	21	5	683.8			12000.0		98.4	
	2/26/85	22	6	901.2			3000.0		98.2	
	2/27/85	23	7	1182.2			6000.0		98.4	
	2/28.85	24	8	587.6			8000.0		98.2	
	3/1.85	25	9	484.2			10000.0		98.2	
	3/2/85	26	10	963.1			4700.0		98.0	
	3/3/85	27	11	488.4			3000.0		98.0	
	3/4.85	28	12	276.4			4000.0		98.0	

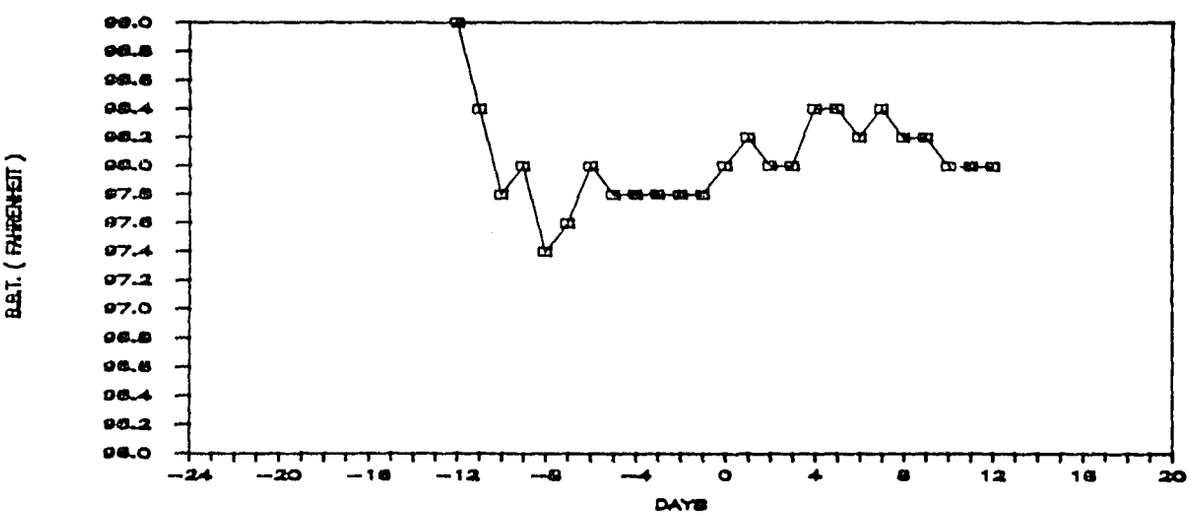

PARFR CYCLE 4
M.C.
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.C

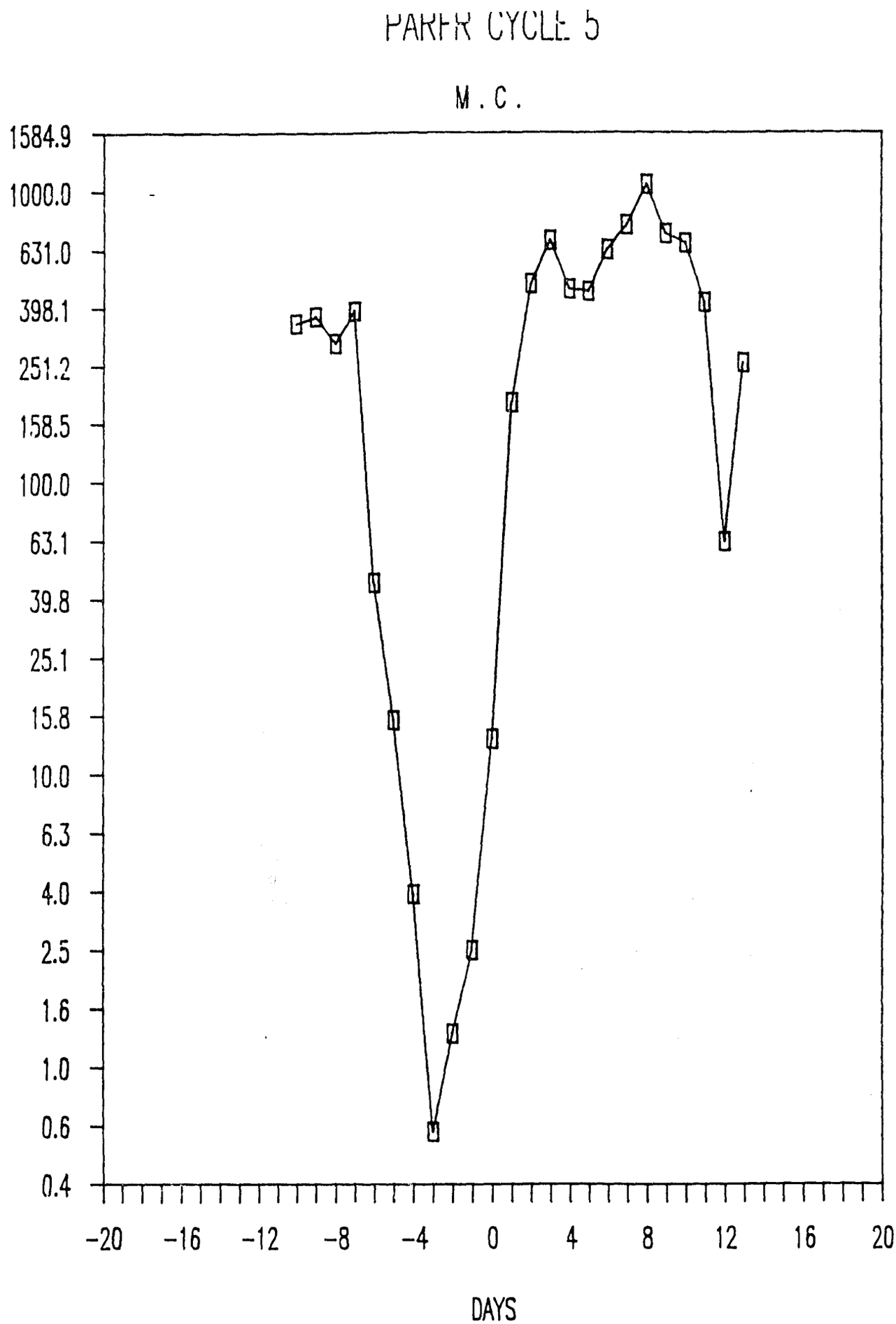
PARFR CYCLE 4



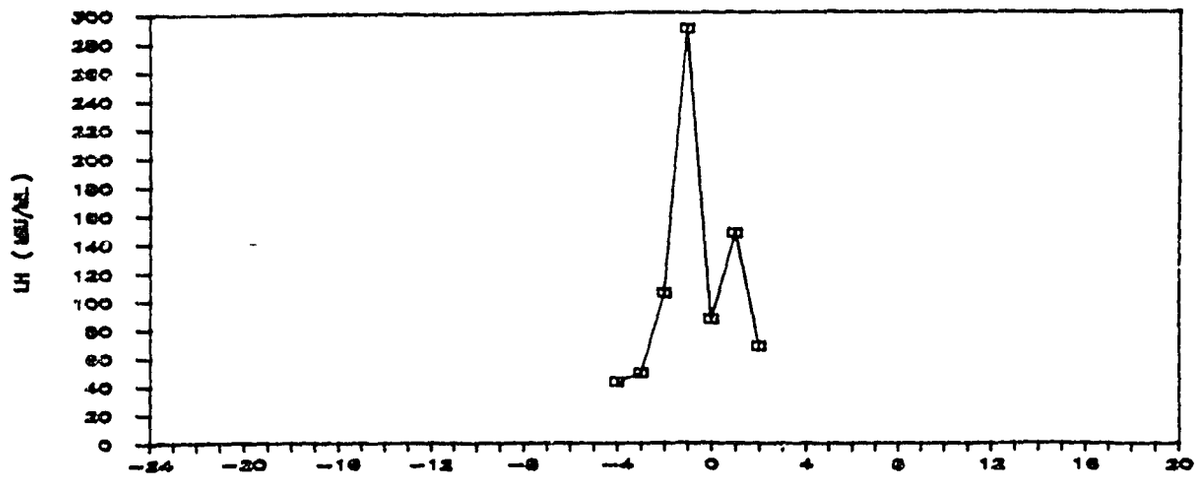


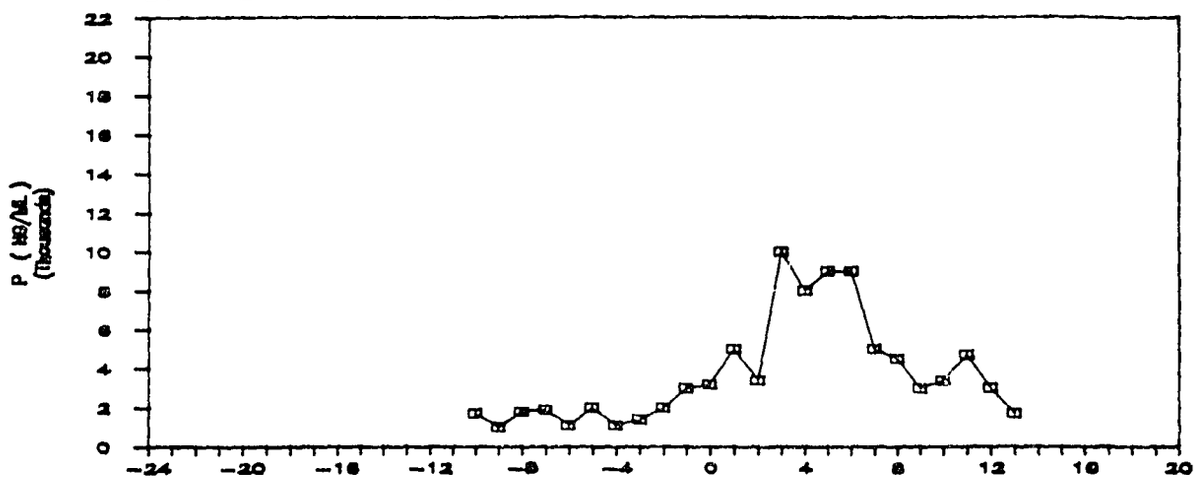


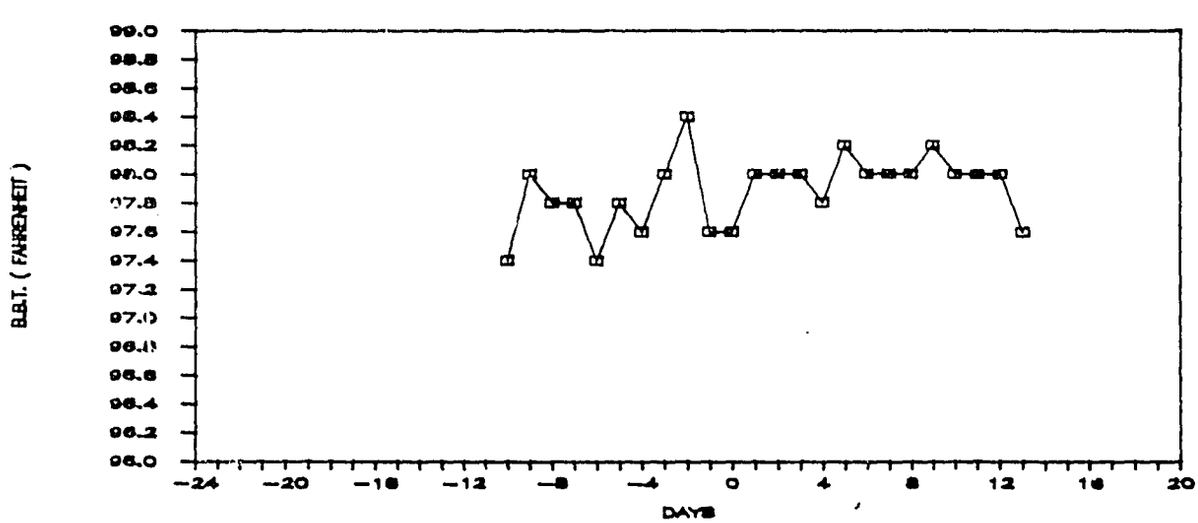
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
M.C.	3/8/85	4	-10	350.3			1700.0		97.4	FARFR CYCLE 5
	3/9/85	5	-9	371.1			1000.0		98.0	
	3/10/85	6	-8	299.6			1800.0		97.8	
	3/11/85	7	-7	389.0			1900.0		97.8	
	3/12/85	8	-6	45.3			1100.0		97.4	
	3/13/85	9	-5	15.3		30.0	2000.0		97.8	
	3/14/85	10	-4	3.9		7.0	1100.0		97.6	
	3/15/85	11	-3	0.6		14.0	1400.0		98.0	
	3/16/85	12	-2	1.3		69.0	2000.0		98.4	
	3/17/85	13	-1	2.5		79.0	3000.0		97.6	
	3/18/85	14	0	13.3		25.0	3200.0		97.6	
	3/19/85	15	1	188.9		42.0	5000.0		98.0	
	3/20/85	16	2	483.4		16.0	3400.0		98.0	
	3/21/85	17	3	678.3			10000.0		98.0	
	3/22/85	18	4	463.3			8000.0		97.8	
	3/23/85	19	5	455.7			9000.0		98.2	
	3/24/85	20	6	632.0			9000.0		98.0	
	3/25/85	21	7	766.1			5000.0		98.0	
	3/26/85	22	8	1048.3			4500.0		98.0	
	3/27/85	23	9	712.0			3000.0		98.2	
	3/28/85	24	10	661.4			3400.0		98.0	
	3/29/85	25	11	414.4			4700.0		98.0	
	3/30/85	26	12	62.7			3000.0		98.0	
	3/31/85	27	13	257.2			1700.0		97.6	


PARFR CYCLE 5
M . C .
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
0.6
0.4
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

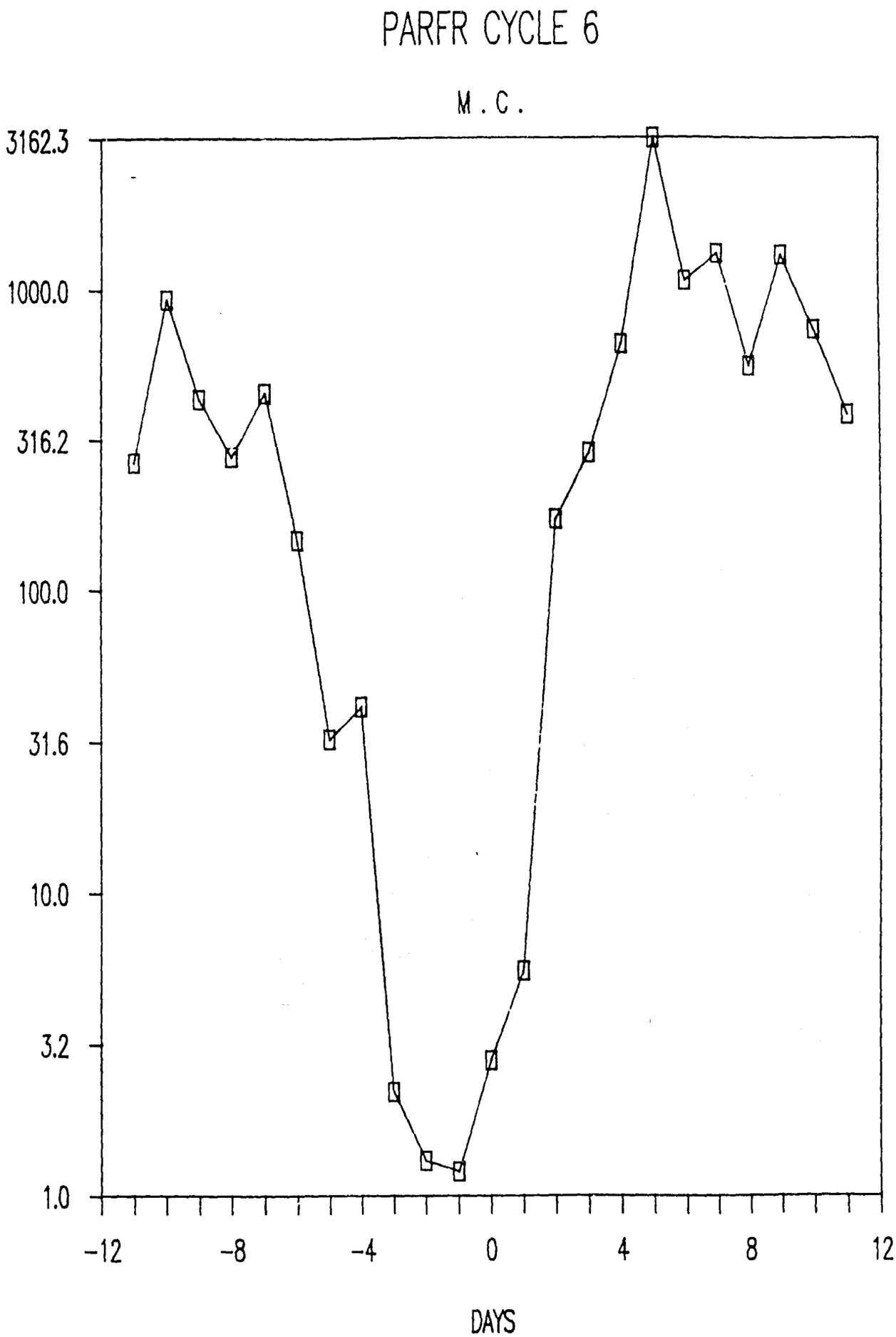
PARFR CYCLE 5







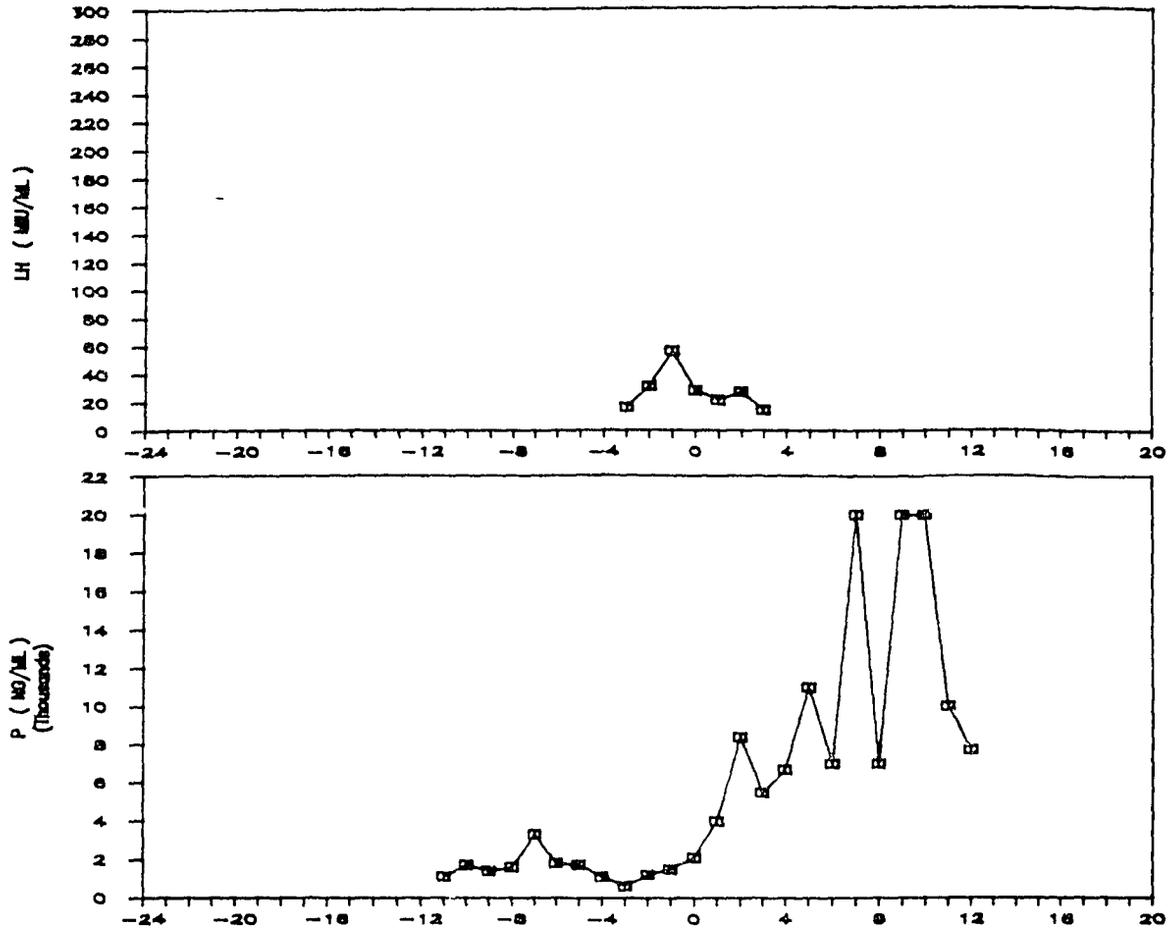
NAME	DATE	DFOM	DVD	GPD	GPV	LH	P	E2	BBT	REMARKS
M.C.	4/4/85	4	-11	265.8			1100.0		97.4	PARFR CYCLE 6
	4/5/85	5	-10	321.9			1700.0		97.4	
	4/6/85	6	-9	430.0			1400.0		97.8	
	4/7/85	7	-8	277.2			1600.0		97.4	
	4/8/85	8	-7	447.6			3300.0		97.2	
	4/9/85	9	-6	146.4			1800.0		97.6	
	4/10/85	10	-5	32.1			1700.0		97.4	
	4/11/85	11	-4	41.1			1100.0		97.6	
	4/12/85	12	-3	2.2		16.0	600.0		97.8	
	4/13/85	13	-2	1.3		31.0	1200.0		97.4	
	4/14/85	14	-1	1.2		56.0	1500.0		98.0	
	4/15/85	15	0	2.8		28.0	2100.0		97.2	
	4/16/85	16	1	5.5		21.0	4000.0		98.0	
	4/17/85	17	2	173.3		27.0	8400.0		98.2	
	4/18/85	18	3	287.4		14.0	5500.0		98.2	
	4/19/85	19	4	658.2			6700.0		98.0	
	4/20/85	20	5	1142.2			11000.0		98.0	
	4/21/85	21	6	1060.6			7000.0		98.2	
	4/22/85	22	7	1706.7			20000.0		98.0	
	4/23/85	23	8	554.4			7000.0		98.0	
	4/24/85	24	9	1214.9			20000.0		98.0	
	4/25/85	25	10	721.3			20000.0		98.2	
	4/26/85	26	11	379.5			10000.0		98.2	
	4/27/85	27	12				7700.0		97.6	

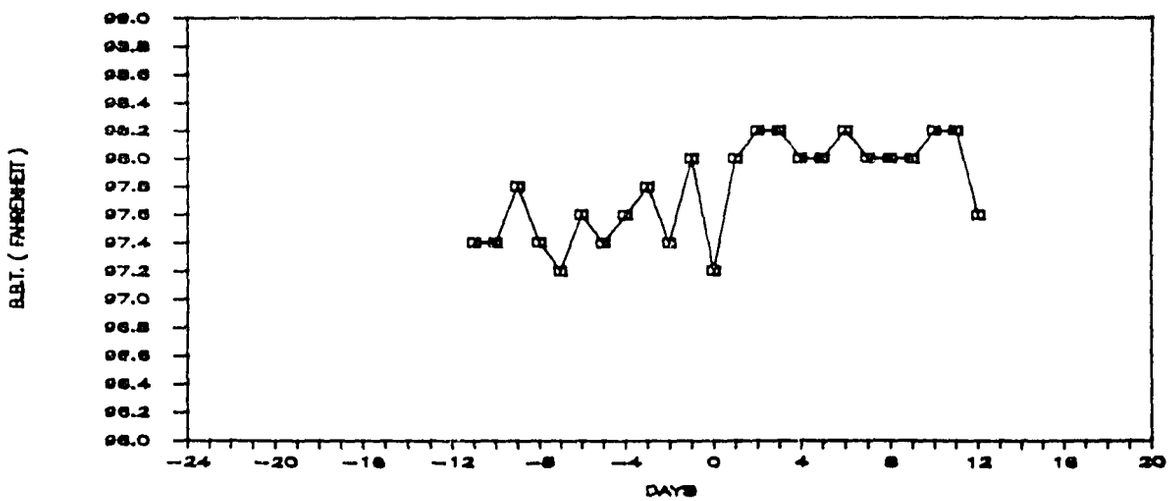

PARFR CYCLE 6
M.C.
3162.3
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
-12
-8
-4
0
4
8
12
DAYS

M.C.

PARFR CYCLE 6

M . C .

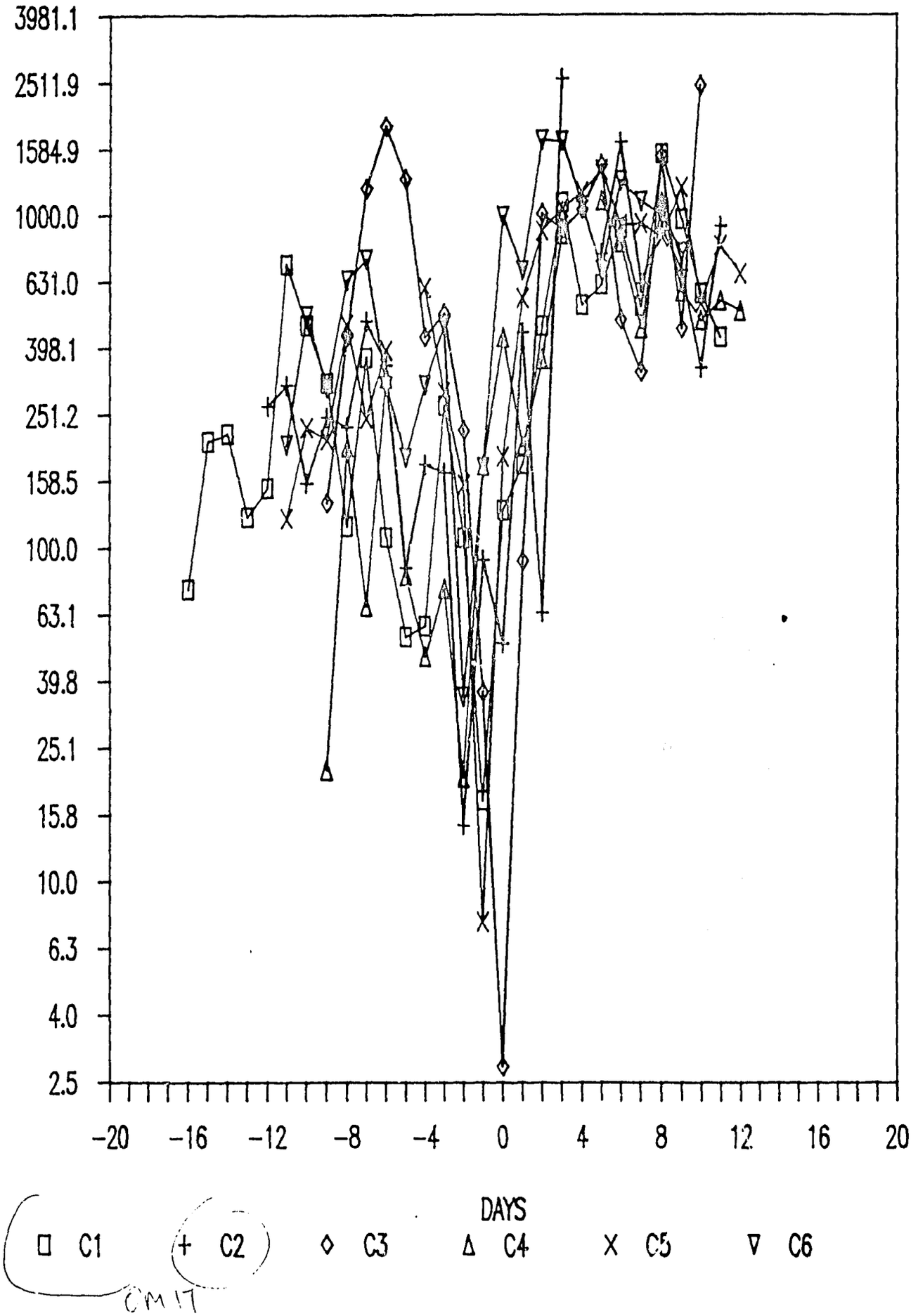




OMITTED from CUSUM
TOO LOW LH in #1 & #2 CY

PARFR CYCLES

M.K.



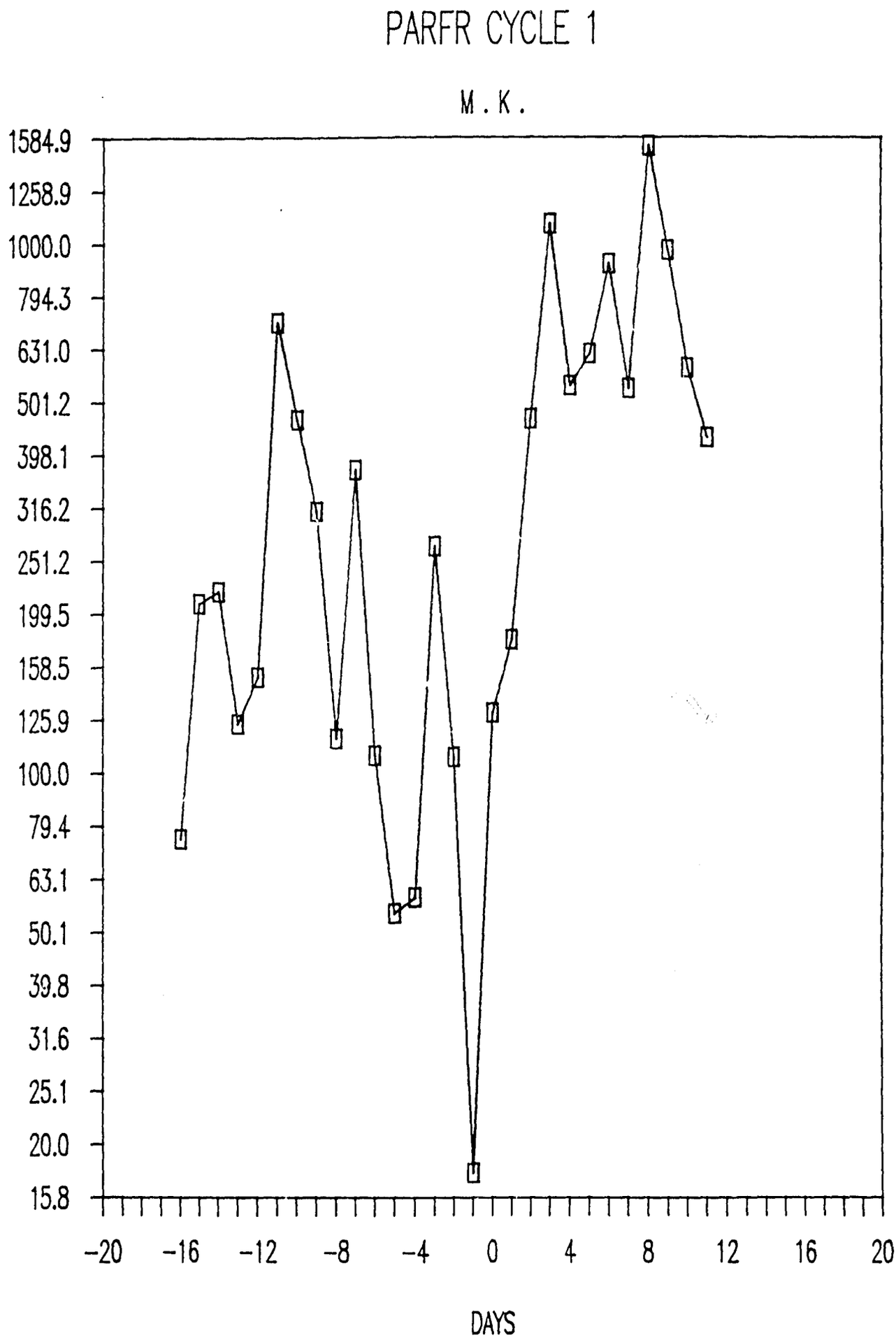
OMIT

OMITTED 1st & 2nd from CUSUM

Too LOW LH!

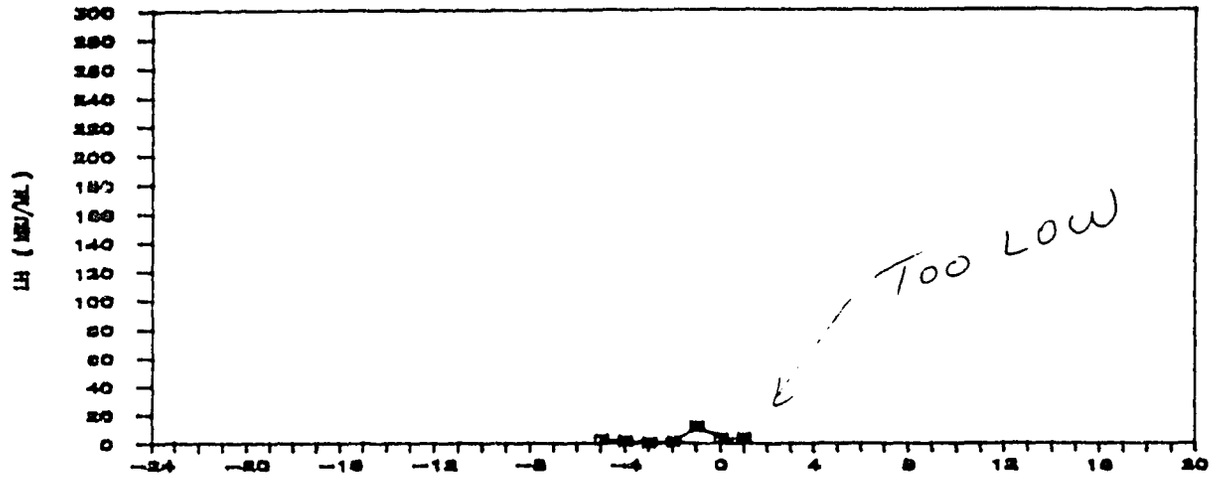
M.K.

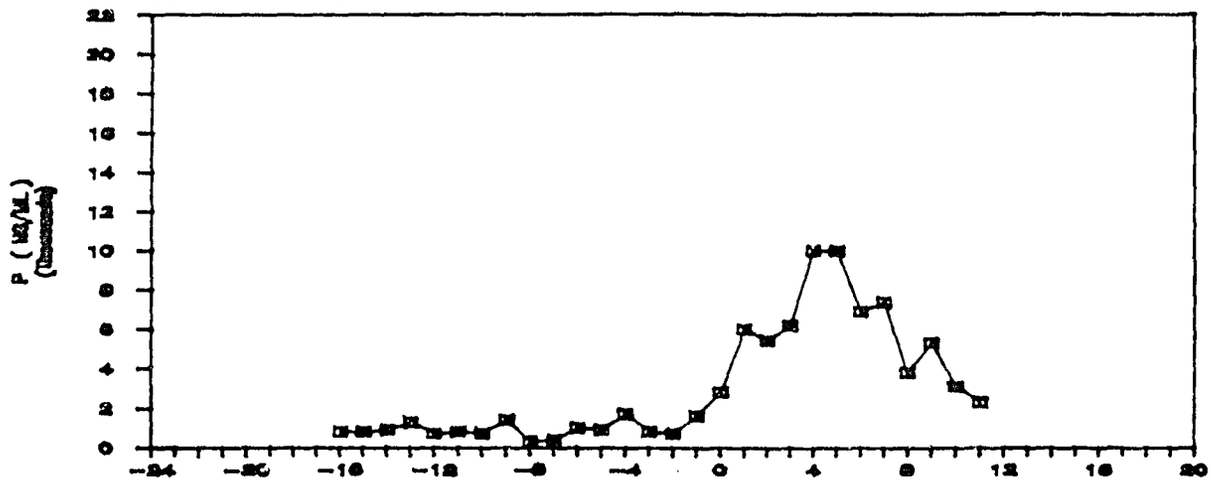
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
	9/20/84	4	-16	75.0			800.0		97.6	PARFR CYCLE 1
	9/21/84	5	-15	208.3			800.0		97.9	
	9/22/84	6	-14	219.8			900.0		98.0	
	9/23/84	7	-13	123.5			1300.0		97.6	
	9/24/84	8	-12	151.5			700.0		97.2	
	9/25/84	9	-11	708.8			800.0		97.6	
	9/26/84	10	-10	463.8			700.0		97.9	
	9/27/84	11	-9	311.7			1400.0		97.2	
	9/28/84	12	-8	115.9			300.0		97.6	
	9/29/84	13	-7	373.2			400.0		97.4	
	9/30/84	14	-6	107.9			1000.0		97.4	
	10/1/84	15	-5	54.3		2.7	900.0		97.4	
	10/2/84	16	-4	58.3		1.7	1700.0		97.2	
	10/3/84	17	-3	267.5		0.5	800.0		97.6	
	10/4/84	18	-2	107.1		1.1	700.0		97.2	
	10/5/84	19	-1	17.6		11.9	1600.0		97.6	
	10/6/84	20	0	130.3		3.3	2800.0		98.2	
	10/7/84	21	1	179.2		3.7	6000.0		98.2	
	10/8/84	22	2	467.3			5400.0		98.0	
	10/9/84	23	3	1092.6			6200.0		98.2	
	10/10/84	24	4	538.9			10000.0		97.6	
	10/11/84	25	5	619.8			10000.0		98.0	
	10/12/84	26	6	915.6			6900.0		98.2	
	10/13/84	27	7	531.6			7400.0		98.0	
	10/14/84	28	8	1529.3			3800.0		98.0	
	10/15/84	29	9	970.7			5300.0		98.2	
	10/16/84	30	10	581.3			3100.0		97.8	
	10/17/84	31	11	429.6			2300.0		97.7	

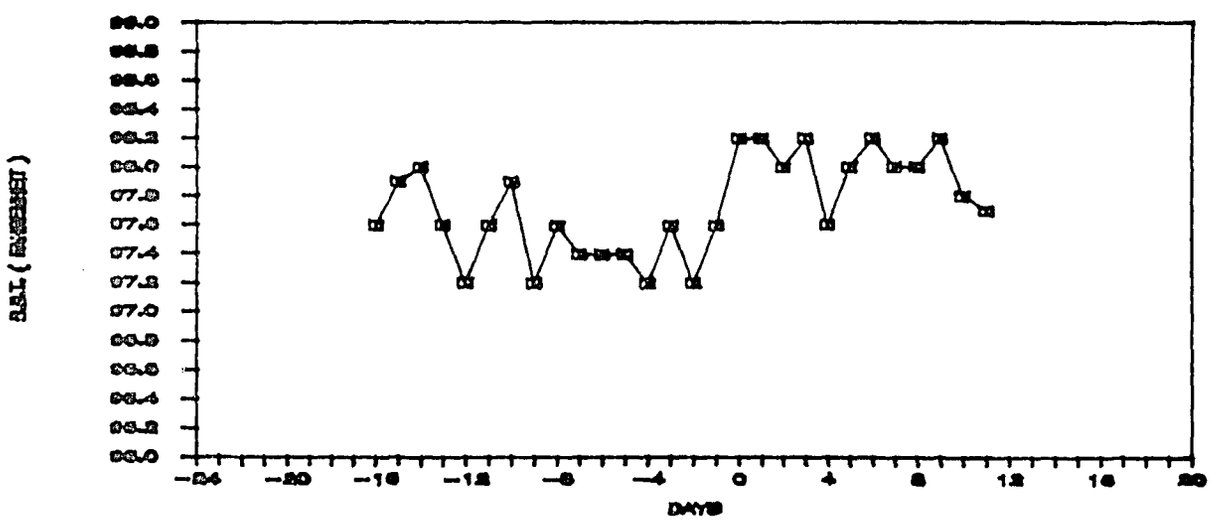

PARFR CYCLE 1
M.K.
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.K.

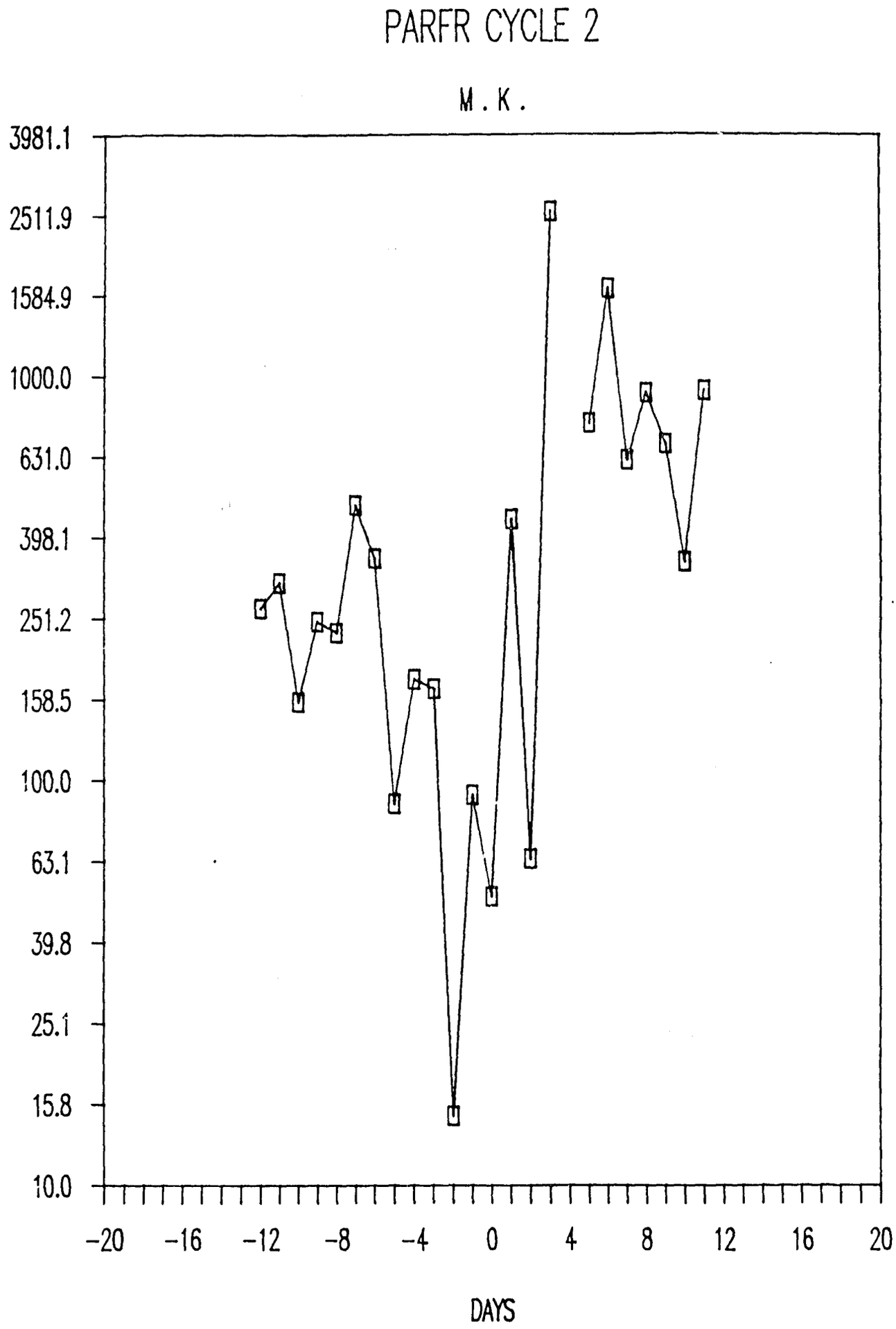
PARFR CYCLE 1





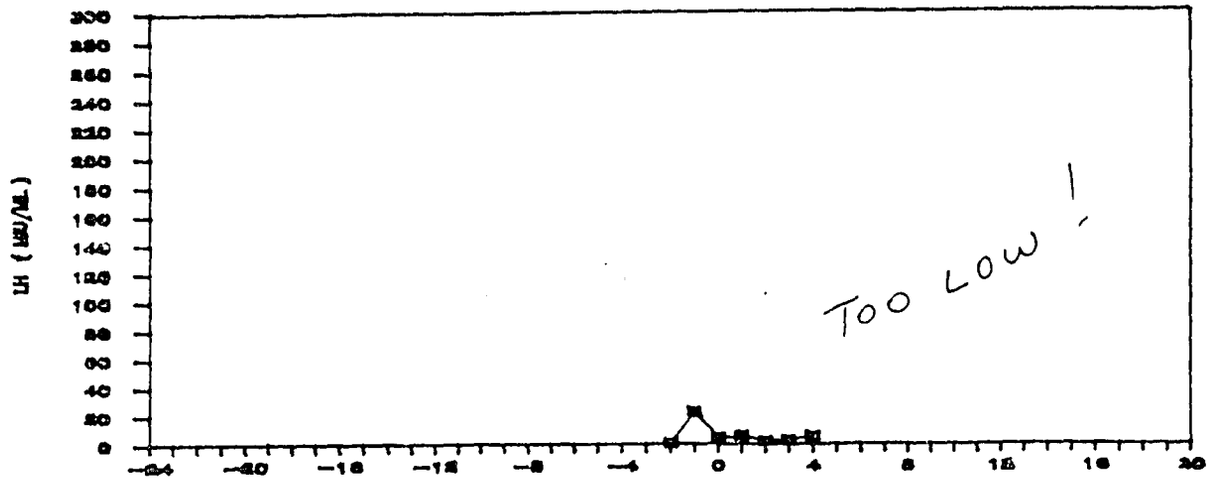


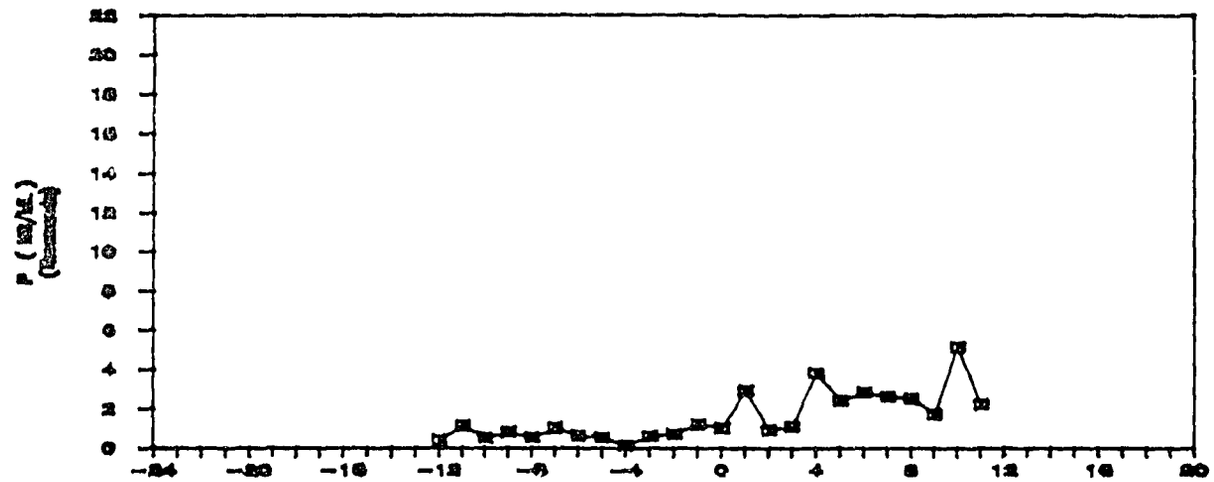
NAME	DATE	DFOM	DVD	GPC	GPV	LH	P	E2	BBT	REMARKS
M.K.	10/22/84	5	-12	265.5			400.0		97.2	PARFR CYCLE 2
	10/23/84	6	-11	307.3			1100.0		97.4	
	10/24/84	7	-10	156.2			500.0		97.4	
	10/25/84	8	-9	246.9			800.0		---	
	10/26/84	9	-8	231.1			500.0		97.6	
	10/27/84	10	-7	478.5			1000.0		97.6	
	10/28/84	11	-6	353.1			600.0		97.8	
	10/29/84	12	-5	87.4			500.0		97.6	
	10/30/84	13	-4	177.9			100.0		97.6	
	10/31/84	14	-3	168.5			600.0		97.6	
	11/1/84	15	-2	14.8		0.7	700.0		97.6	
	11/2/84	16	-1	91.9		22.2	1200.0		97.6	
	11/3/84	17	0	51.7		4.7	1000.0		97.6	
	11/4/84	18	1	443.1		5.9	2900.0		98.0	
	11/5/84	19	2	64.1		2.2	900.0		98.0	
	11/6/84	20	3	2572.1		3.2	1100.0		98.2	
	11/7/84	21	4	---		5.6	3800.0		98.2	
	11/8/84	22	5	768.0			2400.0		98.2	
	11/9/84	23	6	1656.1			2800.0		98.0	
	11/10/84	24	7	622.2			2600.0		98.2	
	11/11/84	25	8	913.7			2500.0		98.2	
	11/12/84	26	9	680.3			1700.0		98.2	
	11/13/84	27	10	346.1			5100.0		98.0	
	11/14/84	28	11	922.3			2200.0		98.0	

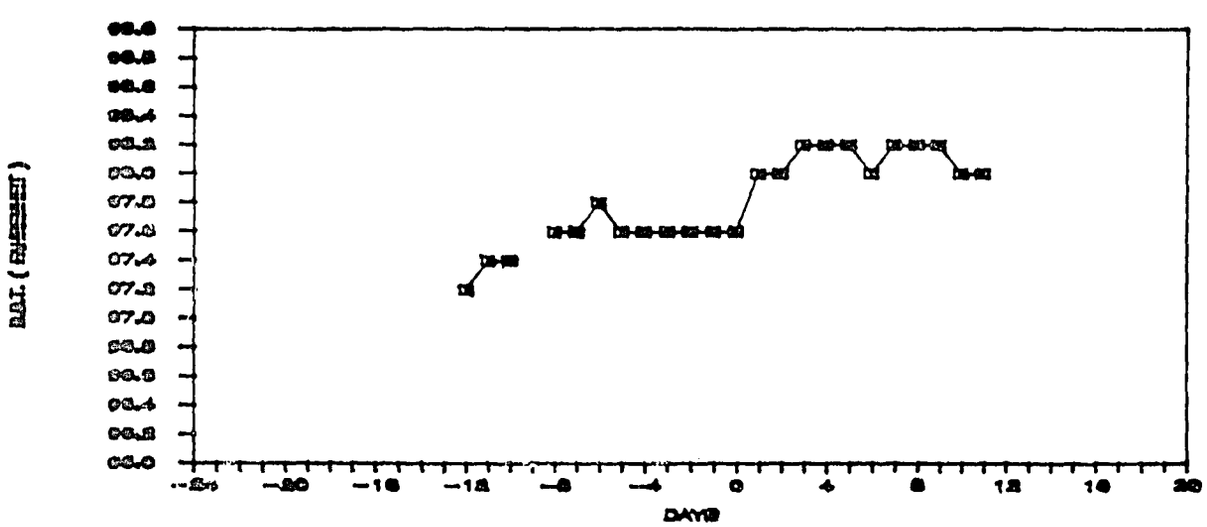

PARFR CYCLE 2
M.K.
3981.1
2511.9
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.K.

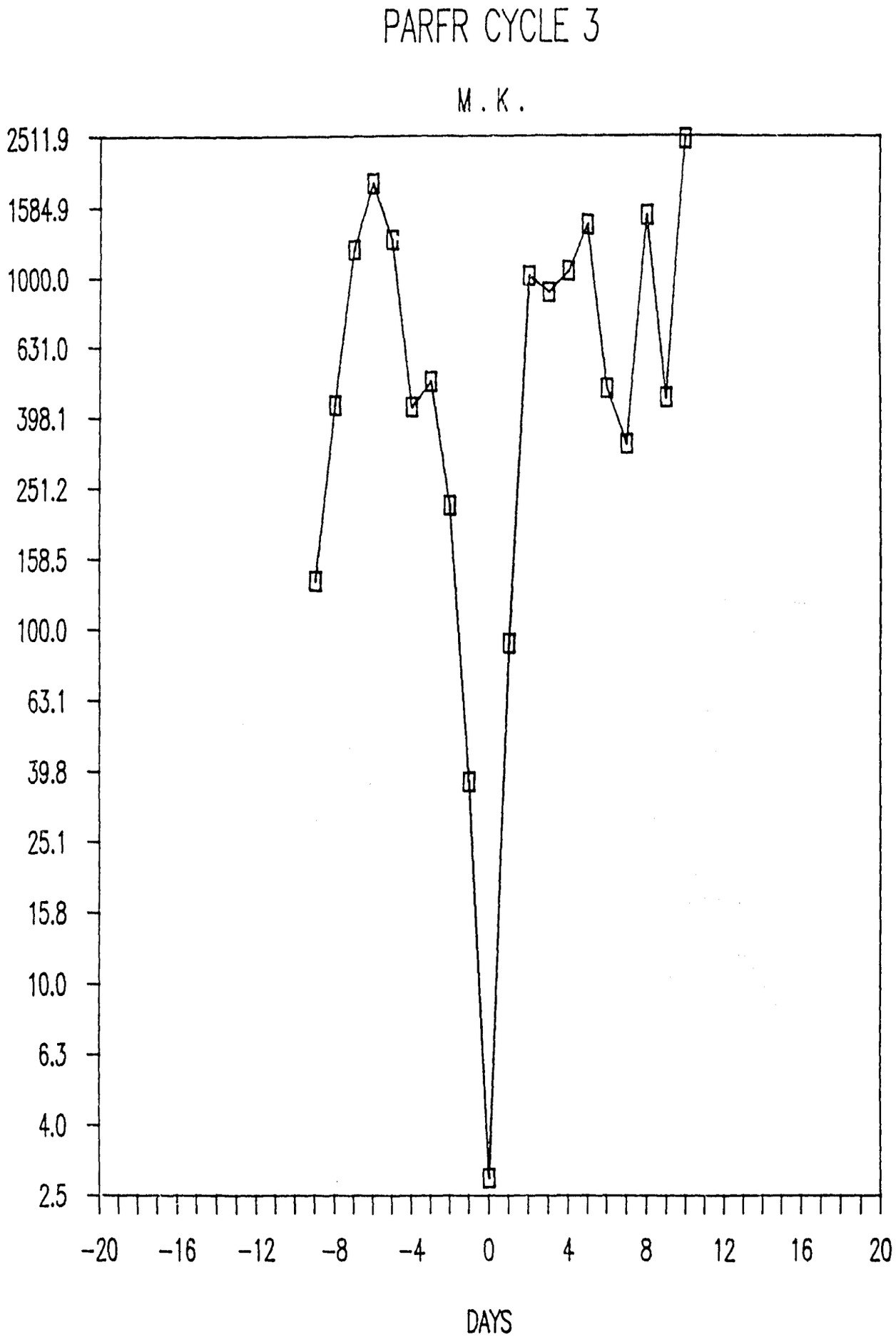
PARFR CYCLE 2





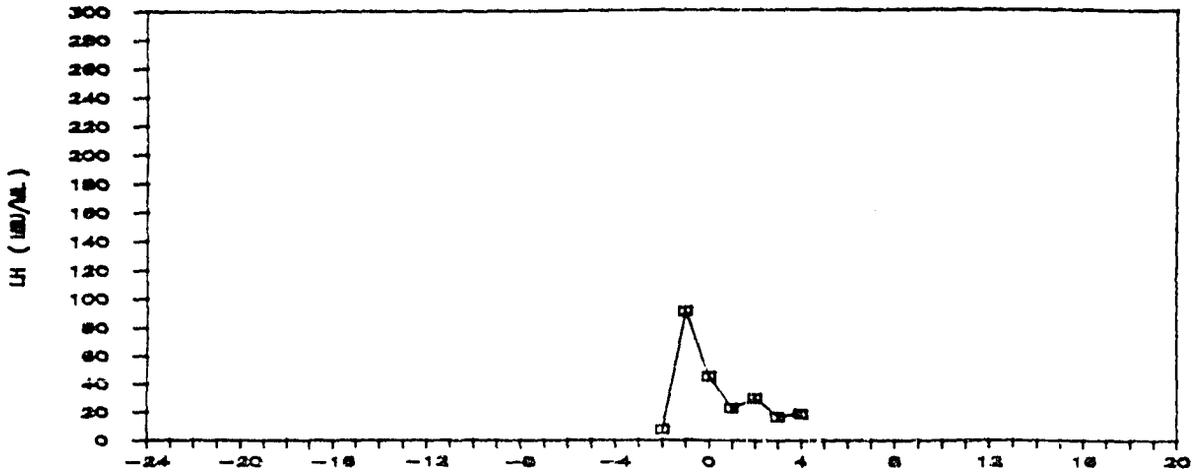


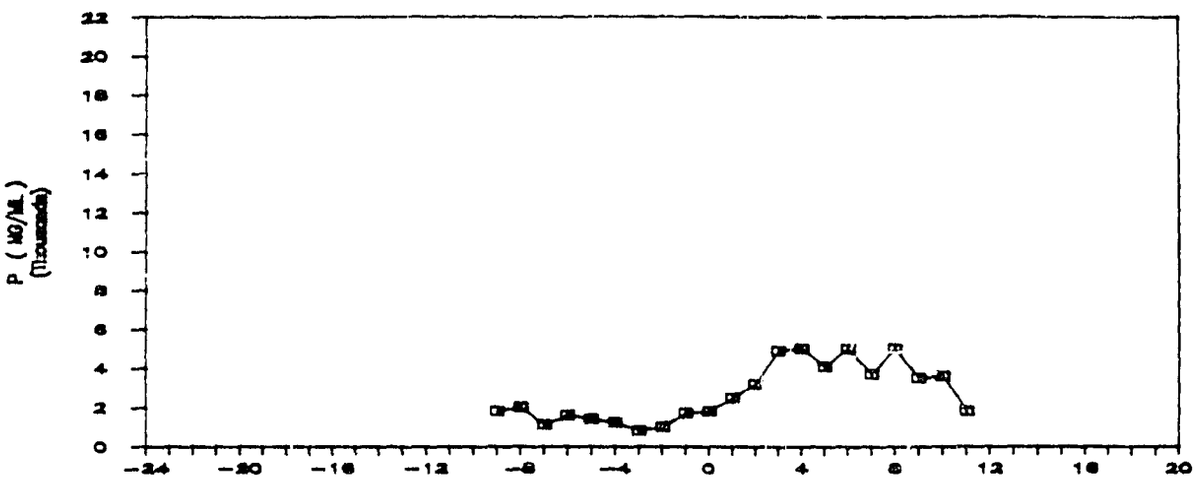
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
M.K.	11/19/84	5	-9	136.5			1800.0		97.2	PARFR CYCLE 3
	11/20/84	6	-8	431.9			2000.0		97.2	
	11/21/84	7	-7	1203.9			1100.0		97.6	
	11/22/84	8	-6	1851.5			1600.0		98.0	
	11/23/84	9	-5	1283.9			1400.0		97.7	
	11/24/84	10	-4	428.8			1200.0		97.7	
	11/25/84	11	-3	505.6			800.0		98.0	
	11/26/84	12	-2	225.3		8.0	1000.0		97.6	
	11/27/84	13	-1	37.1		91.0	1700.0		97.6	
	11/28/84	14	0	2.8		45.0	1800.0		97.6	
	11/29/84	15	1	91.5		23.0	2500.0		98.0	
	11/30/84	16	2	1012.2		30.0	3200.0		98.2	
	12/1/84	17	3	908.2		17.0	4900.0		98.4	
	12/2/84	18	4	1043.3		19.0	5000.0		98.4	
	12/3/84	19	5	1415.1			4100.0		98.2	
	12/4/84	20	6	484.4			5000.0		98.2	
	12/5/84	21	7	338.0			3700.0		98.0	
	12/6/84	22	8	1502.2			5000.0		98.2	
	12/7/84	23	9	455.9			3500.0		---	
	12/8/84	24	10	2451.1			3600.0		98.0	
	12/9/84	25	11				1800.0		98.0	

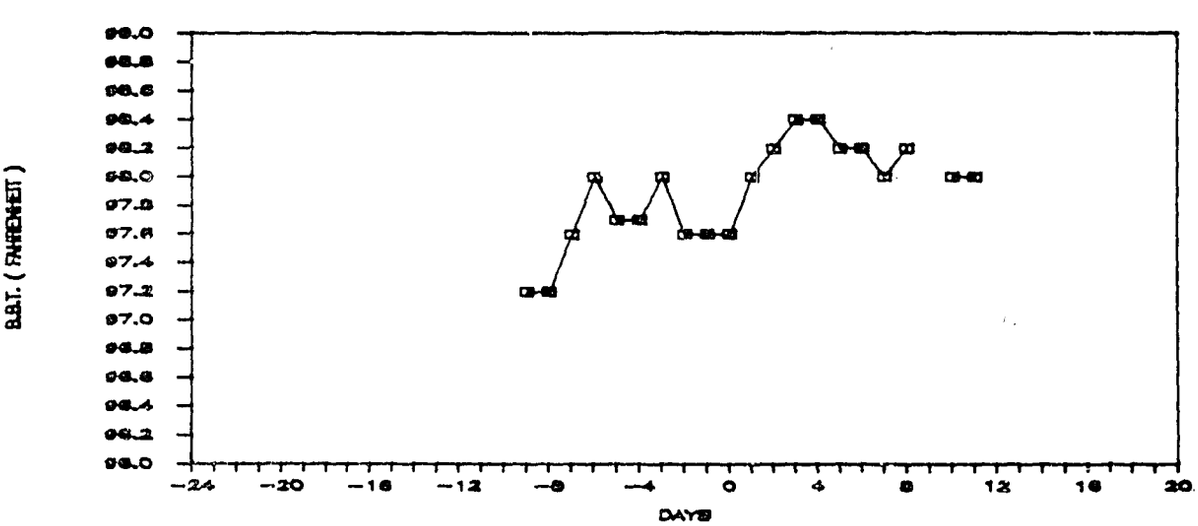

PARFR CYCLE 3
M.K.
2511.9
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.K.

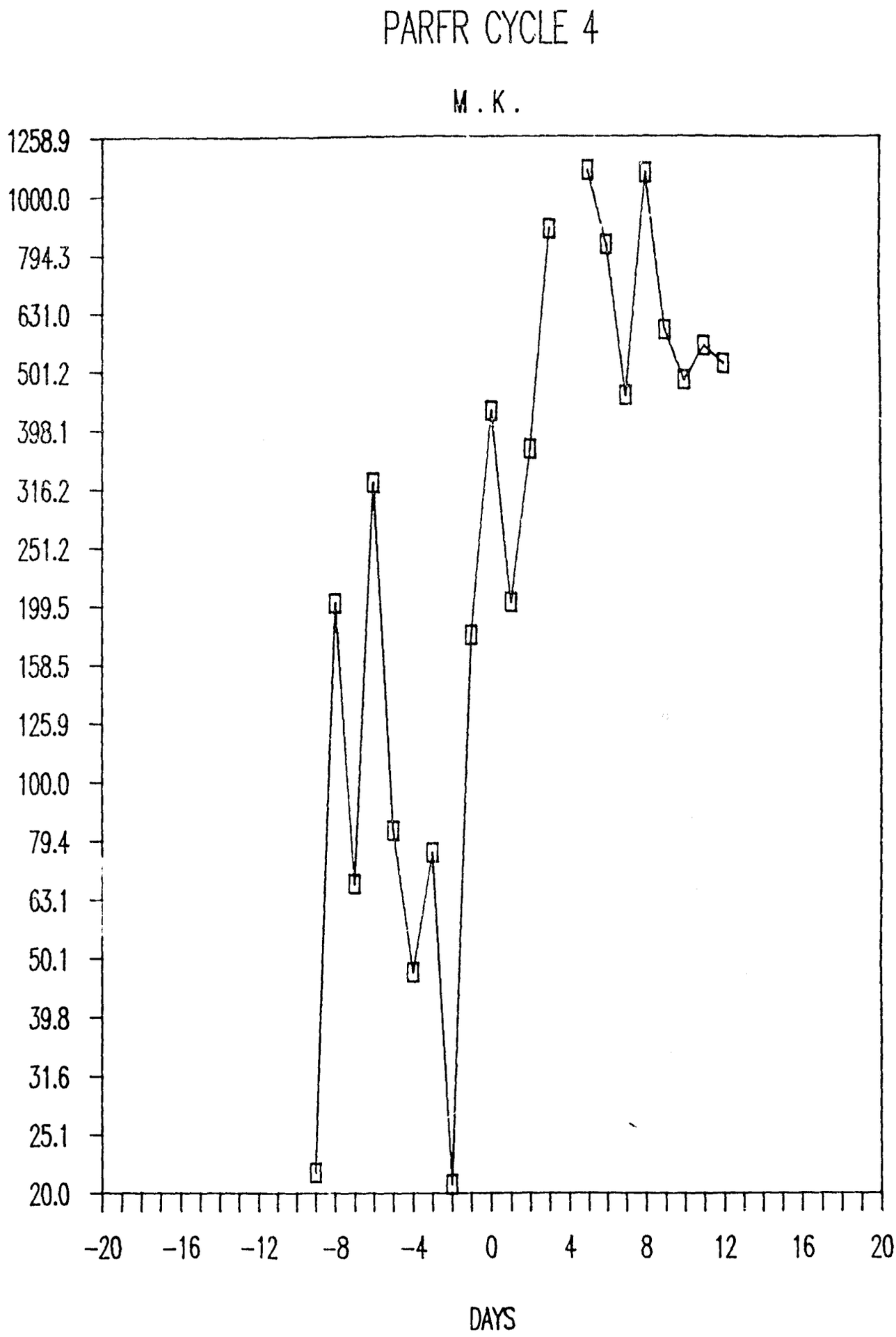
PARTA CYCLE 3





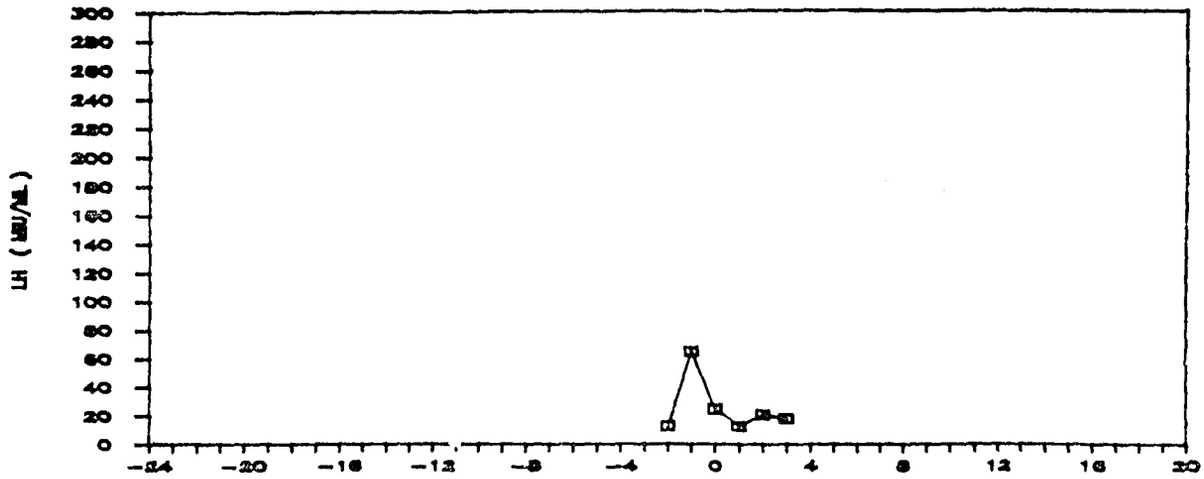


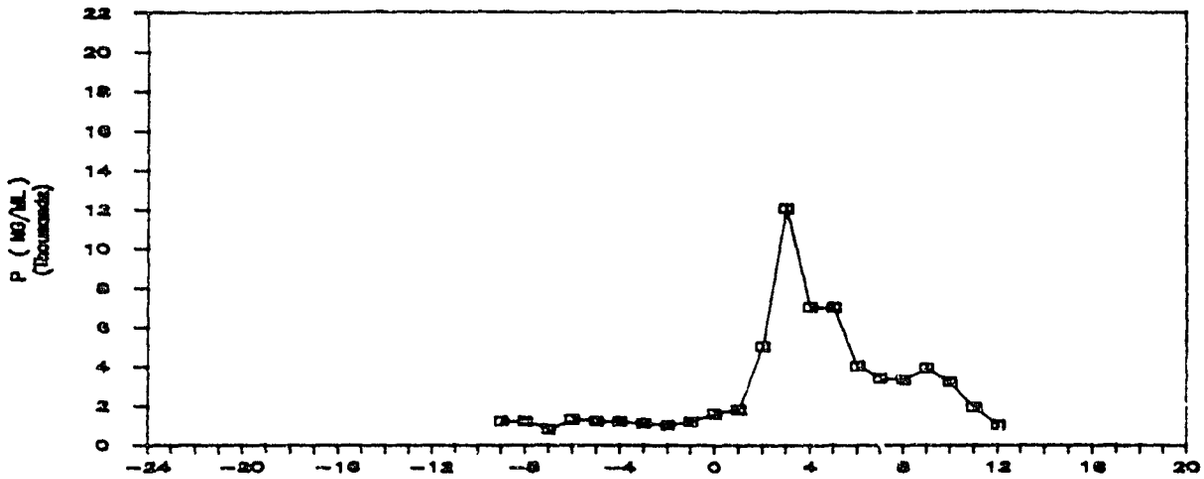
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
M.K.	1/8/85	4	-9	21.6			1200.0		97.6	PARFR CYCLE 4
	1/9/85	5	-8	201.4			1200.0		98.2	
	1/10/85	6	-7	67.0			800.0		97.6	
	1/11/85	7	-6	324.0			1300.0		97.4	
	1/12/85	8	-5	82.5			1200.0		97.8	
	1/13/85	9	-4	47.3			1200.0		97.6	
	1/14/85	10	-3	75.7			1100.0		97.6	
	1/15/85	11	-2	20.6		13.0	1000.0		97.4	
	1/16/85	12	-1	177.5		65.0	1200.0		97.6	
	1/17/85	13	0	428.6		25.0	1600.0		97.6	
	1/18/85	14	1	202.3		13.0	1800.0		98.0	
	1/19/85	15	2	370.0		21.0	5000.0		98.4	
	1/20/85	16	3	880.4		18.0	12000.0		98.2	
	1/21/85	17	4	---			7000.0		98.2	
	1/22/85	18	5	1106.0			7000.0		98.0	
	1/23/85	19	6	828.7			4000.0		98.0	
	1/24/85	20	7	456.3			3400.0		98.2	
	1/25/85	21	8	1095.0			3300.0		98.4	
	1/26/85	22	9	589.3			3900.0		98.4	
	1/27/85	23	10	483.1			3200.0		98.4	
	1/28/85	24	11	553.5			1900.0		98.0	
	1/29/85	25	12	515.9			1000.0		98.2	

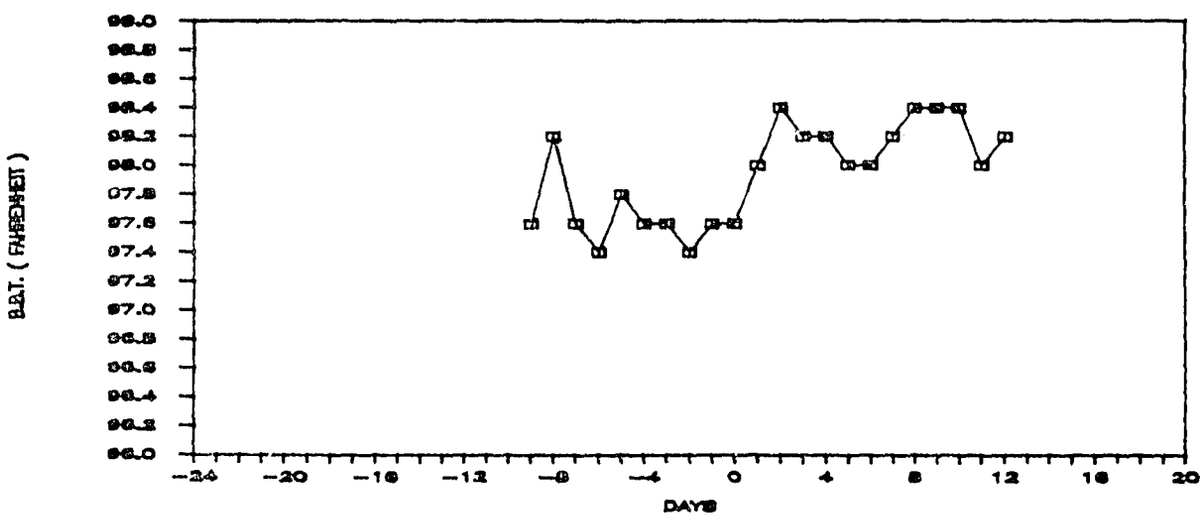

PARFR CYCLE 4
M.K.
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.K

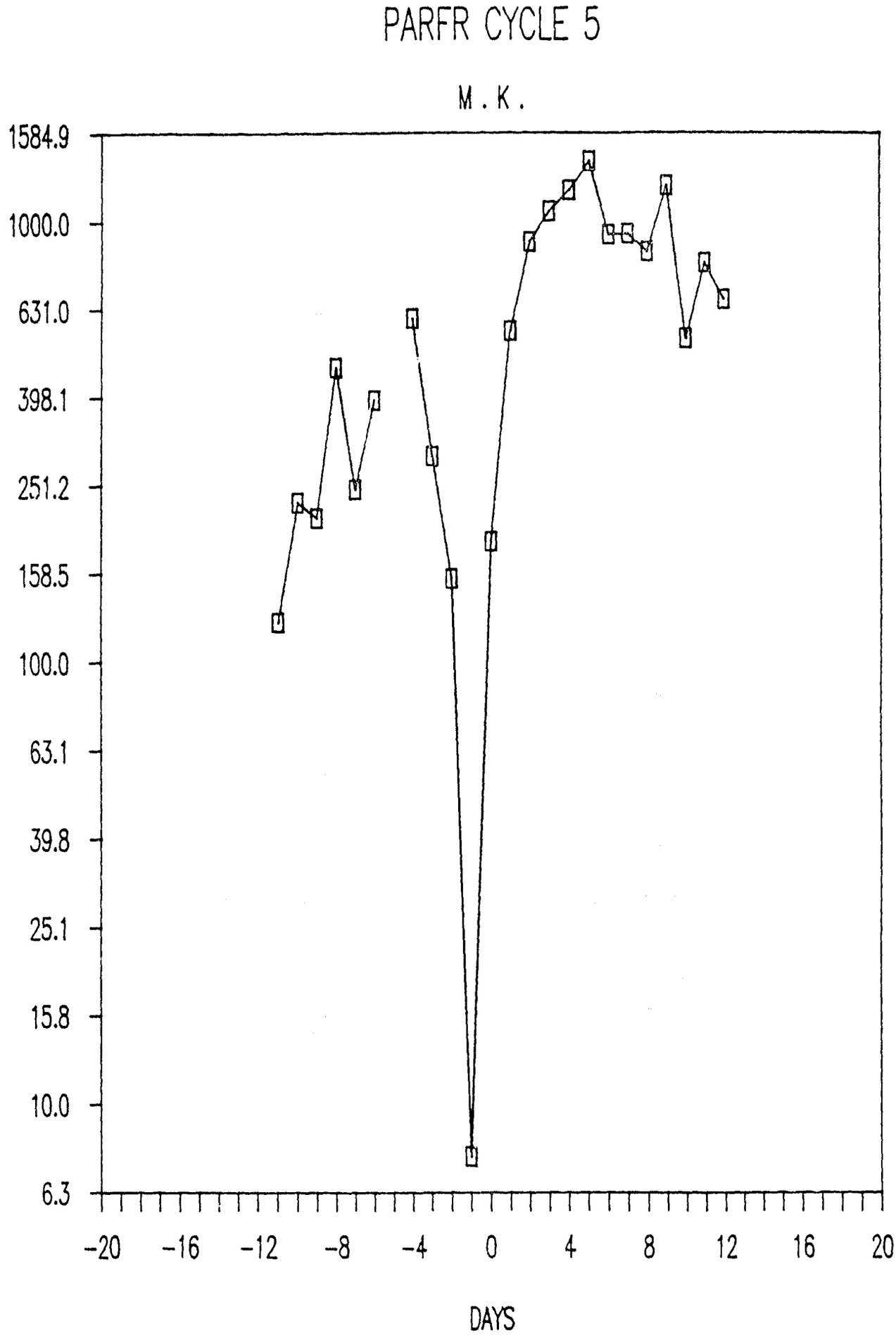
PARFR CYCLE 4





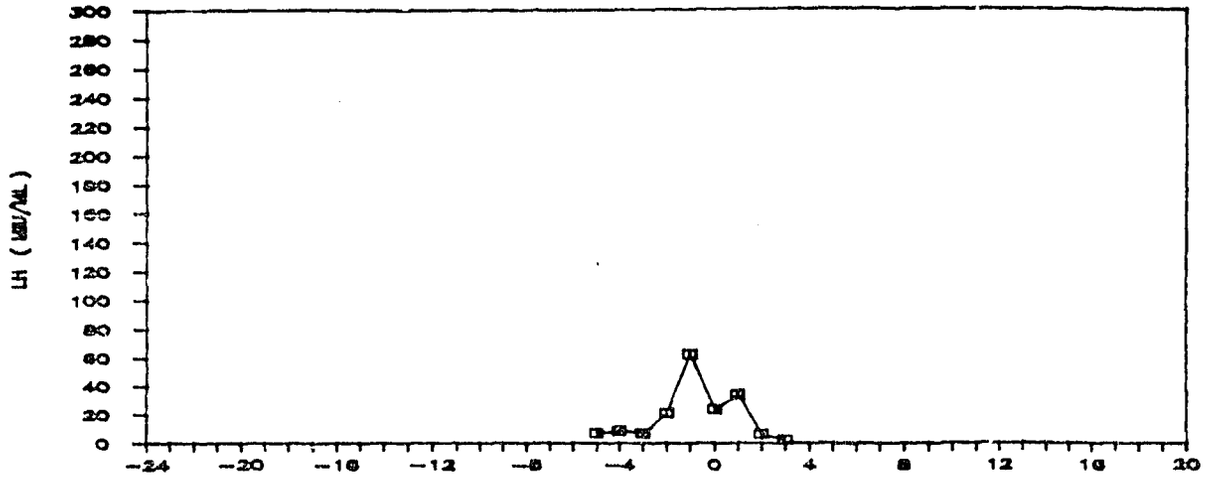


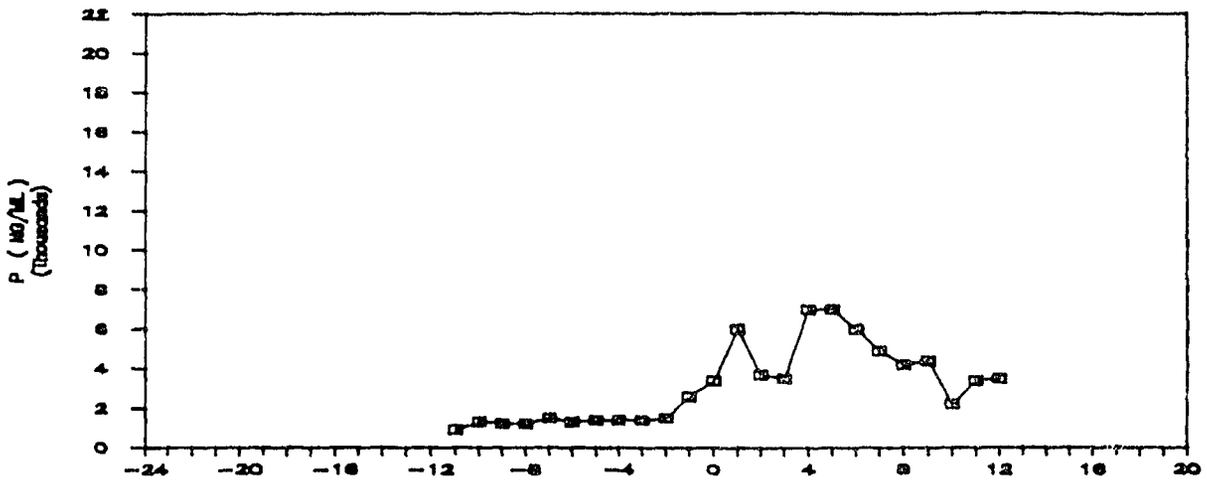
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
M.K.	2/2/85	4	-11	122.6			900.0		98.0	PARFR CYCLE 5
	2/3/85	5	-10	229.7			1300.0		97.6	
	2/4/85	6	-9	211.8			1200.0		97.4	
	2/5/85	7	-8	465.5			1200.0		97.6	
	2/6/85	8	-7	246.0			1500.0		97.6	
	2/7/85	9	-6	391.6			1300.0		97.6	
	2/8/85	10	-5	---		7.0	1400.0		97.6	
	2/9/85	11	-4	602.0		9.0	1400.0		97.8	
	2/10/85	12	-3	292.8		7.0	1400.0		98.0	
	2/11/85	13	-2	155.1		21.0	1500.0		97.6	
	2/12/85	14	-1	7.6		62.0	2600.0		98.0	
	2/13/85	15	0	188.3		24.0	3400.0		---	
	2/14/85	16	1	564.6		34.0	6000.0		97.8	
	2/15/85	17	2	899.6		6.0	3700.0		98.2	
	2/16/85	18	3	1056.0		2.0	3500.0		98.2	
	2/17/85	19	4	1176.3			7000.0		98.2	
	2/18/85	20	5	1370.6			7000.0		98.2	
	2/19/85	21	6	933.9			6000.0		98.2	
	2/20/85	22	7	941.9			4900.0		98.0	
	2/21/85	23	8	857.3			4200.0		98.0	
	2/22/85	24	9	1207.5			4400.0		98.2	
	2/23/85	25	10	541.8			2200.0		98.4	
	2/24/85	26	11	807.2			3400.0		98.2	
	2/25/85	27	12	666.4			3500.0		97.6	

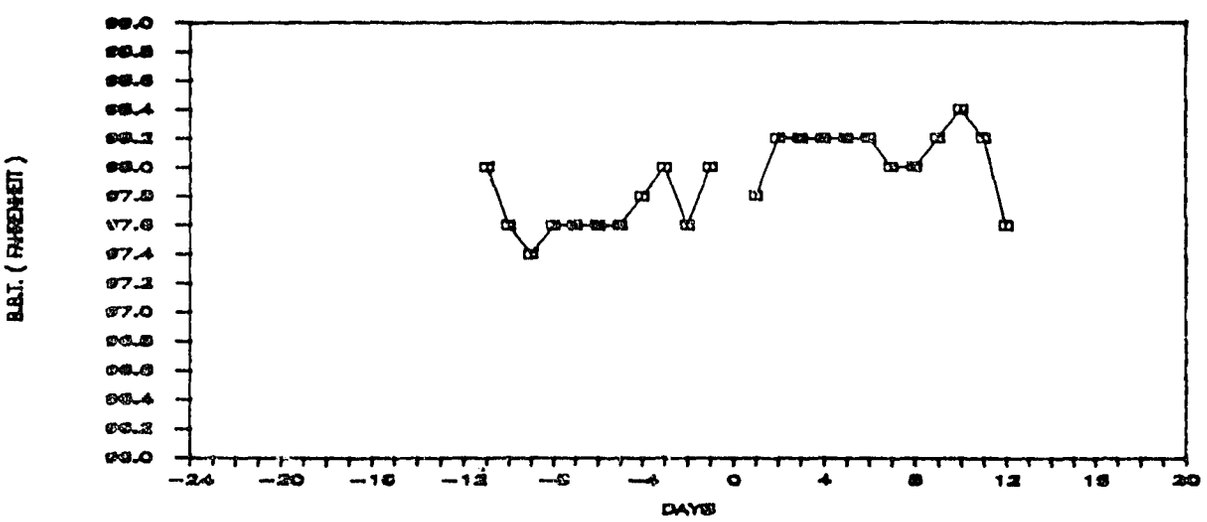

PARFR CYCLE 5
M.K.
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.K.

PARFR CYCLE 5

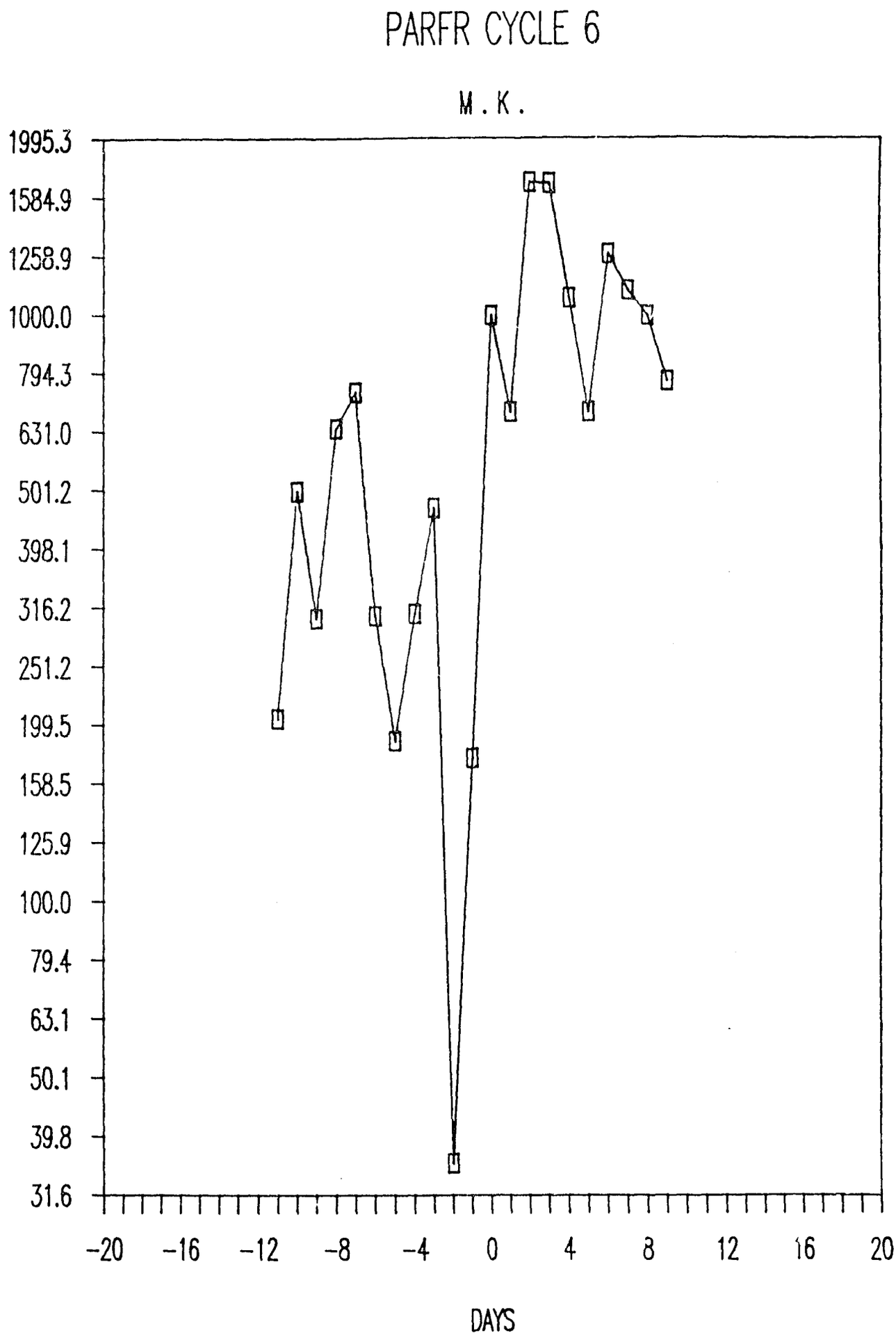






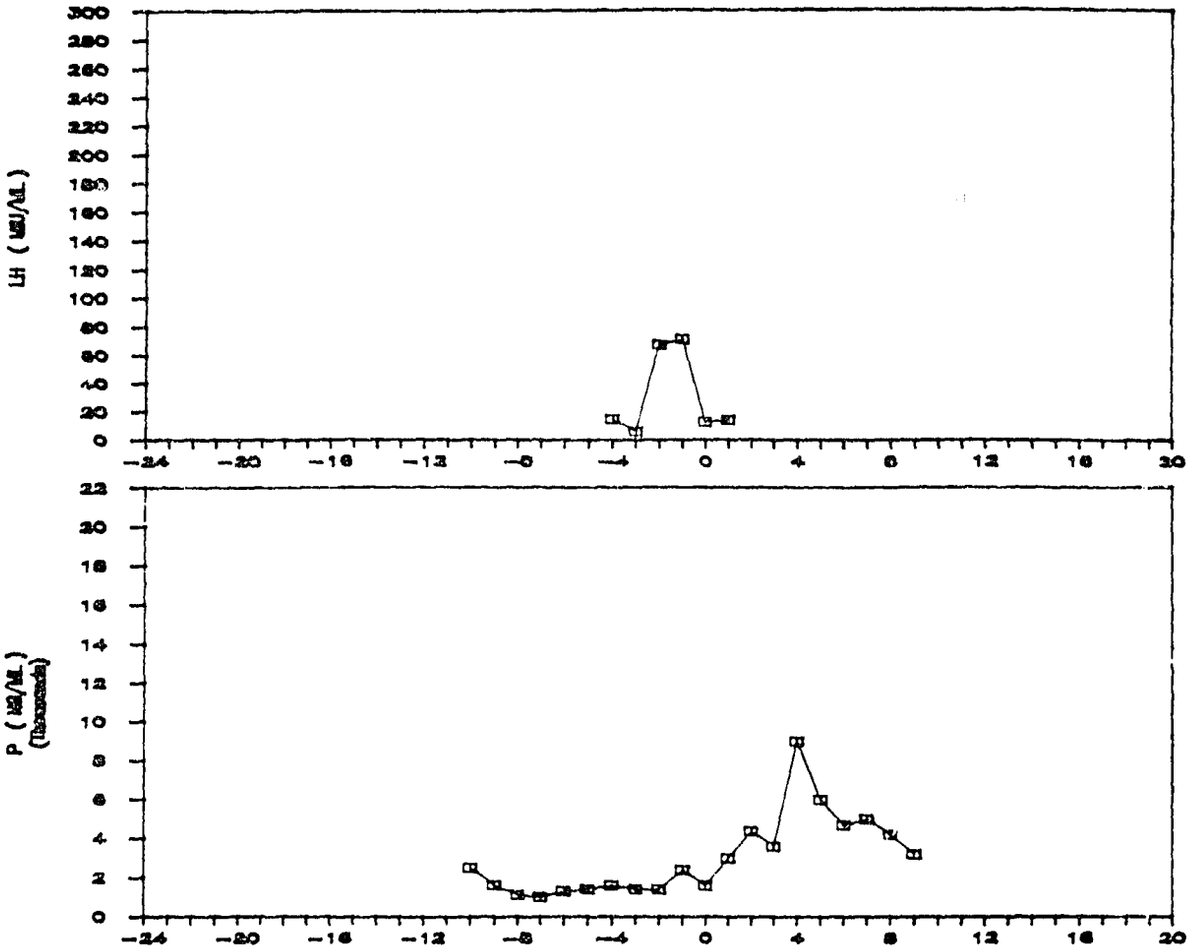
M.K.

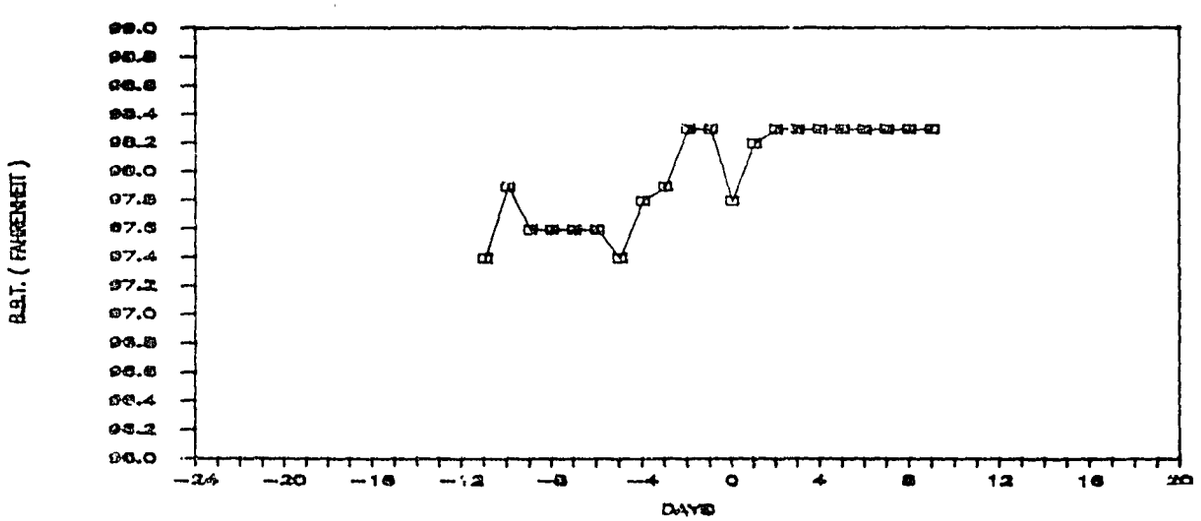
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
	3/1/85	4	-11	203.7					97.4	PARFR CYCLE 6
	3/2/85	5	-10	496.8			2500.0		97.9	
	3/3/85	6	-9	302.2			1600.0		97.6	
	3/4/85	7	-8	635.6			1100.0		97.6	
	3/5/85	8	-7	735.3			1000.0		97.6	
	3/6/85	9	-6	305.3			1300.0		97.6	
	3/7/85	10	-5	186.9			1400.0		97.4	
	3/8/85	11	-4	308.3		15.0	1600.0		97.8	
	3/9/85	12	-3	466.4		6.0	1400.0		97.9	
	3/10/85	13	-2	35.8		67.0	1400.0		98.3	
	3/11/85	14	-1	175.1		71.0	2400.0		98.3	
	3/12/85	15	0	996.5		13.0	1600.0		97.8	
	3/13/85	16	1	681.5		14.0	3000.0		98.2	
	3/14/85	17	2	1681.3			4400.0		98.3	
	3/15/85	18	3	1671.0			3600.0		98.3	
	3/16/85	19	4	1067.0			9000.0		98.3	
	3/17/85	20	5	682.7			6000.0		98.3	
	3/18/85	21	6	1271.0			4700.0		98.3	
→	3/19/85	22	7	1100.0			5000.0		98.3	
	3/20/85	23	8	994.0			4200.0		98.3	
	3/21/85	24	9	768.2			3200.0		98.3	

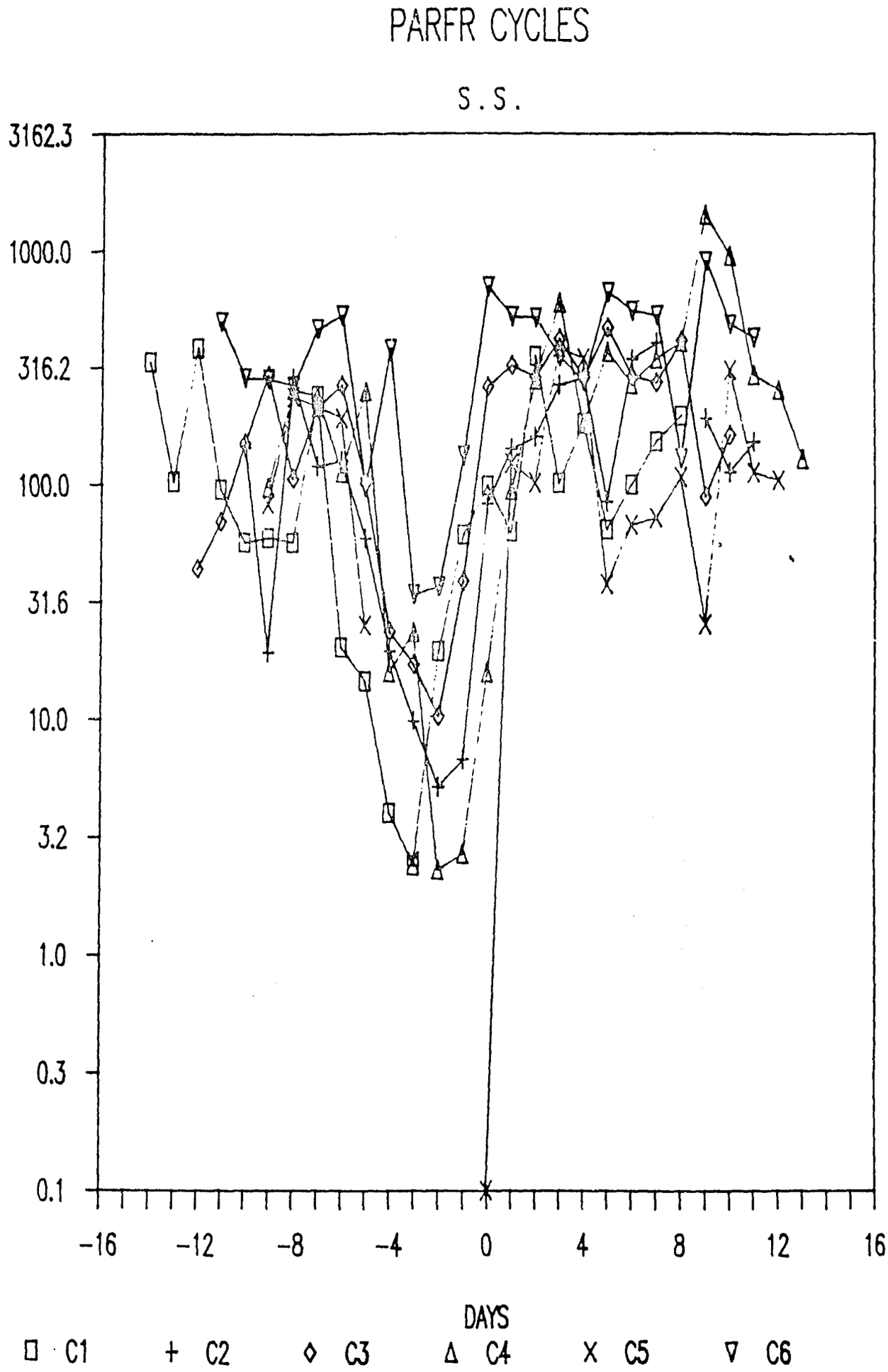

PARFR CYCLE 6
M.K.
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

M.K.

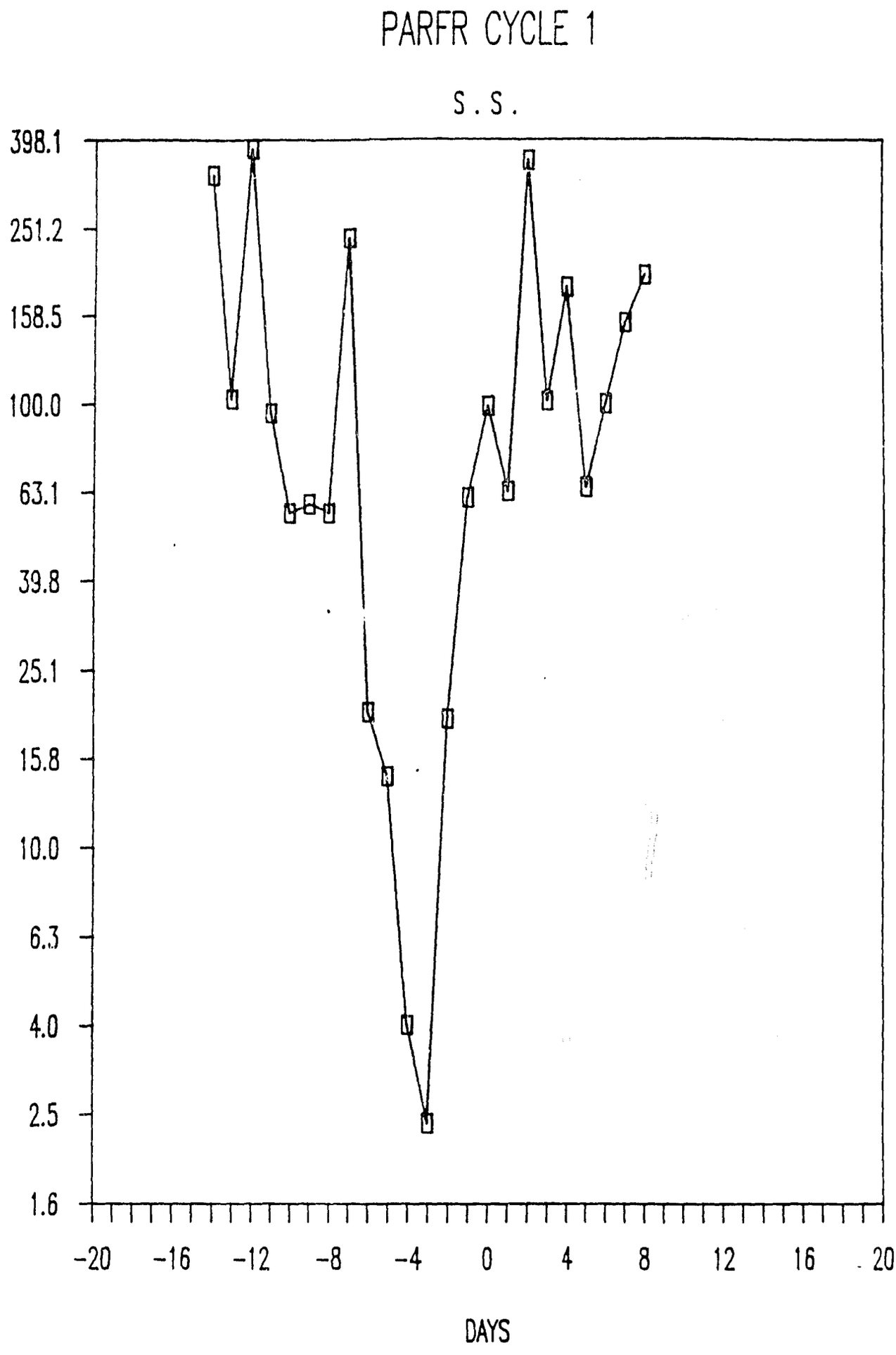
PARFR CYCLE 6



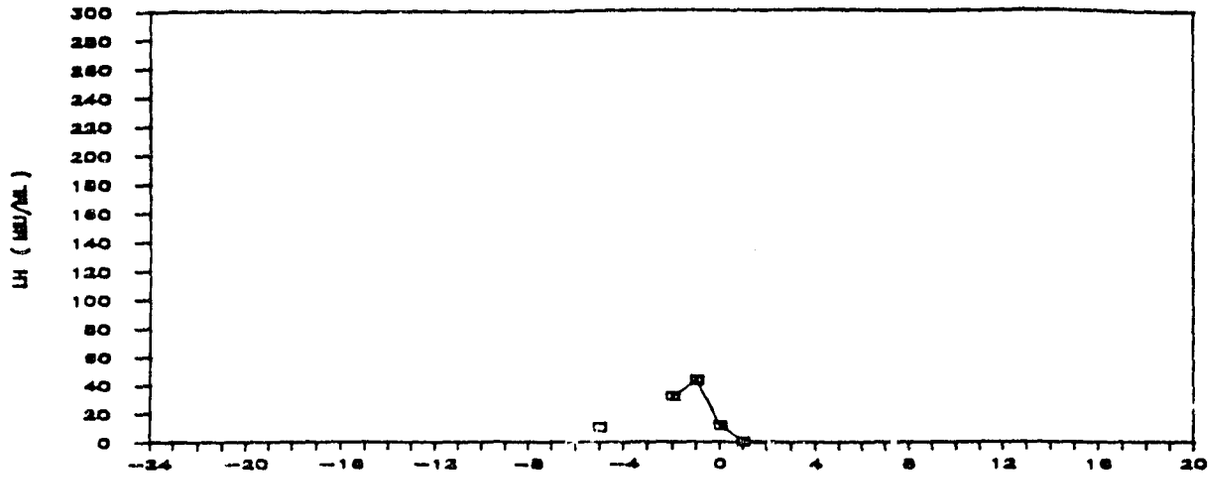


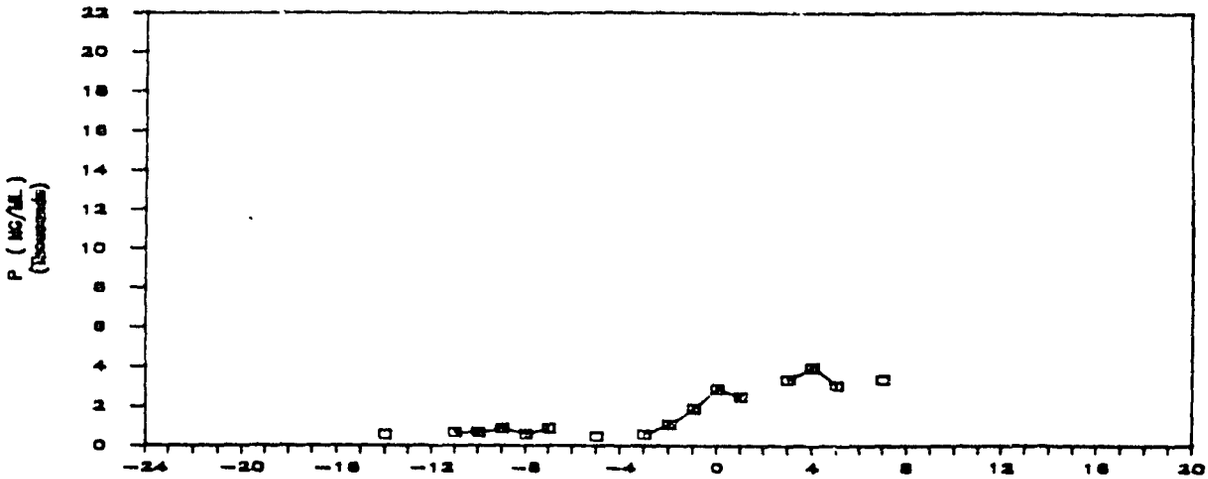

PARFR CYCLES
S.S.
3162.3
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-16
-12
-8
-4
0
4
8
12
16
DAYS
C1
C2
C3
C4
C5
C6

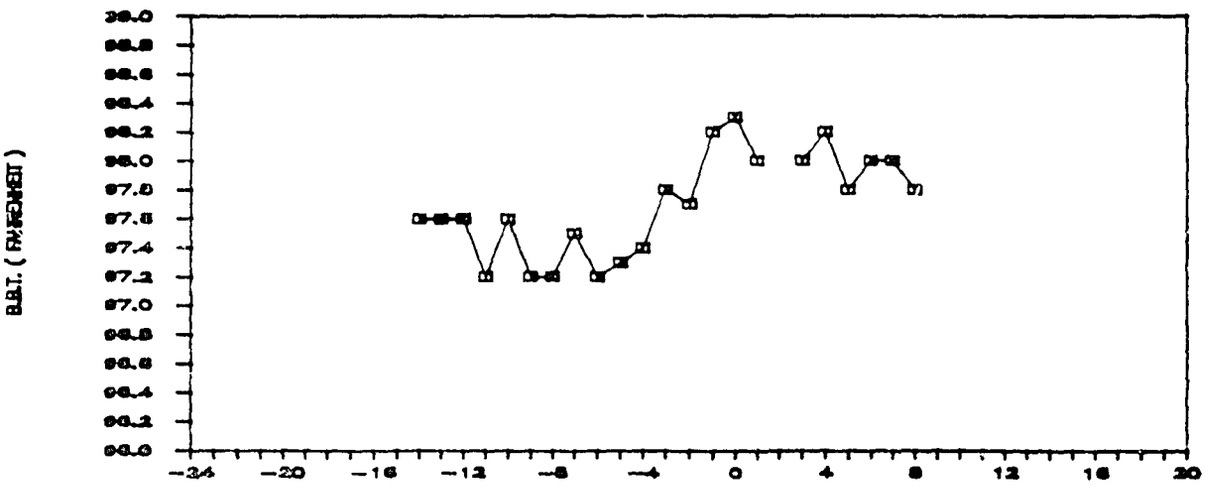
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
S.S.	7/11/84	5	-14	331.6	27.2		600.1		97.6	PARFR CYCLE 1
	7/12/84	6	-13	102.3	60.9		---		97.6	
	7/13/84	7	-12	379.6	72.6		---		97.6	
	7/14/84	8	-11	95.3	125.6		700.1		97.2	
	7/15/84	9	-10	56.6	131.9		700.2		97.6	
	7/16/84	10	-9	59.3	55.1		900.2		97.2	
	7/17/84	11	-8	56.6	57.7		600.2		97.2	
	7/18/84	12	-7	238.7	35.2		900.2		97.5	
	7/19/84	13	-6	20.2	43.6		---		97.2	
	7/20/84	14	-5	14.5	91.9	10.9	200.2		97.3	
	7/21/84	15	-4	4.0	8.3	---	---		97.4	
	7/22/84	16	-3	2.4	4.3	---	600.2		97.8	
	7/23/84	17	-2	19.6	19.4	32.3	1100.2		97.7	
	7/24/84	18	-1	61.7	64.6	43.7	1900.2		98.2	
	7/25/85	19	0	99.4	53.8	11.6	2900.3		98.3	
	7/26/84	20	1	63.8	132.5	0.3	2500.3		98.0	
	7/27/84	21	2	356.9	27.8		---		---	
	7/28/84	22	3	102.3	158.3		3400.3		98.0	
	7/29/84	23	4	185.0	68.2		4000.3		98.2	
	7/30/84	24	5	65.2	54.7		3100.3		97.8	
	7/31/84	25	6	100.7	36.9		---		98.0	
	8/1/84	26	7	153.5	64.0		3400.0		98.0	
	8/2/84	27	8	196.6	71.4		---		97.8	


PARFR CYCLE 1
S.S.
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

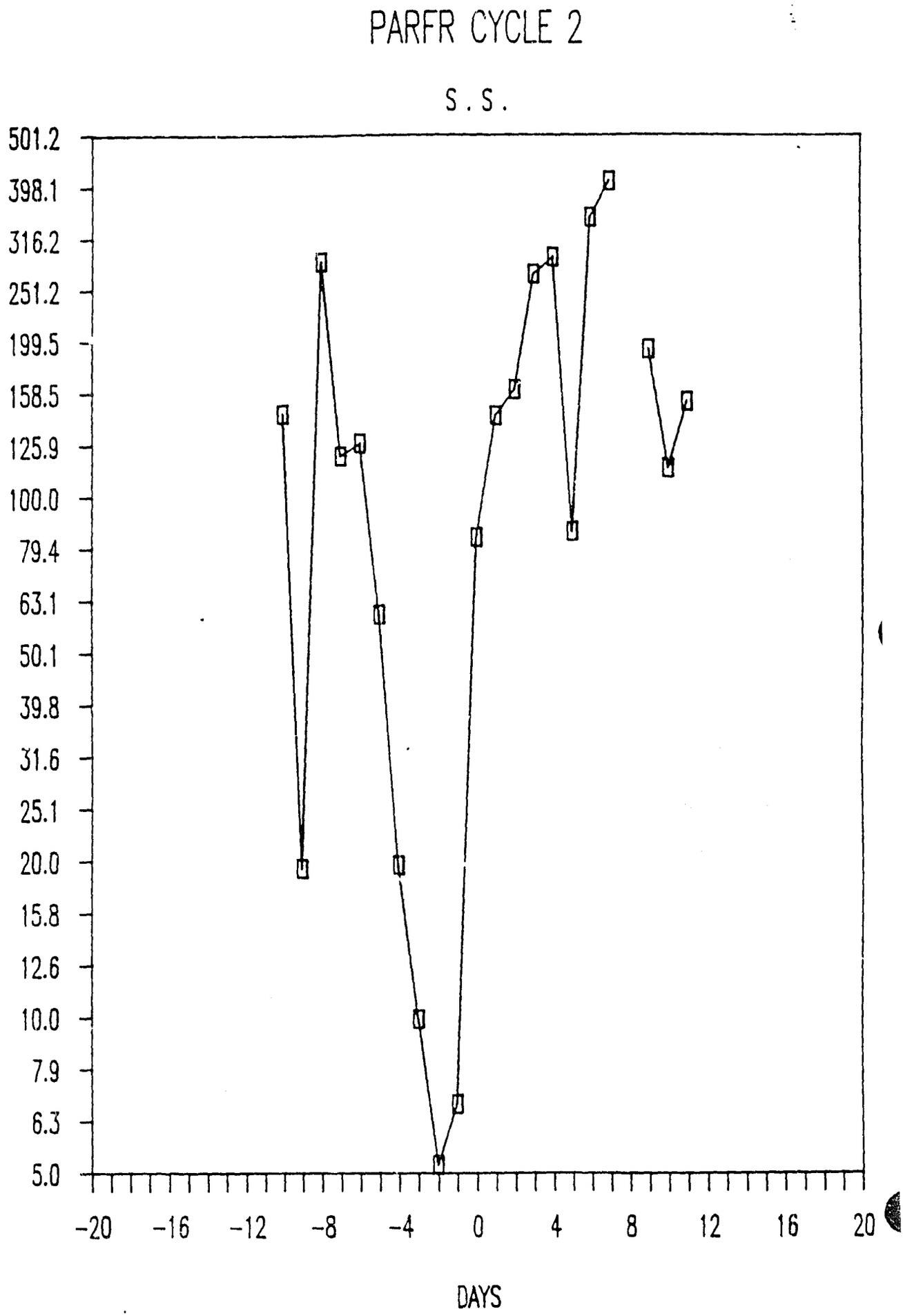
PARFR CYCLE 1





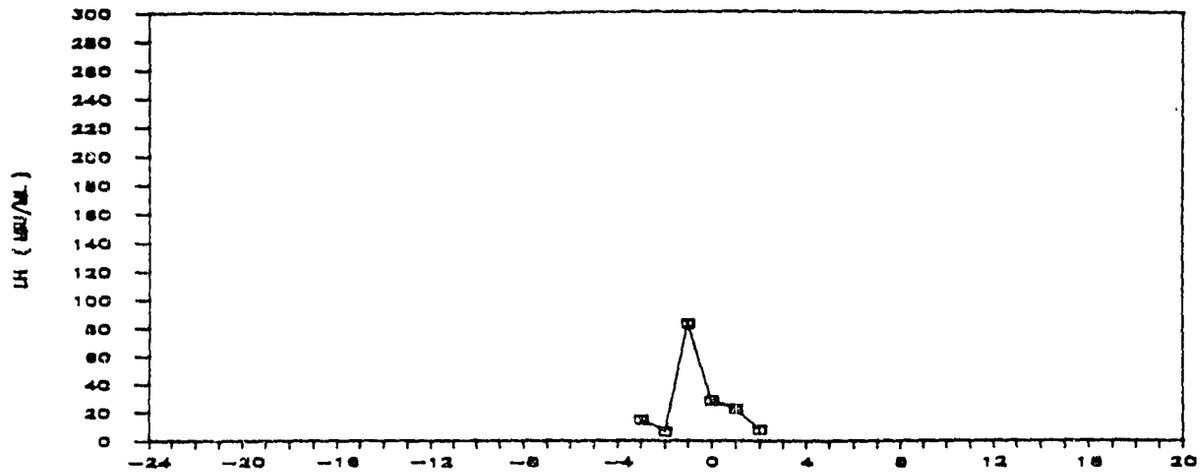


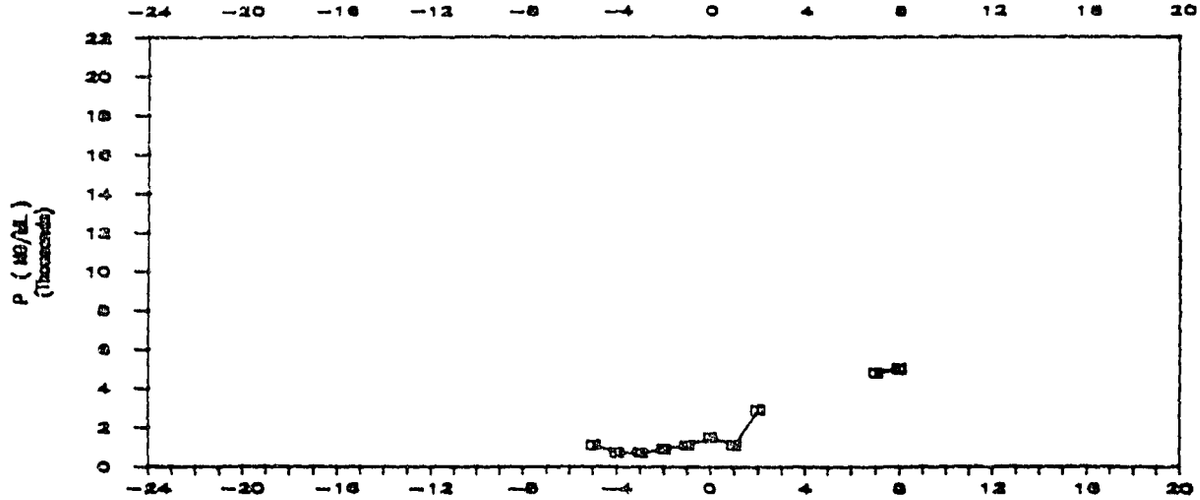
NAME	DATE	DFOM	OVD	SPC	GPV	LH	P	E2	BBT	REMARKS
S.S.	8/9/84	5	-10	144.5	62.3				97.3	PARFR CYCLE 2
	8/10/84	6	-9	19.3	44.0				97.5	
	8/11/84	7	-8	284.6	53.3				97.6	
	8/12/84	8	-7	119.9	69.3				97.8	
	8/13/84	9	-6	126.8	44.5				97.6	
	8/14/84	10	-5	59.4	42.7		1100.0		97.2	
	8/15/84	11	-4	19.6	69.8		700.0		97.6	
	8/16/84	12	-3	9.9		14.9	700.0		97.6	
	8/17/84	13	-2	5.2		6.5	900.0		97.6	
	8/18/84	14	-1	6.8		82.6	1100.0		97.7	
	8/19/84	15	0	83.7		28.1	1500.0		97.6	
	8/20/84	16	1	143.5		22.5	1100.0		98.0	
	8/21/84	17	2	161.3		7.8	2900.0		98.0	
	8/22/84	18	3	269.1			---		97.7	
	8/23/84	19	4	290.1			---		97.7	
	8/24/84	20	5	85.8			---		98.3	
	8/25/84	21	6	345.9			---		98.4	
	8/26/84	22	7	406.0			4800.0		98.0	
	8/27/84	23	8	---			5000.0		98.0	
	8/28/84	24	9	192.1					98.2	
	8/29/84	25	10	113.1					98.1	
	8/30/84	26	11	152.2					97.8	

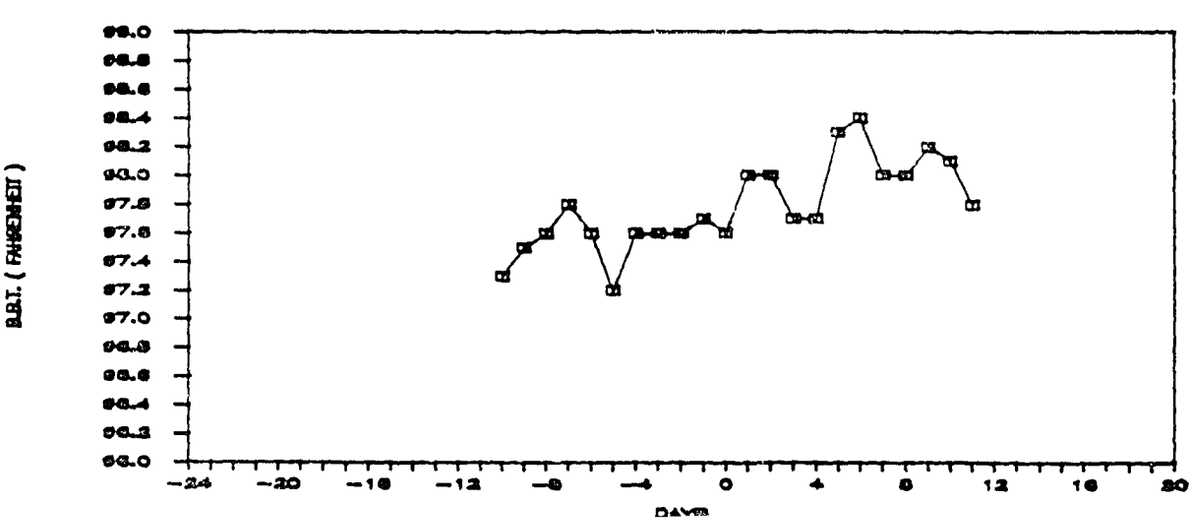

PARFR CYCLE 2
S.S.
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
7.9
6.3
5.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

S.S

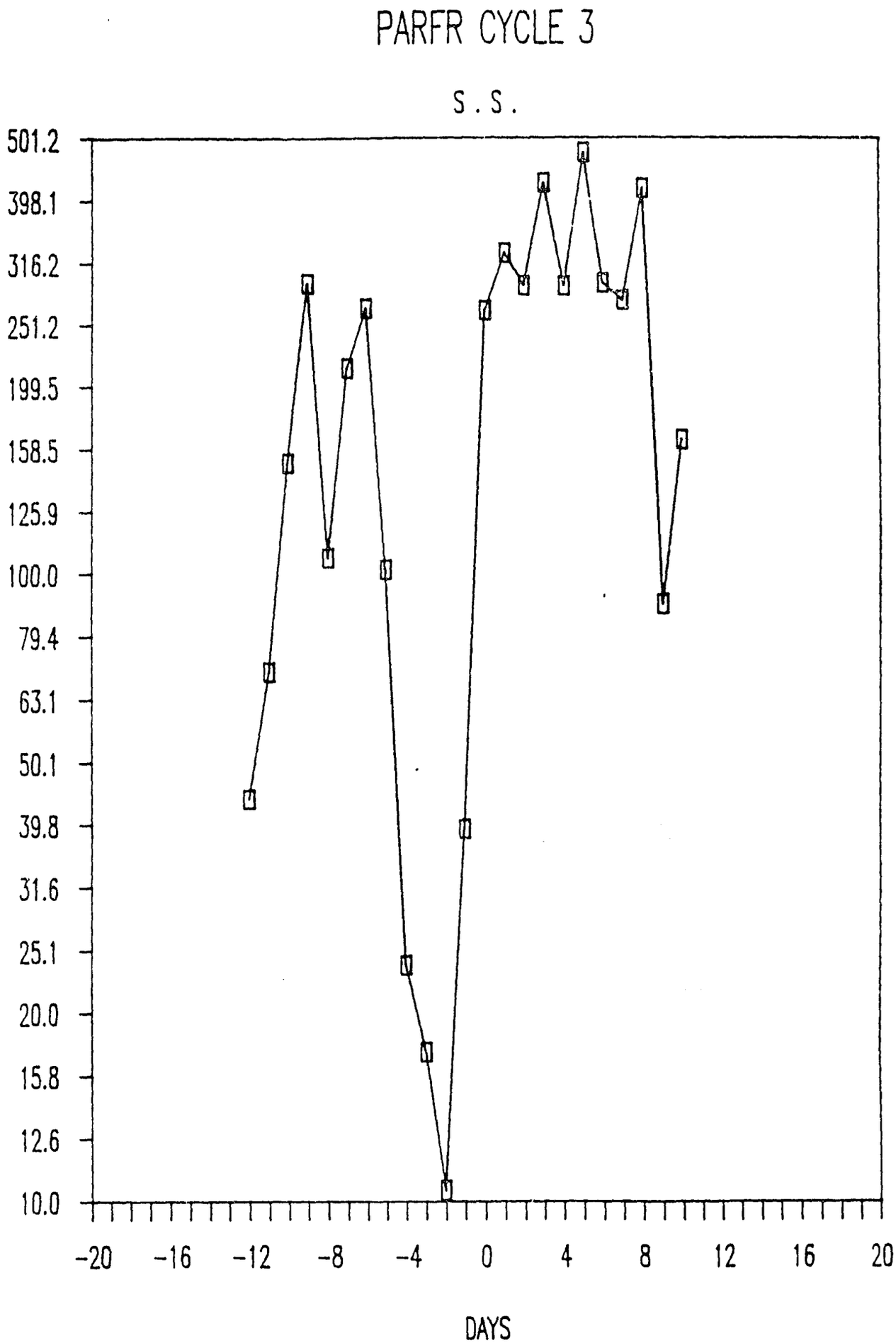
PARFR CYCLE 2





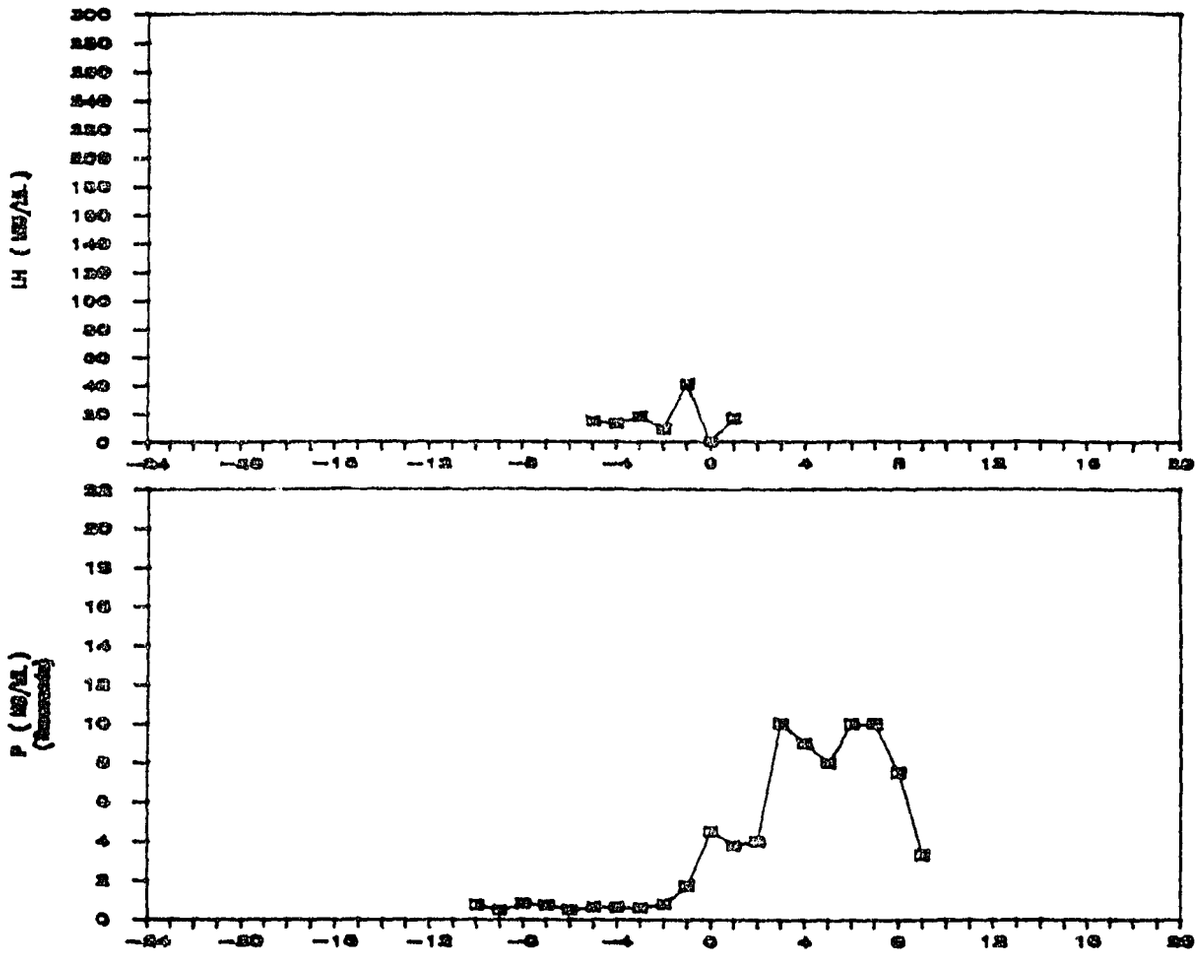


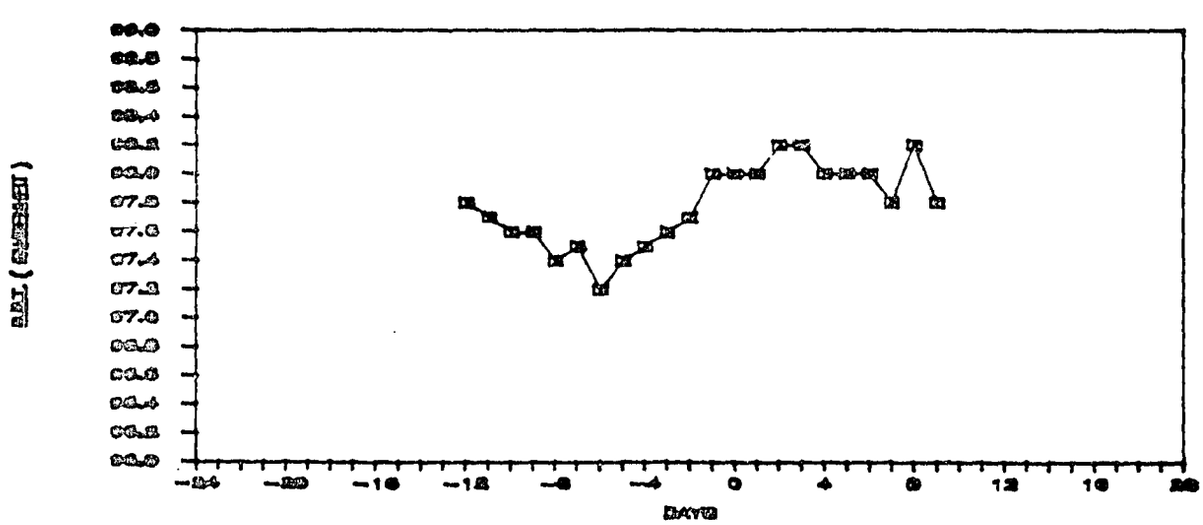
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
S.S.	9/5/84	6	-12	43.8					97.8	PARFR CYCLE 3
	9/6/84	7	-11	69.8					97.7	
	9/7/84	8	-10	150.5			720.0		97.6	
	9/8/84	9	-9	291.8			450.0		97.6	
	9/9/84	10	-8	106.2			790.0		97.4	
	9/10/84	11	-7	213.7			700.0		97.5	
	9/11/84	12	-6	266.5			470.0		97.2	
	9/12/84	13	-5	101.8		15.2	620.0		97.4	
	9/13/84	14	-4	23.8		13.4	600.0		97.5	
	9/14/84	15	-3	17.3		18.6	590.0		97.6	
	9/15/84	16	-2	10.4		9.4	770.0		97.7	
	9/16/84	17	-1	39.3		41.0	1700.0		98.0	
	9/17/84	18	0	264.3		0.5	4500.0		98.0	
	9/18/84	19	1	327.2		16.9	3800.0		98.0	
	9/19/84	20	2	290.1			4000.0		98.2	
	9/20/84	21	3	423.9			10000.0		98.2	
	9/21/84	22	4	289.3			9000.0		98.0	
	9/22/84	23	5	473.4			8000.0		98.0	
	9/23/84	24	6	291.6			10000.0		98.0	
	9/24/84	25	7	273.7			10000.0		97.8	
	9/25/84	26	8	415.0			7500.0		98.2	
	9/26/84	27	9	89.3			3300.0		97.8	
	9/27/84	28	10	163.7					---	


PARFR CYCLE 3
S.S.
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

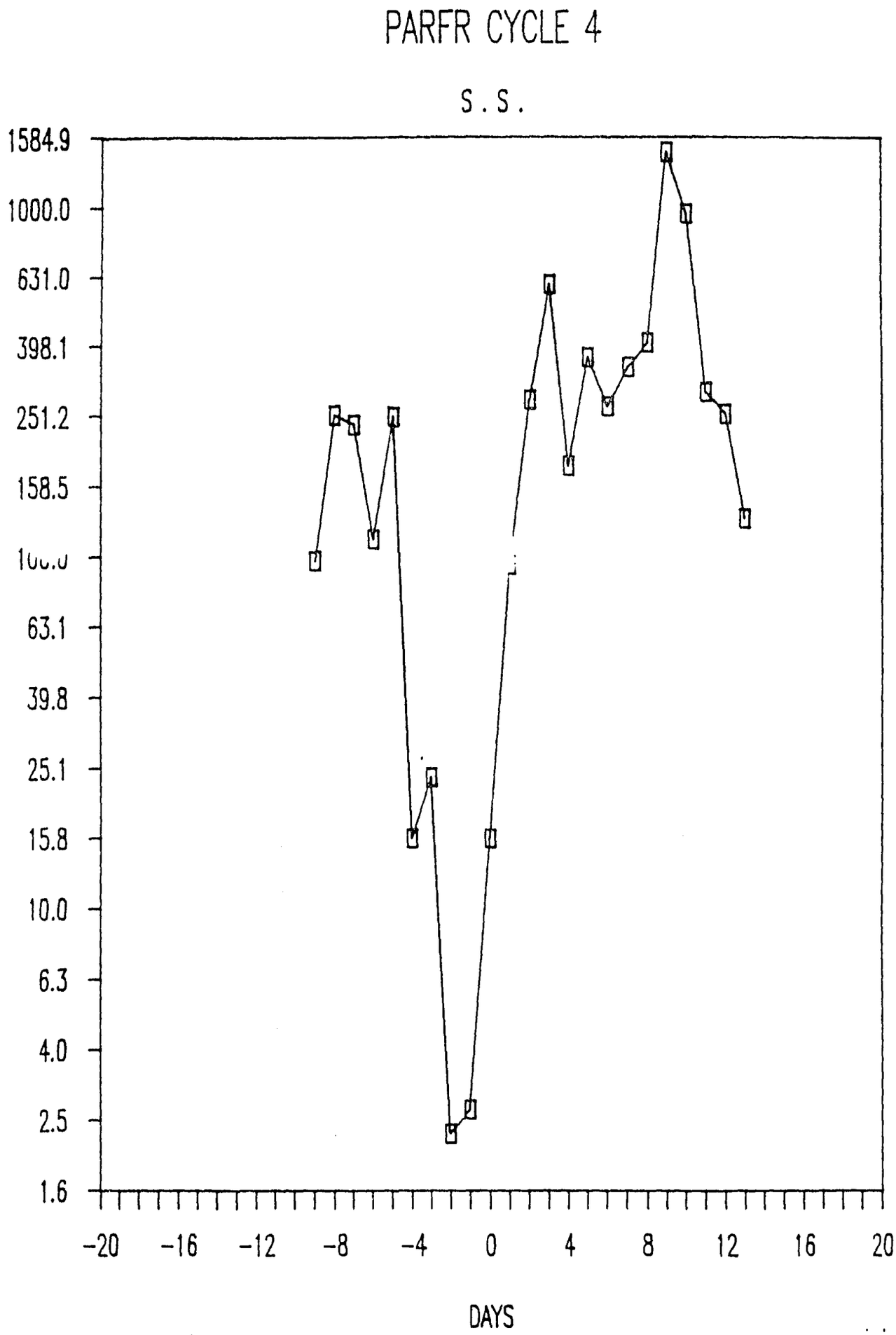
S.S.

PARFR CYCLE 3



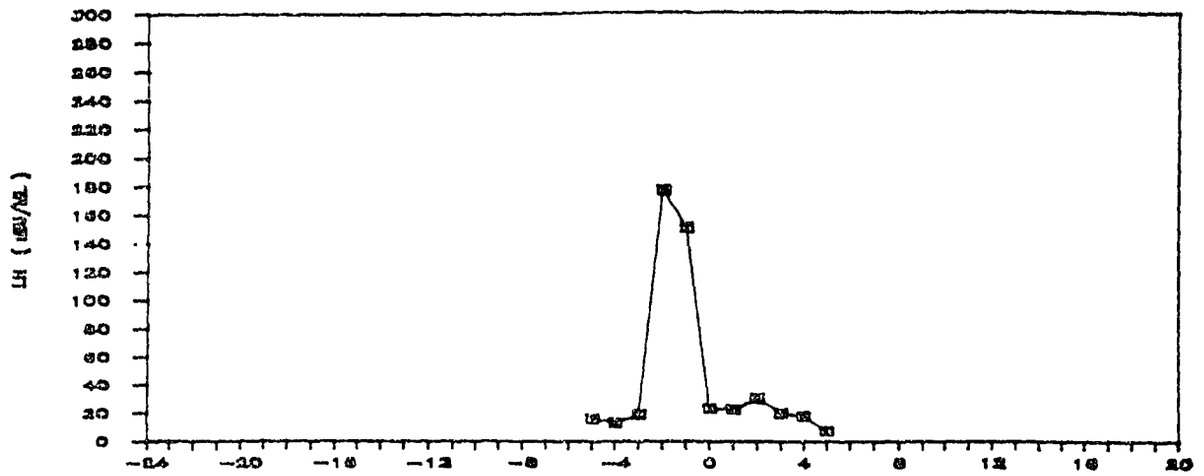


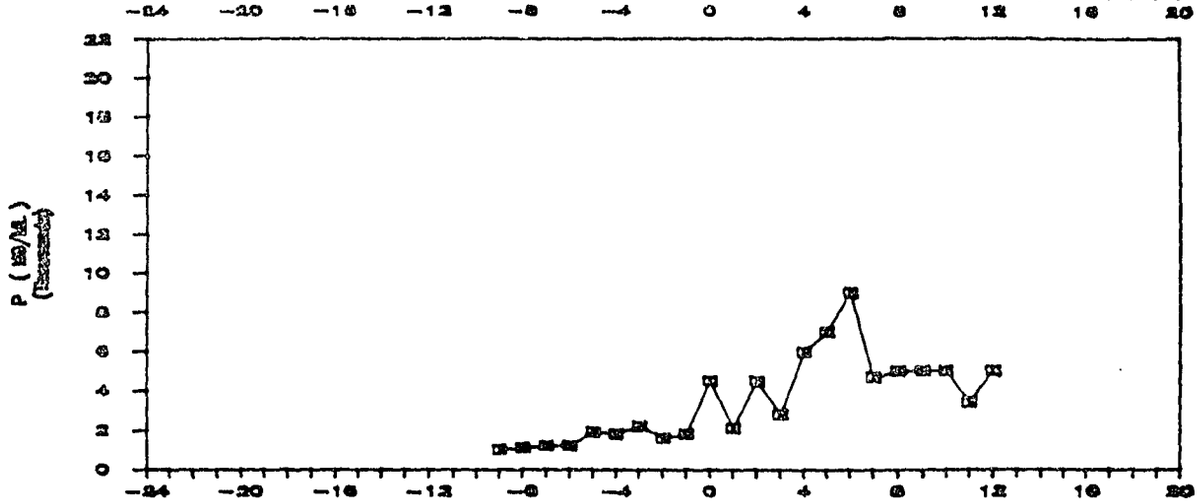
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
S.S.	10/2/84	5	-9	97.3			1000.0		---	PARFR CYCLE 4
	10/3/84	6	-8	253.3			1100.0		97.7	
	10/4/84	7	-7	237.4			1200.0		97.8	
	10/5/84	8	-6	112.5			1200.0		---	
	10/6/84	9	-5	250.6		16.3	1900.0		97.5	
	10/7/84	10	-4	15.9		13.6	1800.0		97.6	
	10/8/84	11	-3	23.7		19.3	2200.0		97.4	
	10/9/84	12	-2	2.3		177.0	1600.0		97.6	
	10/10/84	13	-1	2.7		150.8	1800.0		97.8	
	10/11/84	14	0	15.9		23.3	4500.0		97.8	
	10/12/84	15	1	95.6		22.9	2100.0		98.0	
	10/13/84	16	2	281.9		30.8	4500.0		---	
	10/14/84	17	3	601.2		20.1	2800.0		98.4	
	10/15/84	18	4	182.6		18.1	6000.0		98.2	
	10/16/84	19	5	371.5		7.6	7000.0		98.0	
	10/17/84	20	6	268.8			9000.0		98.2	
	10/18/84	21	7	347.1			4700.0		98.0	
	10/19/84	22	8	406.7			5000.0		98.0	
	10/20/84	23	9	1429.4			5000.0		98.6	
	10/21/84	24	10	951.5			5000.0		98.0	
	10/22/84	25	11	294.1			3400.0		98.2	
	10/23/84	26	12	254.3			5000.0		97.8	
	10/24/84	27	13	128.4			---		97.9	

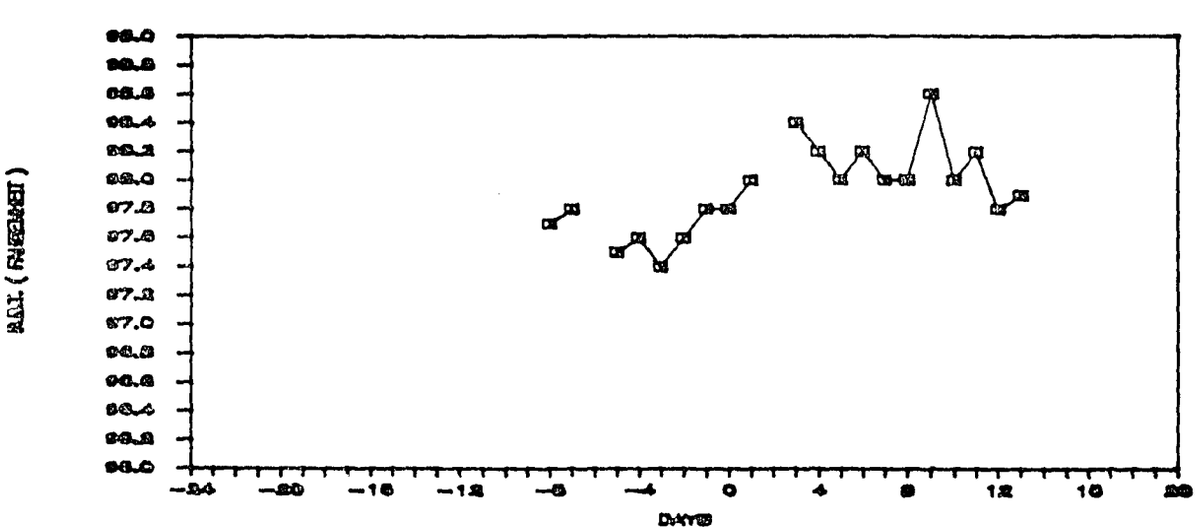

PARFR CYCLE 4
S.S.
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

S.S

PARFR CYCLE 4

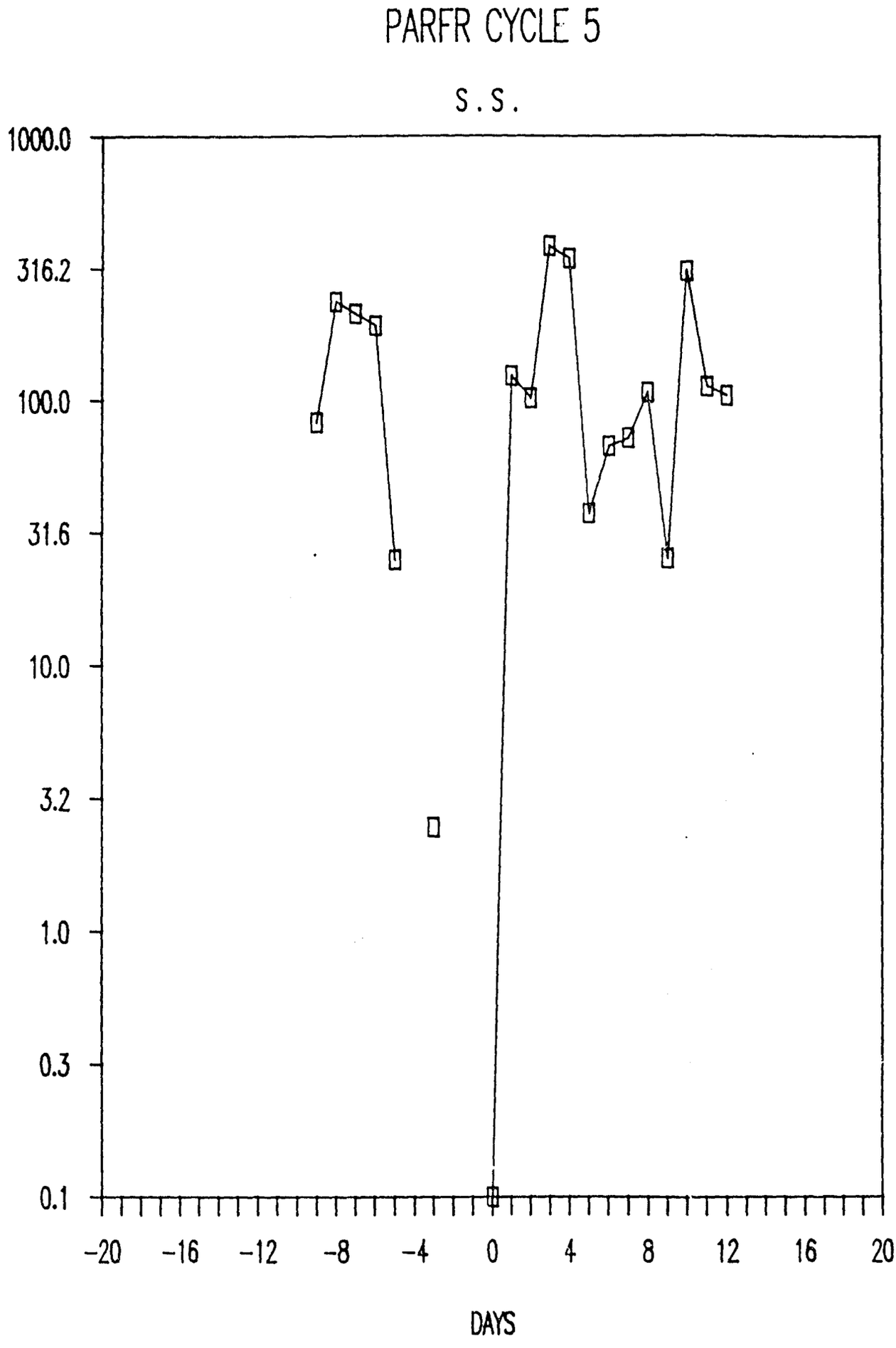




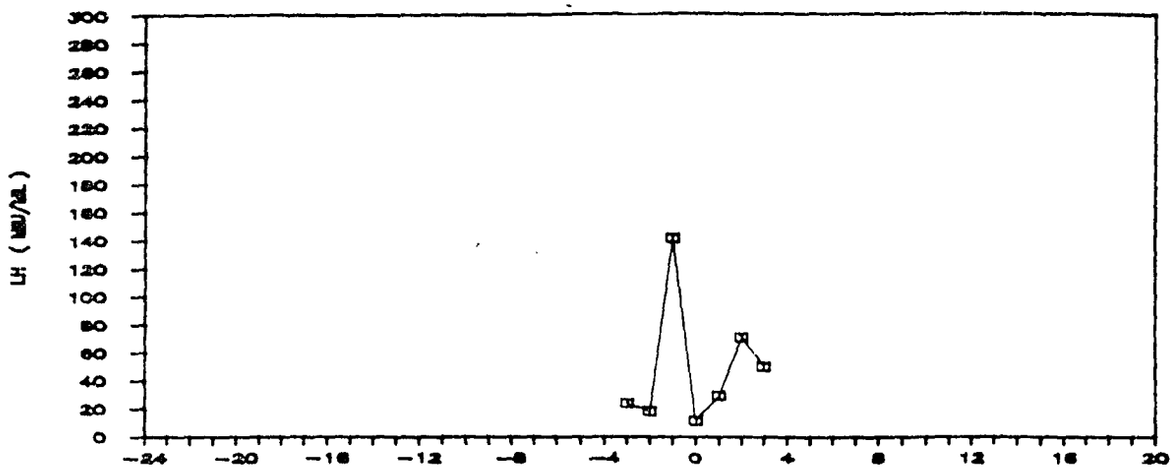


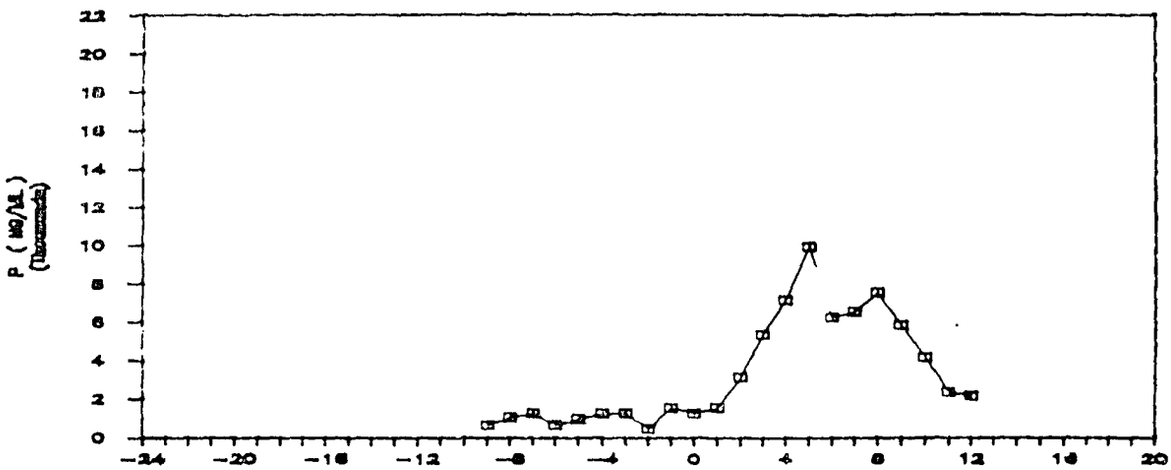
S.S.

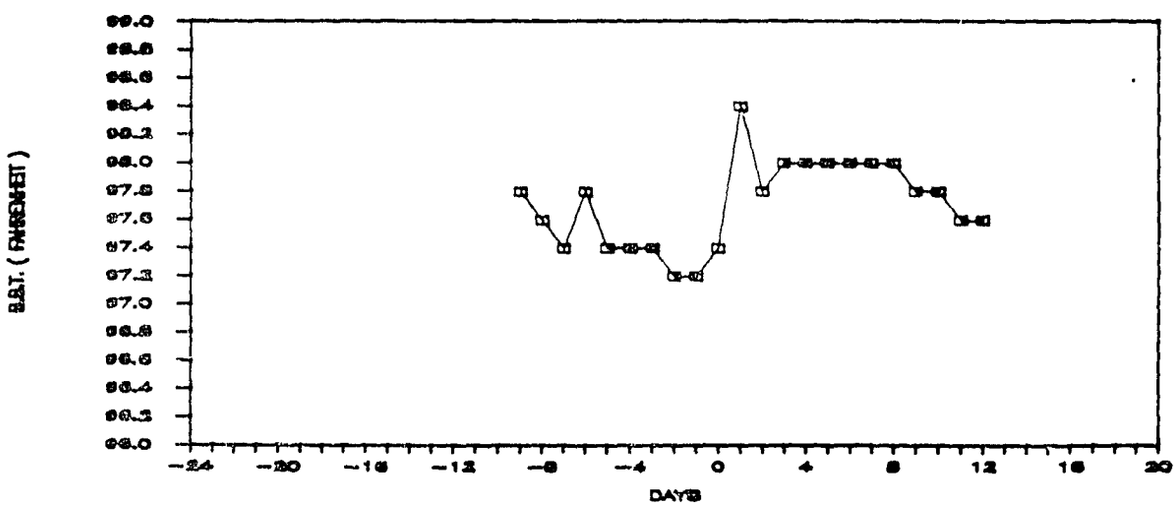
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
	10/29/84	5	-9	82.6			700.0		97.8	PARFR CYCLE 5
	10/30/84	6	-8	235.5			1100.0		97.6	
	10/31/84	7	-7	213.0			1300.0		97.4	
	11/1/84	8	-6	191.5			700.0		97.8	
	11/2/84	9	-5	25.1			1000.0		97.4	
	11/3/84	10	-4	---			1300.0		97.4	
	11/4/84	11	-3	2.5		24.6	1300.0		97.4	
	11/5/84	12	-2	---		18.8	500.0		97.2	
	11/6/84	13	-1	---		142.3	1600.0		97.2	
	11/7/84	14	0	0.0		12.1	1300.0		97.4	
	11/8/84	15	1	124.4		30.1	1600.0		98.4	
	11/9/84	16	2	102.5		71.5	3200.0		97.8	
	11/10/84	17	3	388.6		50.9	5400.0		98.0	
	11/11/84	18	4	347.4			7200.0		98.0	
	11/12/84	19	5	37.9			10000.0		98.0	
	11/13/84	20	6	67.8			6300.0		98.0	
	11/14/84	21	7	72.5			6600.0		98.0	
	11/15/84	22	8	107.5			7600.0		98.0	
	11/16/84	23	9	25.4			5900.0		97.8	
	11/17/84	24	10	308.8			4200.0		97.8	
	11/18/84	25	11	112.6			2400.0		97.6	
	11/19/84	26	12	104.1			2200.0		97.6	


PARFR CYCLE 5
S.S.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

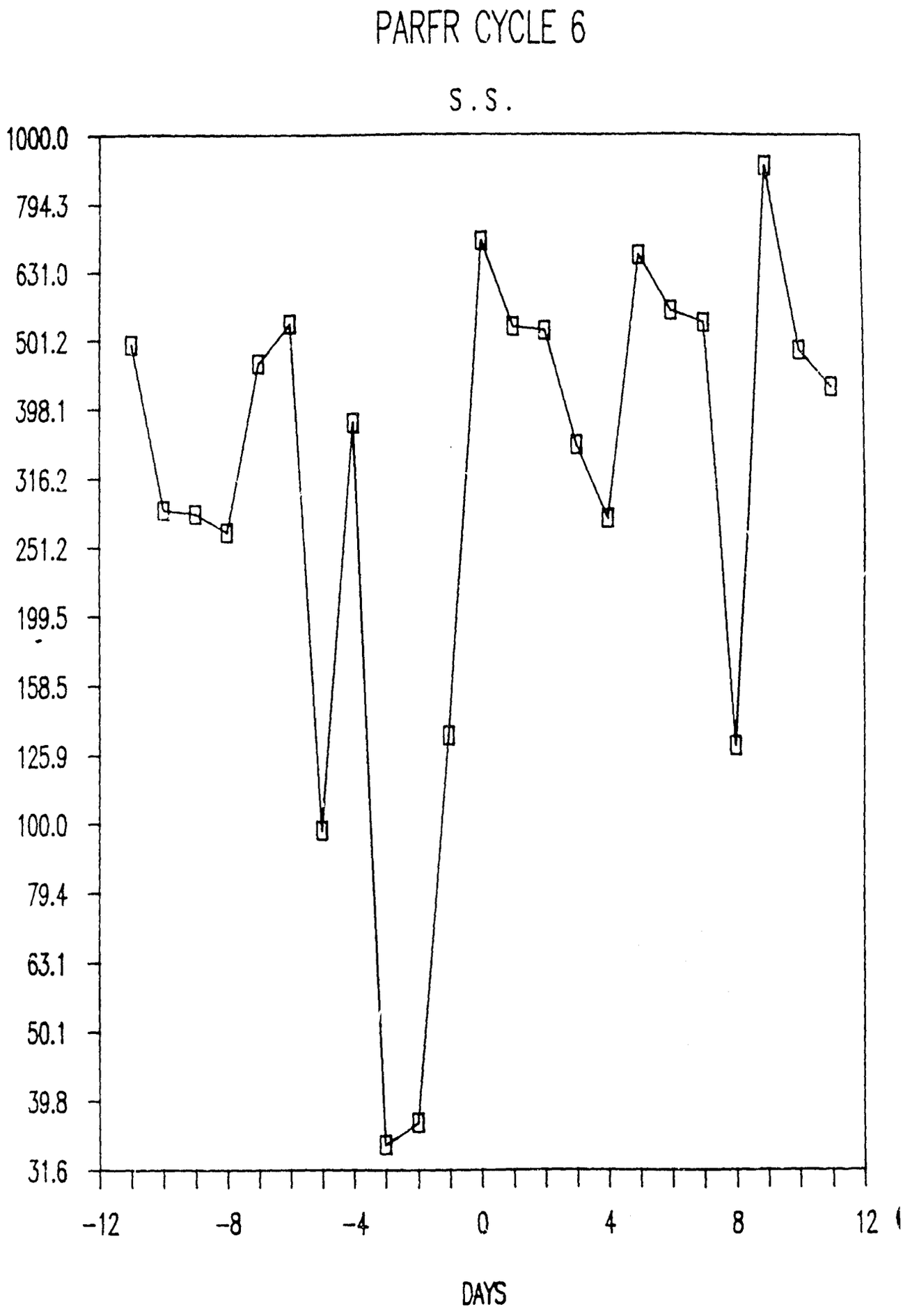
PARFR CYCLE 5 S.S. S.S.







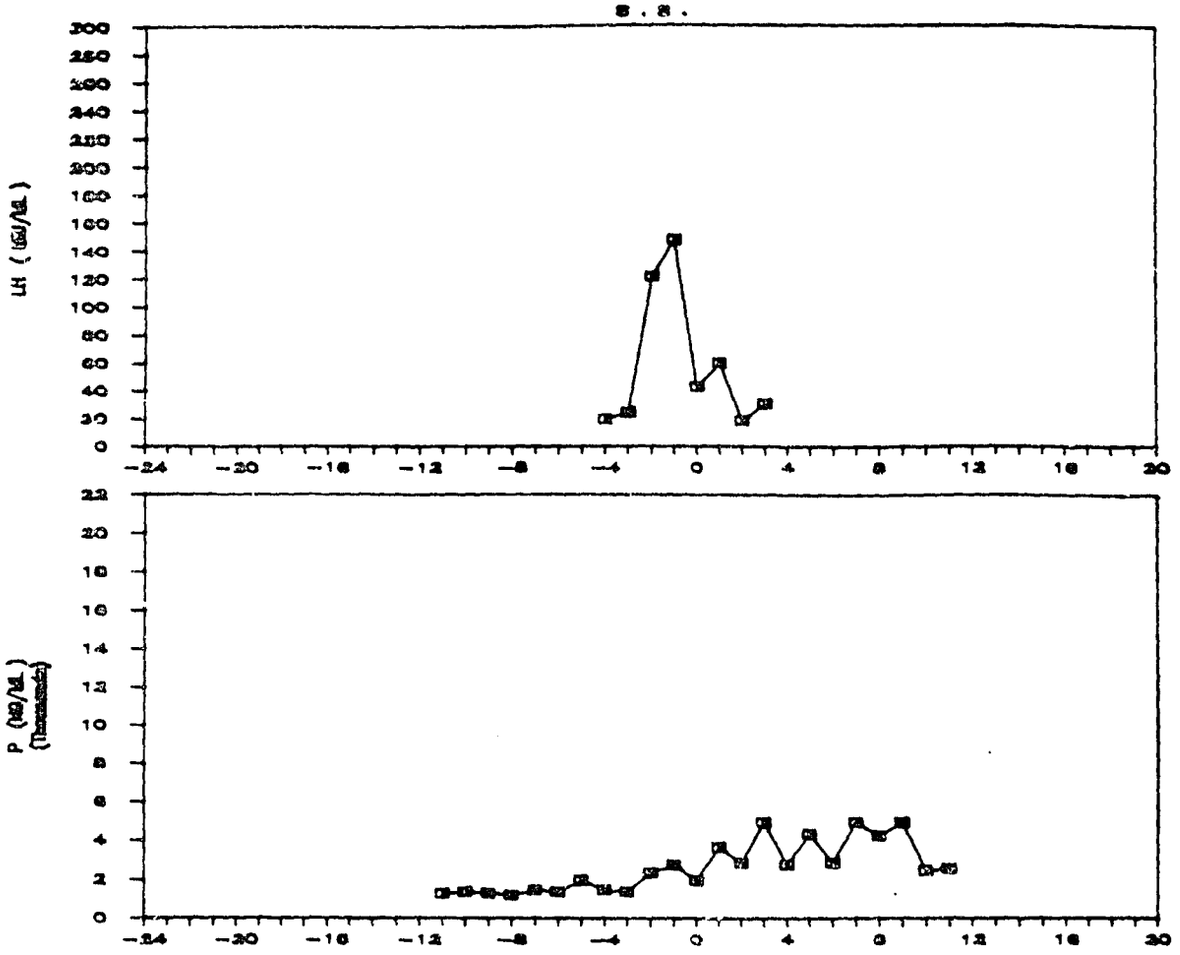
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
S.S.	3/7/85	4	-11	494.5			1300.0			PARFR CYCLE 6
	3/8/85	5	-10	284.3			1400.0		96.7	
	3/9/85	6	-9	280.4			1300.0		97.0	
	3/10/85	7	-8	263.8			1200.0		97.6	
	3/11/85	8	-7	462.8			1500.0		98.0	
	3/12/85	9	-6	529.3			1400.0		97.2	
	3/13/85	10	-5	97.9			2000.0		97.7	
	3/14/85	11	-4	380.5		20.0	1500.0		97.0	
	3/15/85	12	-3	34.4		25.0	1400.0		97.0	
	3/16/85	13	-2	37.0		122.0	2400.0		97.6	
	3/17/85	14	-1	134.4		148.0	2800.0		98.0	
	3/18/85	15	0	703.0		43.0	2000.0		98.0	
	3/19/85	16	1	526.3		60.0	3700.0		97.6	
	3/20/85	17	2	519.2		19.0	2900.0		98.2	
	3/21/85	18	3	353.9		31.0	5000.0		97.8	
	3/22/85	19	4	276.9			2800.0		98.0	
	3/23/85	20	5	667.3			4400.0		98.0	
	3/24/85	21	6	553.7			2900.0		98.2	
	3/25/85	22	7	531.0			5000.0		98.2	
	3/26/85	23	8	129.5			4300.0			
	3/27/85	24	9	906.6			5000.0		98.4	
	3/28/85	25	10	484.0			2500.0		98.4	
	3/29/85	26	11	427.5			2600.0		97.8	

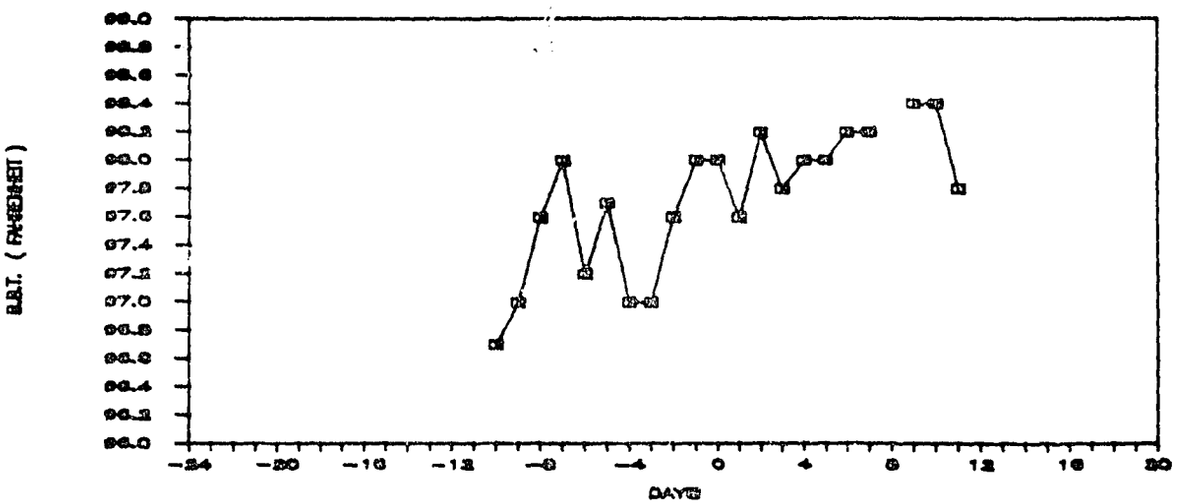

PARFR CYCLE 6
S.S.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
-12
-8
-4
0
4
8
12
DAYS

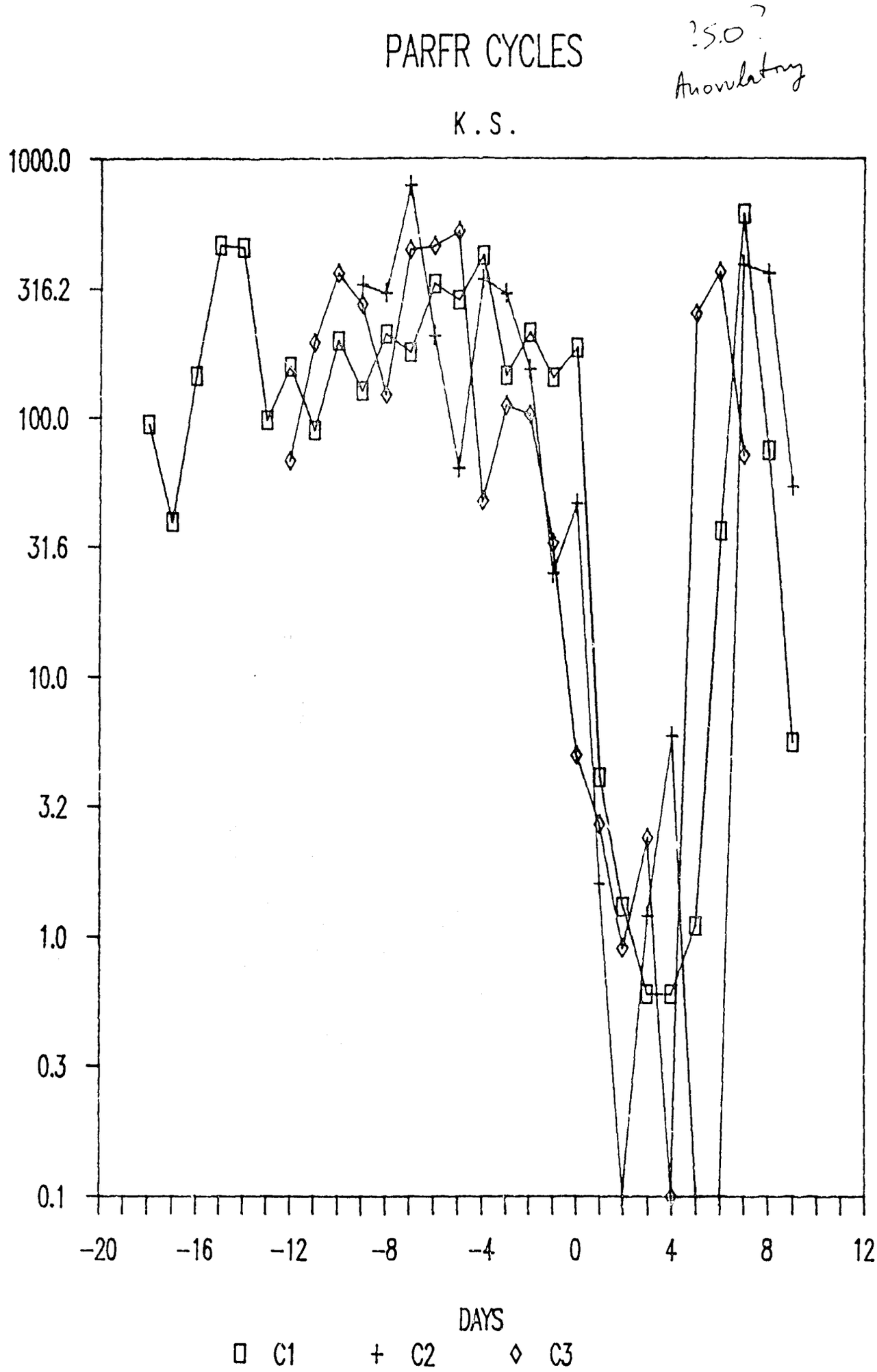
S.S.

PARFR CYCLE 6

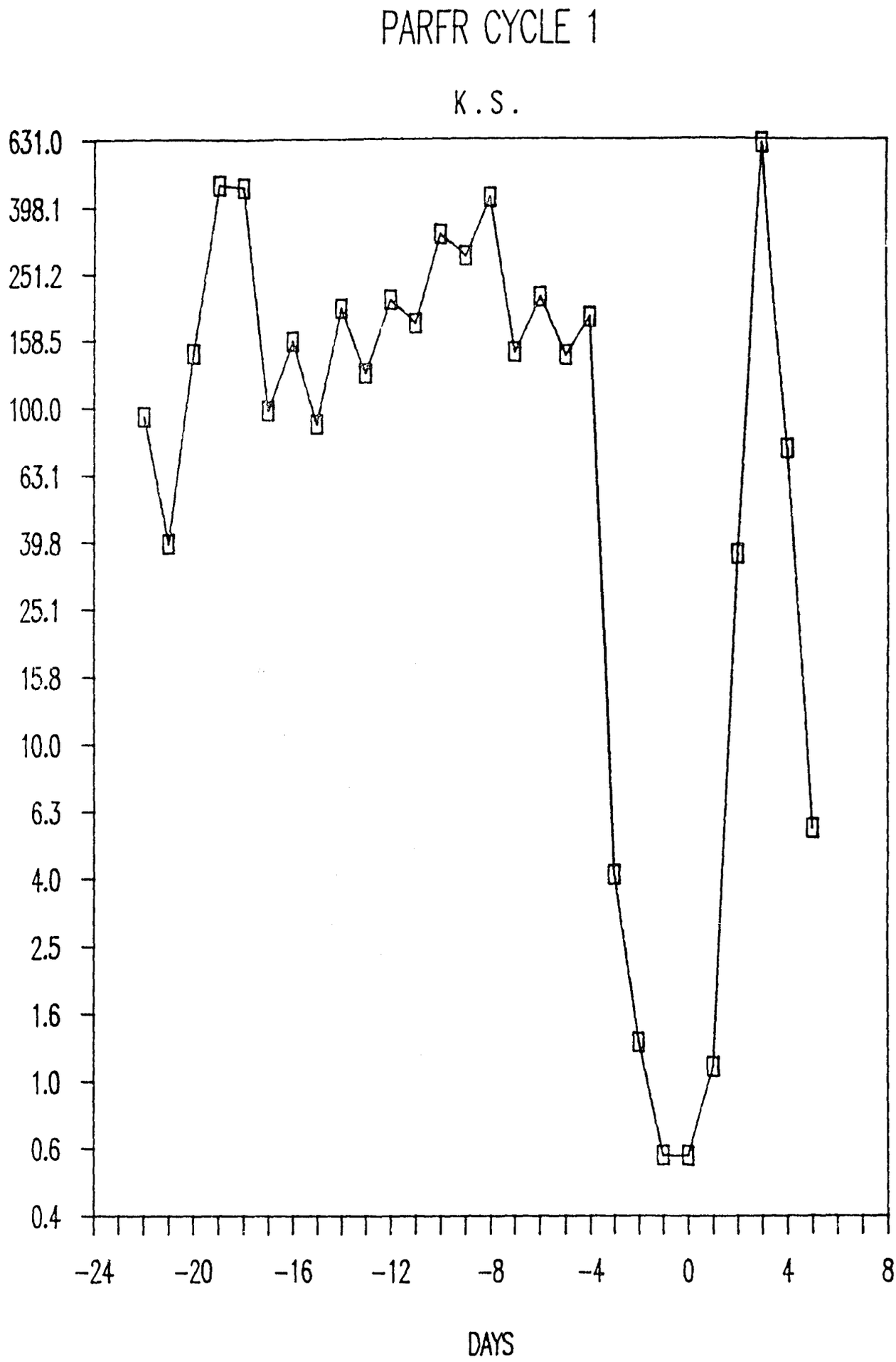
S.S.





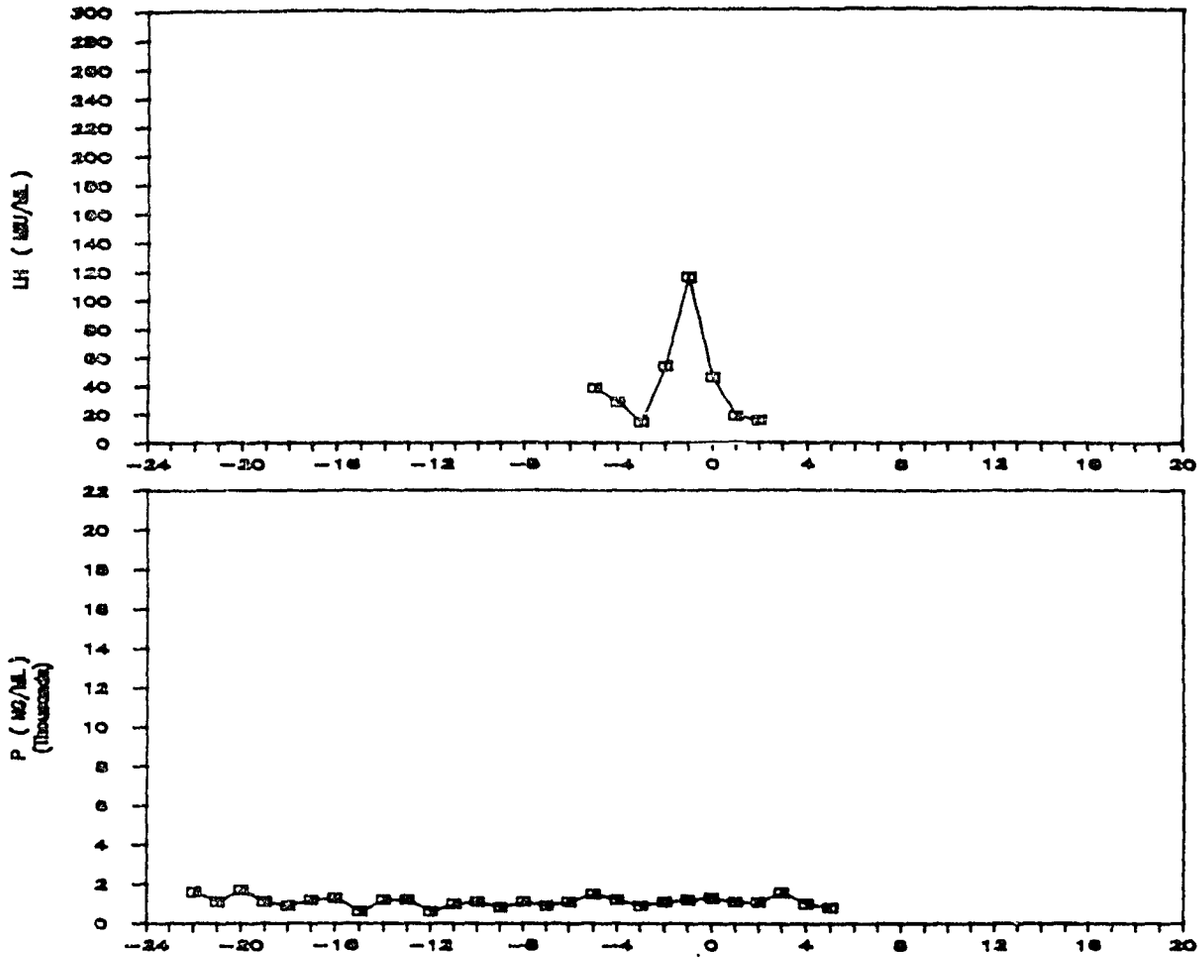

PARFR CYCLES
?5.0?
Anovulatory
K.S.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-20
-16
-12
-8
-4
0
4
8
12
DAYS
C1
C2
C3

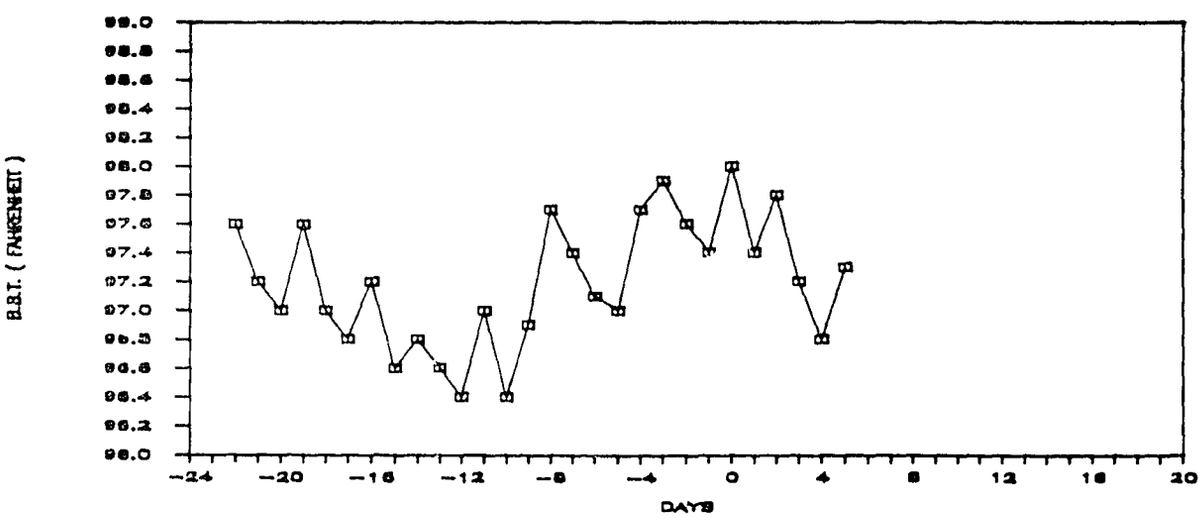
NAME	DATE	DFOM	"OVD"	GPC	GPV	LH	P	E2	BBT	REMARKS
K.S.	9/22/84	4	-22	93.9			1600.0		97.6	PARFR CYCLE 1
	9/23/84	5	-21	39.4			1100.0		97.2	
	9/24/84	6	-20	144.3			1700.0		97.0	
	9/25/84	7	-19	463.0			1100.0		97.6	
	9/26/84	8	-18	454.0			900.0		97.0	
	9/27/84	9	-17	97.6			1200.0		96.8	
	9/28/84	10	-16	156.8			1300.0		97.2	
	9/29/84	11	-15	89.0			600.0		96.6	
	9/30/84	12	-14	197.2			1200.0		96.8	
	10/1/84	13	-13	125.9			1200.0		96.6	
	10/2/84	14	-12	209.4			600.0		96.4	
	10/3/84	15	-11	178.8			1000.0		97.0	
	10/4/84	16	-10	329.2			1100.0		96.4	
	10/5/84	17	-9	283.9			800.0		96.9	
	10/6/84	18	-8	423.8			1100.0		97.7	
	10/7/84	19	-7	146.2			900.0		97.4	
	10/8/84	20	-6	213.8			1100.0		97.1	
	10/9/84	21	-5	143.6		38.0	1500.0		97.0	
	10/10/84	22	-4	186.7		28.0	1200.0		97.7	
	10/11/84	23	-3	4.1		14.0	900.0		97.9	
	10/12/84	24	-2	1.3		53.0	1100.0		97.6	
	10/13/84	25	-1	0.6		115.0	1200.0		97.4	
	10/14/84	26	0	0.6		45.0	1300.0		98.0	
	10/15/84	27	1	1.1		18.0	1100.0		97.4	
	10/16/84	28	2	36.5		15.0	1100.0		97.8	
	10/17/84	29	3	613.3			1600.0		97.2	
	10/18/84	30	4	75.0			1000.0		96.8	
	10/19/84	31	5	5.6			800.0		97.3	


PARFR CYCLE 1
K.S.
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
0.6
0.4
-24
-20
-16
-12
-8
-4
0
4
8
DAYS

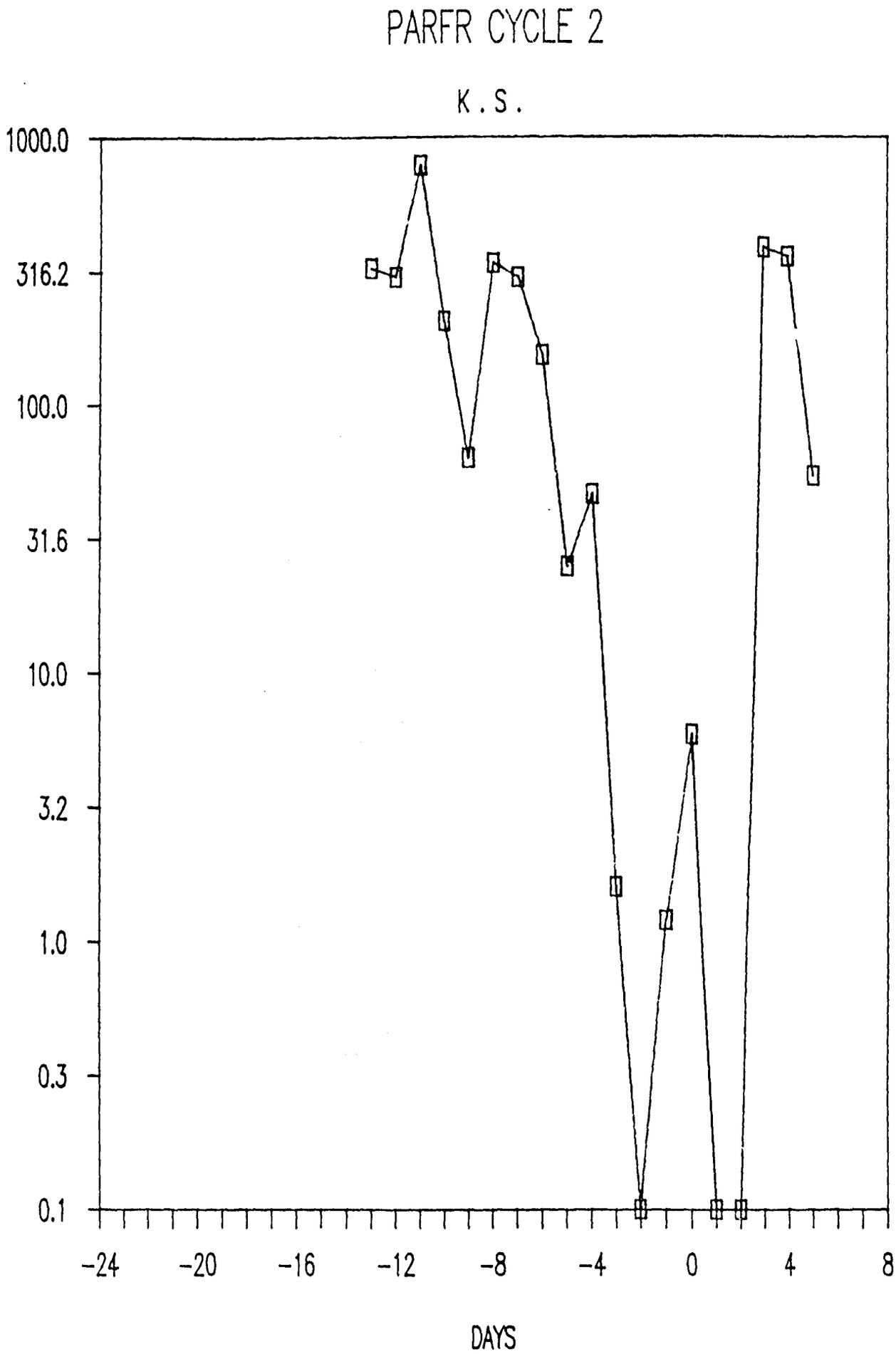
K.S.

PARFR CYCLE 1



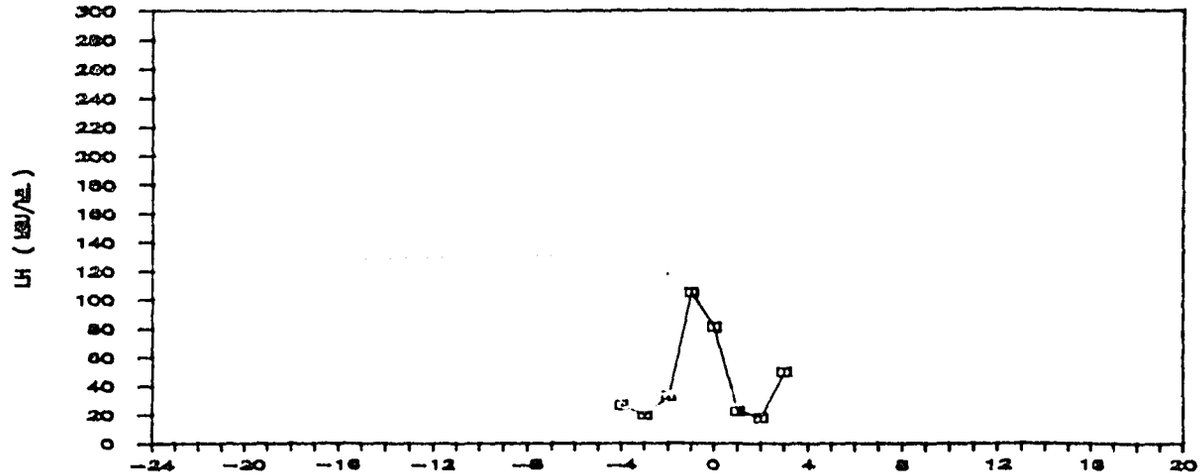


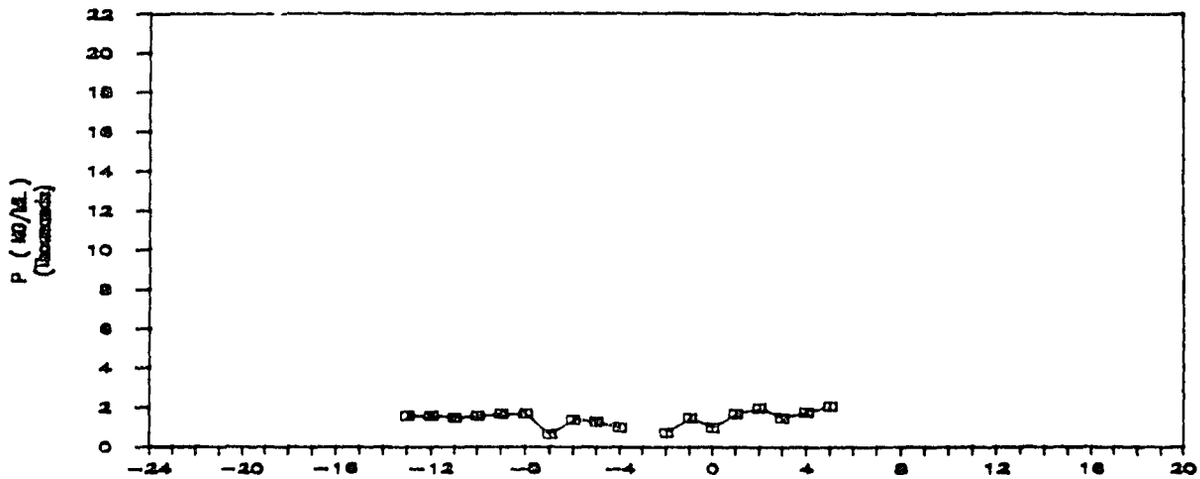
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
K.S.	10/22/84	4	-13	324.9			1600.0		96.2	PARFR CYCLE 2
	10/23/84	5	-12	302.6			1600.0		97.1	
	10/24/84	6	-11	789.6			1500.0		96.8	
	10/25/84	7	-10	207.8			1600.0		97.1	
	10/26/84	8	-9	63.6			1700.0		97.1	
	10/27/84	9	-8	343.4			1700.0		97.2	
	10/28/84	10	-7	302.8			640.0		97.1	
	10/29/84	11	-6	154.3			1400.0		97.4	
	10/30/84	12	-5	25.1			1300.0		97.0	
	10/31/84	13	-4	46.7		26.0	1000.0		97.0	
	11/1/84	14	-3	1.6		19.0	---		97.6	
	11/2/84	15	-2	0.0		32.0	730.0		96.6	
	11/3/84	16	-1	1.2		104.0	1500.0		97.2	
	11/4/84	17	0	5.9		80.0	1000.0		97.2	
	11/5/84	18	1	0.0		22.0	1700.0		97.6	
	11/6/84	19	2	0.0		17.0	2000.0		97.2	
	11/7/84	20	3	392.4		49.0	1500.0		97.5	
	11/8/84	21	4	361.9			1800.0		97.2	
	11/9/84	22	5	54.2			2100.0		97.5	

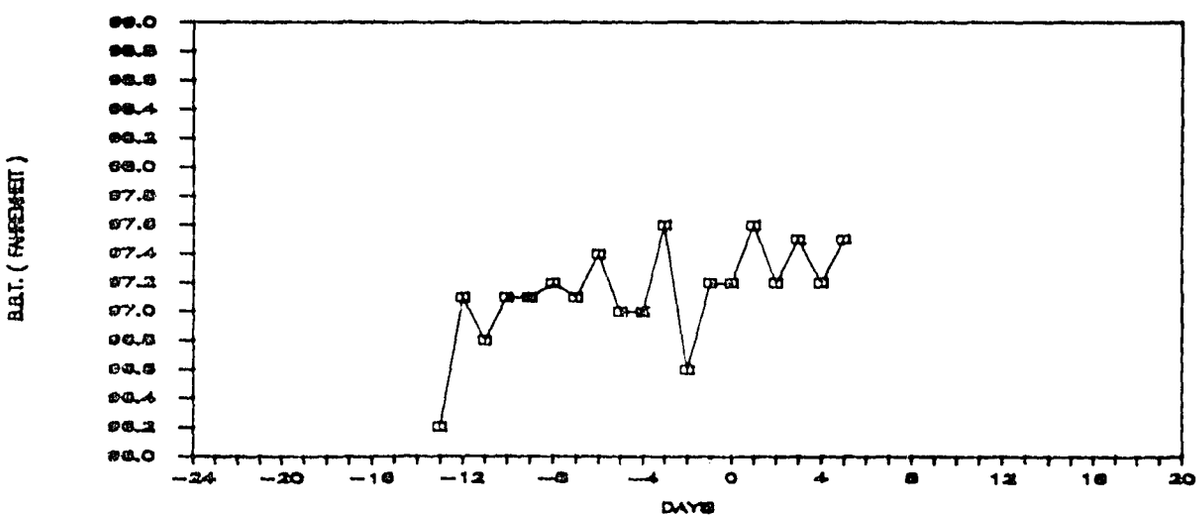

PARFR CYCLE 2
K.S.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-24
-20
-16
-12
-8
-4
0
4
8
DAYS

K.S.

PARFR CYCLE 2



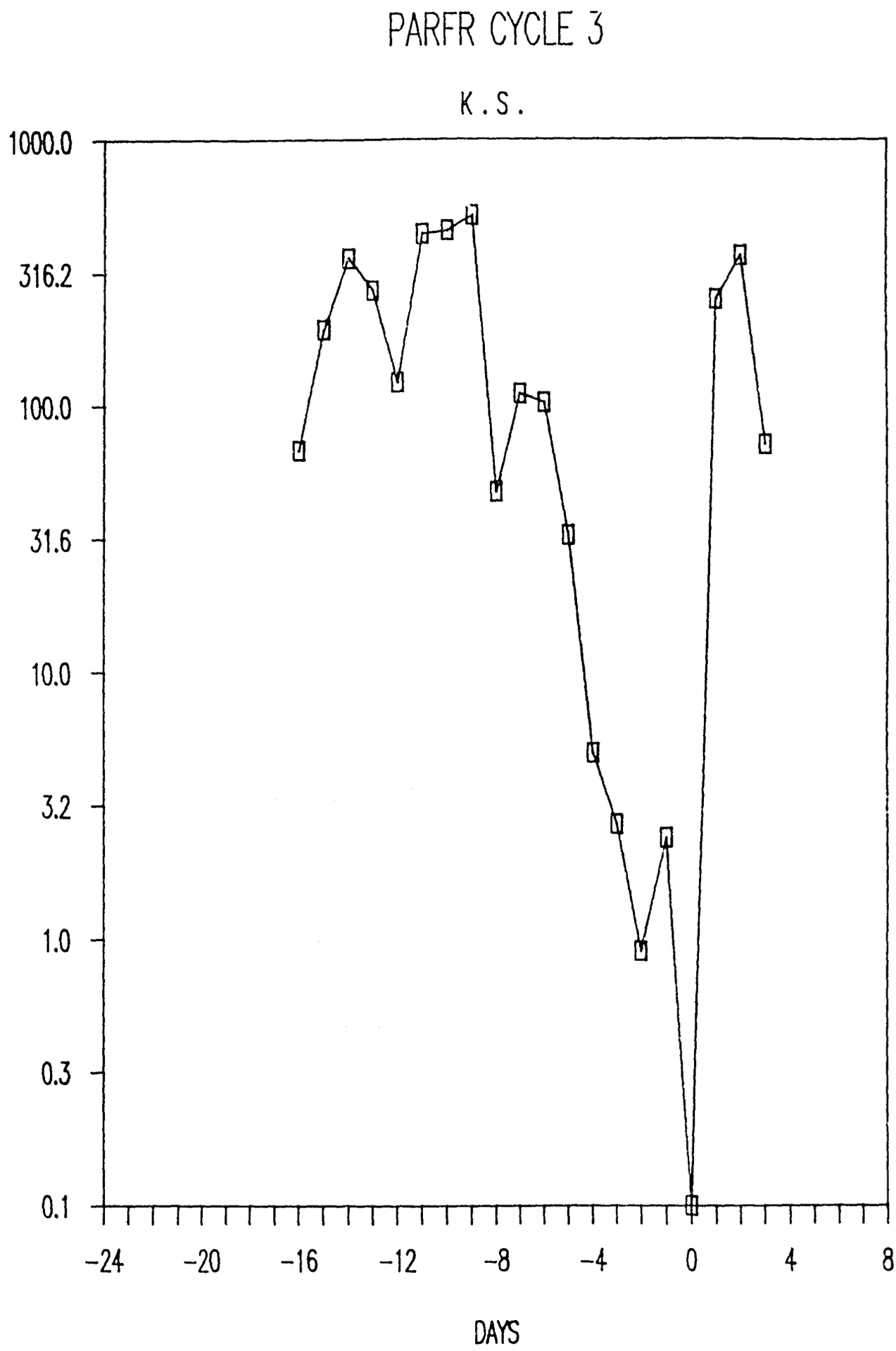




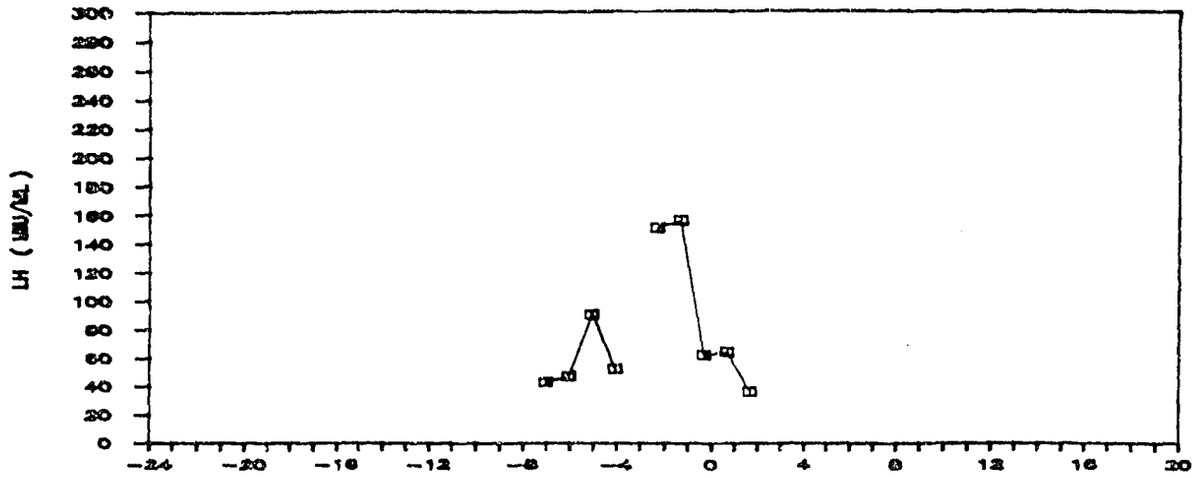
54

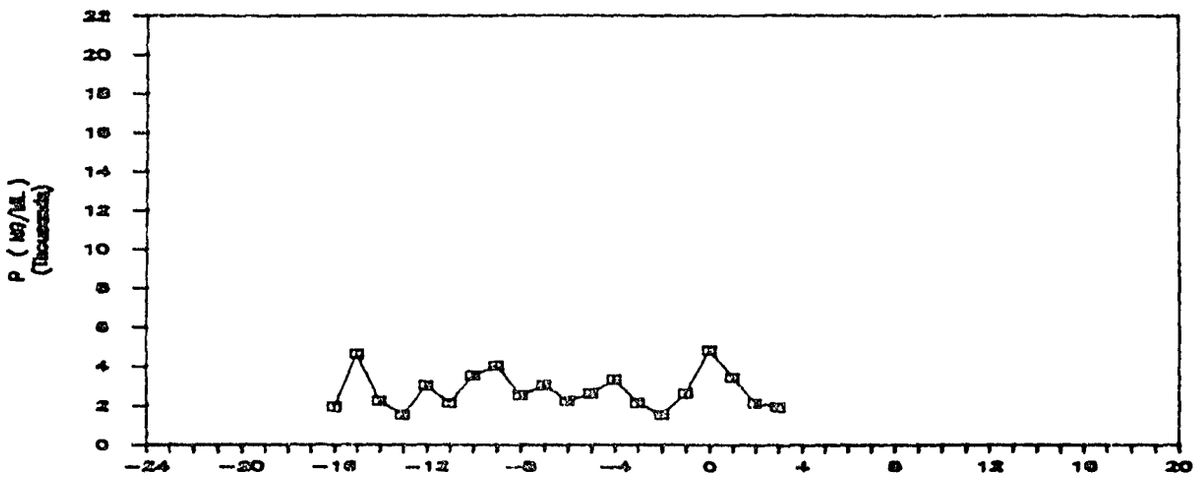
K.S.

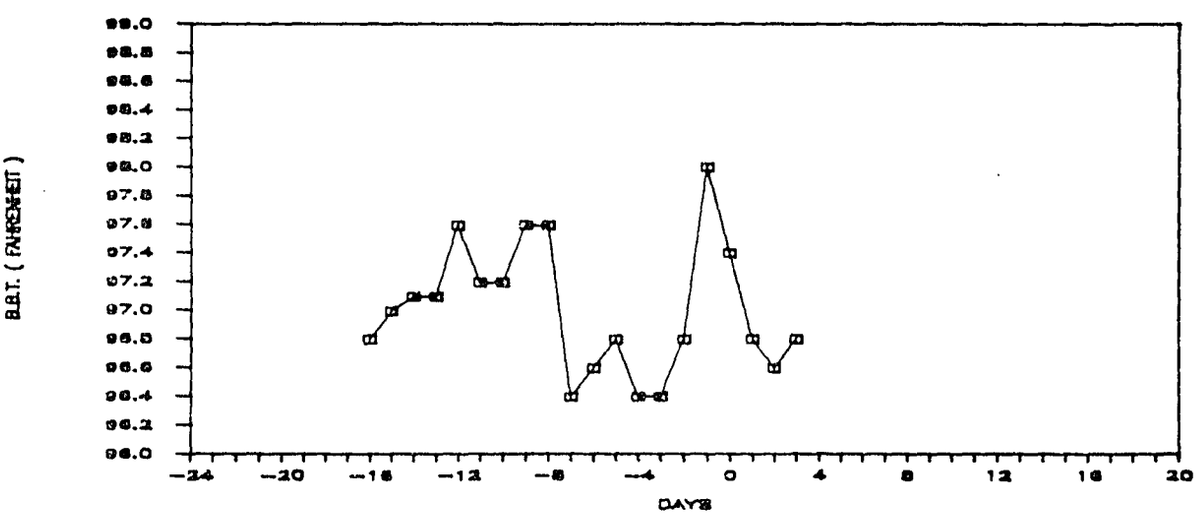
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
	1/26/85	4	-16	68.0			1900.0		96.8	PARFR CYCLE 3
	1/27/85	5	-15	193.5			4600.0		97.0	
	1/28/85	6	-14	360.9			2200.0		97.1	
	1/29/85	7	-13	272.4			1500.0		97.1	
	1/30/85	8	-12	122.5			3000.0		97.6	
	1/31/85	9	-11	446.7			2100.0		97.2	
	2/1/85	10	-10	459.6			3500.0		97.2	
	2/2/85	11	-9	524.8			4000.0		97.6	
	2/3/85	12	-8	47.7			2500.0		97.6	
	2/4/85	13	-7	111.6		43.0	3000.0		96.4	
	2/5/85	14	-6	103.4		47.0	2200.0		96.6	
	2/6/85	15	-5	32.8		90.0	2600.0		96.8	
	2/7/85	16	-4	5.0		52.0	3300.0		96.4	
	2/8/85	17	-3	2.7		---	2100.0		96.4	
	2/9/85	18	-2	0.9		150.0	1500.0		96.8	
	2/10/85	19	-1	2.4		155.0	2600.0		98.0	
	2/11/85	20	0	0.0		62.0	4800.0		97.4	
	2/12/85	21	1	252.9		64.0	3400.0		96.8	
	2/13/85	22	2	368.9		36.0	2100.0		96.6	
	2/14/85	23	3	71.4			1900.0		96.8	

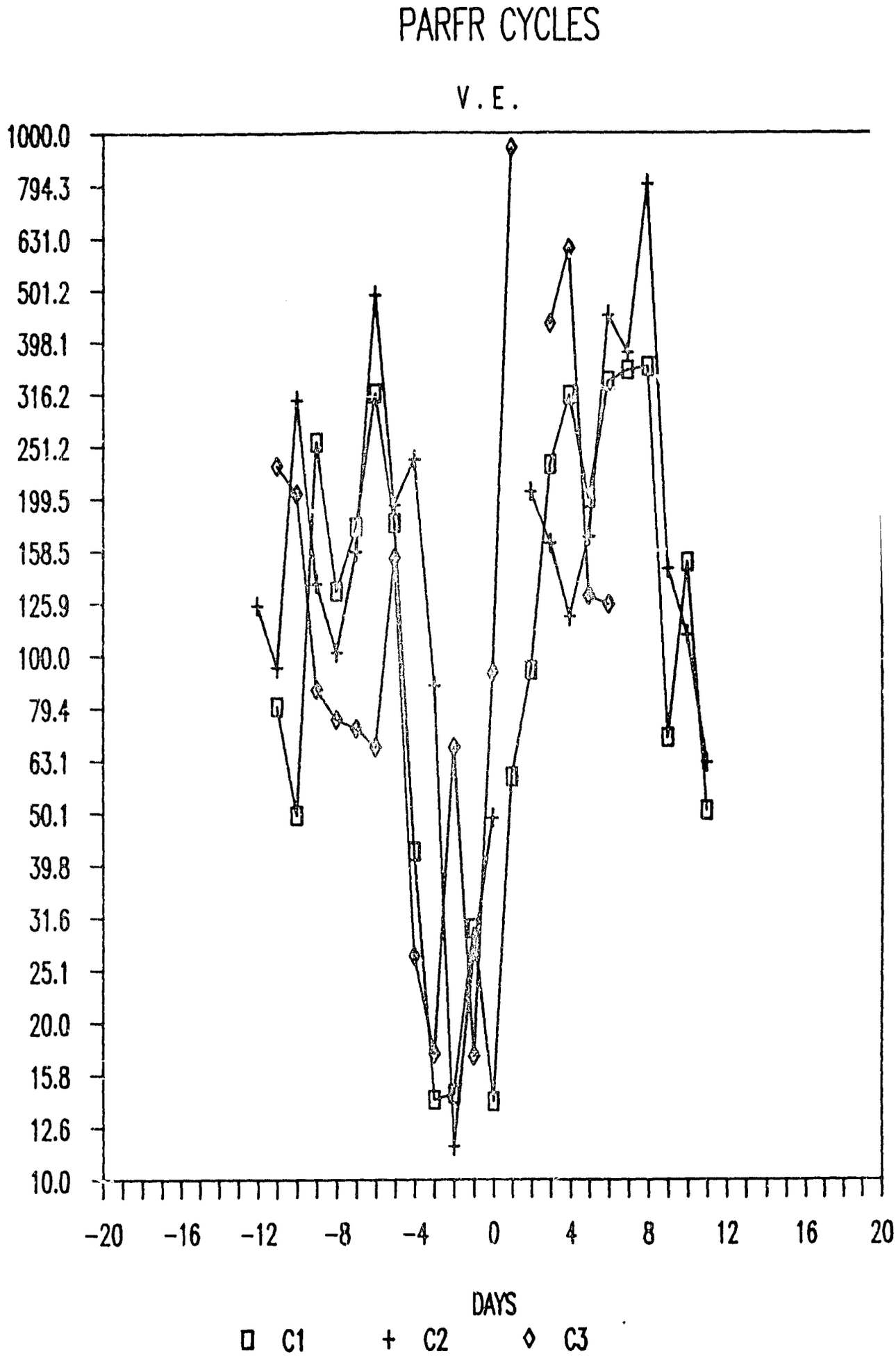

PARFR CYCLE 3
K.S.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-24
-20
-16
-12
-8
-4
0
4
8
DAYS

PARFR CYCLE 3

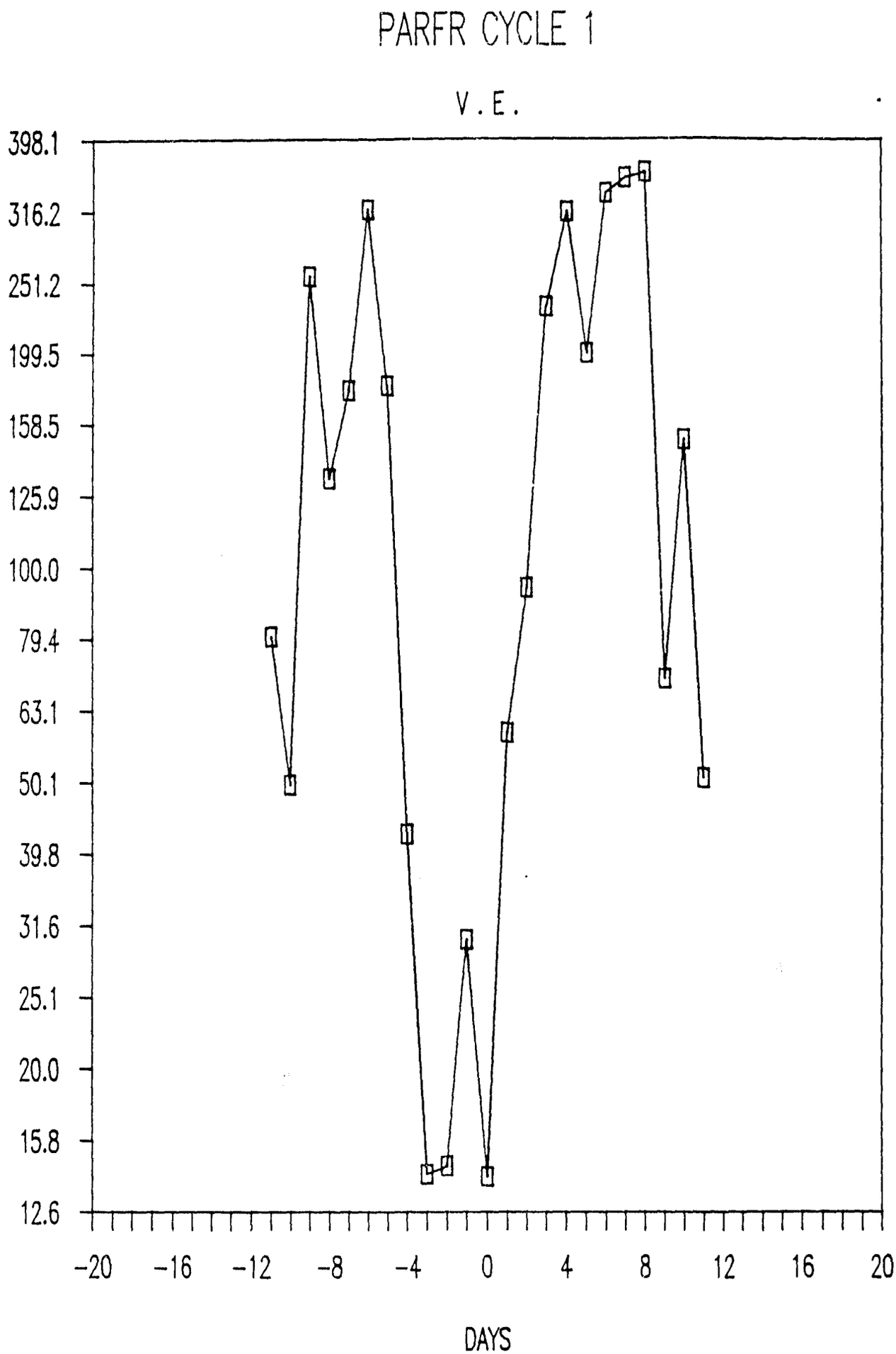






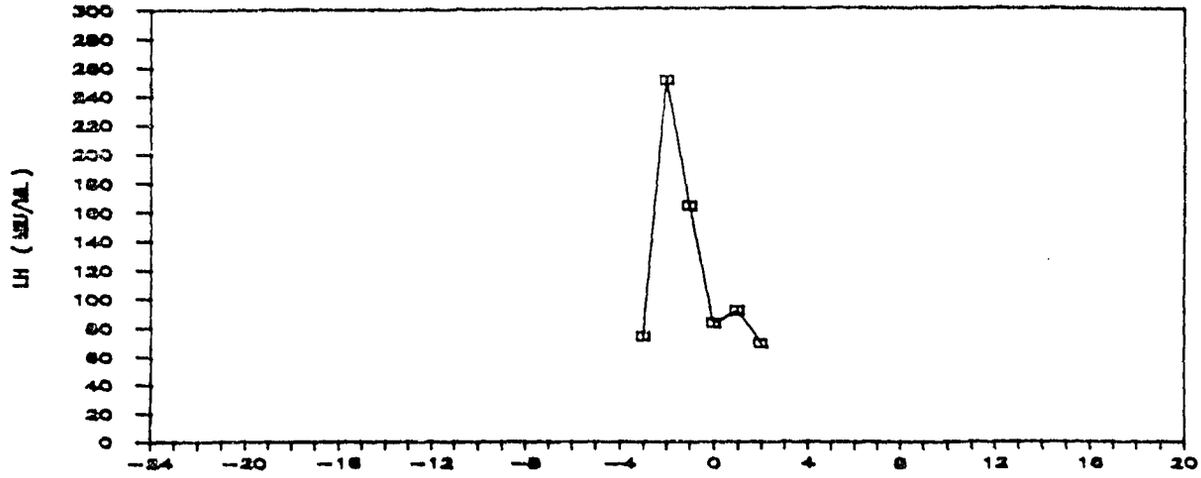

PARFR CYCLES
V.E.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS
C1
C2
C3

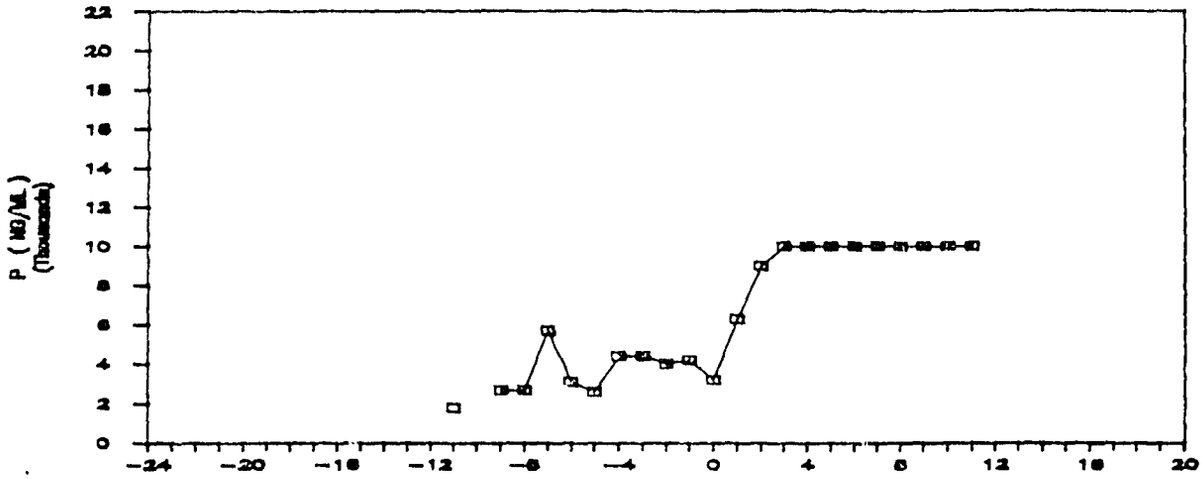
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
V.E.	10/2/84	5	-11	80.0			1800.0		98.4	PARFR CYCLE 1
	10/3/84	6	-10	49.6			---		97.3	
	10/4/84	7	-9	256.2			2700.0		97.2	
	10/5/84	8	-8	132.9			2700.0		97.3	
	10/6/84	9	-7	176.9			5700.0		98.0	
	10/7/84	10	-6	317.7			3100.0		98.0	
	10/8/84	11	-5	179.8			2600.0		98.0	
	10/9/84	12	-4	42.4			4400.0		98.0	
	10/10/84	13	-3	14.2		73.0	4400.0		97.8	
	10/11/84	14	-2	14.6		250.0	4000.0		97.8	
	10/12/84	15	-1	30.2		163.0	4200.0		98.0	
	10/13/84	16	0	14.1		82.0	3200.0		98.2	
	10/14/84	17	1	58.9		91.0	6300.0		98.2	
	10/15/84	18	2	93.9		68.0	9000.0		98.2	
	10/16/84	19	3	232.5			10000.0		98.2	
	10/17/84	20	4	315.7			10000.0		98.4	
	10/18/84	21	5	200.0			10000.0		98.4	
	10/19/84	22	6	334.7			10000.0		98.4	
	10/20/84	23	7	351.9			10000.0		98.2	
	10/21/84	24	8	357.5			10000.0		98.6	
	10/22/84	25	9	69.8			10000.0		98.0	
	10/23/84	26	10	150.9			10000.0		98.2	
	10/24/84	27	11	50.7			10000.0		98.2	

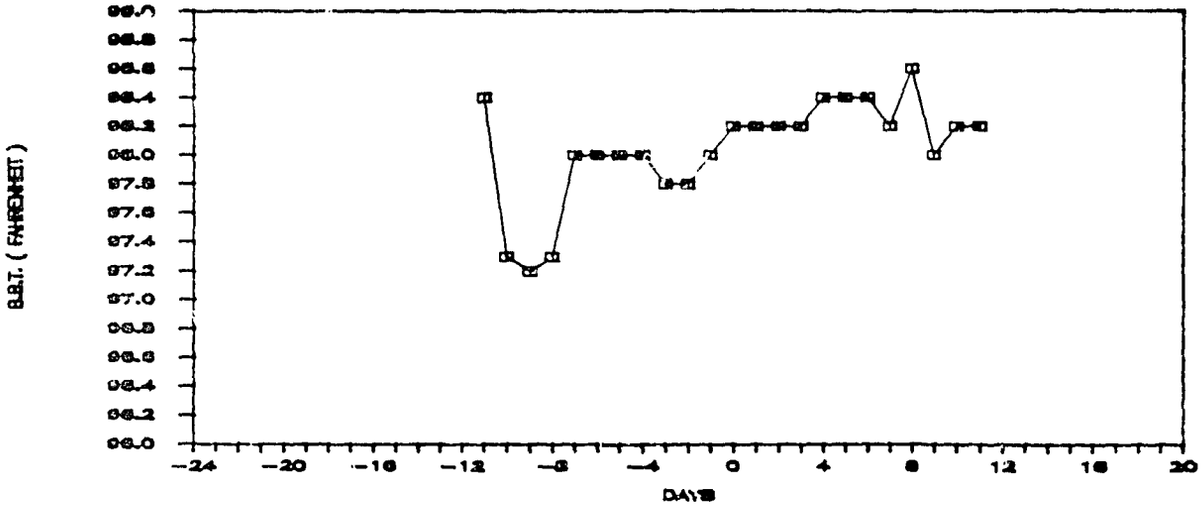

PARFR CYCLE 1
V.E.
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

V.E.

PARFR CYCLE 1

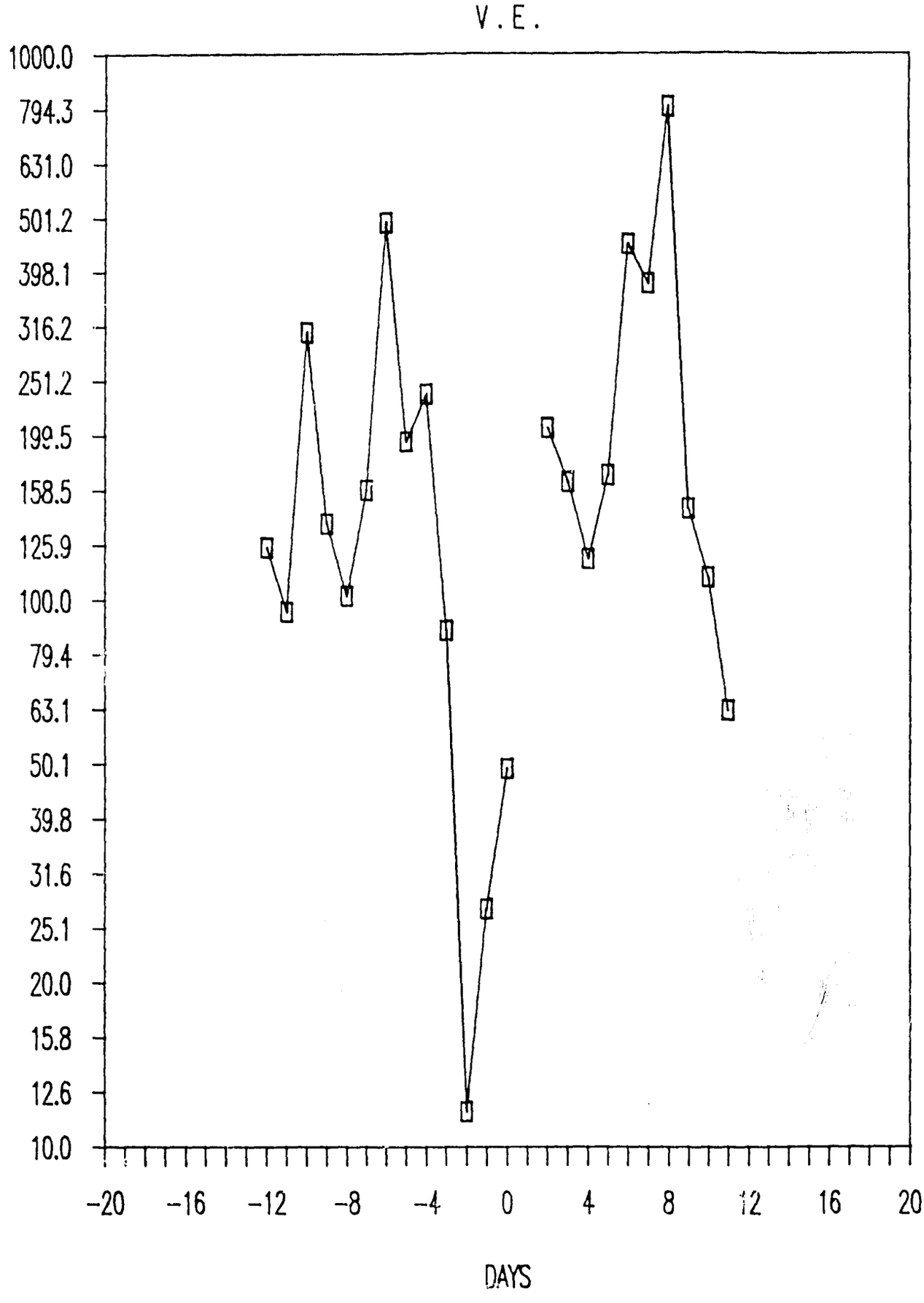






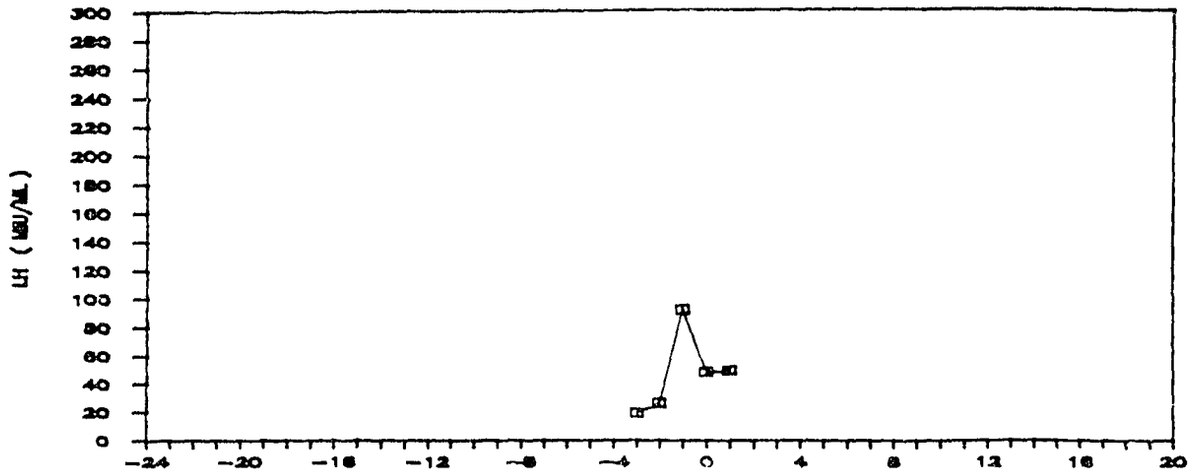
NAME	DATE	DFOM	DVD	GPC	GPV	LH	P	E2	BBT	REMARKS
V.E.	10/29/84	5	-12	124.7			3000.0		98.0	FARFR CYCLE 2
	10/30/84	6	-11	94.9			2500.0		97.8	
	10/31/84	7	-10	308.4			2600.0		97.8	
	11/1/84	8	-9	137.4			1700.0		97.6	
	11/2/84	9	-8	101.5			4100.0		97.8	
	11/3/84	10	-7	158.5			2000.0		98.0	
	11/4/84	11	-6	490.7			2100.0		98.2	
	11/5/84	12	-5	194.3			2500.0		97.6	
	11/6/84	13	-4	237.6			2400.0		97.8	
	11/7/84	14	-3	87.8		19.0	700.0		97.6	
	11/8/84	15	-2	11.6		26.0	1300.0		97.8	
	11/9/84	16	-1	27.2		92.0	1800.0		98.0	
	11/10/84	17	0	49.1		48.0	2300.0		98.0	
	11/11/84	18	1	---		49.0	6600.0		---	
	11/12/84	19	2	206.1			6500.0		98.0	
	11/13/84	20	3	164.4			1200.0		97.8	
	11/14/84	21	4	118.8			5000.0		98.2	
	11/15/84	22	5	168.9			7000.0		98.4	
	11/16/84	23	6	448.0			10000.0		98.2	
	11/17/84	24	7	380.0			10000.0		98.4	
	11/18/84	25	8	800.0			10000.0		98.2	
	11/19/84	26	9	146.7			---		98.2	
	11/20/84	27	10	109.8			10000.0		98.4	
	11/21/84	28	11	62.6			8400.0		98.4	

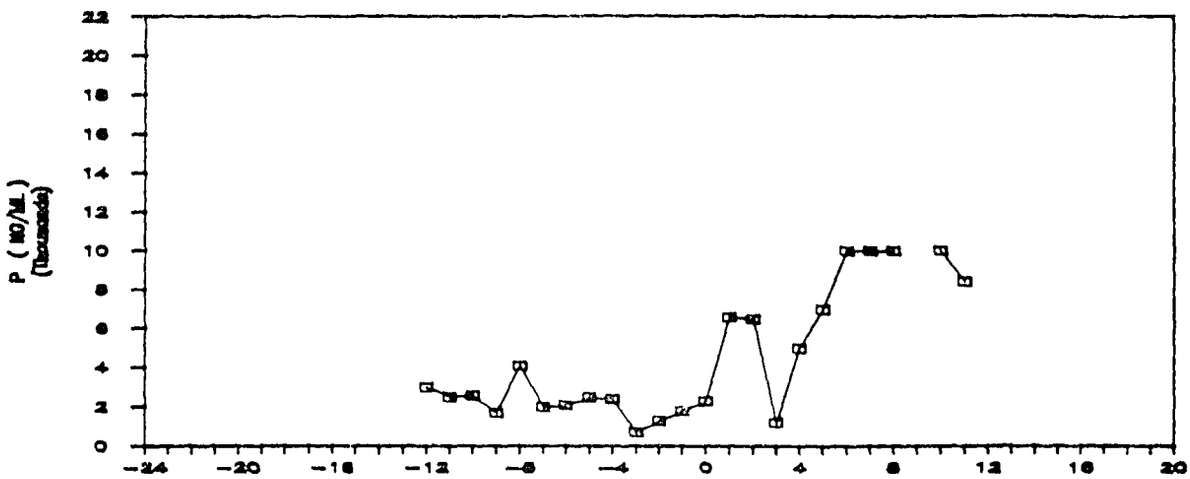
PARFR CYCLE 2

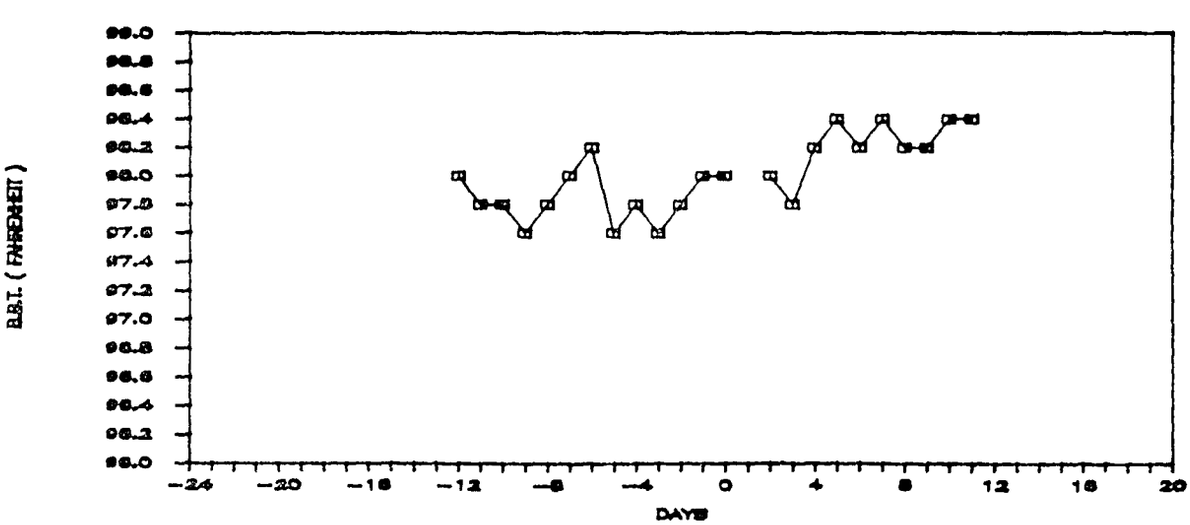


V.E

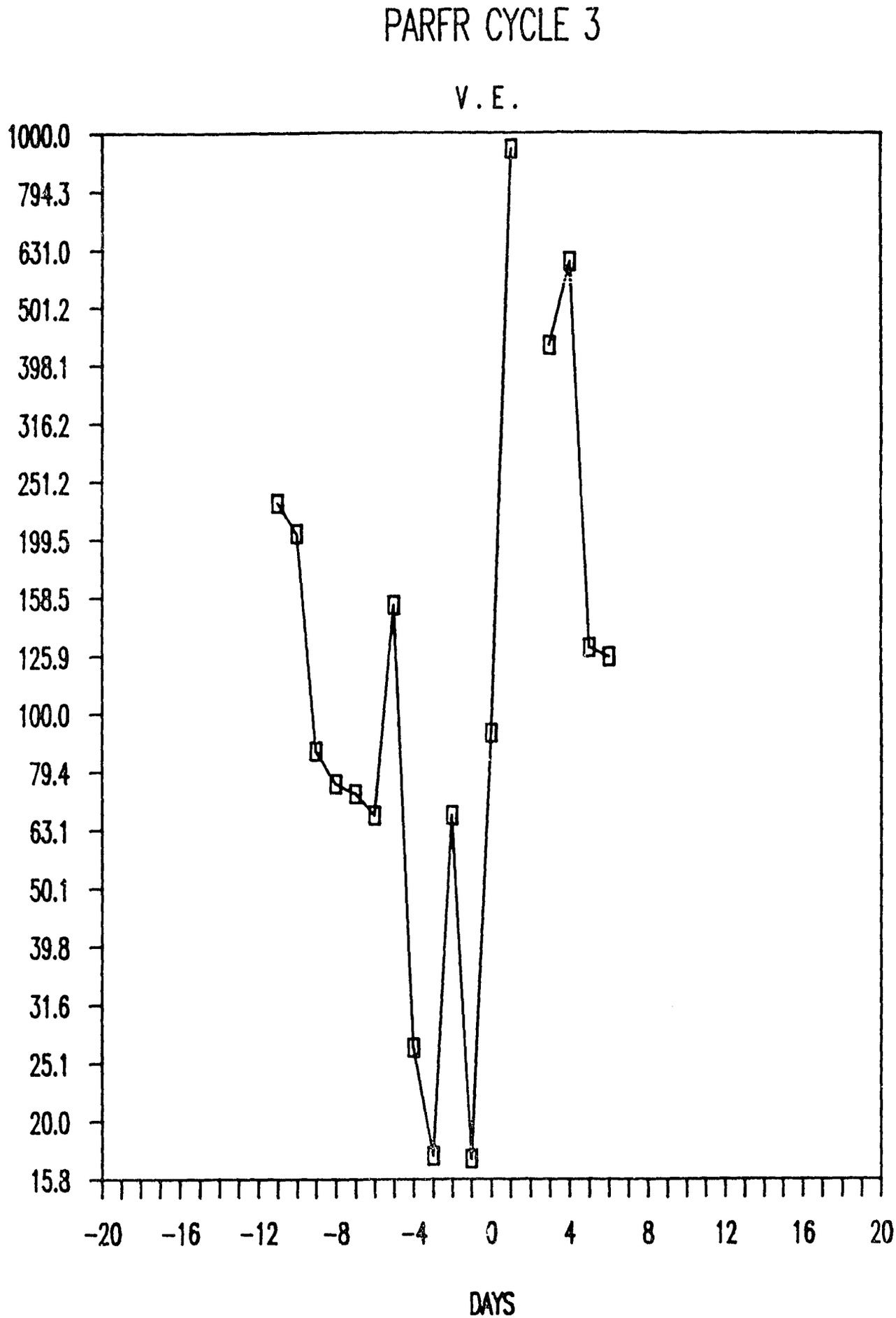
PARFR CYCLE 2





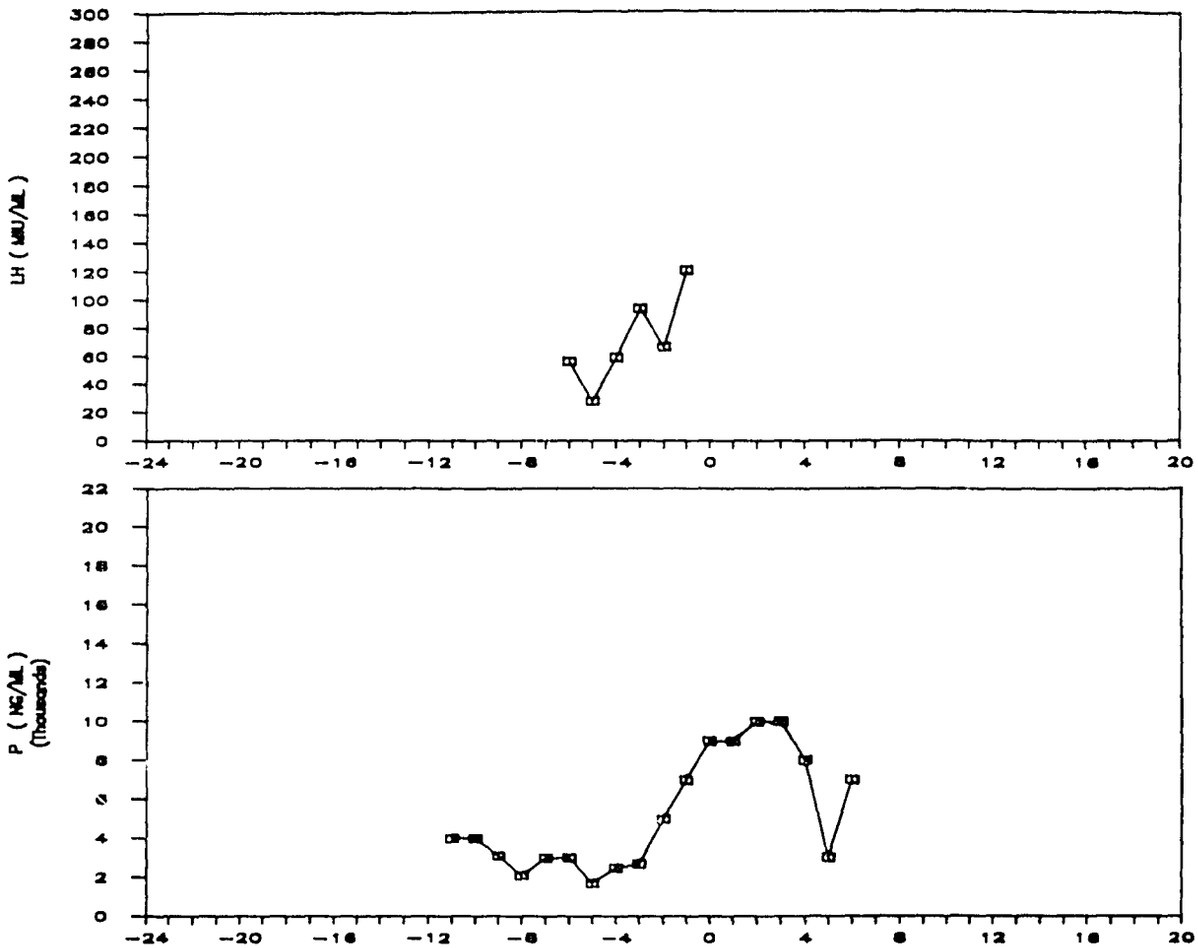


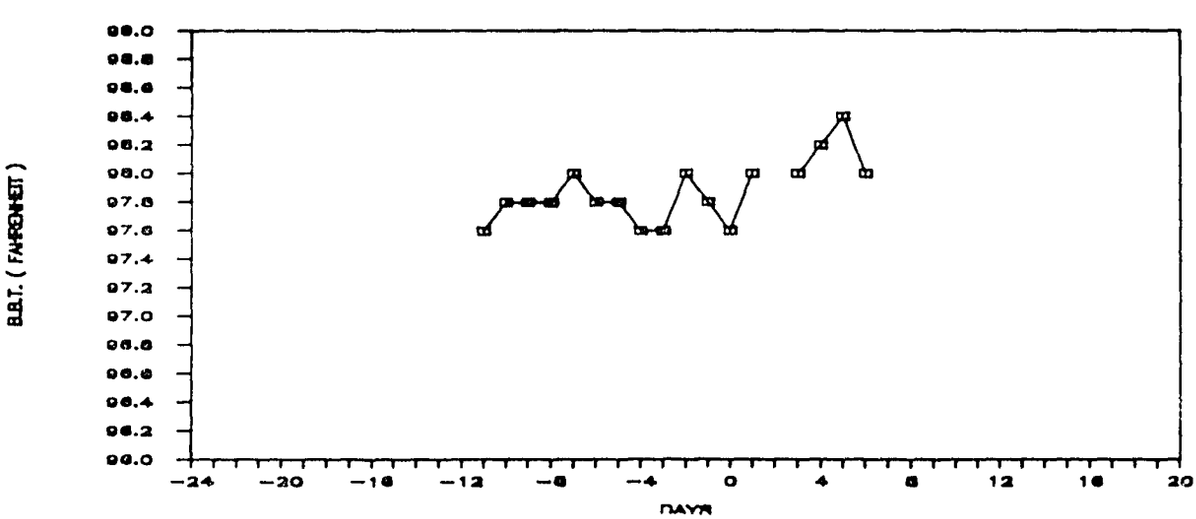
NAME	DATE	DFOM	OVD	GPC	LH	P	BBT	REMARKS
V.E.	11/26/84	5	-11	230.8		4000.0	97.6	PARFR CYCLE 3
	11/27/84	6	-10	204.1		4000.0	97.8	
	11/28/84	7	-9	86.3		3100.0	97.8	
	11/29/84	8	-8	75.7		2100.0	97.8	
	11/30/84	9	-7	72.8		3000.0	98.0	
	12/1/84	10	-6	67.0	56.0	3000.0	97.8	
	12/2/84	11	-5	154.0	28.0	1700.0	97.8	
	12/3/84	12	-4	26.7	59.0	2500.0	97.6	
	12/4/84	13	-3	17.4	93.0	2700.0	97.6	
	12/5/84	14	-2	67.0	66.0	5000.0	98.0	
	12/6/84	15	-1	17.2	120.0	7000.0	97.8	
	12/7/84	16	0	92.9		9000.0	97.6	
	12/8/84	17	1	938.5		9000.0	98.0	
	12/9/84	18	2	---		10000.0	---	
	12/10/84	19	3	432.0		10000.0	98.0	
	12/11/84	20	4	601.5		8000.0	98.2	
	12/12/84	21	5	130.2		3000.0	98.4	
	12/13/84	22	6	125.3		7000.0	98.0	

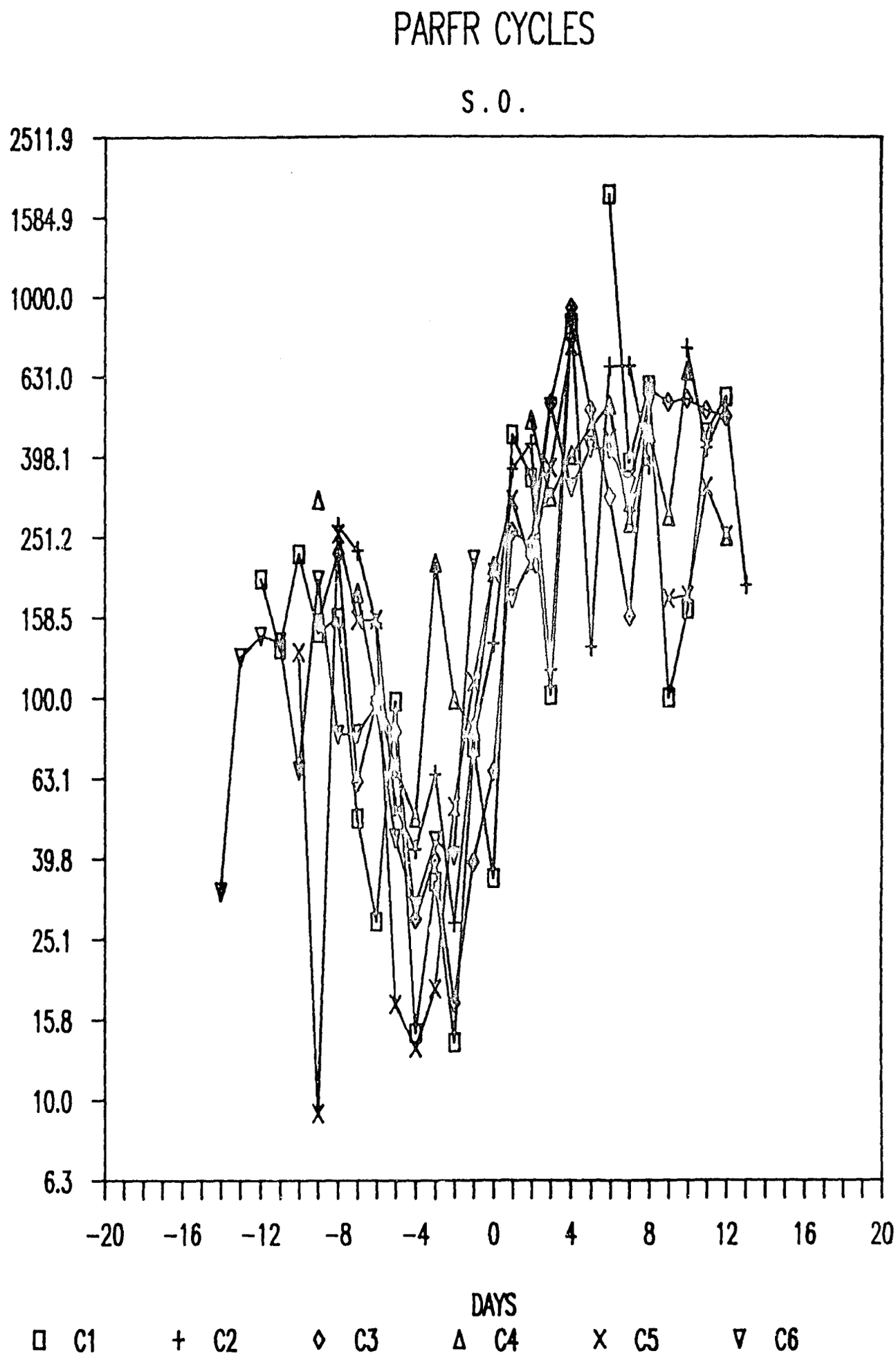

PARFR CYCLE 3
V.E.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

V.E.

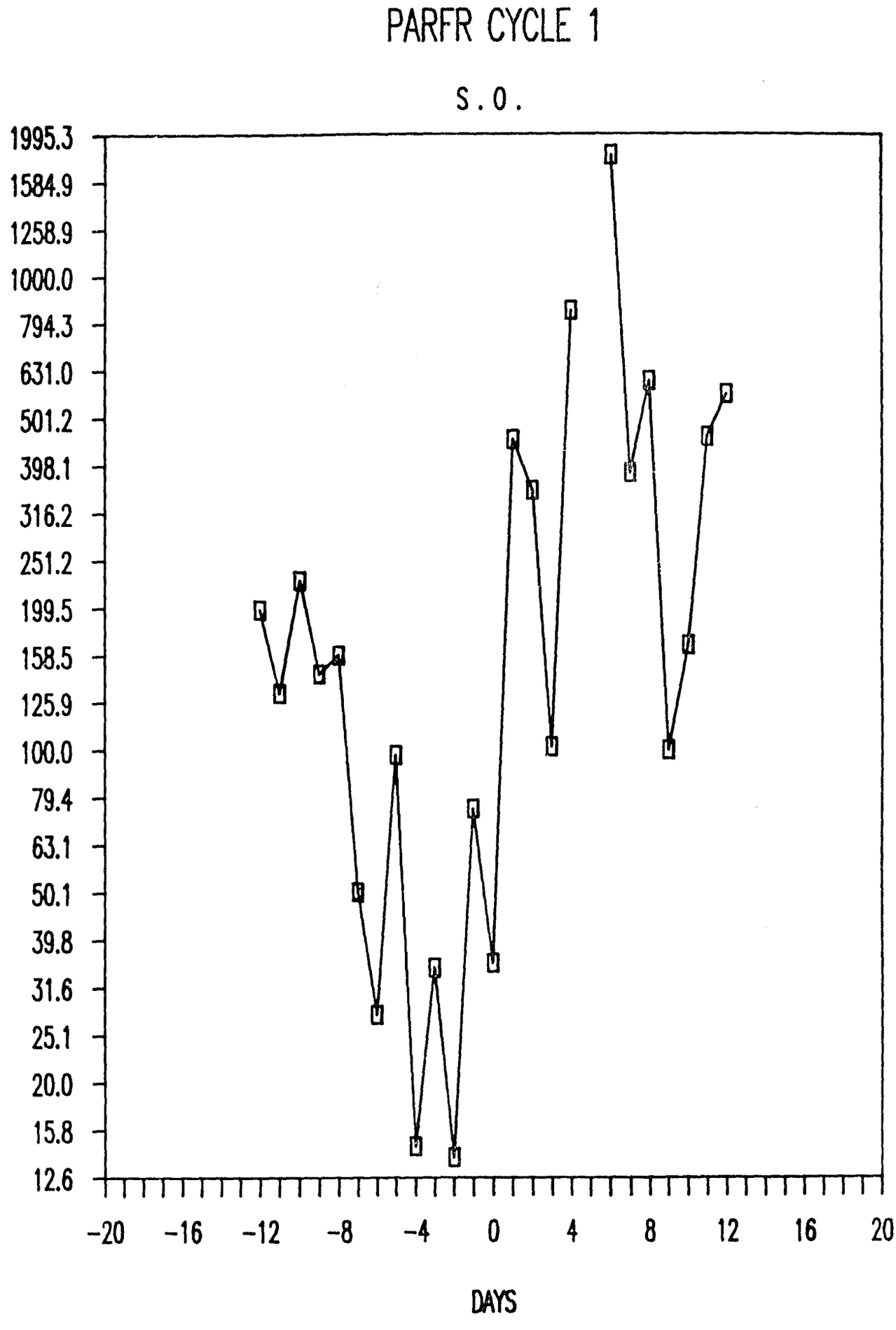
PARFR CYCLE 3





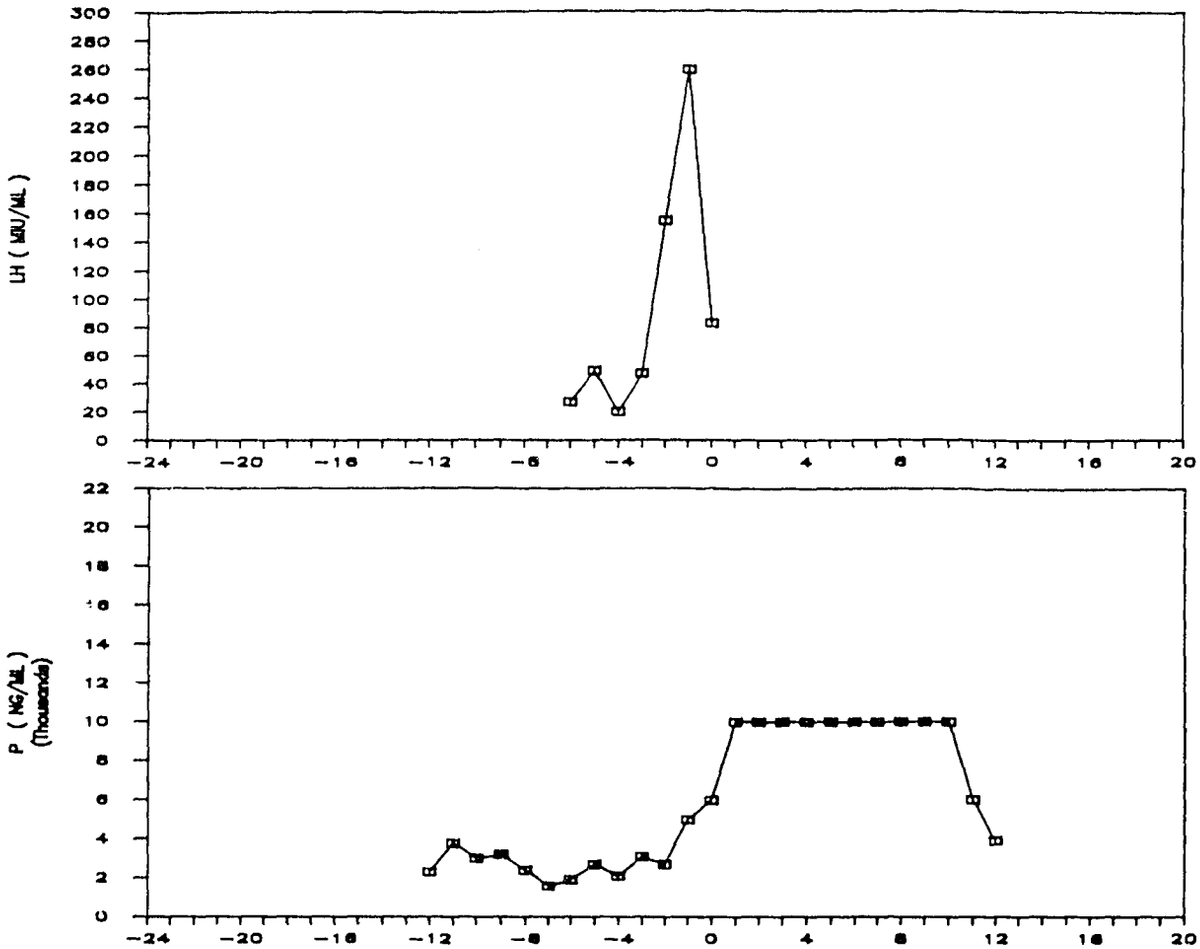

PARFR CYCLES
S.O.
2511.9
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS
C1
C2
C3
C4
C5
C6

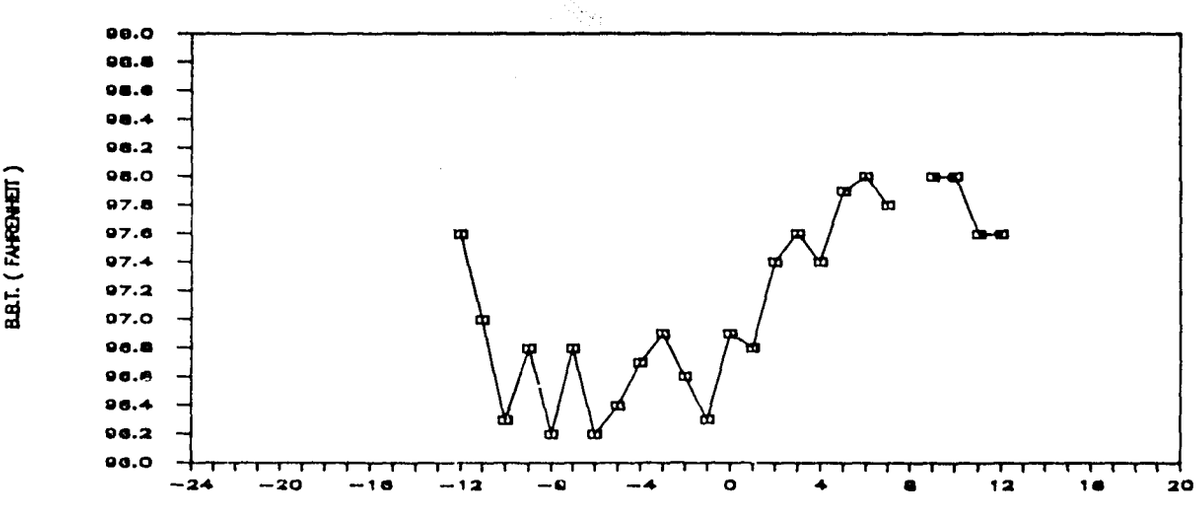
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
S.O.	11/7/84	5	-12	197.9			2300.0		97.6	PARFR CYCLE 1
	11/8/84	6	-11	132.2			3800.0		97.0	
	11/9/84	7	-10	228.0			3000.0		96.3	
	11/10/84	8	-9	145.3			3200.0		96.8	
	11/11/84	9	-8	158.8			2400.0		96.2	
	11/12/84	10	-7	50.3			1600.0		96.8	
	11/13/84	11	-6	27.8		27.0	1900.0		96.2	
	11/14/84	12	-5	98.1		49.0	2700.0		96.4	
	11/15/84	13	-4	14.7		20.0	2100.0		96.7	
	11/16/84	14	-3	34.9		47.0	3100.0		96.9	
	11/17/84	15	-2	13.9		154.0	2700.0		96.6	
	11/18/84	16	-1	75.4		259.0	5000.0		96.3	
	11/19/84	17	0	35.7		82.0	5000.0		96.9	
	11/20/84	18	1	454.4			10000.0		96.8	
	11/21/84	19	2	354.2			10000.0		97.4	
	11/22/84	20	3	101.9			10000.0		97.6	
	11/23/84	21	4	850.3			10000.0		97.4	
	11/24/84	22	5				10000.0		97.9	
	11/25/84	23	6	1808.0			10000.0		98.0	
	11/26/84	24	7	385.6			10000.0		97.8	
	11/27/84	25	8	601.9			10000.0			
	11/28/84	26	9	100.3			10000.0		98.0	
	11/29/84	27	10	166.6			10000.0		98.0	
	11/30/84	28	11	458.9			6000.0		97.6	
	12/1/84	29	12	565.1			3900.0		97.6	


PARFR CYCLE 1
S.O.
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

S.O.

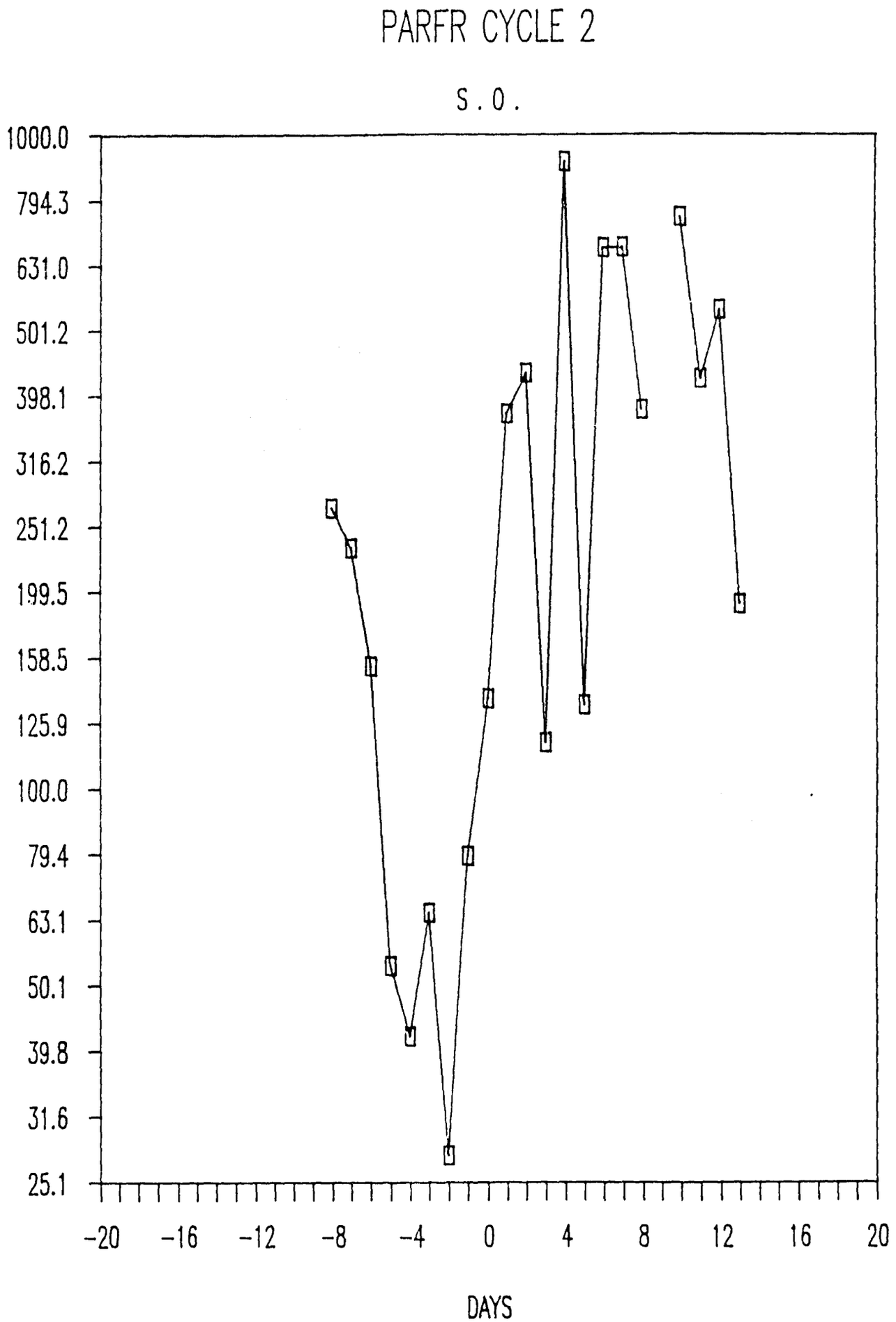
PARFR CYCLE 1



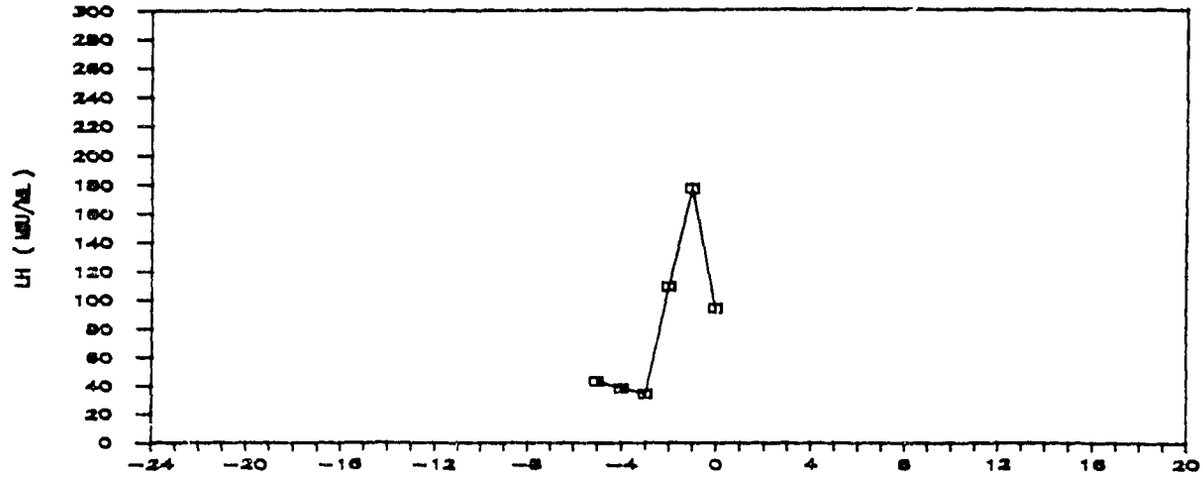


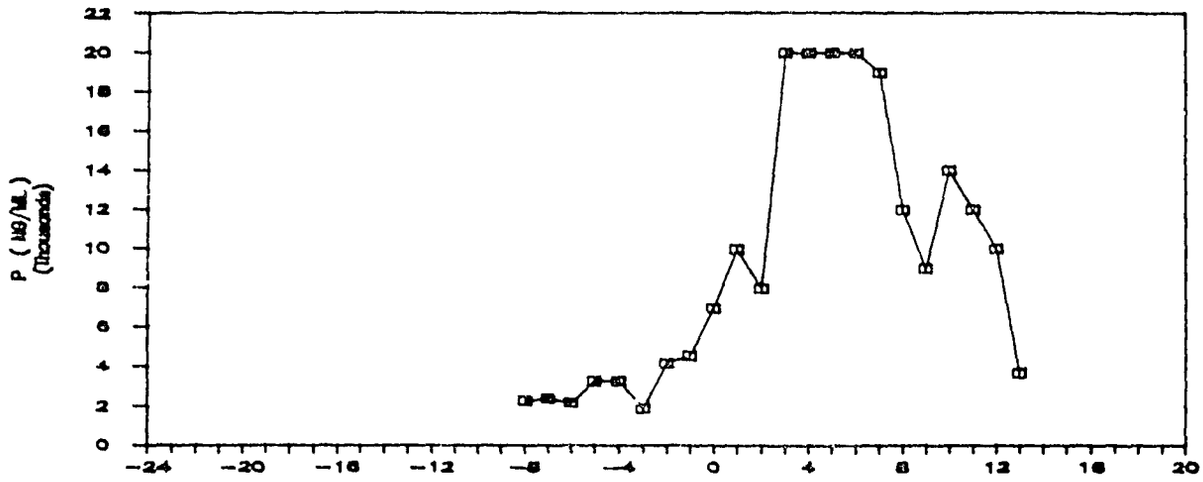
S.O.

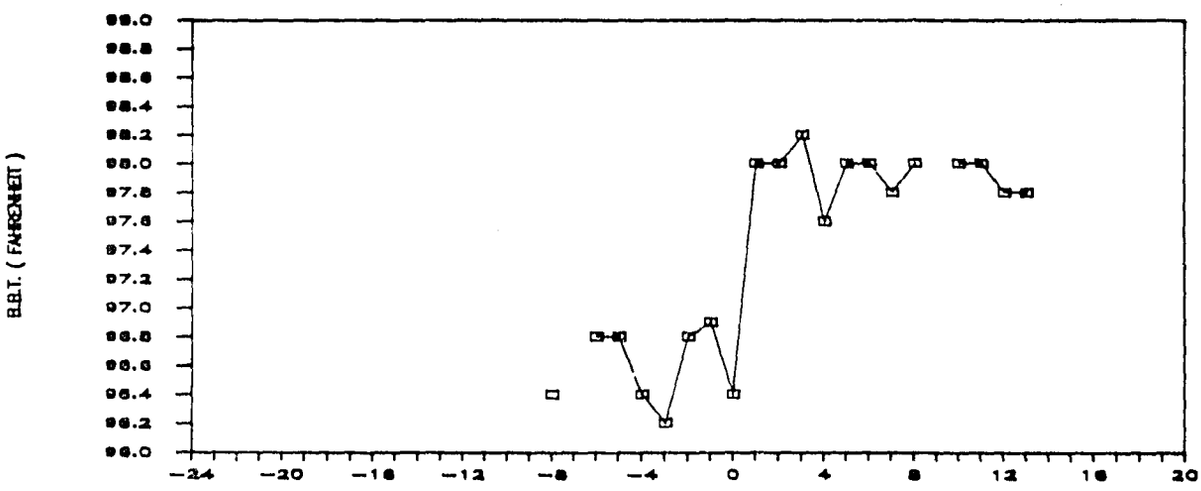
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
	1/2/85	5	-8	268.0			2300.0		96.4	PARFR CYCLE 2
	1/3/85	6	-7	232.9			2400.0		---	
	1/4/85	7	-6	153.7			2200.0		96.8	
	1/5/85	8	-5	53.7		43.0	3300.0		96.8	
	1/6/85	9	-4	42.0		38.0	3300.0		96.4	
	1/7/85	10	-3	64.7		34.0	1900.0		96.2	
	1/8/85	11	-2	27.6		109.0	4200.0		96.8	
	1/9/85	12	-1	79.1		177.0	4600.0		96.9	
	1/10/85	13	0	137.3		94.0	7000.0		96.4	
	1/11/85	14	1	374.4			10000.0		98.0	
	1/12/85	15	2	431.2			8000.0		98.0	
	1/13/85	16	3	117.7			20000.0		98.2	
	1/14/85	17	4	907.6			20000.0		97.6	
	1/15/85	18	5	134.4			20000.0		98.0	
	1/16/85	19	6	670.1			20000.0		98.0	
	1/17/85	20	7	671.4			19000.0		97.8	
	1/18/85	21	8	378.9			12000.0		98.0	
	1/19/85	22	9	---			9000.0		---	
	1/20/85	23	10	748.1			14000.0		98.0	
	1/21/85	24	11	421.6			12000.0		98.0	
	1/22/85	25	12	538.1			10000.0		97.8	
	1/23/85	26	13	190.8			3700.0		97.8	


PARFR CYCLE 2
S.O.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

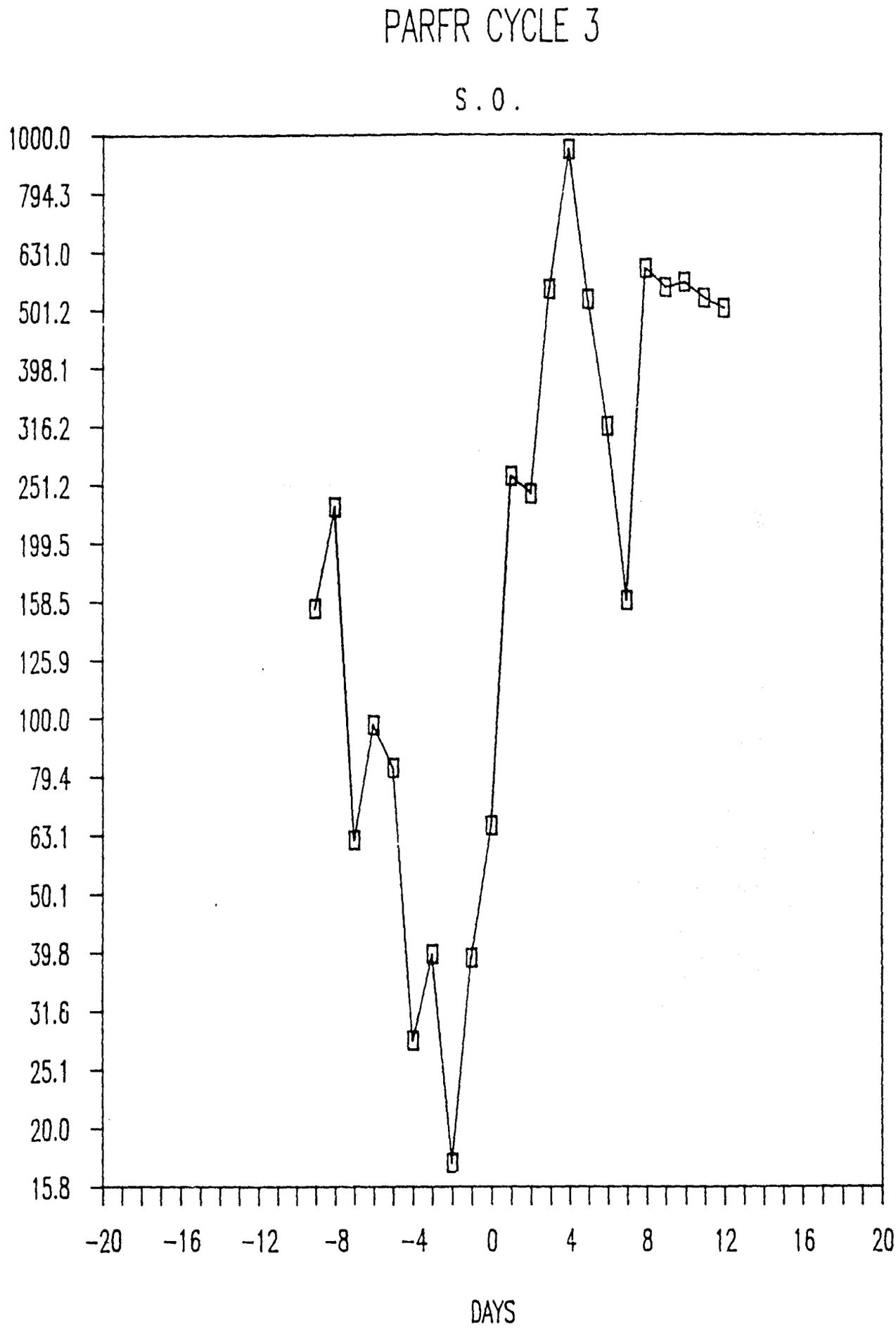
PARFR CYCLE 2



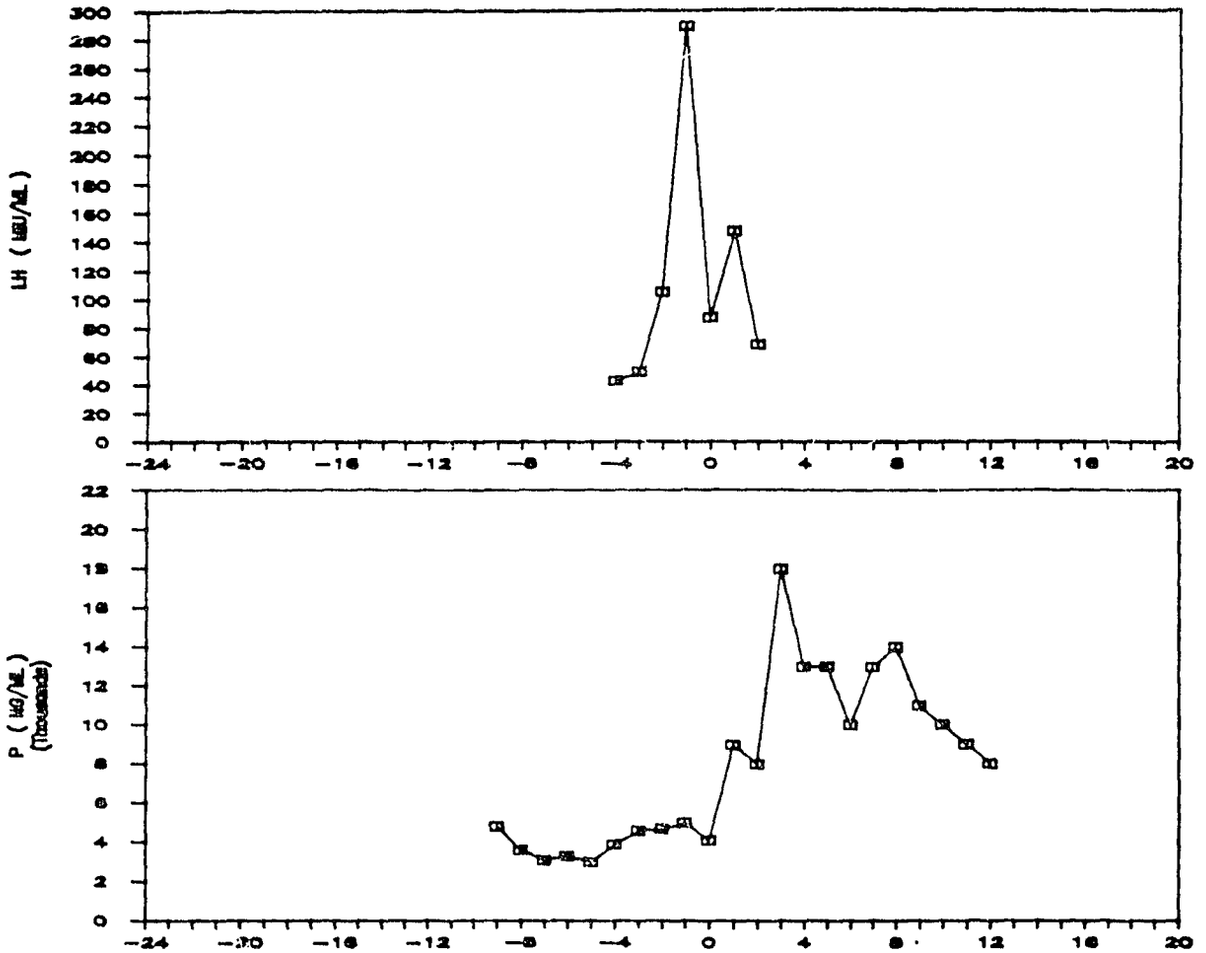


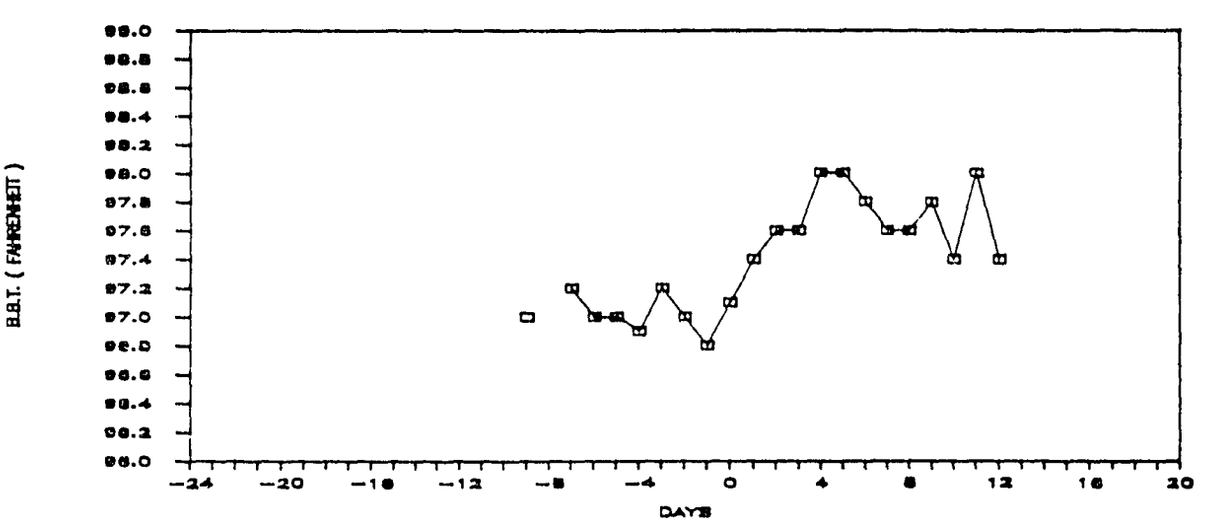


NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BST	REMARKS
S.O.	1/28/85	5	-9	154.0			4800.0		97.0	PARFR CYCLE 3
	1/29/85	6	-8	229.9			3600.0		---	
	1/30/85	7	-7	62.0			3100.0		97.2	
	1/31/85	8	-6	97.4			3300.0		97.0	
	2/1/85	9	-5	82.4			3000.0		97.0	
	2/2/85	10	-4	28.2		43.0	3900.0		96.9	
	2/3/85	11	-3	39.6		49.0	4600.0		97.2	
	2/4/85	12	-2	17.4		105.0	4700.0		97.0	
	2/5/85	13	-1	39.0		289.0	5000.0		96.8	
	2/6/85	14	0	65.7		07.0	4100.0		97.1	
	2/7/85	15	1	260.5		147.0	9000.0		97.4	
	2/8/85	16	2	242.6		68.0	8000.0		97.6	
	2/9/85	17	3	544.0			18000.0		97.6	
	2/10/85	18	4	940.3			13000.0		98.0	
	2/11/85	19	5	522.3			13000.0		98.0	
	2/12/85	20	6	316.5			10000.0		97.8	
	2/13/85	21	7	159.3			13000.0		97.6	
	2/14/85	22	8	589.5			14000.0		97.6	
	2/15/85	23	9	545.3			11000.0		97.8	
	2/16/85	24	10	557.6			10000.0		97.4	
	2/17/85	25	11	523.1			9000.0		98.0	
	2/18/85	26	12	502.7			8000.0		97.4	

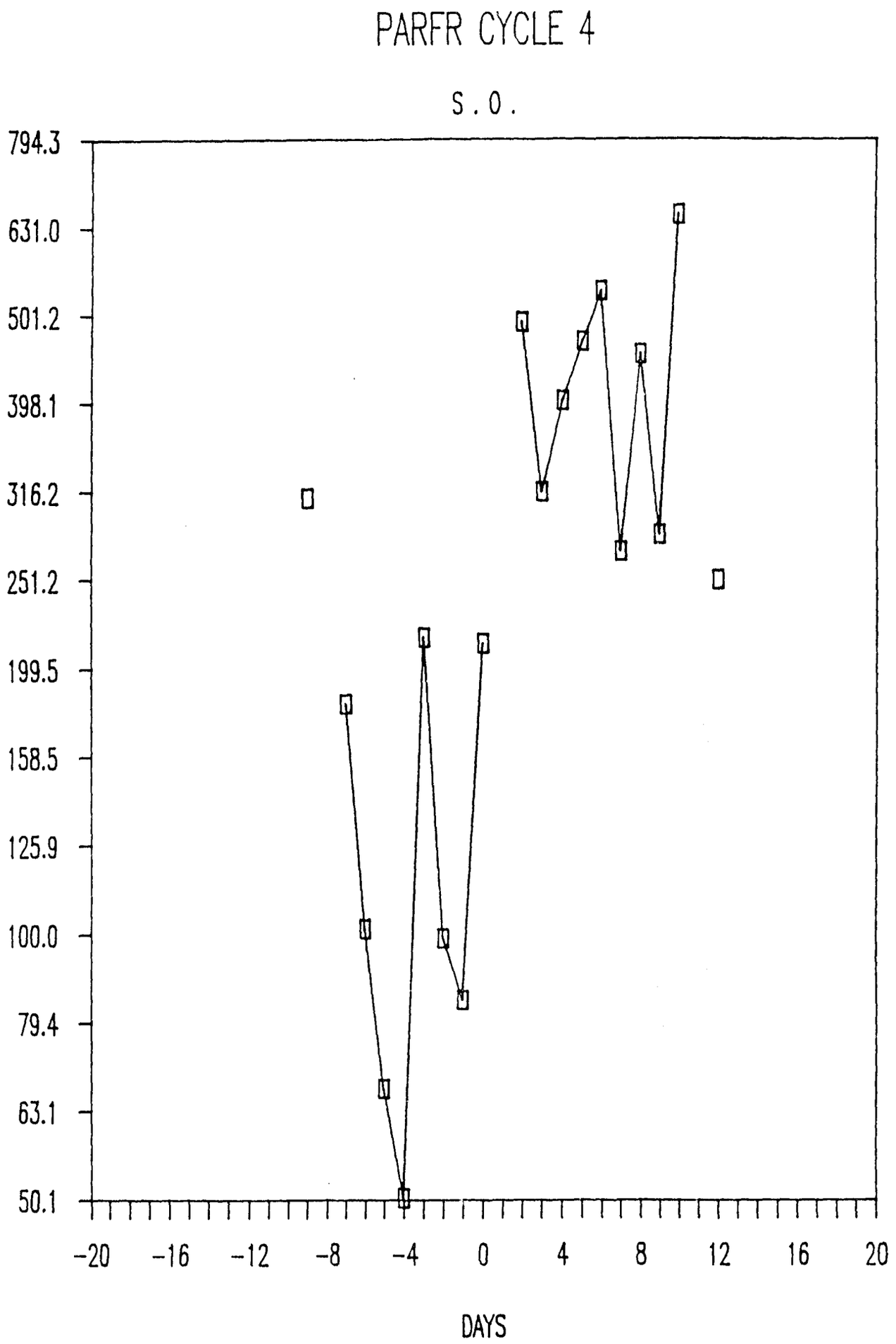

PARFR CYCLE 3
S.O.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

PARFR CYCLE 3

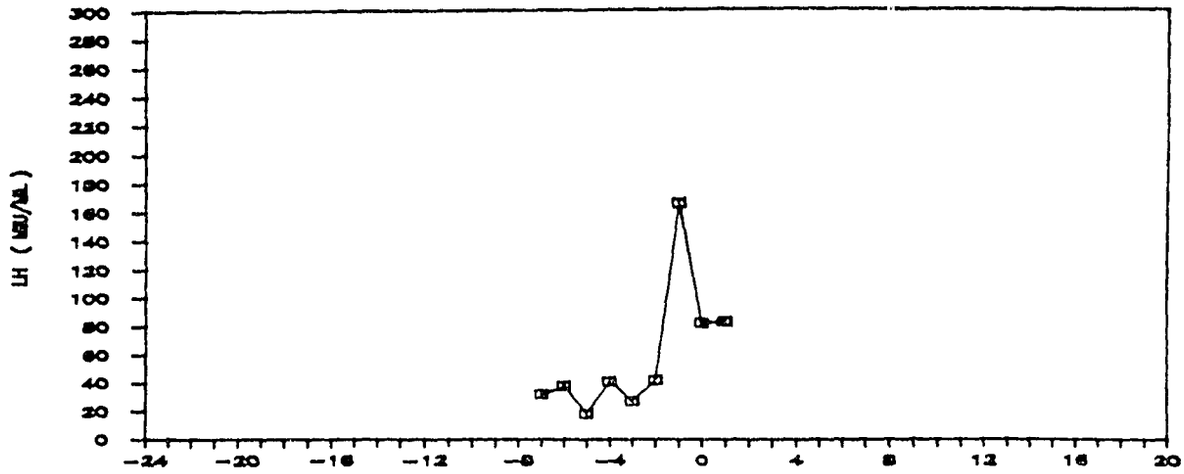


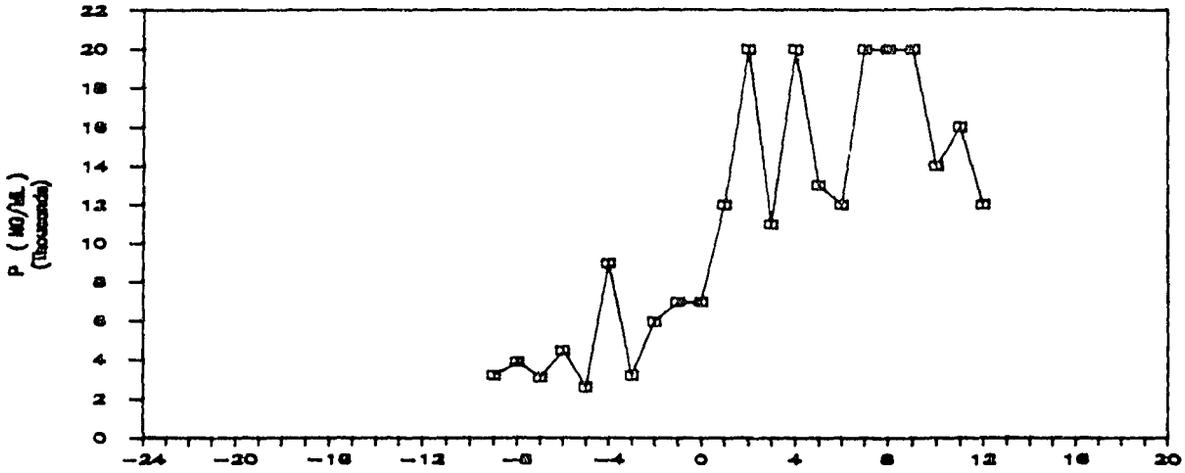


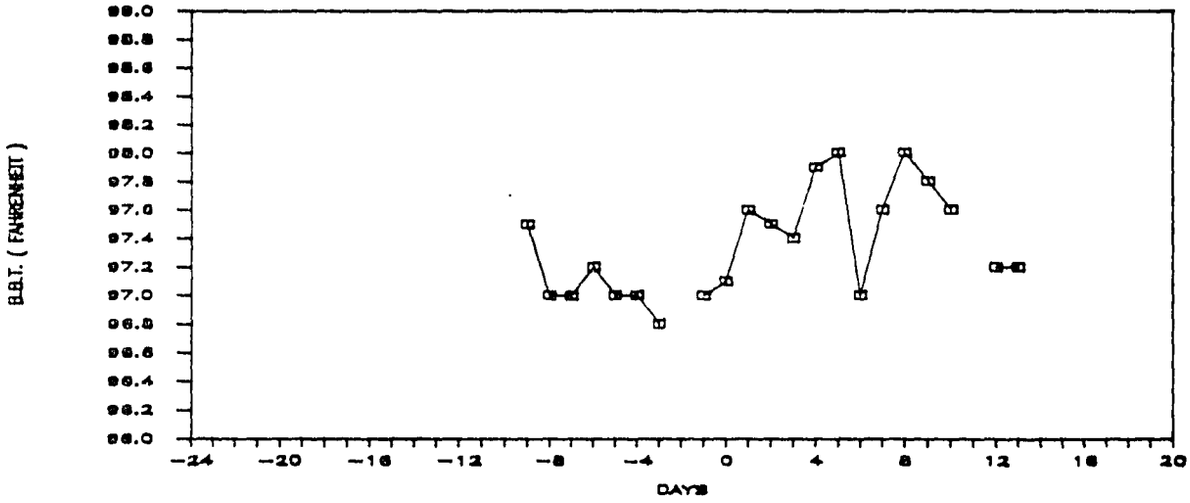
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
S.O.	2/23/85	5	-9	312.0			3200.0		97.5	PARFR CYCLE 4
	2/24/85	6	-8	---			3900.0		97.0	
	2/25/85	7	-7	182.7		32.0	3100.0		97.0	
	2/26/85	8	-6	101.7		38.0	4500.0		97.2	
	2/27/85	9	-5	67.1		18.0	2600.0		97.0	
	2/28/85	10	-4	50.5		41.0	9000.0		97.0	
	3/1/85	11	-3	217.2		27.0	3200.0		96.8	
	3/2/85	12	-2	99.2		42.0	6000.0		---	
	3/3/85	13	-1	84.5		165.0	7000.0		97.0	
	3/4/85	14	0	214.1		82.0	7000.0		97.1	
	3/5/85	15	1	---		83.0	12000.0		97.6	
	3/6/85	16	2	494.4			20000.0		97.5	
	3/7/85	17	3	317.9			11000.0		97.4	
	3/8/85	18	4	402.8			20000.0		97.9	
	3/9/85	19	5	470.0			13000.0		98.0	
	3/10/85	20	6	536.7			12000.0		97.0	
	3/11/85	21	7	271.8			20000.0		97.6	
	3/12/85	22	8	454.9			20000.0		98.0	
	3/13/85	23	9	283.6			20000.0		97.8	
	3/14/85	24	10	654.0			14000.0		97.6	
	3/15/85	25	11	---			16000.0		---	
	3/16/85	26	12	252.2			12000.0		97.2	
	3/17/85	27	13	---			---		97.2	

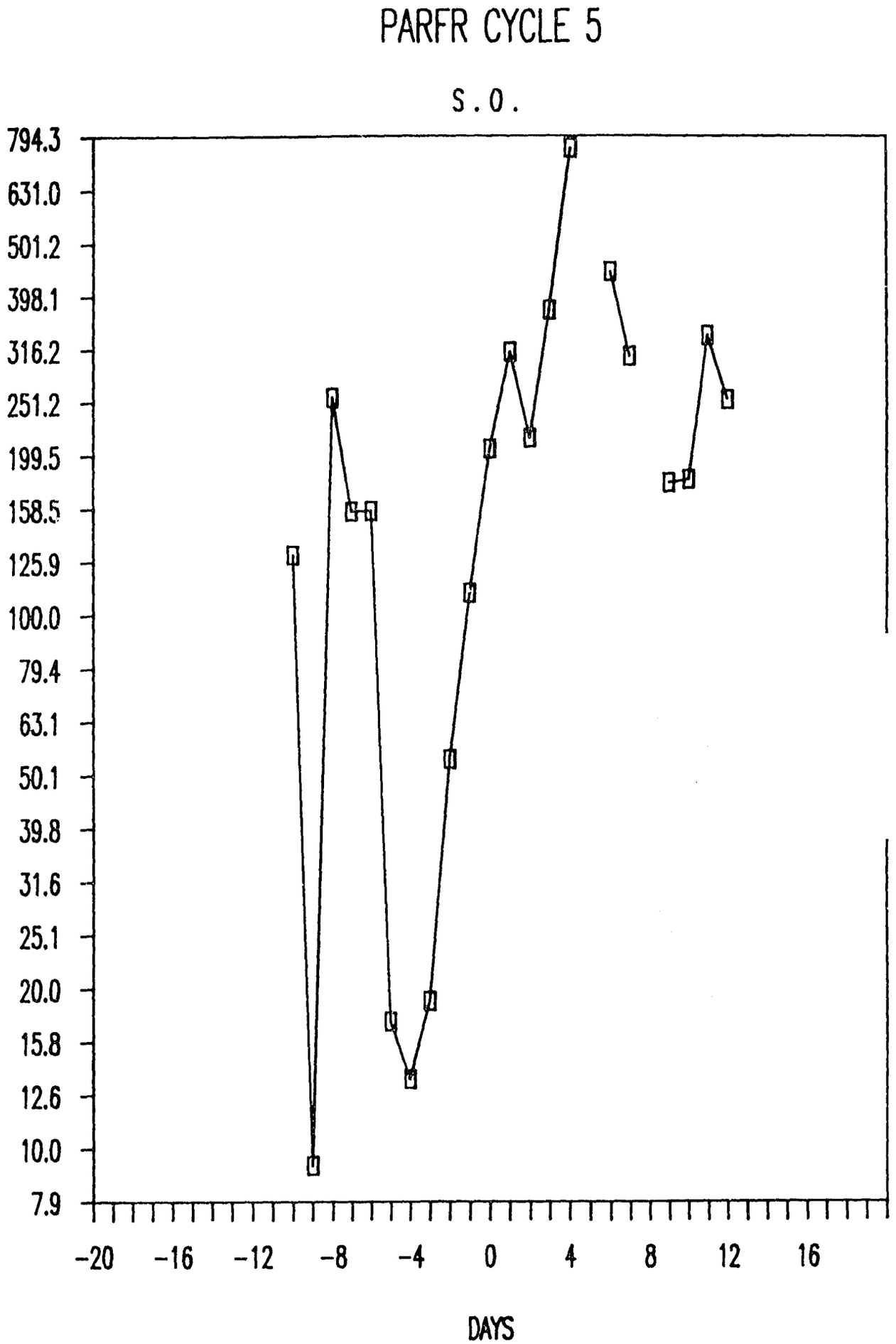

PARFR CYCLE 4
S.O.
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

PARFR CYCLE 4





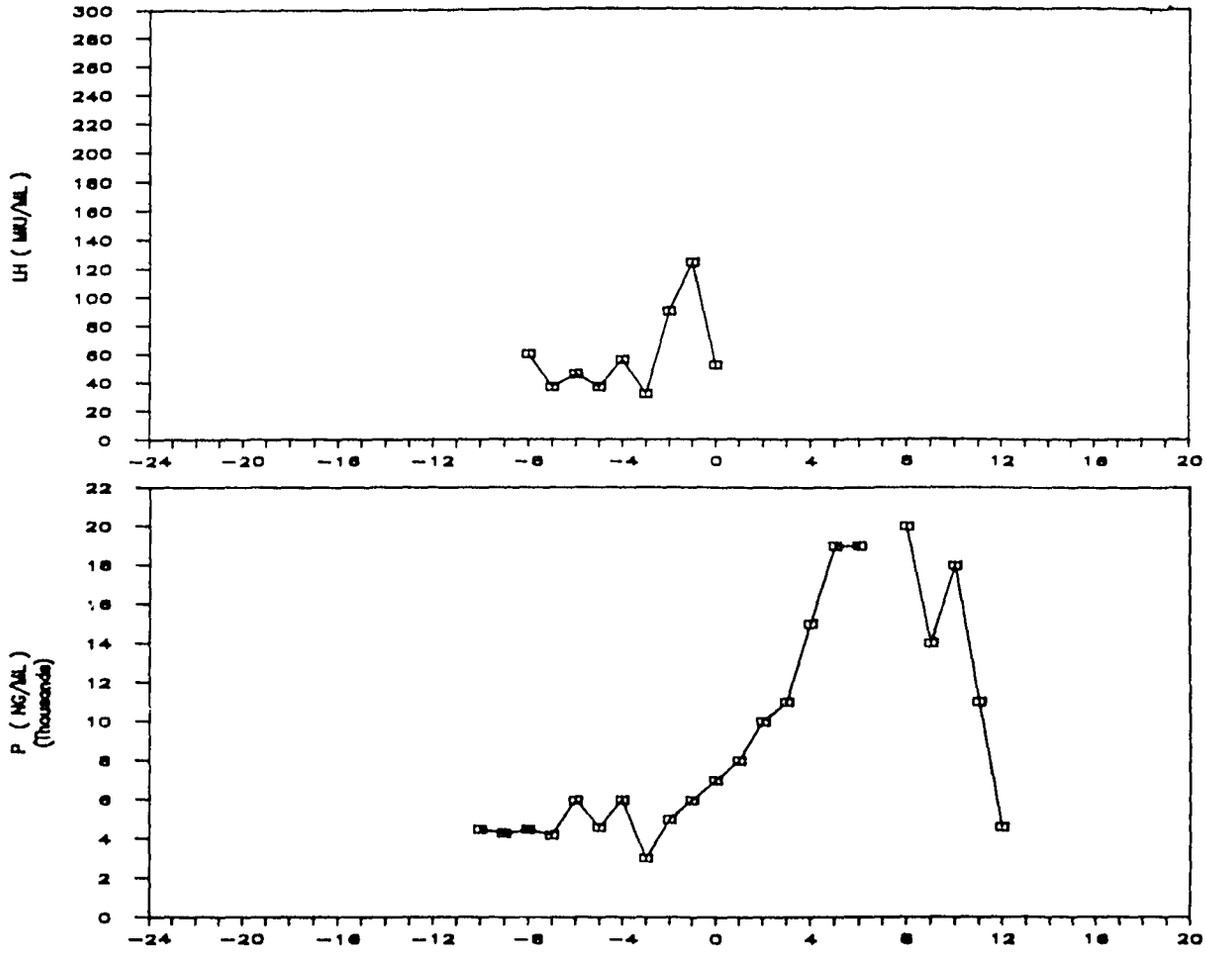


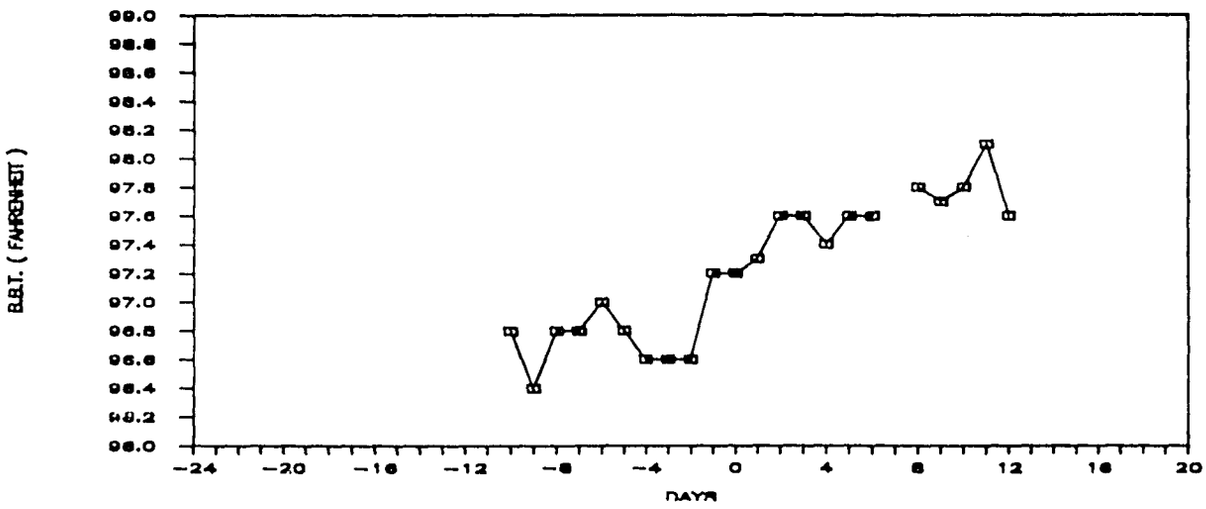

PARFR CYCLE 5
S.O.
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
7.9
-20
-16
-12
-8
-4
0
4
8
12
16
DAYS

NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
S.O.	3/21/85	5	-10	130.0			4500.0		96.8	PARFR CYCLE 5
	3/22/85	6	-9	9.3			4300.0		96.4	
	3/23/85	7	-8	256.6		60.0	4500.0		96.8	
	3/24/85	8	-7	156.7		37.0	4200.0		96.8	
	3/25/85	9	-6	157.2		46.0	6000.0		97.0	
	3/26/85	10	-5	17.3		37.0	4600.0		96.8	
	3/27/85	11	-4	13.5		56.0	6000.0		96.6	
	3/28/85	12	-3	18.9		32.0	3000.0		96.6	
	3/29/85	13	-2	53.8		90.0	5000.0		96.6	
	3/30/85	14	-1	110.2		124.0	6000.0		97.2	
	3/31/85	15	0	206.0		52.0	7000.0		97.2	
	4/1/85	16	1	313.1			8000.0		97.3	
	4/2/85	17	2	215.6			10000.0		97.6	
	4/3/85	18	3	375.3			11000.0		97.6	
	4/4/85	19	4	755.2			15000.0		97.4	
	4/5/85	20	5				19000.0		97.6	
	4/6/85	21	6	442.9			19000.0		97.6	
	4/7/85	22	7	306.7						
	4/8/85	23	8				20000.0		97.8	
	4/9/85	24	9	177.1			14000.0		97.7	
	4/10/85	25	10	179.7			18000.0		97.8	
	4/11/85	26	11	336.0			11000.0		98.1	
	4/12/85	27	12	253.9			4600.0		97.6	

S.O.

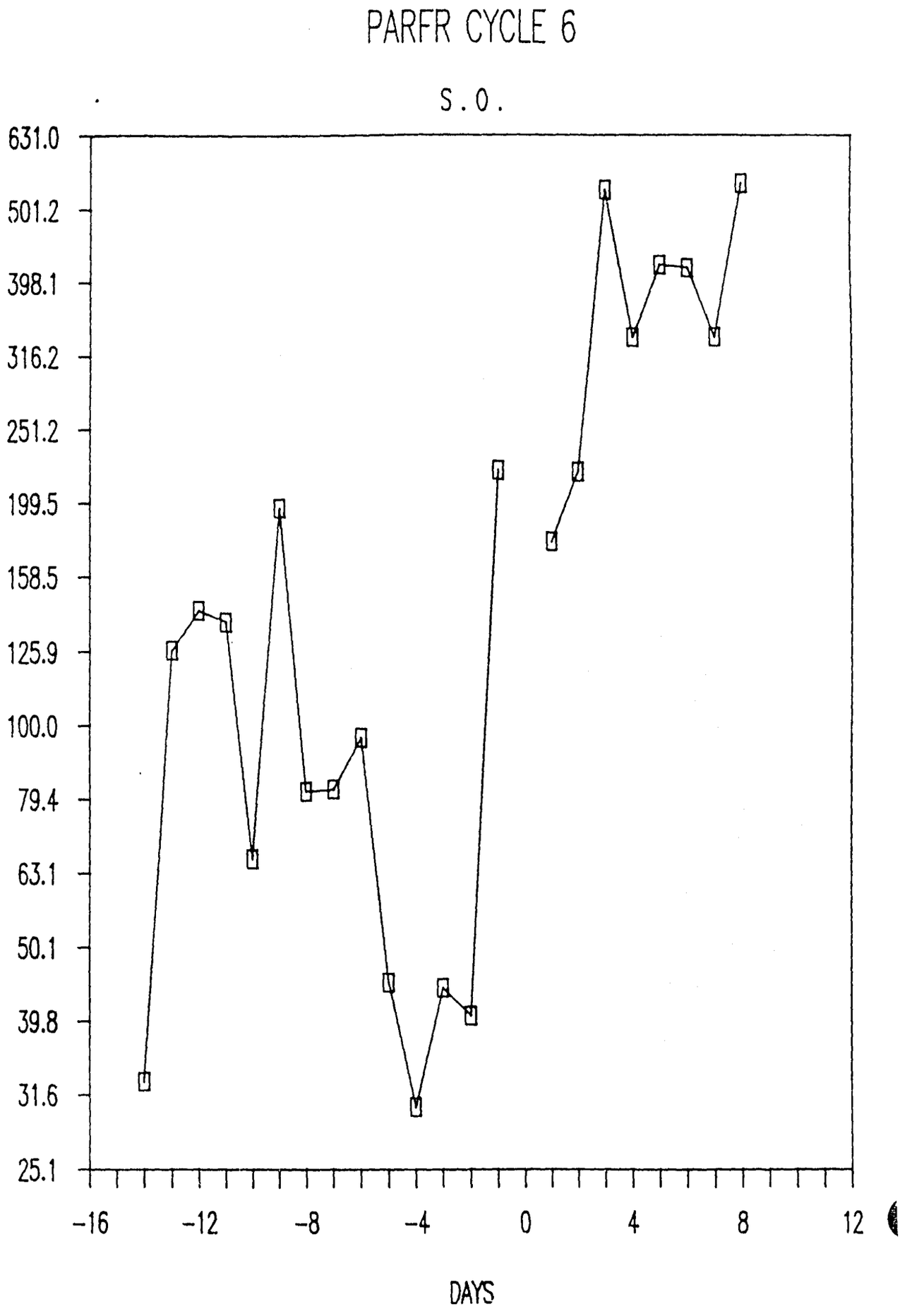
PARFR CYCLE 5





S.O.

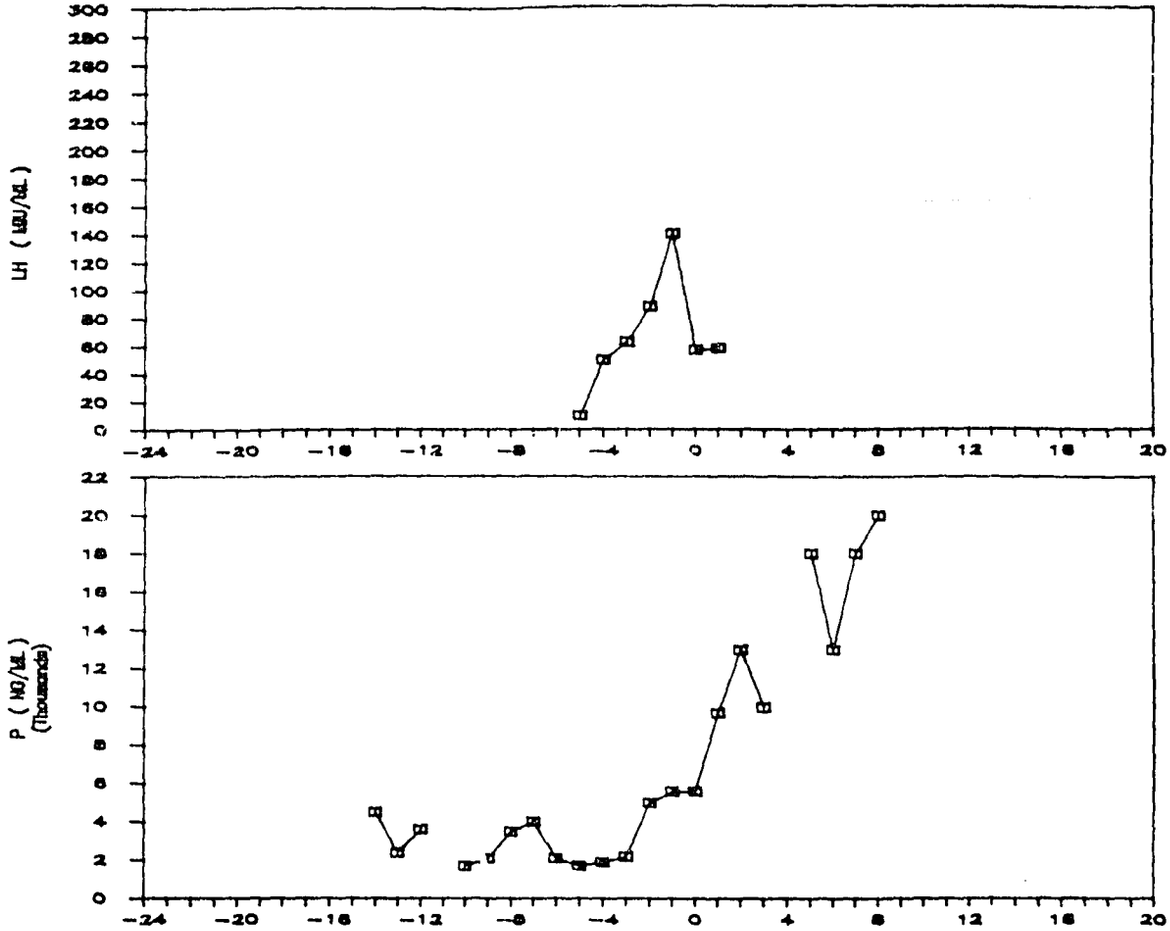
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
	4/17/85	5	-14	33.0			4500.0		96.7	PARFR CYCLE 6
	4/18/85	6	-13	126.4			2400.0		97.3	
	4/19/85	7	-12	142.8			3600.0		97.2	
	4/20/85	8	-11	137.9					97.4	
	4/21/85	9	-10	66.0			1700.0		97.3	
	4/22/85	10	-9	196.6			2100.0		96.3	
	4/23/85	11	-8	81.3			3500.0		97.1	
	4/24/85	12	-7	81.9			4000.0		97.3	
	4/25/85	13	-6	96.3			2100.0		96.6	
	4/26/85	14	-5	44.9		10.0	1700.0		97.0	
	4/27/85	15	-4	30.5		50.0	1900.0			
	4/28/85	16	-3	44.2		63.0	2200.0		96.6	
	4/29/85	17	-2	40.5		88.0	5000.0		96.8	
	4/30/85	18	-1	221.8		140.0	5600.0		96.8	
	5/1/85	19	0			57.0	5600.0		97.4	
	5/2/85	20	1	177.5		58.0	9700.0		96.8	
	5/3/85	21	2	220.6			13000.0		97.2	
	5/4/85	22	3	533.3			10000.0		97.6	
	5/5/85	23	4	335.5					98.0	
	5/6/85	24	5	420.2			18000.0		98.0	
	5/7/85	25	6	417.0			13000.0		98.0	
	5/8/85	26	7	335.9			18000.0		97.8	
	5/9/85	27	8	544.6			20000.0		98.0	

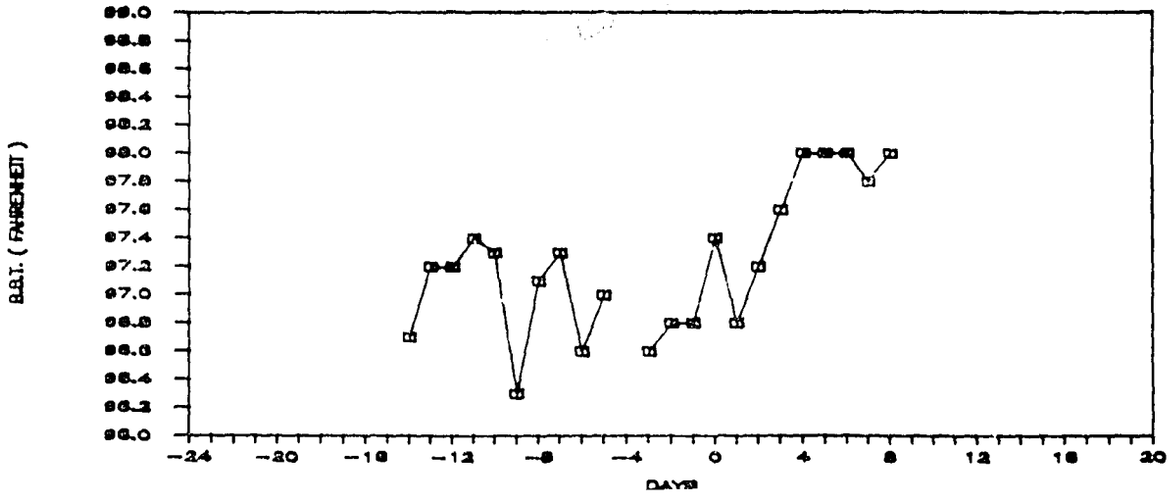

PARFR CYCLE 6
S.O.
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
-16
-12
-8
-4
0
4
8
12
DAYS

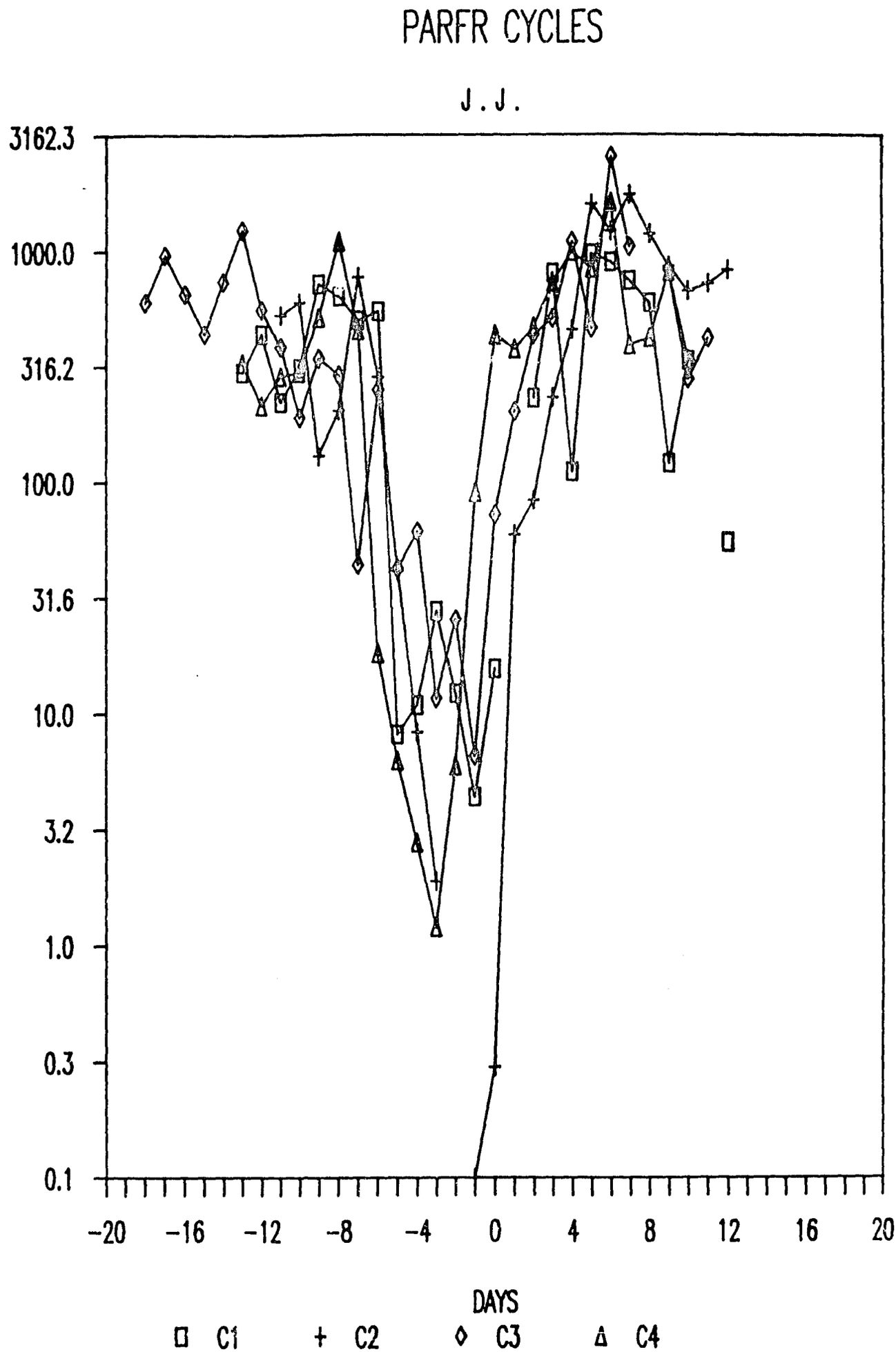
S.O.

PARFR CYCLE 6

S.O.

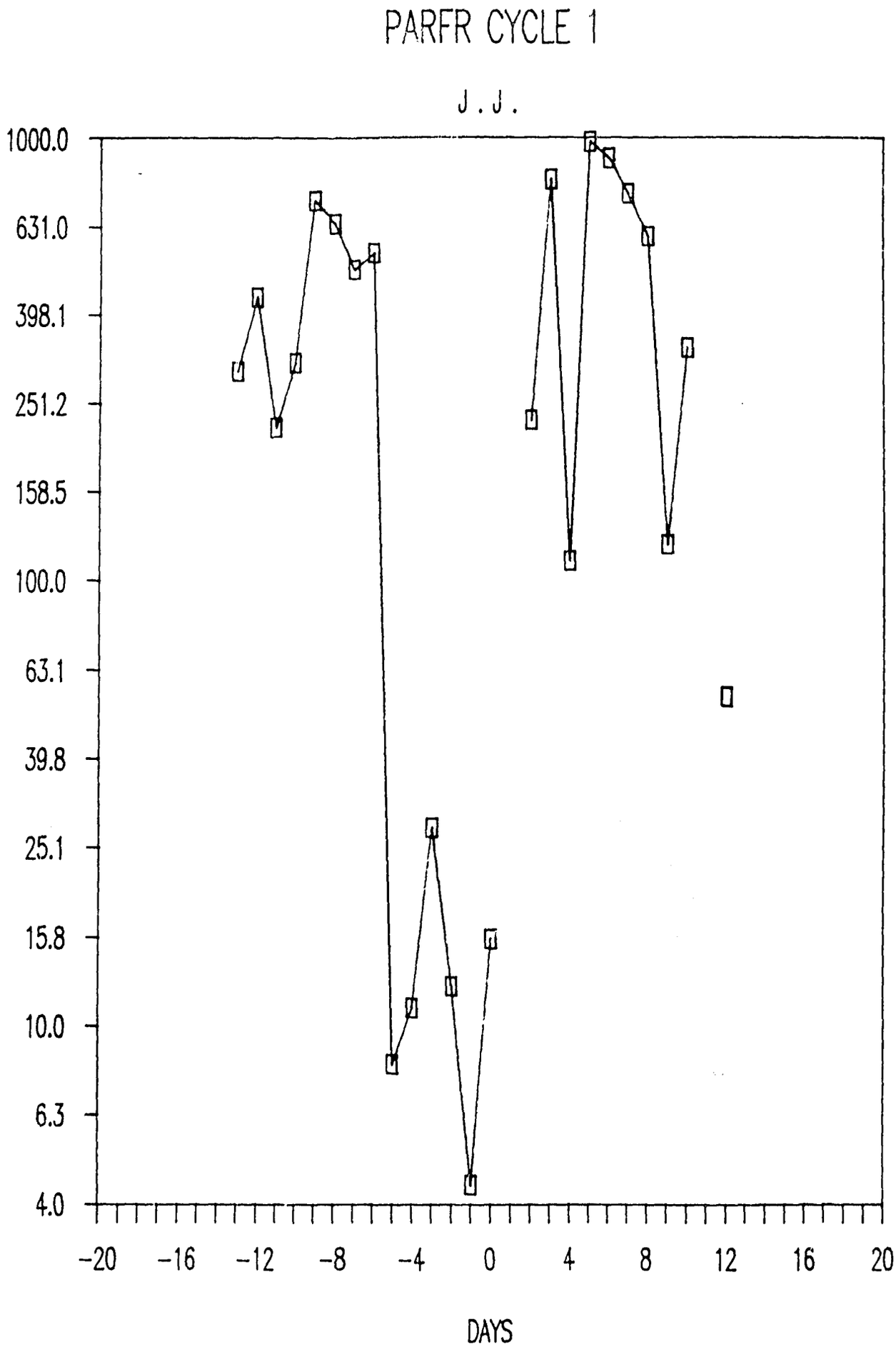





PARFR CYCLES
J.J.
3162.3
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS
C1
C2
C3
C4

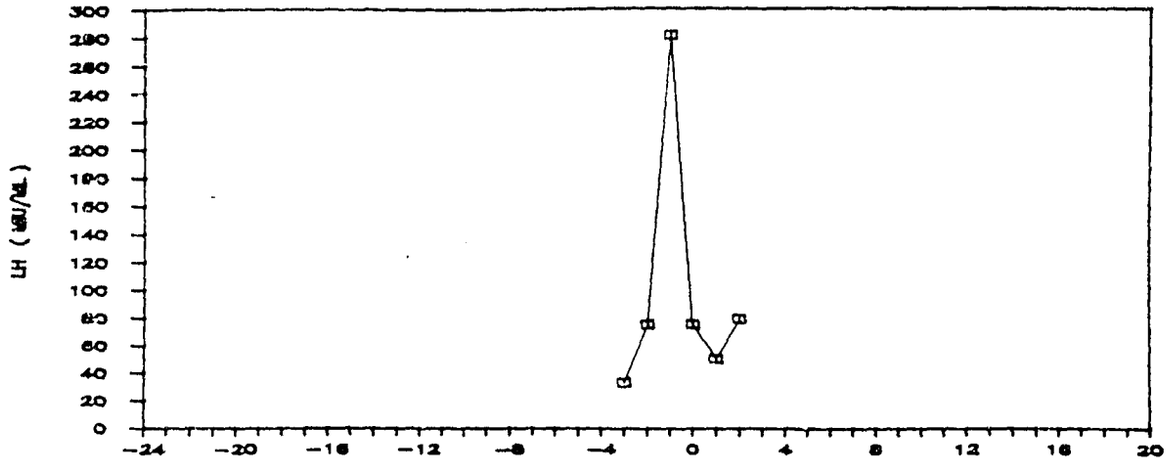
Total: 4 cycles

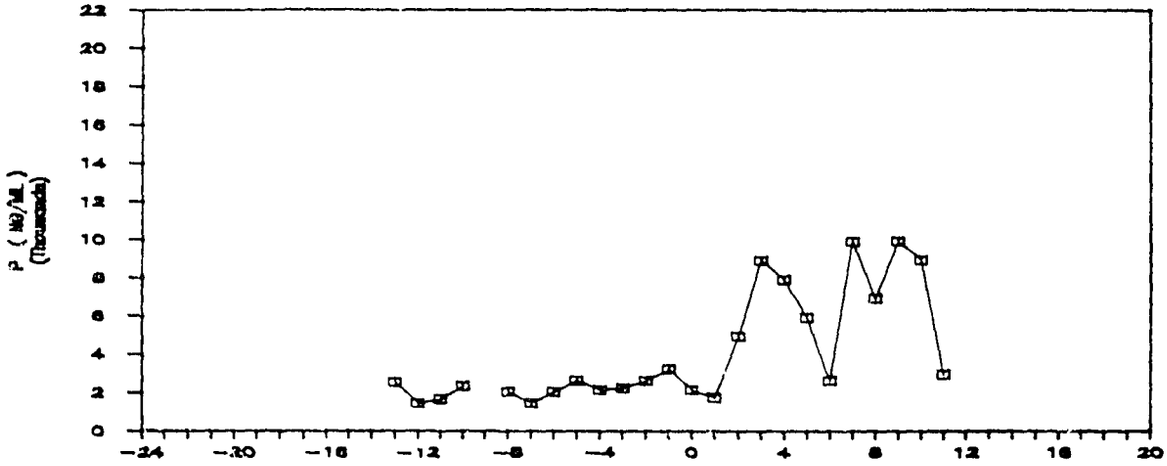
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
J.J.	1/14/85	4	-13	295.7			2600.0		97.4	PARFR CYCLE 1
	1/15/85	5	-12	434.6			1500.0		98.0	
	1/16/85	6	-11	221.1			1700.0		97.7	
	1/17/85	7	-10	309.5			2400.0		---	
	1/18/85	8	-9	721.9			---		98.0	
	1/19/85	9	-8	639.0			2100.0		98.4	
	1/20/85	10	-7	503.6			1500.0		98.3	
	1/21/85	11	-6	548.8			2100.0		98.0	
	1/22/85	12	-5	8.2			2700.0		97.6	
	1/23/85	13	-4	11.0			2200.0		97.7	
	1/24/85	14	-3	27.9		33.0	2300.0		97.9	
	1/25/85	15	-2	12.3		75.0	2700.0		97.5	
	1/26/85	16	-1	4.4		281.0	3300.0		97.8	
	1/27/85	17	0	15.7		75.0	2200.0		98.0	
	1/28/85	18	1	---		50.0	1800.0		98.0	
	1/29/85	19	2	231.3		79.0	5000.0		97.6	
	1/30/85	20	3	806.0			9000.0		97.7	
	1/31/85	21	4	111.4			8000.0		98.0	
	2/1/85	22	5	977.4			6000.0		98.0	
	2/2/85	23	6	900.7			2700.0		97.3	
	2/3/85	24	7	748.1			10000.0		97.8	
	2/4/85	25	8	600.0			7000.0		97.5	
	2/5/85	26	9	120.9			10000.0		---	
	2/6/85	27	10	335.7			9000.0		97.7	
	2/7/85	28	11	---			3000.0		97.0	
	2/8/85	29	12	54.9			---		97.4	

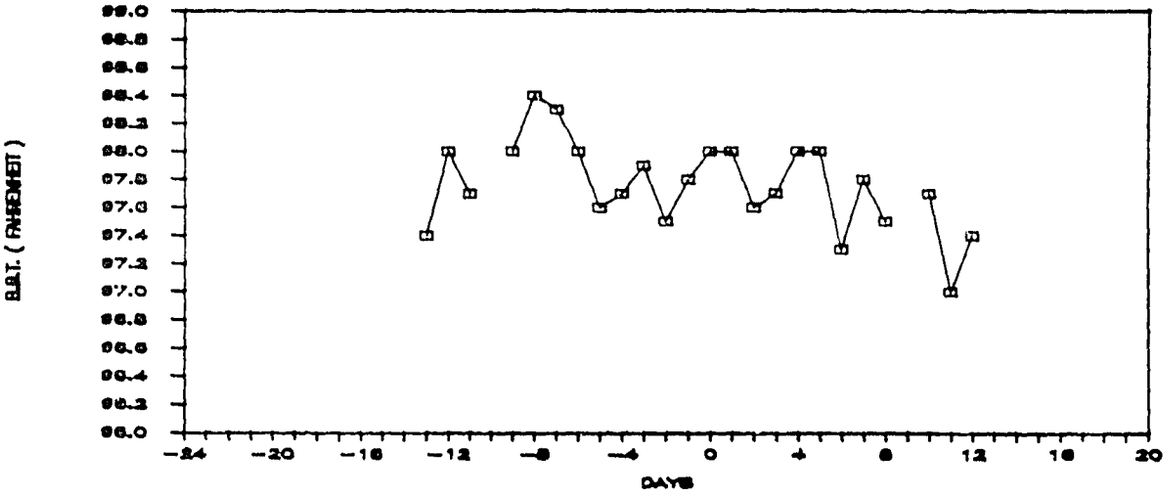

PARFR CYCLE 1
J.J.
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

J.J.

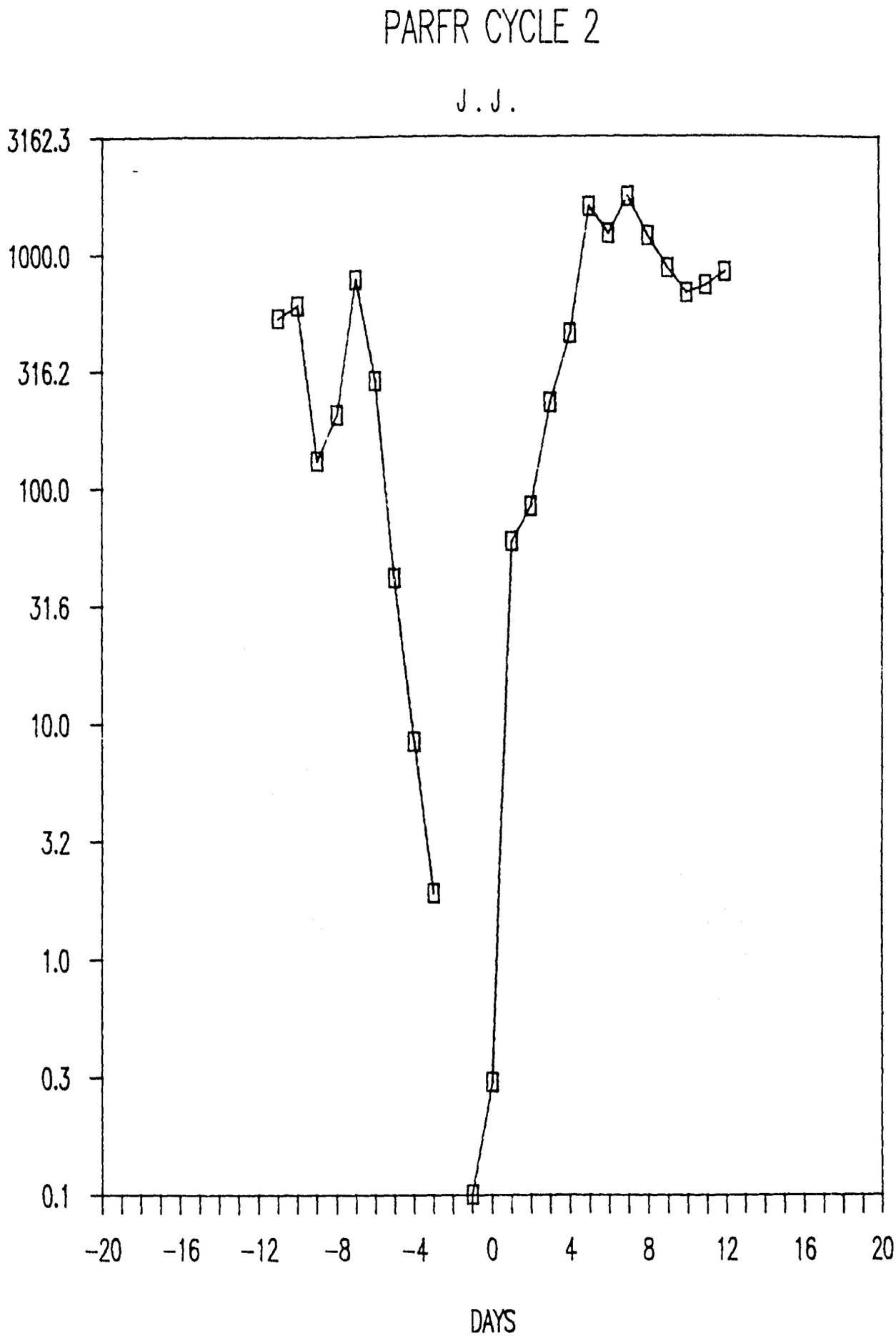
PARFR CYCLE 1





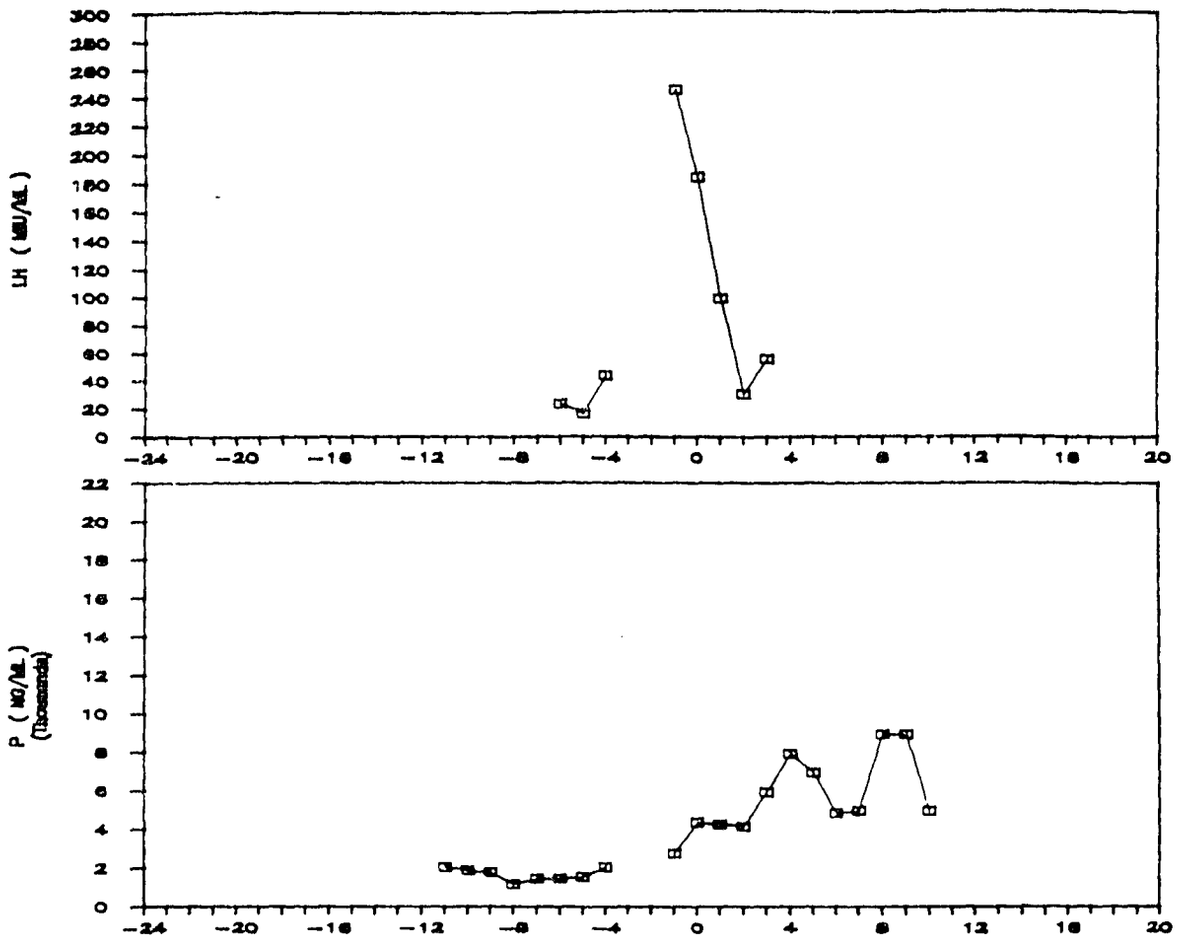


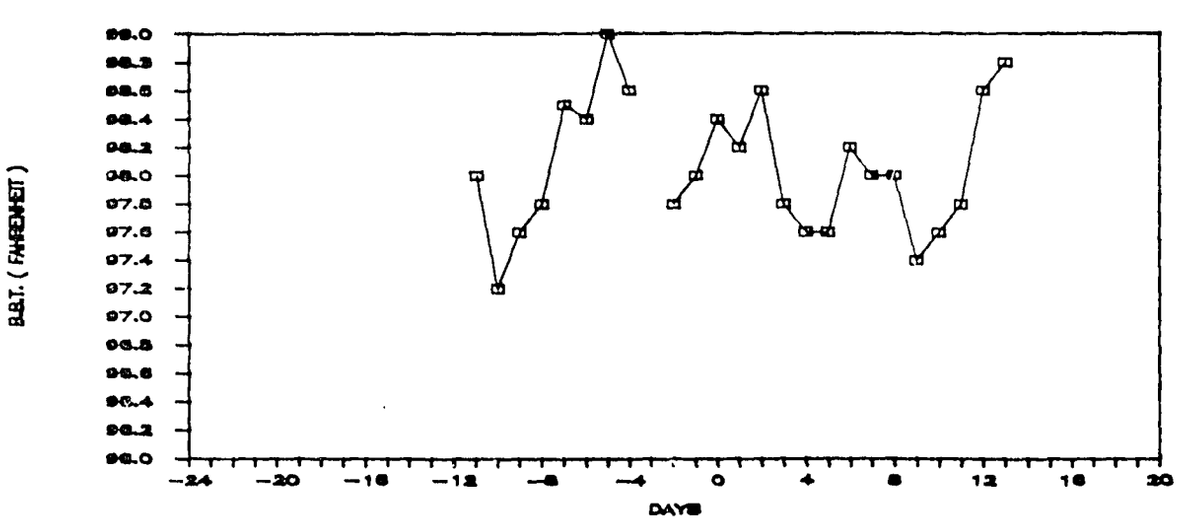
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
J.J.	2/13/85	5	-11	526.2			2100.0		98.0	PARFR CYCLE 2
	2/14/85	6	-10	599.2			1900.0		97.2	
	2/15/85	7	-9	130.1			1800.0		97.6	
	2/16/85	8	-8	204.5			1200.0		97.8	
	2/17/85	9	-7	771.4			1500.0		98.5	
	2/18/85	10	-6	284.9		23.0	1500.0		98.4	
	2/19/85	11	-5	41.6		16.0	1600.0		99.0	
	2/20/85	12	-4	8.4		43.0	2100.0		98.6	
	2/21/85	13	-3	1.9		---	---		---	
	2/22/85	14	-2	---		---	---		97.8	
	2/23/85	15	-1	0.1		244.0	2800.0		98.0	
	2/24/85	16	0	0.3		183.0	4400.0		98.4	
	2/25/85	17	1	59.7		98.0	4300.0		98.2	
	2/26/85	18	2	84.3		30.0	4200.0		98.6	
	2/27/85	19	3	232.6		55.0	6000.0		97.8	
	2/28/85	20	4	457.4			8000.0		97.6	
	3/1/85	21	5	1600.1			7000.0		97.6	
	3/2/85	22	6	1225.0			4900.0		98.2	
	3/3/85	23	7	1753.0			5000.0		98.0	
	3/4/85	24	8	1192.0			9000.0		98.0	
	3/5/85	25	9	863.5			9000.0		97.4	
	3/6/85	26	10	674.7			5000.0		97.6	
	3/7/85	27	11	731.3					97.8	
	3/8/85	28	12	833.9					98.6	


PARFR CYCLE 2
J.J.
3162.3
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

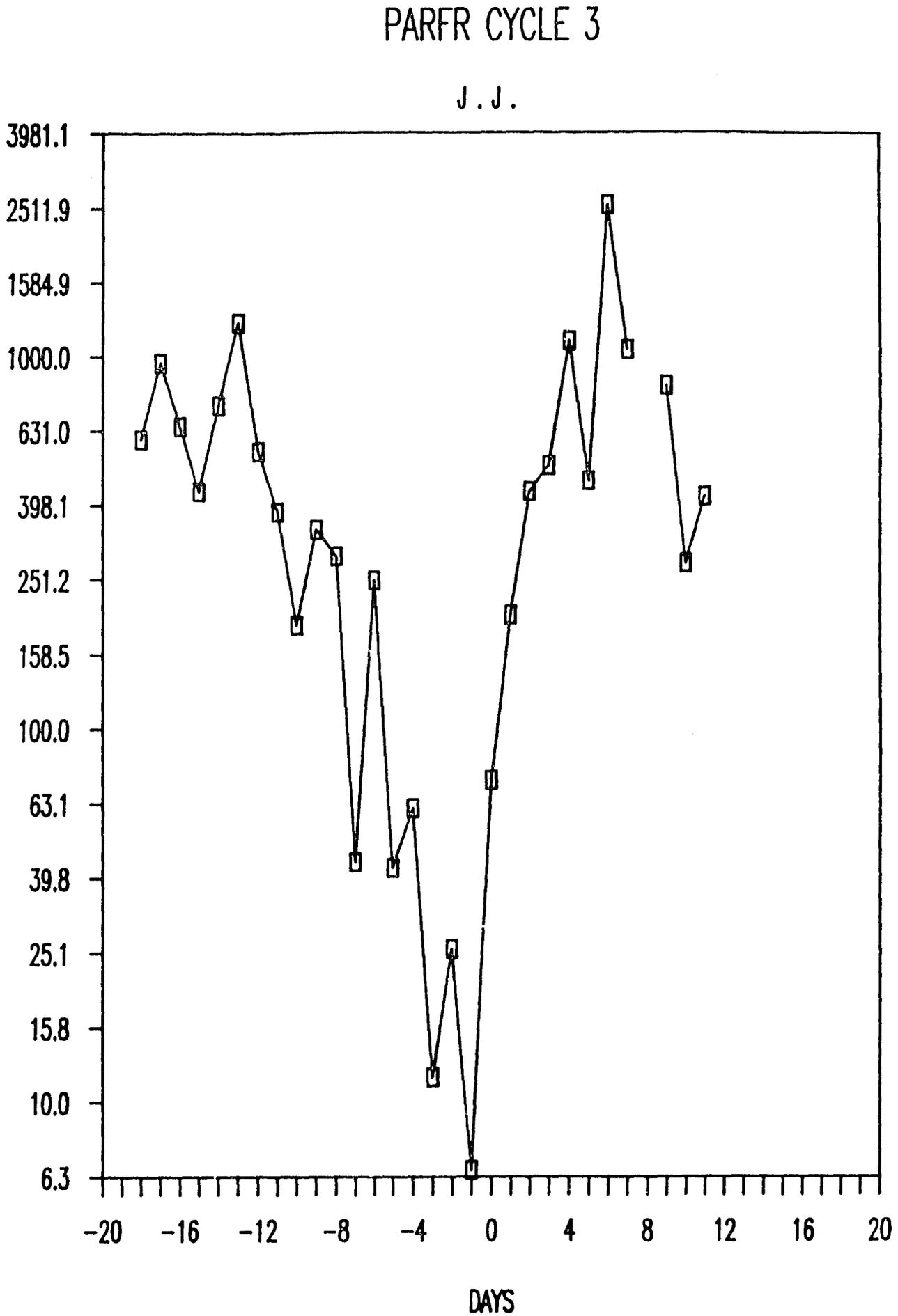
J.J.

PARFR CYCLE 2

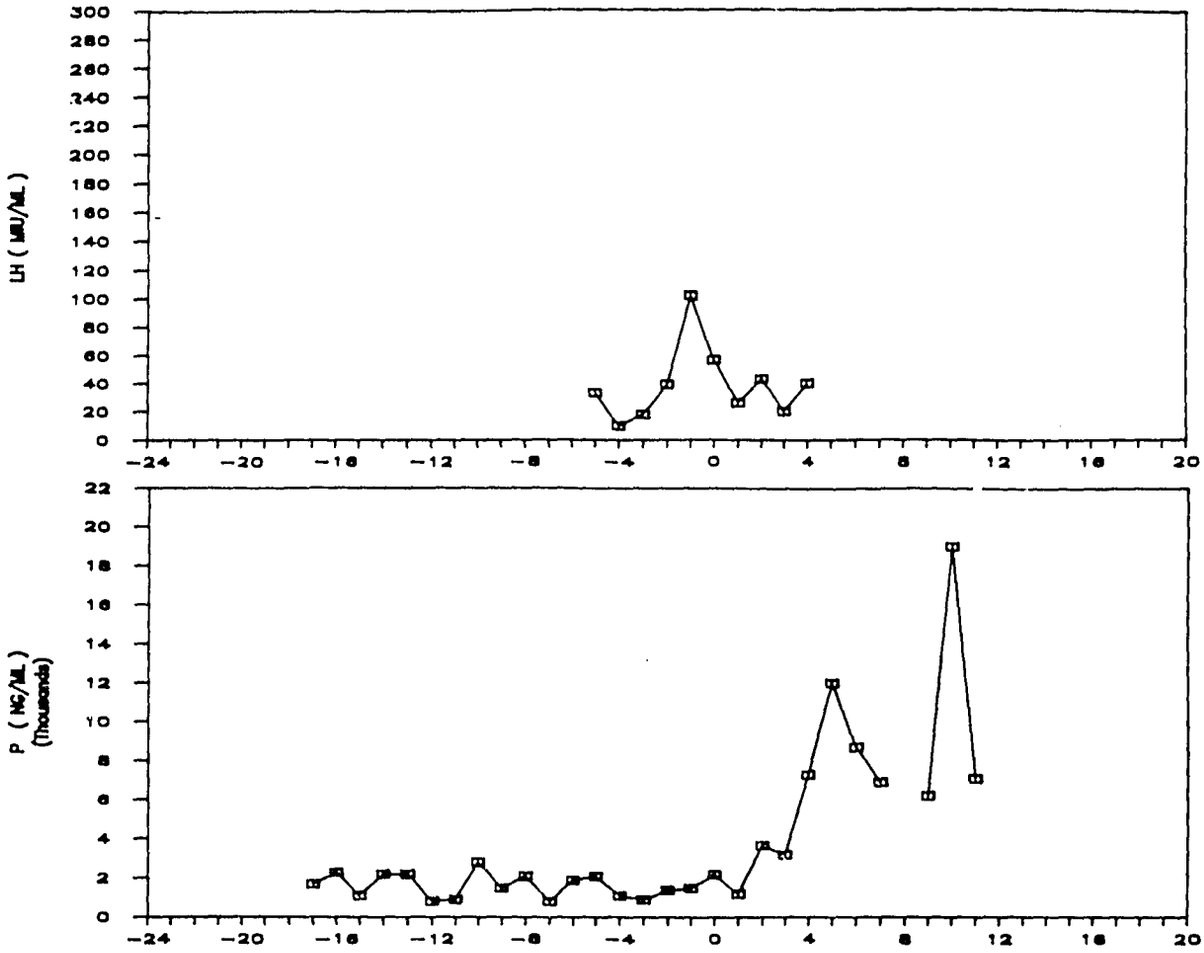




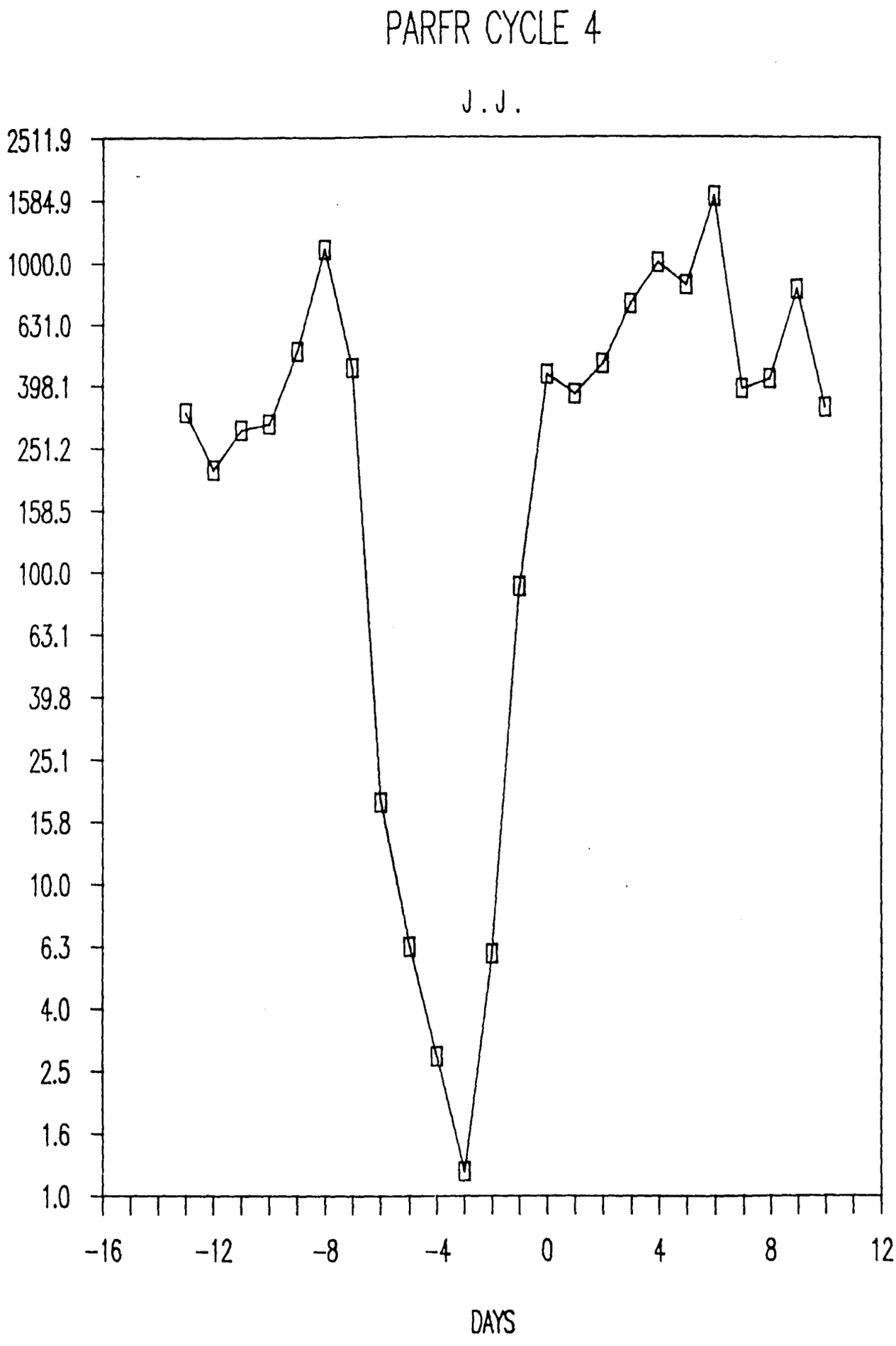
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
J.J.	3/12/85	4	-18	599.0						PARFR CYCLE 3
	3/13/85	5	-17	959.2			1700.0			
	3/14/85	6	-16	648.9			2300.0			
	3/15/85	7	-15	434.1			1100.0			
	3/16/85	8	-14	735.1			2200.0			
	3/17/85	9	-13	1228.0			2200.0			
	3/18/85	10	-12	555.6			800.0			
	3/19/85	11	-11	381.9			900.0			
	3/20/85	12	-10	190.1			2800.0			
	3/21/85	13	-9	342.4			1500.0			
	3/22/85	14	-8	291.4			2100.0			
	3/23/85	15	-7	44.1			800.0			
	3/24/85	16	-6	251.3			1900.0			
	3/25/85	17	-5	42.6		33.0	2100.0			
	3/26/85	18	-4	61.3		10.0	1100.0			
	3/27/85	19	-3	11.7		18.0	900.0			
	3/28/85	20	-2	25.7		39.0	1400.0			
	3/29/85	21	-1	6.6		101.0	1500.0			
	3/30/85	22	0	72.9		56.0	2200.0			
	3/31/85	23	1	203.0		26.0	1200.0			
	4/1/85	24	2	434.7		43.0	3700.0			
	4/2/85	25	3	510.9		20.0	3200.0			
	4/3/85	26	4	1101.2		40.0	7300.0			
	4/4/85	27	5	463.8			12000.0			
	4/5/85	28	6	2555.5			8700.0			
	4/6/85	29	7	1043.8			6900.0			
	4/7/85	30	8							
	4/8/85	31	9	836.0			6200.0			
	4/9/85	32	10	278.9			19000.0			
	4/10/85	33	11	421.5			7100.0			


PARFR CYCLE 3
J.J.
3981.1
2511.9
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

PARFR CYCLE 3



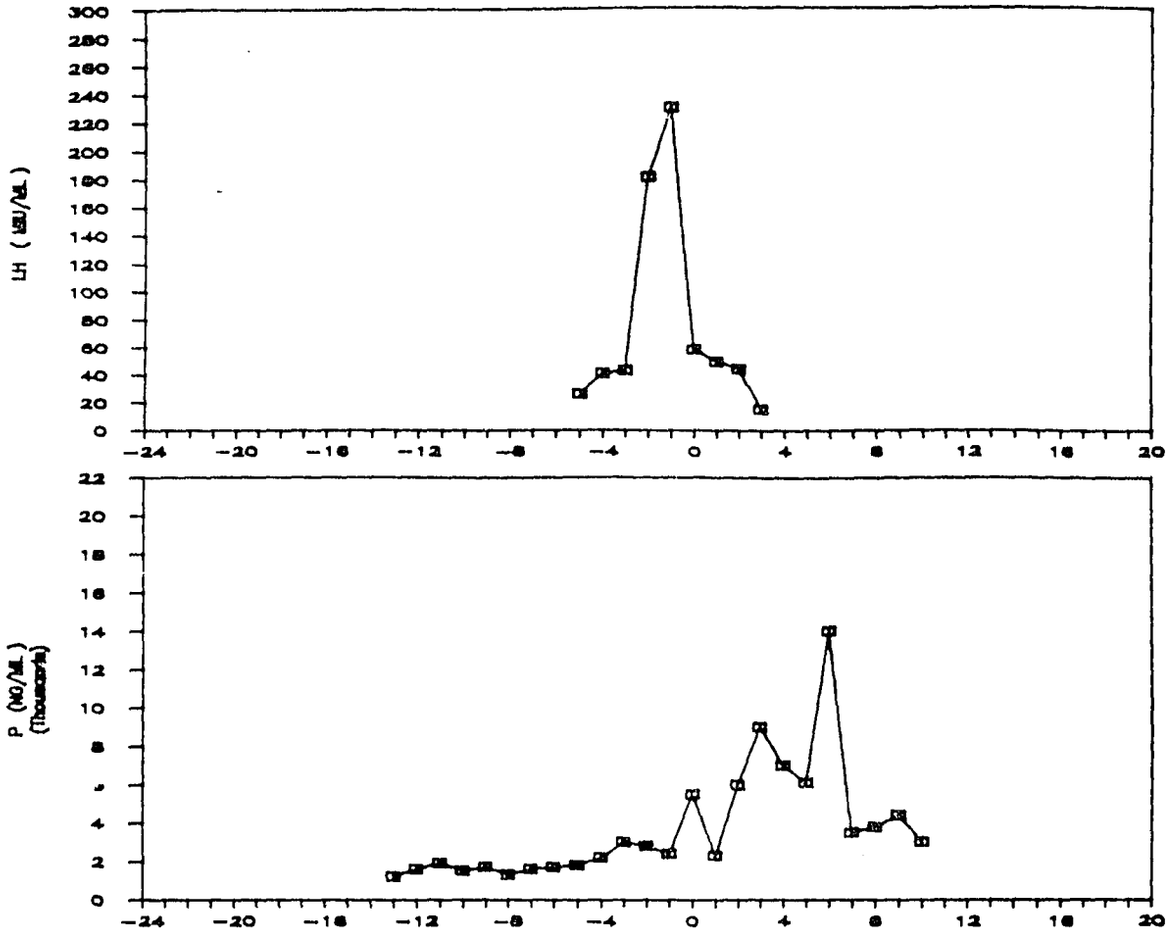
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	EZ	BBT	REMARKS
J.J.	4/15/85	4	-13	324.3			1200.0			PARFR CYCLE 4
	4/16/85	5	-12	213.4			1600.0			
	4/17/85	6	-11	285.9			1900.0			
	4/18/85	7	-10	298.3			1500.0			
	4/19/85	8	-9	513.0			1700.0			
	4/20/85	9	-8	1104.5			1300.0			
	4/21/85	10	-7	454.2			1600.0			
	4/22/85	11	-6	18.3			1700.0			
	4/23/85	12	-5	6.3		26.0	1800.0			
	4/24/85	13	-4	2.8		41.0	2200.0			
	4/25/85	14	-3	1.2		43.0	3000.0			
	4/26/85	15	-2	6.0		181.0	2800.0			
	4/27/85	16	-1	90.7		230.0	2400.0			
	4/28/85	17	0	435.6		58.0	5500.0			
	4/29/85	18	1	377.0		49.0	2300.0			
	4/30/85	19	2	471.6		44.0	6000.0			
	5/1/85	20	3	734.8		15.0	9000.0			
	5/2/85	21	4	997.8			7000.0			
	5/3/85	22	5	844.8			6100.0			
	5/4/85	23	6	1632.0			14000.0			
	5/5/85	24	7	391.6			3500.0			
	5/6/85	25	8	421.7			3800.0			
	5/7/85	26	9	820.9			4400.0			
	5/8/85	27	10	740.0			3000.0			

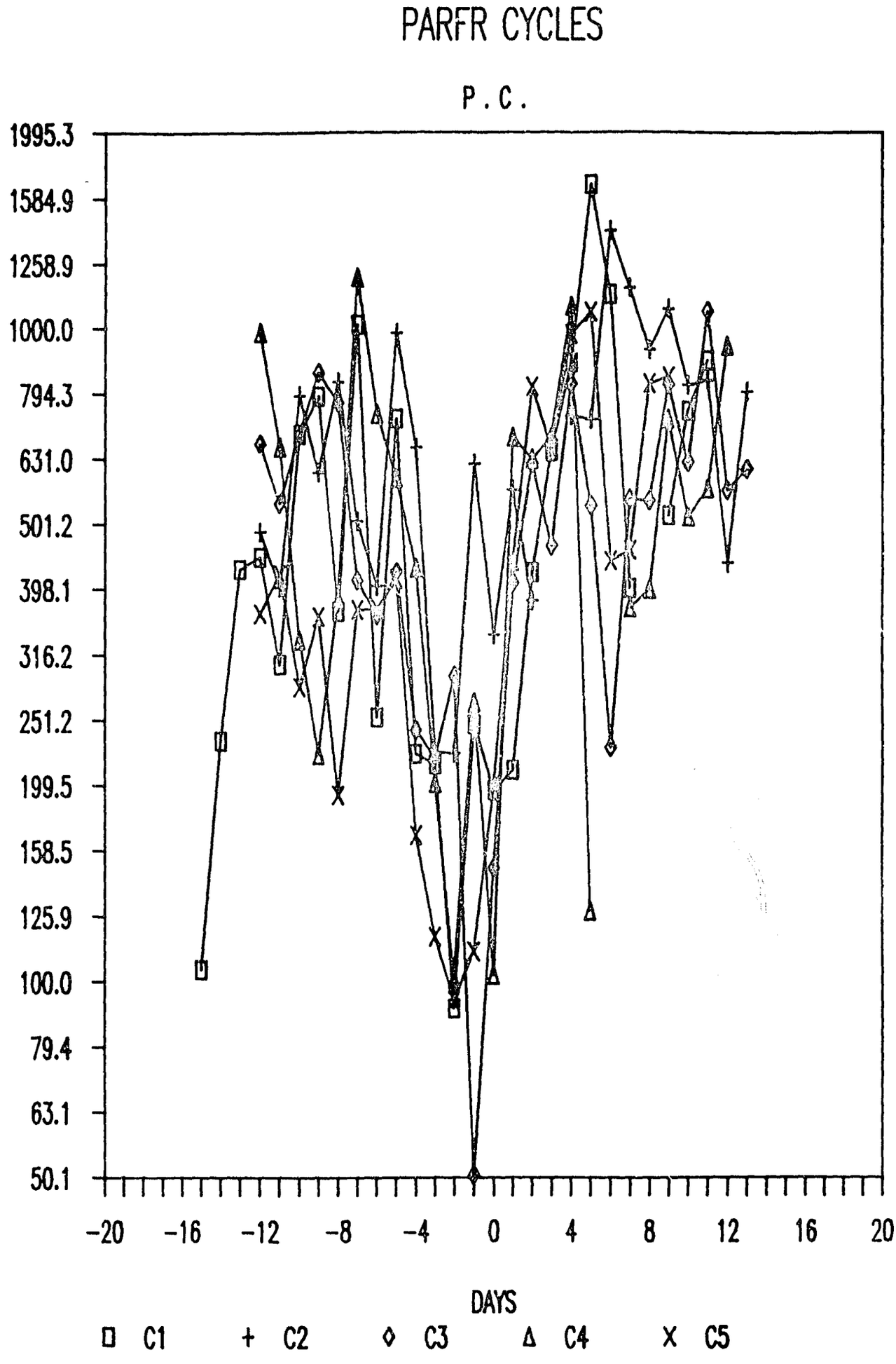

PARFR CYCLE 4
J.J.
2511.9
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
-16
-12
-8
-4
0
4
8
12
DAYS

J.J.

PARFR CYCLE 4

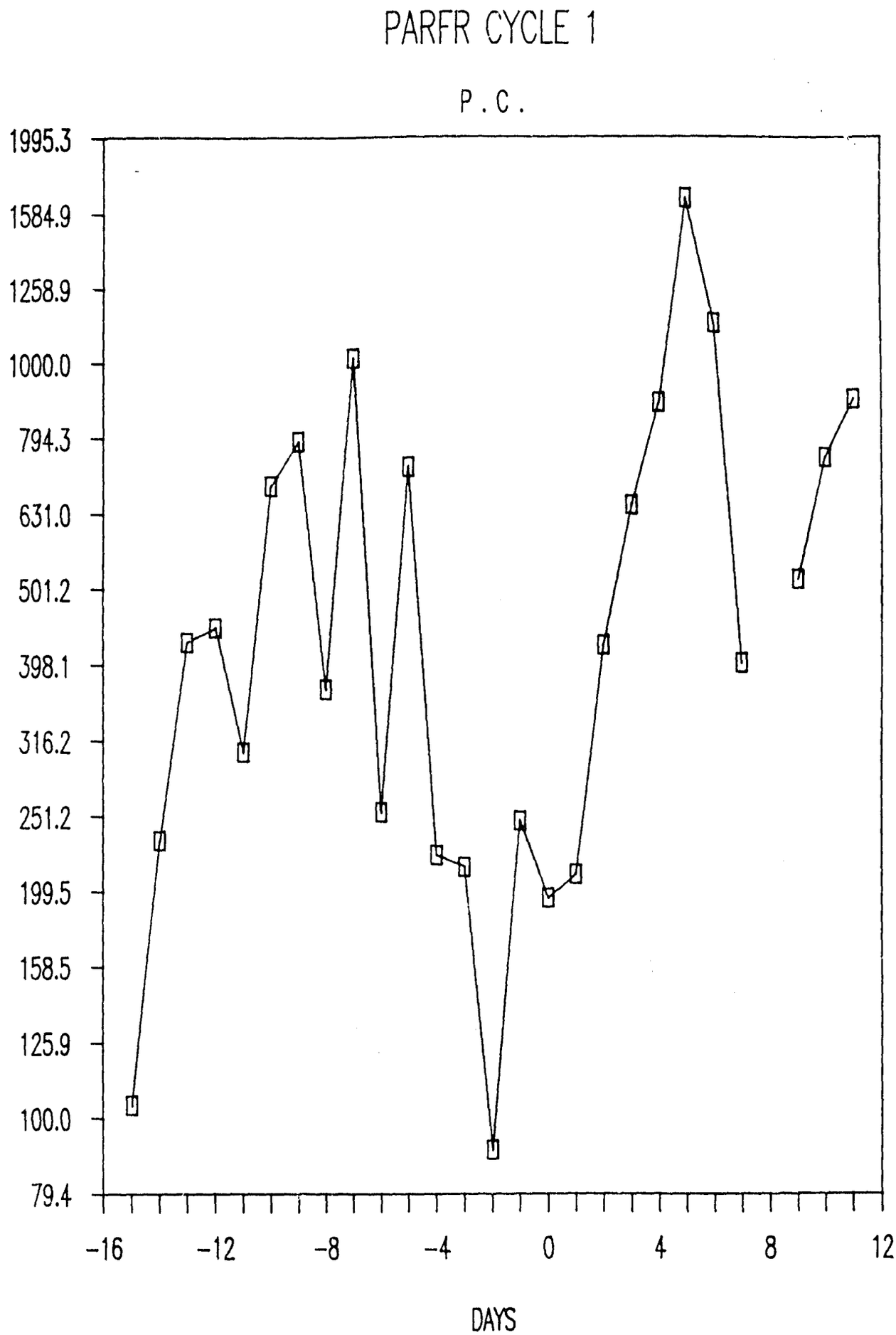
J . J .




PARFR CYCLES
P.C.
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS
C1
C2
C3
C4
C5

Total
5 cycles

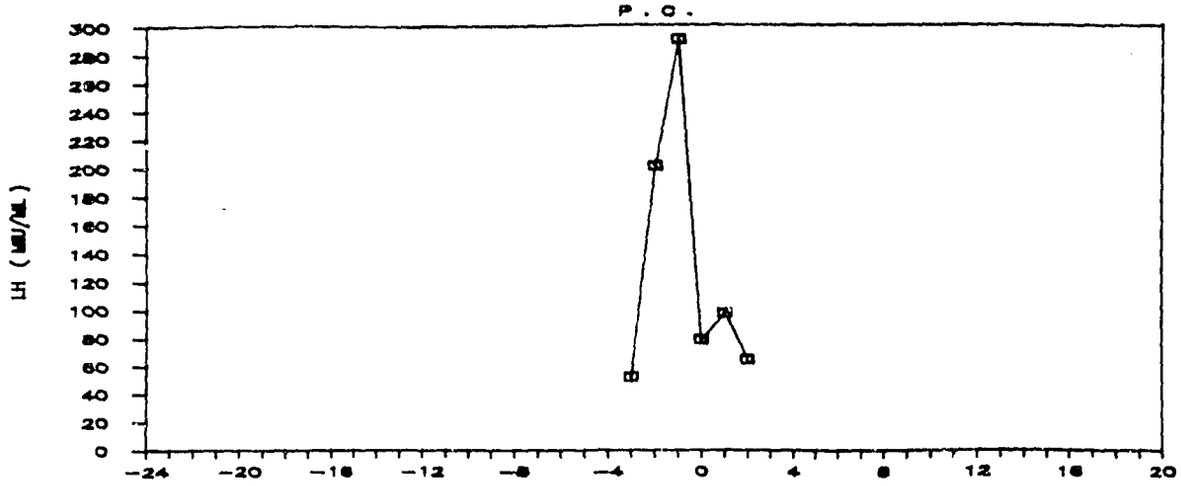
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
P.C.	2/5/85	4	-15	104.3			4200.0		98.2	FARFR CYCLE 1
	2/6/85	5	-14	233.3			2300.0		97.8	
	2/7/85	6	-13	426.8			2300.0		97.6	
	2/8/85	7	-12	445.5			2500.0		97.8	
	2/9/85	8	-11	305.3			3200.0		98.0	
	2/10/85	9	-10	687.0			3100.0		98.0	
	2/11/85	10	-9	786.2			2100.0		97.8	
	2/12/85	11	-8	368.7			2800.0		97.8	
	2/13/85	12	-7	1013.3			2900.0		97.9	
	2/14/85	13	-6	254.0			2800.0		97.8	
	2/15/85	14	-5	728.6			2700.0		97.9	
	2/16/85	15	-4	223.2			3800.0		98.1	
	2/17/85	16	-3	215.2		51.0	2400.0		97.8	
	2/18/85	17	-2	90.9		201.0	3900.0		98.0	
	2/19/85	18	-1	247.8		290.0	4600.0		97.8	
	2/20/85	19	0	195.9		78.0	3300.0		98.1	
	2/21/85	20	1	210.6		97.0	6000.0		98.0	
	2/22/85	21	2	423.5		64.0	7000.0		98.0	
	2/23/85	22	3	649.1			9000.0		98.1	
	2/24/85	23	4	886.5			9000.0		98.2	
	2/25/85	24	5	1662.7			13000.0		98.0	
	2/26/85	25	6	1130.3			13000.0		98.2	
	2/27/85	26	7	399.3			15000.0		98.1	
	2/28/85	27	8				8000.0		98.0	
	3/1/85	28	9	516.0			14000.0		98.1	
	3/2/85	29	10	748.2			9000.0		98.3	
	3/3/85	30	11	894.4			5000.0		98.4	

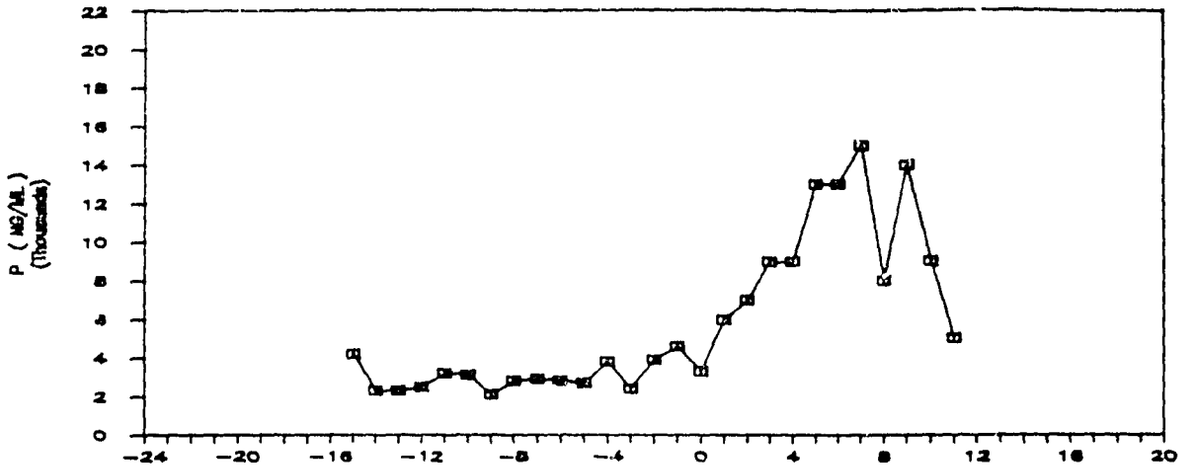

PARFR CYCLE 1
P.C.
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
-16
-12
-8
-4
0
4
8
12
DAYS

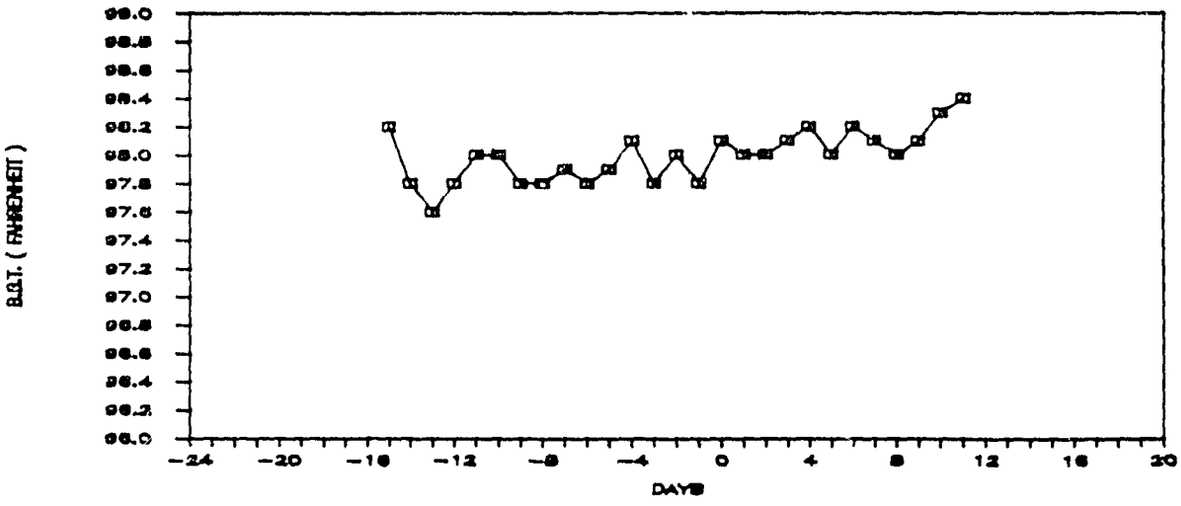
P.C.

PARFR CYCLE 1

P . C .

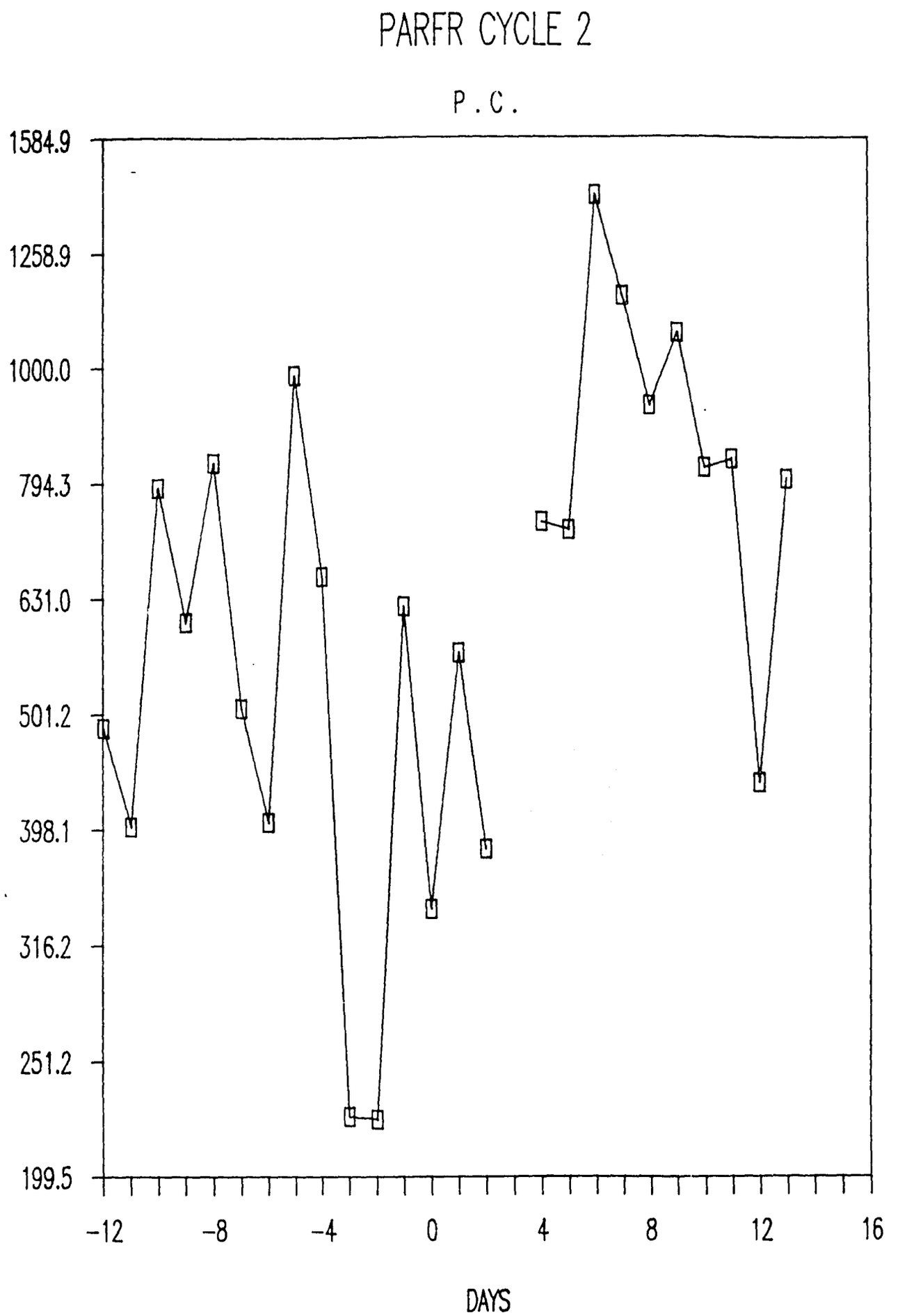






P.C.

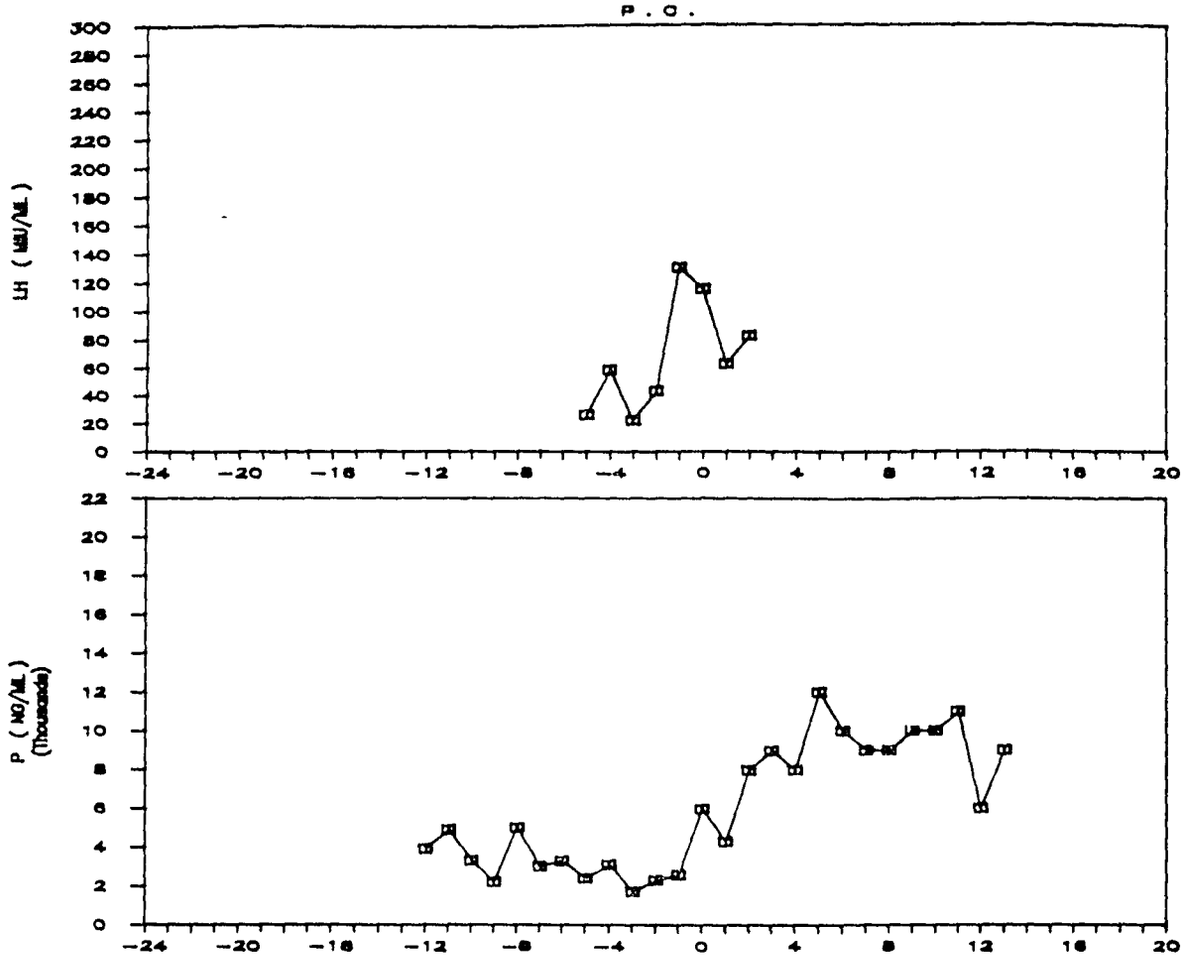
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
	3/7/85	4	-12	487.6			3900.0		98.0	PARFR CYCLE 2
	3/8/85	5	-11	400.9			4900.0		98.0	
	3/9/85	6	-10	787.5			3300.0		98.1	
	3/10/85	7	-9	601.7			2200.0		98.1	
	3/11/85	8	-8	827.3			5000.0		97.6	
	3/12/85	9	-7	506.7			3000.0		97.8	
	3/13/85	10	-6	404.0			3300.0		97.9	
	3/14/85	11	-5	984.7		26.0	2400.0		98.0	
	3/15/85	12	-4	658.6		58.0	3100.0		97.8	
	3/16/85	13	-3	224.9		22.0	1700.0		97.8	
	3/17/85	14	-2	223.7		43.0	2300.0		97.7	
	3/18/85	15	-1	621.2		130.0	2600.0		98.0	
	3/19/85	16	0	339.9		115.0	6000.0		97.7	
	3/20/85	17	1	967.2		62.0	4300.0		98.0	
	3/21/85	18	2	383.6		82.0	8000.0		98.1	
	3/22/85	19	3				9000.0			
	3/23/85	20	4	736.5			8000.0		98.2	
	3/24/85	21	5	724.7			12000.0		98.0	
	3/25/85	22	6	1414.9			10000.0		98.0	
	3/26/85	23	7	1155.6			9000.0		98.0	
	3/27/85	24	8	927.3			9000.0		98.2	
	3/28/85	25	9	1072.0			10000.0		98.2	
	3/29/85	26	10	819.3			10000.0		98.3	
	3/30/85	27	11	833.7			11000.0		98.0	
	3/31/85	28	12	436.8			6000.0		98.2	
	4/1/85	29	13	300.0			9000.0		97.9	

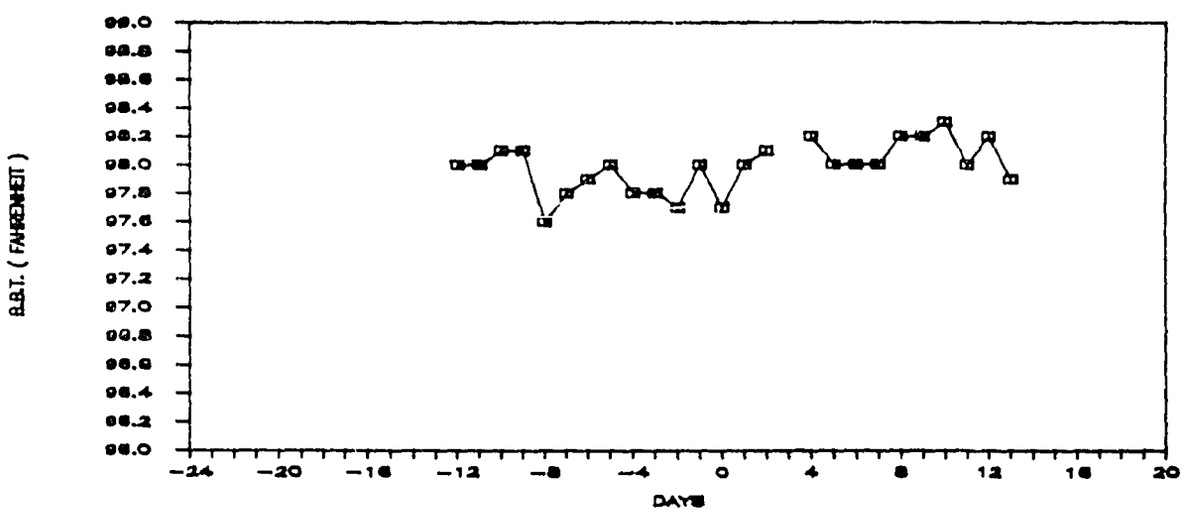

PARFR CYCLE 2
P.C.
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
-12
-8
-4
0
4
8
12
16
DAYS

P.C.

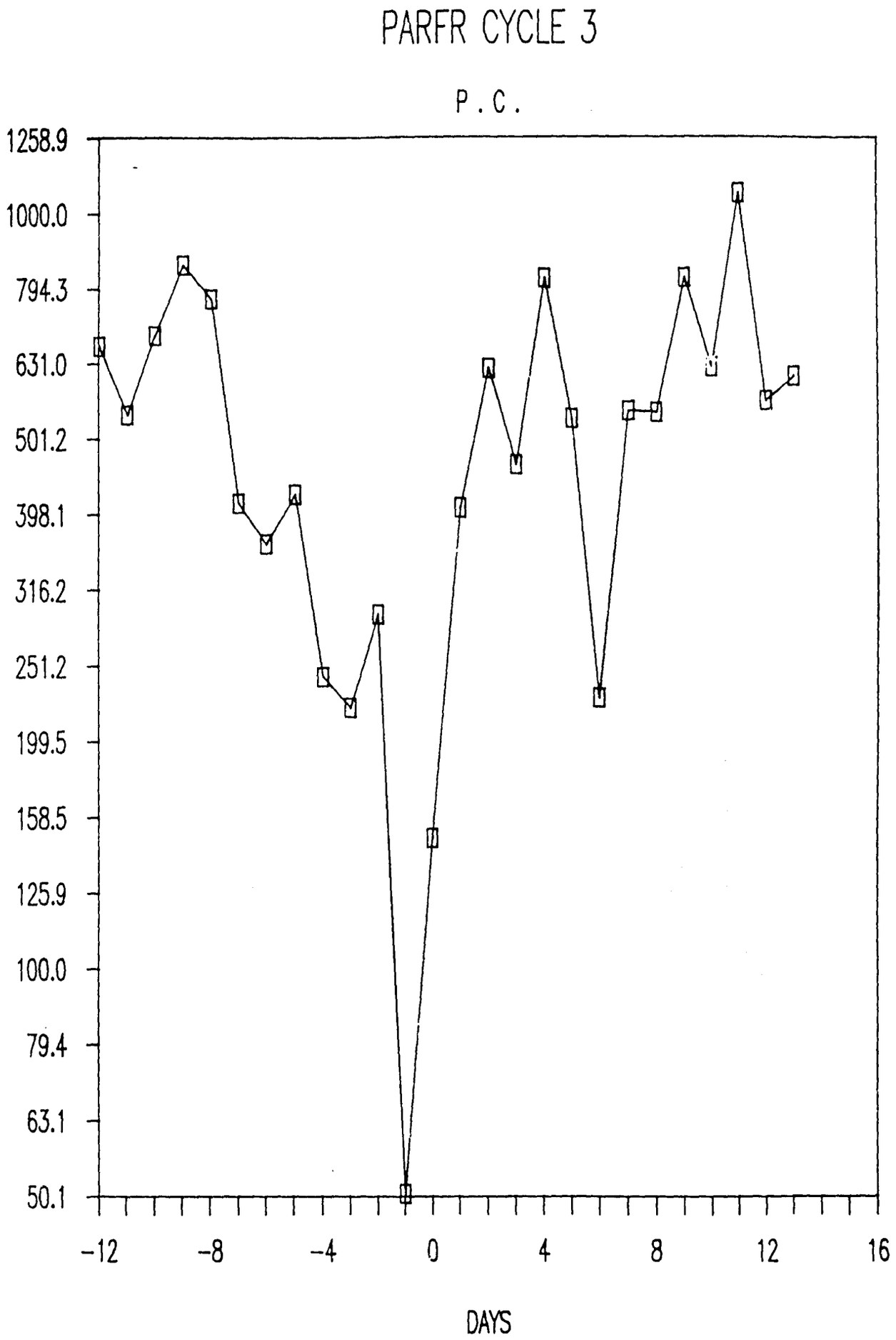
PARFR CYCLE 2

P . C .





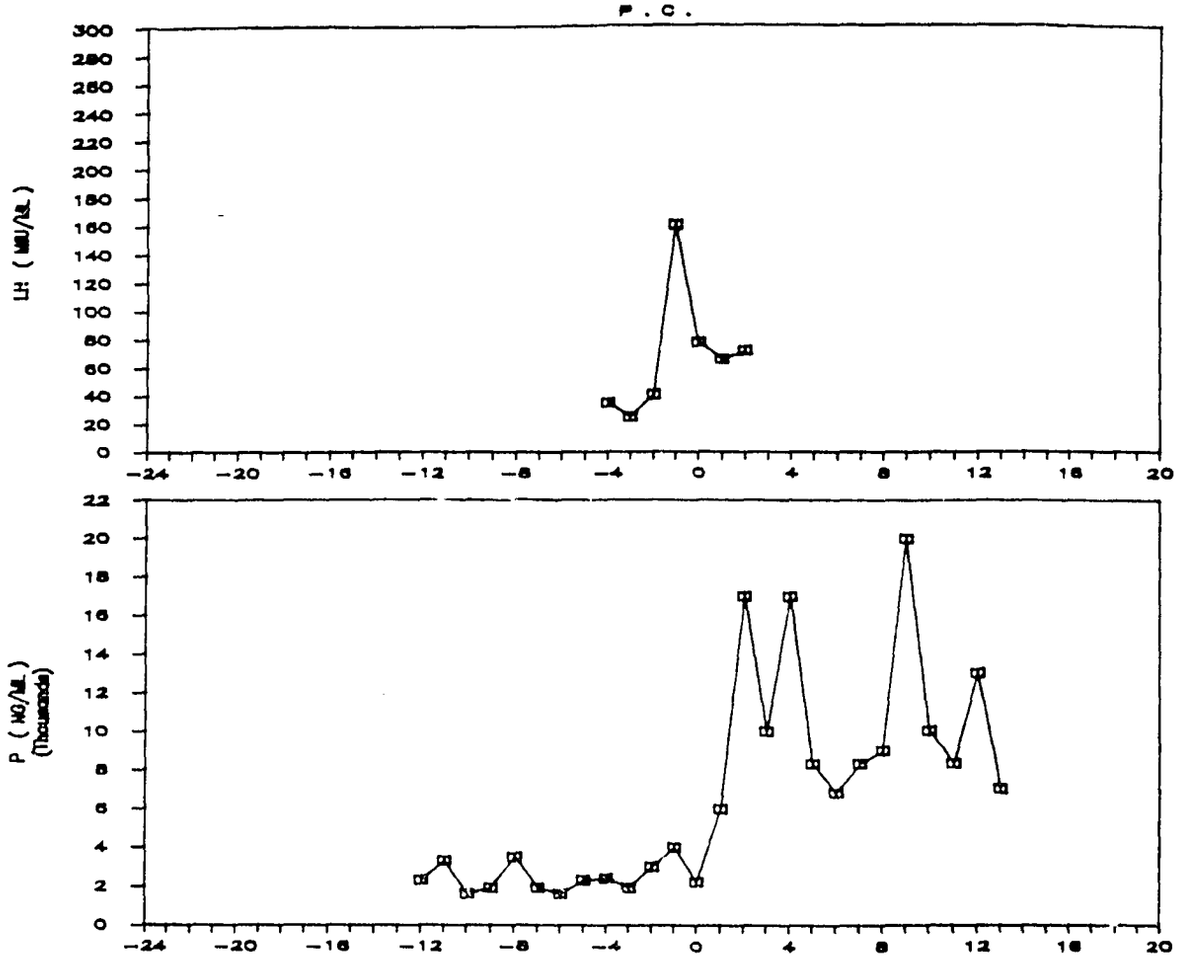
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
P.C.	4/5/85	4	-12	665.0			2300.0		98.4	PARFR CYCLE 3
	4/6/85	5	-11	539.3			3300.0		97.9	
	4/7/85	6	-10	698.0			1600.0		98.0	
	4/8/85	7	-9	854.7			1900.0		98.1	
	4/9/85	8	-8	769.1			3500.0		98.0	
	4/10/85	9	-7	411.6			1900.0		97.9	
	4/11/85	10	-6	363.8			1600.0		98.0	
	4/12/85	11	-5	423.2			2300.0		97.6	
	4/13/85	12	-4	242.5		35.0	2400.0		97.6	
	4/14/85	13	-3	220.7		25.0	1900.0		97.6	
	4/15/85	14	-2	293.9		41.0	3000.0		98.0	
	4/16/85	15	-1	50.5		161.0	4000.0		98.0	
	4/17/85	16	0	149.1		78.0	2200.0		98.0	
	4/18/85	17	1	407.5		66.0	6000.0		98.0	
	4/19/85	18	2	622.4		72.0	17000.0		98.0	
	4/20/85	19	3	465.0			10000.0		98.0	
	4/21/85	20	4	822.6			17000.0		98.2	
	4/22/85	21	5	535.2			8300.0		97.9	
	4/23/85	22	6	228.1			6800.0		98.4	
	4/24/85	23	7	547.0			8300.0		98.2	
	4/25/85	24	8	544.0			9000.0		98.1	
	4/26/85	25	9	820.5			20000.0		98.3	
	4/27/85	26	10	624.1			10000.0		98.0	
	4/28/85	27	11	1064.3			8300.0		98.1	
	4/29/85	28	12	564.1			13000.0		98.4	
	4/30/85	29	13	607.6			7000.0		98.0	

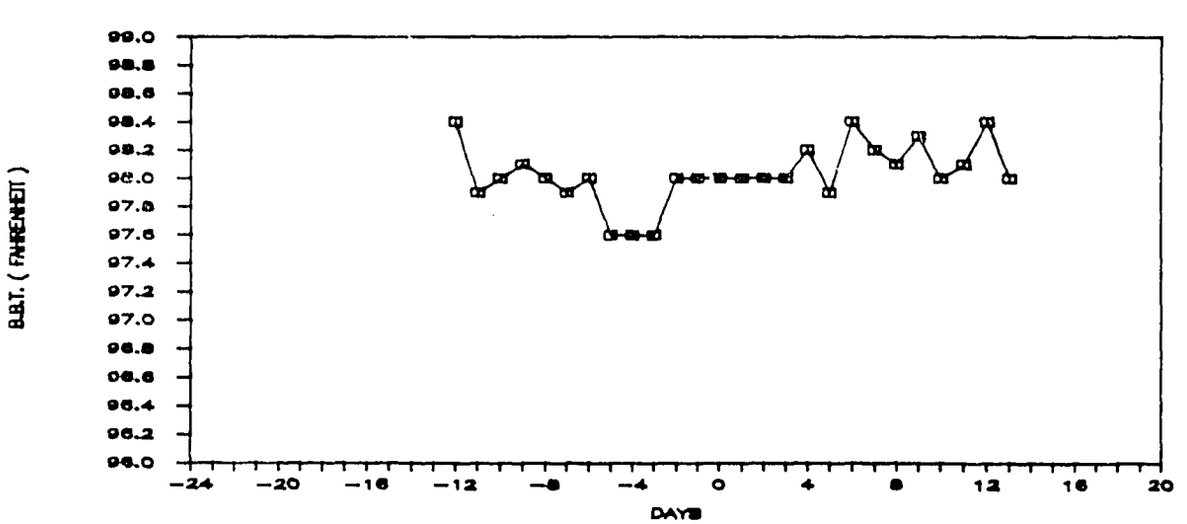

PARFR CYCLE 3
P.C.
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
-12
-8
-4
0
4
8
12
16
DAYS

P.C.

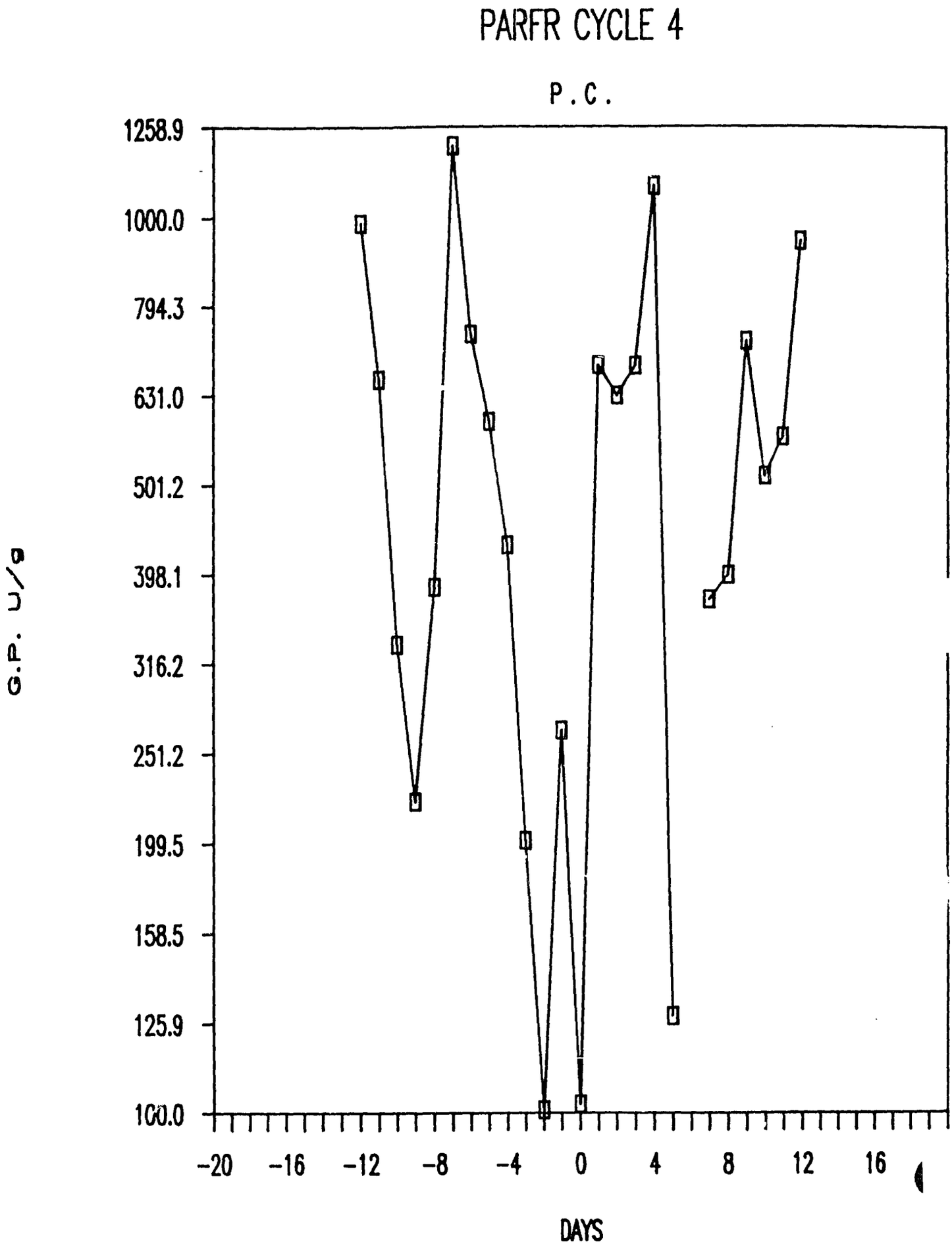
PARFR CYCLE 3

P . C .



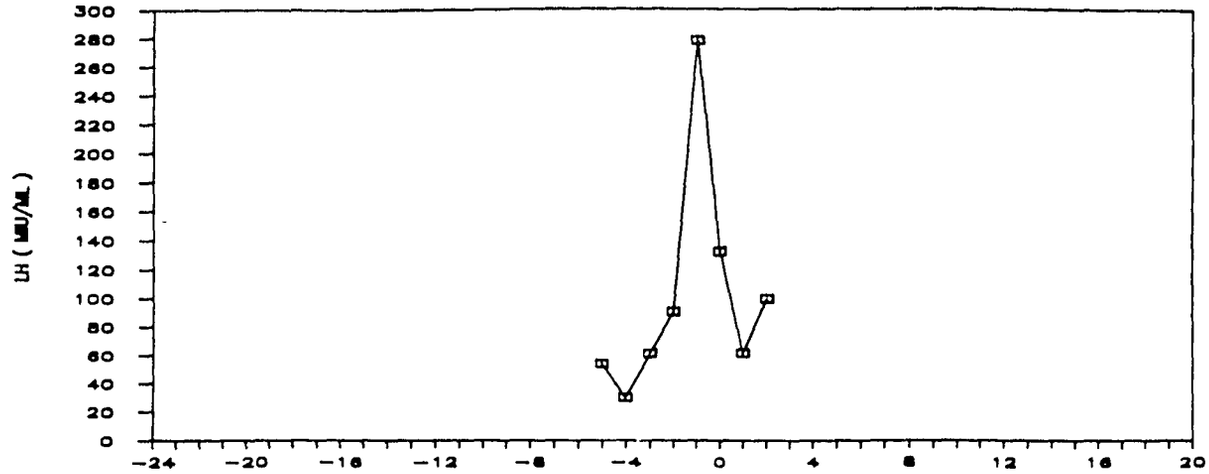


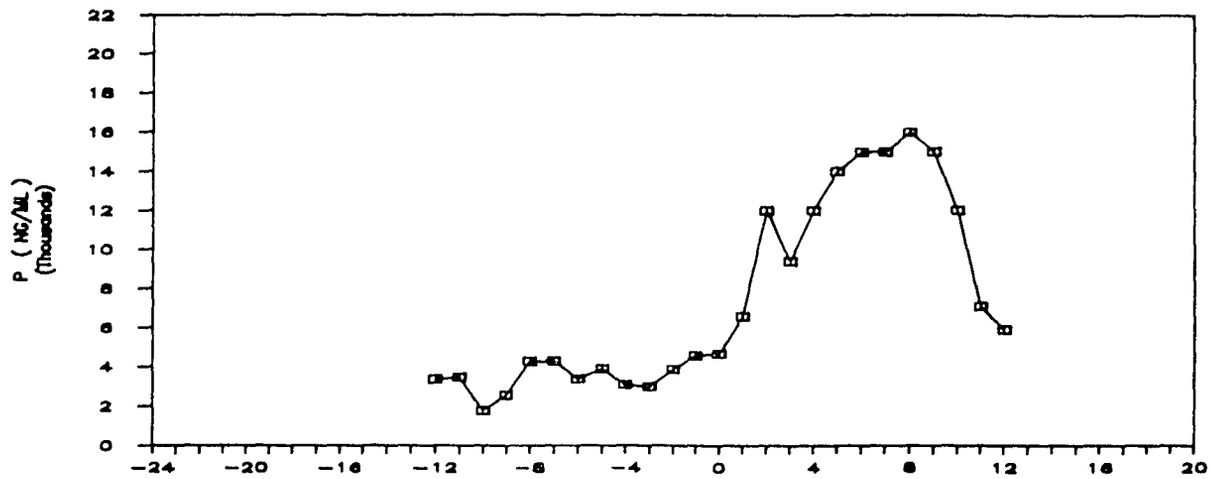
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
P.C.	5/4/85	4	-12	986.0			3400.0			PARFR CYCLE 4
	5/5/85	5	-11	658.1			3500.0		98.2	
	5/6/85	6	-10	332.8			1800.0		97.8	
	5/7/85	7	-9	222.4			2600.0		98.0	
	5/8/85	8	-8	385.7			4300.0		97.8	
	5/9/85	9	-7	1199.4			4300.0		98.0	
	5/10/85	10	-6	740.4			3400.0		97.8	
	5/11/85	11	-5	591.3		54.0	3900.0		98.1	
	5/12/85	12	-4	430.7		30.0	3100.0		97.9	
	5/13/85	13	-3	201.3		61.0	3000.0		97.9	
	5/14/85	14	-2	100.8		90.0	3900.0		98.0	
	5/15/85	15	-1	267.4		278.0	4600.0		98.0	
	5/16/85	16	0	102.3		132.0	4700.0		98.0	
	5/17/85	17	1	683.6		61.0	6600.0		98.0	
	5/18/85	18	2	632.2		99.0	12000.0		98.0	
	5/19/85	19	3	682.9			9400.0		98.2	
	5/20/85	20	4	1084.0			12000.0		98.2	
	5/21/85	21	5	128.3			14000.0		98.0	
	5/22/85	22	6				15000.0		98.4	
	5/23/85	23	7	373.3			15000.0		98.1	
	5/24/85	24	8	397.5			16000.0		98.2	
	5/25/85	25	9	726.6			15000.0		98.2	
	5/26/85	26	10	513.9			12000.0		98.2	
	5/27/85	27	11	567.9			7100.0		98.4	
	5/28/85	28	12	941.7			5900.0		98.2	

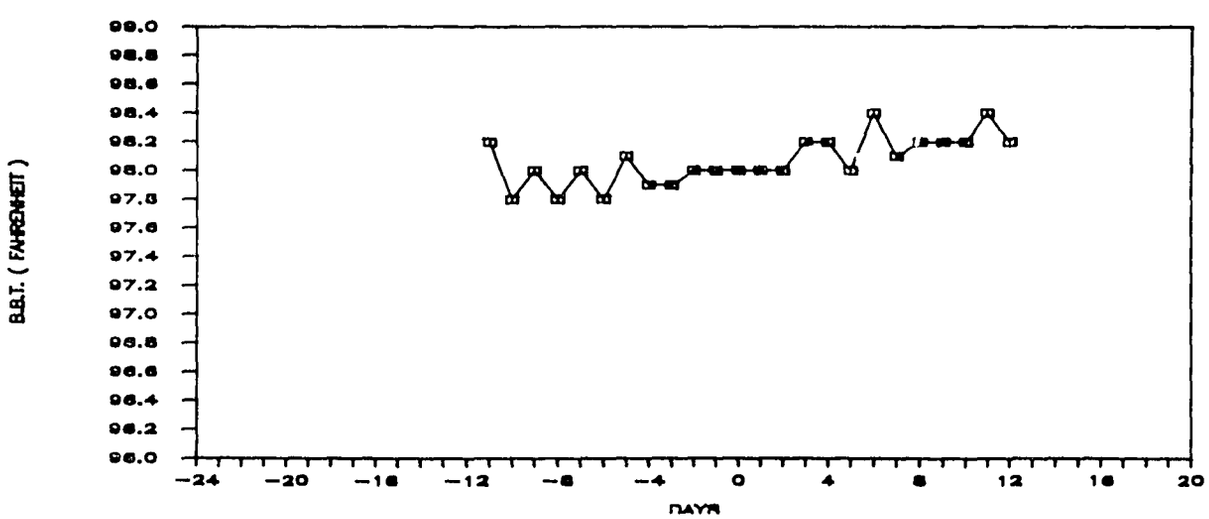

PARFR CYCLE 4
P.C.
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
G.P. U/g
-20
-16
-12
-8
-4
0
4
8
12
16
DAYS

P.C.

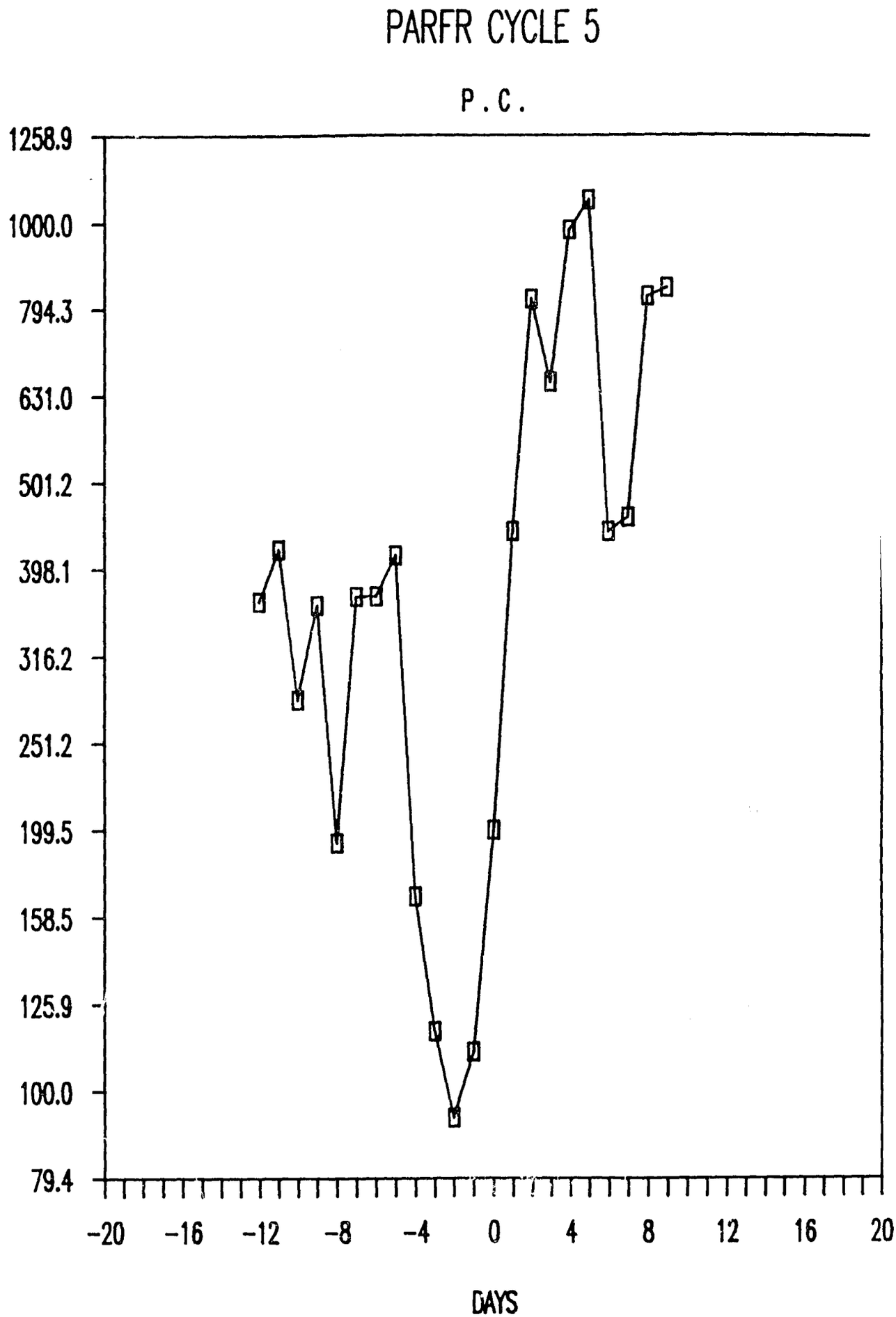
PARFR CYCLE 4





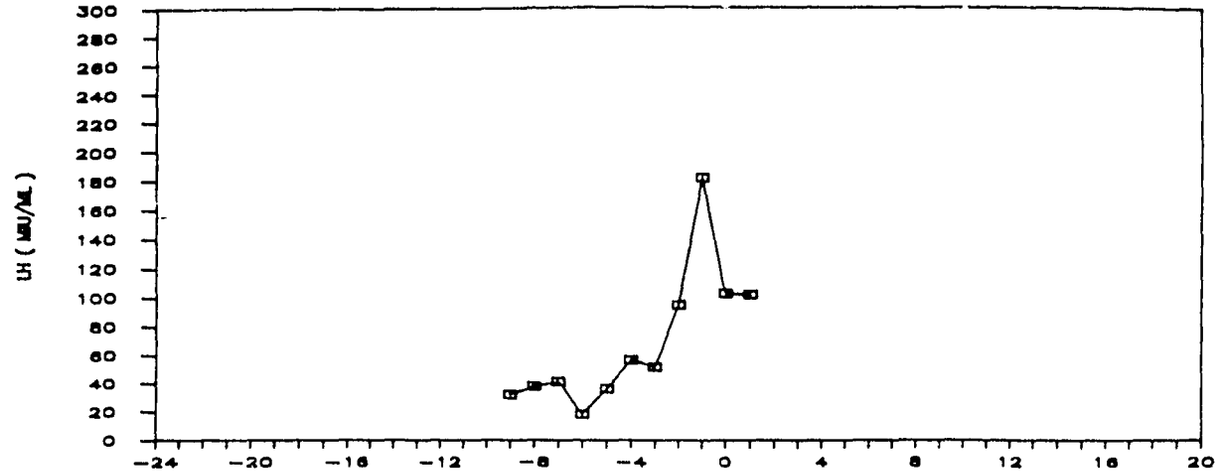


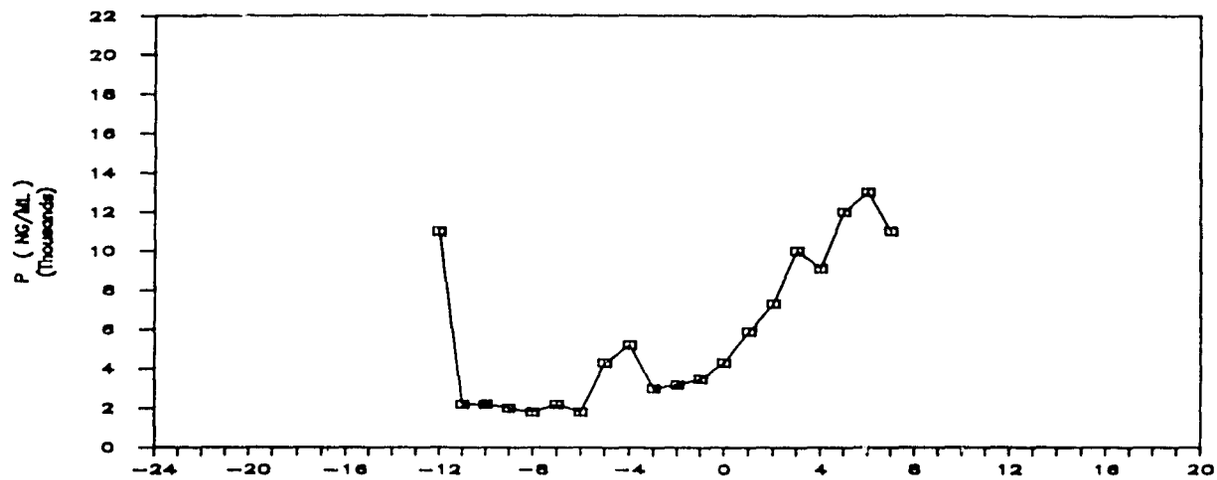
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	EZ	BBT	REMARKS
P.C.	6/2/85	5	-12	365.3			11000.0		98.0	PARFR CYCLE 5
	6/3/85	6	-11	419.7			2200.0		98.0	
	6/4/85	7	-10	282.1			2200.0		98.2	
	6/5/85	8	-9	362.1		32.0	2000.0		97.9	
	6/6/85	9	-8	193.0		38.0	1800.0		97.9	
	6/7/85	10	-7	370.5		41.0	2200.0		98.0	
	6/8/85	11	-6	371.3		18.0	1800.0		97.7	
	6/9/85	12	-5	413.8		36.0	4300.0		97.9	
	6/10/85	13	-4	167.5		56.0	5200.0		98.0	
	6/11/85	14	-3	117.2		51.0	3000.0		97.8	
	6/12/85	15	-2	93.4		94.0	3200.0		97.6	
	6/13/85	16	-1	111.1		182.0	3500.0		98.0	
	6/14/85	17	0	199.6		102.0	4300.0		97.6	
	6/15/85	18	1	440.7		101.0	5900.0		97.8	
	6/16/85	19	2	816.5			7700.0		98.2	
	6/17/85	20	3	654.7			10000.0		98.0	
	6/18/85	21	4	980.6			9100.0		98.0	
	6/19/85	22	5	1062.4			12000.0		98.0	
	6/20/85	23	6	440.0			13000.0		98.3	
	6/21/85	24	7	457.1			11000.0		98.2	
	6/22/85	25	8	823.1					98.0	
	6/23/85	26	9	842.7					98.2	

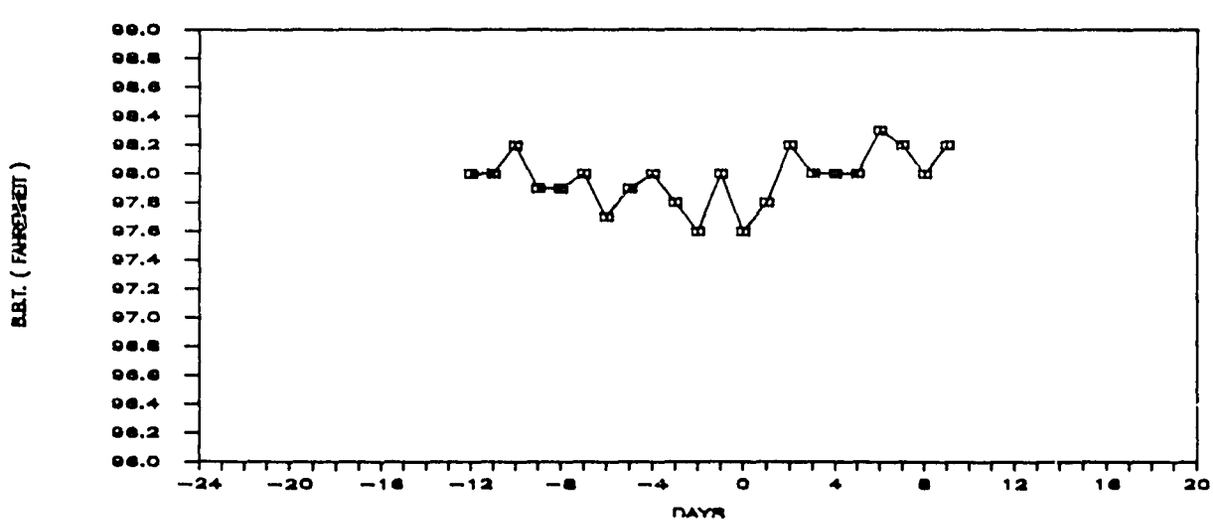

PARFR CYCLE 5
P.C.
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

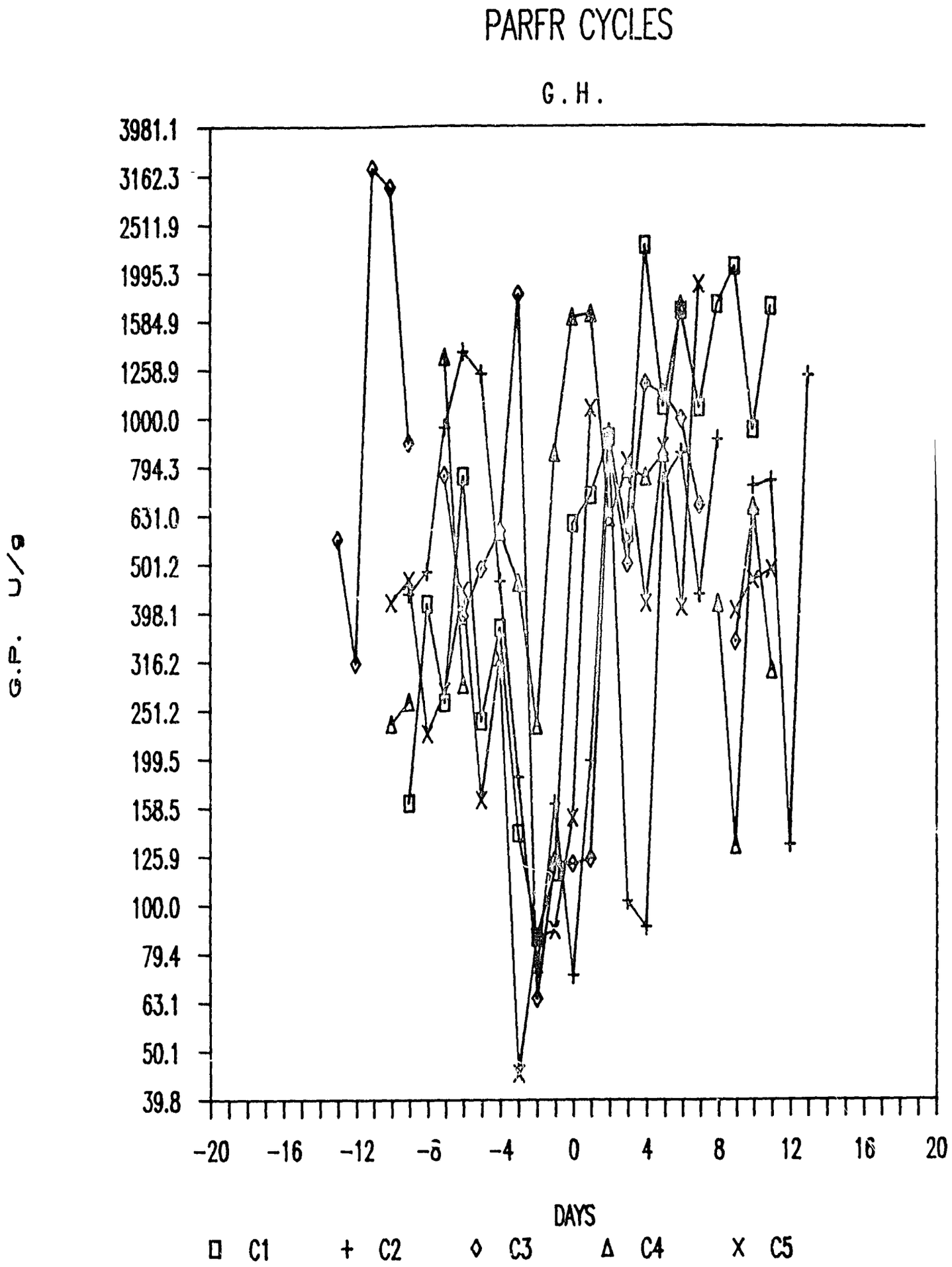
P.C.

PARFR CYCLE 5

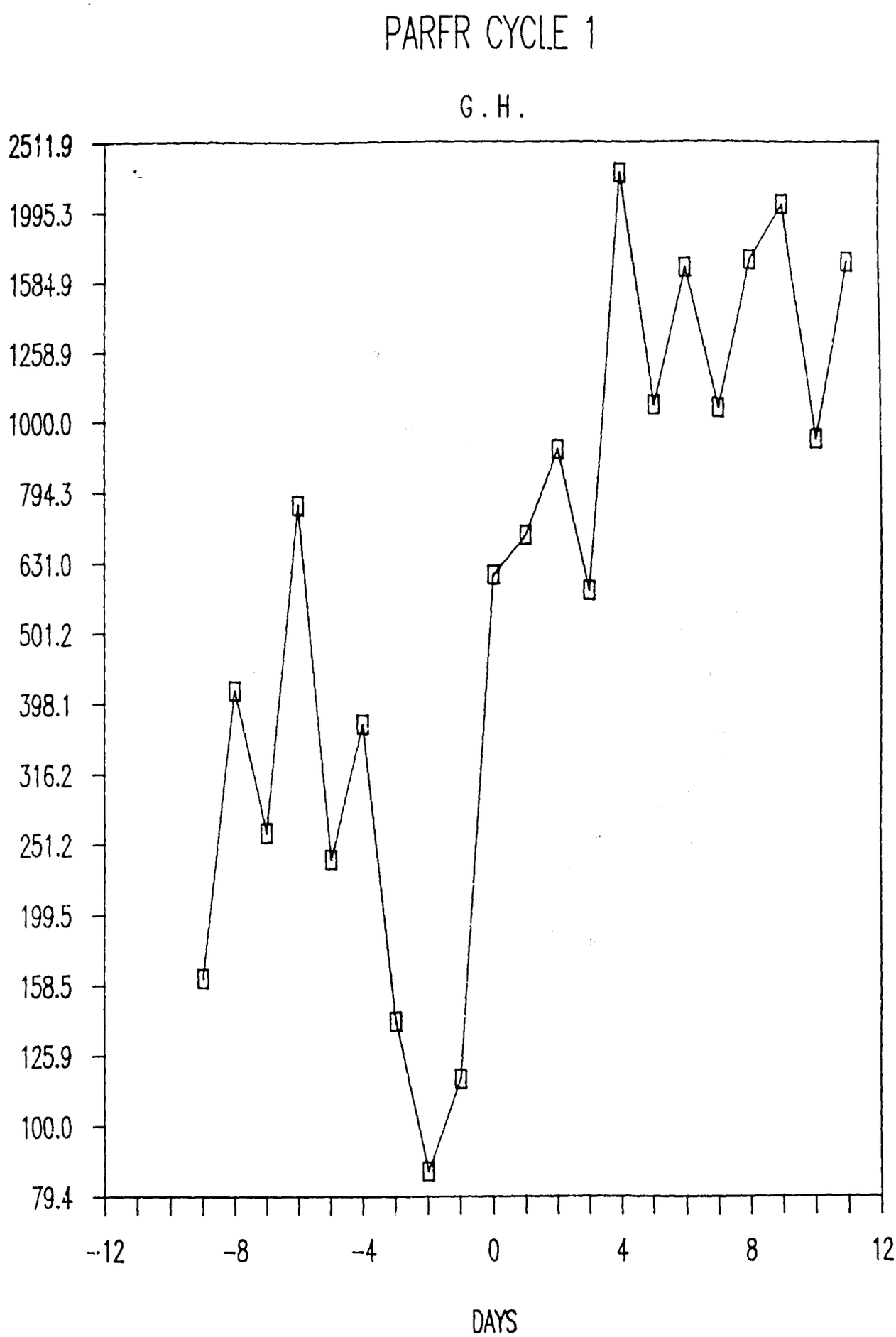







PARFR CYCLES
G.H.
3981.1
3162.3
2511.9
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
G.P. U/g
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS
C1
C2
C3
C4
C5

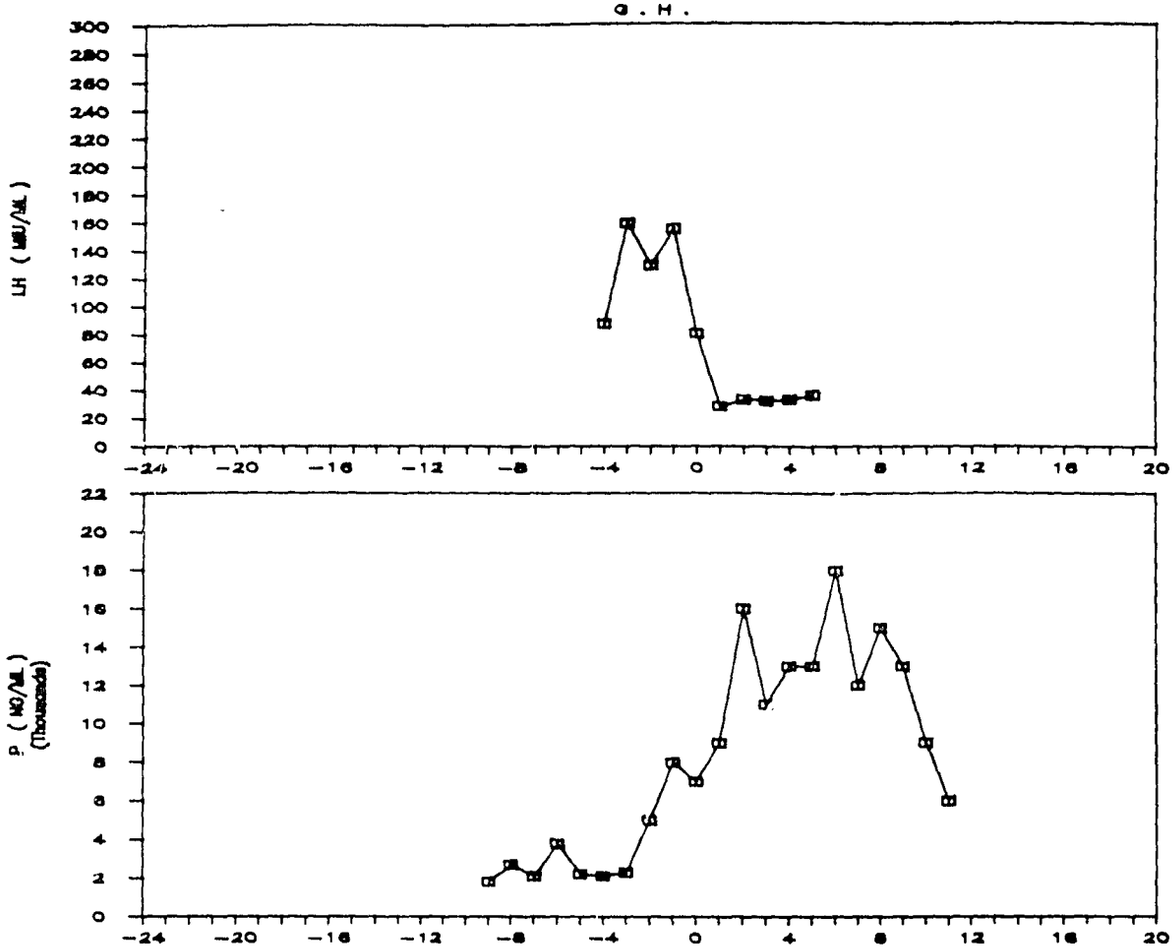
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
G.H.	2/12/85	4	-9	162.0			1800.0			PARFR CYCLE 1
	2/13/85	5	-8	415.2			2700.0		97.1	
	2/14/85	6	-7	269.7			2100.0		98.2	
	2/15/85	7	-6	760.0			3800.0		98.0	
	2/16/85	8	-5	238.5			2200.0		97.2	
	2/17/85	9	-4	371.5		97.0	2100.0		98.0	
	2/18/85	10	-3	140.4	TOO BROAD	159.0	2300.0		98.0	
	2/19/85	11	-2	86.1		129.0	5000.0		98.0	
	2/20/85	12	-1	116.4		155.0	8000.0		98.0	
	2/21/85	13	0	607.0		80.0	7000.0		99.0	
	2/22/85	14	1	691.7		28.0	9000.0		99.0	
	2/23/85	15	2	914.3		33.0	16000.0		98.0	
	2/24/85	16	3	577.6		32.0	11000.0		98.2	
	2/25/85	17	4	2269.0		33.0	13000.0		98.4	
	2/26/85	18	5	1056.7		36.0	13000.0		98.4	
	2/27/85	19	6	1662.4			18000.0		98.6	
	2/28/85	20	7	1047.5			12000.0		98.6	
	3/1/85	21	8	1707.0			15000.0		98.2	
	3/2/85	22	9	2047.0			13000.0		98.5	
	3/3/85	23	10	943.6			9000.0		98.8	
	3/4/85	24	11	1694.0			6000.0		98.5	

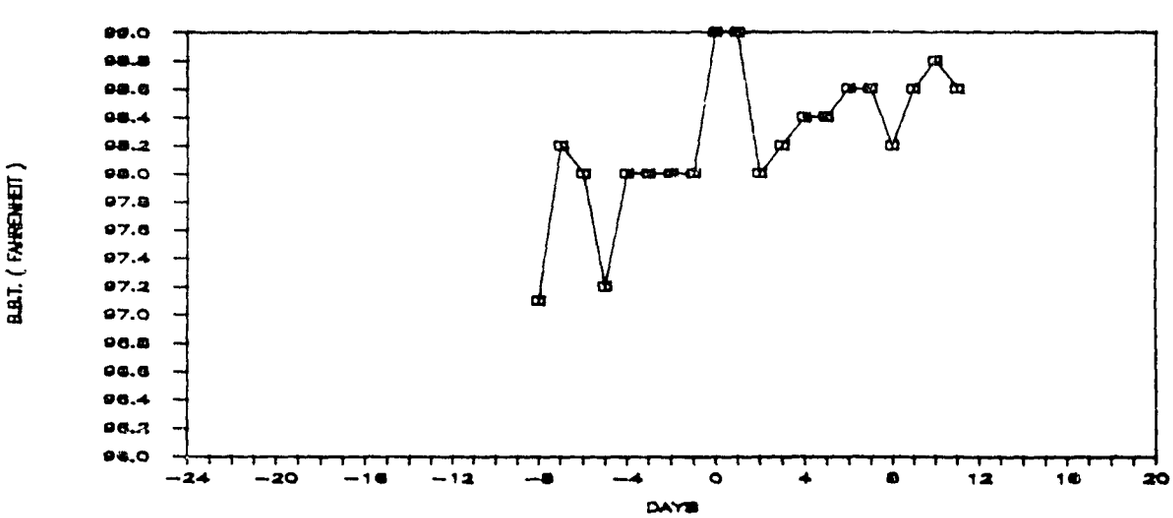

PARFR CYCLE 1
G.H.
2511.9
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
-12
-8
-4
0
4
8
12
DAYS

G.H.

PARFR CYCLE 1

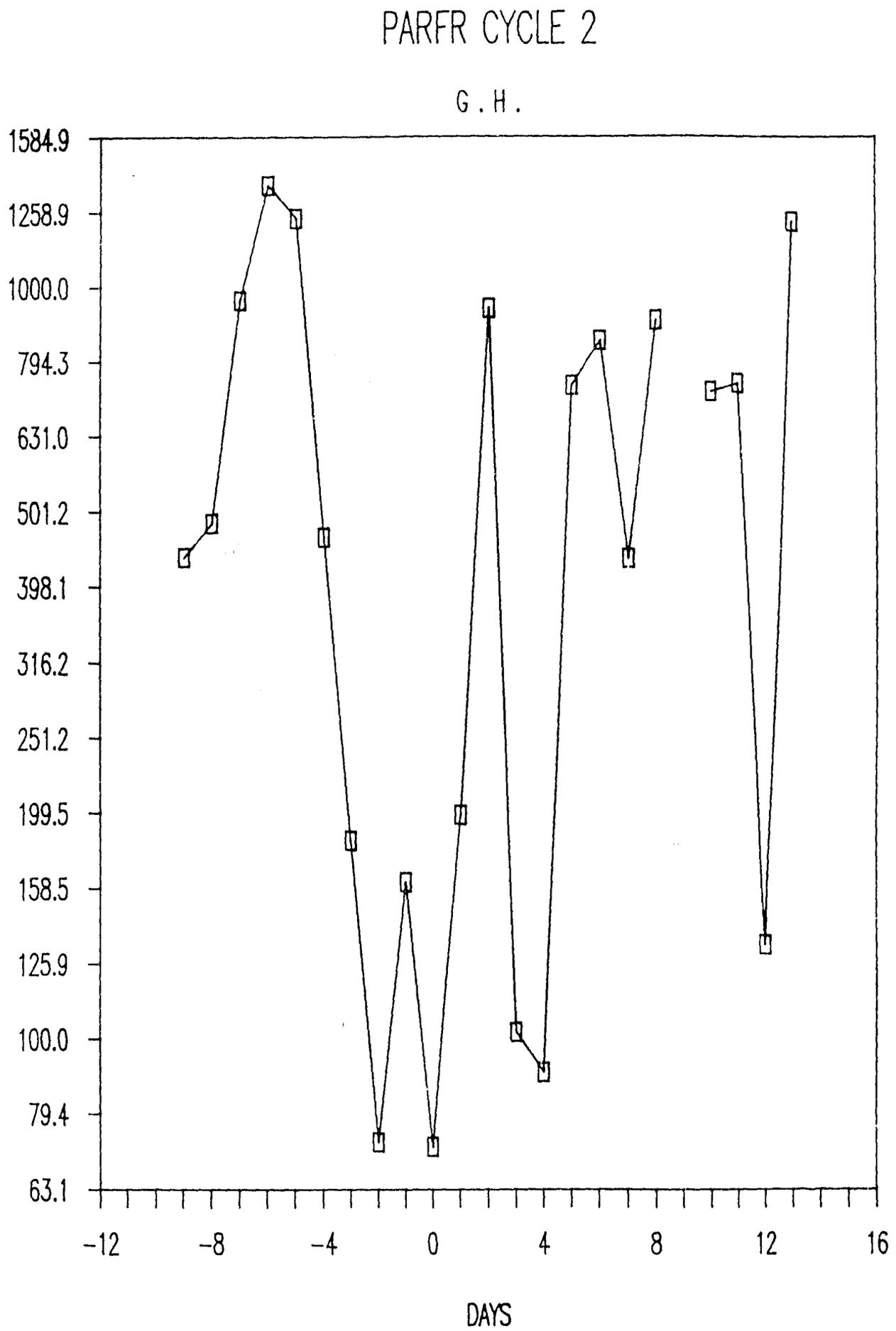
G . H .





G.H.

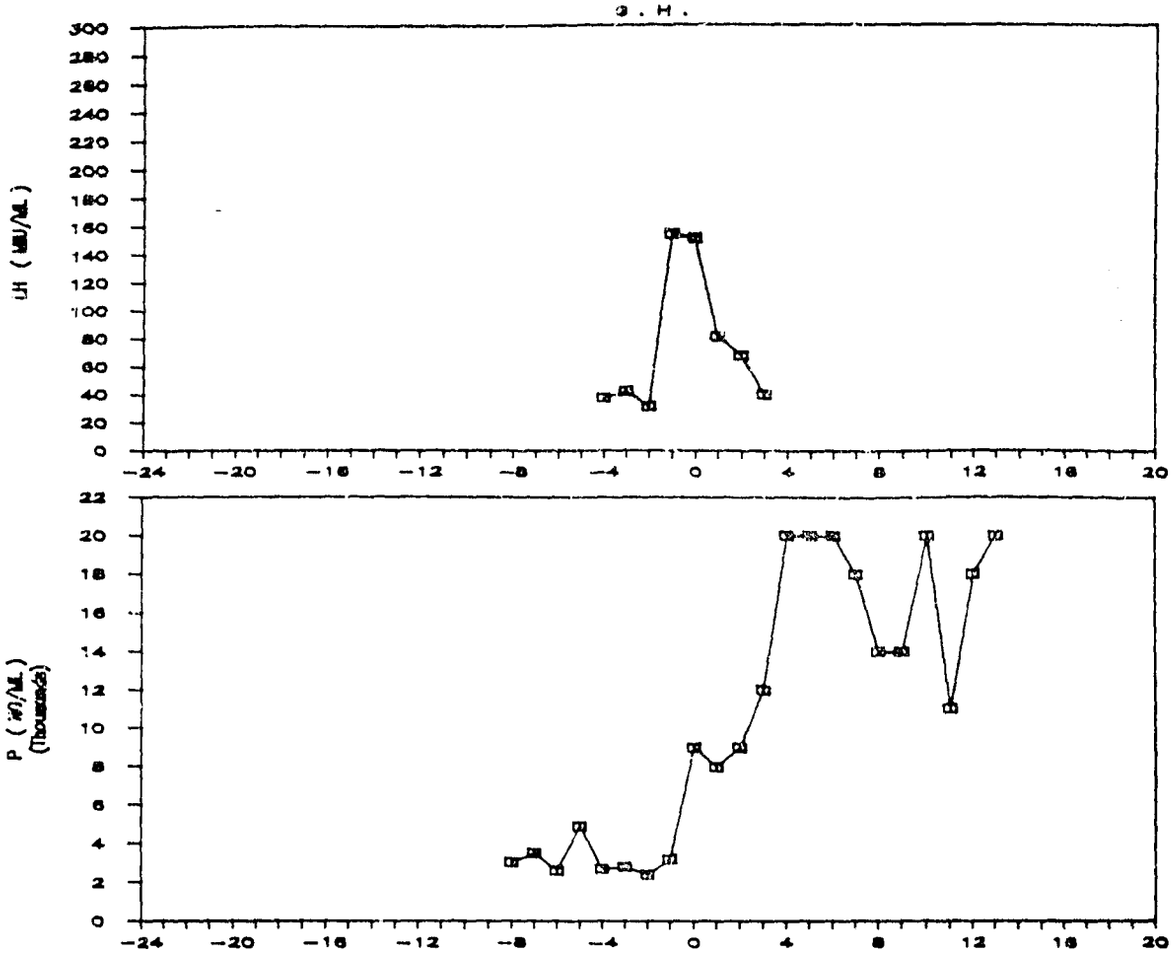
NAME	DATE	DFOM	DVD	GPC	GPV	LH	P	E2	BBT	REMARKS
	3/9/85	5	-9	434.3					97.4	PARFR CYCLE 2
	3/10/85	6	-8	483.3			3000.0		98.0	
	3/11/85	7	-7	956.0			3500.0		98.0	
	3/12/85	8	-6	1366.0			2600.0		98.0	
	3/13/85	9	-5	1233.3			4900.0		98.0	
	3/14/85	10	-4	462.6		37.0	2700.0		97.6	
	3/15/85	11	-3	183.1		42.0	2800.0		98.0	
	3/16/85	12	-2	72.8		31.0	2400.0		98.0	
	3/17/85	13	-1	161.2		154.0	3200.0		98.0	
	3/18/85	14	0	71.8		151.0	9000.0		97.8	
	3/19/85	15	1	198.1		81.0	8000.0		98.2	
	3/20/85	16	2	935.0		68.0	9000.0		99.0	
	3/21/85	17	3	101.9		40.0	12000.0		98.2	
	3/22/85	18	4	90.2			20000.0		98.0	
	3/23/85	19	5	739.6			20000.0		98.0	
	3/24/85	20	6	848.3			20000.0		98.2	
	3/25/85	21	7	434.1			18000.0		98.0	
	3/26/85	22	8	901.1			14000.0		98.0	
	3/27/85	23	9				14000.0		98.0	
	3/28/85	24	10	722.3			20000.0		98.2	
	3/29/85	25	11	742.7			11000.0		98.2	
	3/30/85	26	12	133.0			18000.0		98.0	
	3/31/85	27	13	1220.0			20000.0		98.0	

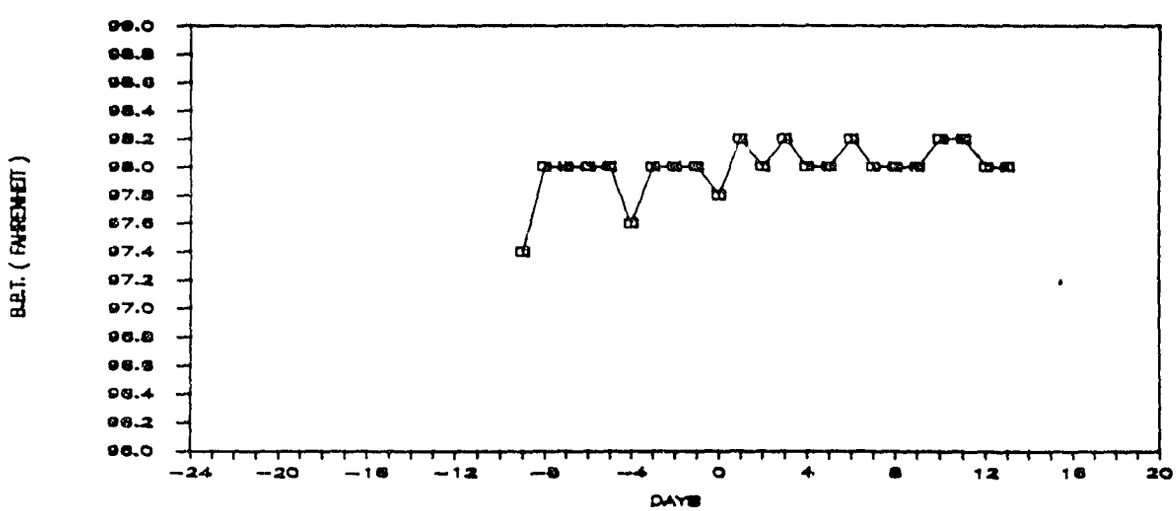

PARFR CYCLE 2
G.H.
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
-12
-8
-4
0
4
8
12
16
DAYS

G.H.

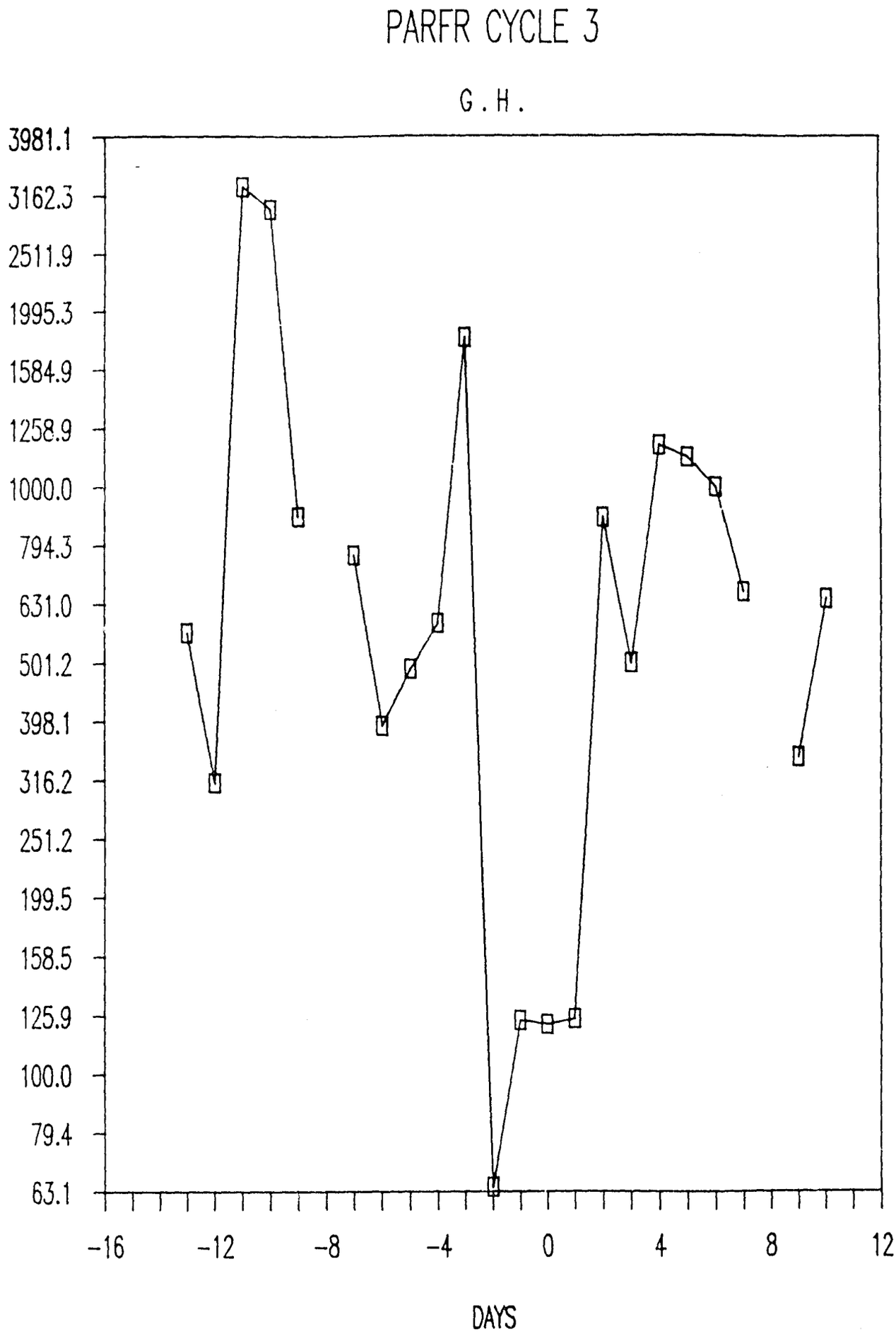
PARFR CYCLE 2

G.H.





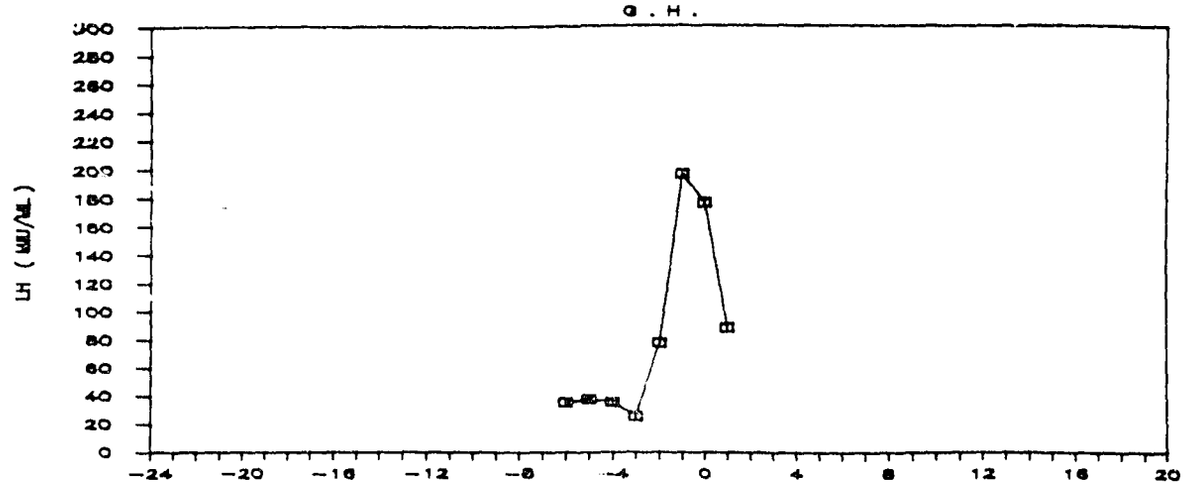
NAME	DATE	DFOM	DVD	GPC	GPV	LH	P	E2	BBT	REMARKS
G.H.	4/3/85	4	-13	562.9			1690.0		98.0	PARFR CYCLE 3
	4/4/85	5	-12	313.3			2200.0		97.6	
	4/5/85	6	-11	3277.3			2400.0		98.0	
	4/6/85	7	-10	2986.7			3000.0		98.0	
	4/7/85	8	-9	888.3			2100.0		97.8	
	4/8/85	9	-8				1700.0		97.6	
	4/9/85	10	-7	764.4			2500.0		98.0	
	4/10/85	11	-6	391.6		35.0	2400.0		98.0	
	4/11/85	12	-5	489.2		37.0	3000.0		98.0	
	4/12/85	13	-4	385.7		35.0	2800.0		98.0	
	4/13/85	14	-3	1800.0		35.0	2800.0		98.0	
	4/14/85	15	-2	64.2		37.0	3400.0		97.8	
	4/15/85	16	-1	123.4		136.0	5600.0		98.0	
	4/16/85	17	0	121.4		176.0	6000.0		98.2	
	4/17/85	18	1	124.3		88.0	5500.0		98.0	
	4/18/85	19	2	882.9			8100.0		98.0	
	4/19/85	20	3	501.0					98.0	
	4/20/85	21	4	1177.5			6000.0		98.2	
	4/21/85	22	5	1122.4			14000.0		98.2	
	4/22/85	23	6	996.0			18000.0		98.0	
	4/23/85	24	7	659.0			18000.0		98.0	
	4/24/85	25	8				11000.0		98.2	
	4/25/85	26	9	345.8			7000.0		98.2	
	4/26/85	27	10	642.0			11000.0		98.0	
	4/27/85	28	11				8400.0		98.0	

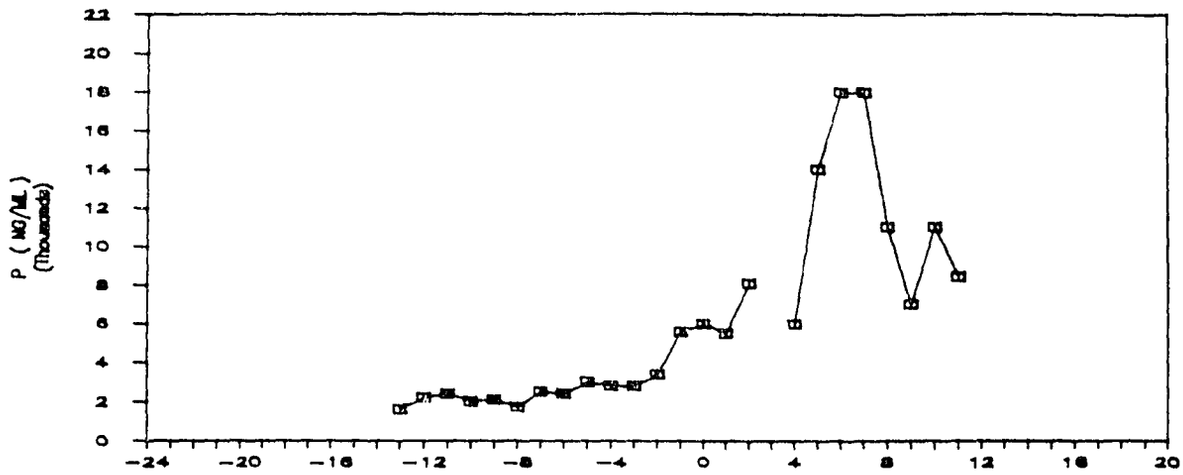

PARFR CYCLE 3
G.H.
3981.1
3162.3
2511.9
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
-16
-12
-8
-4
0
4
8
12
DAYS

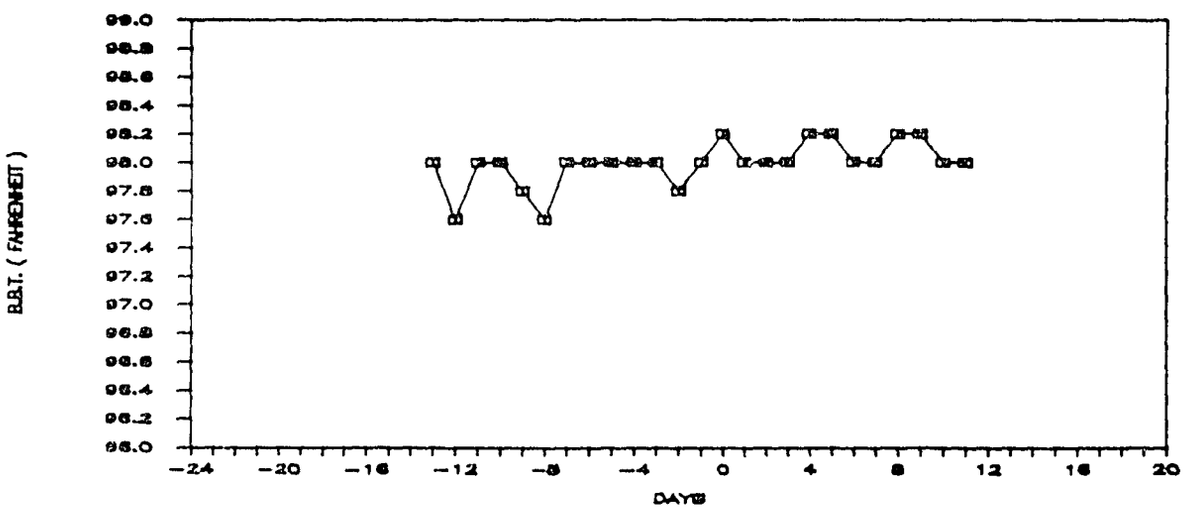
G.H.

PARFR CYCLE 3

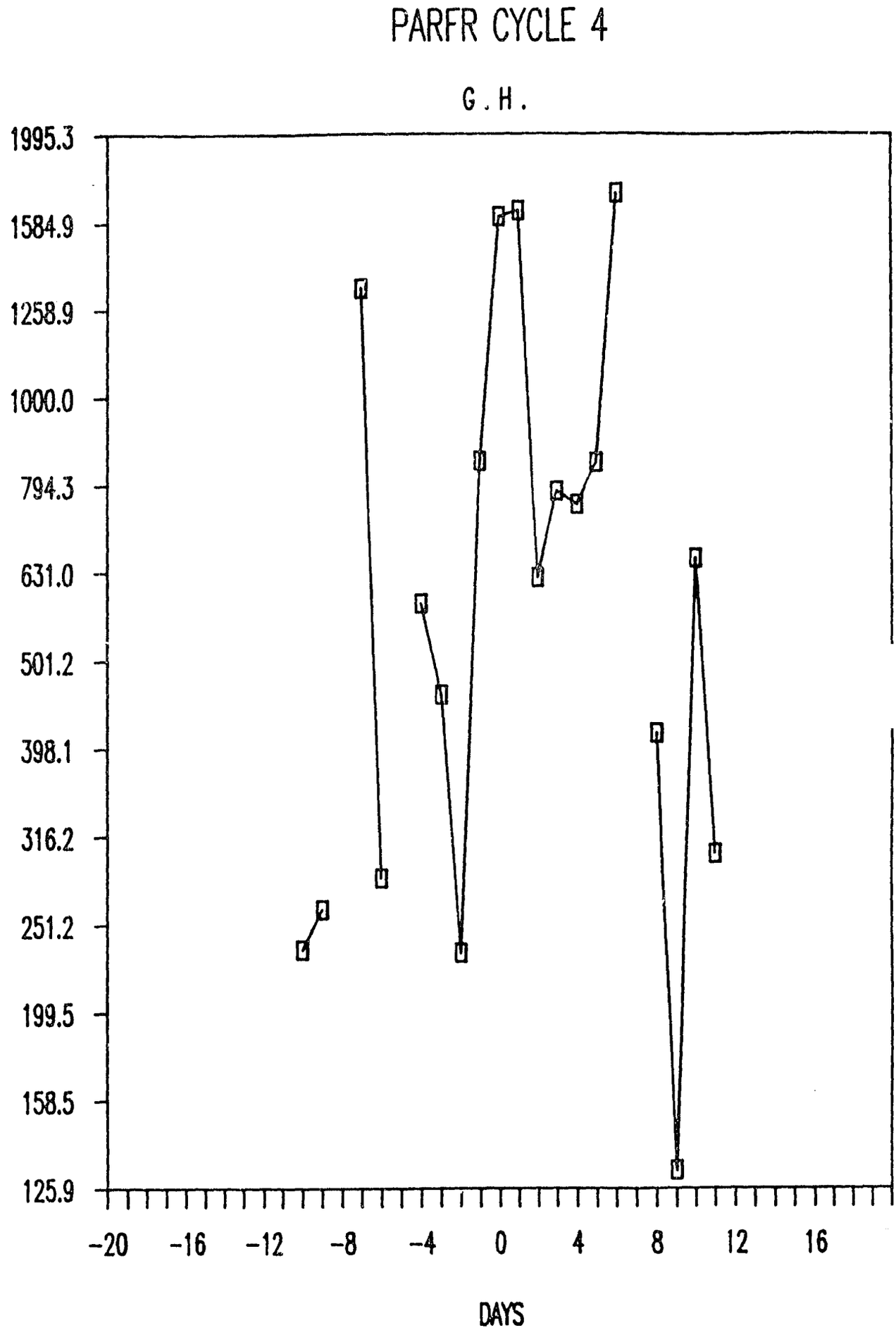
G . H .





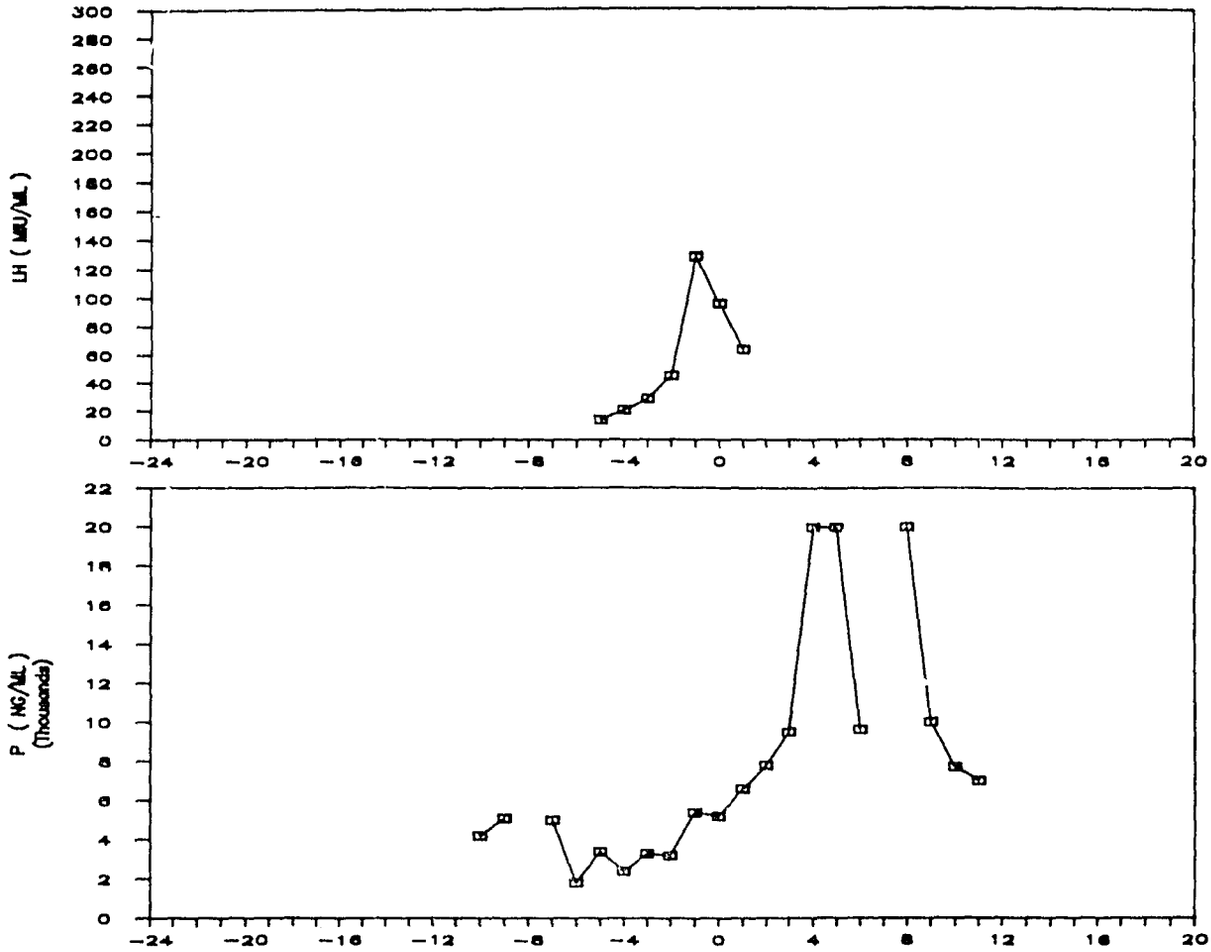


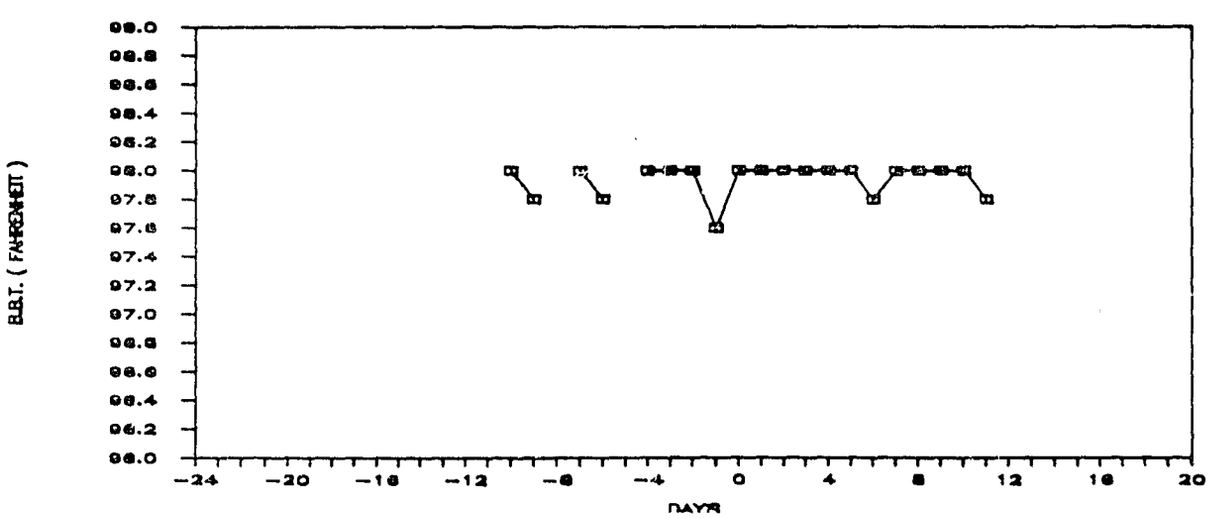
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
G.H.	5/2/85	5	-10	235.4			4200.0		98.0	PARFR CYCLE 4
	5/3/85	6	-9	261.4			5100.0		97.8	
	5/4/85	7	-8							
	5/5/85	8	-7	1335.2			5000.0		98.0	
	5/6/85	9	-6	284.0			1800.0		97.8	
	5/7/85	10	-5			14.0	3400.0			
	5/8/85	11	-4	583.8		21.0	2400.0		98.0	
	5/9/85	12	-3	459.5		29.0	3300.0		98.0	
	5/10/85	13	-2	233.4		45.0	3200.0		98.0	
	5/11/85	14	-1	848.3		128.0	5400.0		97.6	
	5/12/85	15	0	1614.1		95.0	5200.0		98.0	
	5/13/85	16	1	1637.9		63.0	6600.0		98.0	
	5/14/85	17	2	624.9			7800.0		98.0	
	5/15/85	18	3	784.2			9500.0		98.0	
	5/16/85	19	4	756.3			20000.0		98.0	
	5/17/85	20	5	844.4			20000.0		98.0	
	5/18/85	21	6	1710.0			9600.0		97.8	
	5/19/85	22	7						98.0	
	5/20/85	23	8	414.4			20000.0		98.0	
	5/21/85	24	9	132.1			10000.0		98.0	
	5/22/85	25	10	655.3			7700.0		98.0	
	5/23/85	26	11	302.5			7000.0		97.8	


PARFR CYCLE 4
G.H.
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
-20
-16
-12
-8
-4
0
4
8
12
16
DAYS

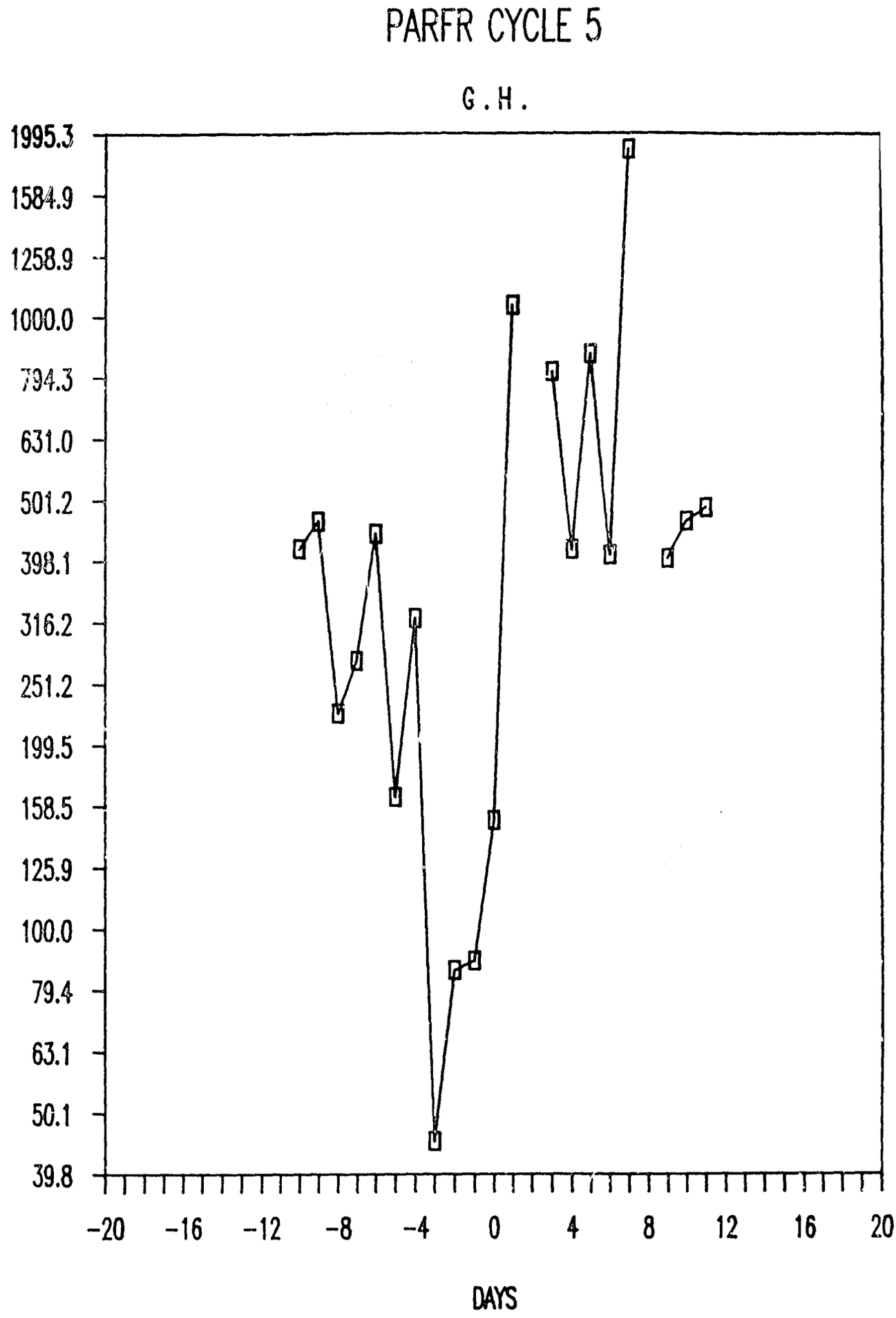
G.H.

PARFR CYCLE 4



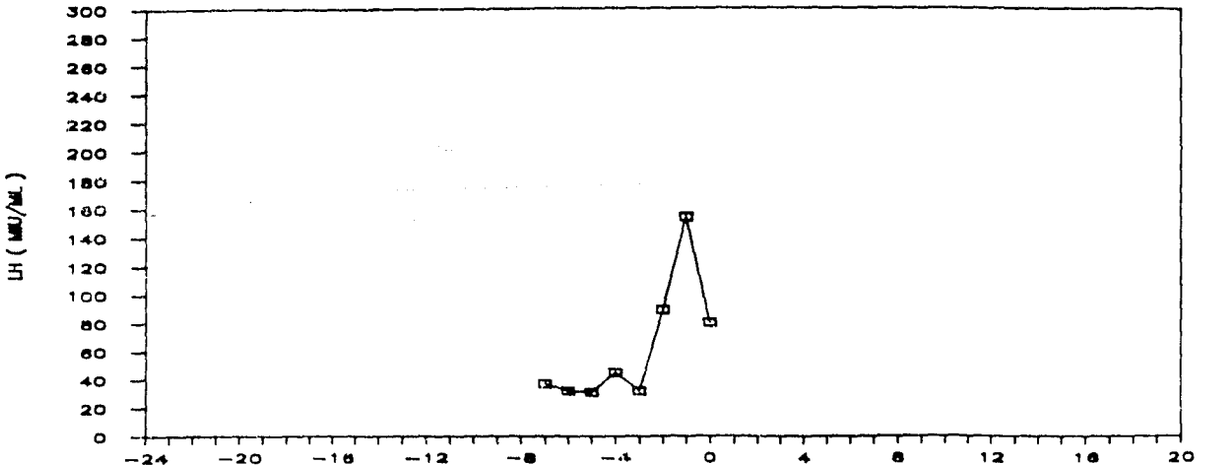


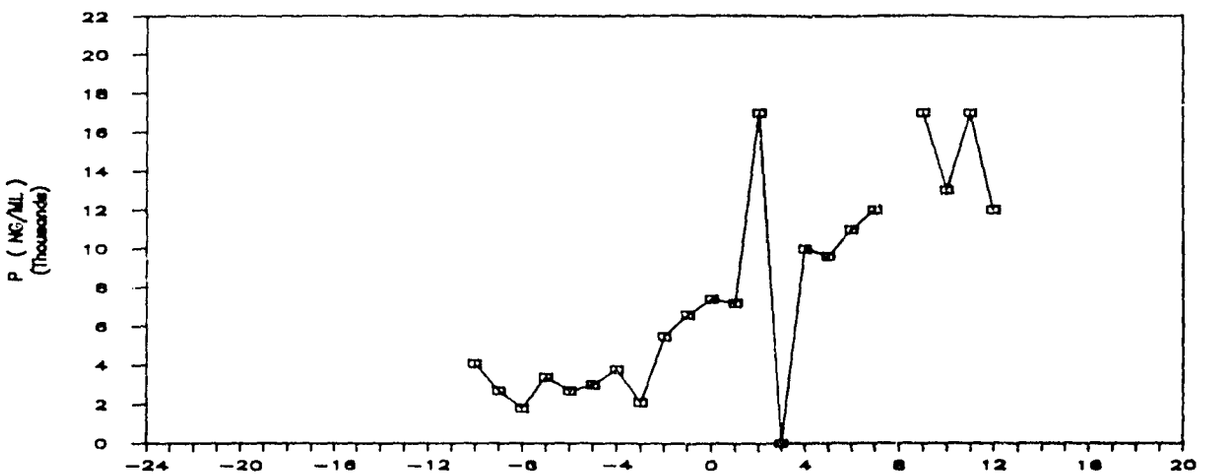
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
G.H.	5/27/85	4	-10	417.5			4100.0		97.6	PARFR CYCLE 5
	5/28/85	5	-9	463.7			2700.0		98.0	
	5/29/85	6	-8	225.0			1800.0		98.0	
	5/30/85	7	-7	274.3		37.0	3400.0		97.4	
	5/31/85	8	-6	442.2		32.0	2700.0		98.0	
	6/1/85	9	-5	164.3		31.0	3000.0		97.8	
	6/2/85	10	-4	322.2		45.0	3800.0		98.0	
	6/3/88	11	-3	45.1		32.0	2100.0		98.0	
	6/4/85	12	-2	95.7		89.0	5500.0		97.6	
	6/5/85	13	-1	88.8		154.0	6600.0		97.8	
	6/6/85	14	0	150.7		80.0	7400.0		98.0	
	6/7/85	15	1	1047.3			7200.0		98.0	
	6/8/85	16	2				17000.0		98.0	
	6/9/85	17	3	315.7			0.0		98.0	
	6/10/85	18	4	416.5			10000.0		97.9	
	6/11/85	19	5	871.5			9600.0		98.0	
	6/12/85	20	6	407.7			11000.0		98.0	
	6/13/85	21	7	1880.0			12000.0		97.8	
	6/14/85	22	8							
	6/15/85	23	9	402.4			17000.0		98.0	
	6/16/85	24	10	463.2			13000.0		98.0	
	6/17/85	25	11	487.1			17000.0		98.0	

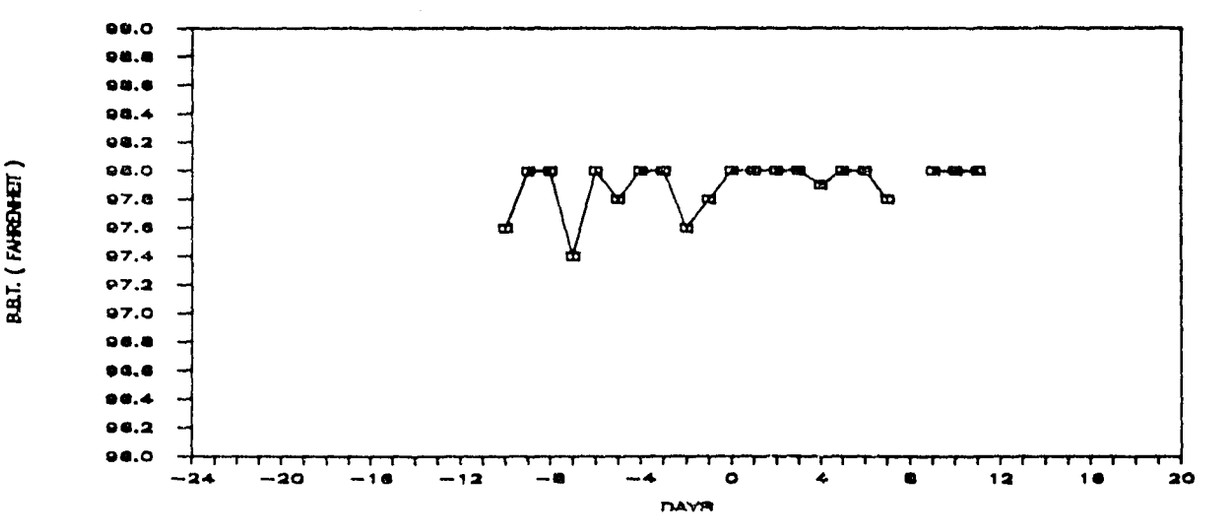

PARFR CYCLE 5
G.H.
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

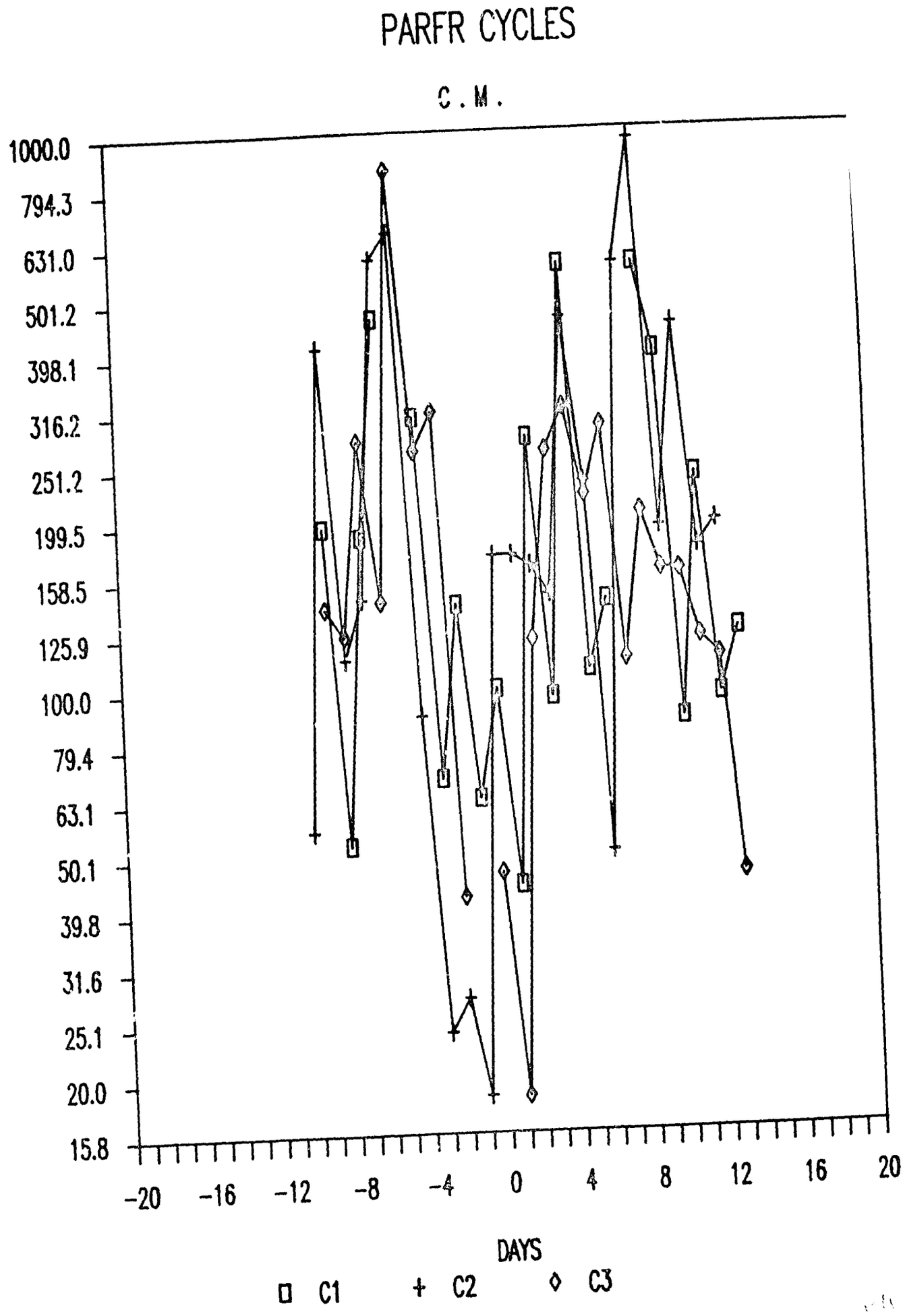
G.H.

PARFR CYCLE 5





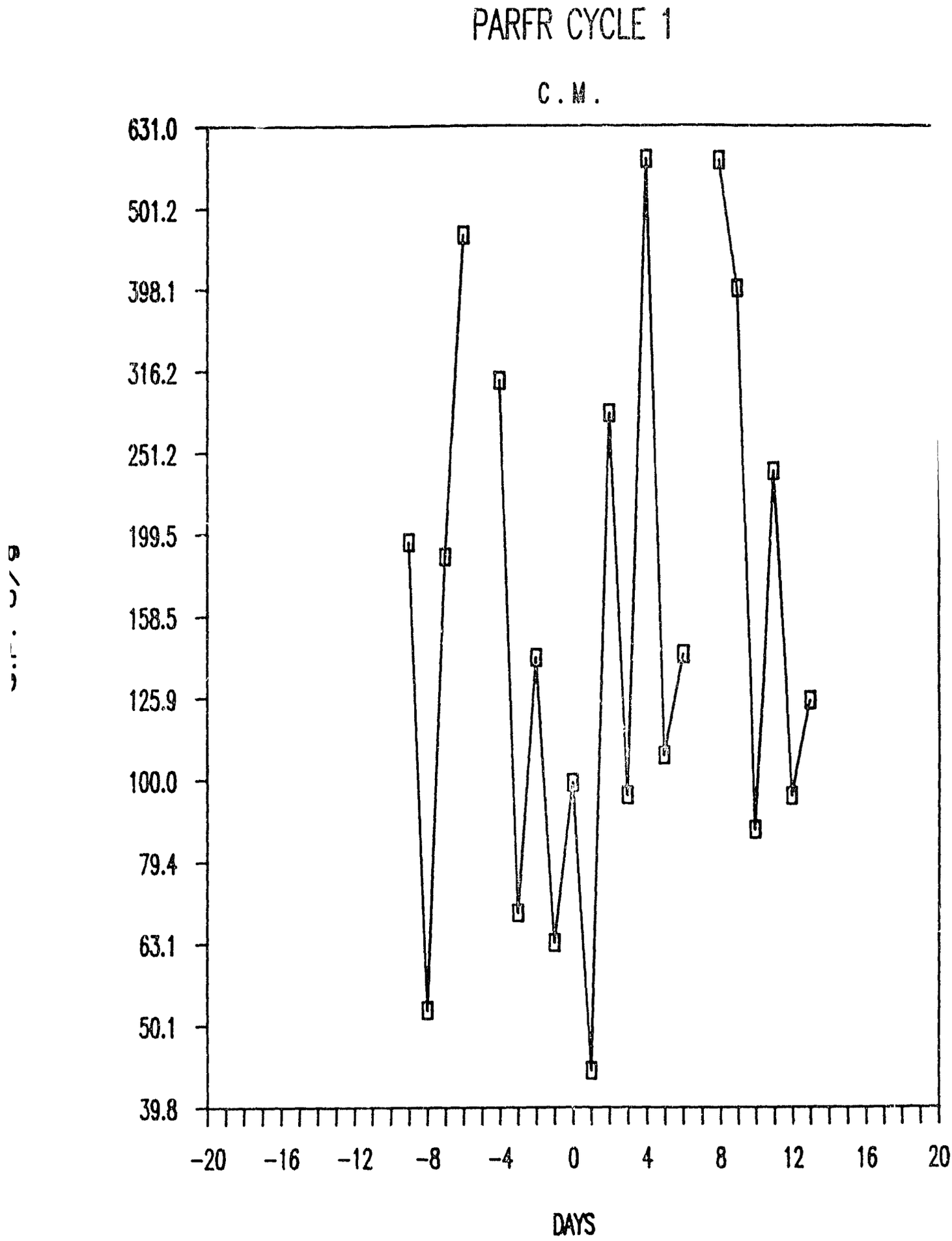



PARFR CYCLES
C.M.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS
C1
C2
C3

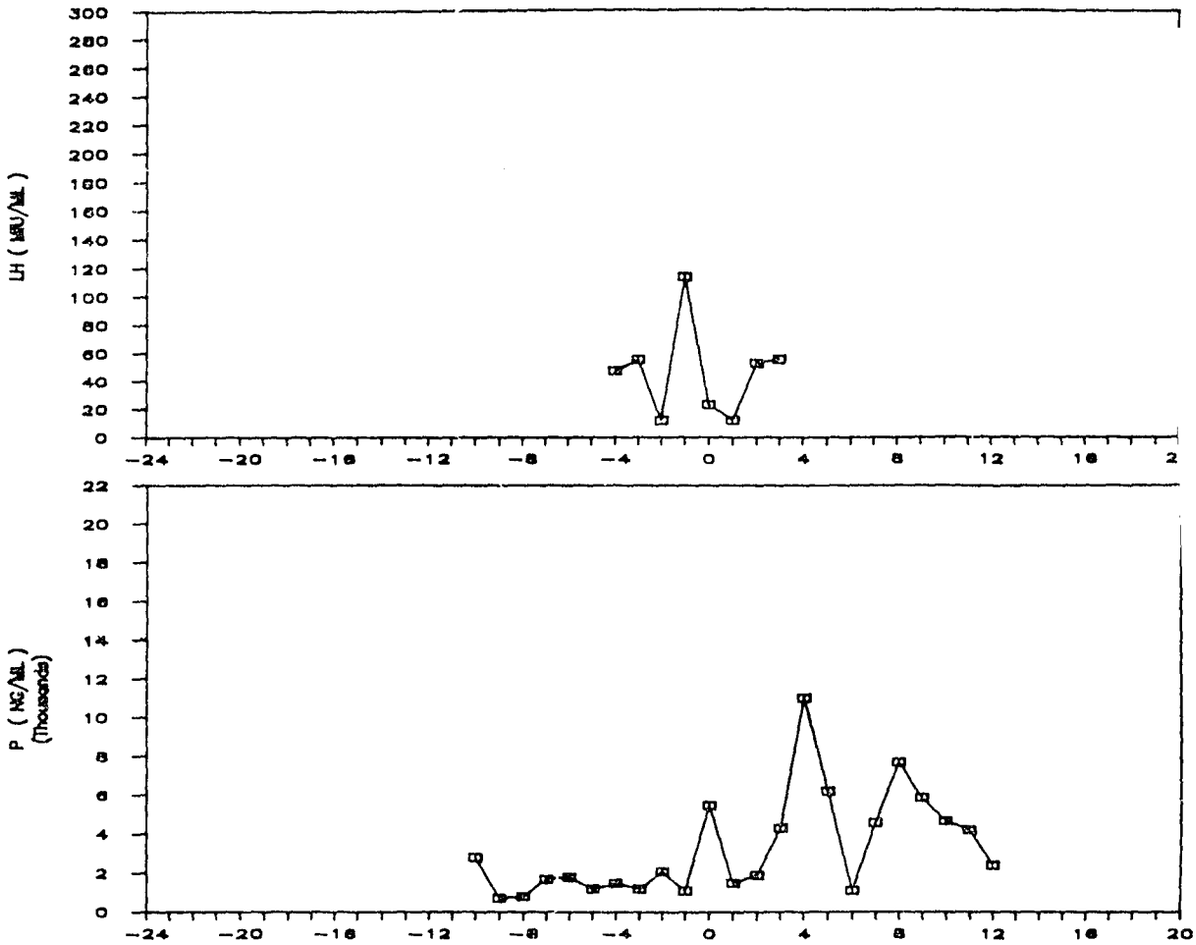
Total: 3 cycles

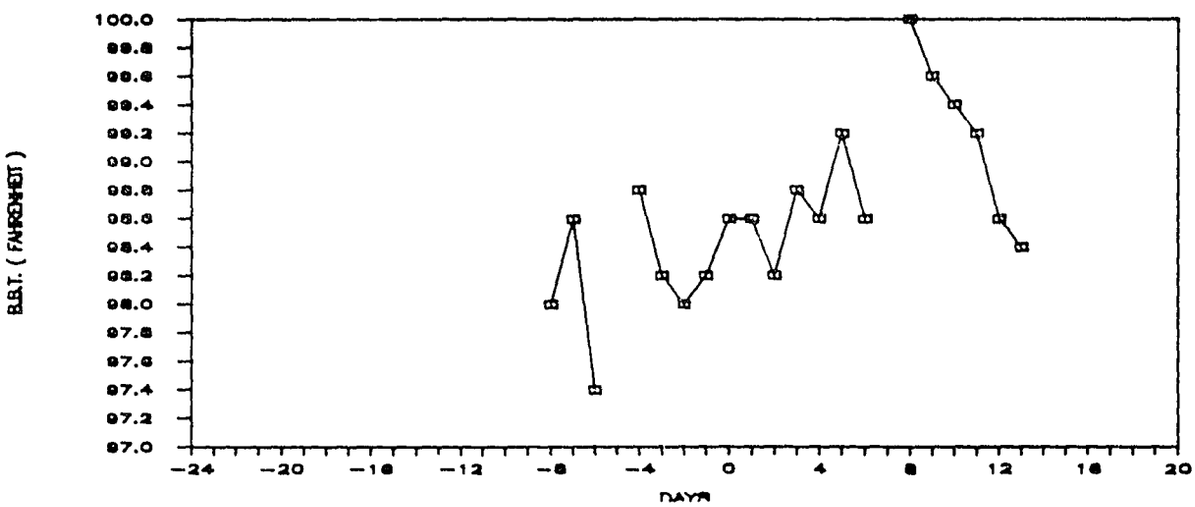
C.M.

NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMAR...
	3/21/85	6	-9	195.3						PARFR CYCLE I
	3/22/85	7	-8	52.4			3300.0		98.0	
	3/23/85	8	-7	187.7			2800.0		98.6	
	3/24/85	9	-6	464.4			3900.0		97.4	
	3/25/85	10	-5				1700.0			
	3/26/85	11	-4	308.4		47.0	3800.0		98.8	
	3/27/85	12	-3	68.9		55.0	3700.0		98.2	
	3/28/85	13	-2	141.4		12.0	1400.0		98.0	
	3/29/85	14	-1	63.3		113.0	3900.0		98.2	
	3/30/85	15	0	99.4		23.0	2200.0		98.6	
	3/31/85	16	1	44.2		12.0	2200.0		98.6	
	4/1/85	17	2	281.0		52.0	4400.0		98.2	
	4/2/85	18	3	95.8		55.0	7000.0		98.8	
	4/3/85	19	4	575.1			8000.0		98.6	
	4/4/85	20	5	107.2			9000.0		99.2	
	4/5/85	21	6	142.4			20000.0		98.6	
	4/6/85	22	7							
	4/7/85	23	8	573.3			10000.0		100.0	
	4/8/85	24	9	399.4			8000.0		99.6	
	4/9/85	25	10	86.9			8000.0		99.4	
	4/10/85	26	11	238.4			10000.0		99.2	
	4/12/85	27	12	95.6			2900.0		98.6	
	4/13/85	28	13	124.9			2000.0		98.4	

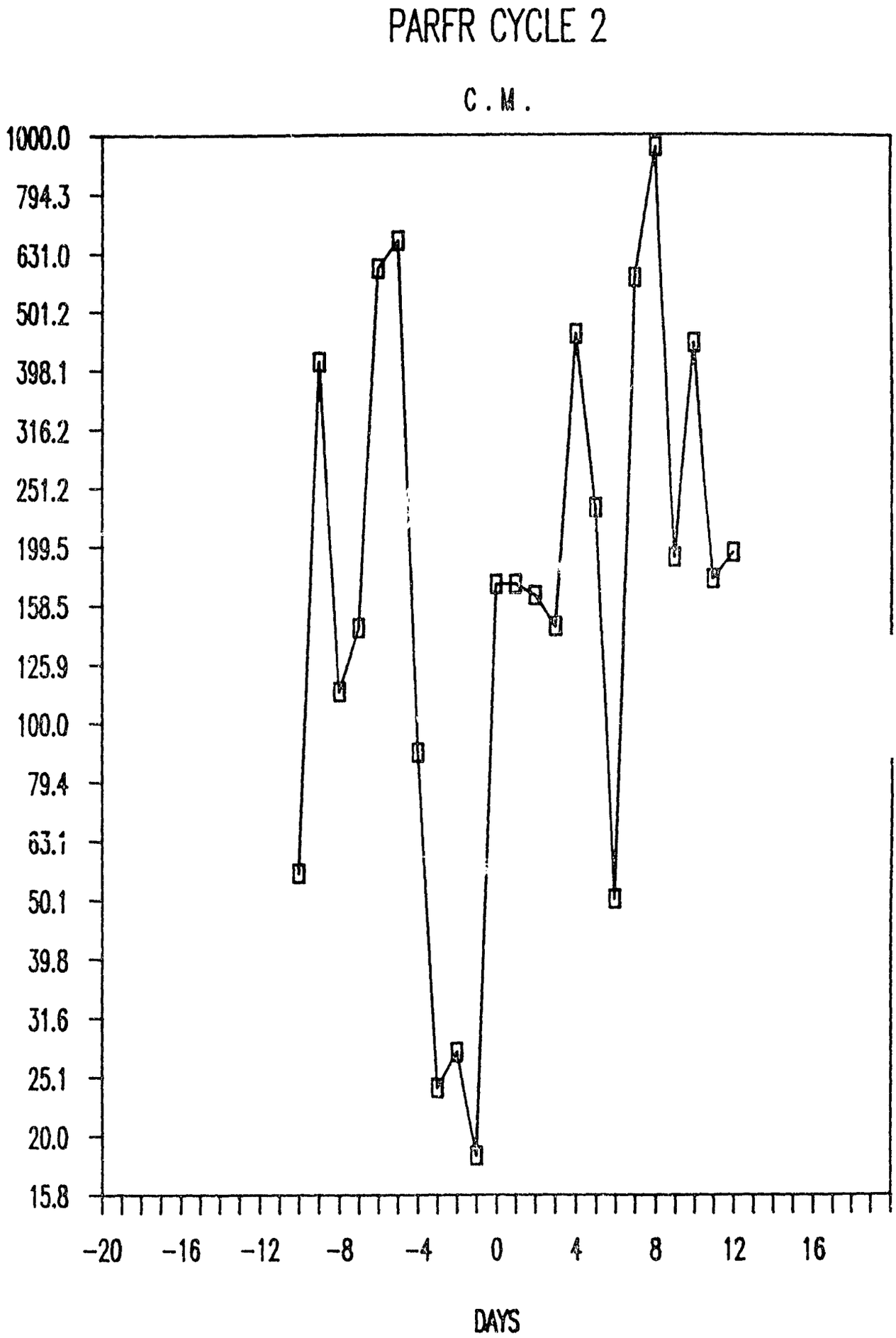

PARFR CYCLE 1
C.M.
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

PARFR CYCLE 1



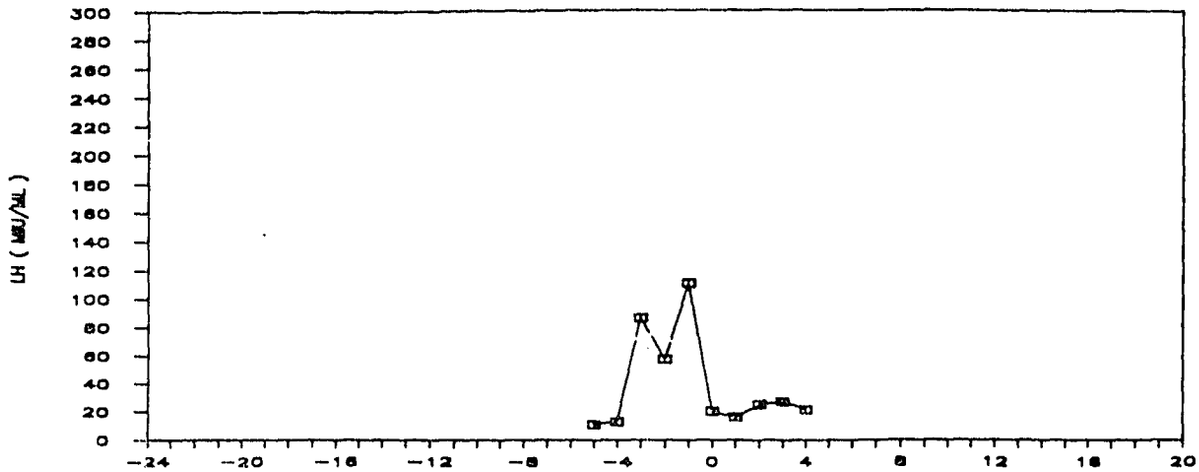


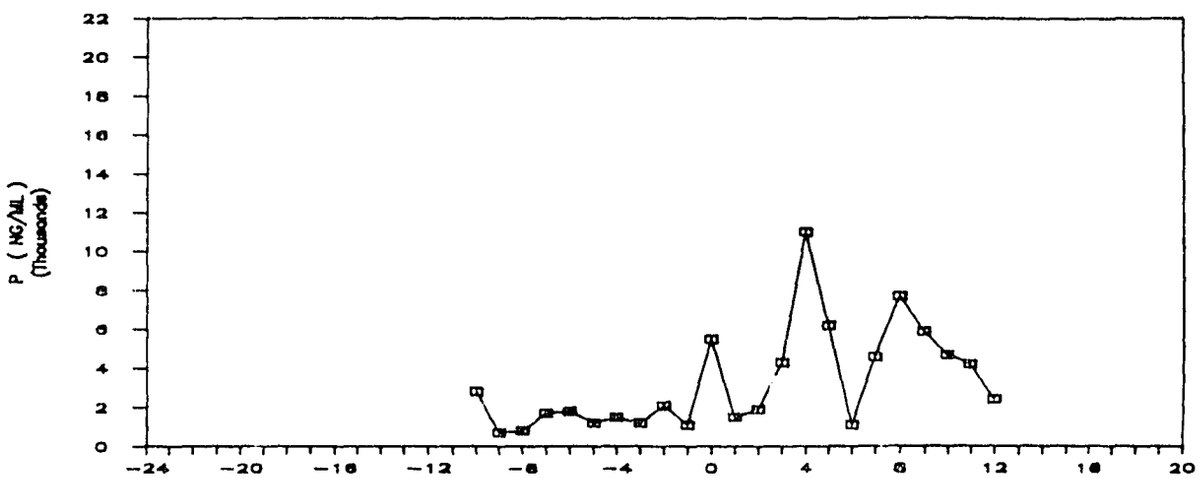
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
C.M.	4/17/85	5	-10	55.7			2800.0			PARFR CYCLE 2
	4/18/85	6	-9	411.5			700.0		98.6	
	4/19/85	7	-8	113.5			800.0		98.2	
	4/20/85	8	-7	145.6			1700.0		99.0	
	4/21/85	9	-6	594.4			1800.0		98.8	
	4/22/85	10	-5	663.3		11.0	1200.0		98.6	
	4/23/85	11	-4	89.5		13.0	1500.0		98.2	
	4/24/85	12	-3	24.1		86.0	1200.0		98.6	
	4/25/85	13	-2	27.7		57.0	2100.0		98.6	
	4/26/85	14	-1	18.5		110.0	1100.0		98.6	
	4/27/85	15	0	172.9		20.0	5500.0		98.6	
	4/28/85	16	1	172.6		16.0	1500.0		98.6	
	4/29/85	17	2	165.5		25.0	1900.0		99.2	
	4/30/85	18	3	146.3		27.0	4300.0		98.4	
	5/1/85	19	4	460.3		21.0	11000.0		98.6	
	5/2/85	20	5	232.7			6200.0		98.6	
	5/3/85	21	6	50.4			1100.0		98.6	
	5/4/85	22	7	574.5			4600.0		98.6	
	5/5/85	23	8	954.7			7700.0		98.2	
	5/6/85	24	9	192.1			5900.0		99.0	
	5/7/85	25	10	443.5			4700.0		98.4	
	5/8/85	26	11	176.4			4200.0		99.2	
	5/9/85	27	12	195.3			2400.0		99.0	

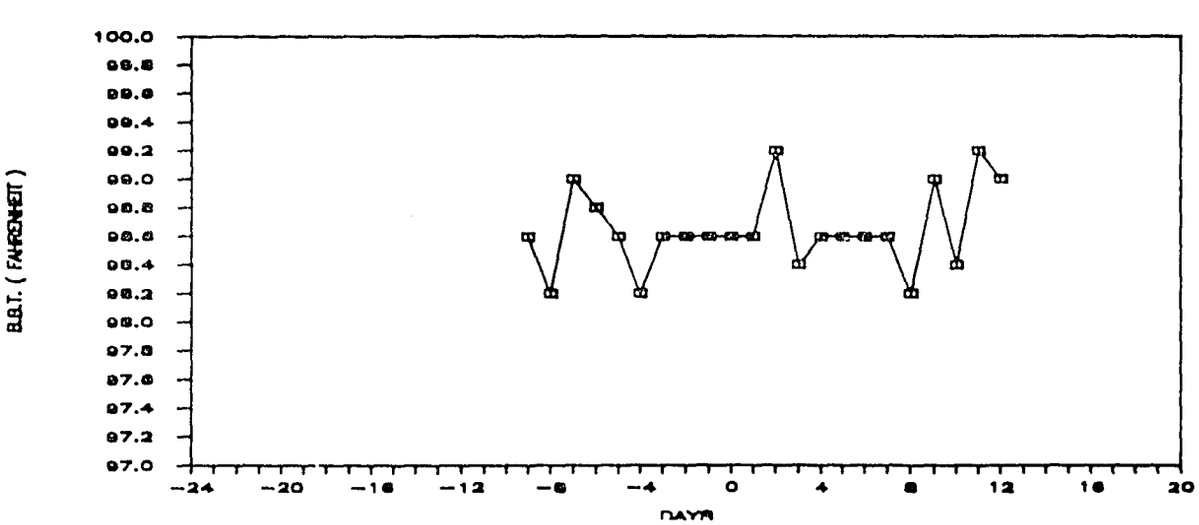

PARFR CYCLE 2
C.M.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
-20
-16
-12
-8
-4
0
4
8
12
16
DAYS

C.M.

PARFR CYCLE 2

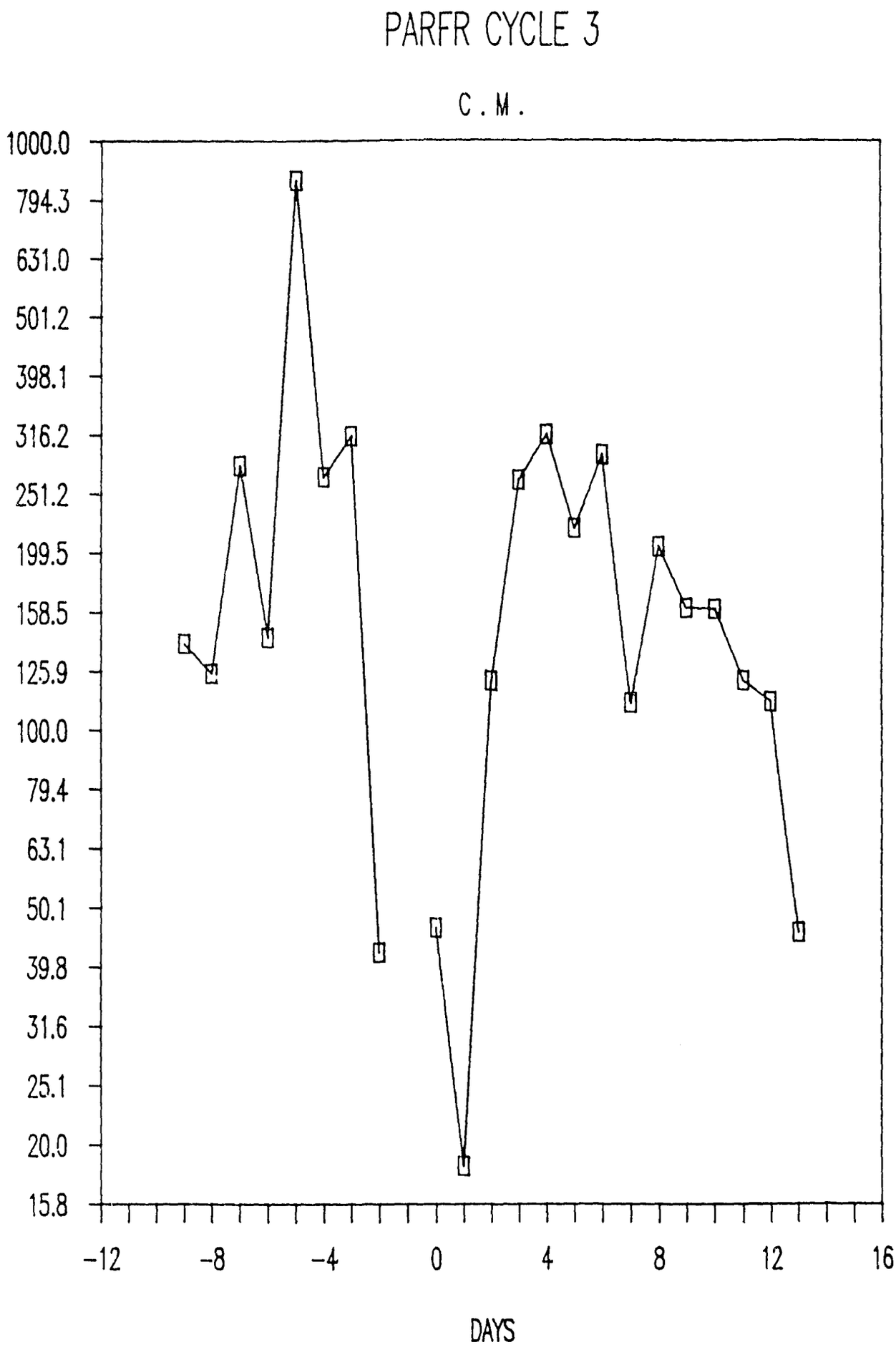






C.M.

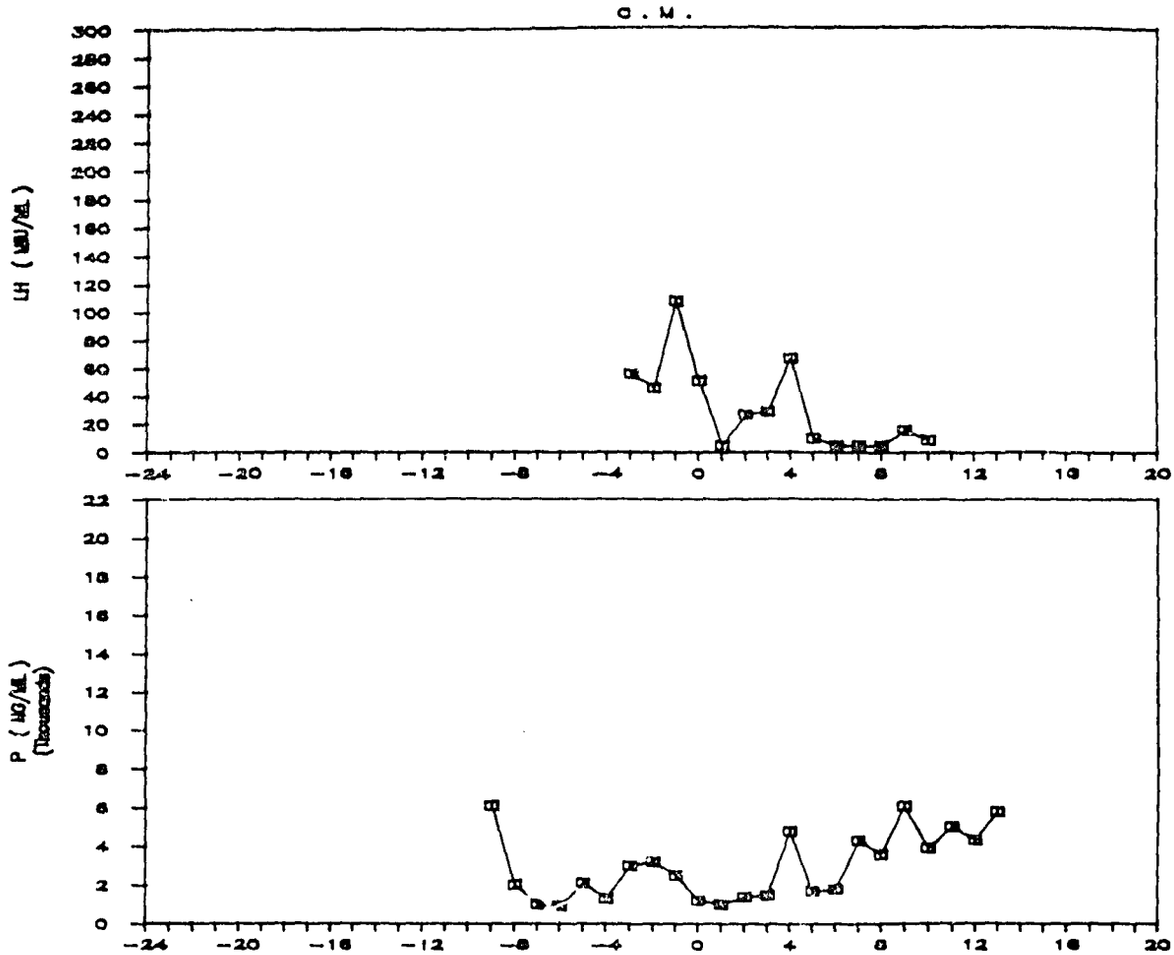
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
	5/14/85	5	-9	140.0			6100.0		98.0	PARFR CYCLE 3
	5/15/85	6	-8	124.6			2000.0		98.0	
	5/16/85	7	-7	280.0			1000.0		97.6	
	5/17/85	8	-6	143.3			900.0		98.2	
	5/18/85	9	-5	857.4			2100.0		98.0	
	5/19/85	10	-4	267.4			1300.0		97.8	
	5/20/85	11	-3	314.5		56.0	3000.0		98.2	
	5/21/85	12	-2	42.1		46.0	3200.0		98.0	
	5/22/85	13	-1			108.0	2500.0		98.0	
	5/23/85	14	0	46.5		51.0	1200.0		98.0	
	5/24/85	15	1	18.4		5.0	1000.0		97.8	
	5/25/85	16	2	121.7		27.0	1400.0		97.6	
	5/26/85	17	3	265.7		29.0	1500.0		98.4	
	5/27/85	18	4	317.3		67.0	4800.0		98.6	
	5/28/85	19	5	220.0		10.0	1700.0		98.6	
	5/29/85	20	6	293.3		5.0	1800.0		98.6	
	5/30/85	21	7	111.4		5.0	4300.0		98.6	
	5/31/85	22	8	204.2		5.0	3600.0		98.6	
	6/1/85	23	9	160.0		16.0	6100.0		98.6	
	6/2/85	24	10	160.0		9.0	3700.0		99.2	
	6/3/85	25	11	121.3			5000.0		98.2	
	6/4/85	26	12	111.7			4300.0		98.6	
	6/5/85	27	13	45.6			5800.0		98.8	

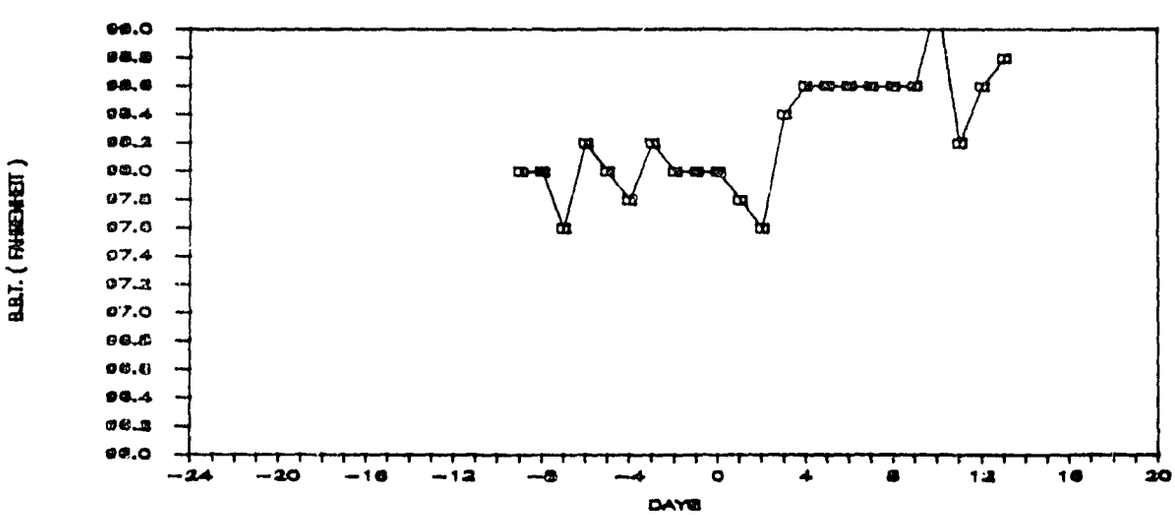

PARFR CYCLE 3
C.M.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
-12
-8
-4
0
4
8
12
16
DAYS

C.M.

PARFR CYCLE 3

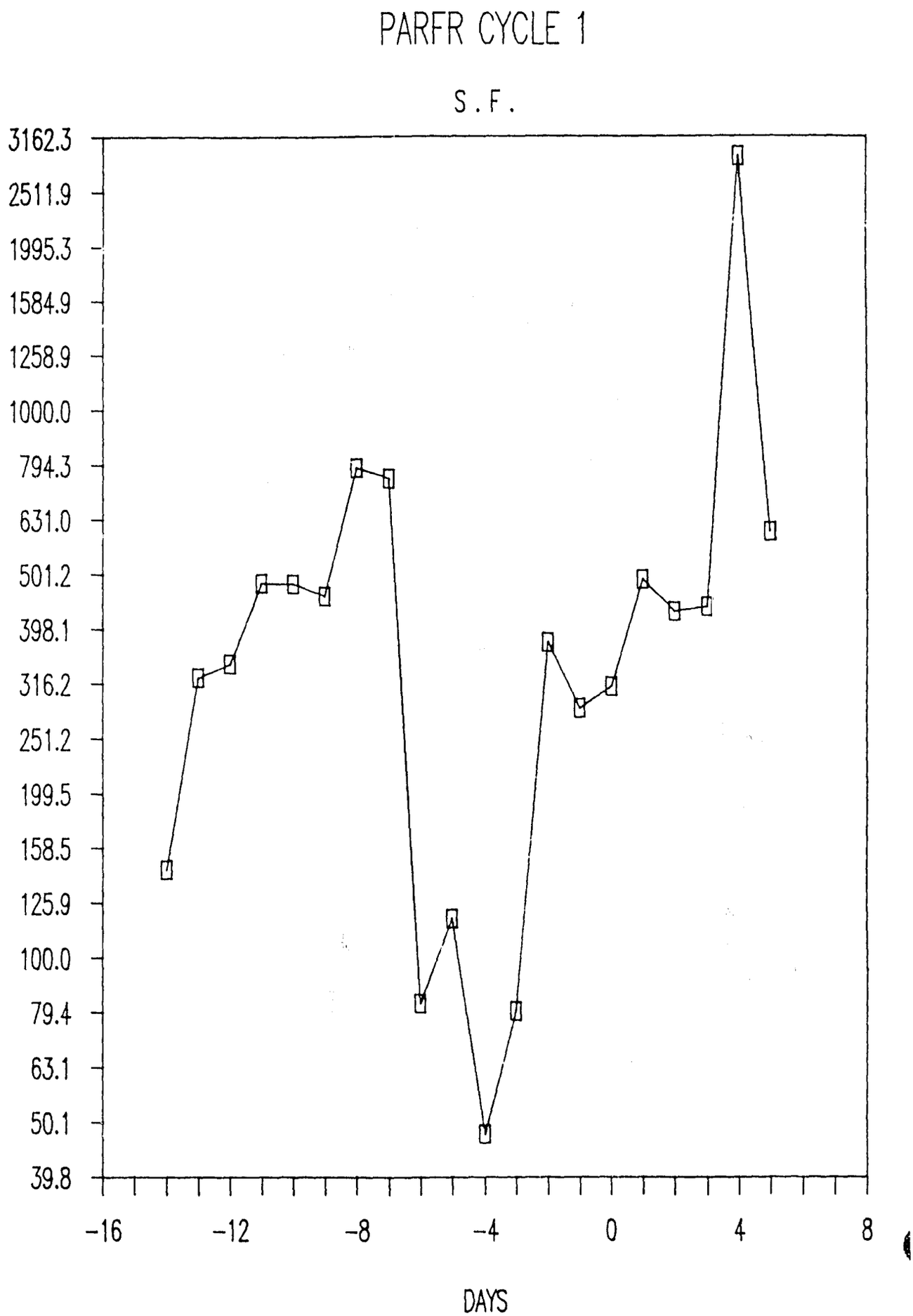
C.M.





Total
1 cycle

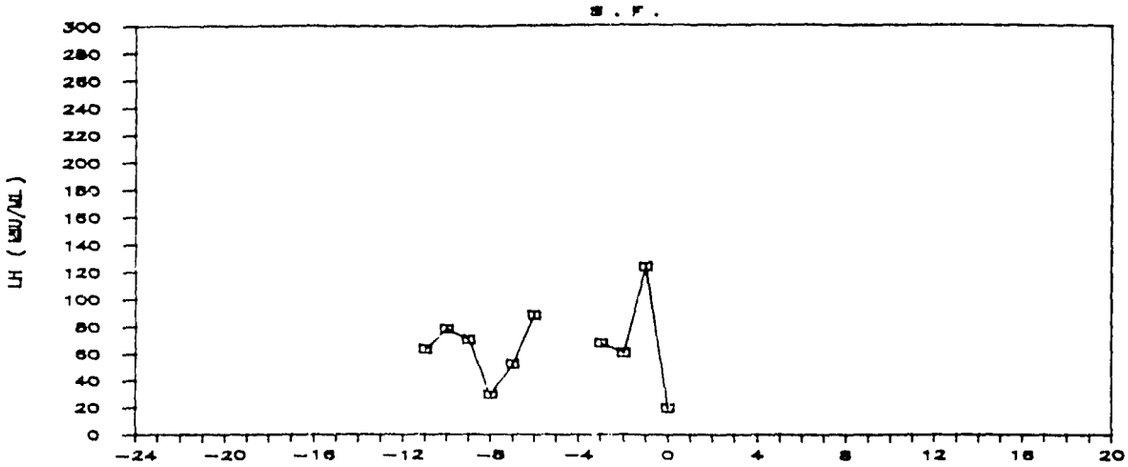
NAME	DATE	DFDM	DVD	GFC	GFS	LH	P	E2	BBT	REMARKS
S.F.	2/25/85	4	-14	145.1						PAPER CYCLE 1
	2/26/85	5	-13	324.8	0.0		3300.0			
	2/27/85	6	-12	343.3	39.2		3500.0		97.8	
	2/28/85	7	-11	482.4	578.1	64.0	3200.0		97.8	
	3/1/85	8	-10	481.6	367.0	79.0	4300.0		98.0	
	3/2/85	9	-9	457.1	548.3	71.0	4600.0		97.8	
	3/3/85	10	-8	384.4	658.2	73.0	3400.0		98.0	
	3/4/85	11	-7	350.5	483.2	53.0	4000.0		98.0	
	3/5/85	12	-6	32.7		57.0	6000.0		97.4	
	3/6/85	13	-5	117.9	511.0		5000.0		97.6	
	3/7/85	14	-4	47.8	302.5		4700.0		97.6	
	3/8/85	15	-3	90.0	608.4	63.0	4310.0		97.9	
	3/9/85	16	-2	377.6	302.3	61.0	4300.0		97.9	
	3/10/85	17	-1	385.6	636.7	124.0	5000.0		98.2	
	3/11/85	18	0	312.8	197.4	20.0	3000.0		98.3	
	3/12/85	19	1	491.2	577.0		7000.0		97.9	
	3/13/85	20	2	429.2	761.1		7000.0		98.4	
	3/14/85	21	3	437.5	1047.3		5000.0		98.6	
	3/15/85	22	4	2917.0	1166.0		6300.0		99.0	
	3/16/85	23	5	600.3	478.2		4106.0		97.4	

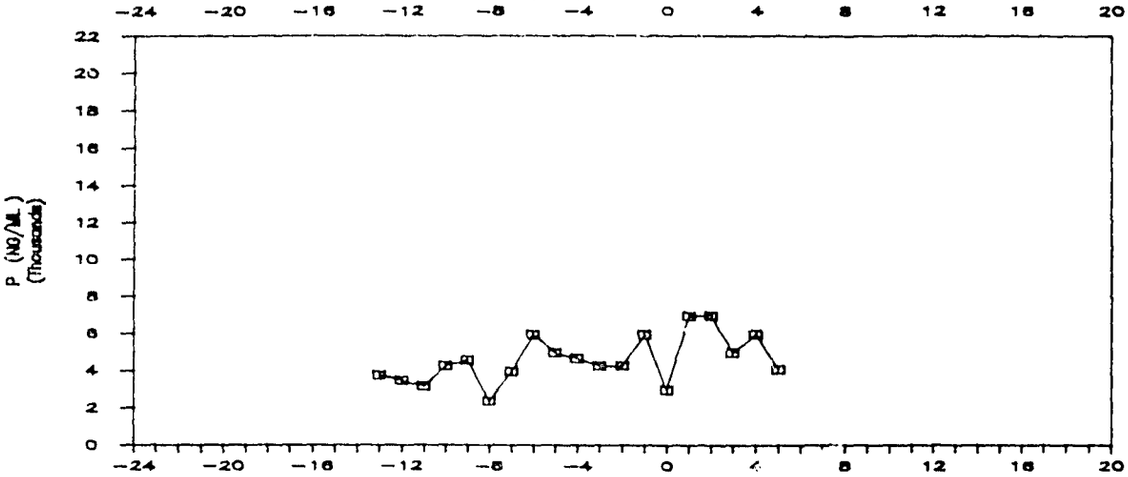

PARFR CYCLE 1
S.F.
3162.3
2511.9
1995.3
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
-16
-12
-8
-4
0
4
8
DAYS

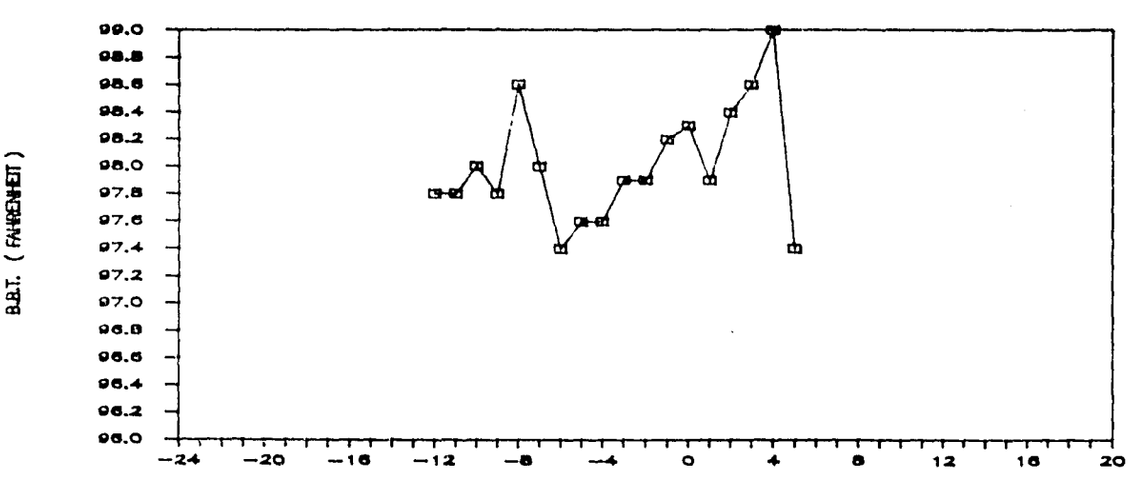
S.F.

PARFR CYCLE 1

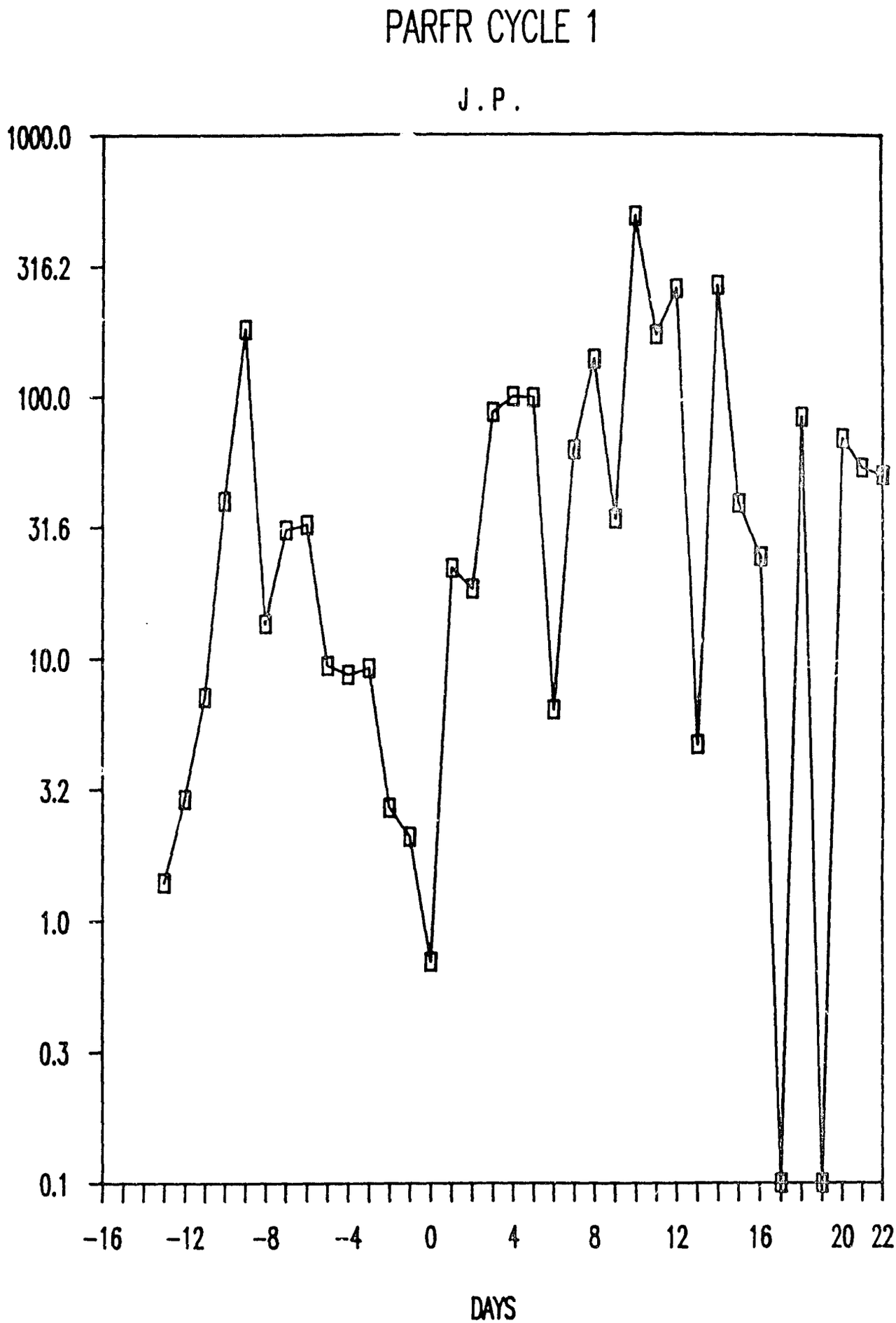
S.F.



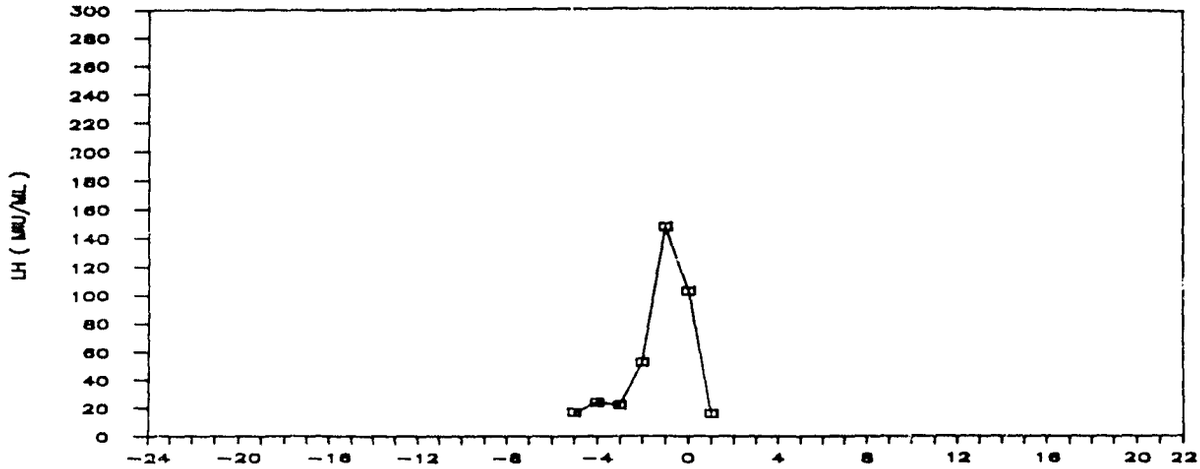


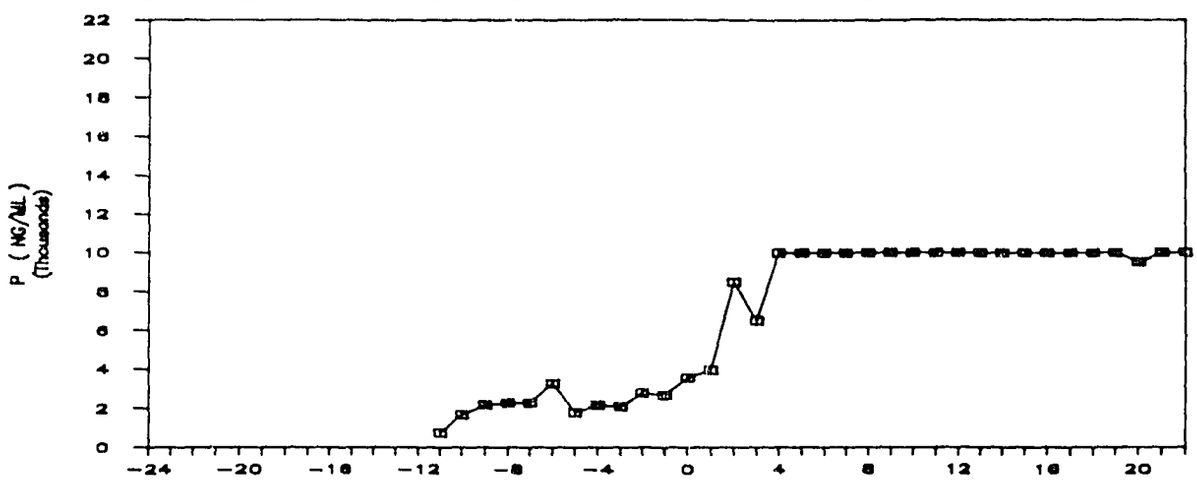


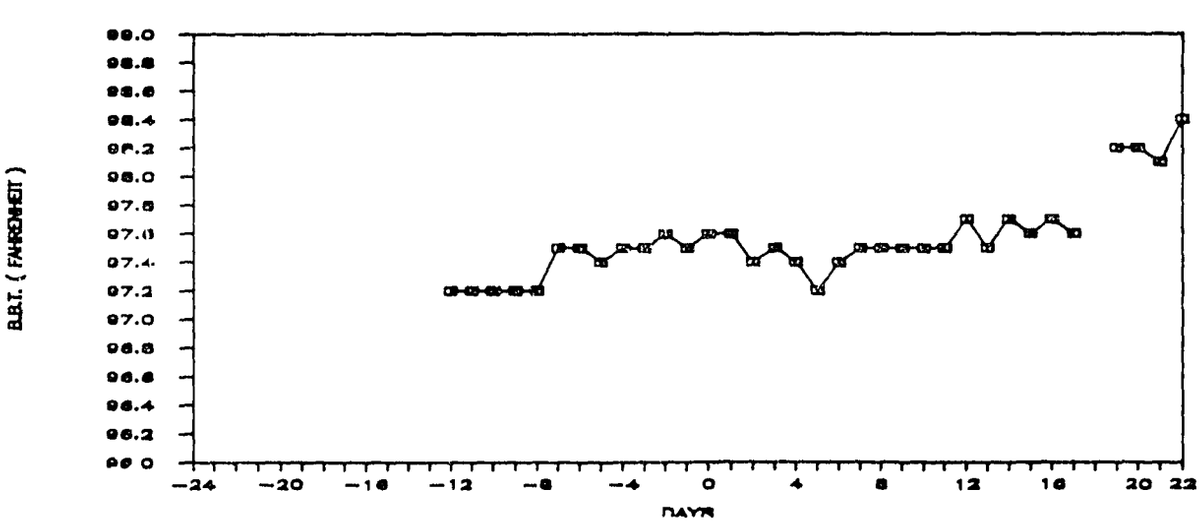
NAME	DATE	DFOM	OVD	GPC	LH	P	BBT	REMARK
J.P.	9/14/84	5	-13	1.4				PARFR CYC
	9/15/84	6	-12	2.9			97.2	
	9/16/84	7	-11	7.1		760.0	97.2	
	9/17/84	8	-10	39.9		1700.0	97.2	
	9/18/84	9	-9	180.0		2200.0	97.2	
	9/19/84	10	-8	13.6		2300.0	97.2	
	9/20/84	11	-7	30.9		2300.0	97.5	
	9/21/84	12	-6	32.4		3300.0	97.5	
	9/22/84	13	-5	9.4	17.0	1800.0	97.4	
	9/23/84	14	-4	8.7	24.0	2200.0	97.5	
	9/24/84	15	-3	9.2	22.0	2100.0	97.5	
	9/25/84	16	-2	2.7	52.0	2800.0	97.6	
	9/26/84	17	-1	2.1	147.0	2700.0	97.5	
	9/27/84	18	0	0.7	102.0	3600.0	97.6	
	9/28/84	19	1	22.2	16.0	4000.0	97.6	
	9/29/84	20	2	18.5		8500.0	97.4	
	9/30/84	21	3	87.4		9500.0	97.5	
	10/1/84	22	4	100.7		10000.0	97.4	
	10/2/84	23	5	99.6		10000.0	97.2	
	10/3/84	24	6	6.4		10000.0	97.4	
	10/4/84	25	7	62.9		10000.0	97.5	
	10/5/84	26	8	139.1		10000.0	97.5	
	10/6/84	27	9	33.9		10000.0	97.5	
	10/7/84	28	10	493.3		10000.0	97.5	
	10/8/84	29	11	172.1		10000.0	97.5	
	10/9/84	30	12	258.5		10000.0	97.7	
	10/10/84	31	13	4.7		10000.0	97.5	
	10/11/84	32	14	266.7		10000.0	97.7	
	10/12/84	33	15	38.9		10000.0	97.6	
	10/13/84	34	16	24.3		10000.0	97.7	
	10/14/84	35	17	0.0		10000.0	97.6	
	10/15/84	36	18	83.0		10000.0	---	
	10/16/84	37	19	0.0		10000.0	98.2	
	10/17/84	38	20	68.5		9500.0	98.2	
	10/18/84	39	21	52.9		10000.0	98.1	
	10/19/84	40	22	49.6		10000.0	98.4	

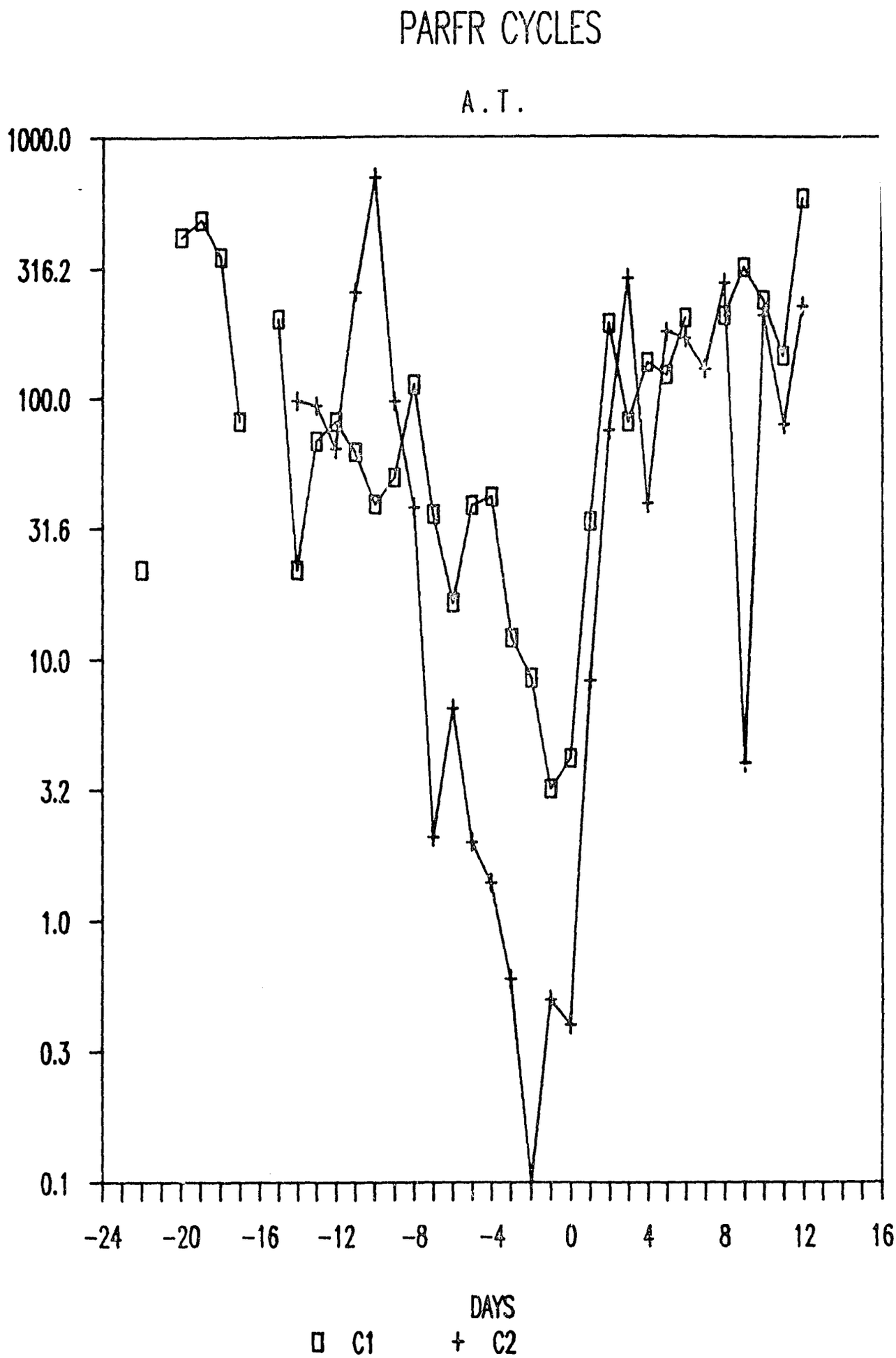

PARFR CYCLE 1
J.P.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-16
-12
-8
-4
0
4
8
12
16
20
22
DAYS

PARFR CYCLE 1





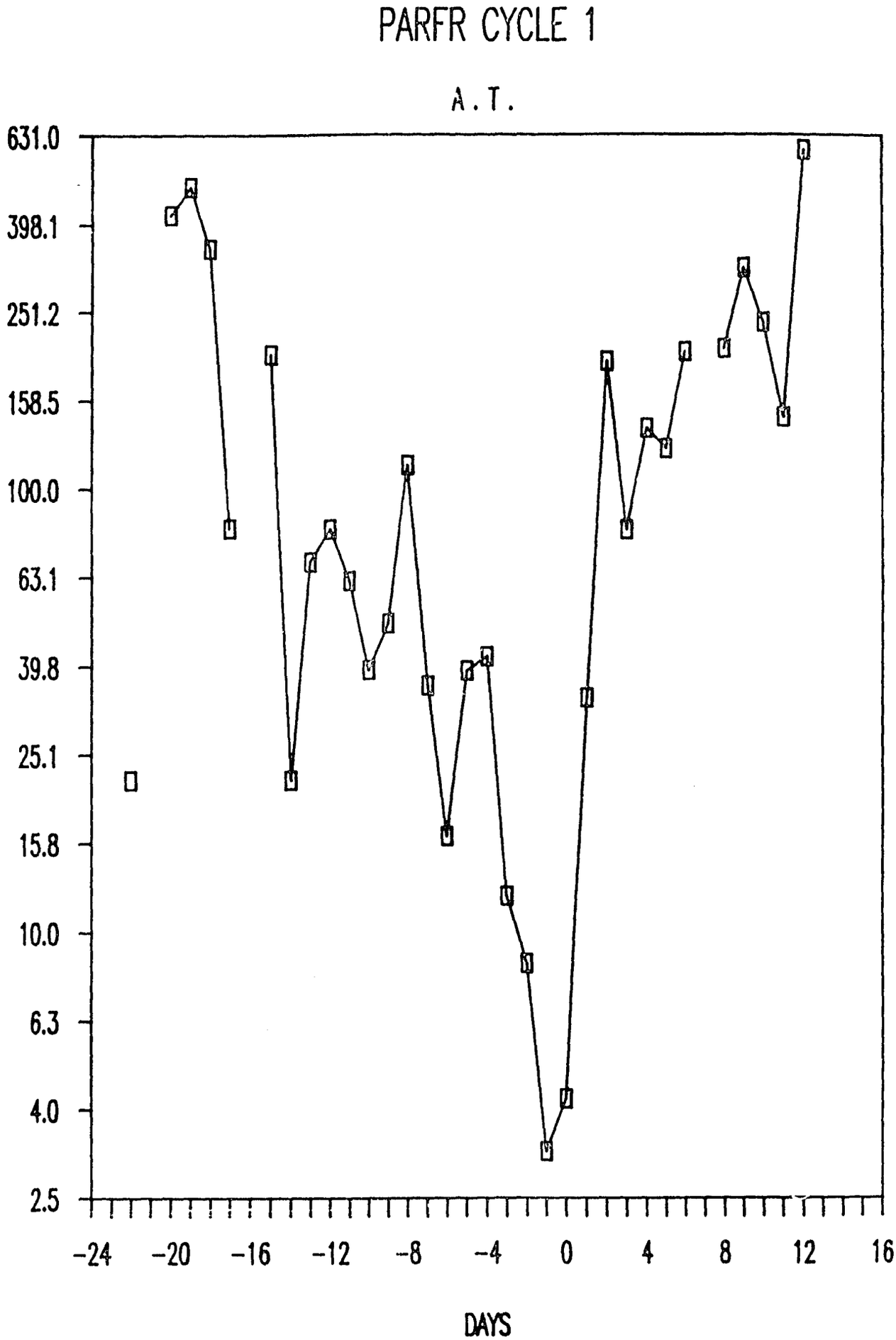



PARFR CYCLES
A.T.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
-24
-20
-16
-12
-8
-4
0
4
8
12
16
DAYS
C1
C2

Total:
2 cycles

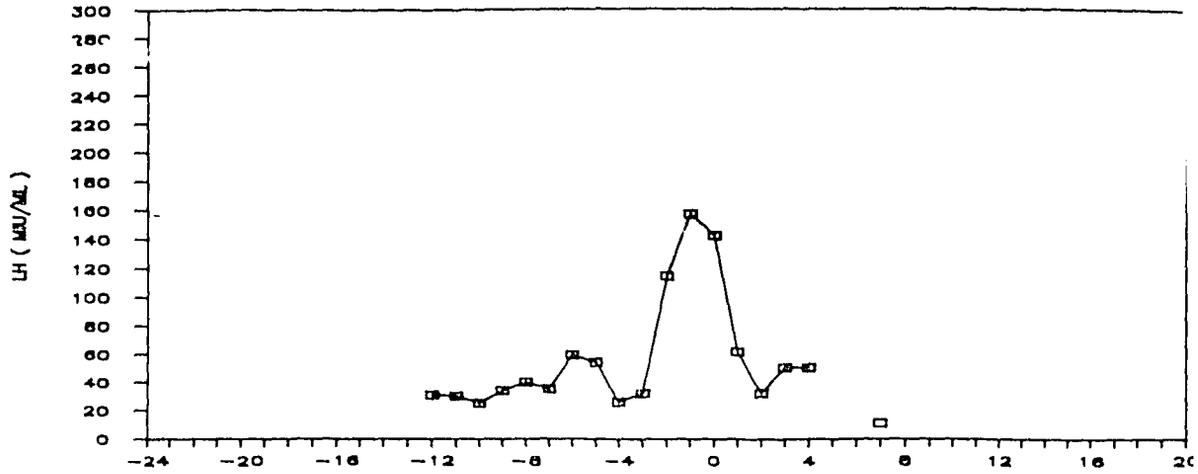
A.T.

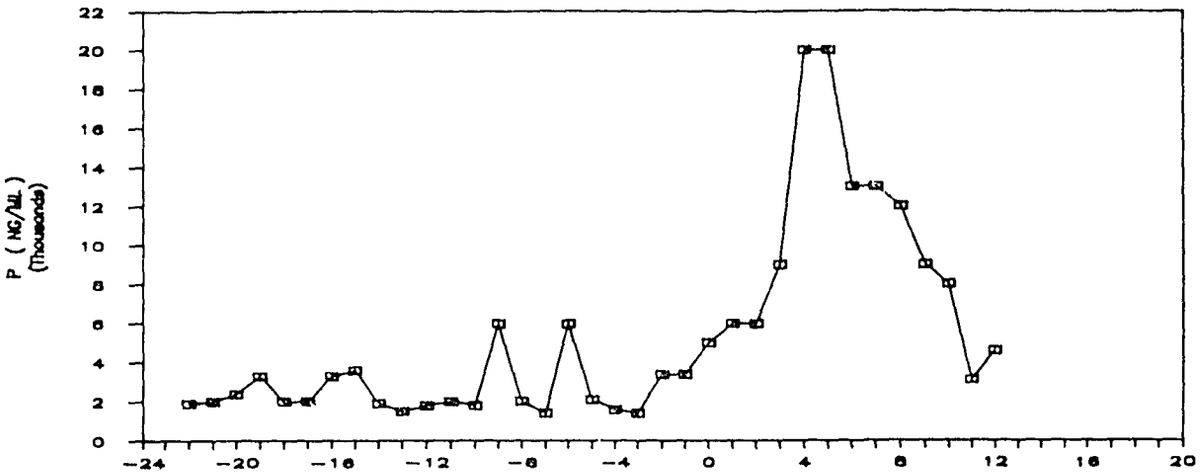
NAME	DATE	DFOM	OVD		GPC	GPV	LH	P	E2	BBT	REMARKS
	3/25/85	4	-22	-24	22.0			1900.0		97.2	PARFR CYCLE 1
	3/26/85	5	-21	-23				2000.0		97.4	
	3/27/85	6	-20	-22	417.3			2400.0		97.8	
	3/28/85	7	-19	-21	483.8			3300.0		97.6	
	3/29/85	8	-18	-20	349.7			2000.0		96.2	
	3/30/85	9	-17	-19	81.4			2000.0		97.4	
	3/31/85	10	-16	-18				3300.0		97.4	
	4/1/85	11	-15	-17	200.9			3600.0		97.8	
	4/2/85	12	-14	-16	22.0			1900.0		98.2	
	4/3/85	13	-13	-15	68.4			1500.0		98.2	
	4/4/85	14	-12	-14	81.1		31.0	1800.0		97.6	
	4/5/85	15	-11	-13	62.0		30.0	2000.0		98.0	
	4/6/85	16	-10	-12	39.1		25.0	1800.0		97.4	
	4/7/85	17	-9	-11	49.8		34.0	6000.0		97.8	
	4/8/85	18	-8	-10	113.3		40.0	2000.0		97.2	
	4/9/85	19	-7	-9	36.1		35.0	1400.0		97.2	
	4/10/85	20	-6	-8	16.5		59.0	6000.0		97.4	
	4/11/85	21	-5	-7	38.9		54.0	2100.0		97.4	
	4/12/85	22	-4	-6	42.0		26.0	1600.0		97.3	
	4/13/85	23	-3	-5	12.1		32.0	1400.0		98.0	
	4/14/85	24	-2	-4	8.5		114.0	3400.0		97.7	
	4/15/85	25	-1	-3	3.2		157.0	3400.0		97.5	
	4/16/85	26	0	-2	4.2		142.0	5000.0		97.7	
	4/17/85	27	1	-1	33.8		61.0	6000.0		97.8	
	4/18/85	28	2	0	194.3		32.0	6000.0		98.4	
	4/19/85	29	3	1	80.7		50.0	9000.0		97.8	
	4/20/85	30	4	2	137.0		50.0	20000.0		98.2	
	4/21/85	31	5	3	123.3			20000.0		98.4	
	4/22/85	32	6	4	204.7			13000.0		98.3	
	4/23/85	33	7	5				13000.0		98.2	
	4/24/85	34	8	6	208.6			12000.0		98.2	
	4/25/85	35	9	7	317.9		11.0	9000.0		98.2	
	4/26/85	36	10	8	238.6			8000.0		7.5	
	4/27/85	37	11	9	145.5			3100.0		97.8	
	4/28/85	38	12	10	584.5			4600.0			

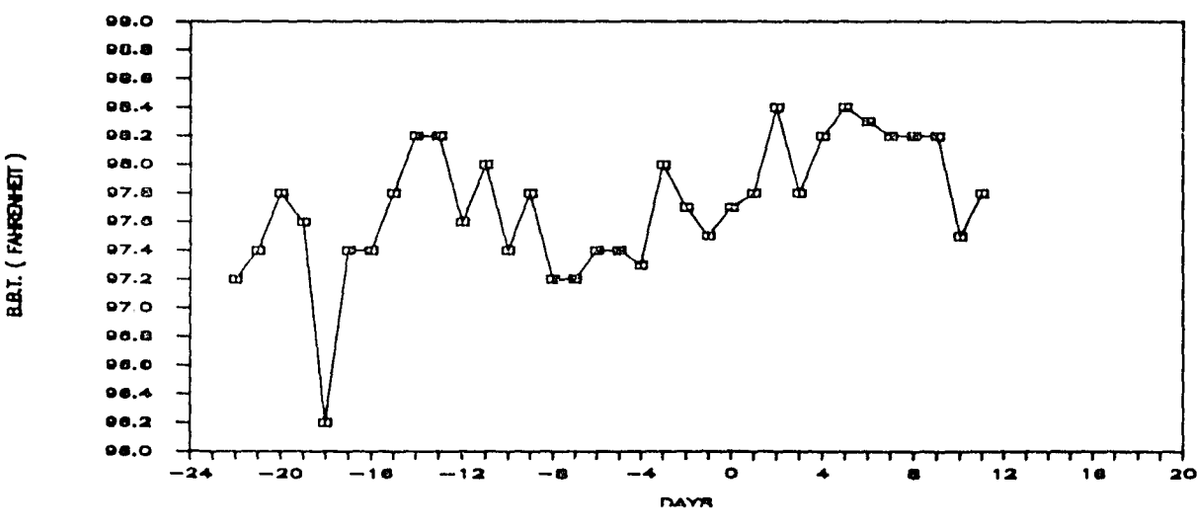

PARFR CYCLE 1
A.T.
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
-24
-20
-16
-12
-8
-4
0
4
8
12
16
DAYS

A.T

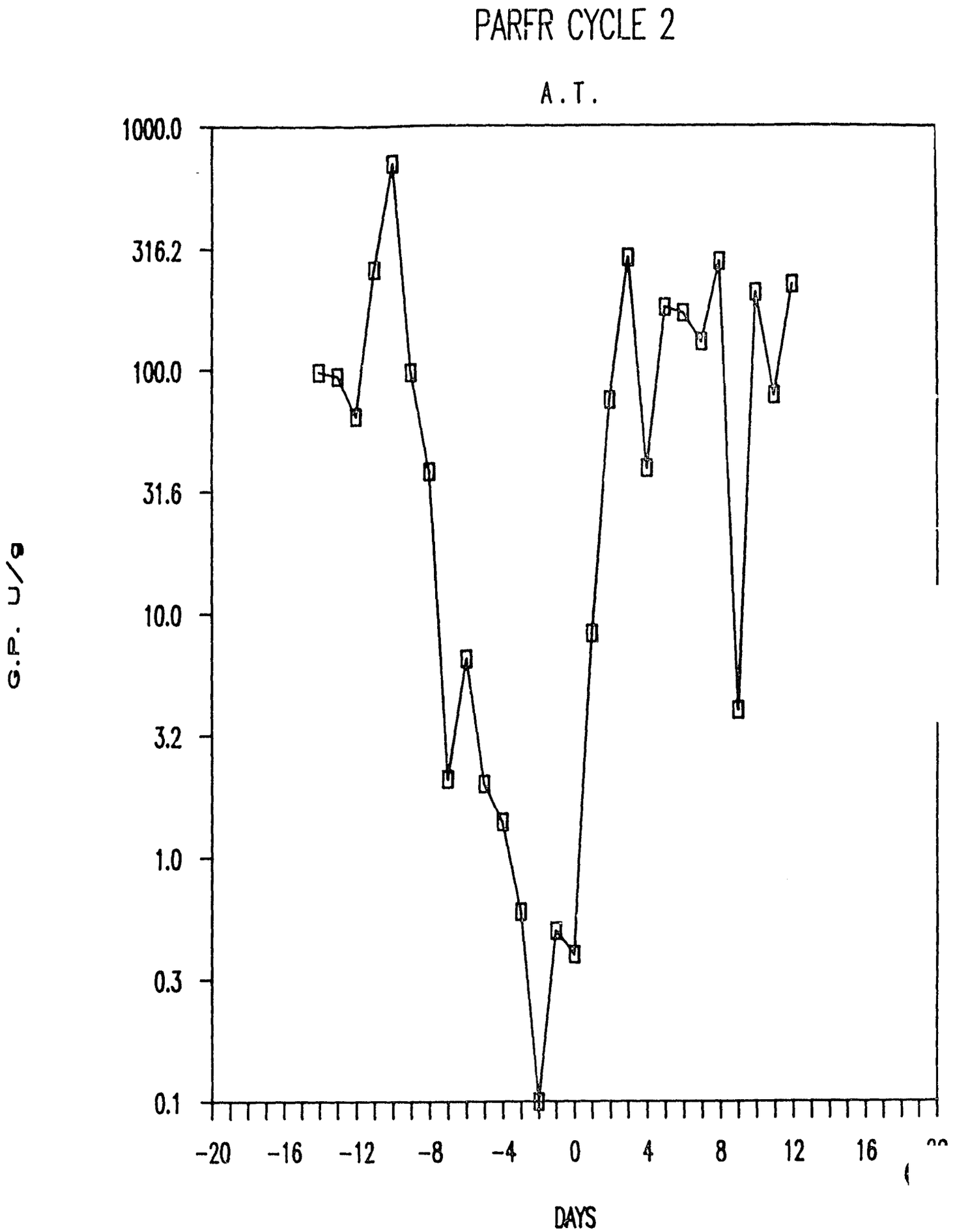
PARFR CYCLE 1





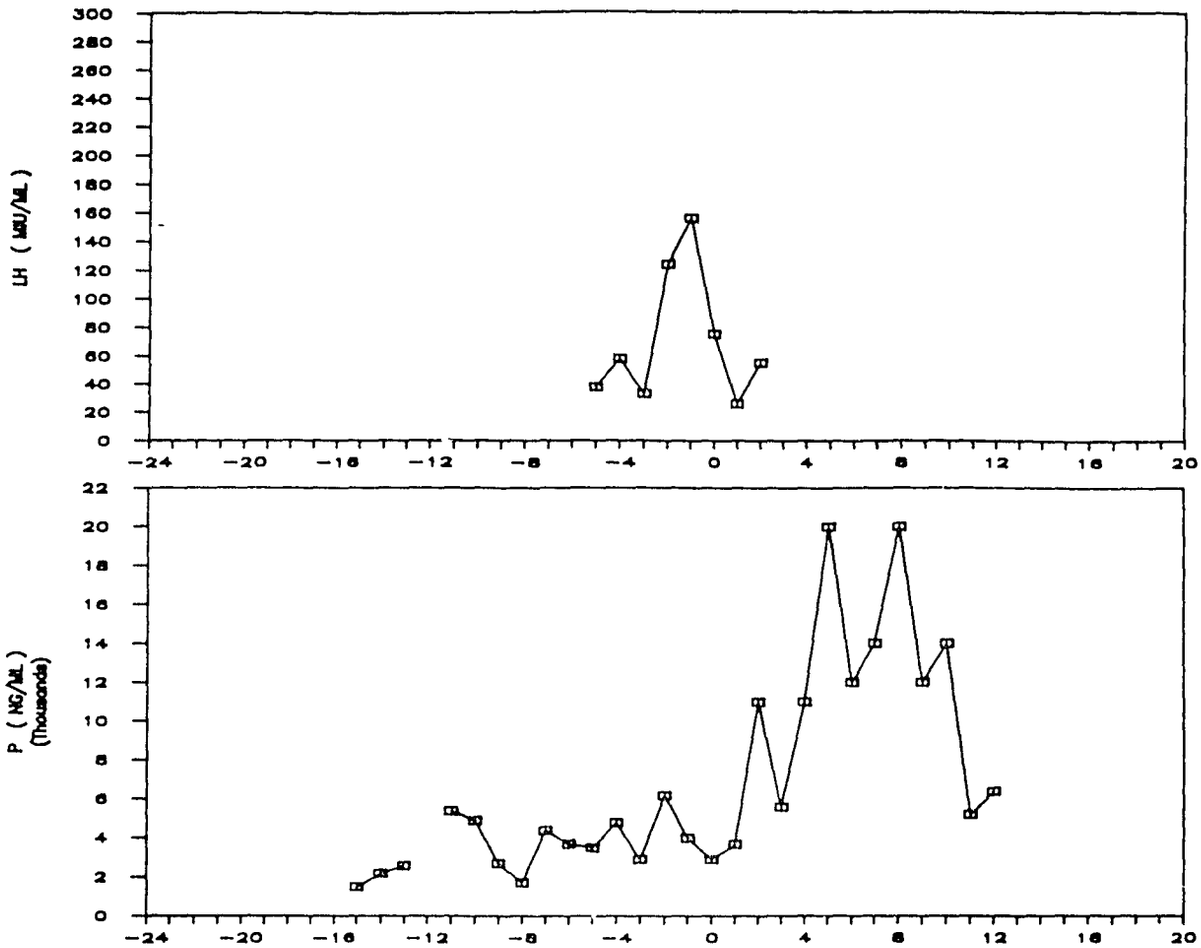


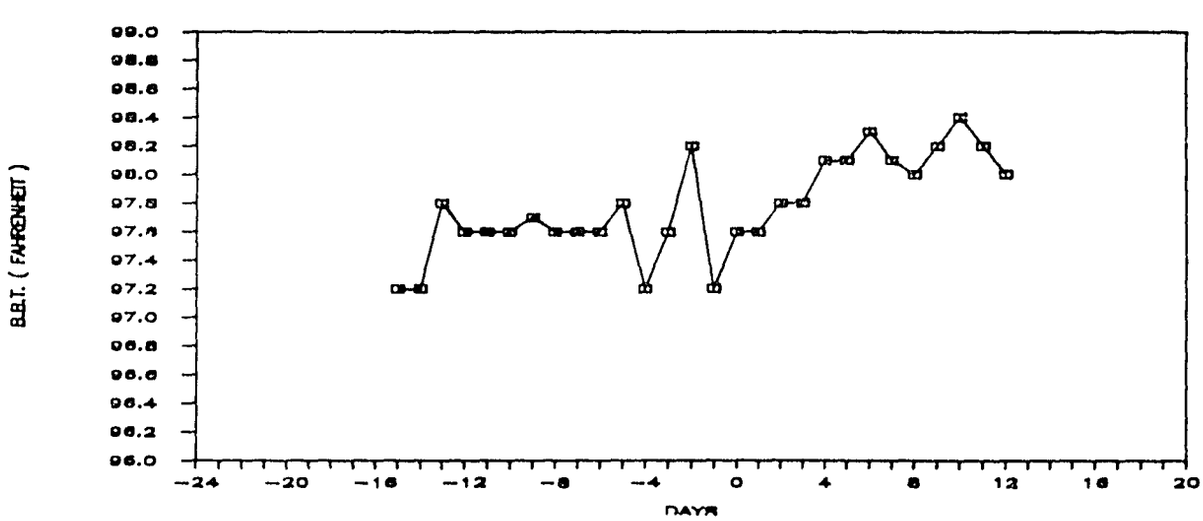
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARKS
A.T.	5/2/85	4	-15				1500.0		97.2	PARFR CYCLE 2
	5/3/85	5	-14	97.5			2200.0		97.2	
	5/4/85	6	-13	93.7			2600.0		97.8	
	5/5/85	7	-12	64.0					97.6	
	5/6/85	8	-11	256.1			3400.0		97.6	
	5/7/85	9	-10	704.0			4900.0		97.6	
	5/8/85	10	-9	97.6			2700.0		97.7	
	5/9/85	11	-8	38.1			1700.0		97.6	
	5/10/85	12	-7	2.1			4400.0		97.6	
	5/11/85	13	-6	6.5			3700.0		97.6	
	5/12/85	14	-5	2.0		38.0	3500.0		97.8	
	5/13/85	15	-4	1.4		58.0	4800.0		97.2	
	5/14/85	16	-3	0.6		33.0	2900.0		97.6	
	5/15/85	17	-2	0.0		124.0	6200.0		98.2	
	5/16/85	18	-1	0.5		156.0	4000.0		97.2	
	5/17/85	19	0	0.4		75.0	2900.0		97.6	
	5/18/85	20	1	8.3		26.0	3700.0		97.6	
	5/19/85	21	2	75.3		55.0	11000.0		97.8	
	5/20/85	22	3	288.9			5600.0		97.8	
	5/21/85	23	4	39.3			11000.0		98.1	
	5/22/85	24	5	180.8			20000.0		98.1	
	5/23/85	25	6	170.7			12000.0		98.3	
	5/24/85	26	7	129.6			14000.0		98.1	
	5/25/85	27	8	278.4			20000.0		98.0	
	5/26/85	28	9	4.0			12000.0		98.2	
	5/27/85	29	10	208.1			14000.0		98.4	
	5/28/85	30	11	78.9			5200.0		98.2	
	5/29/85	31	12	225.6			6400.0		98.0	


PARFR CYCLE 2
A.T.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
G.P. U/g
-20
-16
-12
-8
-4
0
4
8
12
16
DAYS

A.T.

PARFR CYCLE 2

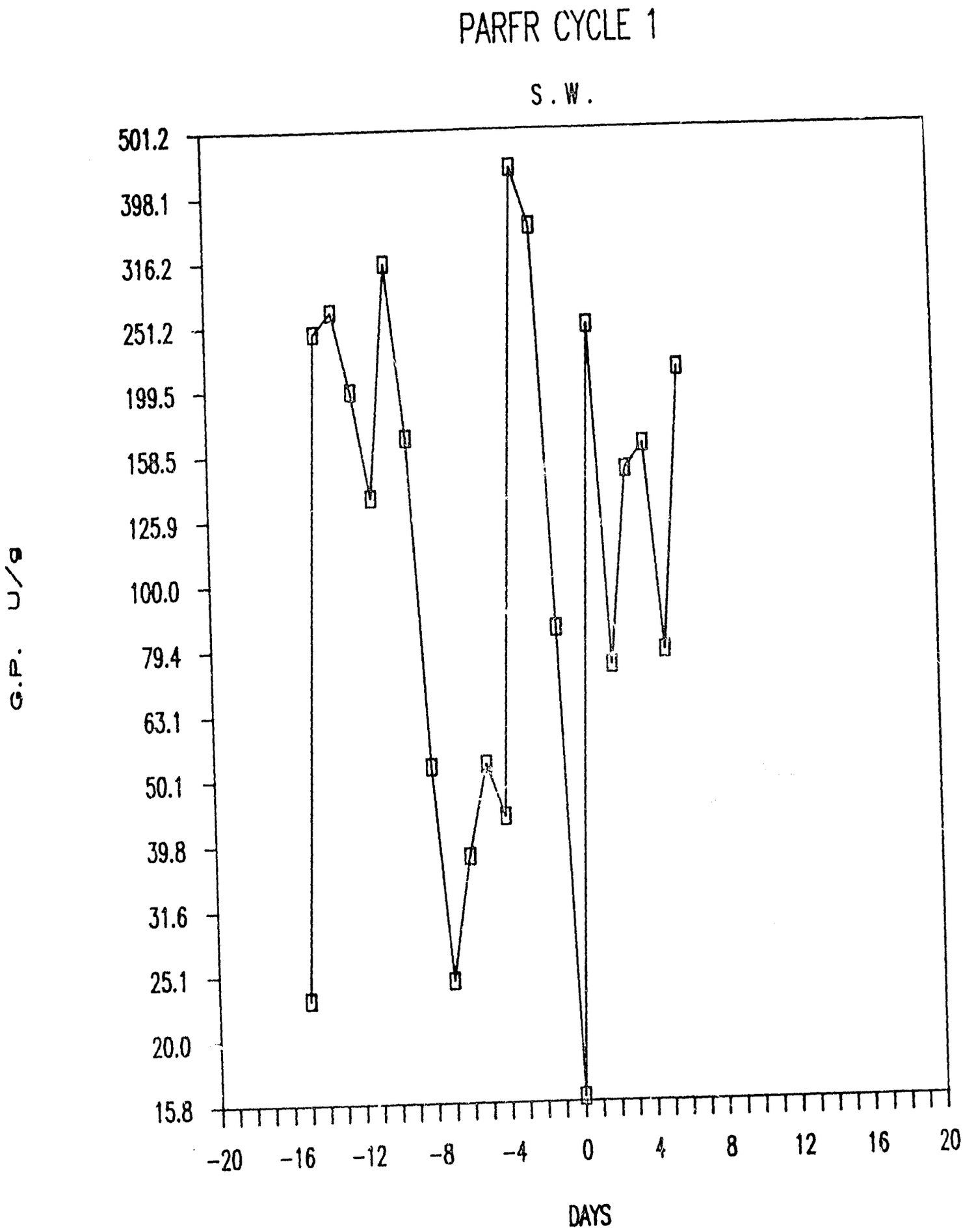




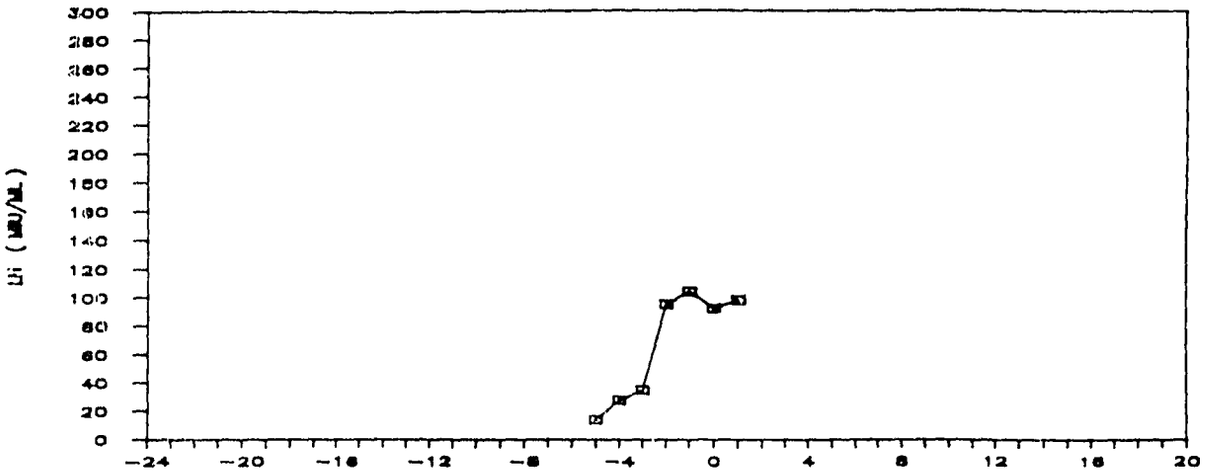
Not [illegible]

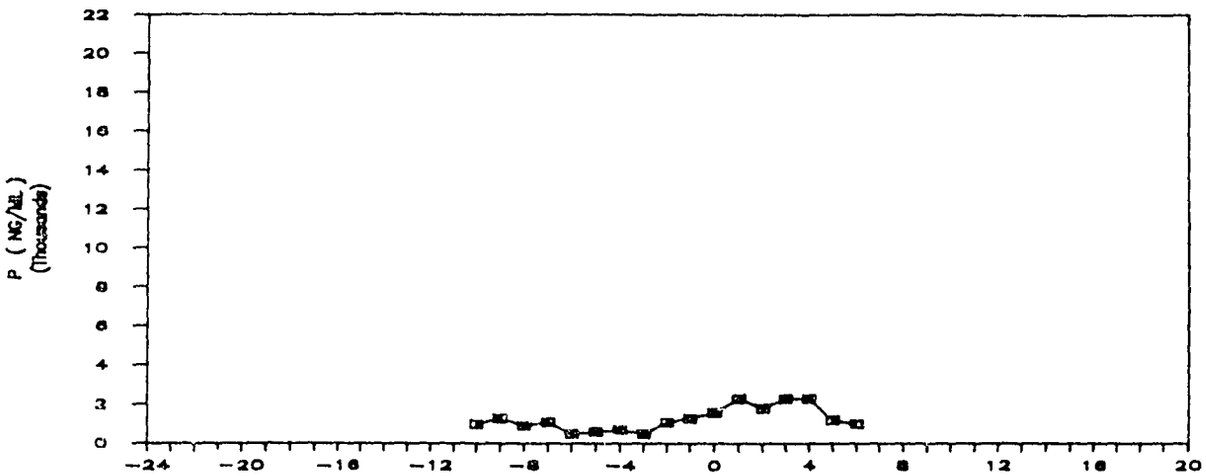
S.W.

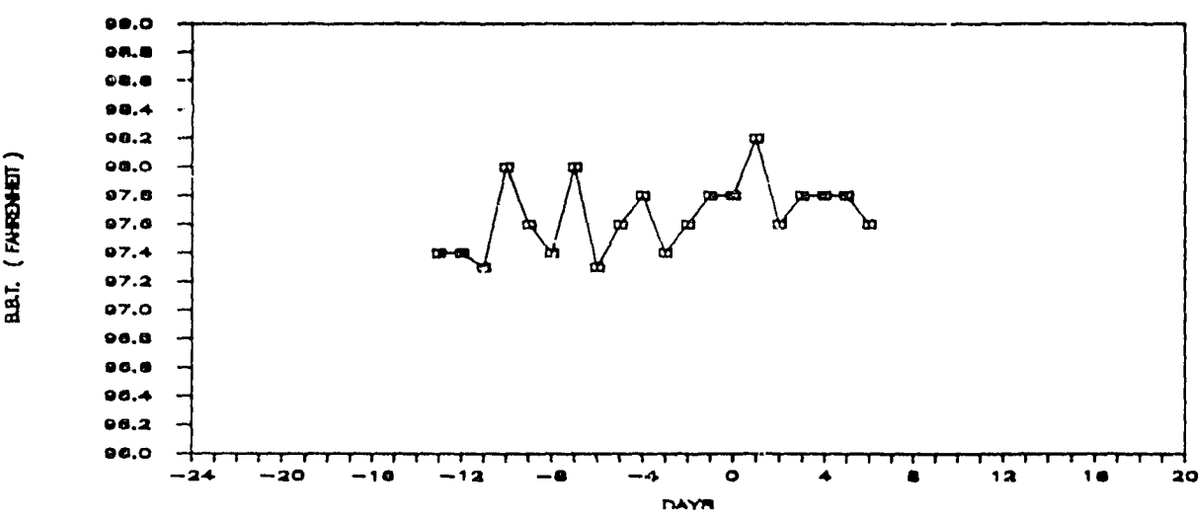
NAME	DATE	DFOM	OVD	GPC	GPV	LH	P	E2	BBT	REMARK
	6/29/84	5	-15	23.0	5.8					CYCLE 1
	6/30/84	6	-14	244.4	54.1					
	7/1/84	7	-13	263.6	80.6				97.4	SHE PROBABLY
	7/2/84	8	-12	198.7	84.2				97.4	DID NOT OVULATE
	7/3/84	9	-11	136.1	171.1				97.3	
	7/4/84	10	-10	313.6	56.0		1000.0		98.0	
	7/5/84	11	-9	168.1	58.2		1300.0		97.6	
	7/6/84	12	-8	52.4	7.3		900.0		97.4	
	7/7/84	13	-7	24.5	48.4		1100.0		98.0	
	7/8/84	14	-6	38.0	16.3		500.0		97.3	
	7/9/84	15	-5	52.7	10.8	14.0	600.0		97.6	
	7/10/84	16	-4	43.7	16.3	28.0	700.0		97.8	
	7/11/84	17	-3	437.3	76.8	35.0	500.0		97.4	
	7/12/84	18	-2	356.4	19.6	95.0	1100.0		97.6	
	7/13/84	19	-1	85.3	23.9	104.0	1300.0		97.8	
	7/14/84	20	0	16.1	27.9	92.0	1600.0		97.8	
	7/15/84	21	1	249.8	91.1	98.0	2300.0		98.2	
	7/16/84	22	2	74.5	75.4		1300.0		97.6	
	7/17/84	23	3	149.1	75.0		2300.0		97.8	
	7/18/84	24	4	163.0	118.8		2300.0		97.8	
	7/19/84	25	5	78.2	42.9		1200.0		97.8	
	7/20/84	26	6	213.1	41.0		1000.0		97.6	


PARFR CYCLE 1
S.W.
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
G.P. U/g
-20
-16
-12
-8
-4
0
4
8
12
16
20
DAYS

PARFR CYCLE 1







APPENDIX 2

PARFR-360
(1985-1986)

DYE STUDY

29 Women Studied

No.	Volunteer	Date	Sampling Position		
			Supine	Standing	Squatting
1	AK	11/18/85	U	U	I
2	CM#1	11/18	U	I	I
3	CM#2	11/21	U	U	I
4	AA	11/22	U	A	A
5	LC	11/25	U	U	U
6	DS	11/25	A	U	A
7	MC	11/25	A	I	A
8	MG	11/26	A	A	A
9	PS	11/27	A	A	A
10	LW	11/27	A	I	U
11	PK	12/2	U	A	A
12	KS	12/2	A	A	U
13	DOR	12/2	A	A	A
14	AV	12/3	I	A	A
15	DR	12/3	I	A	A
16	KM	12/3	A	A	A
17	MP	12/3	A	A	A
18	MKL	12/4	A	A	A
19	LP	12/5	A	A	A
20	MK	12/5	A	A	A
21	RB	12/5	A	A	I
22	MW	12/9	A	A	A
23	WN	12/10	A	A	A
24	SA	12/11	A	A	A
25	DC	12/12	A	A	A
26	DB	12/12	U	A	U
27	RG*	12/12	U*	U*	U*
28	PC	12/16	A	A	A
29	SS	12/17	A	A	A
30	BB	12/18	A	A	U

*Failure to sample due to anatomical position of cervix.

Abbreviations used: U=unacceptable
A=acceptable
I=inconclusive

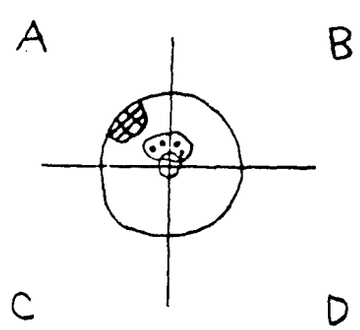
SAMPLING DYE STUDY **DATE** 11/18/85

Name A.K. **Age** 23
Education Background 2 years of college
Medications Ortho Novum 7-7-7 Oral contraceptives
LMP Supposed to be 11/10 **FREQUENCY** 28 days **DURATION** 1-3 days
Problems with menses

Gravida 0
Position of cervix facing down, centered.
Length of vagina Average
Amount of mucus Small

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Methylene Blue	Section A-On edge of cervix.	Unacceptable
STANDING		2	Methylene Blue	Section A-On edge of cervix	Unacceptable
SQUATTING Full Squat		3	Iodine	Section A/B - Near os.	Inconclusive See Examiner's Comments

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** No. Cervix does not feel more sensitive than other part of vagina.

2. **In which position was it easier to reach cervix?**

3. **What position was most comfortable when inserting device?**

Volunteer's Comments- In all three positions, when putting in device she felt a resistance before reaching end of vagina. When she put more pressure on the device, the device went in farther.

Examiner's Comments- The color of the iodine may be too similar to the color of the walls of the vagina to be able to detect. For the next person, I will try using more iodine on the swab.

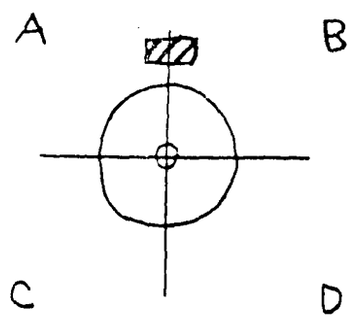
SAMPLING DYE STUDY **DATE** 11/18/85

Name C.M. #1 **Age** 25
Education Background
Medications
LMP 11/9/85 **FREQUENCY** 25 days **DURATION** 4 days
Problems with menses Severe cramps on first day of menses.

Gravida 1
Position of cervix
Length of vagina
Amount of mucus

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Methylene Blue	Section A/B 2 cm from face of cervix.	Unacceptable
STANDING		2	Iodine	No dye found	Inconclusive
SQUATTING Full Squat		3	Methylene Blue	No dye found	Inconclusive

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?**

2. **In which position was it easier to reach cervix?** Supine

3. **What position was most comfortable when inserting device?**

Volunteer's Comments- She could not tell if she was touching cervix, but she did feel pressure after the device was pushed in as far as it would go.

Examiner's Comments-

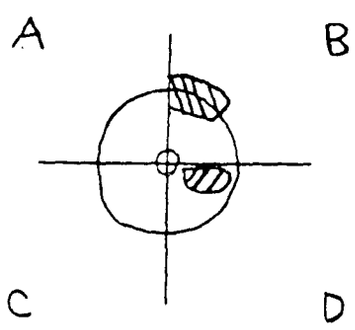
SAMPLING DYE STUDY **DATE** 11/21/85

Name C.M. #2 **Age** 25
Education Background
Medications
LMP 11/9/85 **FREQUENCY** 25 days **DURATION** 4 days
Problems with menses

Gravida
Position of cervix Facing down; centered
Length of vagina Average, approximately 5".
Amount of mucus Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Methylene Blue	Section D-On face of cervix, near os.	Unacceptable
STANDING		2	Gentian Violet	Section B-Partly on face of cervix, and partly on side of cervix.	Unacceptable
SQUATTING Full Squat		3	Lugol's Solution (contains Iodine)	Dye not found	Inconclusive

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. See Volunteer Comments for explanation.

2. **In which position was it easier to reach cervix?** Supine was easiest; squatting was the most difficult position in reaching cervix.

3. **What position was most comfortable when inserting device?** Supine was most comfortable. Squatting was least comfortable.

Volunteer's Comments- The first time she did this experiment on 11/18, she could not tell she was touching specifically her cervix. But now, possibly because of previous practice, she recognizes the feeling of touching her cervix.

Examiner's Comments-

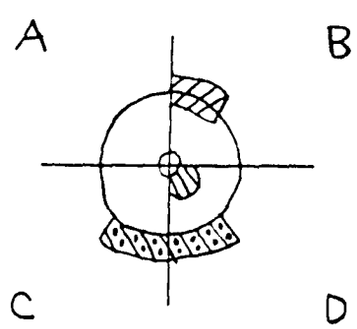
SAMPLING DYE STUDY **DATE** 11/22/85

Name A.A. **Age** 29
Education Background
Medications
LMP 11/12/85 **FREQUENCY** 28 days **DURATION** 3 days
Problems with menses None

Gravida
Position of cervix Facing down.
Length of vagina Average (Approximately 5")
Amount of mucus Small amount

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Section B- On Upper part of side of cervix.	Unacceptable
STANDING		2	Gentian Violet	Section C/D-1cm away from cervix at posterior fornix. Also, a little near os.	Acceptabl
SQUATTING Full Squat		3	Methy-lene violet	Section C/D-1cm away from cervix at posterior fornix.	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. She feels pressure when reaching her cervix.

2. **In which position was it easier to reach cervix?** The device was easier to insert in squatting and standing positions.

3. **What position was most comfortable when inserting device?** In supine position, she did not feel the pressure that she felt in the other two positions when inserting the device.

Volunteer's Comments- The procedure was not difficult.

Examiner's Comments-

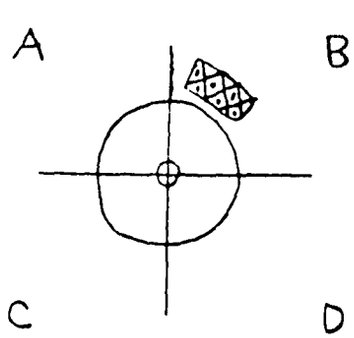
SAMPLING DYE STUDY **DATE** 11/25/85

Name L.C. **Age** 24
Education Background 3rd year in Dental School
Medications Ortho Novum 7-7-7 oral contraceptives. Also just finished taking penicillin 11/22/85
LMP 10/30/85 **FREQUENCY** 28 days **DURATION** 4 days
Problems with menses None

Gravida 0
Position of cervix Facing down; slightly off center on her right side.
Length of vagina Average, approximately 5".
Amount of mucus Small

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	SectionB;1-2cm off from cervix-on neck of cervix.	Unacceptable
STANDING		2	Methylene	Section B-Same as supine	Unaccep
SQUATTING Full Squat		3	Gentian Violet	Section B-Same as supine	Unacceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** No. She just went as far as the device would go. She did not even feel pressure when reaching end of vagina.

2. **In which position was it easier to reach cervix?** All were about the same.

3. **What position was most comfortable when inserting device?** Standing was the most comfortable because she could easily see what she was doing.

Volunteer's Comments- "It was real easy."

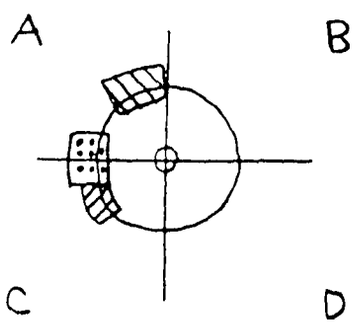
Examiner's Comments- For the standing position, first we used Lugol's Solution, but I could not find this dye. We repeated this position and used Methylene Blue.

SAMPLING DYE STUDY **DATE** 11/25/85

Name D.S. **Age** 28
Education Background 1st year Medical Student
Medications Septra (1 tablet after each sexual intercourse)
LMP 11/17/85 **FREQUENCY** Average 29 days **DURATION** 6 days
Problems with menses Since last couple of months, average frequency of menses has bee 35 days. Severe cramps on first day of period. Mild cramps a day or two before me
Gravida 0
Position of cervix Facing down , to the left. Positioned off center on her left side
Length of vagina Average (approximately 5")
Amount of mucus Moderate

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Section C-Touching edge of cervix, and a little under cervix.	Acceptable
STANDING		2	Lugol's Solution	Section A-Touching edge of cervix, and 1 cm. away from cervix.	Unaccept
SQUATTING		3	Gentian Violet	Section A/C-Touching edge of cervix, and 1 cm. away from cervix.	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. She feels pressure when reaching cervix.

2. **In which position was it easier to reach cervix?** Supine and squatting.

3. **What position was most comfortable when inserting device?** Supine was most comfortable. Squatting was awkward, but it was easy to reach cervix in this position.

Volunteer's Comments- The slight curve in the device makes it easier to insert as opposed to the applicators used to insert medications for vaginal infections. These applicators are straight.

Examiner's Comments-

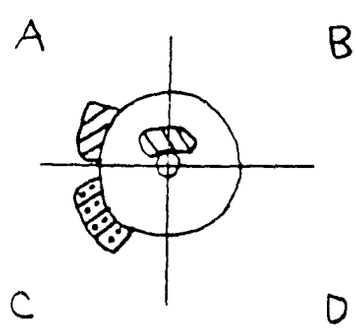
SAMPLING DYE STUDY **DATE** 11/25/85

Name M.C. **Age** 40
Education Background College. She is presently a Gyne Physician's assistant
Medications None
LMP 11/13/85 **FREQUENCY** 28-30 days **DURATION** 3 days
Problems with menses Slight 1 day dysmennorrhea (cramps)

Gravida 1
Position of cervix Facing down; centered. Large cervix.
Length of vagina Average (Approximately 5")
Amount of mucus Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Sections A and C, just off of cervix - mixed in mucus.	Acceptable
STANDING		2	Lugol's Solution	Sections A/B on face of cervix near os. Mixed in mucus.	Inconclus
SQUATTING Full Squat		3	Gentian Violet	Section C, Posterior fornix area- mixed in mucus.	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** She feels like she is touching a more sensitive area when she reaches her cervix with the device.

2. **In which position was it easier to reach cervix?** It was definitely easier to reach cervix in the squatting position.

3. **What position was most comfortable when inserting device?** Lying down.

Volunteer's Comments-

Examiner's Comments- The results from the standing position were inconclusive because the color of the Lugol's Solution blended in too much with the skin.

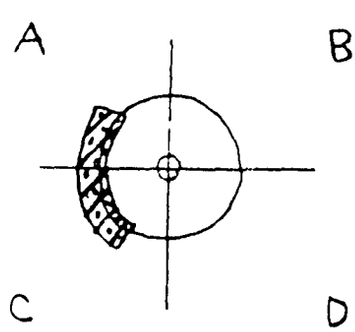
SAMPLING DYE STUDY **DATE** 11/26/85

Name M.G. **Age** 24
Education Background 2nd year Medical Student
Medications None
LMP 11/13/85 **FREQUENCY** 35-42 days **DURATION** 4-5 days
Problems with menses Very irregular

Gravida No comment
Position of cervix probably retroverted
Length of vagina longer than average
Amount of mucus Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Section A/C just off of cervix, and a little on edge of cervix. Dye was mixed in mucus.	Acceptable
STANDING		2	Methylene Blue	Dye not found	Acceptable
SQUATTING full Squat		3	Gentian Violet	Same as Supine	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes, She feels a sharp sensation when she touches cervix. She felt this when squatting and lying down, but not when standing.

2. **In which position was it easier to reach cervix?** Squatting

3. **What position was most comfortable when inserting device?** Standing

Volunteer's Comments- The device tended to push out, especially when squatting. She had to use a little force to keep device in place.

Examiner's Comments- Cervix was hard to find. Vagina was longer than average; And I had to direct speculum upwards a little.

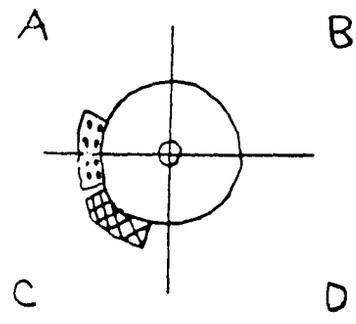
SAMPLING DYE STUDY **DATE** 11/27/85

Name P.S. **Age** 23
Education Background Associates degree, MLT.
Medications Ortho Novum 7-7-7 oral contraceptives
LMP supposed to be 11/1 **FREQUENCY** 28 days **DURATION** 1-3 days
Problems with menses None

Gravida 0
Position of cervix Facing down; a little off center on her left.
Length of vagina Average (approximately 5").
Amount of mucus Small

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Methylene Blue	Section C-in posterior fornix area.	Acceptable
STANDING		2	Indigo Carmine	Section C-in posterior fornix area.	Accepta
SQUATTING Full Squat		3	Gentian Violet	Section A/C-just off of face of cervix. extending a little into fornix area.	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** She feels pressure when reaching back of vagina.

2. **In which position was it easier to reach cervix?** Supine and standing.

3. **What position was most comfortable when inserting device?** Standing

Volunteer's Comments-

Examiner's Comments-

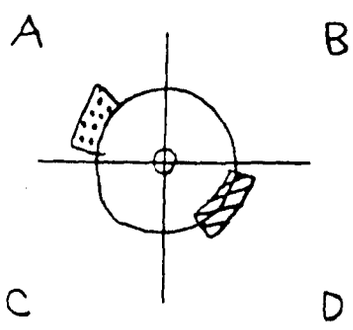
SAMPLING DYE STUDY **DATE** 11/27/85

Name L.W. **Age** 21
Education Background Presently in 2nd year of Nursing School
Medications None
LMP 11/8/85 **FREQUENCY** 28 days **DURATION** 4-5 days
Problems with menses Slight cramps on first day of period.

Gravida 0
Position of cervix Facing down, centered
Length of vagina average, approximately 5 inches
Amount of mucus Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Section D-some just off of cervix; some on cervix	Acceptable
STANDING		2	Methylene Blue	No dye found. see examiner comments.	Inconc
SQUATTING Full Squat		3	Gentian Violet	Section A-On neck of cervix, about 1 cm. away from cervix.	Unacceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** She feels a slight pressure when reaching end of vagina. Also, she feels a more sensitive area which could be the cervix.

2. **In which position was it easier to reach cervix?** standing

3. **What position was most comfortable when inserting device?** Lying down

Volunteer's Comments-

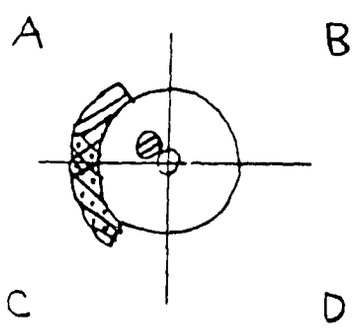
Examiner's Comments- It seemed that it was hard for her to hold herself up in the squatting position. She did the standing position twice. The first time I could not find the dye. The second time I found a little dye in the same area as in the lying down position. This, however, looked more like Indigo Carmine than Methylene Blue.

SAMPLING DYE STUDY **DATE** 12/2/85

Name P.K. **Age** 35
Education Background Master's Degree. She works as a business manager.
Medications Ibuprofen, 600mg - 3 times a day.
LMP 11/25/85 **FREQUENCY** Usually 40 days **DURATION** 3 days
Problems with menses Irregular for last six months. Last interval between periods was 3 weeks. Interval before this was 2 months
Gravida 2
Position of cervix Facing down; a little off center on her left side. Large cervix.
Length of vagina Average length. Above average width.
Amount of mucus Moderate/Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Methylene Blue	Section A - off of cervix. some dye near os mixed in mucus,	Unacceptable
STANDING		2 +4	Indigo Carmine	Could not find dye the first time. Repeated after squatting position and found in same area as squatting position.	Acceptable
SQUATTING Half Squat		3	Gentian Violet	Section A/C, just off of cervix (posterior fornix)	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. She feels pressure when reaching cervix.

2. **In which position was it easier to reach cervix?** They all were equal.

3. **What position was most comfortable when inserting device?** Supine. Squatting was uncomfortable in that it was hard to hold herself up.

Volunteer's Comments-

Examiner's Comments- It was hard to find dye because she was overweight and there were alot of folds of fat. She did the lying down position first and I had not anticipated this problem. I had used a medium speculum and may have been able to see better with a large speculum. Consequently, with the lying down position there may have been dye inbetween the folds in Section C which would have made the result acceptable. I probably could not find the dye with the standing position because of this problem. However, for both the squatting and standing positions I used a large speculum and I used a swab to search for the dye. Also, I am fairly sure that I saw Indigo Carmine the second time she tried the standing position, although it was difficult distinguishing the blue from the Gentian Violet.

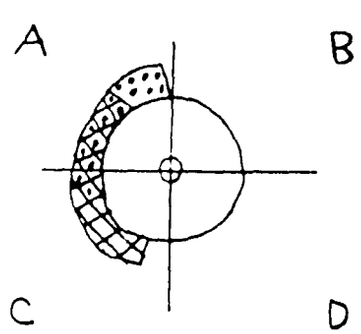
SAMPLING DYE STUDY **DATE** 12/2/85

Name K.S. **Age** 24
Education Background Presently in Medical School
Medications None
LMP 11/15/85 **FREQUENCY** 30 days **DURATION** 5 days
Problems with menses Slight cramping on first day of menses

Gravida 0
Position of cervix Slight retroverted uterus
Length of vagina Average (Approximately 5")
Amount of mucus Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Section A/C in posterior fornix area	Acceptable
STANDING		2	Methylene Blue	Same as supine	Acceptable
SQUATTING Half Squat		3	Gentian Violet	Same as supine; but more towards A section than C.	Unacceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** She did not feel it when she was squatting, but with the other two positions she did.

2. **In which position was it easier to reach cervix?** Supine. In this position s definitely could feel her cervix.

3. **What position was most comfortable when inserting device?** Standing.

Volunteer's Comments- "Squatting and using wall to support yourself makes a person feel like an animal." She suggested sitting on the edge of a stool.

Examiner's Comments- This person squatted slightly, unlike the others before her who squatted all of the way down. Future volunteers will squat halfway down.

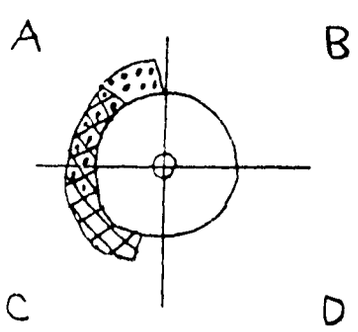
SAMPLING DYE STUDY **DATE** 12/2/85

Name K.S. **Age** 24
Education Background Presently in Medical School
Medications None
LMP 11/15/85 **FREQUENCY** 30 days **DURATION** 5 days
Problems with menses Slight cramping on first day of menses

Gravida 0
Position of cervix Slight retroverted uterus
Length of vagina Average (Approximately 5")
Amount of mucus Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSIO
SUPINE		1	Indigo Carmine	Section A/C in posterior fornix area	Acceptable
STANDING		2	Methylene Blue	Same as supine	Acceptable
SQUATTING Half Squat		3	Gentian Violet	Same as supine; but more towards A section than C.	Unacceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** She did not feel it when she was squatting, but with the other two positions she did.

2. **In which position was it easier to reach cervix?** Supine. In this position she definitely could feel her cervix.

3. **What position was most comfortable when inserting device?** Standing.

Volunteer's Comments- "Squatting and using wall to support yourself makes a person feel like an animal." She suggested sitting on the edge of a stool.

Examiner's Comments- This person squatted slightly, unlike the others before her who squatted all of the way down. Future volunteers will squat halfway down.

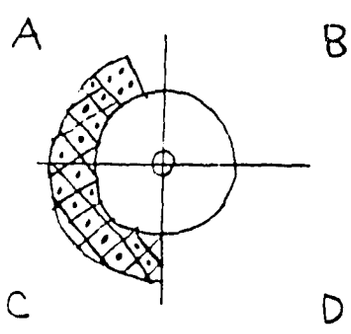
SAMPLING DYE STUDY **DATE** 12/2/85

Name D.O.R. **Age** 29
Education Background 2 years of college
Medications None
LMP 11/21/85 **FREQUENCY** 28 days **DURATION** 5 days
Problems with menses Usually heavy bleeding

Gravida 2
Position of cervix Facing down and centered
Length of vagina Average
Amount of mucus Moderate

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Section A/C Posterior fornix area, near cervix	Acceptable
STANDING		2	Methylene Blue	Same as supine	Acceptable
SQUATTING HALF		3	Gentian Violet	Same as supine but maybe more in Section A than C.	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. It feels like pressure.

2. **In which position was it easier to reach cervix?** Supine

3. **What position was most comfortable when inserting device?** Supine

Volunteer's Comments-

Examiner's Comments-

When squatting, she accidentally started removing self-sampling device before pulling swab back in the tube, and before turning the device. Then she pushed device back in all of the way and repeated the procedure the correct way.

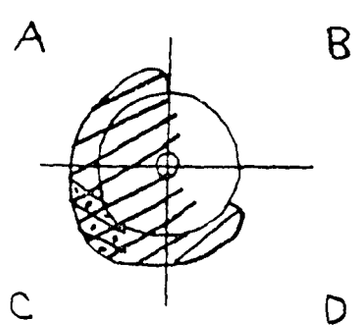
SAMPLING DYE STUDY **DATE** 12/3/85

Name A.V. **Age** 31
Education Background B.S. Degree
Medications Tegretol - 1400 mg/day, Mebaral - 300 mg/day
LMP 11/14/85 **FREQUENCY** average 35 days **DURATION** 8-12 days
Problems with menses Irregular; frequency ranges from 31-38 days.

Gravida 1
Position of cervix facing down; Positioned a little off center. Cervix was facing toward left side.
Length of vagina Average - 5"
Amount of mucus Small

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		3rd	Gentian Violet	Sections A,C, and D. All over right side of cervix, and next to cervix.	Inconclusive
STANDING		2nd	Methylene Blue	Section C- Posterior fornix area; Right side	Acceptal
SQUATTING Half Squat		1st	Indigo Carmine	Section C- Posterior fornix area; Right side	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Cervix feels more sensitive.

2. **In which position was it easier to reach cervix?** Standing was the easiest. She definitely felt she was at cervix when she was standing. Lying down was the most difficult.

3. **What position was most comfortable when inserting device?** Standing was most comfortable; Lying was least comfortable because it was awkward.

Volunteer's Comments-

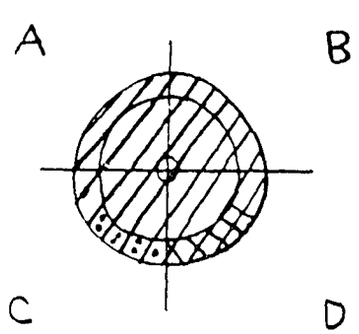
Examiner's Comments- She performed the standing position twice, because the first ti I was not sure I found the Methylene Blue. I did find some blue dye, but I thought this was Indigo Carmine left from previous position. This was the first volunteer that I emphasized the idea of trying to get "underneath" cervix, and to try to push down on device when going in. This technique will probably help volunteers reach the posterior fornix. I will continue to emphasize this for future volunteers.

SAMPLING DYE STUDY **DATE** 12/3/85

Name D.R. **Age** 20
Education Background Presently in 3rd year of college.
Medications None
LMP 11/13/85 **FREQUENCY** 28 days **DURATION** 6 days
Problems with menses Heavy bleeding in beginning of menses. Cramps on first day of menses.
Gravida 0
Position of cervix Facing down, a little off center on her left side.
Length of vagina Average (Approximately 5")
Amount of mucus Small/Moderate

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		3	Gentian Violet	All over and around cervix	Inconclusive
STANDING		2	Methylene Blue	Section D; Posterior fornix	Acceptable
SQUATTING Half Squat		1	Indigo Carmine	Section C; Posterior fornix	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** When lying down, she was able to feel her cervix the most. When squatting, she couldn't tell if she touched her cervix or not; she just pushed the device until it would not go any further.

2. **In which position was it easier to reach cervix?** The device was the easiest to control when she was lying down. It was the hardest to control when she was squatting.

3. **What position was most comfortable when inserting device?** Lying down was most comfortable.

Volunteer's Comments-

Examiner's Comments- Results from supine position were inconclusive because the Gentian Violet may have spread all over cervix due to the high concentration of this dye.

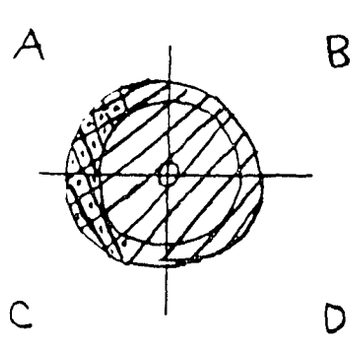
SAMPLING DYE STUDY **DATE** 12/3/85

Name K.M. **Age** 18
Education Background 1st year of college
Medications None
LMP 11/13/85 **FREQUENCY** 23-24 days **DURATION** 3-4 days
Problems with menses Irregular sometimes (sometimes period is delayed 1 week.

Gravida 0
Position of cervix facing down and forward; cervix was positioned off center on her le
Length of vagina Shorter than average s:
Amount of mucus Moderate/Small

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		3rd	Gentian Violet	Sections A,B,& D. All over and around cervix.	Inconclusive
STANDING		2nd	Methylene Blue	Section A/C. just off of cervix. Right side. Extending to posterior fornix.	Acceptabl
SQUATTING Half Squat		1st	Indigo Carmine	Section A/C just off of cervix. Right side. extending into posterior fornix.	Acceptab

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. She can feel around cervix wher she is turning device.

2. **In which position was it easier to reach cervix?** All were equal.

3. **What position was most comfortable when inserting device?** Squatting.

Volunteer's Comments-

Examiner's Comments- Gentian Violet may have spread all over cervix because it is mo concentrated than the other dyes.

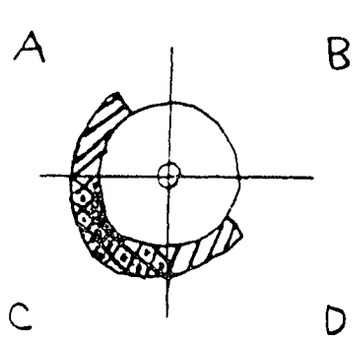
SAMPLING DYE STUDY **DATE** 12-3-85

Name M.P. **Age** 21
Education Background Presently in 4th year of college.
Medications Tetracycline (low dose)
LMP 11/4/85 **FREQUENCY** 23-27 days **DURATION** 6-7 days
Problems with menses Usually moderate cramps first day of period.

Gravida 0
Position of cervix facing down
Length of vagina sl. longer than usual.
Amount of mucus small

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		2	Methylene Blue	Section A, C, and D next to cervix	Acceptable
STANDING		3	Gentian Violet	Section C, next to cervix	Acceptable
SQUATTING Half Squat		1	Indigo Carmine	Section C, next to cervix	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. It feels like pressure; and when she pushes on cervix, it is uncomfortable.

2. **In which position was it easier to reach cervix?** Supine

3. **What position was most comfortable when inserting device?** Supine

Volunteer's Comments- Squatting was uncomfortable.

Examiner's Comments- After dipping swab in Gentian Violet, I wiped off some of it on a tissue, (since Gentian Violet was more concentrated than the other dyes). This was the first person that I tried this with. I obtained much better results-the dye did not spread as much. I will continue doing this for future volunteers. The angle of her vagina was steeper towards her back than the average person.

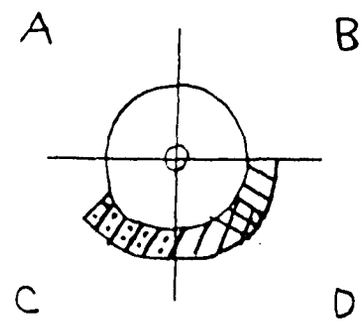
SAMPLING DYE STUDY **DATE** 12/4/85

Name M.K.L. **Age** 31
Education Background Bachelor of Arts degree
Medications None
LMP 11/21/85 **FREQUENCY** 25-28 days **DURATION** 5 days
Problems with menses None

Gravida 1
Position of cervix Facing down; centered. Cervix is larger than average.
Length of vagina Average
Amount of mucus Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		2	Methylene Blue	Sections C and D, Posterior fornix	Acceptable
STANDING		3	Gentian Violet	Section D posterior fornix	Acceptable
SQUATTING Half squat		1	Indigo Carmine	Section C, posterior fornix	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** She feels pressure when reaching cervix.

2. **In which position was it easier to reach cervix?** Supine. "When lyng down there was more room to get underneath cervix. This may be because gravity was not causing organs to push down on cervix.as much as with the other positions."

3. **What position was most comfortable when inserting device?** Supine.

Volunteer's Comments- When lying down it was easier to aim device downward and thus to reach posterior fornix.

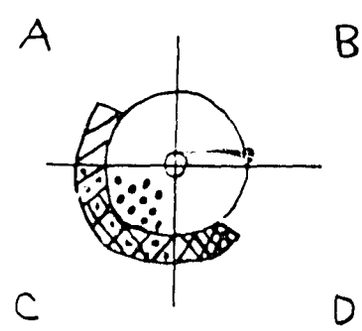
Examiner's Comments- With this person we changed the procedure. She inserted the device with the curve pointing to one side. Then after inserting the swab, she rotated the device so that the curve pointed upward. She continued turning it until the curve pointed to the other side. This was a 180° turn. The rest of the procedure was as usual.

SAMPLING DYE STUDY **DATE** 12/5/85

Name L.P. **Age** 40
Education Background 1 year of college
Medications
LMP 11/12/85 **FREQUENCY** 30 days average **DURATION** 14 days
Problems with menses Irregular: Frequency varies between 28 and 35 days. At end of period, flow is very light. She has spotting for about 5 days.
Gravida 5
Position of cervix Facing down, centered. Large cervix
Length of vagina long
Amount of mucus Moderate

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Section C/D and partly A. Under cervix but not way back in posterior fornix	Acceptable
STANDING		2	Methylene Blue	Section C and D- way back in posterior fornix.	Acceptable See examiner' comments
SQUATTING Half Squat		3	Gentian Violet	Section C-Under cervix and in posterior fornix.	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Feeling cervix feels like a "pin pricking pressure."

2. **In which position was it easier to reach cervix?** When standing and squatting, she felt cervix. She might have been able to feel it when lying down had the device been longer.

3. **What position was most comfortable when inserting device?** Standing was most comfortable. As far as insertion, all were comfortable.

Volunteer's Comments-She thinks that when she inserted the device the first time (lying down), the device was not long enough, and this is why she didn't feel her cervix.

Examiner's Comments- For the squatting and standing postions I lengthened the swab by pulling it farther out. As a result, she was probably able to reach the posterior fornix. She did the standing position two times because the first time I could not find the dye.

SAMPLING DYE STUDY **DATE** 12/5/85

Name M.K. **Age** 22

Education Background 5th year of college

Medications None

LMP 11/18/85 **FREQUENCY** 21 days **DURATION** 5 days

Problems with menses Not usually; but last month she had 2 periods in 1 month due to loss of weight. She has severe cramps in beginning of period.

Gravida 0

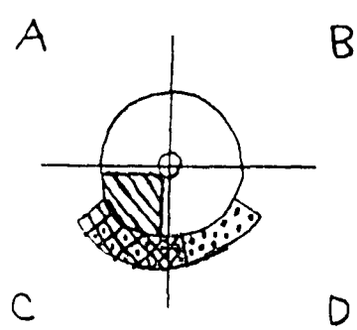
Position of cervix Facing down; centered.

Length of vagina A little shorter than average.

Amount of mucus Small

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		2	Methylene Blue	Section C- Posterior fornix	Acceptable
STANDING		3	Gentian Violet	Section C-On cervix, and in posterior fornix.	Acceptable
SQUATTING Half Squat		1	Indigo Carmine	Section C/D Posterior fornix	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. When reaching cervix, she feels pressure. For all three positions, this pressure sensation felt the same.

2. **In which position was it easier to reach cervix?** Squatting

3. **What position was most comfortable when inserting device?** Squatting

Volunteer's Comments- She thinks squatting is easier because she is accustomed to using this position when she inserts tampons. Also, she thinks that squatting takes the least effort.

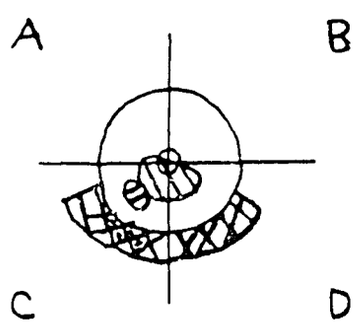
Examiner's Comments- In both squatting and lying down positions, whe was able to get the dye underneath the cervix and far back into the posterior fornix. In the standing position, some of the dye was on the front of the cervix, and also just over the edge of the cervix. She may have reached the back of her posterior fornix. For her, squatting was probably the best.

SAMPLING DYE STUDY **DATE** 12/5/85

Name R.B. **Age** 29
Education Background 2 years of college
Medications None
LMP 11/19/85 **FREQUENCY** 21-28days **DURATION** 5 days
Problems with menses Some severe and some mild cramps in early part of menses.
Gravida 1
Position of cervix Laimed speculum almost straight ahead as opposed to the usual direction (downv
Length of vagina Long. The whole length of device went in.
Amount of mucus Moderate

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Section C-On cervix Section C&D-posterior fornix.	Acceptable
STANDING		3	Gentian Violet	Section C-On cervix. Section C&D-under cervix.	Acceptable
SQUATTING Half Squat		2	Methylene Blue	Section C-posterior fornix	Inconclusive See examiner' comments

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** When lying and squatting, she felt pressure-she could not go further. When standing she did not feel anything with device.

2. **In which position was it easier to reach cervix?** Squatting

3. **What position was most comfortable when inserting device?** Lying down was the most comfortable. Standing was the second most comfortable.

Volunteer's Comments-

Examiner's Comments- I am not sure if I found the Methylene Blue. I found a little blue dye that might have been left over Indigo Carmine. This person was very obese and found this procedure to be more difficult than average person. She was probably able to get underneath cervix better in lying position. In the standing position, she probably got the dye on the bottom part of the face of cervix; t dye might have spread under cervix.

Cervix was large. Also, she needed a longer device. She may have not reached the back of her posterior fornix because the device was not long enough.

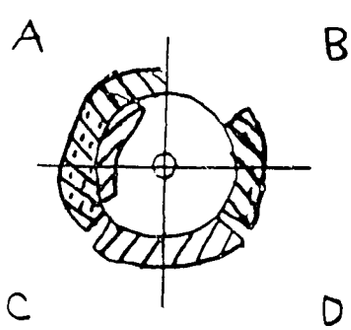
SAMPLING DYE STUDY **DATE** 12/9/85

Name M.W. **Age** 33
Education Background Final year in PhD. program
Medications Prednizone - 2.5 mg 2 times daily
LMP 11/25/85 **FREQUENCY** 26-28 days **DURATION** 5-6 days
Problems with menses Slight cramps during first day of period.

Gravida --
Position of cervix Facing down
Length of vagina Average (approximately 5")
Amount of mucus Moderate / Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		3	Gentian Violet	Section C/D Posterior fornix; also some on edge of cervix in Section A/C.	Acceptable
STANDING		2	Methylene Blue	Section A/C and some in Section B/D - next to cervix; some in posterior fornix.	Acceptable
SQUATTING Half Squat		1	Indigo Carmine	Section A/C next to cervix. Some in posterior fornix	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. She felt her cervix during the lying down position. This is probably because just before this position I put a swab under her cervix at the posterior fornix to show her how the pressure should feel when she does the procedure.
2. **In which position was it easier to reach cervix?** It was easiest to tell where the device was in relation to her cervix when she was in the supine position.

3. **What position was most comfortable when inserting device?** Squatting

Volunteer's Comments- The procedure was not painful.

Examiner's Comments- She did the standing position twice. The first time she bent the device. In section C/D, the Gentian Violet was only found in the back of her posterior fornix, The other dyes were found mixed in mucus next to cervix in Section A/C, and way back in posterior fornix. Lying down was probably the best position for her

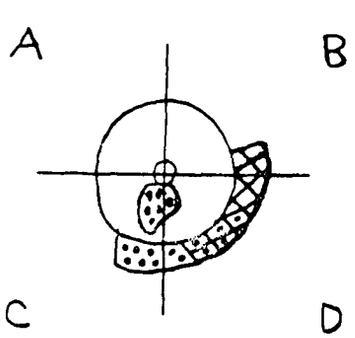
SAMPLING DYE STUDY **DATE** 12/10/85

Name W.N. **Age** 31
Education Background Graduate Student
Medications None
LMP 11/18/85 **FREQUENCY** 26-27 days **DURATION** 4 days
Problems with menses Moderate menstrual cramps on first day of period.

Gravida 0
Position of cervix A little off center on her right side. Cervix was facing to her l
Length of vagina A little longer than average.
Amount of mucus Small

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		2	Methylene Blue	Some mixed in mucus, Section C/D near os, some just under cervix, more dye is found way in posterior fornix.	Acceptable
STANDING		1	Indigo Carmine	Section D; some mixed in mucus just under cervix, some way back in posterior fornix.	Acceptable
SQUATTING Half Squat		3	Gentian Violet	Same as standing	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes; She feels pressure when reaching cervix. For all three positions, she felt the same type of feeling.

2. **In which position was it easier to reach cervix?** Squatting - definitedly. The most difficult was supine. (see volunteer's comments)

3. **What position was most comfortable when inserting device?** Squatting. She was use to this position from using tampons.

Volunteer's Comments- When lying down, at first she felt she reach her cervix, then she pressed down on the device and was able to push device in a little farther.

Examiner's Comments- I had to aim the speculum in a steep downward angle to reach to reach her cervix.
Probably the best position for her is squatting.

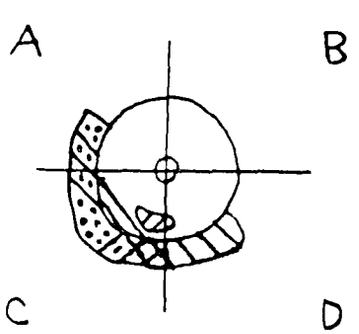
SAMPLING DYE STUDY **DATE** 12/11/85

Name S.A. **Age** 31
Education Background 2 years of college
Medications None
LMP 11/29/85 **FREQUENCY** 28-30 days **DURATION** 2½ days
Problems with menses Severe cramps first day of period

Gravida 3
Position of cervix Uterus is retroverted. Cervix was facing slightly downward; cente
Length of vagina longer than average.
Amount of mucus Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		2	Methylene Blue	Section C - Found a little in back of posterior fornix. Also, a little was mixed in mucus under and on cervix.	Acceptable
STANDING		1	Indigo Carmine	Section C/D Probably did not get way in back of fornix. Mostly mixed in mucus just under cervix, a little on right side and on cervix.	Acceptable See examiner's comments.
SQUATTING Half Squat		3	Gentian Violet	Section A/C found some way in back of posterior fornix and some mixed in mucus.	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. It feels like pressure when she reaches cervix.

2. **In which position was it easier to reach cervix?** Supine

3. **What position was most comfortable when inserting device?** As far as the position itself, standing was most comfortable. However, it was most painful when reaching cervix in this position.

Volunteer's Comments-

Examiner's Comments- Volunteer was obese. It was difficult to find cervix because of angle and length of vagina. She did standing position twice. The first time I coul not get the swab into device because she had bent the device. Also, she did the squatting position twice. The first time I could not find the dye:she probably did not push swab in far enough.

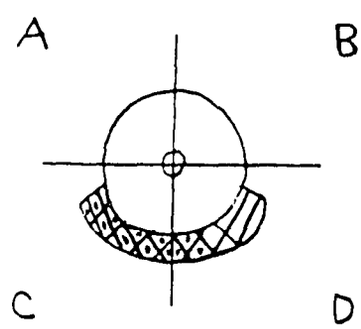
SAMPLING DYE STUDY **DATE** 12/12/85

Name D.C. **Age** 36
Education Background Currently working on Ph.D.
Medications None
LMP 12/5/85 **FREQUENCY** 30 days **DURATION** 5 days
Problems with menses None

Gravida 0
Position of cervix Facing down; centered
Length of vagina Average, approximately 5 inches
Amount of mucus Small

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		1	Indigo Carmine	Section C/D- way back in posterior fornix only.	Acceptable
STANDING		3	Gentian Violet	Section C/D-Same as Supine but not much dye.	Acceptable
SQUATTING Half Squat		2	Methylene Blue	Section C/D-Same as Standing	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. She can feel the pressure when tip of device hits the posterior fornix.

2. **In which position was it easier to reach cervix?** All these positions were equal.

3. **What position was most comfortable when inserting device?** Supine

Volunteer's Comments-

Examiner's Comments-

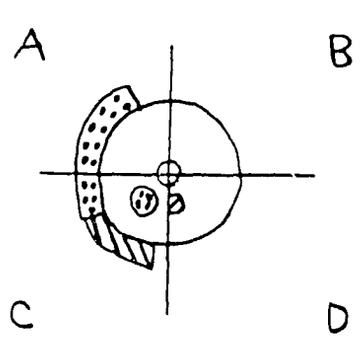
SAMPLING DYE STUDY **DATE** 12/12/85

Name D.B. **Age** 31
Education Background 2 years of college.
Medications None
LMP 12/4/85 **FREQUENCY** 28 days **DURATION** 6 days
Problems with menses Slight cramps in beginning of period.

Gravida 2
Position of cervix
Length of vagina
Amount of mucus

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		2	Methylene Blue	Section D, A little mixed in mucus.	Unacceptable
STANDING		1	Indigo Carmine	Section C, found way back in posterior fornix.	Acceptable
SQUATTING Half Squat		3	Gentian Violet	A little in Section C mixed in mucus on cervix; but mostly in A to right of cervix	Unacceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes "It feels like you are touching something spongy."

2. **In which position was it easier to reach cervix?** Standing. When standing pressure felt different than other positions-she felt more like she got under cervix with device.

3. **What position was most comfortable when inserting device?** Standing

Volunteer's Comments-

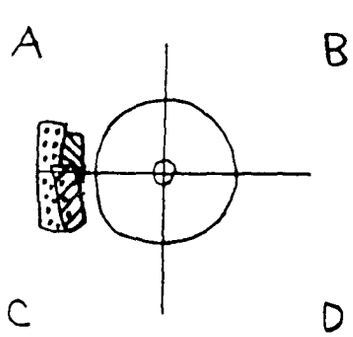
Examiner's Comments-

SAMPLING DYE STUDY **DATE** 12/12/85

Name R.G. **Age** 22
Education Background 1st year in Medical School
Medications Tagemin - taken when needed for ulcer
LMP 11/8/85 **FREQUENCY** 36 days **DURATION** 5 days
Problems with menses Heavy blood flow 1st day of menses with slight cramps; she has increased fatique during menses.
Gravida 0
Position of cervix Extremely off center to her left.
Length of vagina average - Slightly longer.
Amount of mucus Moderate

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		2	Methylene Blue	Section A/C On neck of cervix	Unacceptable
STANDING		1	Indigo Carmine	Section A/C On neck of cervix	Unacceptable
SQUATTING		3	Gentian Violet	Section A/C On neck of cervix	Unacceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** It feels like a barrier-you can't go any further. By the third position she was confident where her cervix was.

2. **In which position was it easier to reach cervix?** Squatting

3. **What position was most comfortable when inserting device?** Supine

Volunteer's Comments- She believes she was able to find cervix easier in the squatting position because she had already had practice with the first two positions.

Examiner's Comments- Because her cervix was positioned so off center, I could not even get the cervix in the speculum. I could only see the side of the cervix. Also, her cervix was facing to her left side. She was extremely uncomfortable when I inserted the speculum and tried to find her cervix.

She may have gotten the dyes under her cervix also, but I was not able to see. However, it seemed that all of the dye was on the right neck of her cervix.

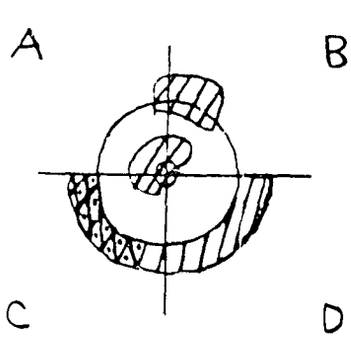
SAMPLING DYE STUDY **DATE** 12/16/85

Name P.C. **Age** 23
Education Background Senior Physical Therapy Student
Medications None
LMP 11/18/85 **FREQUENCY** 28 days **DURATION** 5 days
Problems with menses Occasional severe cramps.

Gravida 0
Position of cervix Facing down, centered.
Length of vagina Average , approximately 5 ".
Amount of mucus Abundant

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		3	Gentian Violet	C/D - under cervix; some in D section way way back in posterior fornix. Section A - at os mixed in mucus. Section B-on neck of cervix.	Acceptable
STANDING		2	Methylene Blue	SectionC-Same as squatting, but not as far back in posterior fornix	Acceptable
SQUATTING Half Squat		1	Indigo Carmine	Section C-Dye was found under cervix but not way back in posterior fornix.	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. It feels like a dull sensatio
   In standing position, she felt less sensation than other two positions.

2. **In which position was it easier to reach cervix?** Squatting.

3. **What position was most comfortable when inserting device?** Lying down.

Volunteer's Comments-

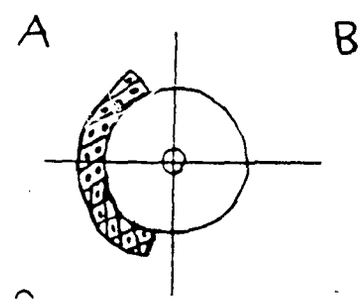
Examiner's Comments- Standing was probably the worst position for this volunteer.

SAMPLING DYE STUDY **DATE** 12/17/85

Name S.S. **Age** 42
Education Background M.A. M.S. degree
Medications None
LMP 12/9/85 **FREQUENCY** 25days **DURATION** 5 days
Problems with menses 2nd day of menses she has very heavy bleeding. Flow is lighter the other days.
Gravida 3
Position of cervix Facing down; centered
Length of vagina Average, But width is above average.
Amount of mucus Moderate

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		2	Methylene Blue	Section A/C; Dye found partly back in posterior fornix, but not all of the way back.	Acceptable
STANDING		1	Indigo Carmine	Section C; same as Supine	Acceptable
SQUATTING Full Squat		3	Gentian Violet	Section A/C; same as Supine	Acceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. She feels slight pressure when she reaches cervix.

2. **In which position was it easier to reach cervix?** It was easier to reach cervix and turn device in supine and squatting positions.

3. **What position was most comfortable when inserting device?** Squatting and Supine

Volunteer's Comments- She thinks that squatting would compress the vagina more and make it more difficult to reach the posterior fornix. However, this may be because she squatted all of the way down.

Examiner's Comments- She squatted almost all of the way down. She felt more comfortable squatting this way than in a half squat position.

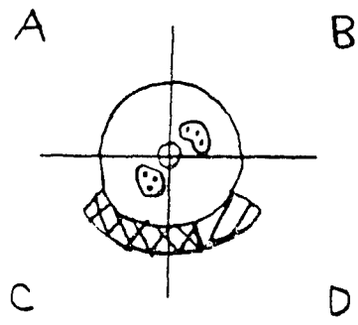
SAMPLING DYE STUDY **DATE** 12/18/85

Name B.B. **Age** 23
Education Background Nursing Student
Medications None
LMP 11/22/85 **FREQUENCY** 28 days **DURATION** 4 days
Problems with menses None

Gravida 0
Position of cervix Facing down; off-centered, facng a little to her left.
Length of vagina Average
Amount of mucus Moderate

POSITION	SYMBOL	SAMPLING ORDER	DYE	LOCATION OF DYE	CONCLUSION
SUPINE		3	Gentian Violet	Section C/D, posterior fornix	Acceptable
STANDING		1	Indigo Carmine	Section C/D, posterior fornix	Acceptable
SQUATTING Half Squat		2	Methylene Blue	Section B and Section C near os, mixed in mucus.	Unacceptable

ILLUSTRATION OF CERVIX AND LOCATION OF DYE



Questions:

1. **Can you feel your cervix using this device?** Yes. She feels pressure when reaching cervix.

2. **In which position was it easier to reach cervix?** Supine

3. **What position was most comfortable when inserting device?** Supine. She found that in the squatting position she was uncomfortable when the device reached her cervix.

Volunteer's Comments-

Examiner's Comments-

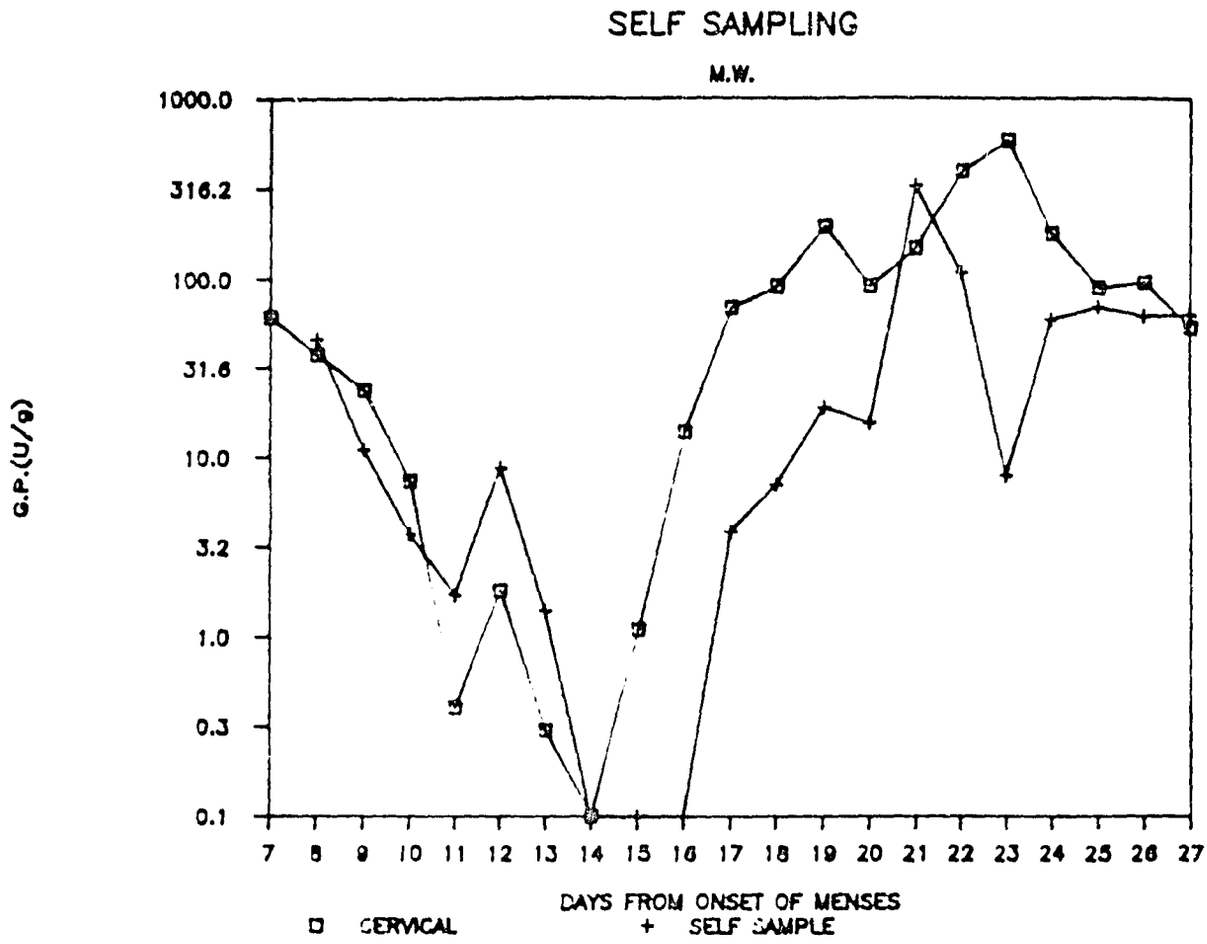
APPENDIX 3

A. Self-Sampling Study PARFR-360 1985-1986

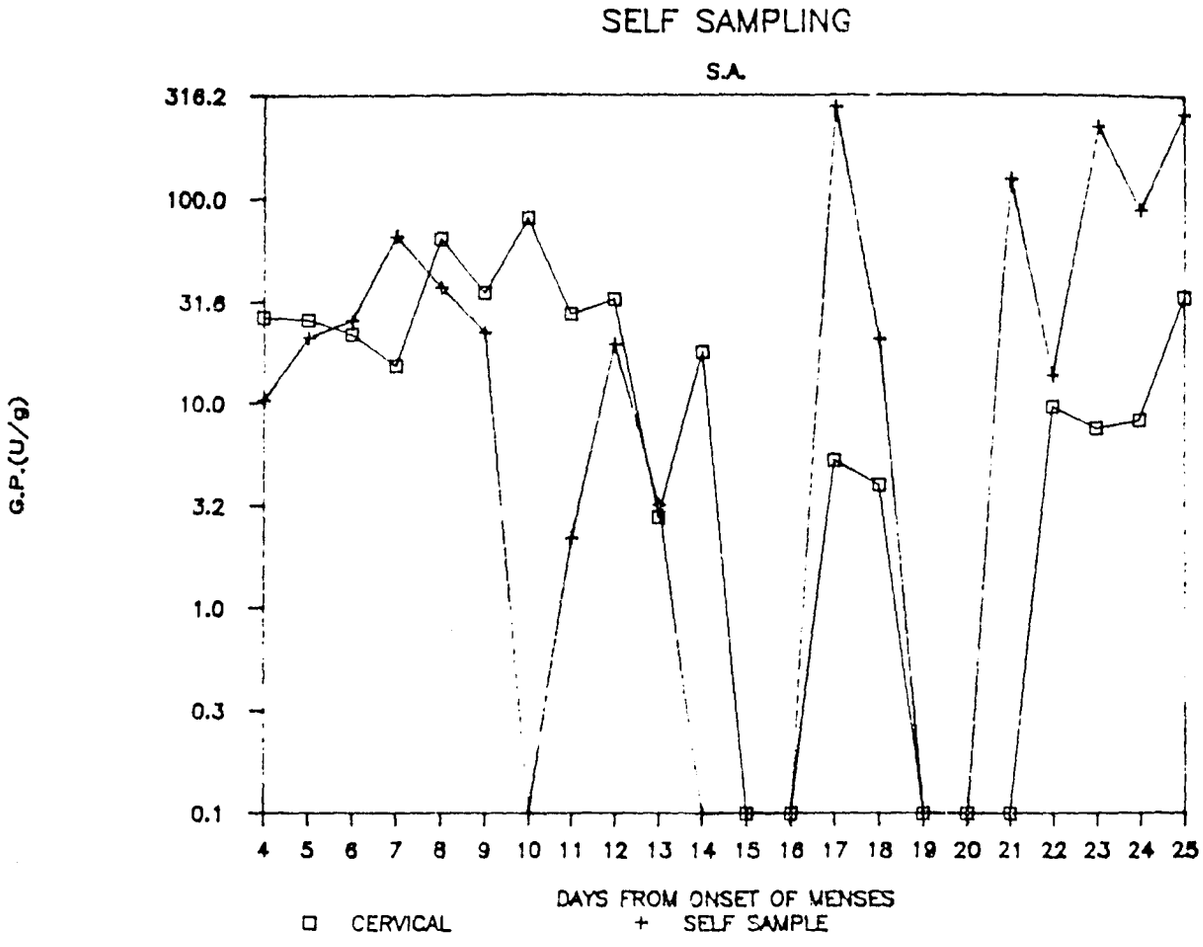
Name	Dates	Satis	Unsatisf	Low Foll GP	Low Lut GP	Low Lut SS
M.W.	12/27/85-1/16/85	Yes	--	Yes	--	Yes
S.A.	12/27/85-1/17/85	--	Yes	--	Yes	--
M.P.	1/3/86-1/19/86	Yes	--	Yes	--	Yes
D.R.	1/17/86-1/21/86	--	Yes	--	--	--
S.S.	1/9/86-1/30/86	--	Yes	--	--	Yes
M.C.	1/9/86-2/5/86	--	Yes	--	--	FollSS too
P.C.	1/10/86-1/28/86	--	Yes	--	--	--
C.V.	1/30/86-2/20/86	--	Yes	--	--	FollSS too
G.G.	2/6/86-2/28/86	--	Yes	Yes	Yes	FollSS too!
M.K.	2/9/86-2/28/86	--	Yes	--	--	--
W.N.	2/15/86-3/7/86	Yes	--	Yes	--	--
L.C.	2/18/86-3/16/86	Yes	--	--	--	Yes
D.S.	2/20/86-3/13/86	--	Yes	Yes	--	Very!
J.M.	3/5/86-3/24/86	Yes	--	Yes	Yes	Yes
D.C.	3/13/86-4/13/86	--	Yes	--	--	Yes
L.A.	3/15/86-4/7/86	--	Yes	--	--	Yes
W.Y.	7/3/86-7/23/86	Yes	--	--	--	Yes
W.B.	7/9/86-7/31/86	--	Yes	--	--	Yes
H.R.	7/13/86-8/6/86	--	Yes	--	--	Yes
K.S.	8/27/86-9/15/86	Yes	--	--	--	Yes
	Total:	7	13	6	3	15
	% of Total: (n=20)	35	65	30	15	75

Abbreviations:

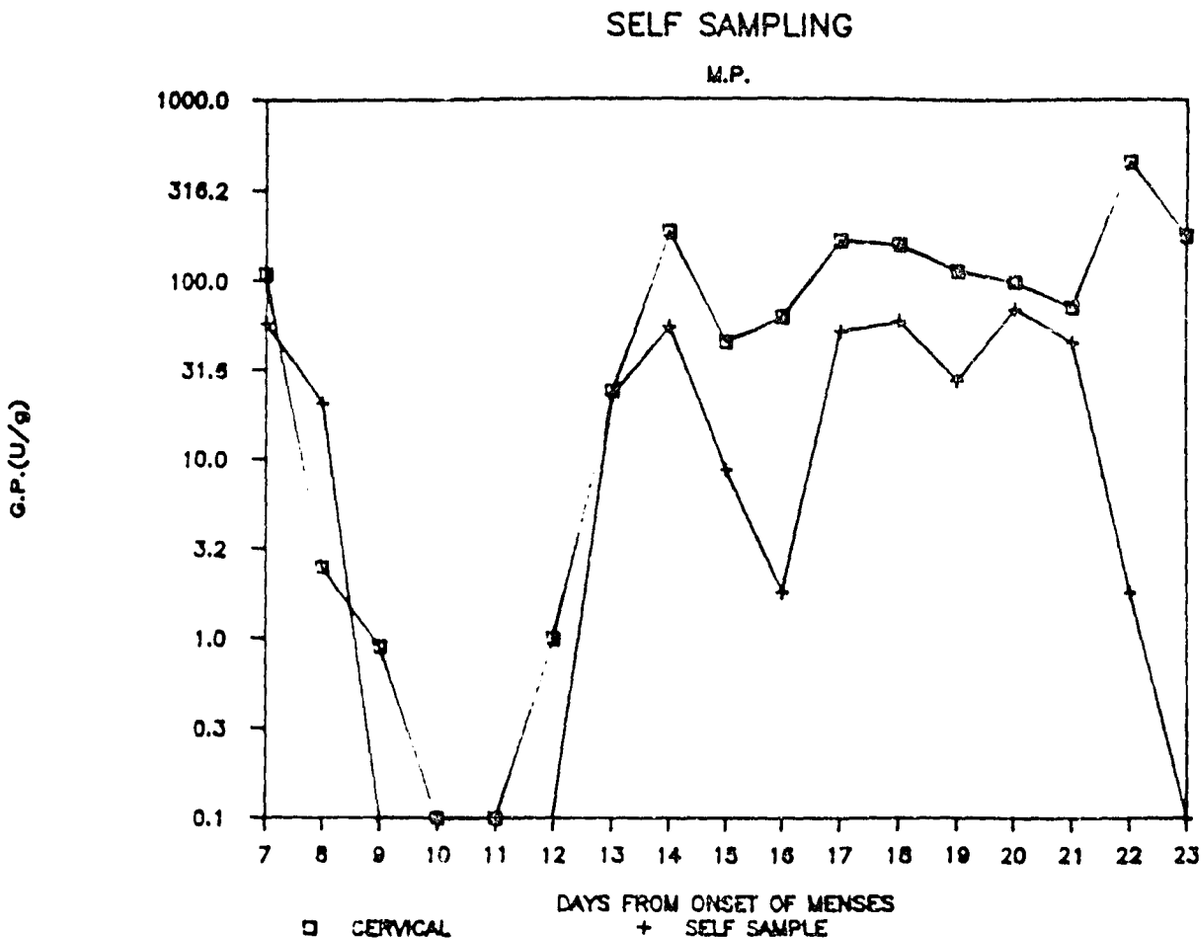
Satisf.: Satisfactory agreement between technician - and self-sampled GP profiles.
Unsat.: Poor correlation between the two GP profiles.
Low Foll GP: Too low (<30 U/g) GP content, follicular phase.
Low LutGP: Too low (<100 U/g) GP content, luteal phase.
Low LutSS: Significantly lower luteal GP content of swab sample compared to that of canula sample.
DFOM: Days from the Onset of Menses.
GPC: GP of cervical mucus sampled with the canula.
GPS: GP of cervical mucus self-sampled with the swab.
SELF-WT: The net weight in g of mucus on the swab.
Cx-Wt: The net weight in g of mucus on the canula.


SELF SAMPLING
M.W.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
G.P.(U/g)
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

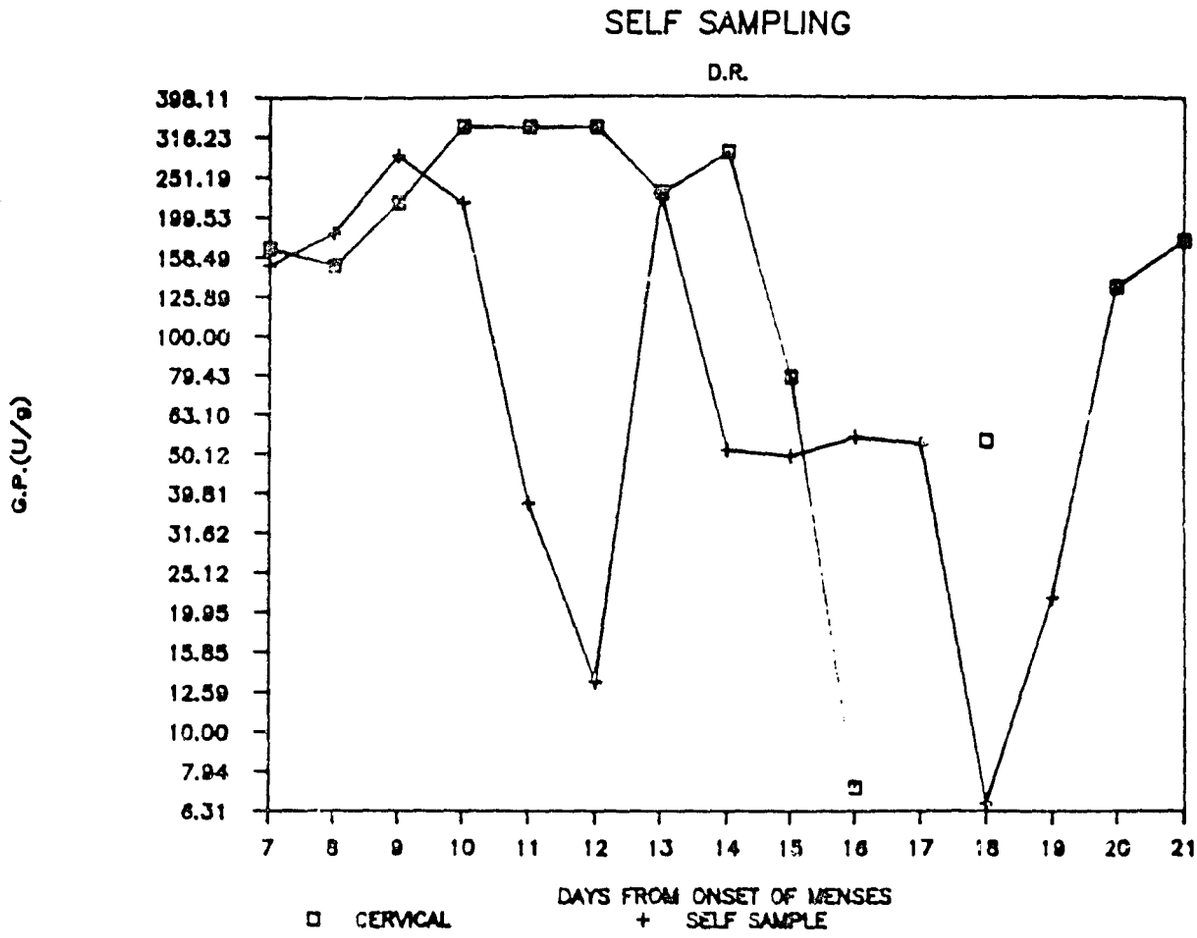
	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
	M.W.	12/27/85	7	60.8			0.034	
		12/28/85	8	37.3	45.5	0.036	0.029	
		12/29/85	9	23.6	11.0	0.109	0.049	
		12/30/85	10	7.3	3.7	0.103	0.049	
7		12/31/85	11	0.4	1.7	0.046	0.034	
8		1/1/86	12	1.8	8.7	0.034	0.037	
9		1/2/86	13	0.3	1.4	0.124	0.168	
10		1/3/86	14	0.1	0.1	0.095	0.108	
11		1/4/86	15	1.1	0.1	0.151	0.110	
12		1/5/86	16	13.9	0.1	0.083	0.033	
13		1/6/86	17	69.1	3.9	0.108	0.014	
14		1/7/86	18	90.7	7.1	0.045	0.021	-EXTRA SWAB=5
15		1/8/86	19	195.2	19.1	0.151	0.025	(posterior fornix)
16		1/9/86	20	90.8	15.5	0.051	0.031	-EXTRA SWAB=7
17		1/10/86	21	148.6	328.1	0.023	0.007	(posterior fornix)
18		1/11/86	22	394.4	109.2	0.056	0.012	
19		1/12/86	23	580.0	8.0	0.068	0.005	
20		1/13/86	24	179.3	60.2	0.102	0.036	
21		1/14/86	25	89.9	70.5	0.043	0.026	
22		1/15/86	26	96.0	62.9	0.045	0.014	
23		1/16/86	27	53.5	63.2	0.039	0.013	
24								


SELF SAMPLING
S.A.
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
G.P.(U/g)
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

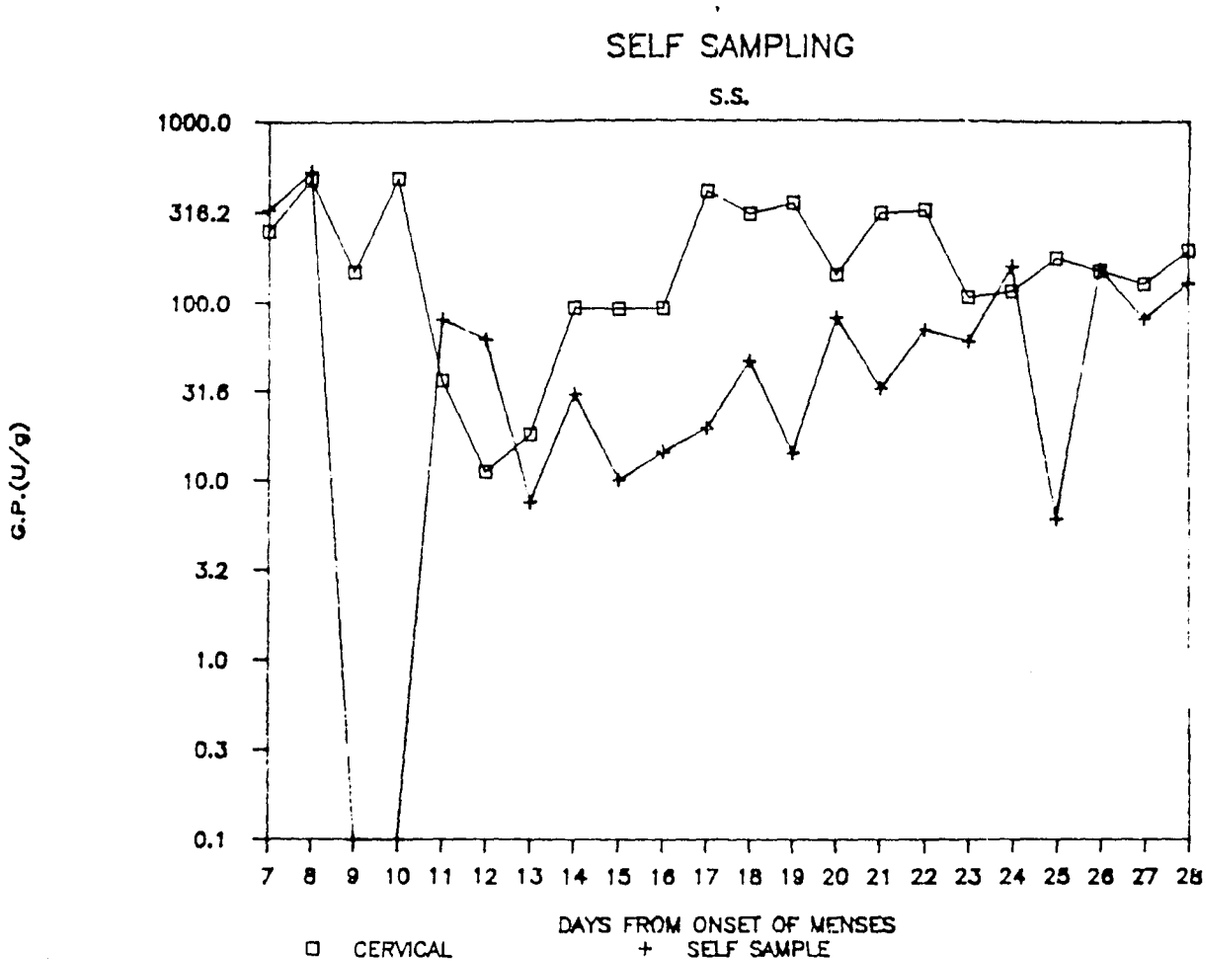
NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARK
S.A.	12/27/85	4	26.7	10.3	0.009	0.009	
	12/28/85	5	26.0	21.2	0.020	0.013	
	12/29/85	6	22.1	25.7	0.017	0.031	
	12/30/85	7	15.4	66.1	0.033	0.014	
	12/31/85	8	64.4	37.2	0.021	0.027	
	1/1/86	9	34.9	22.2	0.016	0.020	
	1/2/86	10	80.0	0.0	0.039	0.024	
	1/3/86	11	27.4	2.2	0.055	0.025	
	1/4/86	12	32.6	19.5	0.016	0.051	
	1/5/86	13	2.8	3.2	0.107	0.048	
	1/6/86	14	18.0	0.0	0.040	0.019	
	1/7/86	15	0.0	0.0	0.086	0.029	
	1/8/86	16	0.0	0.0	0.066	0.015	
	1/9/86	17	5.3	278.2	0.006	0.031	
	1/10/86	18	4.0	20.7	0.043	0.027	
	1/11/86	19	0.0	0.0	0.018	0.019	
	1/12/86	20	0.0	0.0	0.026	0.007	
	1/13/86	21	0.0	124.7	0.030	0.029	
	1/14/86	22	9.8	13.9	0.052	0.026	
	1/15/86	23	7.7	221.9	0.014	0.026	
	1/16/86	24	8.4	87.0	0.025	0.012	
	1/17/86	25	32.9	258.1	0.005	0.012	-SECOND SELF = 92.4


SELF SAMPLING
M.P.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
G.P.(U/g)
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

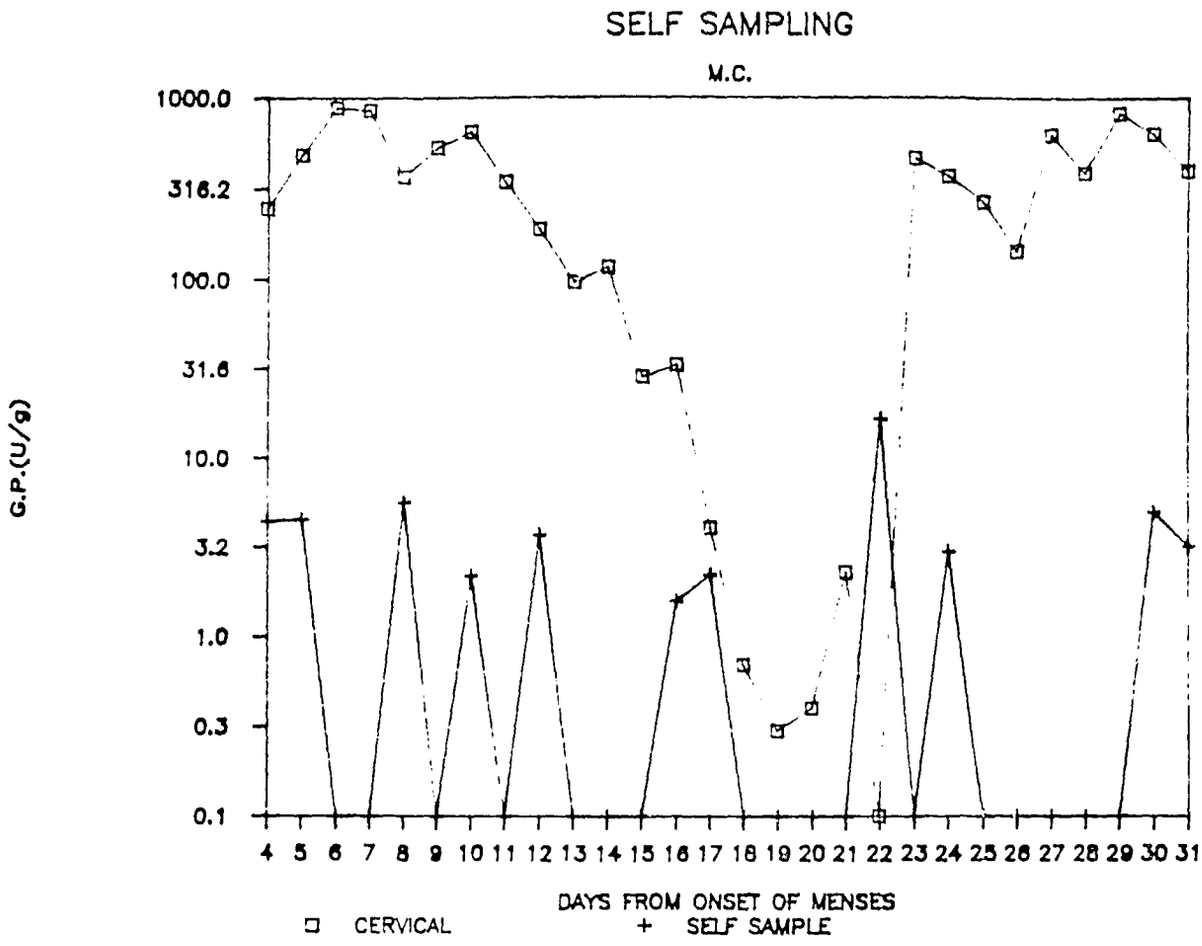
50	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
51								
52	M.P.	1/3/86	7	109.0	57.5	0.037	0.029	
53		1/4/86	8	2.5	20.3	0.054	0.022	
54		1/5/86	9	0.9	0.1	0.009	0.096	
55		1/6/86	10	0.1	0.1	0.021	0.156	
56		1/7/86	11	0.1	0.1	0.034	0.042	
57		1/8/86	12	1.0	0.1	0.024	0.048	
58		1/9/86	13	24.2	23.1	0.027	0.026	
59		1/10/86	14	187.1	55.2	0.032	0.013	
60		1/11/86	15	45.8	8.8	0.012	0.030	
61		1/12/86	16	63.1	1.8	0.030	0.009	
62		1/13/86	17	167.2	52.1	0.051	0.014	
63		1/14/86	18	157.0	59.1	0.027	0.007	
64		1/15/86	19	111.3	27.6	0.021	0.007	
65		1/16/86	20	97.7	68.5	0.020	0.011	
66		1/17/86	21	72.0	45.3	0.030	0.006	
67		1/18/86	22	456.1	1.8	0.026	0.028	
68		1/19/86	23	177.4	0.1	0.004	0.078	-SELF SAMPLE WEIGHT LOW
69								


SELF SAMPLING
D.R.
G.P.(U/g)
398.11
316.23
251.19
199.53
158.49
125.89
100.00
79.43
63.10
50.12
39.81
31.62
25.12
19.95
15.85
12.59
10.00
7.94
6.31
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

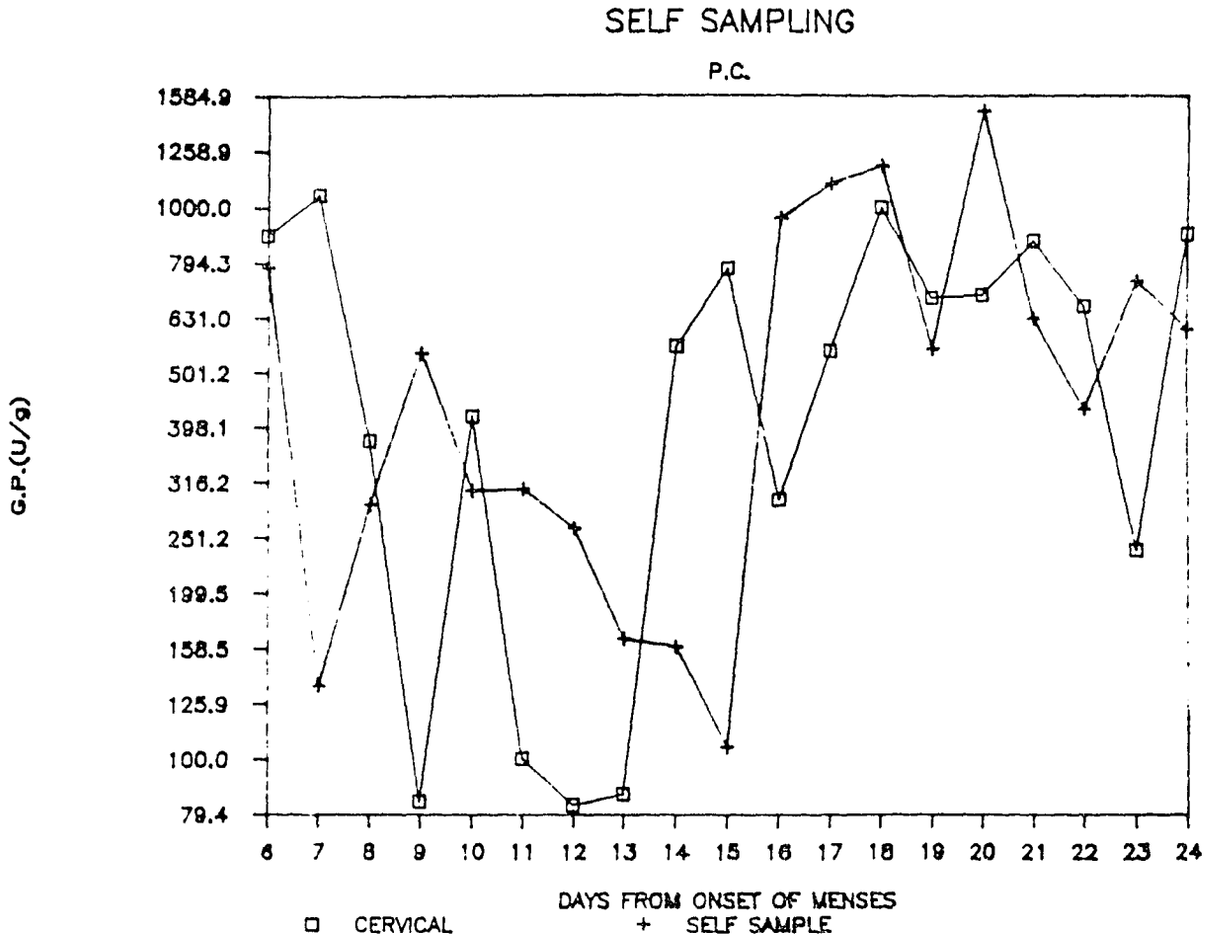
NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
D.R.	1/7/86	7	166.8	151.5	0.047	0.018	
	1/8/86	8	150.4	182.6	0.023	0.025	
	1/9/86	9	216.8	285.6	0.110	0.023	
	1/10/86	10	335.8	216.6	0.132	0.011	
	1/11/86	11	332.4	37.7	0.062	0.035	
	1/12/86	12	332.3	13.4	0.080	0.016	
	1/13/86	13	228.1	229.1	0.169	0.019	
	1/14/86	14	288.5	51.3	0.090	0.088	
	1/15/86	15	78.5	49.7	0.100	0.098	
	1/16/86	16	7.2	55.5	0.173	0.056	
	1/17/86	17	147.2	53.2	0.150	0.037	
	1/18/86	18	54.4	6.6	0.100	0.058	
	1/19/86	19		21.9	0.107		LOST CANNULA
	1/20/86	20	134.0	134.0	0.082	0.046	
	1/21/86	21	173.7	173.7	0.183	0.041	


SELF SAMPLING
S.S.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
G.P.(U/g)
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

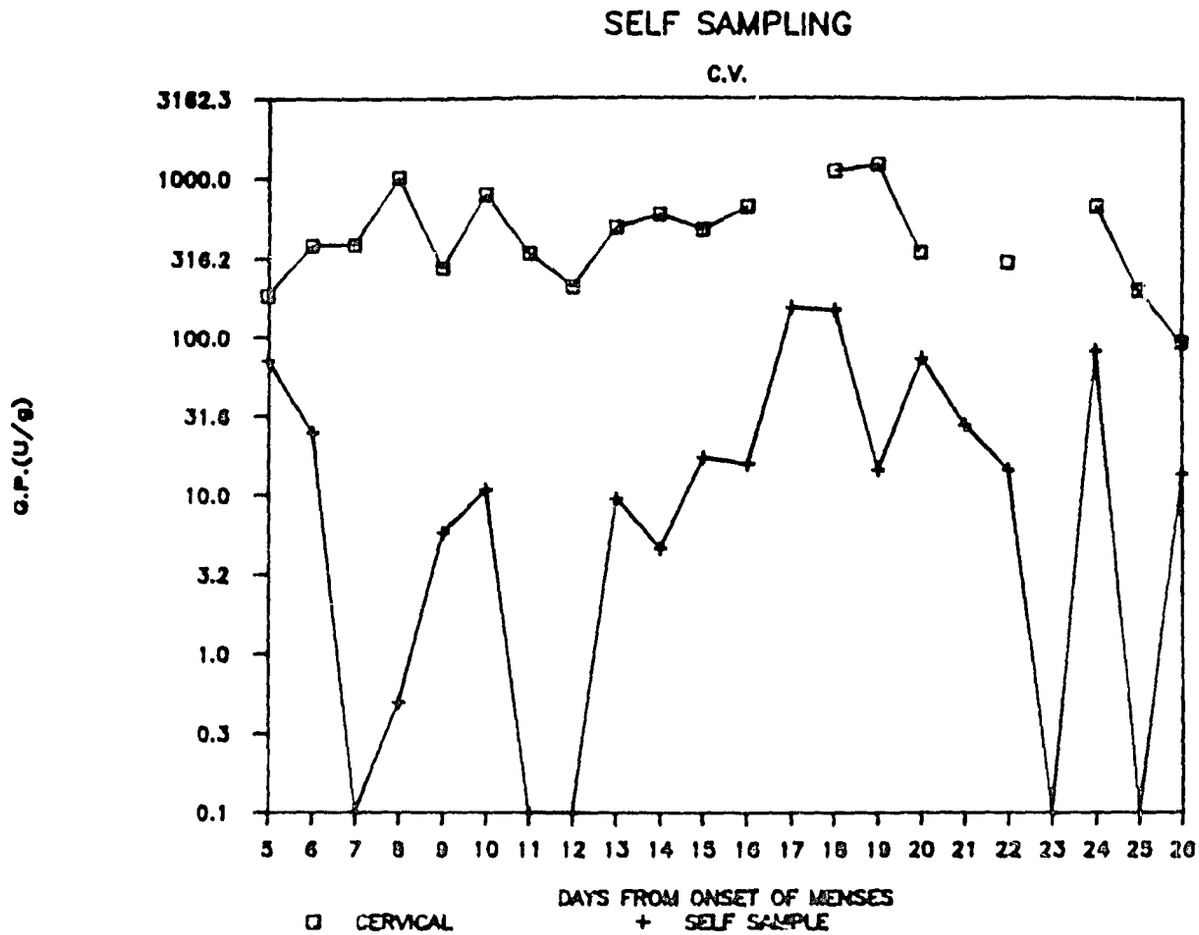
110	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CY-WT	REMARK
120								
121	S.S.	1/9/86	7	252.1	326.4	0.023	0.086	
122		1/10/86	8	486.0	532.1	0.042	0.106	
123		1/11/86	9	149.6	0.0	0.026	0.009	
124		1/12/86	10	491.0	0.0	0.070	0.045	
125		1/13/86	11	37.0	80.9	0.006	0.248	
126		1/14/86	12	11.3	62.0	0.038	0.260	
127		1/15/86	13	18.2	7.6	0.022	0.102	EXTRA SELF =
128		1/16/86	14	93.2	30.4	0.015	0.085	34.0
129		1/17/86	15	91.6	10.0	0.024	0.127	
130		1/18/86	16	92.3	14.4	0.010	0.141	
131		1/19/86	17	410.2	19.7	0.033	0.168	
132		1/20/86	18	309.0	47.2	0.016	0.056	
133		1/21/86	19	354.1	14.3	0.086	0.016	
134		1/22/86	20	142.2	81.2	0.016	0.081	
135		1/23/86	21	310.2	32.5	0.016	0.065	
136		1/24/86	22	324.7	70.1	0.021	0.091	
137		1/25/86	23	108.8	60.7	0.024	0.068	
138		1/26/86	24	117.6	160.3	0.010	0.102	
139		1/27/86	25	179.2	6.2	0.015	0.071	
140		1/28/86	26	152.2	154.3	0.009	0.039	


SELF SAMPLING
M.C.
G.P.(U/g)
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

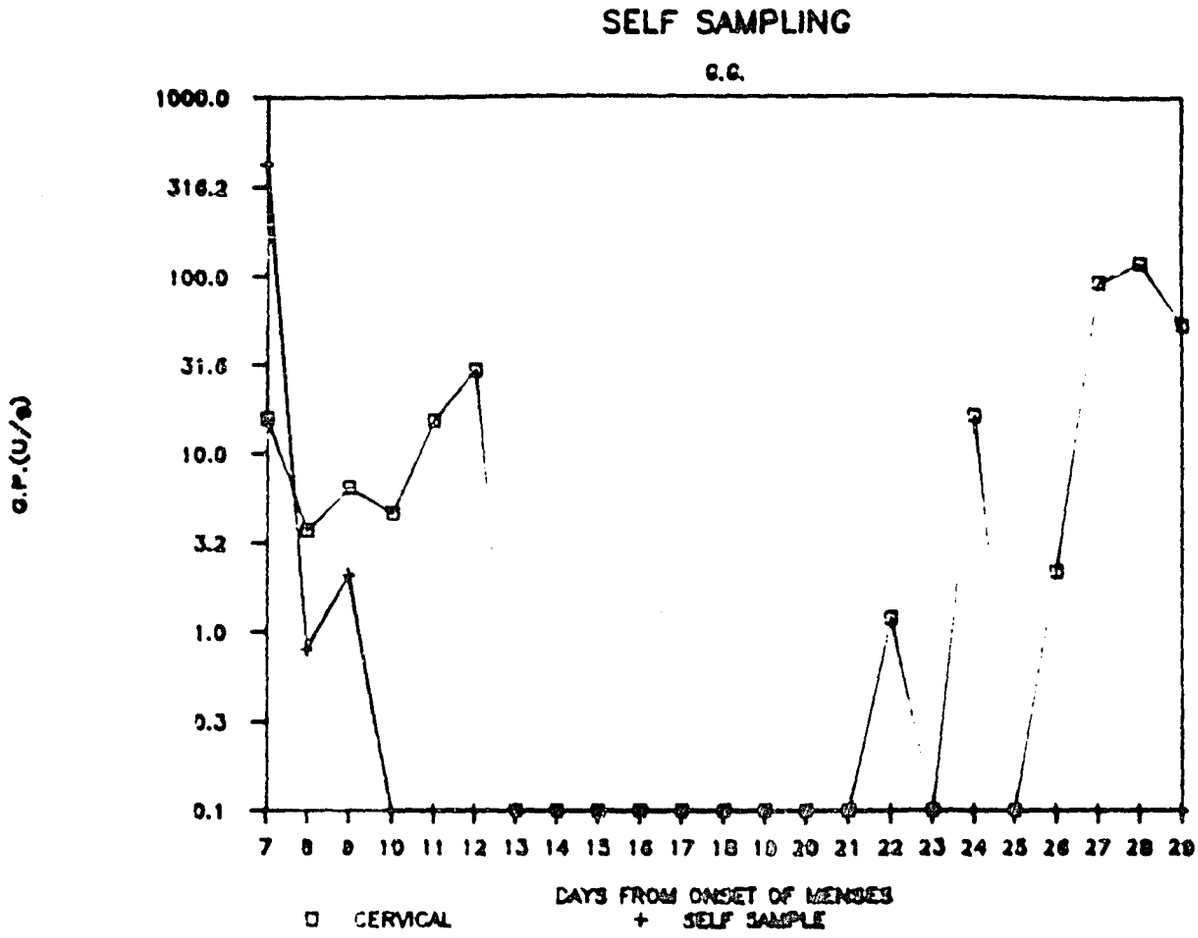
88	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
89								
90	M.C.	1/9/86	4	245.9	4.4	0.011	0.018	
91		1/10/86	5	484.8	4.5	0.016	0.011	
92		1/11/86	6	877.8	0.0	0.036	0.006	
93		1/12/86	7	848.6	0.0	0.014	0.007	
94		1/13/86	8	365.0	5.6	0.030	0.032	
95		1/14/86	9	530.0	0.0	0.058	0.016	
96		1/15/86	10	645.6	2.2	0.054	0.019	
97		1/16/86	11	338.8	0.0	0.038	0.022	
98		1/17/86	12	186.2	3.7	0.048	0.013	
99		1/18/86	13	95.2	0.0	0.074	0.015	
100		1/19/86	14	115.5	0.0	0.050	0.106	
101		1/20/86	15	28.3	0.0	0.050	0.030	
102		1/21/86	16	33.2	1.6	0.094	0.112	
103		1/22/86	17	4.1	2.2	0.086	0.137	
104		1/23/86	18	0.7	0.0	0.086	0.232	
105		1/24/86	19	0.3	0.0	0.095	0.273	
106		1/25/86	20	0.4	0.0	0.050	0.107	
107		1/26/86	21	2.3	0.0	0.057	0.063	
108		1/27/86	22	0.0	16.6	0.043	0.016	
109		1/28/86	23	459.6	0.0	0.091	0.017	
110		1/29/86	24	366.7	3.0	0.053	0.026	
111		1/30/86	25	262.7	0.0	0.058	0.027	
112		1/30/86	26	142.0	0.0	0.075	0.037	
113		2/1/86	27	621.1	0.0	0.086	0.024	
114		2/2/86	28	383.6	0.0	0.075	0.013	
115		2/3/86	29	817.1	0.0	0.062	0.014	
116		2/4/86	30	631.7	5.0	0.067	0.003	-EXTRA swab=25.'
117		2/5/86	31	393.8	3.2	0.049	0.015	(posterior fornix)
118								


SELF SAMPLING
P.C.
G.P.(U/g)
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

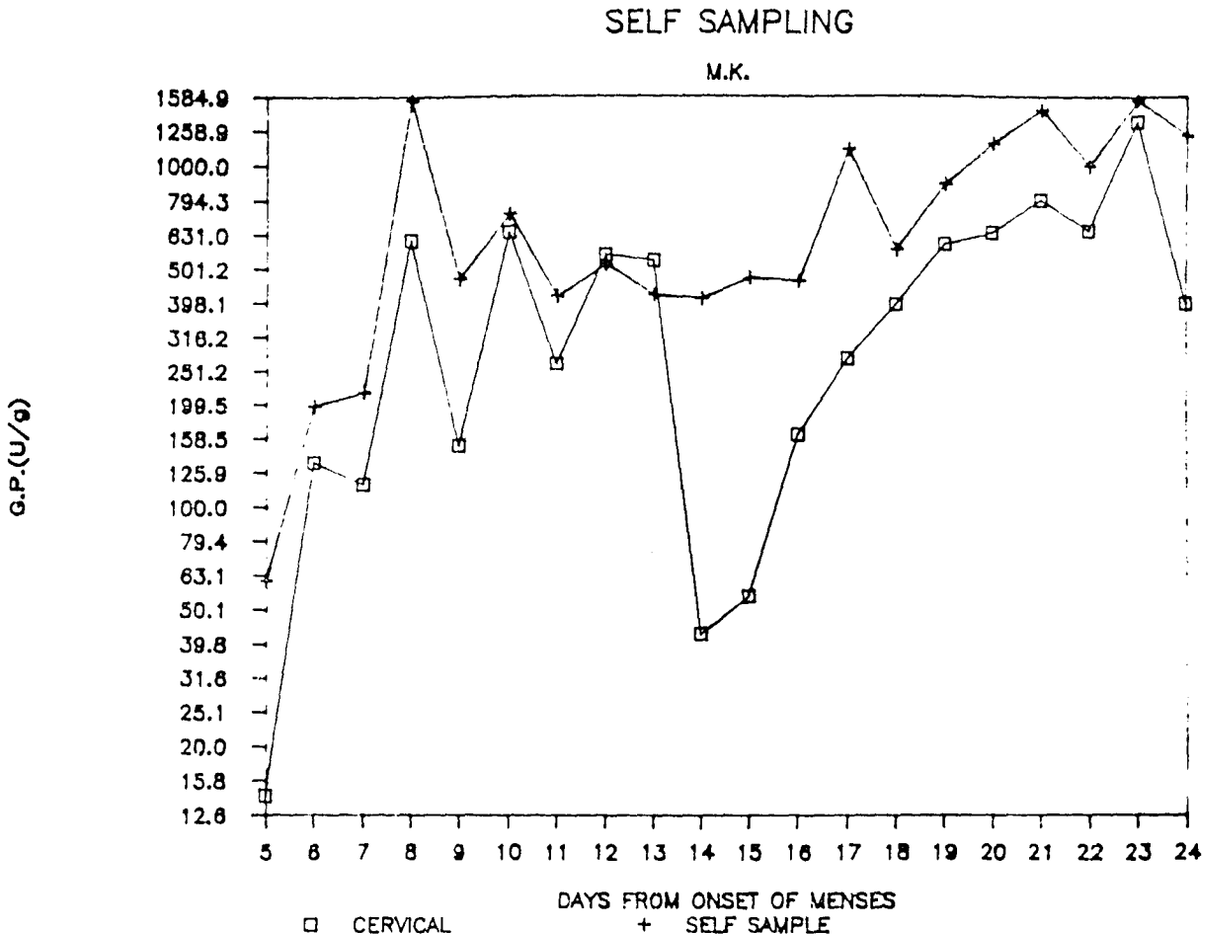
NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
P.C.	1/10/86	6	894.2	782.2	0.057	0.047	
	1/11/86	7	1057.0	135.8	0.027	0.056	
	1/12/86	8	377.8	288.9	0.084	0.131	
	1/13/86	9	84.1	546.6	0.137	0.184	
	1/14/86	10	418.2	305.8	0.032	0.387	
	1/15/86	11	100.5	307.9	0.114	0.380	
	1/16/86	12	82.8	261.7	0.054	0.295	
	1/17/86	13	86.7	165.0	0.040	0.072	
	1/18/86	14	563.3	160.0	0.093	0.142	
	1/19/86	15	779.5	105.4	0.013	0.078	
	1/20/86	16	296.5	955.6	0.012	0.068	
	1/21/86	17	551.1	1102.6	0.036	0.033	
	1/22/86	18	1000.7	1187.3	0.092	0.019	
	1/23/86	19	690.7	555.8	0.019	0.040	
	1/24/86	20	700.7	1489.0	0.049	0.036	
	1/25/86	21	876.8	634.7	0.040	0.034	
	1/26/86	22	670.2	435.2	0.014	0.033	
	1/27/86	23	240.0	740.5	0.043	0.016	
	1/28/86	24	899.4	606.6	0.012	0.025	


SELF SAMPLING
C.V.
3162.3
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
G.P.(U/g)
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

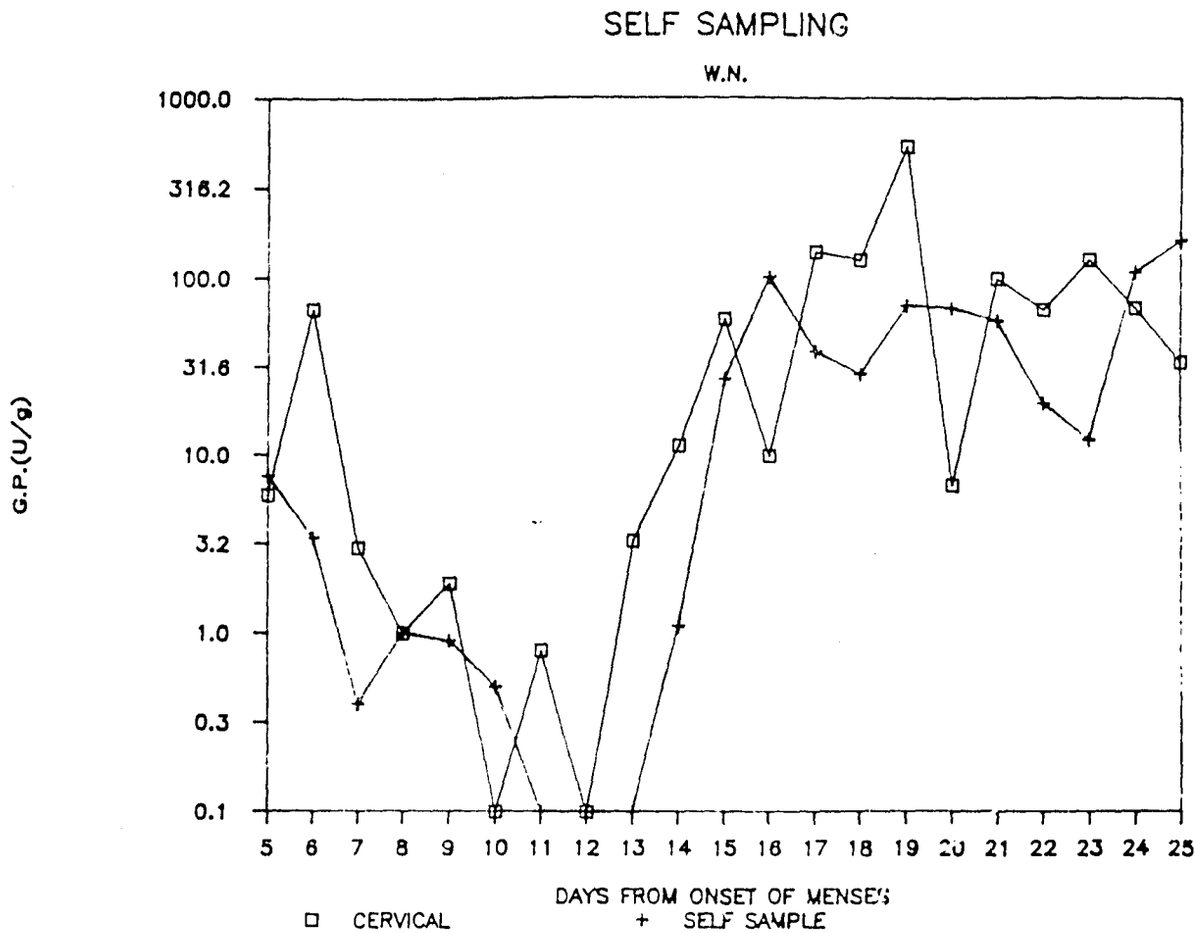
NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
C.V.	1/30/86	5	184.7	71.2	0.046	0.040	
	1/31/86	6	384.9	24.5	0.045	0.030	
	2/1/86	7	389.0	0.0	0.017	0.017	
	2/2/86	8	1016.5	0.5	0.039	0.026	
	2/3/86	9	275.1	5.8	0.077	0.030	
	2/4/86	10	794.8	10.9	0.076	0.041	
	2/5/86	11	337.8	0.0	0.176	0.054	
	2/6/86	12	209.0	0.0	0.211	0.199	
	2/7/86	13	493.0	9.5	0.075	0.062	
	2/8/86	14	598.5	4.6	0.104	0.108	
	2/9/86	15	482.4	17.2	0.045	0.022	
	2/10/86	16	666.7	15.6	0.095	0.034	
	2/11/86	17	___	155.4	0.111	0.017	
	2/12/86	18	1100.7	148.5	0.137	0.036	
	2/13/86	19	1214.0	14.5	0.096	0.021	
	2/14/86	20	348.2	73.3	0.073	0.036	
	2/15/86	21	649.7	27.6	0.044	0.018	
	2/16/86	22	303.6	14.6	0.058	0.022	
	2/17/86	23					NO SHOW
	2/18/86	24	669.5	84.2	0.101	0.038	
	2/19/86	25	200.8	0.0	0.170	0.016	
	2/20/86	26	93.9	13.8	0.038	0.014	


SELF SAMPLING
G.G.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
G.P.(U/g)
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

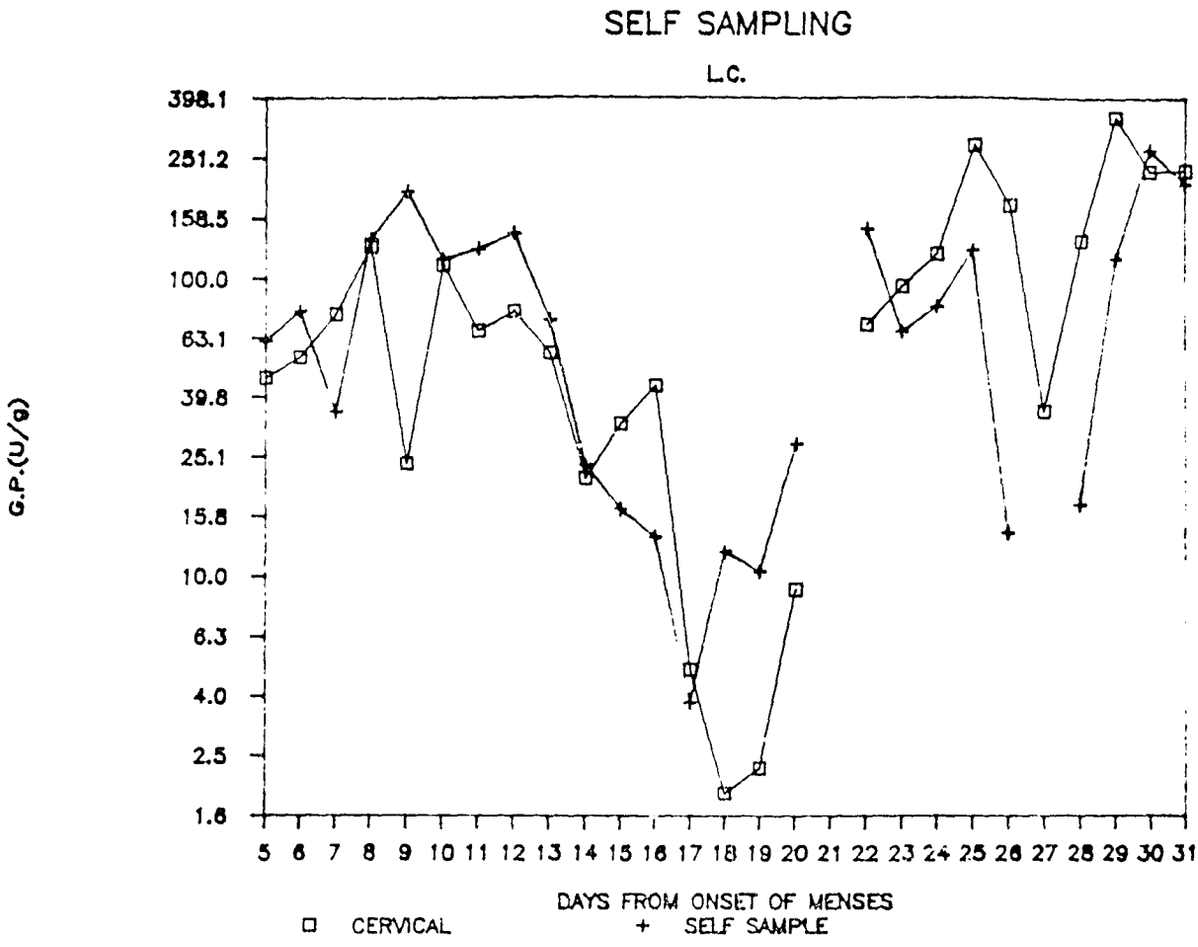
NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
G.G.	2/6/86	7	15.6	423.0	0.022	0.024	
	2/7/86	8	3.7	0.8	0.009	0.021	
	2/8/86	9	6.4	2.1	0.036	0.018	
	2/9/86	10	4.6	0.0	0.024	0.015	
	2/10/86	11	15.3	0.0	0.067	0.026	
	2/11/86	12	29.5	0.0	0.061	0.052	
	2/12/86	13	0.0	0.0	0.056	0.030	
	2/13/86	14	0.0	0.0	0.034	0.034	
	2/14/86	15	0.0	0.0	0.082	0.026	
	2/15/86	16	0.0	0.0	0.077	0.072	
	2/16/86	17	0.0	0.0	0.067	0.075	
	2/17/86	18	0.0	0.0	0.151	0.098	
	2/18/86	19	0.0	0.0	0.085	0.058	
	2/19/86	20	0.0	0.0	0.134	0.131	
	2/20/86	21	0.0	0.0	0.123	0.024	
	2/21/86	22	1.2	0.0	0.062	0.043	
	2/22/86	23	0.0	0.0	0.115	0.026	
	2/23/86	24	16.4	0.0	0.057	0.019	
	2/24/86	25	0.0	0.0	0.053	0.015	
	2/25/86	26	2.2	0.0	0.068	0.013	
	2/26/86	27	90.7	0.0	0.062	0.009	
	2/27/86	28	116.0	0.0	0.066	0.003	
	2/28/86	29	52.6	0.0	0.051	0.011	


SELF SAMPLING
M.K.
G.P.(U/g)
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

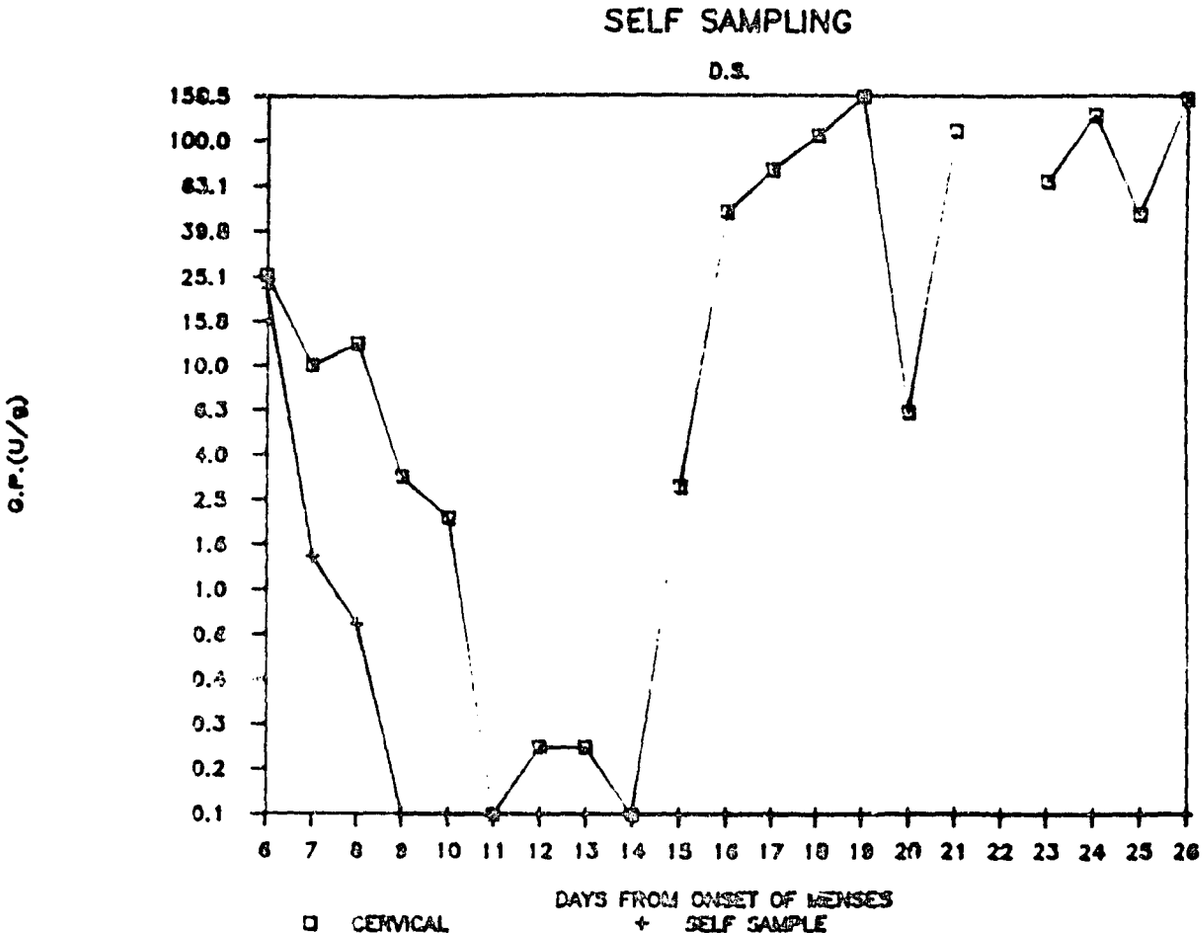
NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
M.K.	2/9/86	5	14.4	61.1	0.011	0.038	
	2/10/86	6	134.7	197.8	0.009	0.024	
	2/11/86	7	116.4	217.6	0.005	0.056	
	2/12/86	8	610.7	1545.0	0.052	0.060	
	2/13/86	9	152.3	471.1	0.018	0.073	
	2/14/86	10	642.9	726.5	0.033	0.056	
	2/15/86	11	264.7	416.0	0.010	0.055	EXTRA CX=143
	2/16/86	12	554.2	519.6	0.037	0.049	
	2/17/86	13	534.9	421.6	0.036	0.070	
	2/18/86	14	42.8	413.8	0.062	0.263	
	2/19/86	15	55.4	475.4	0.058	0.262	
	2/20/86	16	164.6	464.6	0.026	0.076	EXTRA CX=138
	2/21/86	17	275.6	1114.3	0.056	0.045	
	2/22/86	18	395.1	570.5	0.043	0.038	
	2/23/86	19	592.2	883.6	0.022	0.029	
	2/24/86	20	643.3	1158.7	0.020	0.040	
	2/25/86	21	800.0	1453.3	0.016	0.027	
	2/26/86	22	649.7	1000.0	0.008	0.033	
	2/27/86	23	1342.2	1546.7	0.025	0.021	
	2/28/86	24	401.4	1222.6	0.026	0.037	


SELF SAMPLING
W.N.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
G.P.(U/g)
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

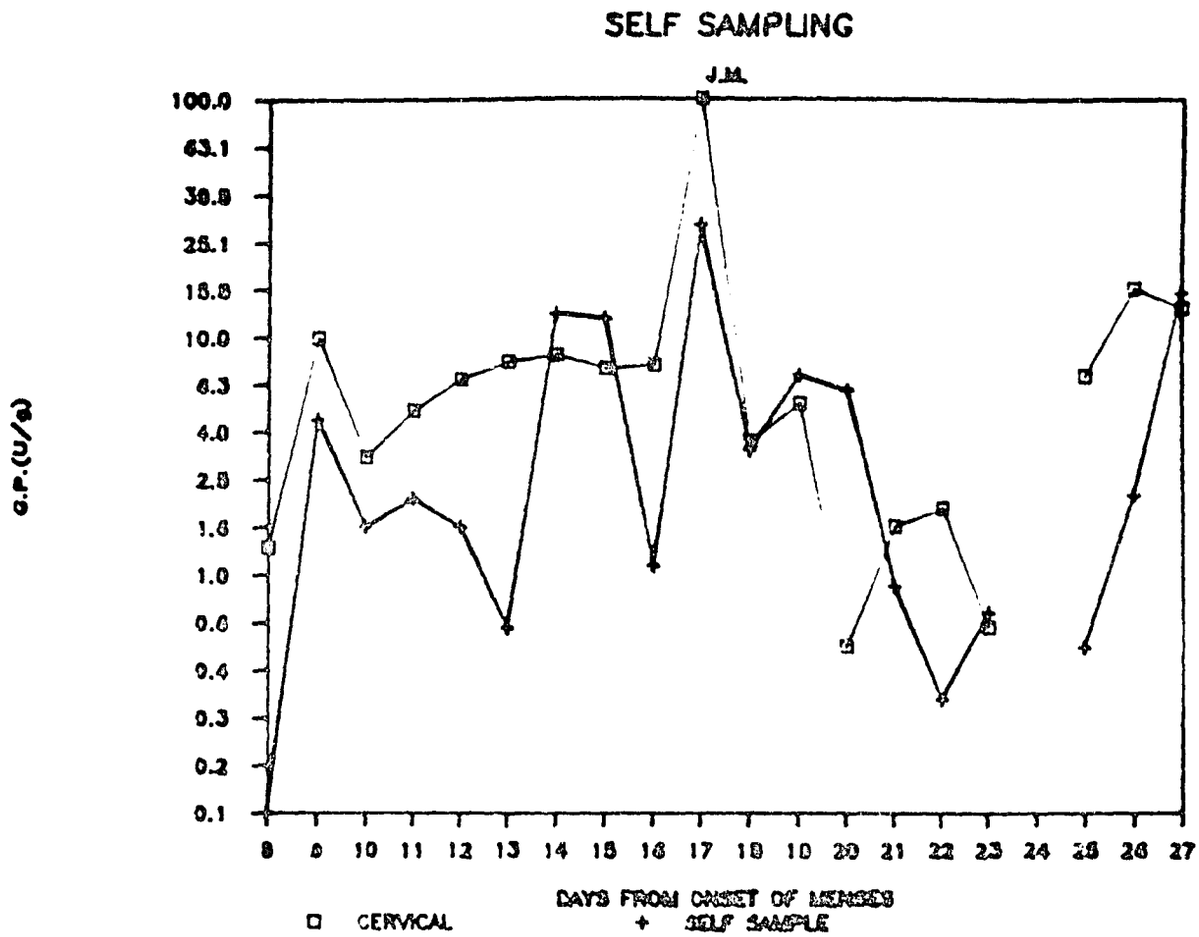
NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
W.N.	2/15/86	5	6.0	7.6	0.022	0.022	
	2/16/86	6	66.5	3.4	0.026	0.009	
	2/17/86	7	3.0	0.4	0.038	0.012	
	2/18/86	8	1.0	1.0	0.040	0.021	
	2/19/86	9	1.9	0.9	0.033	0.031	
	2/20/86	10	0.0	0.5	0.049	0.046	
	2/21/86	11	0.8	0.0	0.050	0.034	
	2/22/86	12	0.0	0.0	0.033	0.078	
	2/23/86	13	3.3	0.0	0.024	0.014	
	2/24/86	14	11.3	1.1	0.058	0.020	
	2/25/86	15	58.9	27.0	0.045	0.010	
	2/26/86	16	10.0	101.3	0.024	0.009	
	2/27/86	17	139.3	38.2	0.020	0.004	
	2/28/86	18	125.3	28.4	0.013	0.002	
	3/1/86	19	541.1	69.5	0.031	0.006	
	3/2/86	20	6.8	67.7	0.024	0.014	
	3/3/86	21	101.0	58.1	0.025	0.004	
	3/4/86	22	68.0	20.0	0.024	0.001	
	3/5/86	23	128.4	12.5	0.017	0.006	
	3/6/86	24	68.7	108.3	0.028	0.003	
	3/7/86	25	34.0	162.2	0.024	0.001	


SELF SAMPLING
L.C.
G.P.(U/g)
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

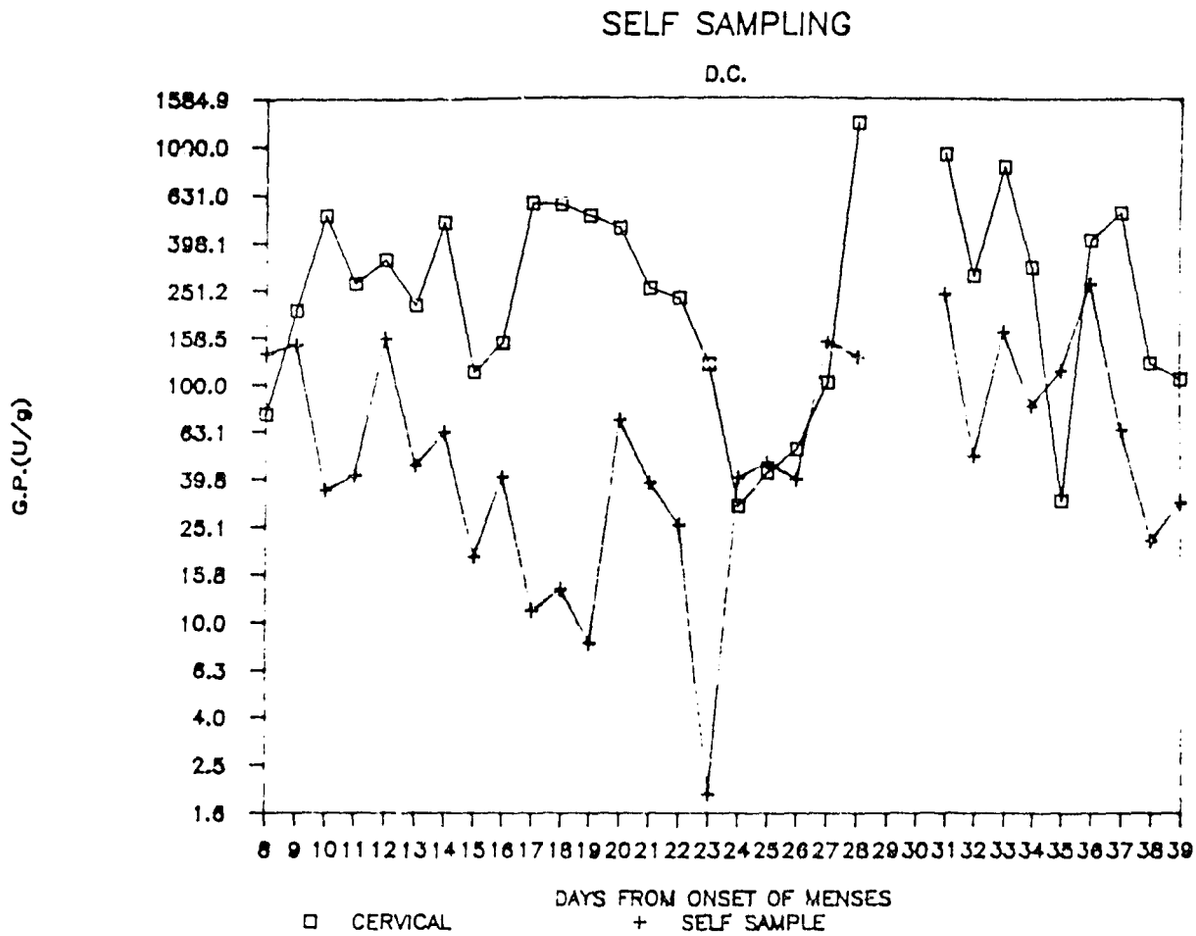
311	NAME	DATE	DFOM	GPC	GFS	SELF-WT	CX-WT	REMARKS
312								
313	L.C	2/18/86	5	46.4	61.7	0.072	0.226	TAKING CLO
314		2/19/86	6	54.6	77.7	0.076	0.176	+ PENNICILLI
315		2/20/86	7	76.1	35.6	0.042	0.117	
316		2/21/86	8	128.9	136.4	0.037	0.113	EXTRA CX=50
317		2/22/86	9	24.0	195.3	0.027	0.054	
318		2/23/86	10	111.6	116.3	0.050	0.107	
319		2/24/86	11	66.7	125.5	0.039	0.067	
320		2/25/86	12	77.6	141.3	0.047	0.048	
321		2/26/86	13	56.3	72.4	0.051	0.087	
322		2/27/86	14	21.4	23.7	0.092	0.022	
323		2/28/86	15	32.6	16.8	0.042	0.113	
324		3/1/86	16	43.6	13.6	0.052	0.132	
325		3/2/86	17	4.9	3.8	0.115	0.249	
326		3/3/86	18	1.9	12.2	0.072	0.089	
327		3/4/86	19	2.3	10.4	0.051	0.137	
328		3/5/86	20	9.1	27.8	0.157	0.025	
329		3/6/86	21	170.3	74.1	0.189	0.052	
330		3/7/86	22	70.5	146.6	0.221	0.026	
331		3/8/86	23	95.1	66.8	0.068	0.038	
332		3/9/86	24	122.8	81.8	0.047	0.017	
333		3/10/86	25	277.8	125.8	0.071	0.048	
334		3/11/86	26	175.6	14.2	0.066	0.029	
335		3/12/86	27	36.3	___		0.022	SELF MISSING
336		3/13/86	28	133.0	17.5	0.059	0.019	
337		3/14/86	29	346.7	116.0	0.067	0.057	
338		3/15/86	30	228.7	268.1	0.028	0.045	
339		3/16/86	31	229.6	206.5	0.078	0.079	
340								


SELF SAMPLING
D.S.
G.P.(U/g)
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
0.6
0.4
0.3
0.2
0.1
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

264	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
265								
266	D.S.	2/20/86	6	25.5	22.4	0.052	0.017	
267		2/21/86	7	10.1	1.4	0.092	0.020	
268		2/22/86	8	12.7	0.7	0.091	0.012	
269		2/23/86	9	3.2	0.0	0.266	0.020	
270		2/24/86	10	2.1	0.0	0.021	0.068	
271		2/25/86	11	0.0	0.0	0.025	0.332	
272		2/26/86	12	0.2	0.0	0.088	0.163	
273		2/27/86	13	0.2	0.0	0.125	0.113	
274		2/28/86	14	0.0	0.0	0.035	0.500	
275		3/1/86	15	2.9	0.0	0.049	0.241	
276		3/2/86	16	48.7	0.0	0.036	0.026	
277		3/3/86	17	74.3	0.0	0.052	0.029	
278		3/4/86	18	105.3	0.0	0.027	0.025	
279		3/5/86	19	157.4	0.0	0.037	0.018	
280		3/6/86	20	6.2	0.0	0.043	0.121	
281		3/7/86	21	111.3	0.0	0.037	0.027	
282		3/8/86	22	94.6	0.0	0.034	0.033	
283		3/9/86	23	66.8	0.0	0.082	0.054	
284		3/10/86	24	130.1	0.0	0.044	0.027	
285		3/11/86	25	48.0	0.0	0.042	0.032	
286		3/12/86	26	154.5	0.0		0.023	
287								


SELF SAMPLING
J.M.
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
0.6
0.4
0.3
0.2
0.1
G.P.(U/g)
8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

288	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARK:
289								
290	J.M.	3/5/86	8	1.3	0.0	0.037	0.022	
291		3/6/86	9	9.9	4.5	0.106	0.102	
292		3/7/86	10	3.1	1.6	0.025	0.089	
293		3/8/86	11	4.9	2.1	0.042	0.051	
294		3/9/86	12	6.7	1.6	0.056	0.085	
295		3/10/86	13	7.9	0.6	0.039	0.035	
296		3/11/86	14	8.4	12.7	0.130	0.057	
297		3/12/86	15	7.4	12.1	0.124	0.046	
298		3/13/86	16	7.7	1.1	0.101	0.041	EXTRA CX=5.5
299		3/14/86	17	99.6	30.2	0.133	0.045	
300		3/15/86	18	3.7	3.4	0.043	0.178	
301		3/16/86	19	5.3	7.0	0.130	0.218	
302		3/17/86	20	0.5	6.0	0.120	0.073	
303		3/18/86	21	1.6	0.9	0.152	0.232	
304		3/19/86	22	1.9	0.3	0.142	0.039	
305		3/20/86	23	0.6	0.7	0.055	0.160	
306		3/21/86	24	2.7	1.7	0.101	0.085	
307		3/22/86	25	7.0	0.5	0.159	0.071	
308		3/23/86	26	16.3	2.2	0.108	0.045	
309		3/24/86	27	13.5	15.8	0.071	0.134	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G.P.(U/g)
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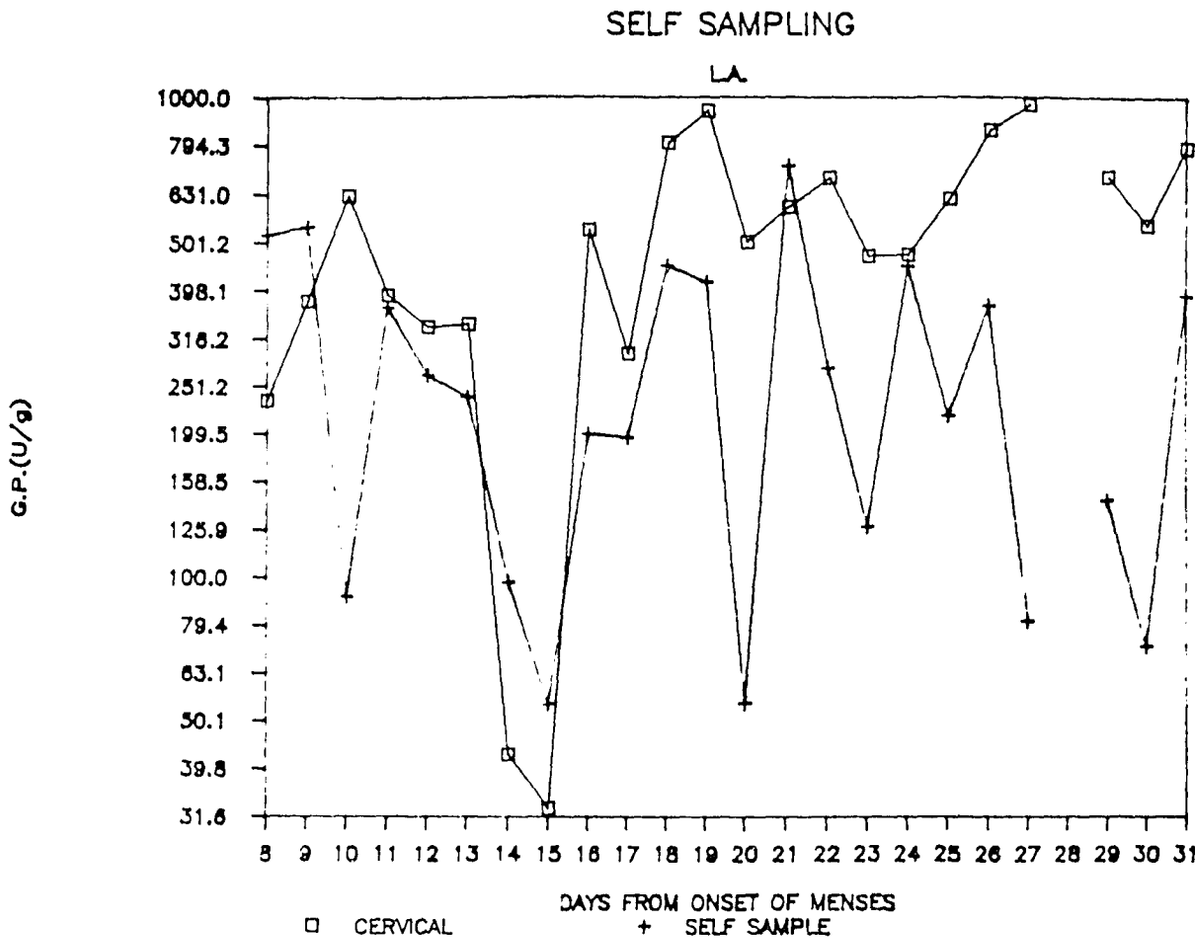
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
343	D.C.	3/13/86	8	75.1	136.0	0.010	0.013	
344		3/14/86	9	207.3	148.7	0.023	0.011	
345		3/15/86	10	521.0	35.9	0.017	0.026	
346		3/16/86	11	270.3	41.6	0.024	0.022	
347		3/17/86	12	339.6	157.7	0.035	0.030	
348		3/18/86	13	219.3	45.6	0.018	0.010	
349		3/19/86	14	486.2	63.5	0.024	0.015	
350		3/20/86	15	113.9	18.9	0.054	0.012	
351		3/21/86	16	151.8	41.1	0.020	0.011	
352		3/22/86	17	585.6	11.3	0.048	0.024	
353		3/23/86	18	577.8	13.9	0.027	0.057	
354		3/24/86	19	516.4	8.2	0.019	0.112	
355		3/25/86	20	460.2	71.3	0.035	0.035	
356		3/26/86	21	256.4	38.8	0.036	0.022	
357		3/27/86	22	235.3	25.7	0.034	0.165	
358		3/28/86	23	123.7	1.9	0.128	0.128	
359		3/29/86	24	31.2	40.9	0.149	0.144	
360		3/30/86	25	43.2	47.7	0.057	0.110	
361		3/31/86	26	54.2	40.4	0.027	0.053	
362		4/1/86	27	103.0	153.1	0.034	0.019	
363		4/2/86	28	1253.3	131.9	0.019	0.013	
364		4/3/86	29	536.7	388.3	0.050	0.006	
365		4/4/86	30	119.7	225.9	0.051	0.008	
366		4/5/86	31	936.5	245.7	0.033	0.017	
367		4/6/86	32	294.7	50.8	0.034	0.010	
368		4/7/86	33	831.4	171.2	0.017	0.007	
[illegible]		4/8/86	34	320.5	83.1	0.030	0.013	
		4/9/86	35	32.9	117.9	0.044	0.012	
		4/10/86	36	415.5	271.9	0.033	0.031	
372		4/11/86	37	538.9	65.8	0.010	0.024	
373		4/12/86	38	126.7	22.3	0.029	0.015	
374		4/13/86	39	108.1	32.3	0.024	0.019	
375								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G.P.(U/g)
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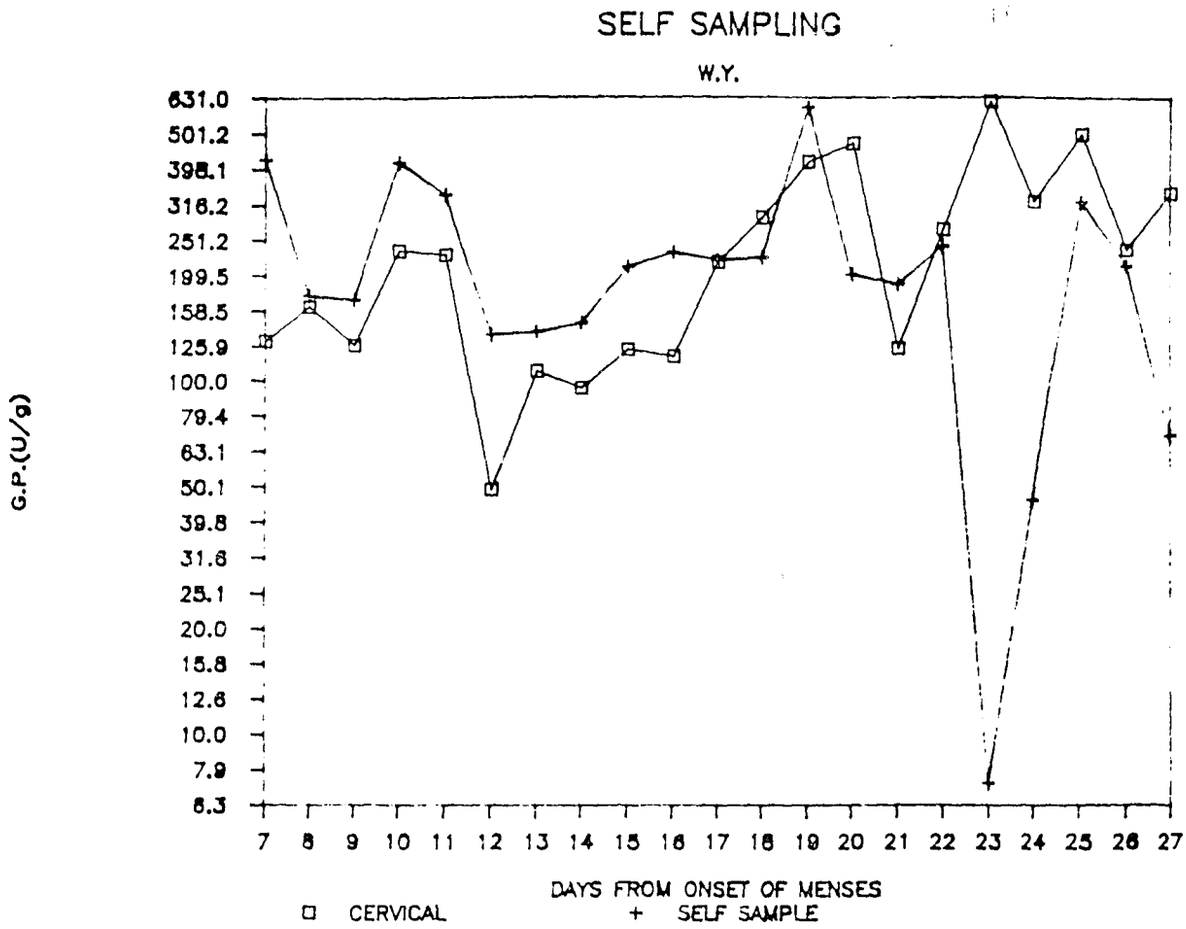
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

376	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
77								
78	L.A.	3/15/86	8	234.7	521.1	0.024	0.085	
79		3/16/86	9	379.0	540.7	0.036	0.121	
30		3/17/86	10	626.6	91.4	0.024	0.044	
381		3/18/86	11	391.1	368.1	0.036	0.033	
382		3/19/86	12	335.2	265.3	0.020	0.070	
383		3/20/86	13	340.2	238.7	0.010	0.095	
384		3/21/86	14	42.7	97.9	0.024	0.160	
385		3/22/86	15	33.0	54.4	0.658	0.033	
386		3/23/86	16	532.3	200.0	0.108	0.039	
387		3/24/86	17	293.7	196.0	0.080	0.004	
388		3/25/86	18	805.3	448.6	0.007	0.025	
389		3/26/86	19	938.3	414.4	0.012	0.027	
390		3/27/86	20	501.8	54.7	0.006	0.044	
391		3/28/86	21	592.6	721.0	0.007	0.027	
392		3/29/86	22	680.8	274.4	0.009	0.017	
393		3/30/86	23	470.1	127.8	0.018	0.023	
394		3/31/86	24	474.5	448.3	0.012	0.039	
395		4/1/86	25	619.1	218.6	0.014	0.014	
396		4/2/86	26	854.5	371.7	0.023	0.028	
397		4/3/86	27	965.3	81.1	0.007	0.025	
398		4/4/86	28	331.1	413.3	0.008	0.018	
399		4/5/86	29	684.2	145.3	0.014	0.064	
400		4/6/86	30	543.8	71.8	0.011	0.102	
401		4/7/86	31	787.7	388.0	0.021	0.039	
402								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398.1
316.2
251.2
199.5
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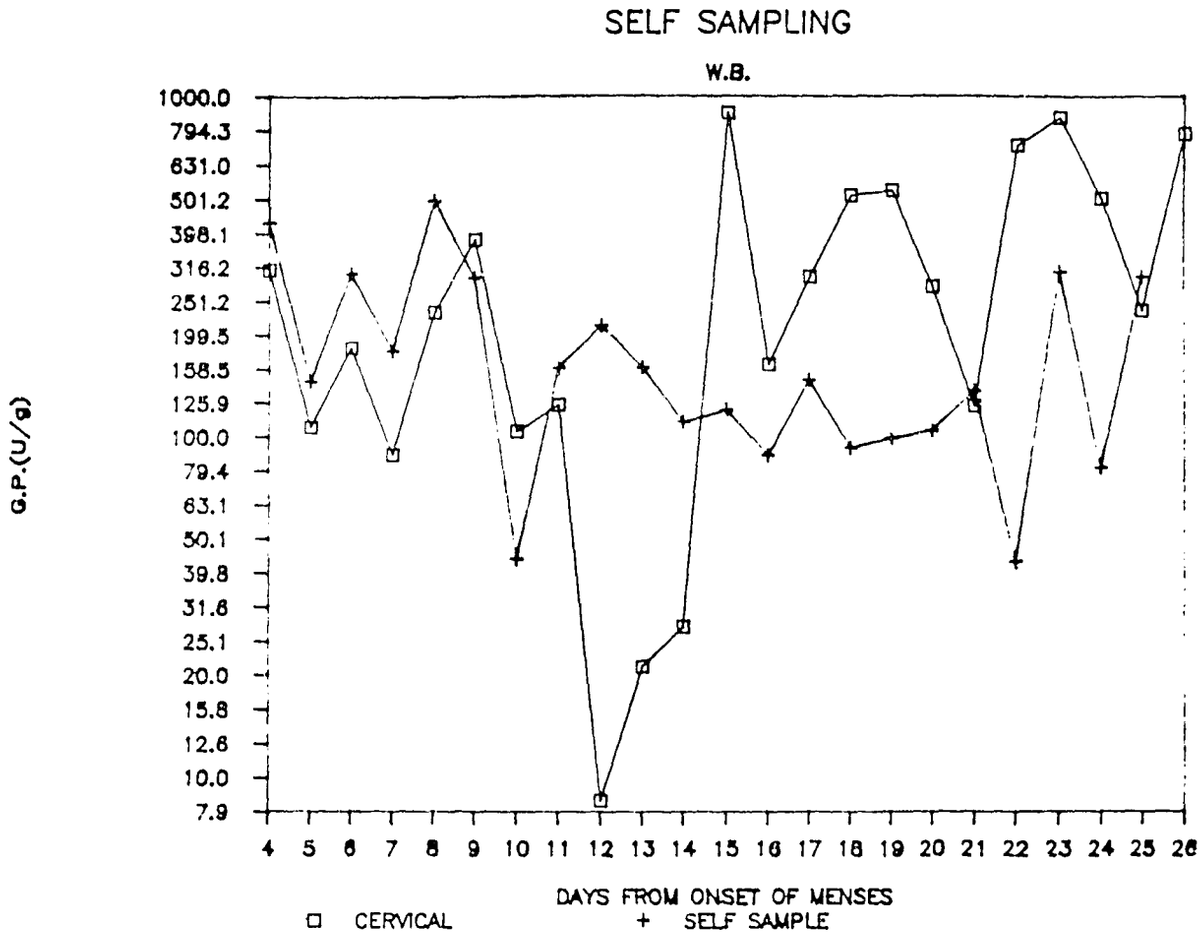
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
7.9
6.3
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
W.Y.	7/3/86	7	130.1	424.2	0.051	0.024	
	7/4/86	8	163.5	175.6	0.082	0.022	
	7/5/86	9	127.0	171.2	0.075	0.019	
	7/6/86	10	235.8	417.4	0.161	0.016	
	7/7/86	11	228.7	337.4	0.181	0.021	
	7/8/86	12	49.3	135.9	0.111	0.024	EXTRA CX=60.
	7/9/86	13	106.9	138.5	0.035	0.079	
	7/10/86	14	95.8	146.9	0.081	0.013	
	7/11/86	15	123.1	212.0	0.036	0.028	
	7/12/86	16	117.6	232.2	0.113	0.033	
	7/13/86	17	218.7	221.5	0.019	0.021	
	7/14/86	18	291.1	225.5	0.032	0.034	
	7/15/86	19	415.5	593.2	0.018	0.033	
	7/16/86	20	468.6	200.9	0.015	0.073	
	7/17/86	21	124.7	189.9	0.033	0.036	
	7/18/86	22	271.2	244.6	0.037	0.022	
	7/19/86	23	614.6	7.3	0.018	0.021	
	7/20/86	24	321.7	46.4	0.087	0.016	
	7/21/86	25	496.4	319.4	0.033	0.044	
	7/22/86	26	236.2	211.6	0.075	0.018	
	7/23/86	27	340.3	70.4	0.033	0.022	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W.B.
G.P.(U/g)
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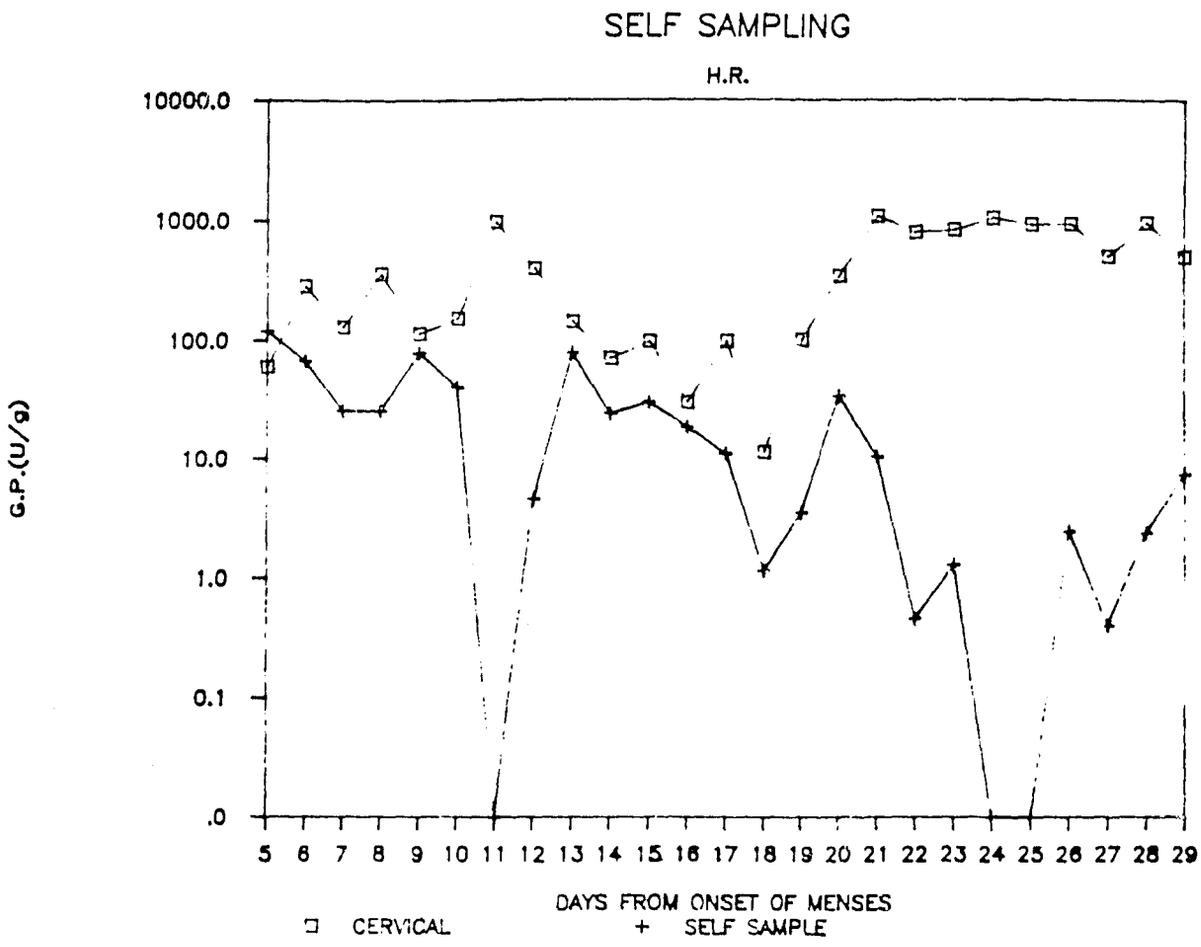
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
7.9
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

427	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
428								
429	W.B.	7/9/86	4	310.9	428.9	0.039	0.007	
430		7/10/86	5	106.7	145.9	0.034	0.017	
431		7/11/86	6	183.1	304.3	0.029	0.024	
432		7/12/86	7	88.3	179.9	0.029	0.019	
433		7/13/86	8	233.9	499.2	0.060	0.017	
434		7/14/86	9	381.3	295.1	0.036	0.032	
435		7/15/86	10	103.7	44.0	0.012	0.043	
436		7/16/86	11	124.1	159.8	0.066	0.140	
437		7/17/86	12	8.5	214.0	0.115	0.200	
438		7/18/86	13	21.1	160.6	0.146	0.279	
439		7/19/86	14	27.8	111.3	0.019	0.187	
440		7/20/86	15	889.4	120.7	0.081	0.130	
441		7/21/86	16	164.1	88.9	0.020	0.030	
442		7/22/86	17	295.2	147.7	0.071	0.040	
443		7/23/86	18	512.0	93.1	0.025	0.021	
444		7/24/86	19	529.9	99.4	0.046	0.044	
445		7/25/86	20	278.0	104.8	0.048	0.016	
446		7/26/86	21	123.9	138.2	0.032	0.028	
447		7/27/86	22	716.9	43.2	0.020	0.043	
448		7/28/86	23	863.0	305.9	0.026	0.011	
449		7/29/86	24	502.8	81.6	0.031	0.031	
450		7/30/86	25	235.6	297.5	0.037	0.017	
451		7/31/86	26	774.3	365.7	0.042	0.028	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1.0
0.1
.0
G.P.(U/g)
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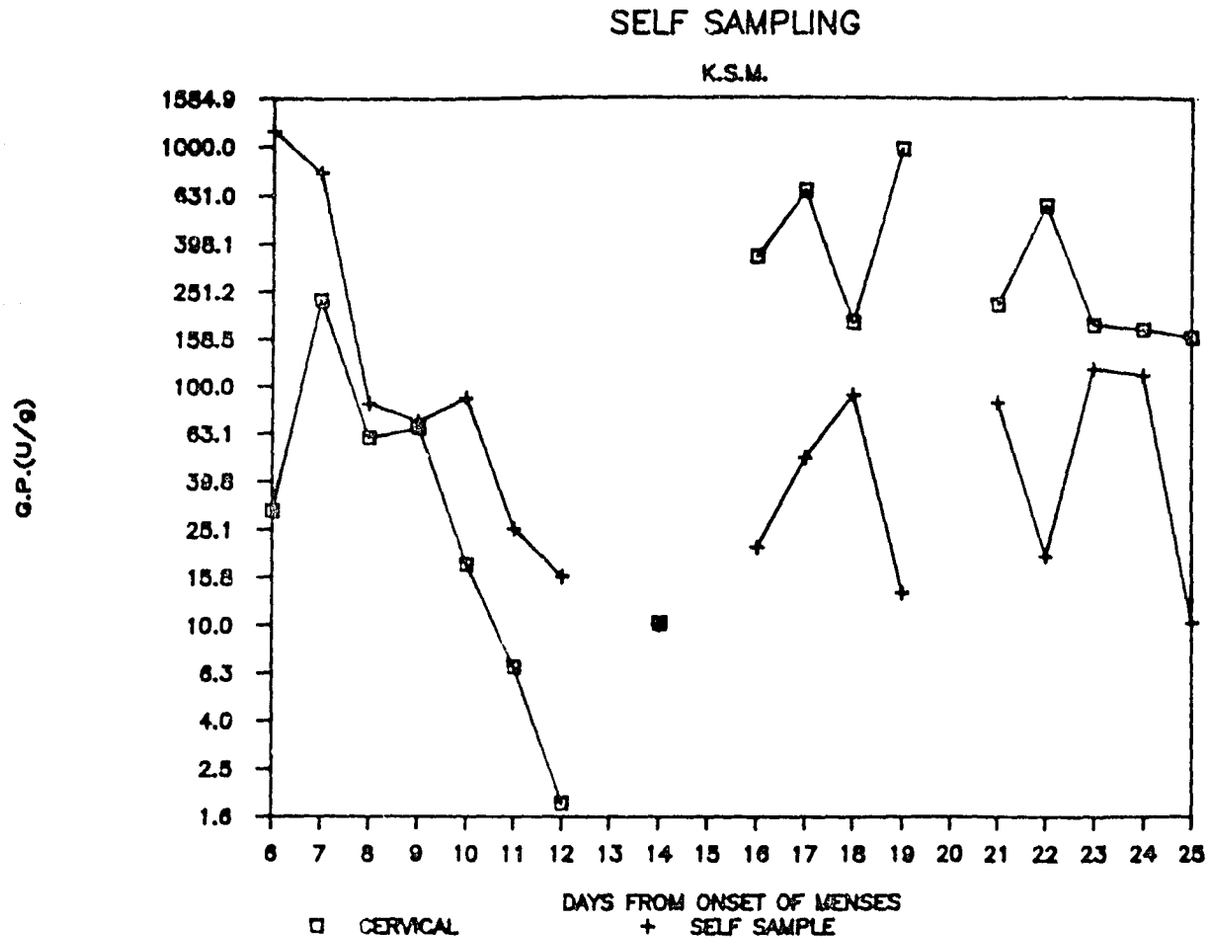
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

453	NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
454								
455	H.R.	7/13/86	5	59.4	120.1	0.019	0.014	
456		7/14/86	6	282.4	66.2	0.011	0.019	
457		7/15/86	7	126.4	25.2	0.012	0.015	
458		7/16/86	8	355.6	25.0	0.013	0.018	
459		7/17/86	9	111.7	77.4	0.053	0.028	
460		7/18/86	10	151.0	39.7	0.058	0.035	
461		7/19/86	11	956.7	0.0	0.008	0.008	
462		7/20/86	12	397.6	4.6	0.074	0.105	
463		7/21/86	13	141.1	78.5	0.020	0.040	
464		7/22/86	14	70.2	24.2	0.064	0.061	
465		7/23/86	15	97.2	30.3	0.068	0.149	
466		7/24/86	16	30.4	18.8	0.107	0.129	
467		7/25/86	17	97.9	11.2	0.083	0.204	
468		7/26/86	18	11.5	1.2	0.091	0.176	
469		7/27/86	19	99.4	3.6	0.040	0.073	
470		7/28/86	20	342.2	33.9	0.017	0.012	
471		7/29/86	21	1085.8	10.4	0.029	0.012	
472		7/30/86	22	795.6	0.5	0.028	0.015	
473		7/31/86	23	835.5	1.3	0.009	0.012	
474		8/01/86	24	1037.3	0.0	0.011	0.010	
475		8/02/86	25	916.2	0.0	0.013	0.014	
476		8/03/86	26	917.3	2.5	0.009	0.010	
477		8/04/86	27	501.7	0.4	0.009	0.027	
478		8/05/86	28	942.9	2.4	0.005	0.014	
479		8/06/86	29	501.0	7.4	0.007	0.012	
480								


SELF SAMPLING
K.S.M.
G.P.(U/g)
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1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
DAYS FROM ONSET OF MENSES
CERVICAL
SELF SAMPLE

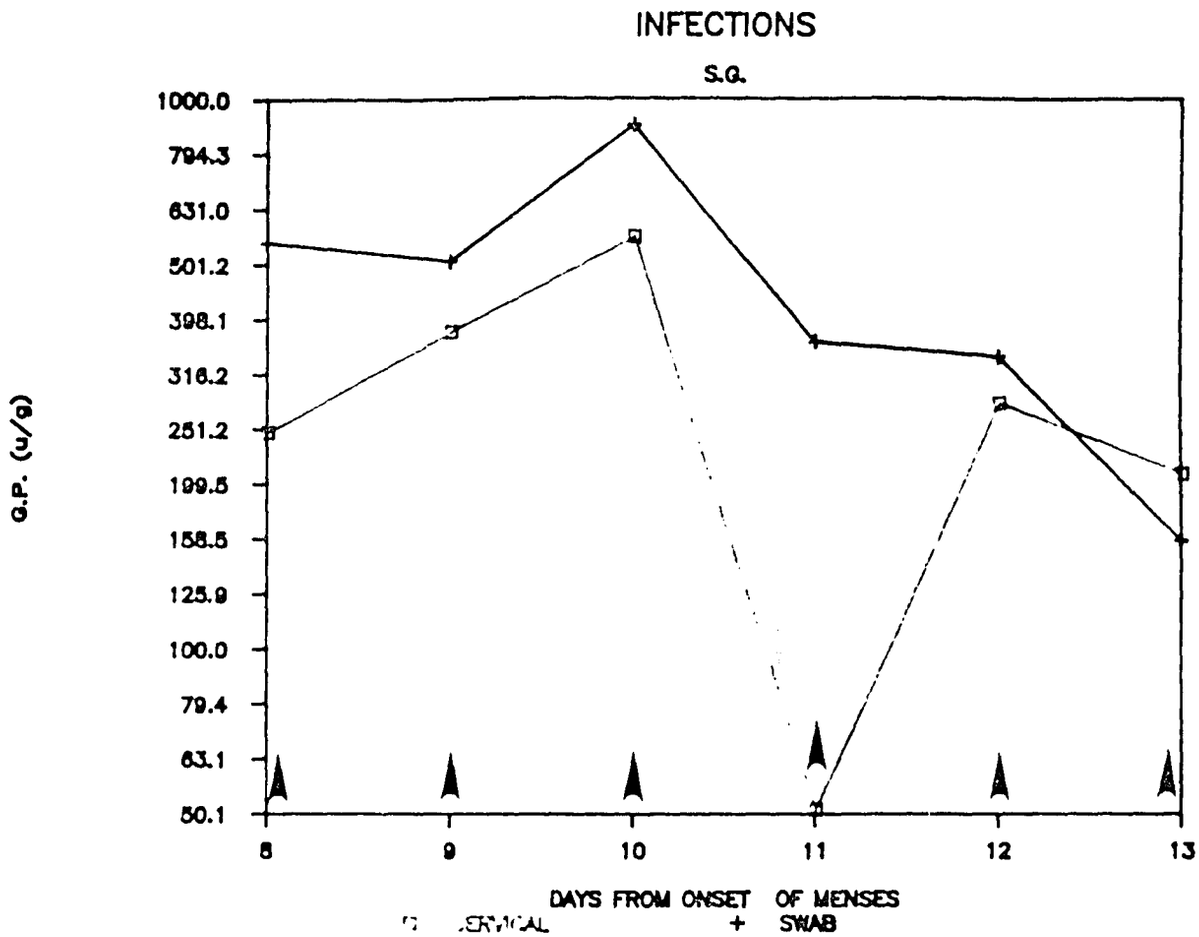
NAME	DATE	DFOM	GPC	GPS	SELF-WT	CX-WT	REMARKS
K.S.M.	8/27/86	6	30.0	1160.0	0.011	0.020	
	8/28/86	7	230.4	779.4	0.022	0.005	
	8/29/86	8	60.7	84.3	0.020	0.035	
	8/30/86	9	66.7	71.3	0.033	0.037	
	8/31/86	10	17.9	89.2	0.039	0.048	
	9/1/86	11	6.7	25.3	0.045	0.100	
	9/2/86	12	1.8	16.0	0.071	0.127	
	9/3/86	13					NO SAMPLE
	9/4/86	14	10.2	10.1	0.033	0.044	
	9/5/86	15					NO SHOW
	9/6/86	16	351.6	21.2	0.046	0.019	
	9/7/86	17	655.5	50.3	0.051	0.024	
	9/8/86	18	185.3	92.0	0.040	0.020	
	9/9/86	19	970.3	13.7	0.043	0.026	
	9/10/86	20					NO SHOW
	9/11/86	21	220.9	85.7	0.052	0.030	
	9/12/86	22	573.0	19.5	0.040	0.035	
	9/13/86	23	182.9	120.1	0.043	0.025	
	9/14/86	24	174.2	112.3	0.045	0.022	
	9/15/86	25	160.0	10.2	0.035	0.043	

APPENDIX 4

B. Infection study

PARFR-360 1985-1986

Name	Date	Infection	Medication
S.G.	3/7/86-3/13	Yeast	Vinegar douche x 7 days
S.S.	3/21/86-3/27	Yeast	Femstat 15 g x 3 days
J.G.	3/26/86-4/1	Gardnerella	Flagyl x 7 days
C.V.	4/8/86-4/14	Gardnerella	None
D.S.	6/10/86-6/16	Yeast	Monistat
G.G.	6/18/86-6/24	Trichomonas	Flagyl 500 mg x 1 day
M.M.	6/18/86-6/24	Yeast	None
G.F.	8/5/86-8/11	Gonorrhea	Ampicillin 300 g + 2 Benamid tablets x 1 day
J.H.	8/8/86-8/14	Yeast (in cervical scrapings)	Monistat x 3 days
T.C.	8/11/86-8/17	Yeast	None
P.H.	8/14/86-8/20	Trichomonas + possible gardnerella	None
R.J.	8/19/86-8/25	Gardnerella	None
J.R.	9/4/86-9/10	Gardnerella	Flagyl 500 mg x 5 days
A.B.Y.	9/8/86-9/12	Gardnerella	Flagyl 250 mg x 7 days
V.C.	9/15/86-9/21	Gardnerella	Flagyl 500 mg x 5 days
N.W.	9/15/86-9/21	Gardnerella	Flagyl 500 m g x 5 days
J.N.N.	9/18/86-9/24	Yeast	Monistat vag. cream x 7 days
A.H.	9/22/86-9/28	Trichomonas	Flagyl 500 mg x 5 days
A.B.C.	9/24/86-9/30/86	Gardnerella	Flagyl 500 mg x 5 days
T.S.	9/25/86-10/1/86	Yeast	Clotrimazole vag. cream x 7 days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1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
G.P. (u/g)
8
9
10
11
12
13
DAYS FROM ONSET OF MENSES
CERVICAL
+ SWAB

SL AGE 31 G3 P3

LMP 2/28/86 Frequency 30 Days Lasts 5 days

Problems with Menses NONE

BC Method condoms ~~Number of partners~~ _____

Gyn Hx July '85 - removed cervical polyps. Presently has Gardenerella inf
and had endometritis in 1982 after 3rd baby. 1 year ago - yeast infec

Med Dis Hx Cyst was removed from right breast 7 years ago.

Medications NONE, But will use vinegar douche (1qt. water a
2 tsp. vinegar) qhs 7 days for infection

Allergies NONE

Habits Cigs NO ETOH occasionally Exercise NO

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ med _____ low _____

lacto _____ WBC sm ___ med ____ large _____

bact sm _____ med _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

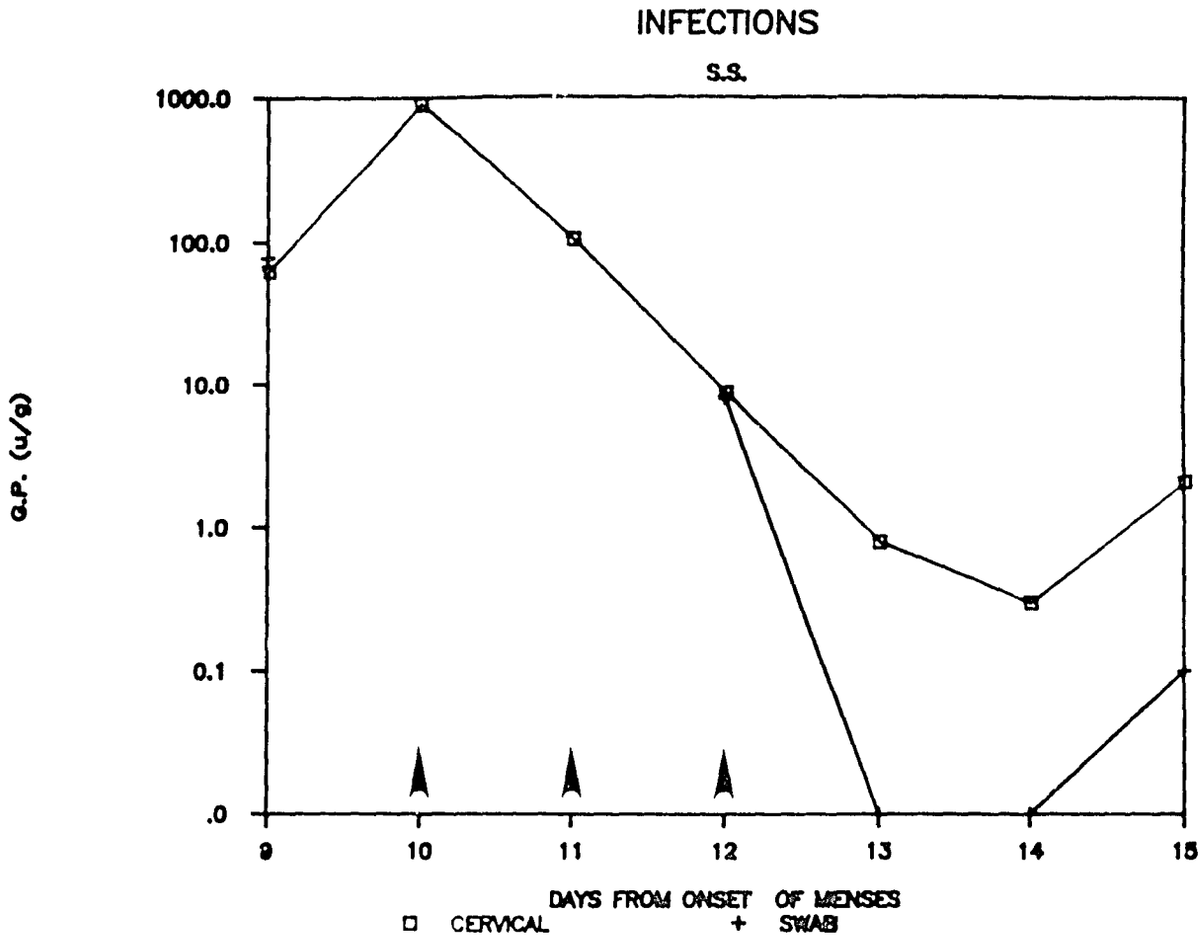
Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____


INFECTIONS
S.S.
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100.0
10.0
1.0
0.1
.0
G.P. (u/g)
9
10
11
12
13
14
15
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

S.S. AGE 37 G2 P0

LMP 3/13/86 Frequency 28-30 days Lasts 4-5 days

Problems with Menses [illegible] sometimes during whole period.

BC Method diaphragm ~~Number of partners~~

Gyn Hx NONE IN PAST Presently has yeast infection.

Med Dis Hx Hospitalized for mono hepatitis ✓

Medications Femstat (butoconazole nitrate), approximately 5 grams of cream in vagina at bedtime for 3 days.

Allergies NONE

Habits Cigs [illegible] ETOH [illegible] Exercise 1-2x/week

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ med ____ large _____

bact sm _____ med _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

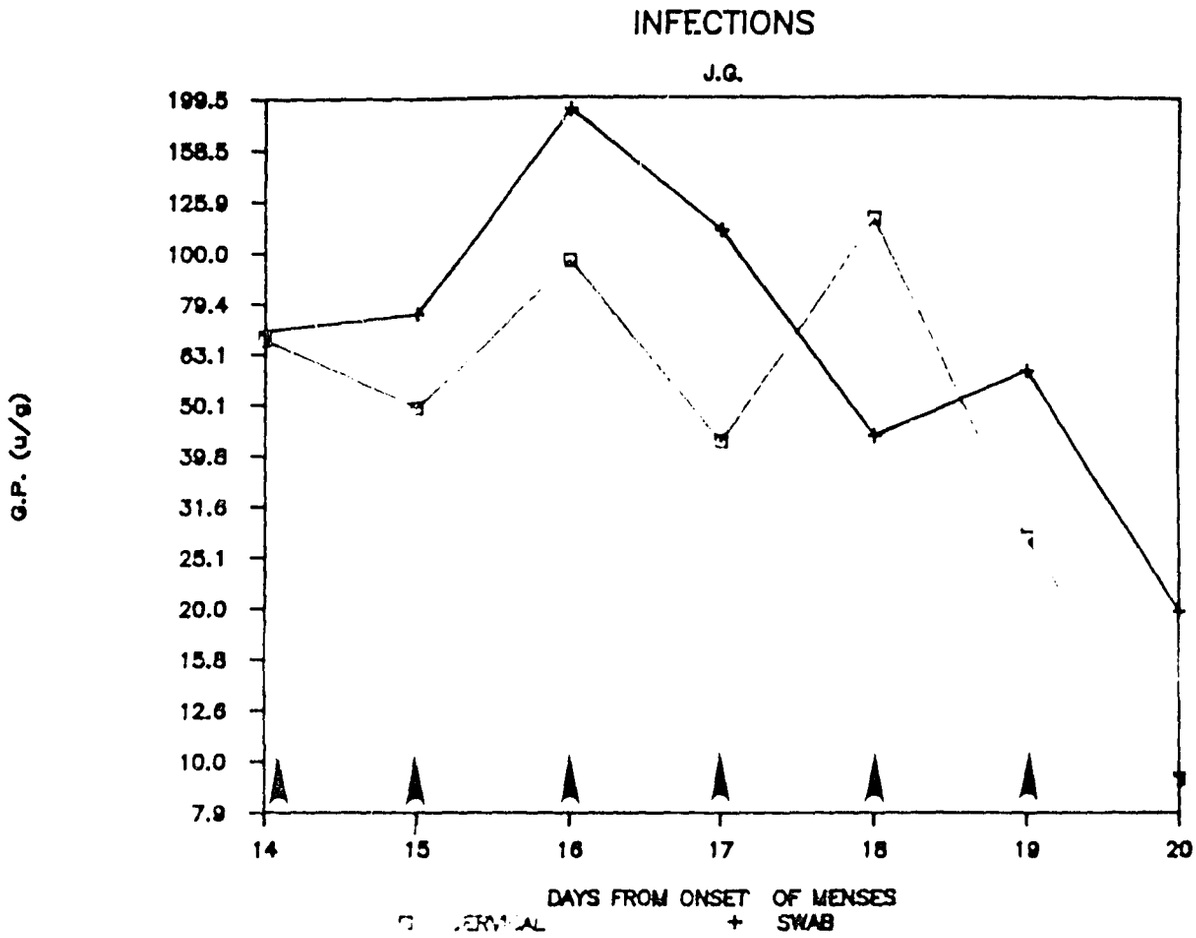
Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____


INFECTIONS
J.Q.
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
7.9
G.P. (u/g)
14
15
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19
20
DAYS FROM ONSET OF MENSES
+ SWAB

J.G. AGE 24 G1 P0

LMP 3/13/86 Frequency 28-31 days Lasts 3-5 days.

Problems with Menses Sometimes she bloats + gets frequency of urination one week before period. always get cramps before per

BC Method abstinence ~~Number of partners~~

Gyn Hx 2 years ago had yeast infection and had urinary tract infection 3 abnormal pap smears in one year. 2 were class 2 + one was.

Med Dis Hx ~~Migraines occasionally~~ 2 years ago hospitalized for migraines (possible aneurysm) but found nothing

Medications None. Will take Flagyl now for 7 days for yeast infection

Allergies most decongestants (nervous reaction) Cannot take motrin (make pain worse), Nervous reaction to Ergotamine tt

Habits Cigs 1/2 ETOH ~~occasional~~ Exercise Karate everyday + yoga. Safeway

Physical - Ext Exam

Bimanual

pH epi clean dry "clue none

rare med low

lacto WBC sm med large

bact sm med large

trich yeast hyphae

Told of : BBT

Collection blood mucus [redacted] tip

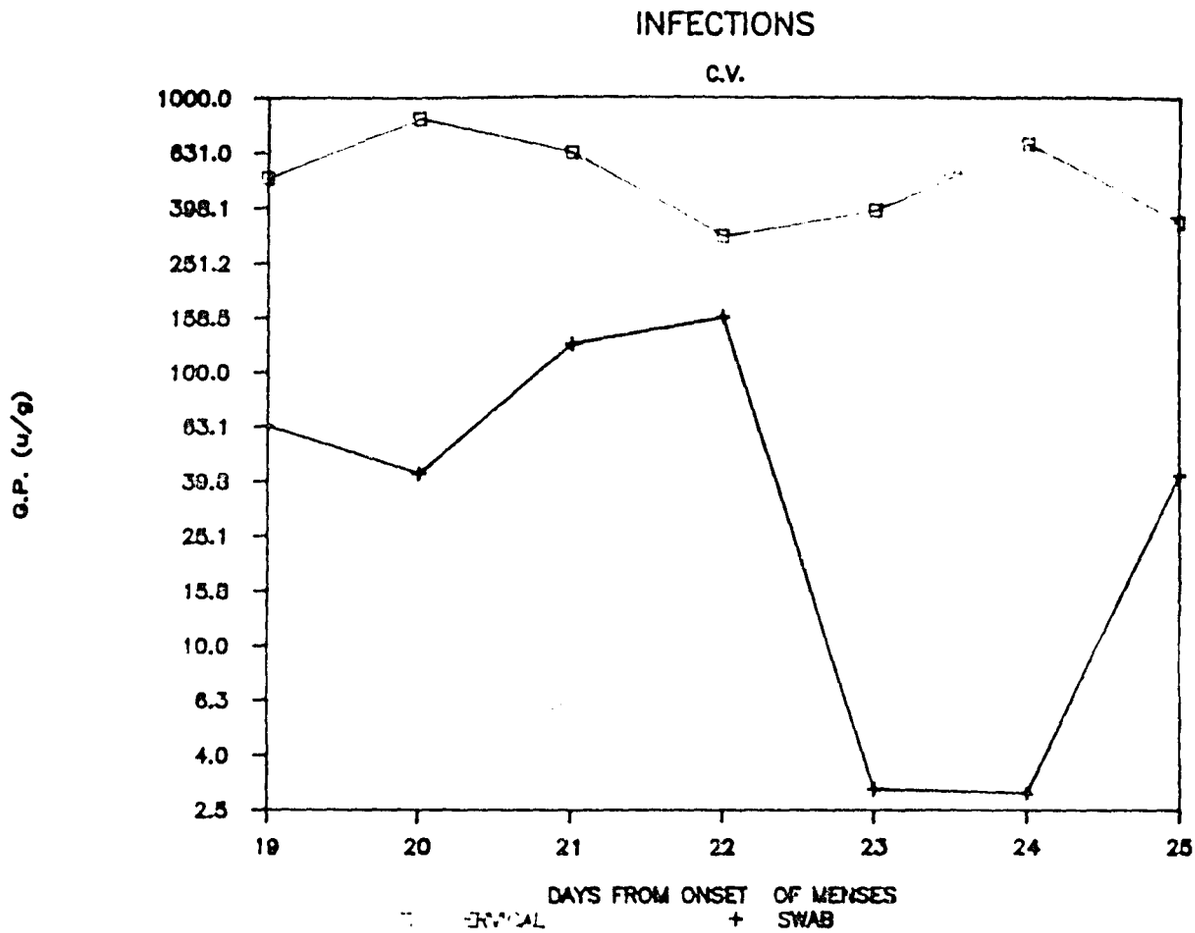
Weekends

BC method

Money Employee

Vag itching risk Blood drawing risk

Permit Signed


INFECTIONS
C.V.
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
G.P. (u/g)
19
20
21
22
23
24
25
DAYS FROM ONSET OF MENSES
+ SWAB

C.V. AGE 25 G1 P0 ___

LMP 3/21/86 Frequency 28 days Lasts 3-4 days

Problems with Menses Severe cramps 1st day of period.

BC Method NONE ~~[illegible]~~ ___

Gyn Hx Had Urinary Tract infection 1½ year ago. Trichomonas ~~[illegible]~~ ~~[illegible]~~ when she was on the Pill. Presently has ~~[illegible]~~ Nonspecific vaginitis.

Med Dis Hx Has hemorrhoids

Medications NONE. Will take Flagyl after next period for inf[illegible]

Allergies NONE

Habits Cigs ~~Occasionally~~ ETOH ~~Occasionally~~ Exercise ~~Occasionally~~ Regularly 2x/week aerobics + jogging

Physical - Ext Exam ___

Bimanual ___

pH ___ epi clean ___ dry ___ "clue none

rare ___ med ___ low ___

lacto ___ WBC sm ___ med ___ large ___

bact sm ___ med ___ large ___

trich ___ yeast ___ hyphae ___

Told of : BBT ___

Collection blood ___ mucus ___ [redacted] tip ___

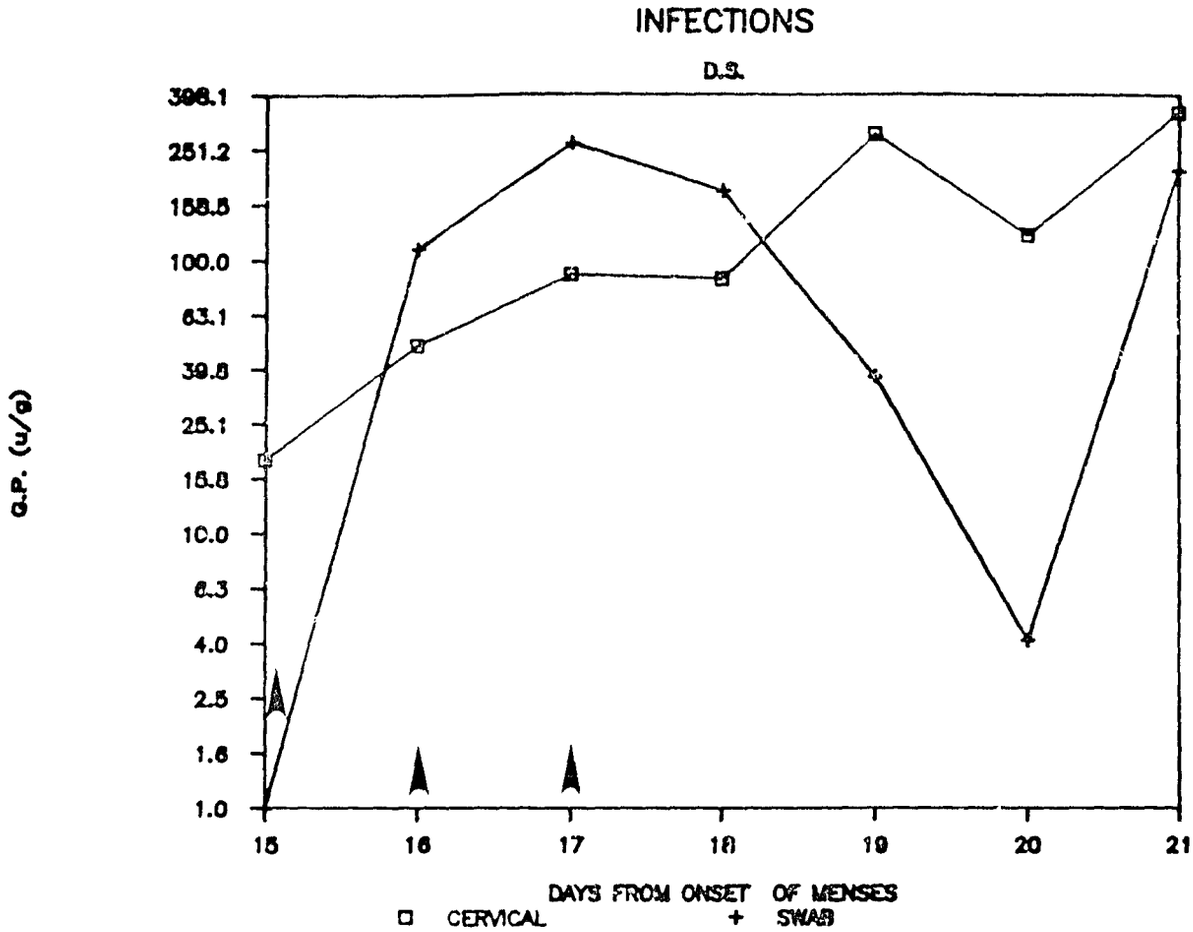
Weekends ___

BC method ___

Money Employee ___

Vag itching risk ___ Blood drawing risk ___

Permit Signed ___


INFECTIONS
D.S.
398.1
251.2
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63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
G.P. (u/g)
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16
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21
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

D.S. AGE 31 G1 P1

LMP 5/27/86 Frequency 22 days ± 1 day Lasts 5 days

Problems with Menses about 4 yrs ago had some heavy bleeding during periods.

BC Method NONE ~~Number of partners~~

Gyn Hx ≈ 1 yeast infection / year since pregnancy. Presently has yeast infection. 4 years ago had Trichomonas.

Med Dis Hx Mother is diabetic

Medications NONE except for present infection - Monistat

Allergies NONE

Habits Cigs NO ETOH NO Exercise NO

Ht. 4'10" wt. 100 lb.

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ med _____ large _____

bact sm _____ mod _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip _____

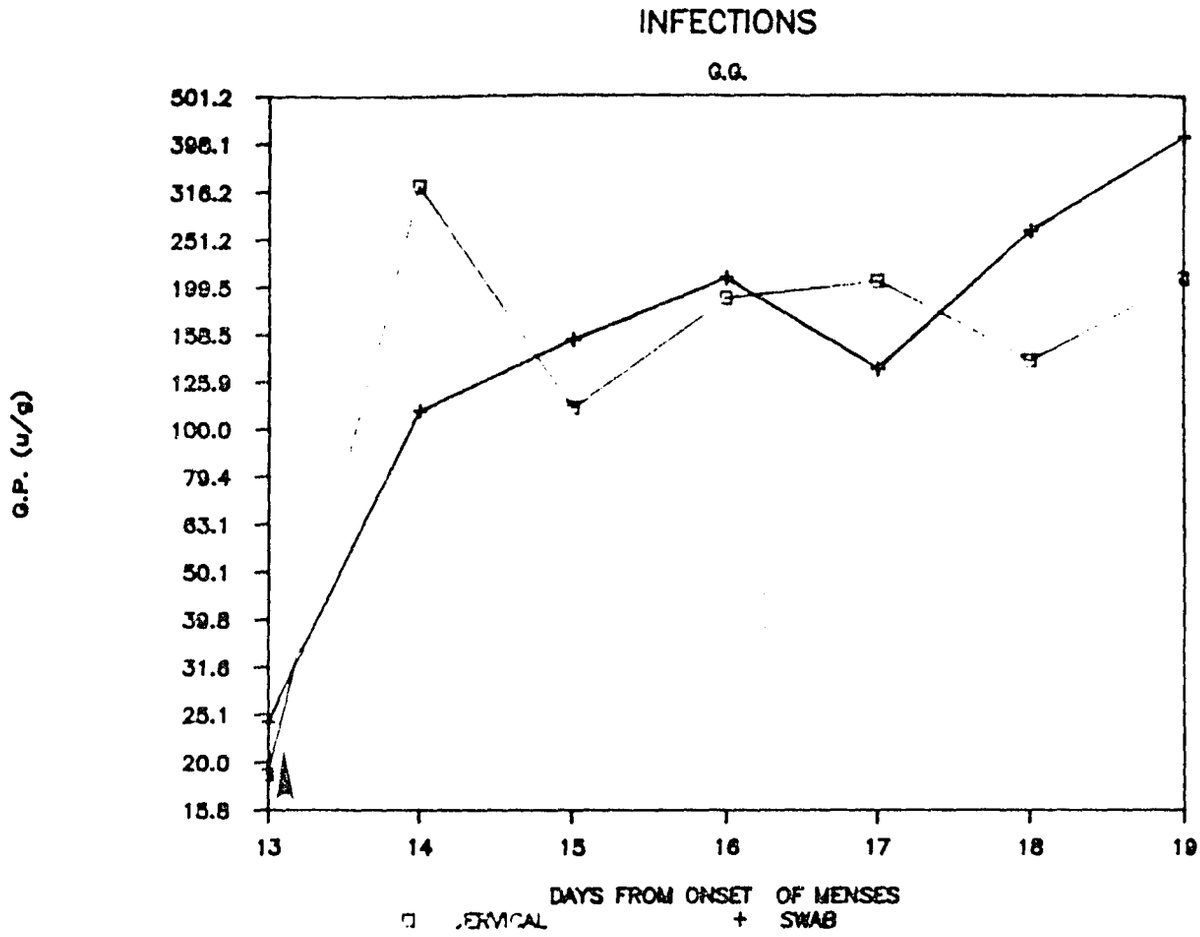
Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____


INFECTIONS
Q.Q.
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
Q.P. (u/g)
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DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

GG. AGE 23 G3 P2 AB1

LMP 6/6/86 Frequency 26 days Lasts 4 days

Problems with Menses moderate-Severe cramps just before period star and lasts for 1-2 days.

BC Method abstinence Number of partners _____

Gyn Hx Trichomonas infection in 1978. Has Trichomonas presently.

Med Dis Hx NONE

Medications NONE except multivitamin 1/day. Will take Flag (500mg) 4 tab/da

Allergies NONE

Habits Cigs NO ETOH NO Exercise 4-6 blocks/day walking

Ht 5'6" wt 145 lbs.

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ clue none

rare _____ med _____ low _____

lacto _____ WBC sm ___ med _____ large _____

bact sm _____ med _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip _____

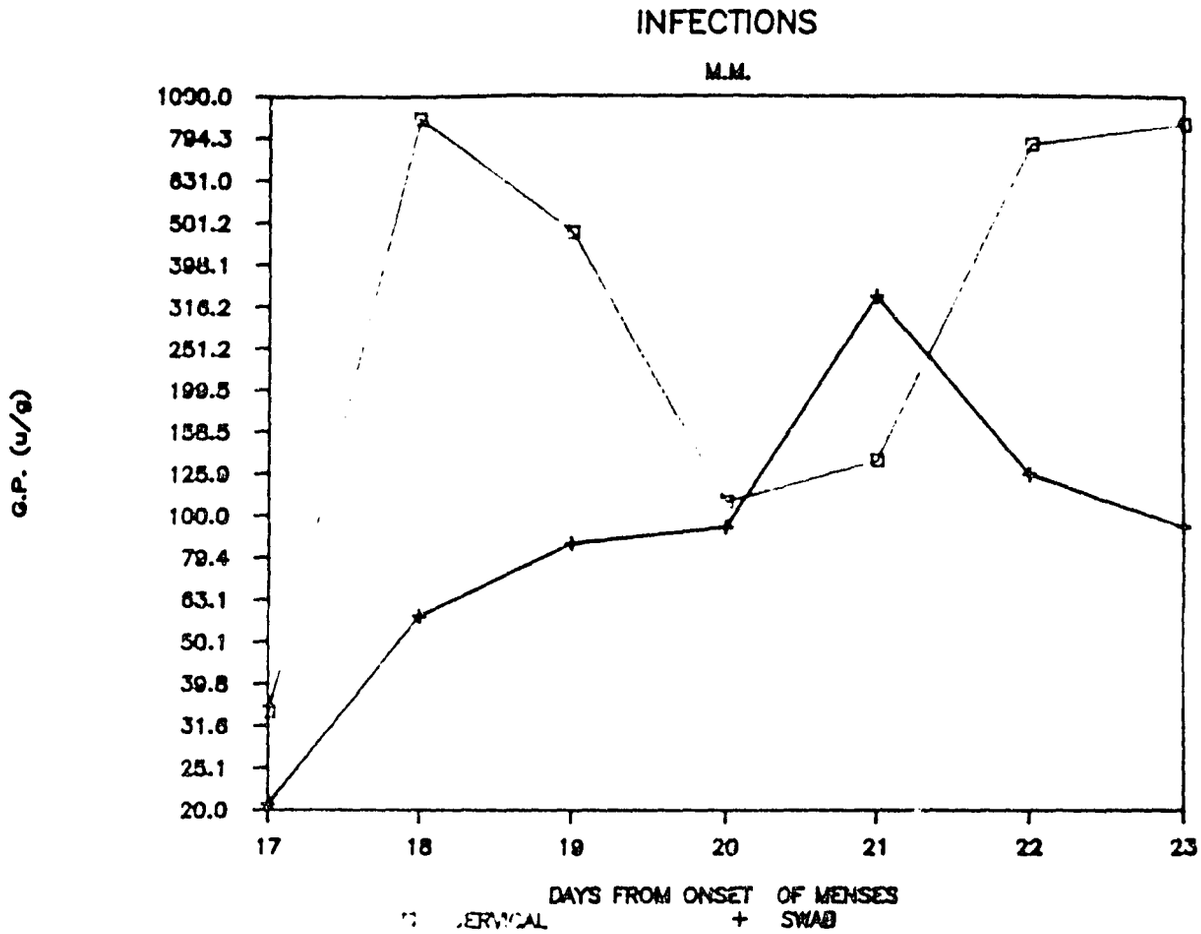
Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____


INFECTIONS
M.M.
G.P. (u/g)
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
17
18
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21
22
23
DAYS FROM ONSET OF MENSES
CERVICAL
+ SWAB

A.L.M. AGE 22 G0 P0

LMP 6/2/86 Frequency 25 days Lasts 5-6 days

Problems with Menses mild-moderate cramps in beginning of period - just before 1st day and Day 1 & 2. Every other per is heavy bleed and more severe c

BC Method diaphragm Number of partners _____

Gyn Hx Yeast infection at age 11 or 12. Has had Gardnerella + nonspecific 2 years ago. It spread to tube.

Med Dis Hx Had tonsils

Medications NONE

Allergies Hayfever, Cats + dogs, pets.

Habits Cigs NO ETOH usually occasionally but not last week. Exercise NO

Ht. 5'7" wt. 130 lb.

Physical - Ext Exam _____

Bimanual _____

pH _____ opi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ mod ____ large _____

bact sm _____ mod _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____

Cook County Hospital
1835 W. Harrison St., Chicago, Illinois 60612 Telephone 312/633 6000

CERTIFICATE OF PROOF OF ILLNESS

TO: _____ Insurance Co. in Lieu of Form _____

Patient's Name M, M Address _____

Date of Birth _____ Sex _____ Race _____ Marital Status _____

Date of Onset Present Illness _____

History of Injury: No. _____ Yes _____ Date _____

Hospitalization: Date _____ To: _____

Clinic Visits: First _____ Last 6/20/86 Next Appt. To Gyne

Final Diagnosis: (1) Condyloma Accuminata
(2) yeast vaginitis

Prognosis: Good

Disability: Total _____ 19 ___ To _____ 19 _____
Partial _____ 19 ___ To _____ 19 _____

Remarks:

Fantus Health Center
Emergency Department

E.C. DASEN PA-C
Clinic Physician

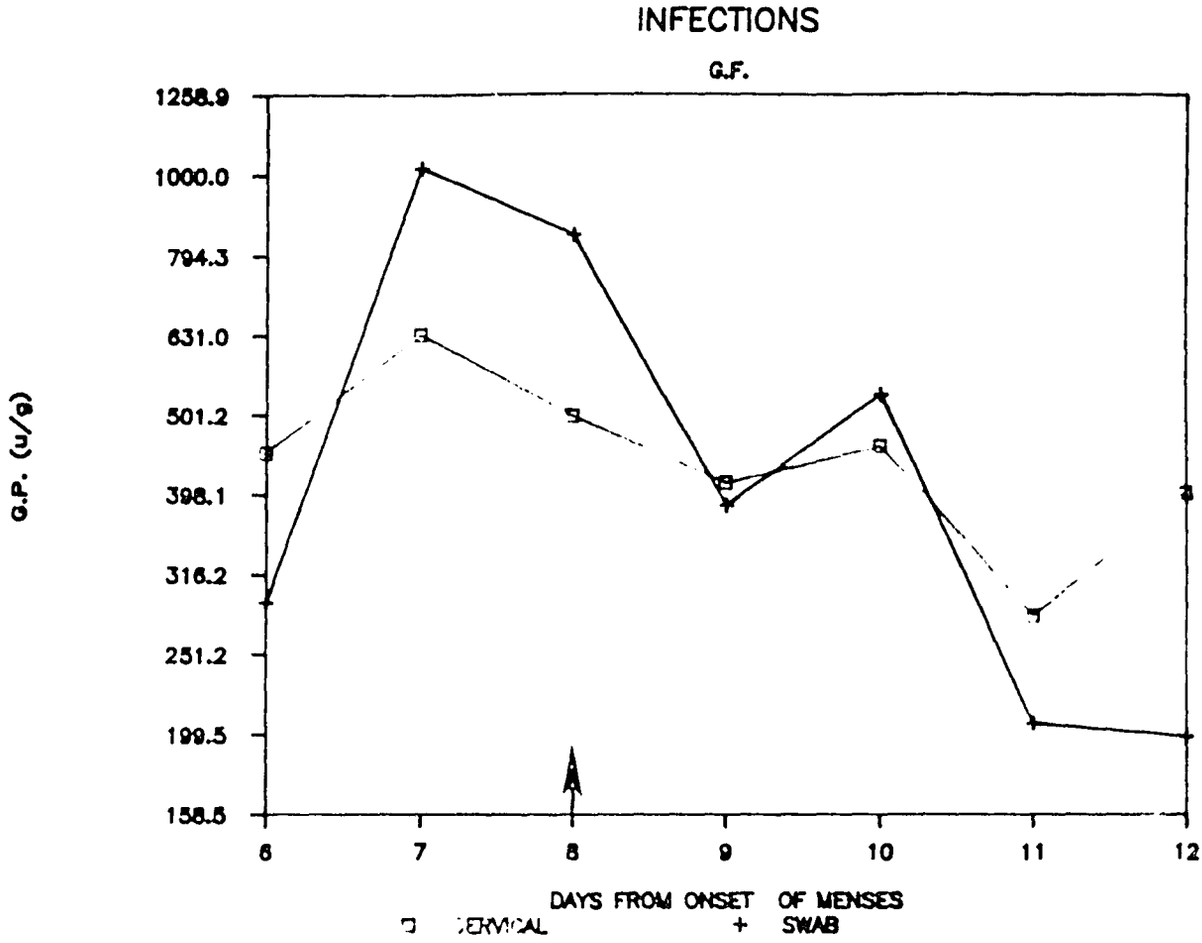
_____
Signature

(Imprint Plate)

Increase in the number and variet of insurance forms, and shortage of personnel make it necessary to submit this form in lieu of your form.
We sincerely trust it will meet your needs.

L.C.Edwards, M.D.
Associate Medical Director
Cook County Hospital

FAMILY PLANNING


INFECTIONS
G.F.
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
G.P. (u/g)
6
7
8
9
10
11
12
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

G.E. AGE 23 G1 P1

LMP 7/31/86 Frequency 28-29 days Lasts 2 days moderate 3rd day light

Problems with Menses ~~No cramps~~ for past 13 yrs. Periods real regular

BC Method ~~Diaphragm~~ Condoms + jelly. ~~Number of partners~~ _____

Gyn Hx yeast infection when takes antibiotics about 2x/year. Cuts'ag had Gardnerella. Had C-section.

Med Dis Hx ~~Sickle cell anemia trait~~

Medications Vitamin

Allergies ~~pets allergic symptoms in spring~~. She does not know what she is allergic to.

Habits Cigs NO ETOH NO Exercise ~~Bike riding in summer~~ 7-100 miles/week

Ht 5'9" wt 135 lb

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ mod ____ large _____

bact sm _____ mod _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____

UNIVERSITY OF ILLINOIS
CHICAGO CIRCLE CAMPUS
HEALTH SERVICE

Phone 996-3388 University Hall

FOR G.F. DATE 8-4-86

Rx ADDRESS

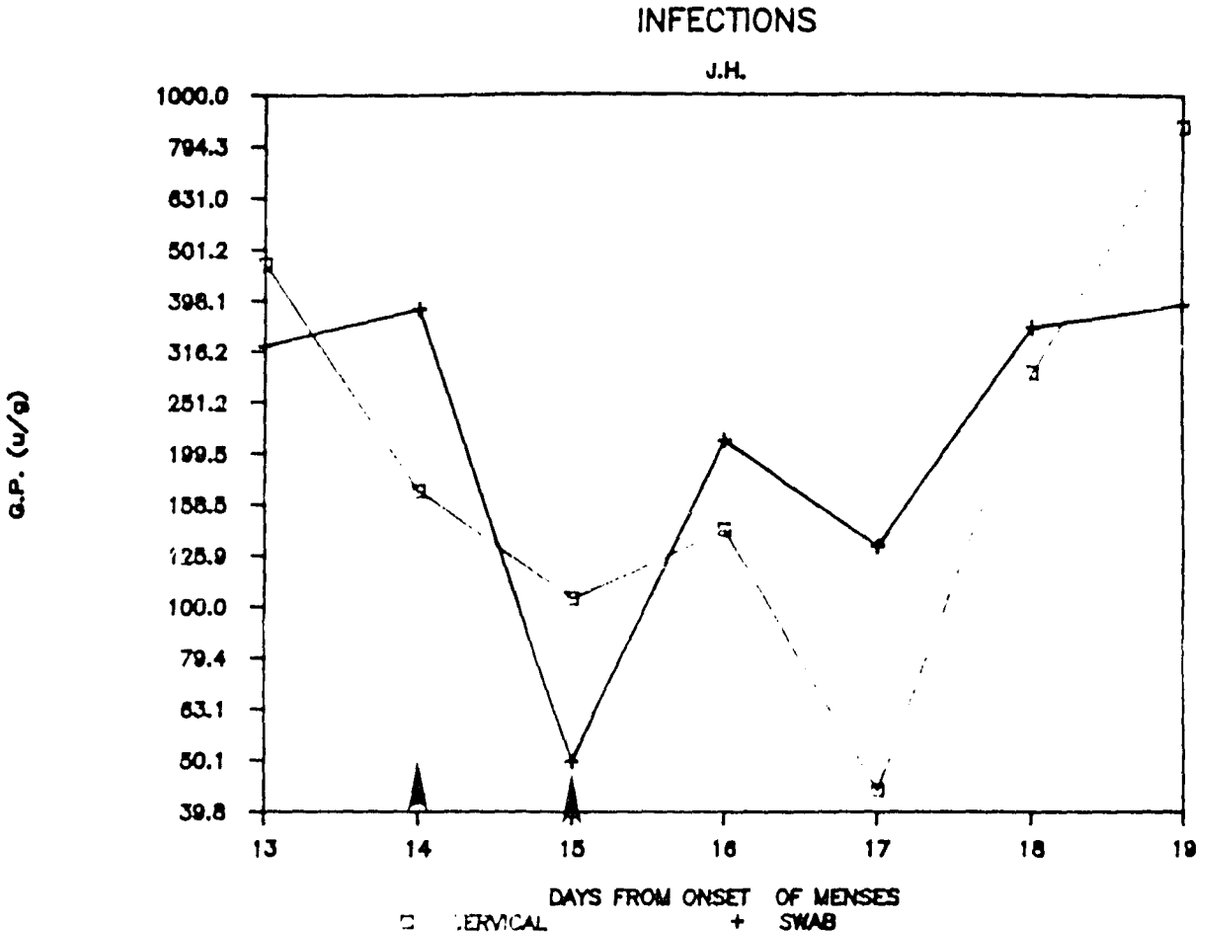
Smear - G/+/Rods

℞ Sultrin vag. cr. 78g
Sig. insert bid

PLEASE LABEL PRESCRIPTION

BNDD No _____ M.D.

Non Repetatur 10-11-17250


INFECTIONS
J.H.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
G.P. (u/g)
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14
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DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

J.H. AGE 30 G0 P0

LMP 7/27/86 Frequency 26 Lasts 5-7 days

Start with thick brown small amount clumps. Starting 3rd day heavy w/ clots. Lasts 24 hr then tapers. + feeling like D

Problems with Menses always has cramps. Sometimes more much though. Once a single yrs ago had spotting inbetween per

BC Method Condoms Number of partners

Gyn Hx One time had Abs 1 almond occ smears, possibly due to infection, ≈ 2x/year gets monilial infection, Had trichomonas ≈ 7 yrs ago

Med Dis Hx 3 yrs old had tonsilectomy Has retroverted uterus

Medications Will take 1 supp. each night x 3 nights, then use cream for (Monistat) monilial vagin

Allergies NONE

Habits Cigs ≈ 1-2 cigs/day ETOH socially Exercise Last 2 days - [illegible] Volleyball 1 x/week

Ht 5'7½" wt 164.5 lb.

Physical - Ext Exam

Bimanual

pH _____ opi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC on ___ mod _____ large _____

bact on _____ mod _____ large _____

trich _____ yeast _____ hyphae _____

send check in mail

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip _____

Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____

MED RECORD NO BIRTH DATE

PATIENT NAME J. H.

ADDRESS

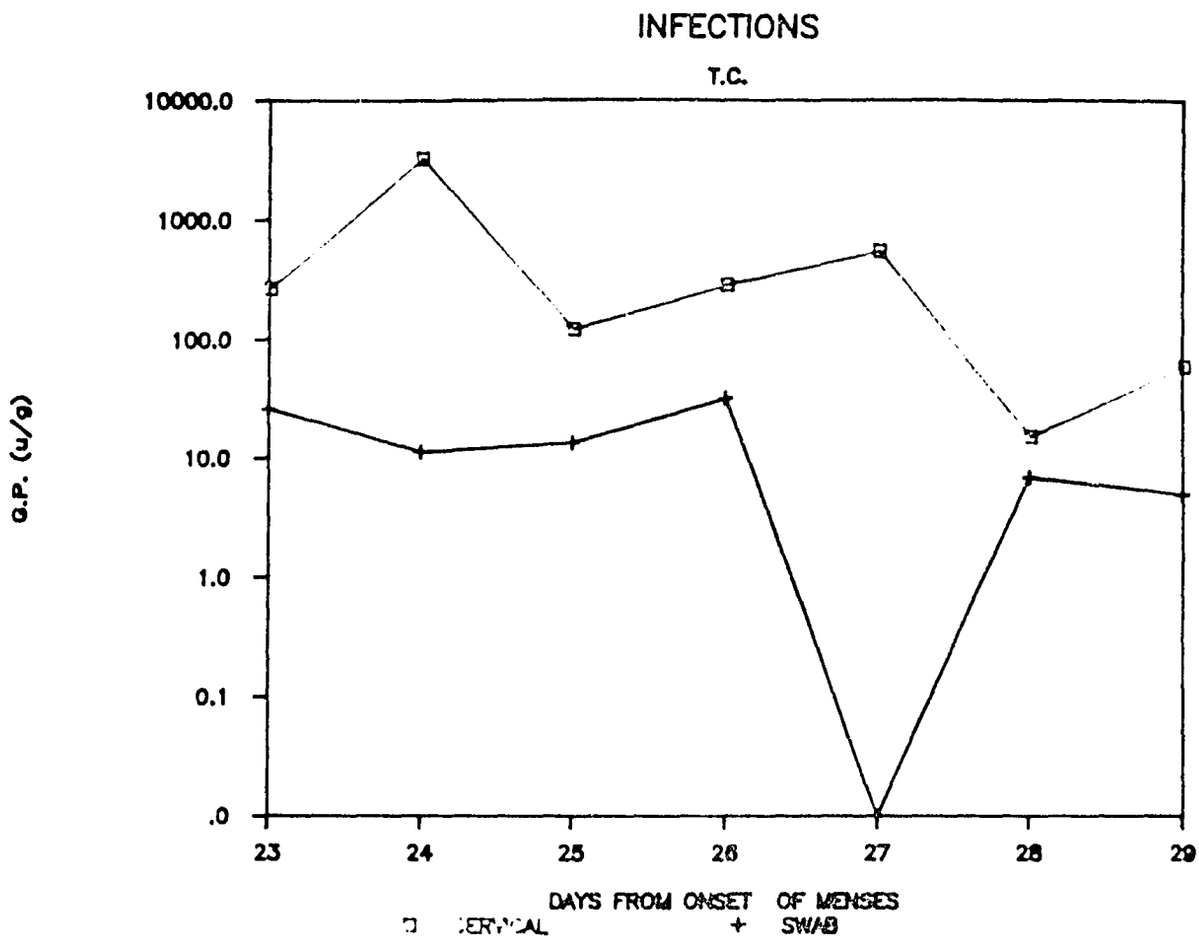
PROGRESS NOTES

DATE WRITTEN Date	Problem No.	
08/04		LMP = 7/27/86 BP 112/70
8/7/86		Here for evaluation of vaginitis c/o vaginal discharge and itching & burning
		Mod-C Nulliparous
		c/o vaginal itchiness frequently following menses (+) antibiotic use 2 months ago used acid douche over weekend symptoms improved since then but not resolved
		exam: perineum neg vag No erythema (+) cheesy white discharge PH 4.5 dark wet mount pH 5.6 No trich or clue cells - normal bacterial morphology KOH test - No hyphae seen
		otherwise Normal size No adnexal masses.
Imp		symptoms most consistent with monilial vaginitis resolving with self treatment
Plan		Monistat as empiric treatment

LOYOLA UNIVERSITY
MULCAHY OUTPATIENT CENTER
Maywood, Illinois 60153

- Signature and professional discipline of recorder is to be shown
- Show date and time of entry

30931


INFECTIONS
T.C.
10000.0
1000.0
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10.0
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.0
G.P. (u/g)
23
24
25
26
27
28
29
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

T.C. AE-25 G0 P0

LMP 7/20 [redacted] Frequency 25-26 days. Lasts 4-5 day

Problems with Menses None No cramps.

BC Method Condoms ~~Number of partners~~ _____

Gyn Hx ~6 months ago - yeast infection. Had several yeast infections before.

Med Dis Hx Mother died of heart attack + stroke. She was diabetics (diet controlled) + hypertension, and enlarged

Medications None Father + mother has had hypertension.

Allergies No Toy has been hospitalized for Scoliosis in her

Habits Cigs No ETOH occasionally Exercise Jog 2 miles every other day.

Ht 5'7" wt 155 lb.

Tonsilectomy Pneumonia and March 5C. presently has yeast infec

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ med ____ large _____

bact sm _____ med _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

Weekends _____

BC method _____

Money Employes _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____

NORTHWESTERN MEMORIAL HOSPITAL
SUPERIOR & FAIRBANKS COURT, CHICAGO ILLINOIS 60611

℞ No.

908-2000

FOR

DATE 8/8/86

ADDRESS CITY STATE

THIS MAY NOT BE USED FOR SCHEDULE II DRUGS

℞ I C. was seen in ER today. Appears to have varicella infection with white lesions and drainage

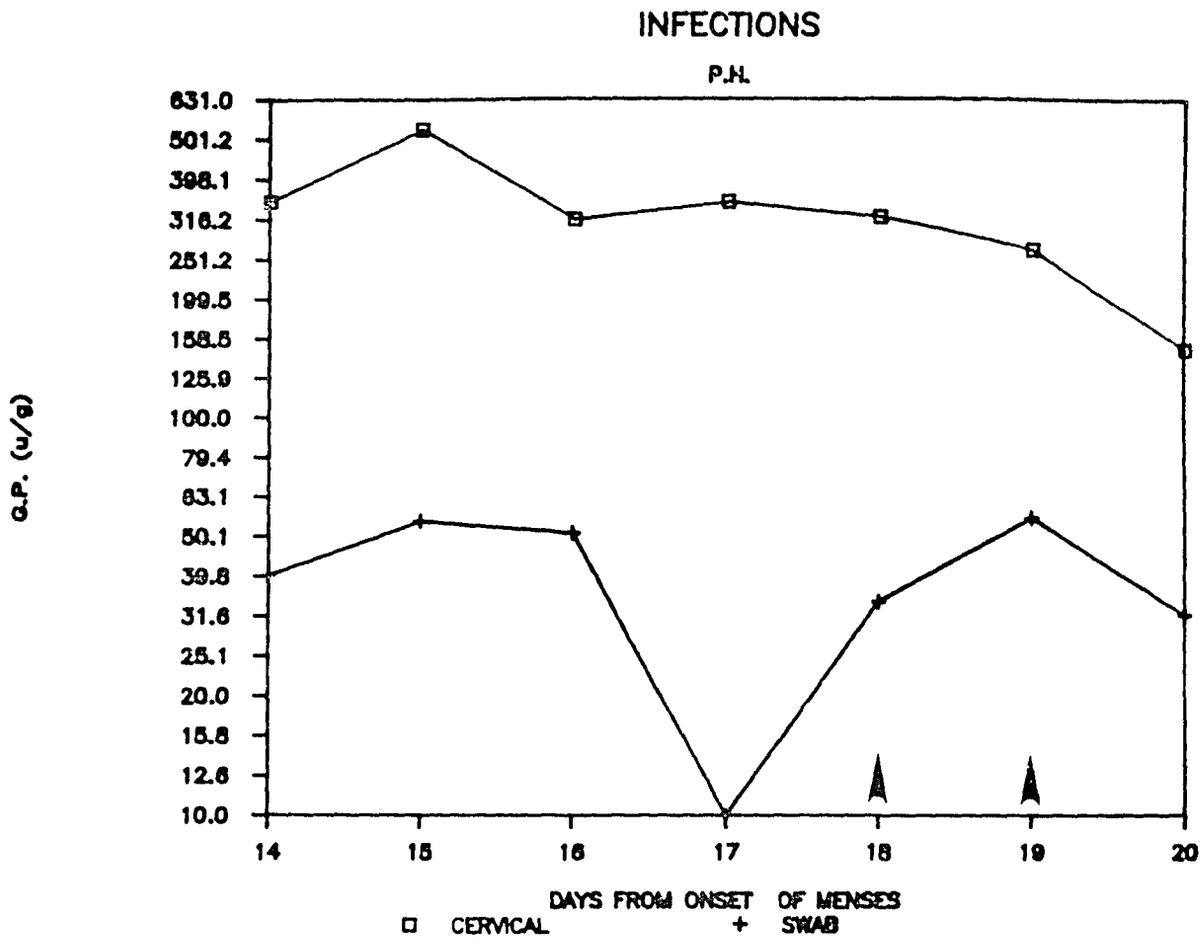
☐ MAY SUBSTITUTE
☐ MAY NOT SUBSTITUTE

Dr

REFILL _____ TIMES

416008 Rev 2 82

HOSPITAL NO OR D E A NO


INFECTIONS
P.H.
631.0
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20.0
15.8
12.6
10.0
G.P. (u/g)
14
15
16
17
18
19
20
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

ALMAR MOLDY CLINICAL LABORATORY, INC.
2514 W. Pratt Blvd. - Chicago, Ill. 60659
Phone: 743-7118

URINALYSIS

Attending Physician: Dr Paulos

- ☐ Volume
- ☐ Color
- ☐ pH 6.0
- ☐ Specific Gravity 1.002
- ☐ Protein 0
- ☐ Sugar 0
- ☐ Acetone 0
- ☐ Diacetic Acid
- ☐ Bile
- ☐ Urobilinogen

Urine Specimen: Voided ☐ Catheterized ☐

Additional Urine Tests (Specify)

P.H.

- ☐ Cells (HPF)
  - ☐ R.B.C. 0-1
  - ☐ W.B.C. 0-1
  - ☐ Epithelial mod.
- ☐ Bacteria
- ☐ Crystals
- ☐ Mucus
- ☐ Casts Hyaline / Granular

Comments: Clue cells present

TECHNICIAN: Samuel Lopez

DIRECTOR

Date 8/12/86

URINALYSIS

PRESS FIRMLY
PRINT WITH BALL POINT PEN OR PENCIL
ALL COPIES MUST BE LEGIBLE

ALMAR MOLDY CLINICAL LABORATORY, INC.
2514 W. Pratt Blvd. - Chicago, Ill. 60659
Phone: 743-7118

MISCELLANEOUS

ATTENDING PHYSICIAN: Dr Paulos

SPECIMEN:

REPORT:

Wet prep - many bact + epith. cells, neg for yeast, positive for trich + Clue cells.

Gram Stain = neg for gm. neg. intra + extracellular diplococci, many gm. neg. rods + cocci, moderate gm. (+) cocci, clue cells present.

DIRECTOR

TECHNICIAN

MISCELLANEOUS

DATE

PRESS FIRMLY
PRINT WITH BALL POINT PEN OR PENCIL
ALL COPIES MUST BE LEGIBLE

UNION HEALTH SERVICE, INC.
TELEPHONE 829-4224
1634 W. POLK STREET • CHICAGO, ILLINOIS 60612

DATE 8/12/86

P.H.

℞

FLAGYL 250 mg #30

Sig: 250 mg po TID
No alcohol

REFILL NO TIMES D.E.A. NO. AP1337799

MAY NOT SUBSTITUTE MAY SUBSTITUTE

DR. DR.

200
4 D

P.H. AGE 28 G5 P3 AB2

LMP Aug. 1, '86 Frequency 28 days Lasts 3 days

Problems with Menses For 1 yr. has had mild cramps on 1st day of pe

BC Method Tubal ligation ~~Number of pregnancy~~ _____

Gyn Hx Had UTI when pregnant, Had Trichomonas + yeast infection simulc about every 4 months.

Med Dis Hx Tonsilectomy in '76

Has present trichomonas vaginal infection.

Medications NONE, except Flagyl for present infection (250 mg)

Allergies NONE

Habits Cigs 10 cig/day ETOH moderately Exercise 15 min/night of exercis

Ht 5'1" wt. 150 lb.

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ med ____ large _____

bact sm _____ med _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

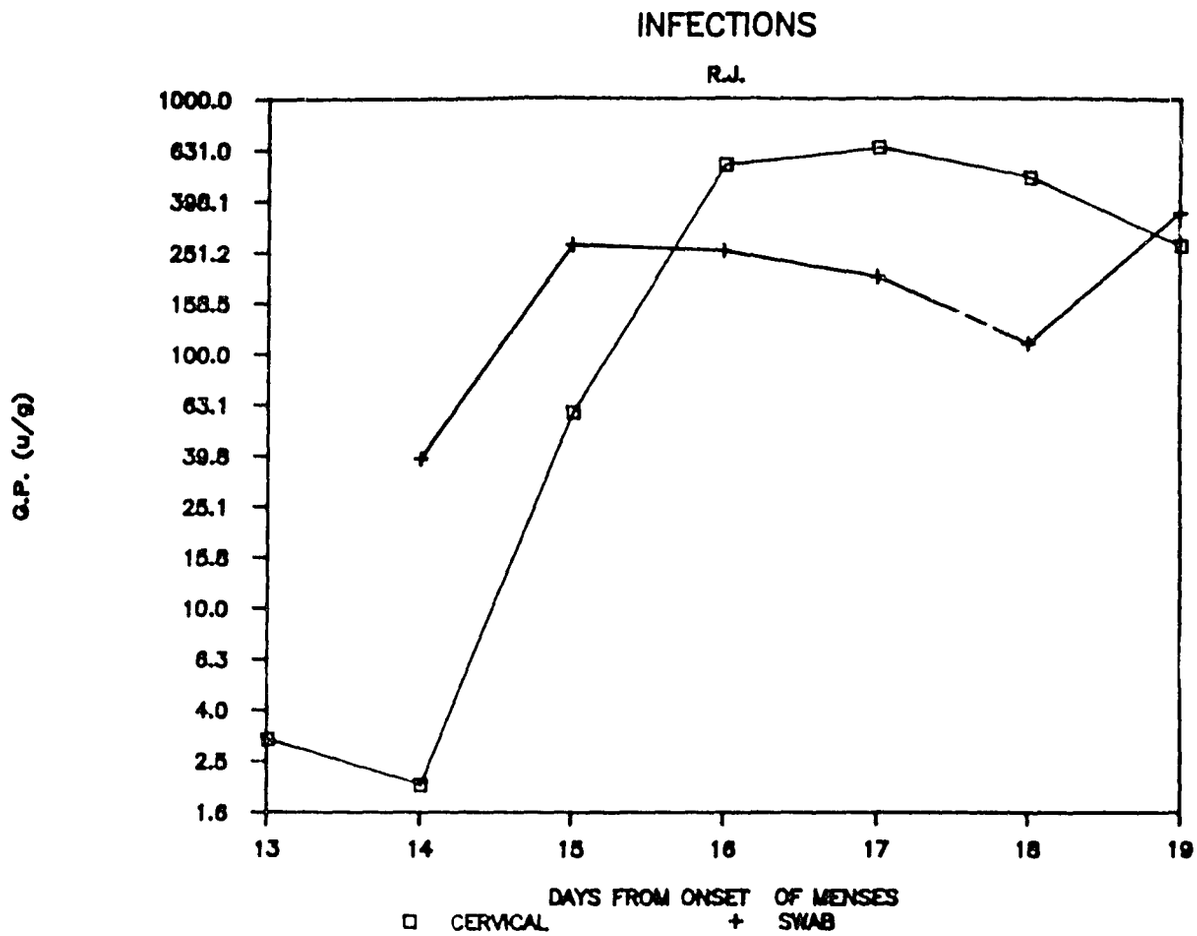
Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____


INFECTIONS
R.J.
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63.1
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15.8
10.0
6.3
4.0
2.5
1.6
G.P. (u/g)
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19
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

R.J. AGE 19 G2 P2

LMP 8/7/86 Frequency 23 day cycle Lasts 6 days

Problems with Menses ~~HA~~ slight cramping x 2 wks before period for ~~short~~ time, sometimes has heavy flow + sometimes light.

BC Method None Number of partners 1

Gyn Hx ~~HA~~ Had pelvic infection soon after 1st child born in '83 (F

Med Dis Hx ~~[illegible]~~ Presently has non-specific vaginitis, but will not take prescribed medication (A! V2 Cr

anemia, Tracheotomy at 2

Medications NO

Allergies Wheat Mental

Habits Cigs NO ETOH NO Exercise Sometimes exercising (sit-ups, etc.) off + on, moderately

Ht 5'1" wt 116

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ mod ____ large _____

bact sm _____ mod _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

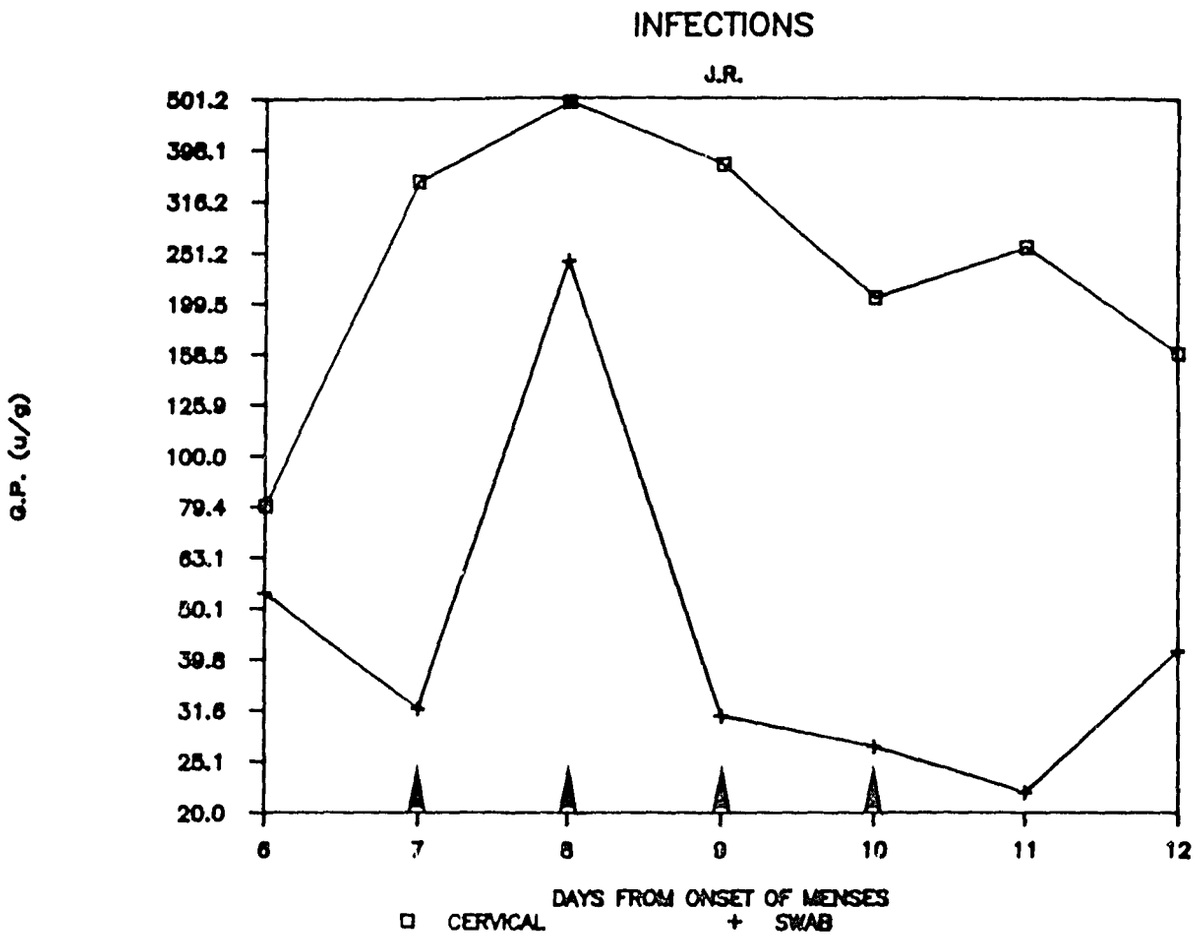
Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____ ✓


INFECTIONS
J.R.
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
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63.1
50.1
39.8
31.6
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20.0
G.P. (u/g)
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DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

J.R. AGE 25 G2 P2

LMP 8/29/86 Frequency 28-31 days Lasts 5 days

Problems with Menses During period moderate cramps. ≈ 5 months ago had extra heavy bleeding during period

BC Method tubal ligation

Gyn Hx pts. yeast infections (vaginal) ≈ 1/yr. Once had trichomonas. Presently has bacterial infection in vagina.

Med Dis Hx NONE

Medications Flagyl 500 mg tabs - (2 tab/Day x 5 days) for present infection

Allergies NONE

Habits Cigs NO ETOH NO Exercise NO

Ht 5'4" wt 185 lbs.

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ mod ____ large _____

bact sm _____ mod _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed

UNIVERSITY OF ILLINOIS HOSPITAL

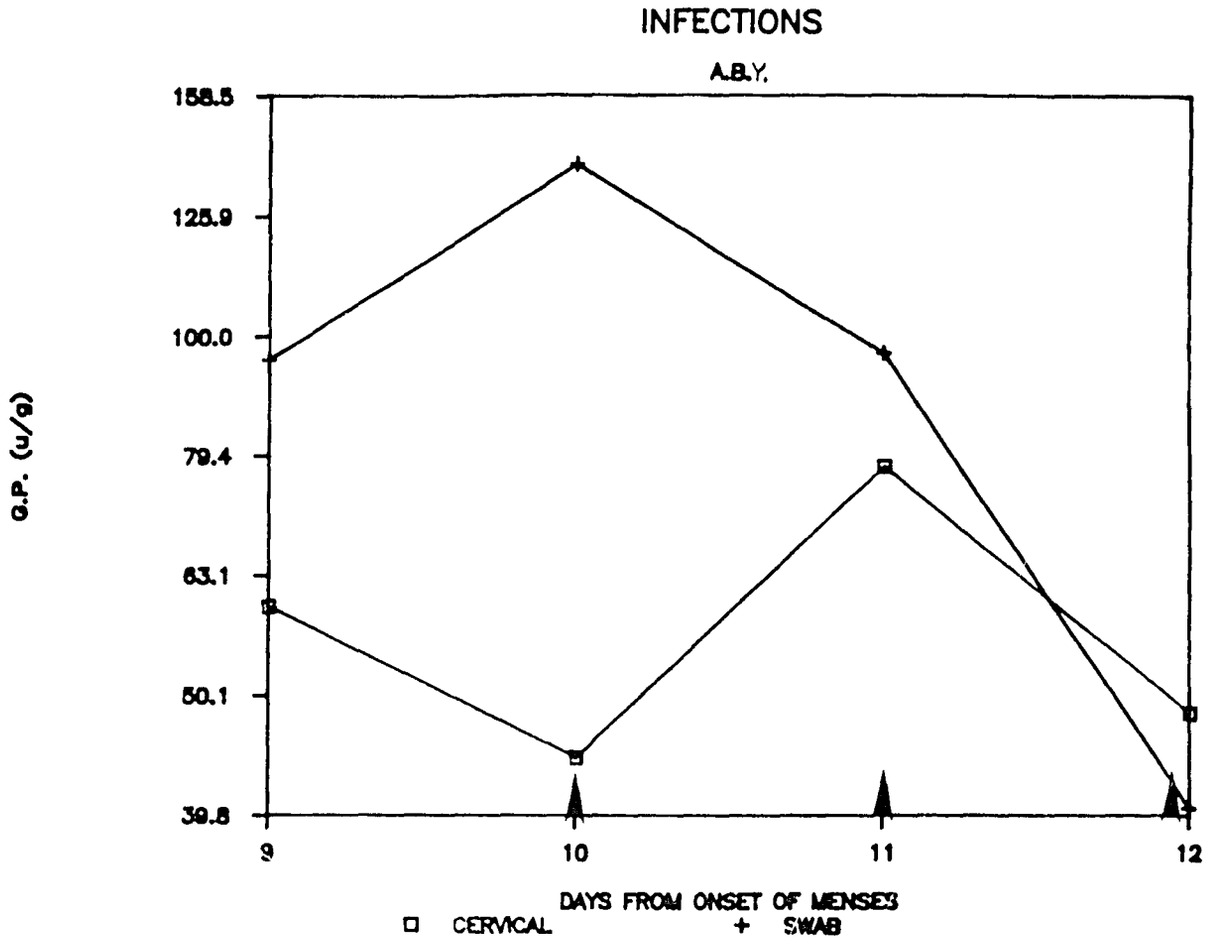
J.R.

9/3/86 - 2[illegible]062P2002
c̄ LMP 8/31/86 here because of vaginal itching + burning x 3wks
No disch. Denies antibiotics or medication intake.
PE - Pasty discharge
Smear: (+) Clue cells. (+) Amine release c̄ KOH

Imp - Bacterial vaginosis
Plan - Flagyl x 5 days

BE SURE TO SIGN ALL YOUR NOTATIONS.

FORM UI-408 (OVER) CLINIC PROGRESS NOTES


INFECTIONS
A.B.Y.
158.5
125.9
100.0
79.4
63.1
50.1
39.8
G.P. (u/g)
9
10
11
12
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

ABY AGE 24 G3 P3

LMP 8-31-86 Frequency 28 days Lasts 5-7 days

Problems with Menses Since tubal ligation, periods have gotten a little and because of infection he had spotting. Normally not have spotting between periods

BC Method tubal ligation ~~Number of periods~~ _____

Gyn Hx ~~Had several yeast infections during pregnancy~~ Presently has gardenerella vaginalis infection. ≈ 2 yrs ago.

Med Dis Hx Tonsilectomy long ago.

Medications Flagyl 250 mg Sig + Po tid x 7d. #21.

Allergies NONE

Habits Cigs ½ pk/day ETOH ~~no~~ Exercise ~~jogs~~ ≈ 1 mile/day + works out for 20 min/d

Ht 5'6" wt 135 lbs

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ mod ____ large _____

bact sm _____ mod _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

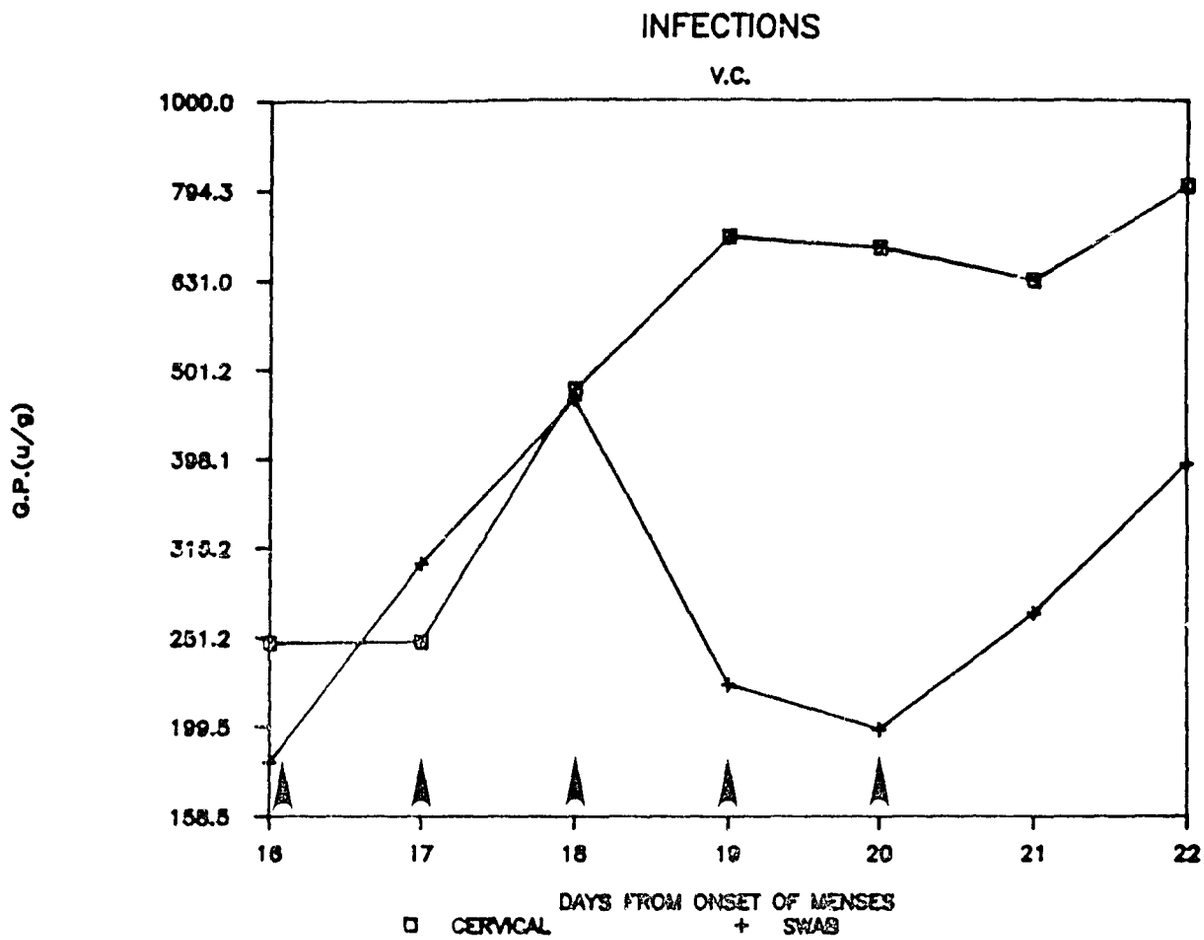
Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____


INFECTIONS
V.C.
1000.0
794.3
631.0
501.2
398.1
315.2
251.2
199.5
158.5
G.P.(u/g)
16
17
18
19
20
21
22
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

25 yo G1 P1 MP 8/31/86 | V.C.
here c̄ vaginal discharge
x 3 wks, thick, not malodorous. No pruritus
Diaphragm for contraception
PE - Vagina - Pasty gray disch
Cervix - Clean
Smear - + Clue cells + Amine release

Imp - Bacterial vaginosis
Plan - Flagyl 500 mg bid x 5 days

V.C. AGE 25 G0 P0

LMP 8/31/86 Frequency 28-30 days Lasts 5-6 days

Problems with Menses moderate cramps on 1st day of period usually.

BC Method DIAPHRAGM [redacted]

Gyn Hx Presently has bacterial vaginosis (non-specific vaginitis)
Had yeast infections frequently when on the pill. Had non-specific vaginitis

Med Dis Hx at age 18 had period 2 months straight... had D&C to stop it
[redacted]. Appendix removed at age 13.

Medications Flagyl 500 mg BID x 5 days for infection

Allergies NONE

Habits Cigs occasionally ETOH NONE Exercise stairs etc. ≈ 20 min/day
Ht 5'[illegible] 0-10 cigs/day [illegible] [redacted]s, 150 lbs.

Physical - Ext Exam

Bimanual

pH cpi clean dry "clue none

rare mod low

lacto WBC sm med large

bact sm med large

trich yeast hyphae

Told of : BBT

Collection blood mucus [redacted] tip

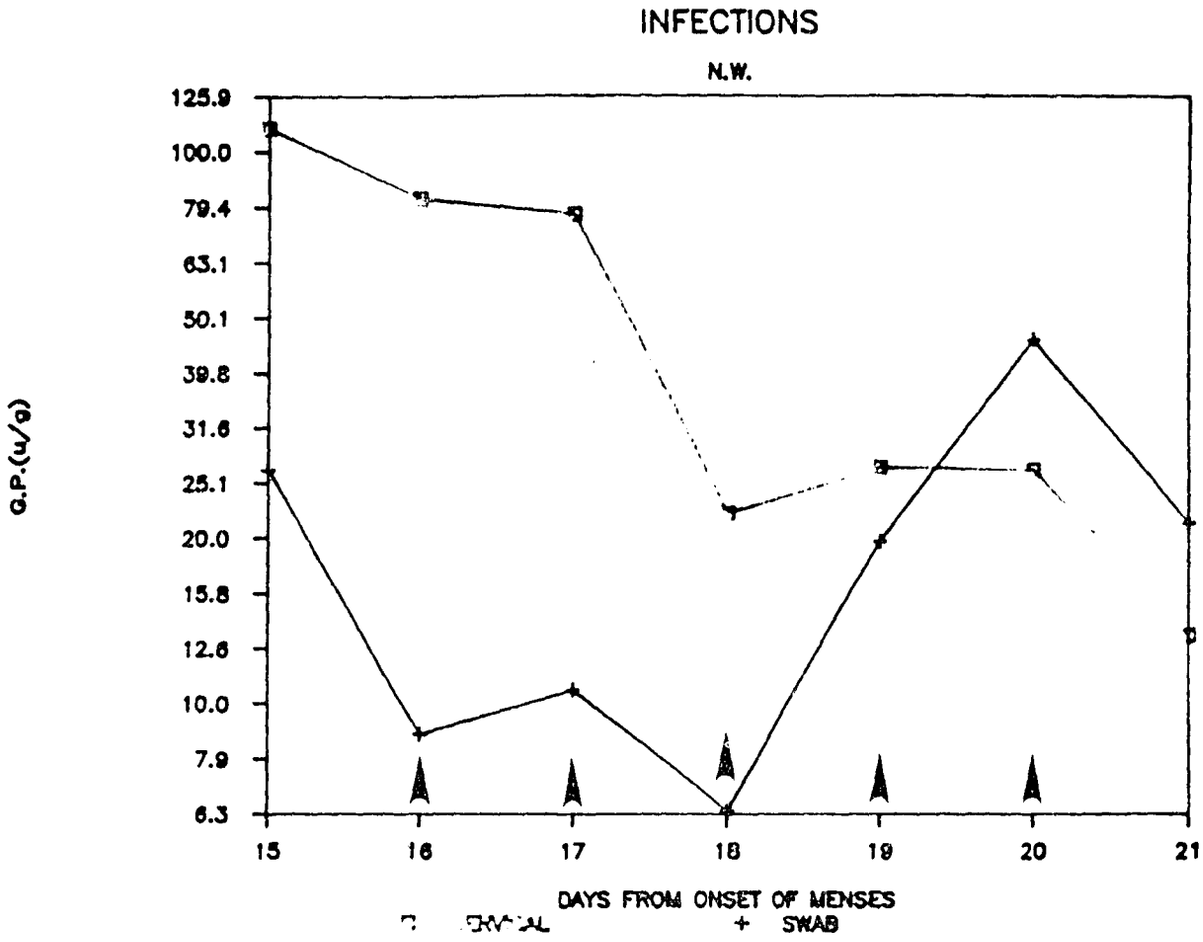
Weekends

BC method

Money Employee

Vag itching risk Blood drawing risk

Permit Signed


INFECTIONS
N.W.
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
7.9
6.3
G.P.(u/g)
15
16
17
18
19
20
21
DAYS FROM ONSET OF MENSES
+ SWAB

N.W. AGE 29 G1 P1

LMP 9/1/86 Frequency 28-29 days Lasts 4-6 days

Problems with Menses Occasionally has severe cramps before period. Usually has heavy periods when she has cramps

BC Method Tubal Ligation ~~Number of pregnancies~~

Gyn Hx ~~D/C suppositories for pain~~ Has had vaginal discharge intermittently x few years. Treated c̄ ampicill. Also has had pelvic pain, some dysfunction. Presently has non-specific vaginitis. Has had it previously. Also had yeast infections prev.

Med Dis Hx ~~≈4 yrs ago hospitalized for frequent blackouts~~

Medications Flagyl (500 mg) bid x 5 days for present non-specific vaginitis

Allergies ACNE

Habits Cigs ≈ ½ Pack/day ETOH Socially Exercise NO

Ht 5'8" wt 118 lbs.

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ med _____ low _____

lacto _____ WBC sm ___ med ____ large _____

bact sm _____ med _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed ____ _____

CLINIC PROGRESS NOTES

UNIVERSITY OF ILLINOIS HOSPITAL

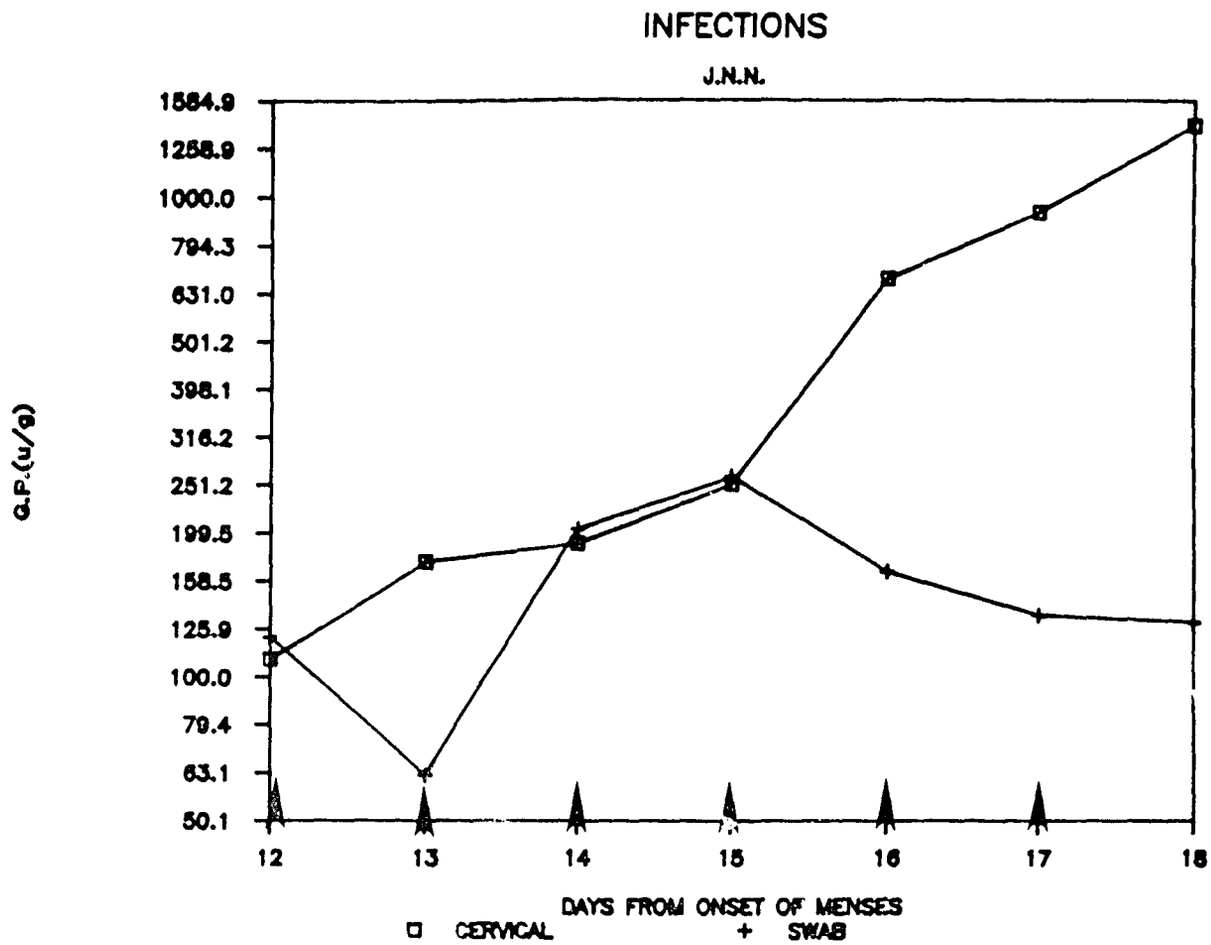
N.U.

29y o G1P1 c̄ LMP 9/1/86
here c̄ vaginal discharge
for intermittently x few years. Treated
c̄ ampicillin, TVC suppositories in
the past. Also has had pelvic
pain, same duration, but is
s/p BTL long before pain began.

Pelvic – Thick pasty discharge
Smear – WBC's, TT Clue cells
++ Amine odor poss some trich

Imp – Bacterial Vaginosis
Plan – Flagyl 500mg bid x 5 days – both partners

BE SURE TO SIGN ALL YOUR NOTATIONS.


INFECTIONS
J.N.N.
1584.9
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
G.P.(u/g)
12
13
14
15
16
17
18
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

J.N.N. AGE 37 G4 P1 AB1

(last pregnancy terminated spt with [illegible]

LMP 9/7/86 Frequency 26-28 days Lasts 2-3 days, usually l

Problems with Menses usually mild cramps 2-3 days before + 1st day of peri
Never has had irregular periods.

BC Method _____

Gyn Hx 3-4 yrs ago Trichomonas - took flagyl. Used to get yeast infection free before 4 yrs ago. Now started getting them again since kidney transplant 1 month ago. Infections in tubes a couple times 6 yrs ago. Recently has yea vaginal infe

Med Dis Hx Kidney transplant 7/10/86. Diabetes. Had congestive heart failure + high blood pressure transplant, but no longer has them. Hospitalized for diabetes several times.

Medications Insulin - 70 Units NPH w/ 18 Units regular in morning. 30 Units NPH w/ 10 regular evening

Allergies Will take Monistat vag cream QHS x 7 days for vag. infection.
Iodine

Habits Cigs NO ETOH NO Exercise walk ½ hr/day

Ht. 5'1½" Wt. 210 lbs.

Physical - Ext Exam _____

Bimanual _____

pH _____ opi clean _____ dry _____ "clue none

rare _____ med _____ low _____

lacto _____ WBC sm ___ med _____ large _____

bact sm _____ med _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip _____

Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____

daniel hale williams health center

5044 SOUTH STATE STREET
CHICAGO ILLINOIS 60609
312 / 538-6700

9/18/86

To Whom it may concern

Ms. J.N.N. was seen

on 9/16/86

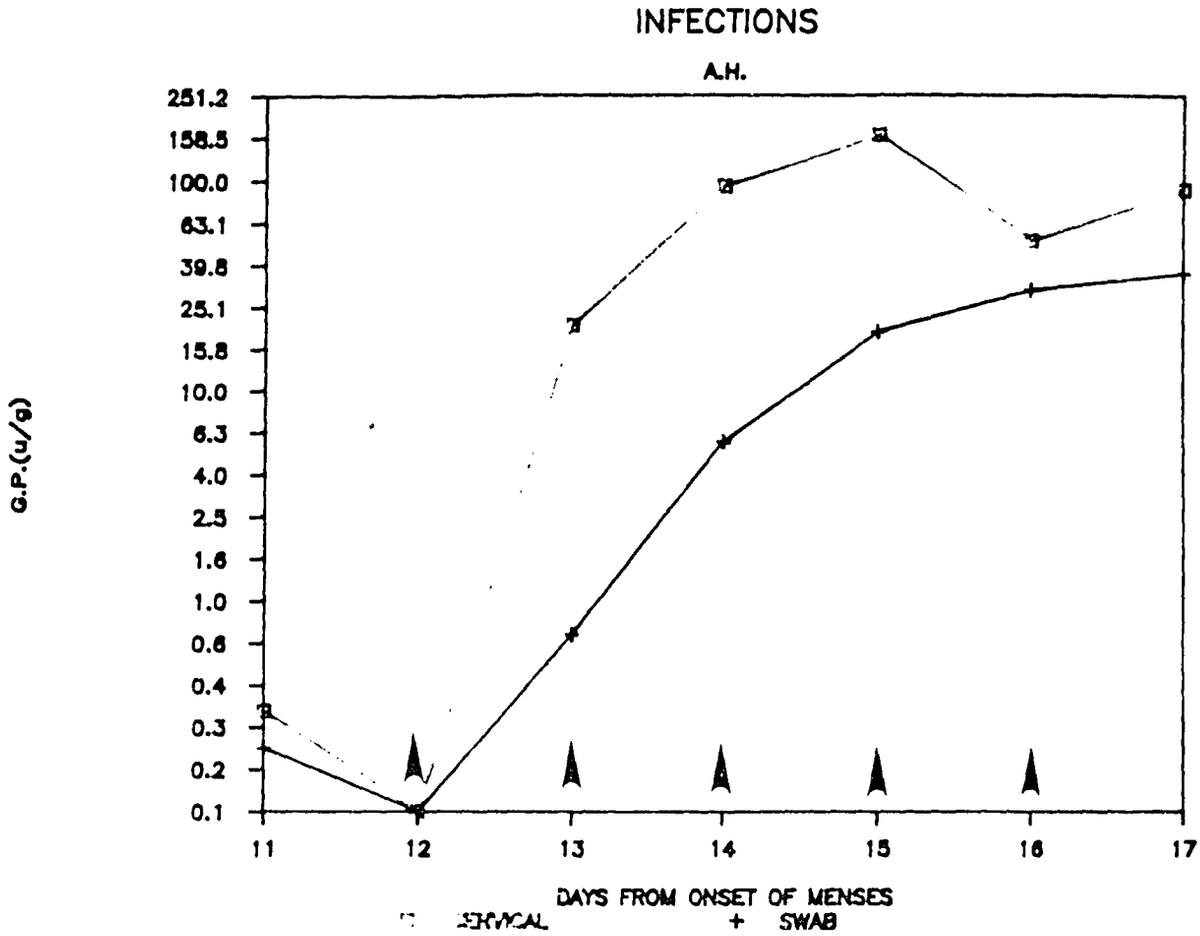
Dx Candidiasis

Rx Monistat 7 & Tegretol

X Tazaga

Sincerely

J. Powell M.D.


INFECTIONS
A.H.
G.P.(u/g)
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
0.6
0.4
0.3
0.2
0.1
11
12
13
14
15
16
17
DAYS FROM ONSET OF MENSES
+ SWAB

A.H. AGE 25 G0 P0

LMP 9/12/86 Frequency 26-27 days Lasts 5-6 days

Problems with Menses Severe cramps first 2 days of period. Slight headache 0 during 3 days after period ends. If she consumes chocolate, the she gets heavy bl during next peri

BC Method NONE

Gyn Hx 2x had class 3 pap. Had tubal infection - took AVC cream. Had yeast infection 1 time before. Trichomas 2x w/in last 2 years.

Med Dis Hx Had pneumonia 3 years ago - hospitalized

Medications NONE. will take Flagyl (500mg) 2/day x 5 days

Presently - Su discharge, + itching + Pains in l va

Allergies Penicillin

Habits Cigs NO ETOH NO Exercise NO

Ht. 5' Wt. 124 lbs

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ med ____ large _____

bact sm _____ mod _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip ____

Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

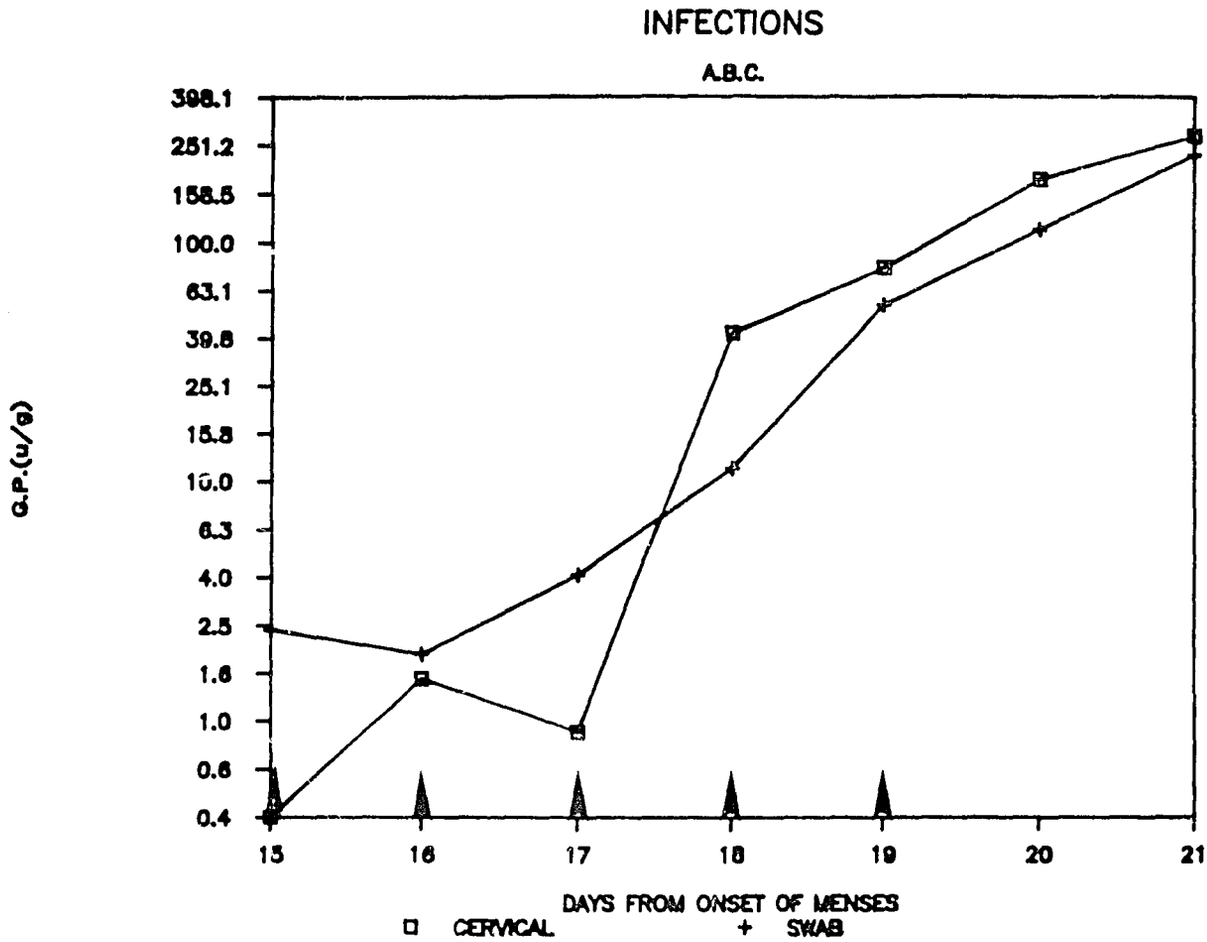
Permit Signed _____

A.H.

9/22/86
28 yr old G0P0 c̄ LMP 9/12/86.
No contraception. c̄ LAP
+ vaginal discharge + dyspareunia x ~2
h/o "PID" treated as outpt. Discharge is
whitish + thick, occas c̄ itching.

PE — Abdomen — Soft Nontender
Pelvic — Ufl, foul disch
Discharge — MWBC's, Trichomonas
+ Amine Release
Imp — Trich Vaginitis
Plan — Flagyl x 5 days — both partners
Explained that pain is probably
unrelated + advised pt to see her
Gynecologist

BE SURE TO SIGN ALL YOUR NOTATIONS.


INFECTIONS
A.B.C.
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
1.0
0.6
0.4
G.P.(u/g)
15
16
17
18
19
20
21
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

ABC. ABE BC G1 P1

LMP 9/10/86 Frequency 29 days Lasts 3-4 days.

Problems with Menses Always has blood clots during period for ≈ 2 yrs Now Starts cramps ≈ 2wks before period due. (moderate cramps). After last day period Has a female flow of blood continuing for 3-4 days foul smell

BC Method Diaphragm

Gyn Hx 2 abnormal pap smears in last year (possibly type C). Had colposcopy done (neg). Has not had another pap smear yet. She may have fibroids because uterus is size of 6 wk pregnancy. Will have ultrasound done. Has right ovary removed in '79 due to dermatoid cyst. Trichomonas 2 yrs ago. Vaginal warts 1 year ago (treatment - they were burned). Had Hemophilus infection years ago. Every couple months itching + douches.

Med Dis Hx NONE

Medications NONE

Allergies Penicillin

Habits Cigs No ETOH No Exercise aerobics + weight machines 2 for ≈ 2 hrs.

Ht. 5'5" Wt. 158 lb.

Physical - Ext Exam _____

Bimanual _____

pH _____ epi clean _____ dry _____ "clue none

rare _____ mod _____ low _____

lacto _____ WBC sm ___ mod _____ large _____

bact sm _____ mod _____ large _____

trich _____ yeast _____ hyphae _____

Told of : BBT _____

Collection blood ___ mucus ___ [redacted] tip _____

Weekends _____

BC method _____

Money Employee _____

Vag itching risk _____ Blood drawing risk _____

Permit Signed _____

OV

9/24/86 - [illegible]

A.B.C.

30y o G1P1 LMP 9/10/86

here because of constant discharge & 2 wks ± odor. No pruritis or burning

Diaphragm = Contraception

PE - Vagina - pasty disch

Cx - Clean mucous

Smear - TT Clue cells No trich or yeast

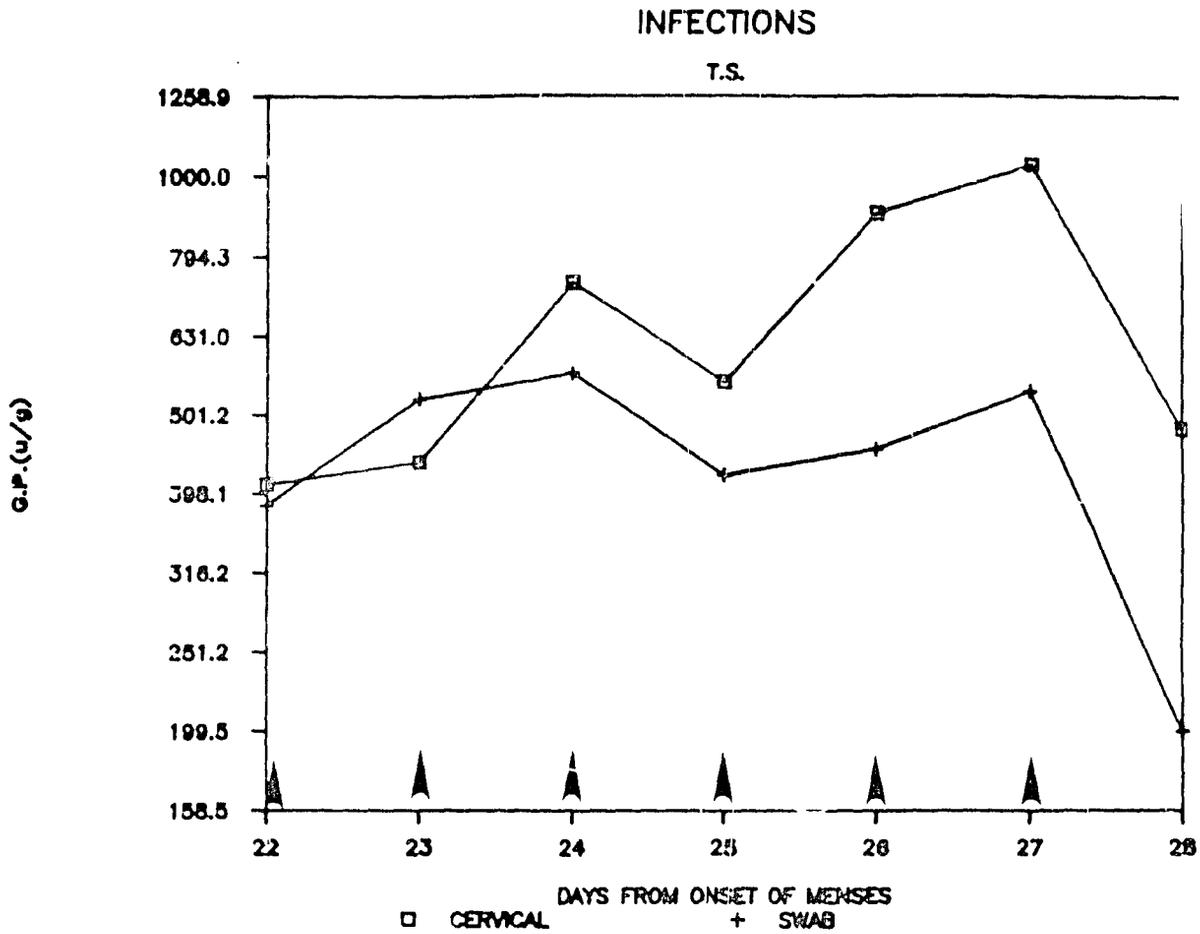
Imp - Bacterial vaginosis

Plan - Flagyl 500mg bid x 5 days

[signature]

BE SURE TO SIGN ALL YOUR NOTATIONS.

FORM U1-4009 (OVER) CLINIC PROGRESS NOTES


INFECTIONS
T.S.
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
G.P.(u/g)
22
23
24
25
26
27
28
DAYS FROM ONSET OF MENSES
CERVICAL
SWAB

T.S. AGE 18 GO PO

Problems with Menses Always severe cramps on 1st day of period. Stops on 5th day and start a spotting a little on 6th day.
Just last month had very heavy flow.

BC Method NONE

Gyn Hx Recently yeast infection probably has listed 2 yrs. ✓

Med Dis Hx In '82 had mono-hospitalized for 5 days.

Medications Clotrimazole cream for 7 days (Stop 1 application)

Allergies NONE

Habits Cigs NO ETOH NO Exercise Regular but no abnormal exercise 30 min - 2x/wk. lift weights 2x/wk. Walks

Ht. 5'9" Wt. 176 lbs

Physical - Ext Exam

Bimanual

pH opt clean dry "clue none

rare mod low

lacto WBC sm mod large

bact sm mod large

trich yeast hyphae

Told of : BBT

Collection blood mucus [redacted] tip

Weekends

BC method

Money Employee

Vag itching risk Blood drawing risk

Permit Signed

UNIVERSITY OF ILLINOIS HOSPITAL

T.S.

9/25/86

18 y/o G[illegible]P0 c̄ LMP 9/4/86.
No contraception. C/o Heavy discharge x 1-2 yrs. Treated for yeast 1 yr ago (bid x 3 days c̄ cream) c̄ relief.

PE - Pelvic: Thick wht disch. Cx: Cl
Smear - Hyphae c̄ buds (mild-mod)

Imp - Candida Vaginitis
Plan - Clotrimazole Vaginal Cream x/[illegible]

BE SURE TO SIGN ALL YOUR NOTATIONS

APPENDIX 5

C. Colorimetric Study

PARFR-360 1985-1986

Name	Date	Net Swab Weight, mg (Mean ± SE)	n (days)
J.J.	4/16/86-5/7	49.2 ± 5.4 (1st swab)	23
		41.8 ± 4.7 (2nd swab)	23
D.S.	4/18/86-5/9	61.6 ± 8.6 (1st swab)	22
		59.4 ± 8.4 (2nd swab)	22
D.C.	4/21/86-5/13	30.2 ± 2.8 (1st swab)	21
		28.2 ± 4.2 (2nd swab)	21
S.S.	5/17/86-6/8	32.6 ± 3.9	22
C.M.	5/24/86-6/11	50.6 ± 3.9	19
L.A.	6/16/86-7/8	37.4 ± 3.9 (1st swab)	23
		29.7 ± 2.9 (2nd swab)	23
W.B.	8/4/86-8/22	62.5 ± 4.5	19
R.R.	8/31/86-9/18	64.5 ± 3.5	17
M.P.	7/12/86-7/28	59.0 ± 7.4	17
J.C.	9/8/86-10/1	47.4 ± 2.5	24

APPENDIX 5

FIGURE 1

J.J.

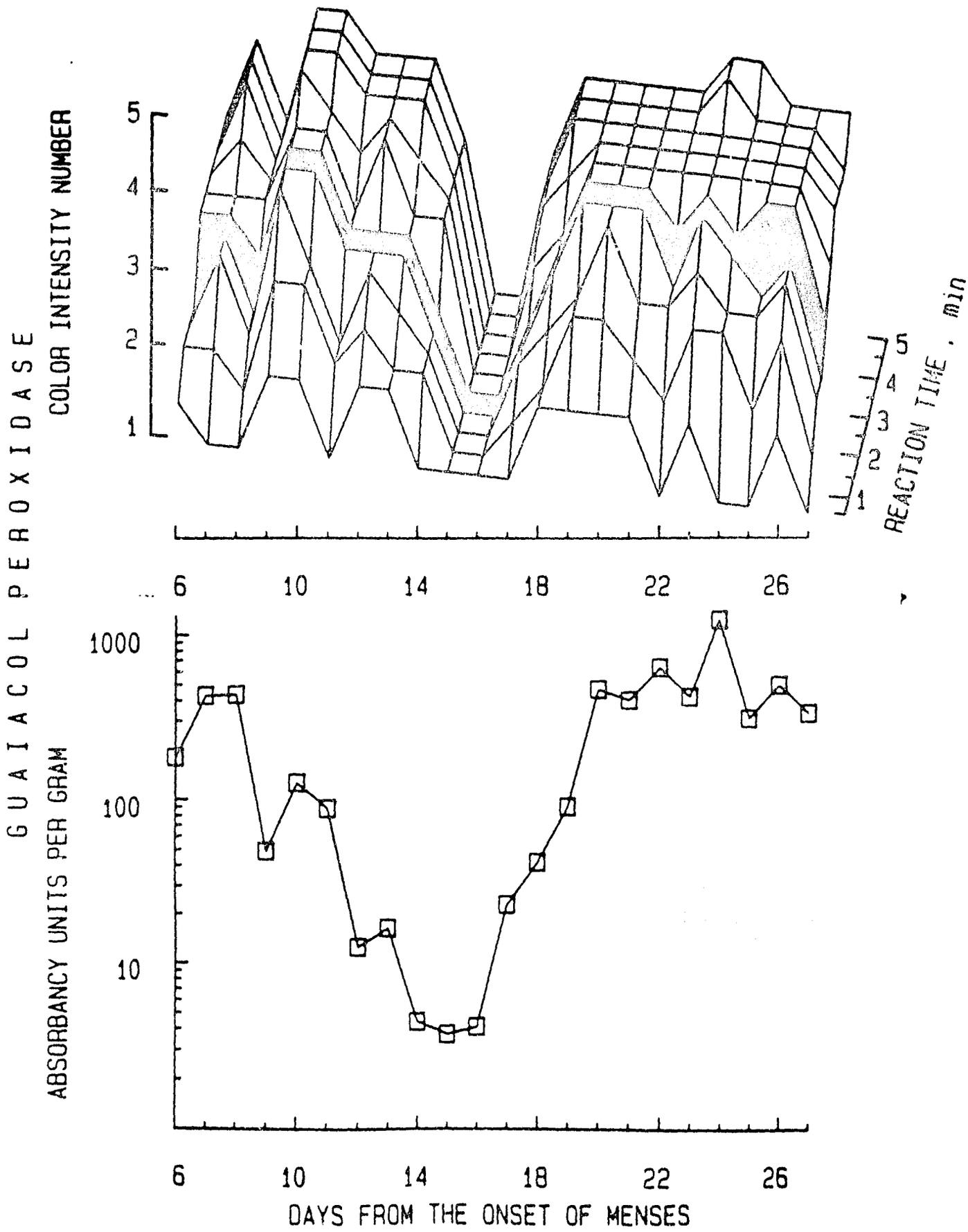
LEGEND TO FIGURE

The guaiacol peroxidase (GP) content of cervical mucus during the menstrual cycle of a volunteer.

The lower panel shows the results from cervical mucus sampled at the external os using a fertility cannula. The rate of the reaction was followed spectrophotometrically.

The upper panel shows the results from vaginal fluids sampled at the posterior fornix with a swab. After dipping the swab in a solution of guaiacol and H_2O_2, the developing color was compared every 30 sec to a set of 5 color standards (standard no. 1 is colorless, standard no. 5 is deep copper-red). As highlighted by the black zone between 2 and 2.5 min, the changes in the intensity/hue of the color are very large and allow the user to easily follow the dramatic pre-ovulation decline and post-ovulation rise in GP.

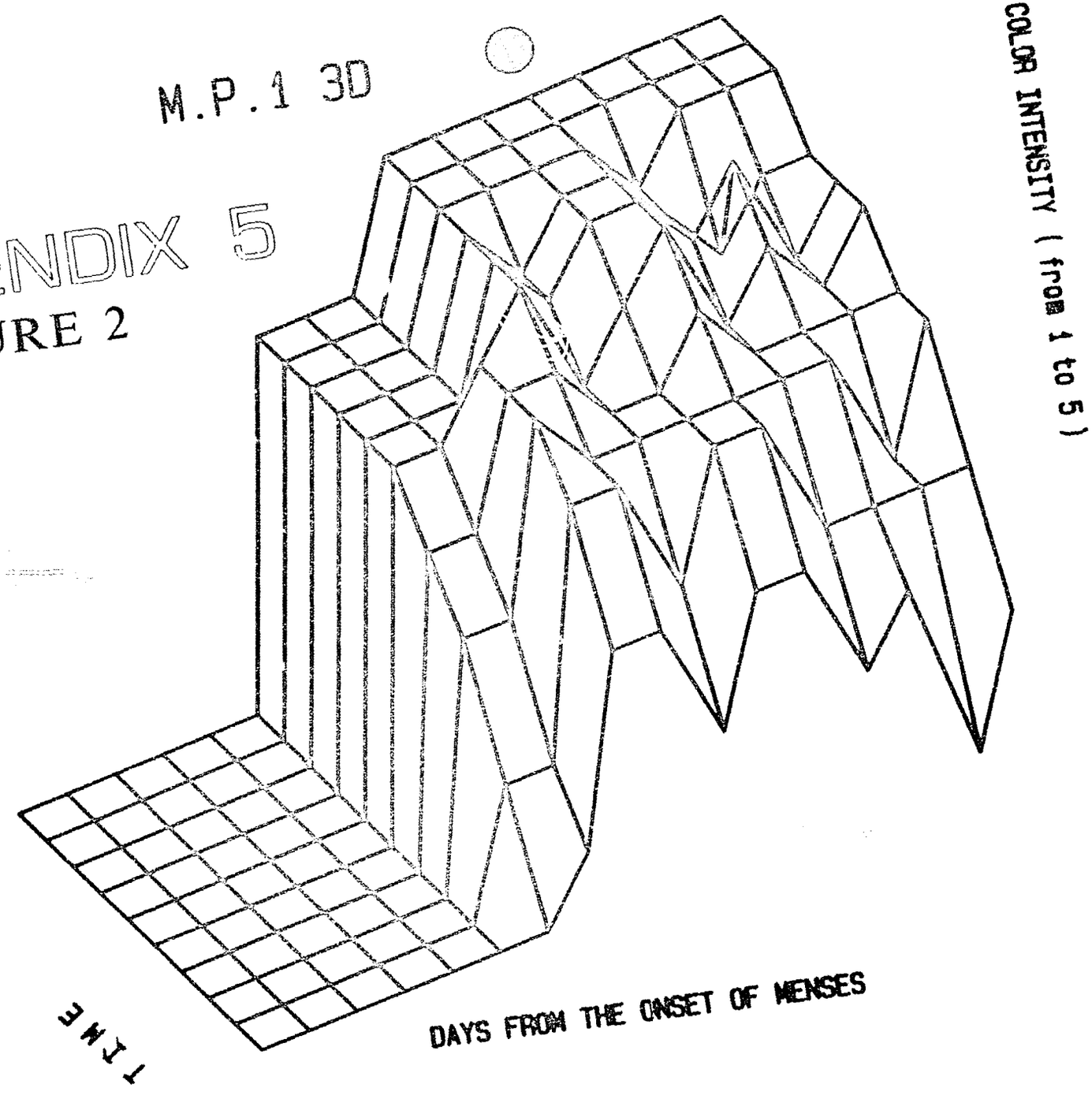
FIGURE 1


GUAIACOL PEROXIDASE
COLOR INTENSITY NUMBER
5
4
3
2
1
REACTION TIME, min
5
4
3
2
1
6
10
14
18
22
26
ABSORBANCY UNITS PER GRAM
1000
100
10
6
10
14
18
22
26
DAYS FROM THE ONSET OF MENSES

M.P.1 3D

APPENDIX 5

FIGURE 2

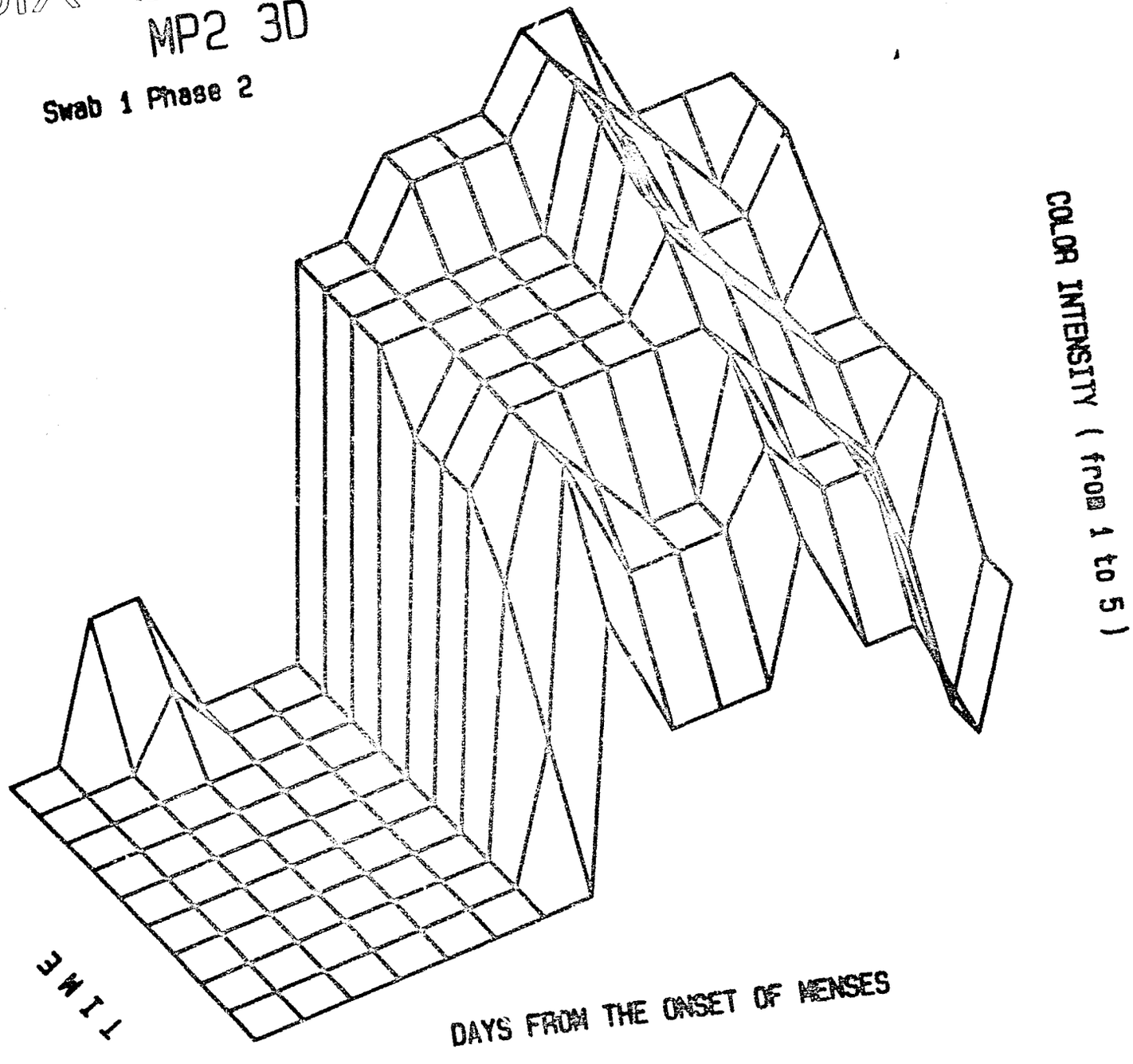


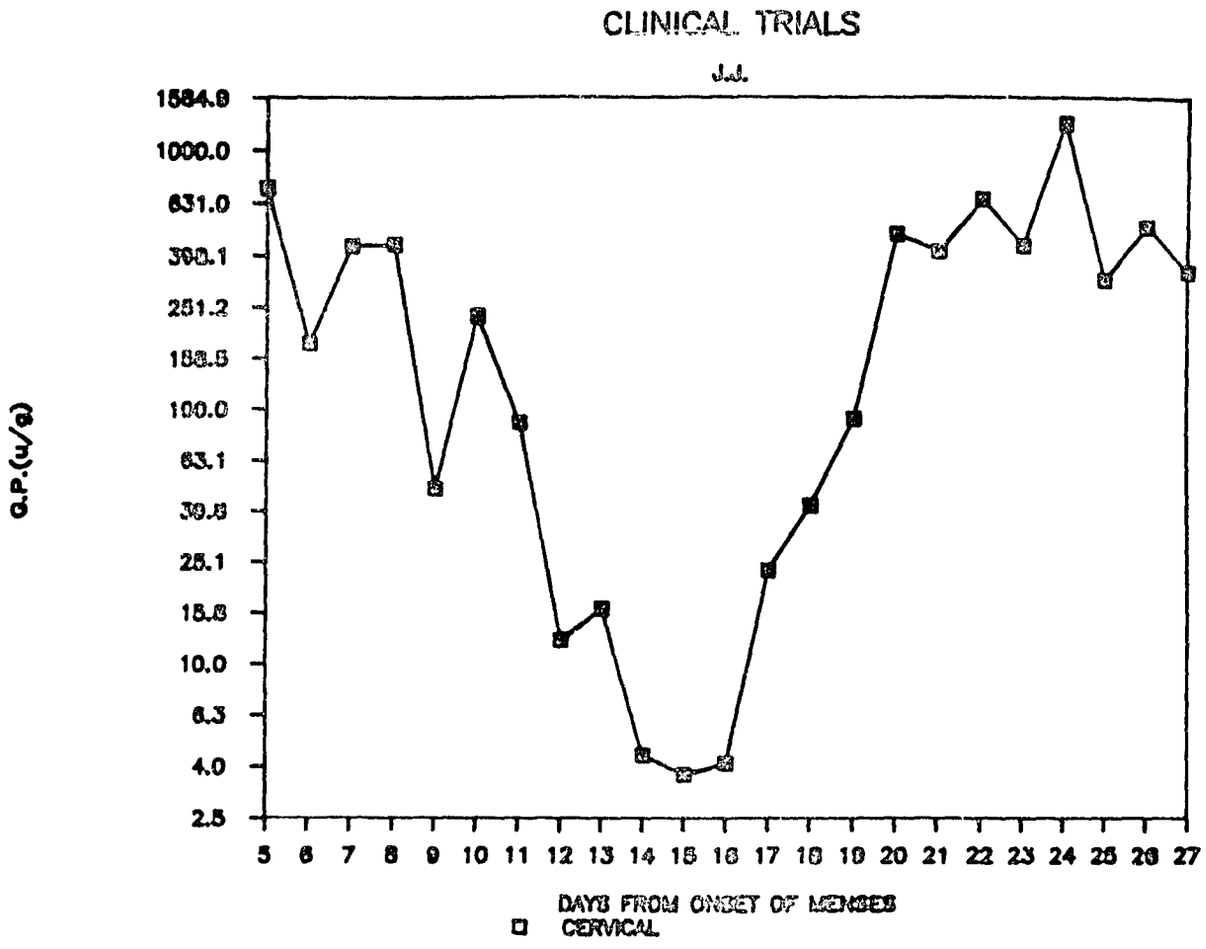
APPENDIX 5

FIGURE 3

MP2 3D

Swab 1 Phase 2




CLINICAL TRIALS
J.J.
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
G.P.(u/g)
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
DAYS FROM ONSET OF MENSES
CERVICAL

JJ1 CLI.3D for SD
JJ1 CLI.2D for GP vs DFOM

Phase 1 - Swab 1

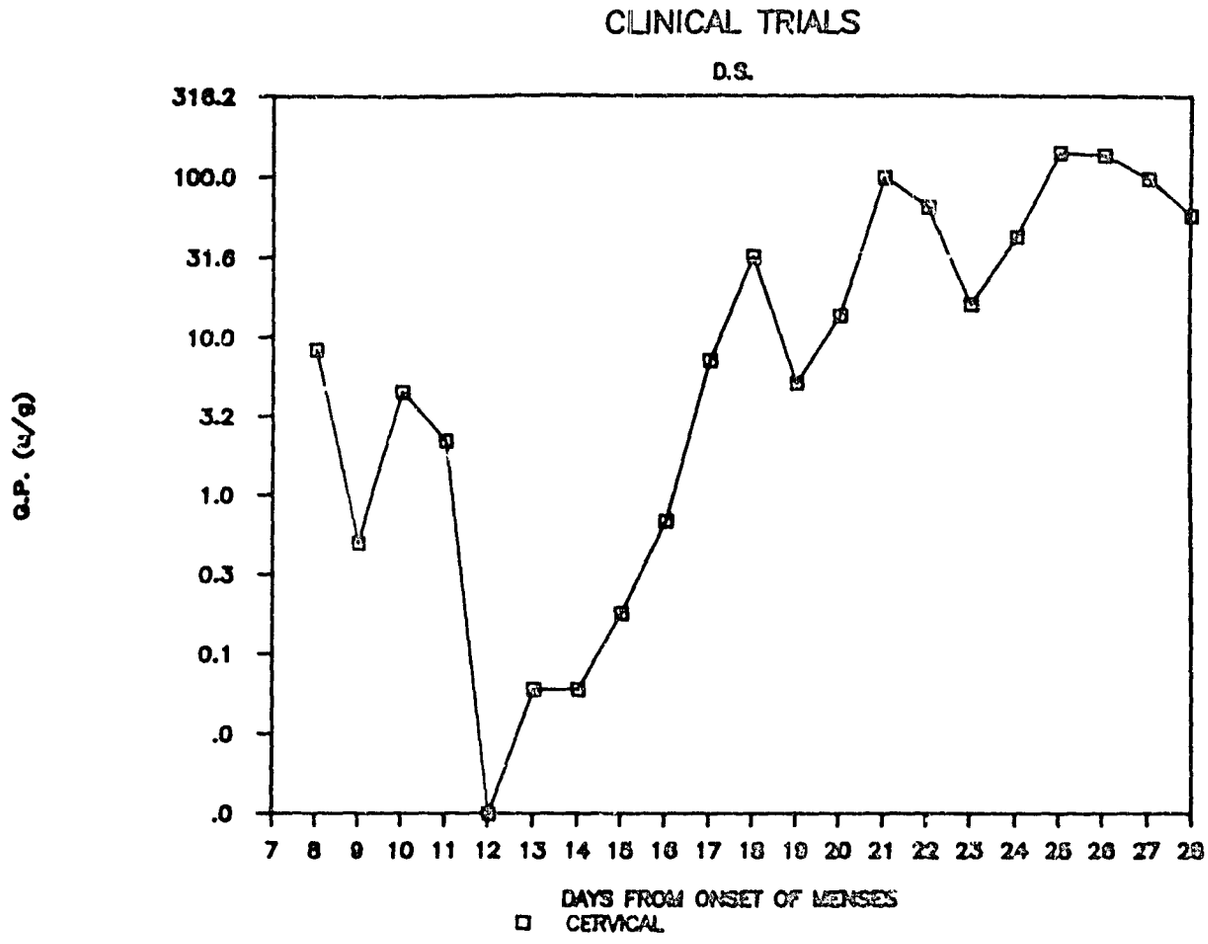
SWAB 1 WEIGHT (g)	Date		1 DFOM	2 Cx u/g	3	SWAB 1 4	5	6	7		
						30" 1	1.5 2	2.5 3	3.5 4	4.5	5
.087	4/15	1	5	716.6							
.039	4/16	2	6	179.7		1.5 2+	3 3	3 3	3 3	3	3
.055	4/17	3	7	428.0		2- 2	2+ 3-	3- 3-	3- 3-	2.5	2.
.060	4/18	4	8	433.2		1 1+	1+ 1+	1+ 1+	1+ 1	1	1
.096	4/19	5	9	45.4		3 4-	4 4	4+ 4.5	4.5 4.5	4.5	4.
.049	4/20	6	10	224.1 224	125.2	2 3-	4- 4	4 4	4 4+	4.5	4.
.041	4/21	7	11	87.6		2 3-	3+ 3.5	4 4	4 4+	4+	4
.047	4/22	8	12	12.4		2.5 4	4 4	4 4	4+ 4+	4.5	4.
.062	4/23	9	13	16.4		1 2	2+ 3	3 3.5	3.5 4	4	4
.079	4/24	10	14	4.4		1.5 2	3- 3	3+ 3+	3.5 4-	4-	4
.061	4/25	11	15	3.7		1 1	1 1	1	1 1		
.054	4/26	12	16	4.1							
.109	4/27	13	17	23.2		1 2-	2 2	2 2+	2+ 2+	2	2
.032	4/28	14	18	4.8		2 3	4 4	4 4	4 4	4	4
.032	4/29	15	19	90.9		2 3	4 4	4 4	4 4	4	4+
.064	4/30	16	20	473.3		2.5 4-	4 4+	4+ 4+	4+ 4+	4+	4+
.020	5/1	17	21	406.6		3- 4	4 4+	4+ 4+	4.5 4.5	4+	4-
.030	5/2	18	22	643.1		2.5 3-	4- 4	4 4	4 4-	4-	4
.033	5/3	19	23	426.7		2.5 3.5	4 4	4.5 4.5	4.5 4.5	5-	5
.017	5/4	20	24	1255.5		2+ 2.5	3+ 4	4 4-	4.5 4.5	4.5	5
.011	5/5	21	25	314.9		2.5 3.5	4 4	4 4.5	4.5 4.5	4.5	4.5
.034	5/6	22	26	504.1		3 4	4- 4-	4+ 4+	4+ 4+	4	4
.020	5/7	23	27	339.2		1.5 3-	3.5 4-	4 4	4 4	4	4
		24									
49.22		25		22 points							
± 25.17 SD											
± 5.37 SEM		26									
		27									
		28									
		29									
		30									
		31									

		1	2	3	4 PHASE I		5		6		7			
SWAB 2 WEIGHT-(g)		DATE	DFOM	Cx u/g	30	1	1.5	2	2.5	3	3.5	4	4.5	[illegible]
.035	1	4/15/86	5											
.032	2	4/16	6	179.7	1.5	3⁻	3⁺	3.5	3.5	3.5	3.5	3.5	3	3
.032	3	4/17	7	428.0	1.5	2.5	3	3	3	3	3	3	3	3
.054	4	4/18	8	433.2	1	1	1	1	1	1	1	1	1	
.077	5	4/19	9	48.4	3	4⁻	4	4	4⁺	4.5	4.5	4.5	4.5	4.
.053	6	4/20	10	229.1 *	2⁻	2.5	3⁺	3.5	3.5	4⁻	4⁻	4⁻	4⁻	4
.069	7	4/21	11	87.6	1	1	1⁺	1.5	2	2	2	2.5	2.5	3
.059	8	4/22	12	12.4	2.5	4⁻	4	4	4	4	4⁺	4⁺	4.5	4.
.057	9	4/23	13	16.4	1.5	2⁺	3	3.5	4	4	4	4	4	4
.058	10	4/24	14	4.4	1	1	1⁺	1.5	2	2	2⁺	2⁺	2.5	2.
.056	11	4/25	15	3.7	1	1	1⁺	2⁻	2	2	2	2⁺	2.5	3
.059	12	4/26	16	4.1	1	1	1	1⁺	1.5	2	2	2	2	2
.089	13	4/27	17	23.2	1	1	1	1⁺	2⁻	2⁻	2⁻	2⁻	2⁻	2
.024	14	4/28	18	41.8	2	2.5	3	3⁺	4	4	4	4	4	4
.014	15	4/29	19	90.9	2	2.5	3	4⁻	4⁻	4	4	4	4	4⁺
.025	16	4/30	20	473.3	3⁻	4	4	4⁺	4⁺	4⁺	4⁺	4⁺	4⁺	4
.020	17	5/1	21	406.6	3	4	4⁺	4.5	5⁻	5⁻	5⁻	4.5	4.5	4.
.022	18	5/2	22	643.1	1.5	2.5	2.5	3⁻	3⁻	3	3⁺	3⁺	3⁺	3.
.025	19	5/3	23	426.7	2⁻	3	4⁻	4⁻	4	4.5	4.5	4.5	5⁻	5
.008	20	5/4	24	1255.5	2	2⁺	3	4⁻	4⁻	4	4	4⁺	4⁺	4.
.024	21	5/5	25	314.9	3⁻	4	4⁺	4.5	4.5	4.5	4.5	4.5	4.5	4.
.027	22	5/6	26	504.1	2	3	3.5	4⁻	4	4	4⁺	4⁺	4	4
—	23	5/7	27	339.2	1⁺	2	3⁻	3⁺	4⁻	4	4	4	4	4
	24													
	25													
*	26	Cx #2 = 21.4 g												
	27													
	28													
	29													
	30													
	31													

JJ1 ...

SWAB 1 PHASE 2

ROOM	DATE	1		2		3		4		5		6	7
		30	1	1.5	2	2.5	3	3.5	4	4.5	5		
5	4/15 (1)												
6 ←	4/16 (2)	1.5	2	2+	2+	3-	3	3	3+	3	3		
7	4/17 (3)	1	2	3-	3	3+	3+	3.5	4-	4-	4-		
8	4/18 (4)	1	1.5	2	2	2.5	3-	3	3	3+	3+		
9	4/19 (5)	2	3	4-	4	4	4+	4.5	4.5	4.5	4.5		
10	4/20 (6)	2	3	3+	4	4	4+	4+	4.5	4.5	4.5		
11	4/21 (7)	1+	2	2+	3-	3	3+	3.5	4+	4	4		
12	4/22 (8)	2	2.5	3	3	3+	4-	4-	4	4	4		
13	4/23 (9)	2-	2	2.5	3-	3	3+	3.5	4-	4	4		
14	4/24 (10)	1	2	2	2	2+	3-	3-	3	3	3		
15	4/25 (11)	1	1	1	1	1				1	1		
16	4/26 (12)	1	1	1	1	1		1		1	1		
17	4/27 (13)	1	1.5	2	2	2	2.5	3-	3-	3	3		
18	4/28 (14)	2-	2	2.5	3	3	3	3.5	4-	4	4		
19	4/29 (15)	2	3	3	4-	4	4	4	4+	4+	4+		
20	4/30 (16)	2+	3+	4-	4	4	4+	4+	4+	4+	4+		
21	5/1 (17)	2-	2.5	3	4-	4-	4	4	4	4	4		
22	5/2 (18)	1+	2+	3-	3	3.5	4-	4	4	4	4		
23	5/3 (19)	2	3	3.5	4	4	4+	4+	4+	4.5	4.5		
24	5/4 (20)	2	3-	3	3.5	3.5	4	4	4	4+	4.5		
25	5/5 (21)	1+	2+	3	3+	4-	4	4	4	4	4		
26	5/6 (22)	2	3-	3.5	3.5	4-	4	4	4	4	4		
27	5/7 (23)	1+	1.5	2	2+	2.5	3	3.5	3.5	3.5	4-		
	24												
=22	25												
	26												
	27												
	28												
	29												
	30												
	31												


CLINICAL TRIALS
D.S.
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
.0
.0
Q.P. (u/g)
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
DAYS FROM ONSET OF MENSES
CERVICAL

DOMINIQUE S.
CLINICAL TRIALS

Swab 1 PAGE 1

	SWAB 1 weight		1 DATE	2 DFOM	3 CX u/g	4 PHASE 1 30"	1'	5 1.5	2.0	6 2.5	30	7 3.5	4.0	4.5	5.0
	.132g	1	4/18/86	7		1	1	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
	.132	2	4/19	8	8.3	1	1	1.5	1	1	1+	1+	1+	1+	1.5
	.129	3	4/20	9	0.5	1	1	1	1	1	1	1	1+	1+	1+
	.075	4	4/21	10	4.5	1	1	1	1	1	1	1	1	1	1
	.053	5	4/22	11	2.2	1	1	1	1	1	1	1	1	1	1
	.092	6	4/23	12	0	1	→								→
	.086	7	4/24	13	0.06	1	→								→
	.062	8	4/25	14	0.06	1	→								→
	.134	9	4/26	15	0.18	1	→								→
	.042	10	4/27	16	0.69	1	1	1	1	1	1+	1.5	2-	2-	2
	.063	11	4/28	17	*7.16 / 1.94	1	→								→
	.034	12	4/29	18	32.0	1	→								→
	.047	13	4/30	19	5.1	1	→								→
	.014	14	5/1	20	136	1	→								→
	.021	15	5/2	21	98.7	1	→								→
	.034	16	5/3	22	64.7	1	→								→
	.011	17	5/4	23	16.1	1	→								→
	.023	18	5/5	24	42.6	1	→								→
	.035	19	5/6	25	141.0	1	→								→
	.02	20	5/7	26	136.3	1	→								→
	.072	21	5/8	27	32.05 / 97.85	1	→								→
	.044	22	5/9	28	577	1	→								→
		23	end												
		24													
		25													
		26													
		27													
		28	* 2 CX samples were		taken										
		29													
		30													
		31													

SWAB 2

	1 PHASE 1		2		3		4		5		6	7	
	30"	1'	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0		Swab 2 weight	
4/18/86 1	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5		.142g	
4/19 2	1	1	1	1	1+	1.5	1.5	1.5	1.5	1.7		.132	
4/20 3	1	1	1	1	1	1	1	1+	1+	1+		.132	
4/21 4	1	1	1	1	1	1	1	1	1	1		.083	
4/22 5	1	—	—	—	—	—	—	—	—	→		.052	
4/23 6	1	—	—	—	—	—	—	—	—	→		.059	
4/24 7	1	—	—	—	—	—	—	—	—	→		.07	
4/25 8	1	—	—	—	—	—	—	—	—	→		.051	
4/26 9	1	—	—	—	—	—	—	—	—	→		.099	
4/27 10	1	1	1	1	1	1	1	1+	1+	1.3		.066	
4/28 11	1	1	1	1	1	1.5	2-	2	2	2		.033	
4/29 12	1	—	—	—	—	—	—	—	—	→		.073	
4/30 13	1	—	—	—	—	—	—	—	—	→		.049	
5/1 14	1	—	—	—	—	—	—	—	—	→		.052	
5/2 15	1	—	—	—	—	—	—	—	—	→		.017	
5/3 16	1	—	—	—	—	—	—	—	—	→		.028	
5/4 17	1	—	—	—	—	—	—	—	—	→		.017	
5/5 18	1	—	—	—	—	—	—	—	—	→		.014	
5/6 19	1	—	—	—	—	—	—	—	—	→		.022	
5/7 20	1	—	—	—	—	—	—	—	—	→		.018	
5/8 21	1	—	—	—	—	—	—	—	—	→		.077	
5/9 22	1	—	—	—	—	—	—	—	—	→		.020	
23													
24													
25													
26													
27													
28													
29													
30													
31													

DOMINIQUE S.

CLINICAL TRIALS

SWAB 1

	1 PHASE 2		2		3		4		5		6	7	
	30"	1'	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0			
4/18/86 1	/	/	/	/		/		/		/			
4/19 2	/	/	/	/	/	/	/	/	/	/			
4/20 3	/	/	/	/	/	/	/	/	/	/			
4/21 4	/	/	/	/	/	/	/	/	/	/			
4/22 5	/	→	→	→	→	→	→	→	→	→			
4/23 6	/	→	→	→	→	→	→	→	→	→			
4/24 7	/	→	→	→	→	→	→	→	→	→			
4/25 8	/	→	→	→	→	→	→	→	→	→			
4/26 9	/	→	→	→	→	→	→	→	→	→			
4/27 10	/	→	→	→	→	→	→	→	→	→			
4/28 11	/	→	→	→	→	→	→	→	→	→			
4/29 12	/	→	→	→	→	→	→	→	→	→			
4/30 13	/	→	→	→	→	→	→	→	→	→			
5/1 14	/	→	→	→	→	→	→	→	→	→			
5/2 15	/	→	→	→	→	→	→	→	→	→			
5/3 16	/	→	→	→	→	→	→	→	→	→			
5/4 17	/	→	→	→	→	→	→	→	→	→			
5/5 18	/	→	→	→	→	→	→	→	→	→			
5/6 19	/	→	→	→	→	→	→	→	→	→			
5/7 20	/	→	→	→	→	→	→	→	→	→			
5/8 21	/	→	→	→	→	→	→	→	→	→			
5/9 22	/	→	→	→	→	→	→	→	→	→			
23													
24													
25													
26													
27													
28													
29													
30													
31													

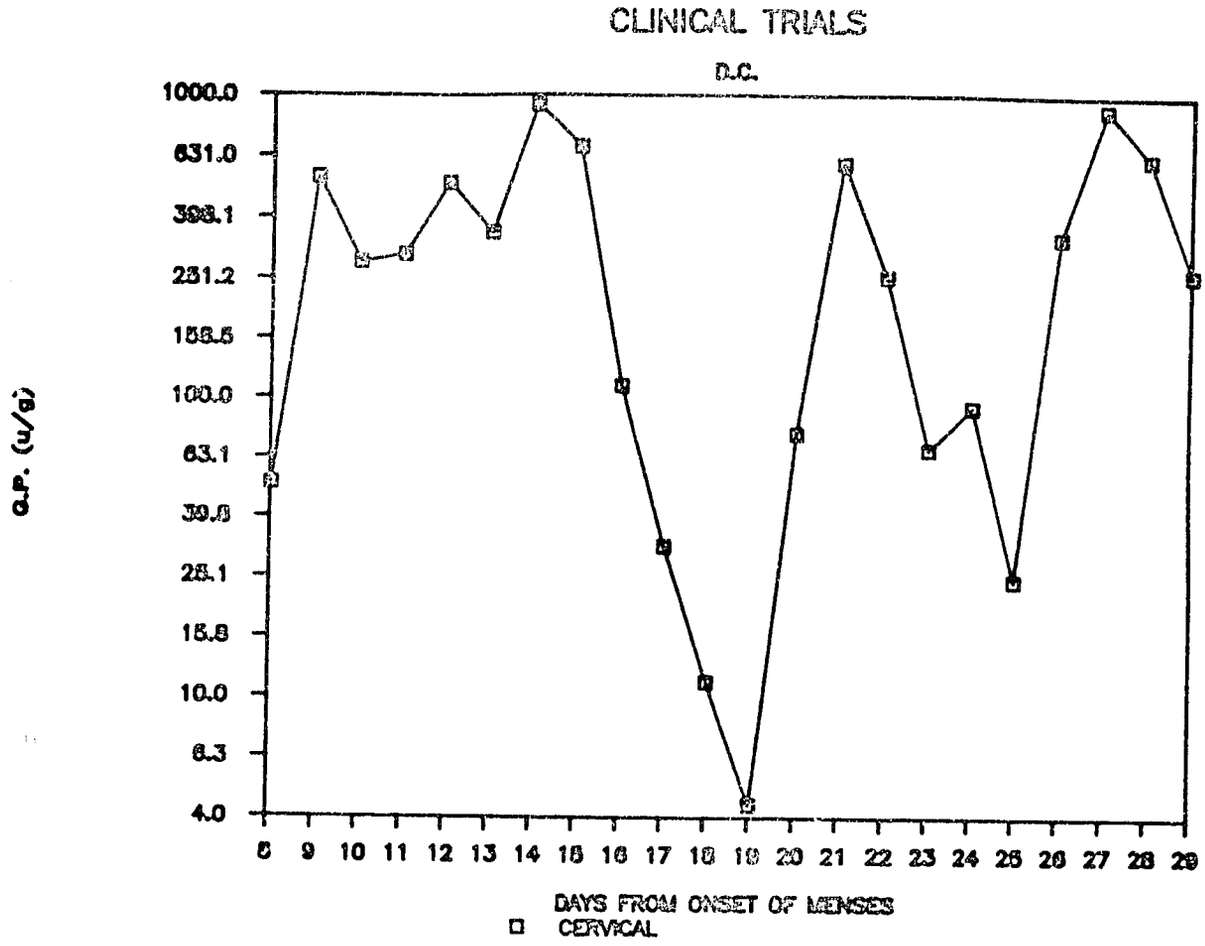
DOMINIQUE S.

CLINICAL TRIALS

PAGE 4

SWAB 2

	1 PHASE 2		2		3		4		5		6	7	
	30"	1'	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0			
4/18/86 1	1	1	-	1	-	1	-	1	-	1			
4/19 2	1	1	1	1	1	1	1	1	1.2	1.2			
4/20 3	1	1	1	1	1	1	1	1	1	1			
4/21 4	1	1	1	1	1	1	1	1	1	1			
4/22 5	1	—	—	—	—	—	—	—	→				
4/23 6	1	—	—	—	—	—	—	—	→				
4/24 7	1	—	—	—	—	—	—	—	→				
4/25 8	1	—	—	—	—	—	—	—	→				
4/26 9	1	—	—	—	—	—	—	—	→				
4/27 10	1	1	1	1	1	1	1	1	1+	1+			
4/28 11	1	—	—	—	—	—	—	—	→				
4/29 12	1	—	—	—	—	—	—	—	→				
4/30 13	1	—	—	—	—	—	—	—	→				
5/1 14	1	—	—	—	—	—	—	—	→				
5/2 15	1	—	—	—	—	—	—	—	→				
5/3 16	1	—	—	—	—	—	—	—	→				
5/4 17	1	—	—	—	—	—	—	—	→				
5/5 18	1	—	—	—	—	—	—	—	→				
5/6 19	1	—	—	—	—	—	—	—	→				
5/7 20	1	—	—	—	—	—	—	—	→				
5/8 21	1	—	—	—	—	—	—	—	→				
5/9 22	1	—	—	—	—	—	—	—	→				
23													
24													
25													
26													
27													
28													
29													
30													
31													


CLINICAL TRIALS
D.C.
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
G.P. (u/g)
8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
DAYS FROM ONSET OF MENSES
CERVICAL

FILE DCIICLL.SD

$\Phi = -105$
$\theta = 55$

DIANE C.
CLINICAL TRIALS

SWAB 1

PAGE 1

swab 1 weight		1 DATE	2 DFOM	3 PHASE 1 Cx U/g	4 30"	4 1'	5 1.5	5 2.0	6 2.5	6 3.0	7 3.5	7 4.0	4.5	[illegible]
.025	1	4/21/86	8	51.7	1	2-	2+	3-	3	3+	4-	4-	4	4
.037	2	4/22	9	540.5	2.5	4-	4	4.5	5-	5-	5-	4.5	4.5	4.5
.036	3	4/23	10	2946	2.5	3.5	4	4+	4.5	4.5	4.5	4+	4	4
.038	4	4/24	11	301.7	2+	4-	4	4.5	5	5-	5-	4.5	4.5	4.5
.036	5	4/25	12	517.7	2	4-	4	4+	4.5	4.5	4.5	4+	4+	4+
.031	6	4/26	13	354.85	3-	4-	4+	4.5	4.5	5-	5-	4.5	4.5	4.5
.060	7	4/27	14	926.96	3	4	4	4.5	4.5	4+	4	4	4	4
.047	8	4/28	15	681.0	3	4	4	4.5	4.5	4+	4	4	4	4
.049	9	4/29	16	110.1	1.5	2+	3	4	4	4	4	4	4	4
.042	10	4/30	17	32.0	3-	4-	4	4+	4+	4+	4.5	4.5	4.5	4.5
.032	11	5/1	18	1.0 * / 11.3	1	2	2.5	3-	3	3.5	4-	4-	4	4
.015	12	5/2	19	4.4	1	2	2	2+	3-	3	3+	3.5	3.5	4-
.020	13	5/3	20	76.68	1.5	2+	3-	3+	4-	4-	4	4	4+	4+
.015	14	5/4	21	597.76	1.5	3-	3	3.5	4-	4	4.5	4.5	5-	[illegible]
.020	15	5/5	22	254.17	1.5	2+	2+	3	3.5	3.5	4-	4	4	4
.021	16	5/6	23	67.3	2+	4	4	4+	4.5	4.5	4.5	4.5	4.5	4.5
.013	17	5/7	24	93.4	1.5	3	3.5	4	4+	4+	4.5	4.5	4.5	4.5
.017	18	5/8	25	24.67	1	2	3	3.5	4-	4-	4-	4-	4-	4-
.021	19	5/9	26	342.22	1+	2.5	3.5	4	4+	4.5	4.5	4.5	4.5	4.5
.038	20	5/10	27	906.7	2-	2.5	3	3.5	4-	4	4.5	4.5	5-	5-
.021	21	5/11	28	622.6	2	3	3.5	4+	4.5	4.5	5-	5-	5-	5-
—	22	5/12	29	259.4	1	1.5	2-	2	2+	3-	3	3	3	3
	23	end												
	24													
	25													
	26													
	27													
	28			* 2 Cx										
	29			Samples										
	30													
	31													

SWAB 2

	1 PHASE I		2		3		4		5		6	7
	30"	1'	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0		Swab 2 weight
4/21/86 1	1	2-	2+	3-	3	3	3.5	4-	4	4		.010
4/22 2	2+	4-	4	4.5	5-	5-	5-	4.5	4.5	4.5		.034
4/23 3	2.5	3.5	4	4+	4.5	4.5	4.5	4+	4	4		.030
4/24 4	2+	4-	4	4.5	5-	5-	5-	4.5	4.5	4.5		.037
4/25 5	3-	4	4	4+	4+	4+	4+	4+	4+	4+		.043
4/26 6	3-	4-	4+	4.5	4.5	5-	5-	4.5	4.5	4.5		.031
4/27 7	2	3-	3	4-	4	4	4	4	4	4		.049
4/28 8	3	4	4	4.5	4.5	4+	4	4	4	4		.037
4/29 9	1+	2	3-	4-	3.5	3.5	4-	4-	4	4		.045
4/30 10	2.5	3+	3.5	4	4+	4+	4.5	4.5	4.5	4.5		.040
5/1 11	1	1	1.5	2-	2	2	2+	2.5	3-	3-		.018
5/2 12	1+	2+	2.5	3-	3.5	4-	4-	4-	4-	4-		.022
5/3 13	1	1	1+	1.5	2	2	2+	2+	2.5	2.5		.007
5/4 14	1	2	3	3+	4-	4	4.5	4.5	5-	5-		.013
5/5 15	1.5	2	3-	3+	4-	4-	4+	4+	4+	4+		.015
5/6 16	2	3	3.5	4-	4	4	4+	4+	4.5	4.5		.015
5/7 17	1	2	2.5	3	4-	4	4	4	4	4		.004
5/8 18	1	1+	1.5	2	2.5	2.5	3-	3-	3-	3-		.089
5/9 19	1+	2+	3	3.5	3.5	4	4.5	4.5	4.5	4.5		.013
5/10 20	1.5	2	2.5	3	3.5	4-	4	4.5	5-	5-		.014
5/11 21	1.5	2	3	3.5	4-	4	5	5	5-	5-		.027
5/12 22	1+	2+	3	3+	3+	3.5	4-	4	4	4		—
23												
24												
25												
26												
27												
28												
29												
30												
31												

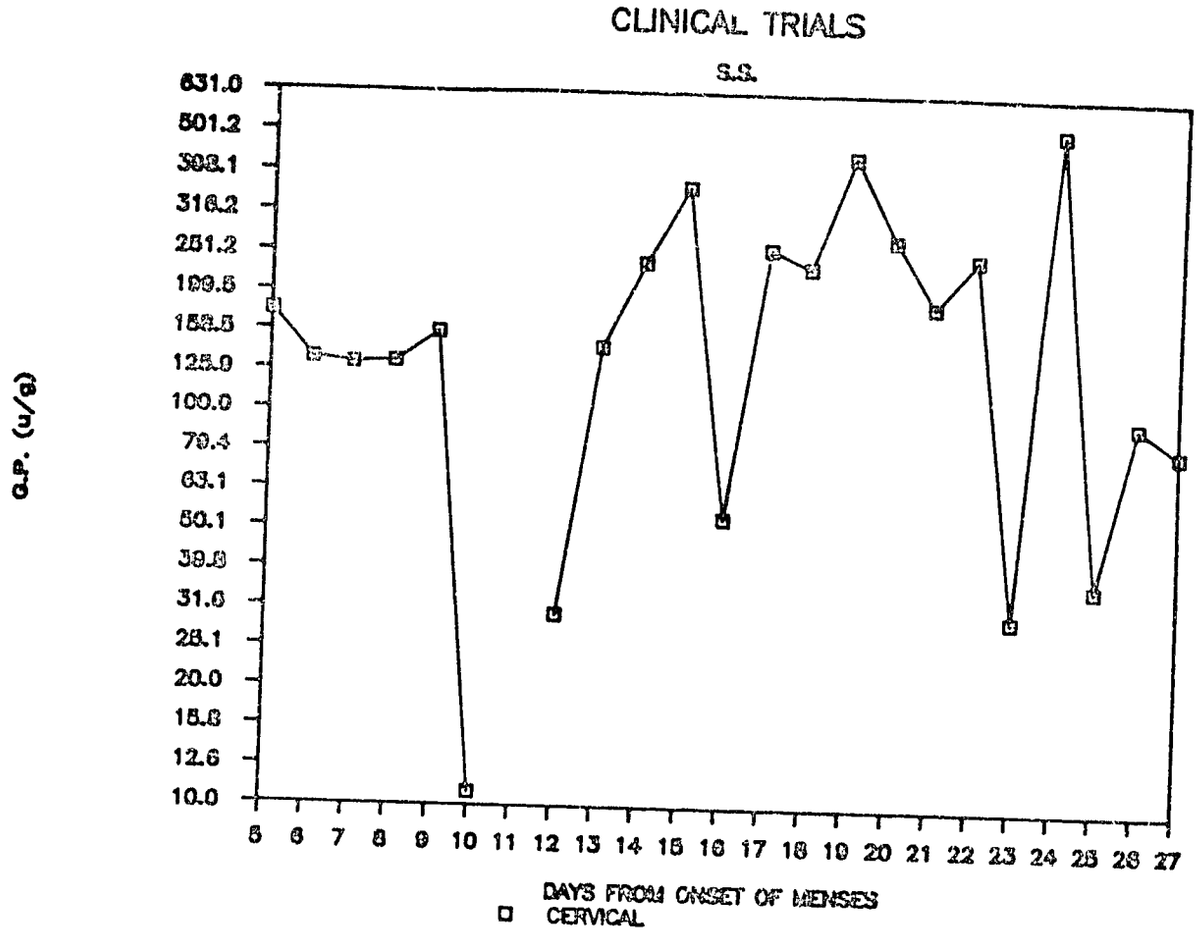
SWAB 1

		1 PHASE 2		2		3		4		5		6	7
		30"	1'	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0		
4/21/86	1	1.5	2	2.2	3-	3	3	3.2	3.5	3.8	4-		
4/22	2	2.8	4	4	4	4+	4.5	4.7	4.5	4+	4+		
4/23	3	2+	3	4	4	4+	4+	4+	4+	4+	4+		
4/24	4	3-	4-	4	4	4.5	4.5	4.5	4.5	4.5	4.5		
4/25	5	2	3	3+	4	4	4	4+	4+	4+	4+		
4/26	6	2	3	4-	4	4	4.5	5-	5-	5-	5-		
4/27	7	2.5	4-	4	4	4	4+	4.3	4.5	4.5	4.5		
4/28	8	3-	4-	4	4.5	4.5	4.5	4.5	4.5	4.5	4.5		
4/29	9	2	3-	3	3.5	4-	4	4	4	4+	4+		
4/30	10	2	3+	4	4	4	4+	4+	4+	4+	4+		
5/1	11	1	2-	2-	2	2.5	2.5	2.5	3-	3	3+		
5/2	12	1	1+	1+	1+	1.5	2-	2	2	2+	2+		
5/3	13	1.5	2	3	3	3+	3.5	4-	4	4	4		
5/4	14	1.5	2	2.5	2.5	3	3+	3+	3.5	4-	4		
5/5	15	1+	2+	2+	3	3.5	3.5	4-	4-	4	4		
5/6	16	2-	2.5	3	3.5	4-	4	4	4	4+	4+		
5/7	17	1.5	2+	2+	3-	3	3.5	4-	4-	4	4		
5/8	18	1+	1.5	2-	2	2	2	2.5	2.5	3-	3-		
5/9	19	1+	2.5	2.5	3+	3.5	4-	4	4+	4.5	4.5		
5/10	20	2-	2.5	3-	3	3.5	3.5	4-	4	4+	4+		
5/11	21	2-	2.5	3	3.5	4-	4	4+	4.5	4.5	5-		
5/12	22	1	1+	1+	1.5	1.5	2-	2-	2	2	2		
	23												
	24												
	25												
	26												
	27												
	28												
	29												
	30												
	31												

DIANE C.
CLINICAL TRIALS
PAGE 4

SWAB 2

	1 PHASE 2		2		3		4		5		6	7
	30"	1'	1.5	2.0	2.5'	3.0'	3.5'	4.0'	4.5'	5.0		
4/21/86 1	1	2	2-	2-	2	2	2	2	2+	2+		
4/22 2	2.5	4-	4-	4	4	4+	4.5	4+	4+	4+		
4/23 3	2	3-	3	3.5	4-	4	4	4	4	4		
4/24 4	2+	3+	4-	4	4.5	4.5	4.5	4.5	4.5	4.5		
4/25 5	3-	4-	4	4+	4+	4+	4.5	4.5	4+	4+		
4/26 6	2	3	4-	4	4	4.5	5-	5-	5-	5-		
4/27 7	2	3-	3	4-	4-	4-	4	4+	4+	4+		
4/28 8	3-	3.5	4	4.5	4.5	4.5	4.5	4.5	4.5	4.5		
4/29 9	2-	2	2	3-	3-	3	3	3	3.5	4-		
4/30 10	2-	3	4-	4	4	4+	4+	4+	4+	4+		
5/1 11	1	1+	1+	1.5	1.5	2-	2-	2-	2-	2-		
5/2 12	1	1+	1.5	1.5	2	2+	2+	2.5	3-	3-		
5/3 13	1	1	1	1+	1+	1.5	2-	2-	2	2		
5/4 14	1+	2-	2	2	2.5	3-	3-	3	3	3		
5/5 15	2	3	3.5	4-	4	4	4+	4+	4.5	4.5		
5/6 16	1.5	2	3+	2.5	2.5	3-	3	3	3.5	3.5		
5/7 17	1+	2	2	2	2+	3	3	3	3	3		
5/8 18	1	1	1	1+	1+	1+	1+	1.5	2-	2-		
5/9 19	1	1.5	2+	2+	2+	3-	3-	3	3+	3+		
5/10 20	1+	2-	2	2	2.5	2.5	3-	3	3+	3+		
5/11 21	2-	2.5	3	3.5	4-	4	4+	4.5	4.5	5-		
5/12 22	1+	1.5	2-	2	2	2+	2+	2+	2+	2+		
23												
24												
25												
26												
27												
28												
29												
30												
31												


CLINICAL TRIALS
S.S.
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
G.P. (u/g)
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
DAYS FROM ONSET OF MENSES
CERVICAL

SWAB 1

PAGE

		1	2	3	4 PH-1		5		6		7		
SWAB 1 Weight		DATE	DFOM	CX u/g	30"	1'	1.5	2	2.5	3	3.5	4	4.5
.008 g	1	5/17/86	5	177.8	2	2.5	3+	3.5	4.0	4+	4+	4+	4+
.007	2	5/18	6	134.5	2-	2.5	3	3.5	4-	4+	4+	4.5	5-
.005	3	5/19	7	131.4	2	2.5	3+	4-	4+	4.5	5-	5	5
.034	4	5/20	8	132.5	3	4	4	4	4+	4+	4.5	4.5	4.5
.014	5	5/21	9	157.9	2	3+	4	4	4+	4.5	4.5	4.5	4.5
.017	6	5/22	10	10.8	2+	3+	4-	4	4+	4+	4+	4+	4-
—	7	5/23	11	NO	SHOW								
.021	8	5/24	12	30.4	1.5	2	2.5	3	3+	4-	4	4	4.5
.014	9	5/25	13	145.3	2	3-	3	3.5	4-	4	4	4.5	4.5
.046	10	5/26	14	238.0	2	3	4+	4+	4.5	4.5	5-	5-	5-
.028	11	5/27	15	367.4	1+	2	3-	3	4-	4	4+	4	4
.034	12	5/28	16	53.9	2	3	3.5	4-	4	4	4-	4+	4+
.032	13	5/29	17	259.1	2	3-	3+	3.5	4-	4	4	4	4
.038	14	5/30	18	232.9	2	3-	3	3.5	4-	4	4	4	4
.032	15	5/31	19	441.5	3	4-	4	4	4+	4+	4+	4	4
.052	16	6/1	20	274.7	3	4	4	4	4	4	4	4	4
.056	17	6/2	21	188.1	2	3	3+	4-	4	4	4+	4+	4+
.033	18	6/3	22	249.3	3-	4-	4	4+	4+	4+	4+	4.5	4.5
.018	19	6/4	23	30.5	1	1.5	2	2+	2+	3-	3	3	3
.027	20	6/5	24	516.2	3-	3+	4	4+	4+	4.5	4.5	4.5	4.5
.047	21	6/6	25	36.9	1	1	1.5	2	2	2.5	3-	3	3
.078	22	6/7	26	96.1	2+	3+	4-	4	4+	4+	4+	4	4
.054	23	6/8	27	81.8	1+	2+	4-	4	4	4	4+	4.5	4.5
31.59	24												
± 18.52 SD	25												
± 3.95 SEM	26												
	27												
	28												
	29												
	30												
	31												

SWAB 2

PAGE

	1 Pit - 1		2		3		4		5		6	7
	30"	1	1.5	2	2.5	3	3.5	4	4.5	5		SWAB 2 WEIGHT
5/17/86 1	2.5	3+	4-	4	4+	4+	4+	4+	4+	4+		.013g
5/18 2	1	1+	1.5	2	2	2+	2.5	2.5	3+	3+		.001
5/19 3	2.5	3+	4+	4.5	5-	5-	5	5	5	5		.004
5/20 4	2.5	4-	4	4	4+	4+	4.5	4.5	4.5	4.5		.014
5/21 5	1.5	3	3+	4-	4+	4.5	4.5	4.5	4.5	4.5		.022
5/22 6	2	3	3+	4-	4	4+	4+	4+	4+	4+		.021
5/23 7		NO	SHOW									—
5/24 8	2	2.5	3+	3.5	4+	4.5	4.5	5-	5-	5-		.014
5/25 9	3	4-	4	4+	4.5	5-	5-	4.5	4.5	4.5		.032
5/26 10	2	3	4+	4+	4.5	4.5	4.5	4.5	4.5	4.5		.040
5/27 11	1	2-	2.5	3-	3	3.5	3.5	4	4	4		.018
5/28 12	2	3	3.5	4-	4	4	4+	4+	4+	4+		.028
5/29 13	3-	3+	4	4+	4+	4+	4+	4+	4	4		.019
5/30 14	3-	4-	4	4	4+	4+	4+	4	4	4		.034
5/31 15	3	4-	4	4	4	4+	4+	4+	4+	4+		.018
6/1 16	3-	4-	4	4+	4+	4+	4	4	4	4		.028
6/2 17	2	3	3.5	4-	4	4	4+	4+	4-	4-		.028
6/3 18	3-	4-	4	4+	4+	4+	4+	4.5	4.5	4.5		.022
6/4 19	1	1.5	2	2+	2.5	2.5	3-	3-	3-	3-		.010
6/5 20	3-	3	4-	4+	4+	4.5	4.5	4.5	4.5	4.5		.023
6/6 21	1	1	1	1	1	1	1	1	1+	1+		.044
6/7 22	2	3-	3	3	3.5	4	4	4	4	4		.054
6/8 23	2+	3+	3	3	4+	4+	4+	4.5	4.5	4.5		.050
24												
25												
26												
27												
28												
29												
30												
31												

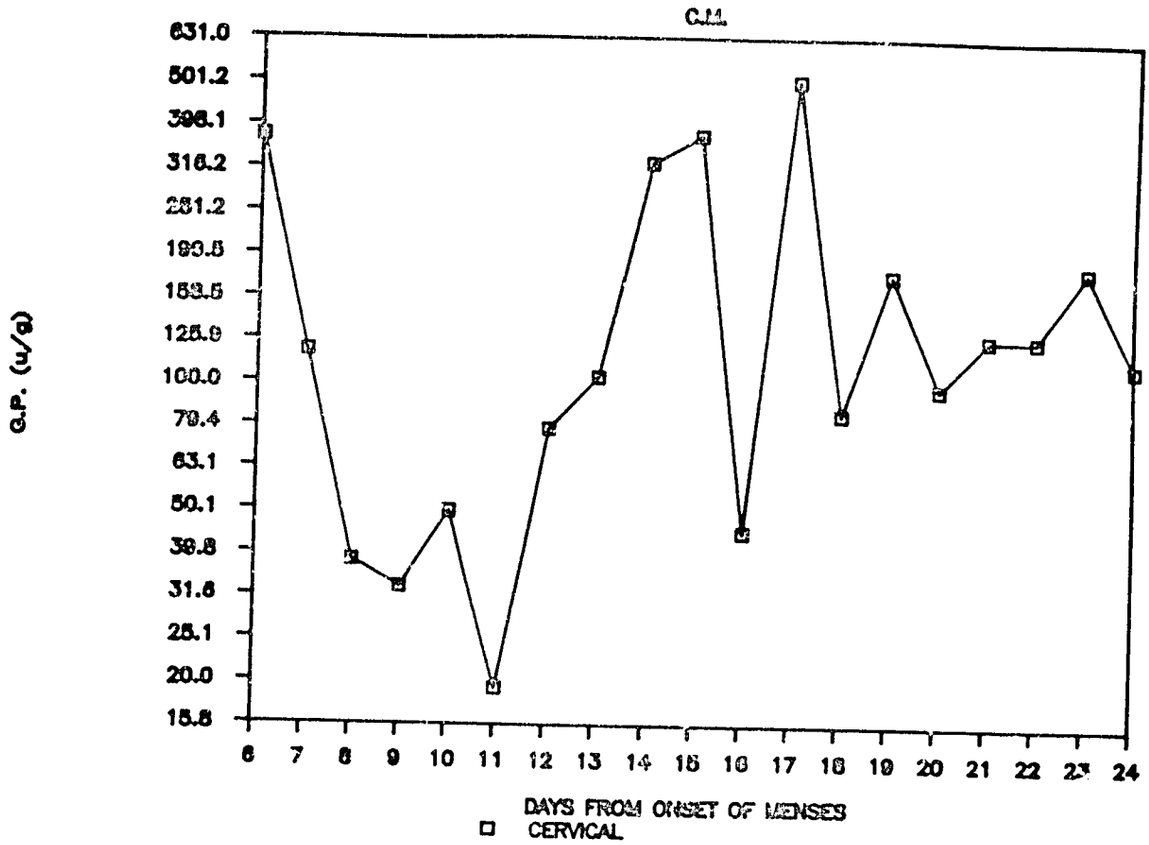
SWAB 1

	1 PH-2		2		3		4		5		6	7
	30"	1	1.5	2	2.5	3	3.5	4	4.5	5		
5/17/80	1+	2-	2+	2.5	3	3	3+	3.5	3.5	3.5		
5/18 2	1.5	1.5	2	2	2+	2+	2.5	2.5	3-	3		
5/19 3	1+	2	2.5	2.5	3-	3-	3	3+	3.5	3.5		
5/20 4	2	3-	3+	4-	4	4	4+	4.5	4.5	4.5		
5/21 5	2-	2.5	3	3+	3.5	4-	4-	4	4	4		
5/22 6	1+	2.5	3-	3	3	3+	3.5	4-	4-	4-		
5/23 7	no show											
5/24 8	1.5	2-	2.5	3-	3-	3.5	4-	4	4	4		
5/25 9	1.5	2	2+	2.5	3-	3-	3.5	4	4	4+		
5/26 10	2	3-	3	3+	3.5	4-	4-	4	4	4		
5/27 11	2	2+	2.5	3-	3	3	3	3+	3+	3.5		
5/28 12	2	3-	3	3+	3.5	4	4	4	4+	4+		
5/29 13	2-	2	3-	3	3+	3.5	4-	4	4	4		
5/30 14	2-	2	3-	3	3	3+	4-	4	4	4		
5/31 15	2	3	3+	4-	4	4	4	4-	4	4		
6/1 16	3	4	4	4	4	4	4	4+	4+	4+		
6/2 17	2	3-	3	3+	4-	4-	4	4	4+	4+		
6/3 18	2-	2	2.5	3-	3	3+	3+	3.5	4-	4-		
6/4 19	1	1	1+	1.5	2-	2-	2	2	2	2		
6/5 20	2	2+	3-	3	3	3.5	3.5	4-	4	4		
6/6 21	1	1	1+	2-	2	2	2	2+	2.5	2.5		
6/7 22	2.5	3+	4	4	4+	4+	4+	4+	4	4		
6/8 23	2-	3-	3	3.5	4-	4	4	4+	4+	4+		
24												
25												
26												
27												
28												
29												
30												
31												

SWAB 2

	1 PH-2		2		3		4		5		6	7
	30"	1	1.5	2	2.5	3	3.5	4	4.5	5		
5/17/86 1	2	3-	3+	4-	4	4	4+	4+	5-	5-		
5/18 2	1	1	1	1	1	1	1	1	1	1		
5/19 3	1+	2	2+	2.5	3-	3-	3	3+	3.5	3.5		
5/20 4	2-	2	3-	3	3.5	4-	4	4	4.5	5		
5/21 5	2-	2	2.5	3-	3	3+	3.5	3.5	3.5	4-		
5/22 6	1+	2+	2+	2.5	3-	3-	3	3	3	3		
5/23 7		NO	SHOW									
5/24 8	2+	3-	3+	3.5	3.5	4-	4+	4.5	5-	5-		
5/25 9	2+	3	3+	4-	4	4	4.5	5	5	4.5		
5/26 10	2-	2+	3-	3	3	3	3	3.5	3.5	3.5		
5/27 11	1+	1.5	2-	2	2	2	2	2+	2+	2+		
5/28 12	2-	2	2+	3-	3	3	3	3+	3+	3.5		
5/29 13	2-	2	3-	3	3	3+	3.5	4-	4-	4		
5/30 14	2+	3	3.5	4	4	4	4+	4+	4+	4+		
5/31 15	2-	2	2+	3	3	3	3+	3+	3.5	4-		
6/1 16	2	2.5	3-	3	3+	4-	4-	4	4	4		
6/2 17	1+	2-	2	2+	2.5	3-	3-	3	3	3		
6/3 18	2-	2	2.5	3-	3	3+	3+	3.5	3.5	4-		
6/4 19	1	1	1+	1.5	2-	2-	2	2	2	2		
6/5 20	2	2	2.5	3-	3-	3	3	3+	4-	4-		
6/6 21	1	1	1	1	1	1	1	1+	1+	1+		
6/7 22	2-	2	2.5	3-	3-	3	3	3+	4-	4-		
6/8 23	2	3	3+	4-	4	4	4	4+	4+	4+		
24												
25												
26												
27												
28												
29												
30												
31												

CLINICAL TRIALS



SWAB 1

	SWAB 1 WEIGHT		1 DATE	2 DFOM	3 CX u/g	4 PHASE 1 30"	1'	5 1.5	2.0	6 2.5	3.0	7 3.5	4.0	4.5	
	.027	1	5/24/86	6	374.6	1	1	1+	1.5	2	2	2.5	2.5	2.5	
	.008	2	5/25	7	118.3	1	1	1+	2	2	2.5	2.5	3-	3	
	.078	3	5/26	8	38.3	1	1+	2-	2	2	2	2+	2.5	3-	
	.067	4	5/27	9	33.2	1	1+	2-	2	2+	3-	3	3+	3+	
	.069	5	5/28	10	49.9	1	1.5	2	2+	2.5	3-	3	3	3	
	.043	6	5/29	11	19.3	1	1	1	1+	2-	2-	2-	2	2	
	.055	7	5/30	12	77.9	1+	2-	2	2+	3-	3	3	3	3	
	.040	8	5/31	13	* 10.1 / 200.7	1	1	1	1	1+	1.5	2-	2	2	
	.060	9	6/1/86	14	325.1	1	1	1+	1.5	2-	2	2	2+	2.5	
	.061	10	6/2	15	377.2	2-	2+	3	3+	4-	4	4	4+	4+	
	.047	11	6/3	16	44.9	1	1.5	2-	2	2+	3-	3-	3	3	
	.045	12	6/4	17	503.7	1	1+	2-	2	2+	2.5	3-	3-	3	
	.044	13	6/5	18	84.76	1	1	1	1+	1.5	2-	2	2+	2.5	
	.038	14	6/6	19	179.8	1	2-	2+	3-	3	3	3	3+	3.5	
	.044	15	6/7	20	97.6	1.5	2+	3-	3	3+	3.5	3.5	4	4+	
	.076	16	6/8	21	127.9	1	1	1+	1+	1.5	2-	2	2	2+	
	.055	17	6/9	22	128.3	1	1+	2-	2+	2.5	3-	3-	3	3	
	.054	18	6/10	23	186.5	1	1	1	1+	2-	2	2	2	2+	
	.050	19	6/11	24	110.8	1	1	1+	1+	2-	2	2	2+	2+	
n=19	50.58	20													
	± 16.80 SD	21													
	± 3.86 SEM	22													
		23													
		24													
		25													
		26													
		27													
		28													
		29													
		30													
		31													

* TWO DIFFERENT CERVICAL MUCUS SAMPLES WERE COLLECTED

CAROL M.

CLINICAL TRIALS

SWAB 2

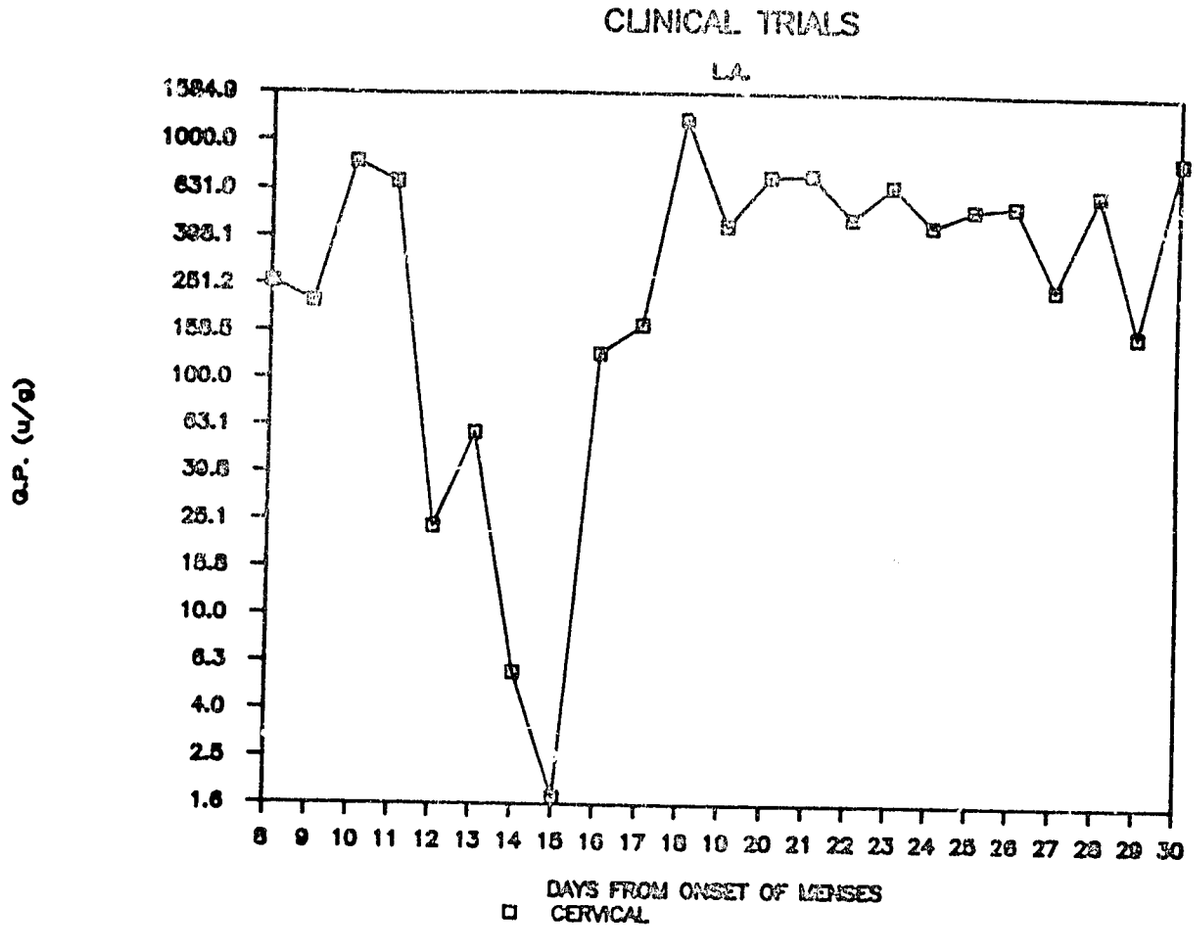
		1 PH-1		2		3		4		5		6	7
		30"	1	1.5	2	2.5	3	3.5	4	4.5	5		SWAB 2 WEIGHT
5/24/86	1	1	1	1.5	2	2+	2+	3	3	3+	3.5		.022
5/25	2	1	1	1+	1.5	2-	2	2	2+	2.5	2.5		.004
5/26	3	1	1+	2	2+	2+	3-	3-	3	3	3		.060
5/27	4	1	1	1	1.5	2-	2	2.5	3-	3	3		.045
5/28	5	1	1	1	1+	1.5	1.5	2-	2	2	2+		.046
5/29	6	1	1	1	1+	2-	2	2	2+	2+	2.5		.074
5/30	7	1+	2	3-	3-	3	3+	3+	3+	3.5	4-		.044
5/31	8	1	1	1	1	1	1	1	1	1	1		.038
6/1/86	9	1	1	1	1	1	1	1	1	1	1+		.064
6/2	10	1	2-	2	2+	2.5	3-	3-	3	3	3		.036
6/3	11	1	1.5	2	2.5	3-	3	3	3+	3+	4-		.036
6/4	12	1	1+	2-	2	2+	2.5	3-	3-	3	3		.029
6/5	13	1	2-	2	2.5	3-	3-	3	3	3	3+		.032
6/6	14	1	1	1	2-	2	2	2	2+	2.5	2.5		.049
6/7	15	1	1+	1.5	2	2	2+	2.5	3-	3-	3-		.051
6/8	16	1	1	1	1	1+	1+	1.5	2-	2	2		.037
6/9	17	1	1+	2-	2	2	2.5	2.5	2.5	3-	3-		.019
6/10	18	1	1+	2-	2	2+	2.5	3-	3-	3	3		.042
6/11	19	1	1+	2-	2	2+	3-	3-	3	3+	3.5		.058
	20												
	21												
	22												
	23												
	24												
	25												
	26												
	27												
	28												
	29												
	30												
	31												

CAROL M.
CLINICAL TRIAL

SWAB 1

		1 PH-2		2		3		4		5		6	7
		30"	1	1.5	2	2.5	3	3.5	4	4.5	5		
5/24/86	1	1	1	1	1	1	1	1	1	1	1		
5/25	2	1	1	1+	1+	1+	1.5	1.5	1.5	1.5	2-		
5/26	3	1	1	1+	2	2	2	2+	2+	3-	3		
*5/27	4	3	3+	3.5	3.5	4	4	4	4+	4+	4+		
5/28	5	1	2-	2	2+	2+	2.5	3-	3-	3-	3		
5/29	6	1	1	1	1	1	1	1	1	1+	1+		
5/30	7	1+	2-	2	2	2	2	2+	3-	3-	3-		
5/31	8	1	1	1	1	1	1	1	1+	1+	1+		
6/1/86	9	1	1+	2-	2	2	2+	2.5	3-	3-	3-		
6/2	10	2	3-	3	3+	3.5	3.5	4-	4	4+	4+		
6/3	11	1+	2-	2	2	2+	2.5	3-	3-	3	3		
6/4	12	1	1	1.5	2-	2	2	2+	2+	2+	2.5		
6/5	13	1	1	1+	1.5	2-	2	2	2	2	2		
6/6	14	1	1.5	2-	2	2	2	2+	2.5	3-	3-		
6/7	15	2	2+	2.5	3-	3	3	3+	3.5	3.5	4-		
6/8	16	1	1	1+	1.5	2-	2	2	2	2+	2+		
6/9	17	1	1.5	2	2	2+	2.5	2.5	3-	3-	3		
6/10	18	1	1	1	1+	1.5	2-	2	2	2	2+		
6/11	19	1	1	1+	2-	2	2	2	2	2.5	2.5		
	20												
	21												
	22												
	23												
	24												
	25												
	26												
	27												
	28												
	29												
	30												
	31												

* SWAB WAS SITTING IN GUAIACOL FOR 5 MINUTES BEFORE H_2O_2 WAS ADDED


CLINICAL TRIALS
L.A.
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
G.P. (u/g)
8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
DAYS FROM ONSET OF MENSES
CERVICAL

SWAB 1

SWAB 1 WEIGHT		1 PH-1 DATE	2. DFCM	3 CX u/g	4 30'	1'	5 1.5	2.0	6 2.5	3.0	7 3.5	4.0	4.5	
.042	1	6/16/86	8	257.45	2	3	3+	4-	4	4+	4.5	4.5	4.5	4.5
.064	2	6/17	9	213.3	3	4-	4+	4+	4.5	4.5	4.5	4+	4+	4+
.022	* 3	6/18	10	818.3	3.5	4	4	5-	5-	5-	5-	5-	5-	5-
.034	4	6/19	11	679.15	3+	4+	5-	5-	5-	5-	5-	5-	4.5	4.5
.050	5	6/20	12	23.5	4-	5-	5-	5-	5-	4.5	4.5	4.5	4+	4+
.043	6	6/21	13	59.0	3	4-	4	4+	4+	5-	5-	5-	4.5	4.5
.063	7	6/22	14	5.7	2	3+	4-	4	4	4	4+	4.5	4.5	4.5
.049	8	6/23	15	1.7	3	4	4	5-	5-	5-	5-	5-	5-	5-
.027	9	6/24	16	128.2	3	3+	4	4	4	4	4	4+	4+	4+
.027	* 10	6/25	17	169.6	2+	3.5	4	4	4.5	4.5	5-	5-	5-	5-
.031	11	6/26	18	1,234	4	4+	4.5	5-	4.5	4.5	4.5	4+	4+	4
.027	12	6/27	19	444.5	4	4.5	5-	5-	5-	4.5	4.5	4.5	4.5	4.5
.006	13	6/28	20	715.9	4.5	5	5+	5+	5+	5+	5+	5+	5+	5+
.005	14	6/29	21	726.2	5-	5	5+	5+	5+	5+	5+	5+	5+	
.033	15	6/30	22	480.1	3	4-	4	5-	5-	5-	5-	5-	4.5	4.5
.034	16	7/1/86	23	655.7	3.5	4	4+	5-	5-	5-	5-	5-	4.5	4.5
.029	17	7/2	24	446.4	3+	4-	4	5-	5-	5-	5-	5-	4.5	4.5
.020	18	7/3	25	526.48	3.5	4	4.5	5-	5-	5-	5-	5-	5-	5-
.041	19	7/4	26	545.52	4-	5-	5-	5-	5-	5-	5-	5-	5-	5-
.041	20	7/5	27	246.4	4-	4	5-	5-	5-	5-	4.5	4.5	4.5	4+
.091	21	7/6	28	613.54	4+	5-	5-	5-	4.5	4.5	4	4	4	4
.032	22	7/7	29	158.46	3.5	4	4.5	5-	5-	4.5	4.5	4.5	4.5	4.5
.044	23	7/8	30	850.72	4-	4+	5-	5-	5-	5-	5-	5-	5-	4.5
	24													
	25													
	26													
	27													
	28													
	29													
	30													
	31													

SWAB 2

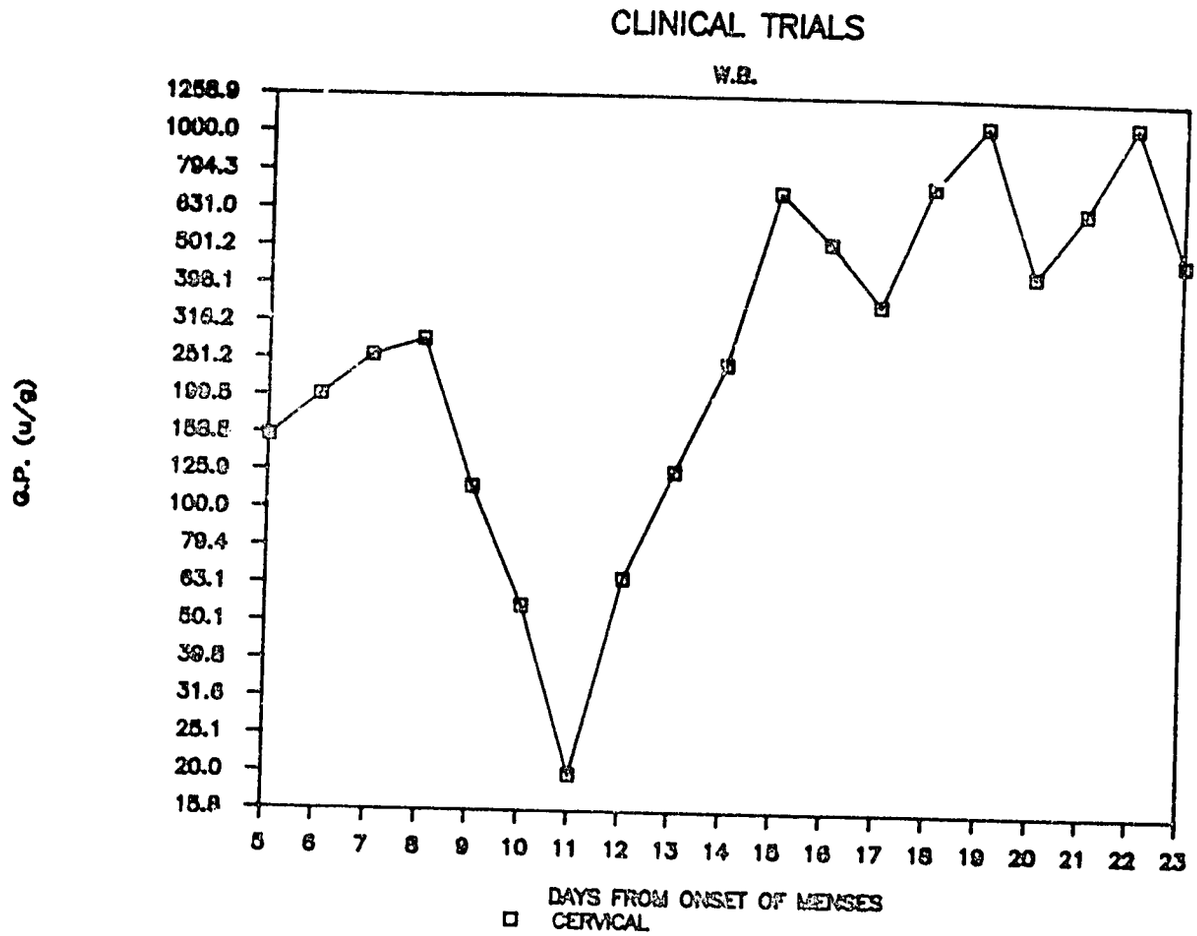
	1 PH-1		2		3		4		5		6	7
	30"	1'	1.5	2	2.5	3	3.5	4	4.5	5		SWAB 2 WEIGHT
6/16/86 1	2+	3+	3.5	4-	4	4	4+	4+	4+	4+		.036
6/17 2	3+	4	4+	4+	4.5	4.5	4.5	4+	4+	4+		.060
*6/18 3	4-	4+	5-	5-	5-	5-	5-	5-	5-	5-		.020
6/19 4	3	4	4+	4+	5-	5-	5-	5-	4.5	4.5		.035
6/20 5	3	4	4	4	5-	4.5	4.5	4.5	4.5	4.5		.037
6/21 6	3	3.5	4-	4	4	4	4+	4.5	4.5	4.5		.043
6/22 7	1	1+	2	2	3	3	3+	3+	3+	3.5		.058
6/23 8	2	3	3.5	3.5	4-	4	4	4	4	4+		.040
6/24 9	3-	3	3+	4-	4	4	4	4	4	4		.024
6/25 10	3	4-	4	4+	4.5	4.5	4.5	4.5	4.5	4.5		.020
6/26 11	3	4-	4+	5-	5-	5-	5-	4.5	4.5	4.5		.013
6/27 12	4	4.5	5-	5-	5-	4.5	4.5	4.5	4.5	4.5		.020
6/28 13	5	5	5+	5+	5+	5+	5+	5+	5+	5+		.008
6/29 14	4-	4.5	5	5	5	5+	5+	5+	5+	5+		.004
6/30 15	4	4.5	5-	5-	5-	5-	5-	4.5	4.5	4.5		.038
7/1/86 16	3.5	4	5-	5-	5-	5-	5-	5-	4.5	4.5		.024
7/2 17	3.5	4	4+	5-	5-	5-	5-	5-	4.5	4.5		.027
7/3 18	3	3.5	4	4+	4.5	5-	5-	5-	5-	5-		.016
7/4 19	4-	5-	5-	5-	5-	5-	5-	5-	5-	5-		.031
7/5 20	4-	4+	5-	5-	5-	5-	4.5	4.5	4.5	4+		.037
7/6 21	4	4.5	5-	5-	4.5	4.5	4+	4+	4+	4+		.038
7/7 22	4	4.5	5-	5-	4.5	4.5	4.5	4.5	4.5	4.5		.027
7/8 23	3+	4+	4+	4.5	5-	5-	5-	5-	5-	5-		.027
24												
25												
26												
27												
28												
29												
30												
31												

SWAB 1

		1 PH-2		2		3		4		5		6	7
		30"	1'	1.5	2	2.5	3	3.5	4	4.5	5		
6/16/86	1	2	2.5	3-	3	3+	3+	4-	4	4	4+		
6/17	2	2	3	3	3+	4-	4	4	4	4	4		
*6/18	3	3+	4-	4-	4+	4.5	4.5	4.5	5-	5-	5-		
6/19	4	2	3	3+	3.5	4-	4	4	4+	4+	4+		
6/20	5	3+	4	4	4.5	5-	5-	5-	5-	5-	4.5		
6/21	6	2	3	3+	3.5	4-	4-	4	4	4	4+		
6/22	7	2	3	3+	3.5	4-	4	4	4	4	4		
6/23	8	2	3-	3	3+	3+	3.5	3.5	3.5	4-	4-		
6/24	9	2	3-	3+	3.5	4	4	4	4	4	4		
6/25	10	2-	2	3	3+	3+	3.5	4-	4-	4-	4		
6/26	11	3	3.5	4-	4	4+	4+	4+	4.5	4.5	4.5		
6/27	12	3+	4	4+	5-	5-	5-	5-	5-	5-	5-		
6/28	13	3	4.5	5	5+	5+	5+	5+	5+	5+	5+		
6/29	14	4+	5	5+	5+	5+	5+	5+	5+	5+	5+		
6/30	15	2	3	3+	3.5	4-	4-	4	4	4	4		
7/1/86	16	2	2+	3	3+	3.5	3.5	3.5	3.5	4-	4-		
7/2	17	3	3+	4-	4-	4-	4	4	4+	4.5	5-		
7/3	18	2	3	3	3+	3.5	4-	4-	4-	4-	4-		
7/4	19	3.5	4-	4+	5-	5-	5-	5-	5-	5-	5-		
7/5	20	2	3-	3	3+	3+	3.5	3.5	4-	4-	4-		
7/6	21	4-	4	4.5	5-	5-	5-	5-	5-	5-	4.5		
7/7	22	3	3.5	4-	4	4	4+	4.5	4.5	4.5	4.5		
7/8	23	3	3.5	4-	4	4+	4+	4.5	5-	5-	5-		
	24												
	25												
	26												
	27												
	28												
	29												
	30												
	31												

SWAB 2

		1 PH-2		2		3		4		5		6	7
		30"	1'	1.5	2	2.5	3	3.5	4	4.5	5		
6/16/86	1	2	3	3+	4-	4	4	4	4+	4+	4+		
6/17	2	2	3+	3.5	4	4	4+	4+	4+	4+	4+		
*6/18	3	4-	4	4	4+	5-	5-	5-	5-	5-	5-		
6/19	4	3	3+	4-	4	4	4.5	5-	5-	5-	5-		
6/20	5	2	3	3+	3+	3.5	4-	4	4	4	4		
6/21	6	2	3-	3	3	3+	3+	3.5	4-	4-	4-		
6/22	7	1	1	2-	2	2	2	2	2	3-	3		
6/23	8	1+	2	2	2	2+	3	3	3	3	3		
6/24	9	2	2	3	3	3.5	4-	4-	4-	4-	4-		
6/25	10	2-	2	3	3+	3+	3.5	4-	4-	4-	4		
6/26	11	2	2	3	3+	3+	3.5	3.5	4-	4-	4-		
6/27	12	3	4-	4	4+	5-	5-	5-	5-	5-	5-		
6/28	13	3+	4.5	5	5+	5+	5+	5+	5+	5+	5+		
6/29	14	4	4.5	5-	5	5	5	5	5	5+	5+		
6/30	15	3.5	4	5-	5-	5-	5-	5-	5-	4.5	4.5		
7/1/86	16	3+	3.5	4-	4	4+	4.5	5-	5-	5-	5-		
7/2	17	3	3+	4-	4-	4	4+	4.5	5-	5-	5-		
7/3	18	2	3	3	3+	3.5	4-	4-	4-	4-	4-		
7/4	19	3	3+	4-	4-	4	4.5	5-	5-	5-	5-		
7/5	20	3	4-	4	4+	4.5	5-	5-	5-	5-	5-		
7/6	21	3	4-	4	4.5	4.5	5-	5-	5-	5-	4.5		
7/7	22	3	3.5	4-	4	4	4+	4.5	4.5	4.5	4.5		
7/8	23	3	3.5	4-	4	4+	4+	4.5	5-	5-	5-		
	24												
	25												
	26												
	27												
	28												
	29												
	30												
	31												


CLINICAL TRIALS
W.B.
1258.9
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.0
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
G.P. (u/g)
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
DAYS FROM ONSET OF MENSES
CERVICAL

SWAB 1

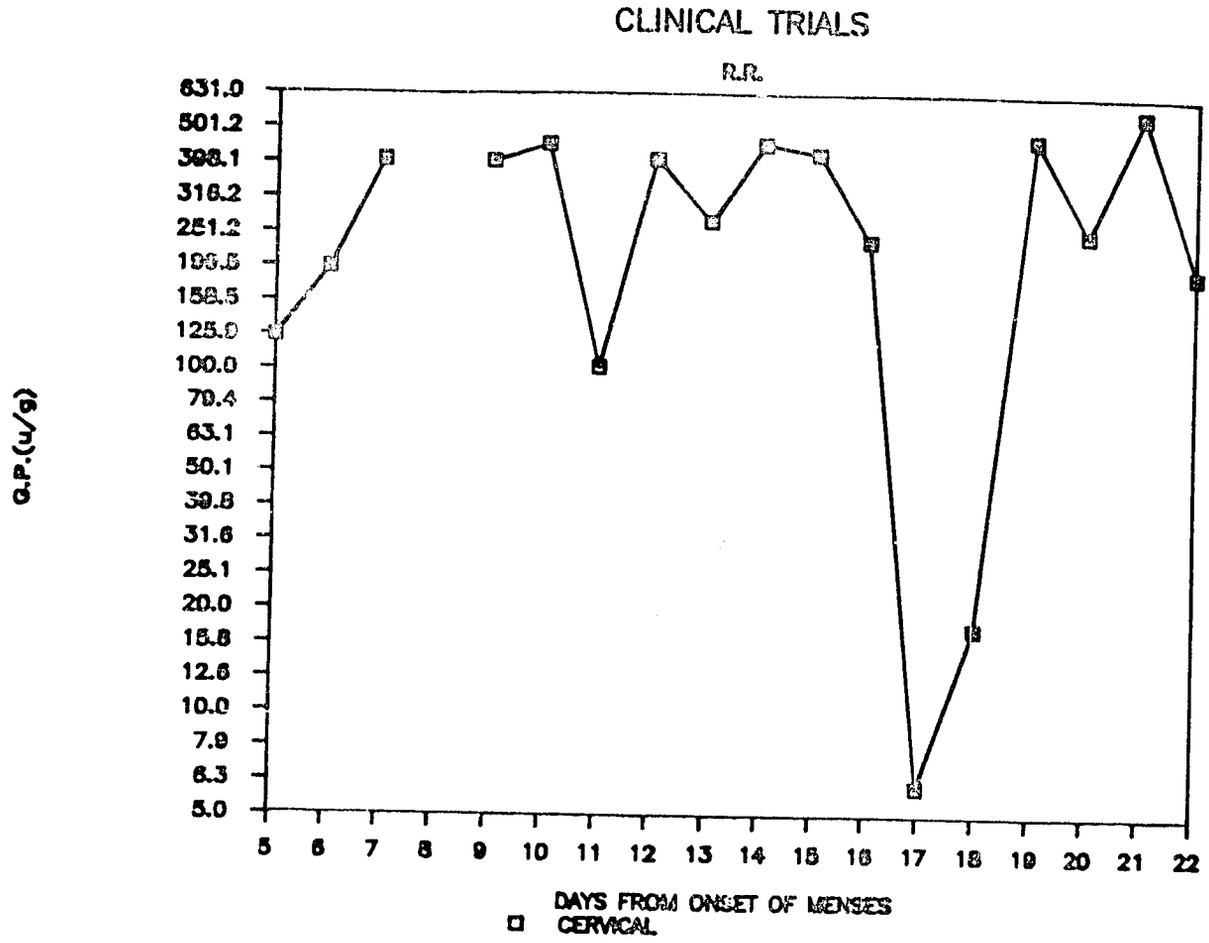
PAGE

SWAB 1 WEIGHT (g)		1 DATE	2 DFOM	3 Cx u/g	4 PHASE 1 30	1	5 1.5	2	6 2.5	3	7 3.5	4	4.5	5
.053	1	8/4/86	5	155.8	4	5-	5-	5-	5-	5-	5-	5-	5-	5
.049	2	8/5	6	201.4	4	5	5	5	5	5	5	5-	5-	5
.053	3	8/6	7	257.5	4-	4.5	5-	5	5	5-	5-	5-	5-	5
.088	4	8/7	8	284.4	4-	4.5	4.5	5-	5-	5-	5-	4.5	4.5	4.
.082	5	8/8	9	115.2	4	4+	4.5	4.5	4+	4+	4+	4+	4+	4
.061	6	8/9	10	55.7	3+	4	4.5	5-	5-	5	5	5-	5-	5
.076	7	8/10	11	19.8	3	4-	4	4.5	5-	5-	5-	5-	5-	5
.070	8	8/11	12	65.9	4-	4+	5-	5-	5-	5-	5-	5-	5-	5
.066	9	8/12	13	127.2	4-	4.5	5	5	5	5	5	5	5-	5
.057	10	8/13	14	246.9	4+	5	5	5	5	5	5-	5-	5-	5
.068	11	8/14	15	708.3	4	5-	5	5	5	5	5	5	5	5
.047	12	8/15	16	522.3	4	5-	5	5	5-	5-	5-	5-	5-	5
.037	13	8/16	17	358.7	4	4.5	5-	5	5	5-	5-	5-	5-	5
.106	14	8/17	18	740.4	4+	5-	5	5-	4.5	4.5	4.5	4.5	4.5	4.
.061	15	8/18	19	1072.3	4	4.5	5+	5+	5	5	5	5	4.5	4.
.037	16	8/19	20	433.9	3.5	5-	5	5	5	5	5	5	5	5
.077	17	8/20	21	642.4	4	5-	5-	5-	5-	5-	5-	4.5	4.5	4.
.076	18	8/21	22	1091.6	4-	4-	4+	4+	4+	4	4	4	4-	4
.024	19	8/22	23	477.5	4	4.5	5-	5-	5-	5-	5-	4.5	4.5	4.5
	20													
	21													
	22													
	23													
	24													
	25													
	26													
	27													
	28													
	29													
	30													
	31													

SWAB 1

PAGE 3

	1	2	3 PHASE 2		4		5		6		7	
	DATE	DFOM	30	1	1.5	2	2.5	3	3.5	4	4.5	5
1	8/4/86	5	3+	4	4.5	5-	5-	5	5	5	5	5
2	8/5	6	3.5	4	5-	5-	5	5	5	5-	5-	5-
3	8/6	7	4-	4+	5-	5	5	5	5-	5-	5-	5-
4	8/7	8	3+	4	4.5	5-	5	5	5	5	5-	5-
5	8/8	9	3+	4	4.5	5-	5-	5	5	5	5-	5-
6	8/9	10	3	3.5	4-	4+	5-	5-	5	5	5	5
7	8/10	11	2+	3+	4-	4	4	4.5	5-	5-	5-	5-
8	8/11	12	3.5	4	4+	5-	5-	5-	5-	5-	5-	5-
9	8/12	13	4	5-	5	5	5	5	5	5	5	5
10	8/13	14	4-	4.5	5	5	5	5	5	5	5-	5-
11	8/14	15	4-	4.5	5-	5	5	5	5	5-	5-	5-
12	8/15	16	4-	4.5	5-	5	5	5-	5-	5-	5-	5-
13	8/16	17	3	4	4.5	5-	5	5	5	5-	5-	5-
14	8/17	18	4.5	5	5	5	5-	5-	4.5	4.5	4.5	4.5
15	8/18	19	3	4-	4.5	5-	5	5	5	5	5	5
16	8/19	20	3+	5-	5	5	5	5	5	5	5	5
17	8/20	21	4-	4.5	5	5	5	4.5	4.5	4.5	4.5	4.5
18	8/21	22	4-	5-	5	5	5	5	5-	5-	5-	5-
19	8/22	23	4-	4.5	5-	5-	5-	5-	5-	5-	5-	5-
20												
21												
22												
23												
24												
25												
26												
27												
28												
29												
30												
31												


CLINICAL TRIALS
R.R.
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.0
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
12.6
10.0
7.9
6.3
5.0
G.P.(u/g)
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
DAYS FROM ONSET OF MENSES
CERVICAL

SWAB 1

PAGE 1

SWAB WEIGHT (g)		1 DATE	2 DFOM	3 CX u/g	4 PHASE 1 30	1	5 1.5	2	6 2.5	3	7 3.5	4	4.5	5
.067	1	8/31/86	5	124.5	3	4-	4	4+	5-	5-	5-	5-	5	5
.057	2	9/1	6	198.2	3	4-	4	5-	5-	5-	5-	5-	5-	5
.064	3	9/2	7	404.8	3.5	4	5-	5-	5-	5-	5-	5-	5-	5
—	4	9/3	8	—										
.061	5	9/4	9	400.7	3	4-	4	4+	4.5	4.5	4.5	4.5	4.5	4.
.046	6	9/5	10	449.0	3+	4-	4.5	5-	5-	5-	5-	5-	5-	5
.057	7	9/6	11	101.3	3+	3.5	4	4.5	4.5	4.5	5-	5-	5-	5
.073	8	9/7	12	406.0	3+	4-	4-	4	4-	4-	4-	4-	4-	3.
.063	9	9/8	13	269.9	3+	4-	4	4	4-	4-	4-	4-	4-	3
.092	10	9/9	14	453.0	3.5	4	4+	4+	4+	4+	4+	4	4	4
.087	11	9/10	15	422.9	3	4-	4+	5-	5-	5-	5-	5-	5-	5
.091	12	9/11	16	237.9	2+	3+	4-	4-	4+	4.5	5-	5-	5-	5
.056	13	9/12	17	6.1	2	3	3+	4-	4-	4	4.5	4.5	5-	5
.062	14	9/13	18	17.7	3-	3.5	4-	4+	4.5	5-	5-	5-	5-	5
.062	15	9/14	19	471.1 *	3+	4-	4.5	5-	5-	5-	5-	5-	5	5
.043	16	9/15	20	250.0	3.5	4	5-	5-	5-	5-	5-	5-	5-	5
.065	17	9/16	21	560.0	4	5-	5-	5-	5-	5-	5-	5-	5-	5
.050	18	9/17	22	192.4	4	5-	5-	5-	5-	5-	5-	5-	5-	5
	19													
	20													
	21													
*	22	Cx #2	= 211.0											
	23													
	24													
	25													
	26													
	27													
	28													
	29													
	30													
	31													

SWAB 1

DATE		1 PHASE 2		2		3		4		5		6	7
		30	1	1.5	2	2.5	3	3.5	4	4.5	5		
8/31/86	1	3	3.5	4-	4	4.5	5-	5-	5-	5-	5-		
9/1	2	3+	4-	4.5	5-	5-	5-	5-	5-	5-	5-		
9/2	3	3+	4-	4.5	5-	5-	5-	5-	5-	5-	5-		
9/3	4												
9/4	5	2+	3+	3.5	4-	4	4.5	5-	5-	5-	5-		
9/5	6	1.5	3+	4-	4-	4	4.5	5-	5-	5-	5-		
9/6	7	2	3	3.5	4-	4-	4	4.5	5-	5-	5-		
9/7	8	3	4-	4-	4+	4.5	5-	5-	5-	5-	5-		
9/8	9	2	3	3.5	4-	4-	4-	4	4+	4.5	5-		
9/9	10	3+	4-	4	4.5	5-	5-	5-	5-	5-	5-		
9/10	11	2.5	3+	4-	4	4.5	5-	5-	5-	5-	5-		
9/11	12	2-	2.5	3+	3.5	4-	4-	4	4+	4.5	5-		
9/12	13	1.5	2+	3	3+	3+	3.5	4-	4-	4-	4		
9/13	14	2	2.5	3	3+	3.5	4-	4-	4-	4	4		
9/14	15	2	3+	3.5	4-	4	4+	4.5	5-	5-	5-		
9/15	16	3	4-	4	4.5	5-	5-	5-	5-	5-	5-		
9/16	17	3.5	4	5-	5-	5-	5-	5-	5-	5-	5-		
9/17	18	3.5	4+	5-	5-	5-	5-	5-	5-	5-	5-		
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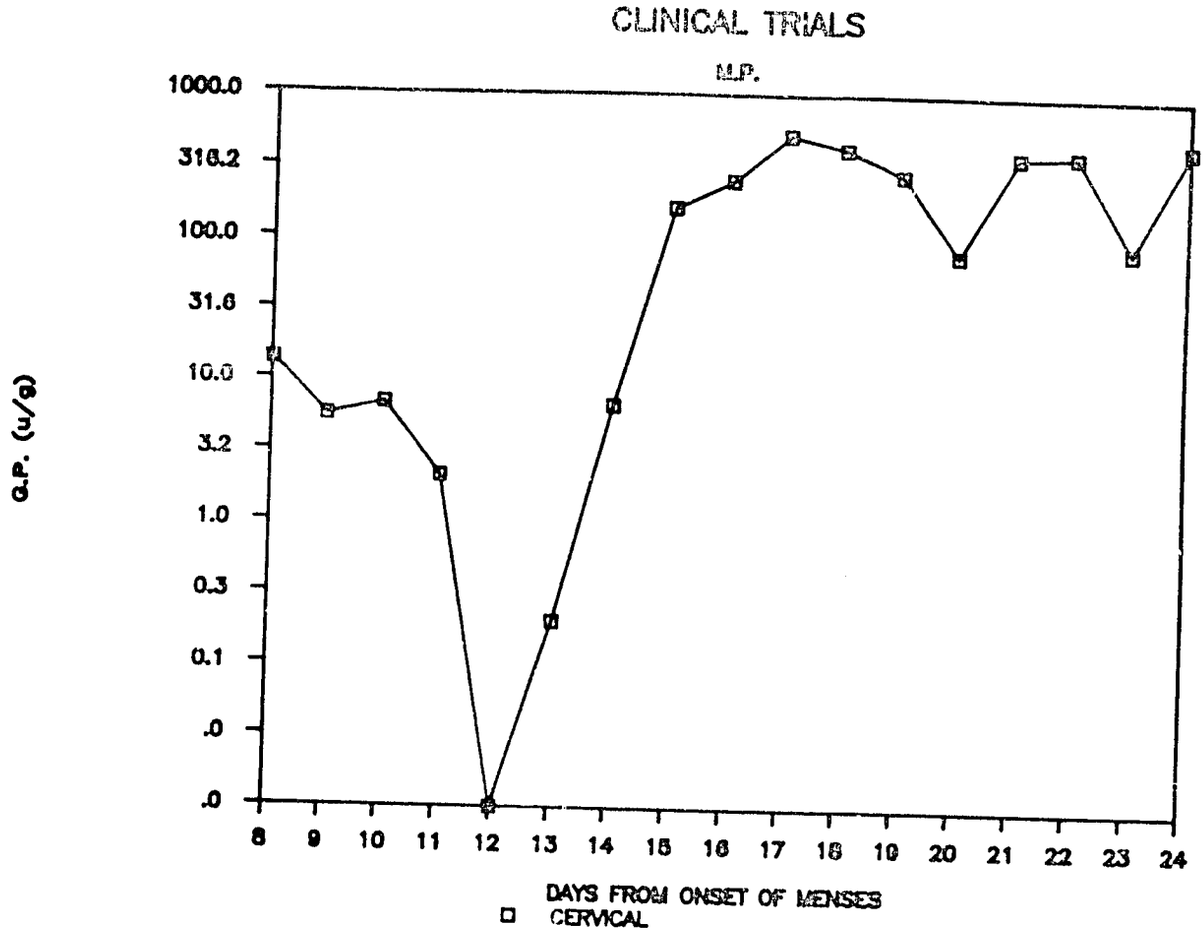
CLINICAL TRIALS
M.P.
1000.0
316.2
100.0
31.6
10.0
3.2
1.0
0.3
0.1
.0
.0
G.P. (u/g)
8
9
10
11
12
13
14
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24
DAYS FROM ONSET OF MENSES
CERVICAL

File MILLL.sr 8=75 MAGGIE M.
CLINICAL TRIALS

swab 1

PAGE 1

	SWAB 1 WEIGHT	1 DATE	2 DFOM	3 CV µ/g	4 PHASE 1 30"	1'	5 1.5	2.0	6 2.5	3.0	7 3.5	4.0	4.5	5.0
1	.066	7/12/86	8	13.75	1	1	1	1	1	1	1	1	1	1
2	.099	7/13/86	9	5.58	1	1	1	1	1	1	1	1	1	1
3	.084	7/14/86	10	6.86	1	1	1	1	1	1+	1+	1+	1+	1+
4	.119	7/15/86	11	2.10	1	1	1	1	1	1	1	1	1	1
5	.093	7/16/86	12	0	1	1	1	1	1	1	1	1	1	1
6	.096	7/17/86	13	0.20	1	1	1	1	1	1	1	1	1	1
7	.059	7/18/86	14	6.65	1	2	3	3.5	4-	4-	4-	4-	4	4
8	.038	7/19/86	15	164.0	1.5	2+	3	3.5	4-	4	4	4+	4+	4+
9	.026	7/20/86	16	254.6	3+	4	4.5	4.5	4.5	4+	4+	4-	4-	4-
10	.056	7/21/86	17	525.6	3+	4	4+	4.5	5-	5-	5-	5-	5-	5-
11	.055	7/22/86	18	422.8	2	3+	4	4+	4+	4.5	4.5	5-	5-	5-
12	.050	7/23/86	19	281.6	3	4-	4	4+	4.5	5-	5-	5-	5-	5-
13	.041	7/24/86	20	19.44	3+	4-	4+	4.5	5-	5-	5-	5-	5-	5-
14	.042	7/25/86	21	385.55	2	3+	4-	4-	4+	4.5	4.5	4.5	5-	5-
15	.01	7/26/86	22	397.94	2.5	3+	4	4+	4.5	5-	5-	5	5	5
16	.017	7/27/86	23	87.5	1+	3-	3+	4-	4	4	4.5	5-	5-	5
17	.053	7/28/86	24	460.95	2+	3+	4-	4	4.5	4.5	4.5	5-	5-	5-
18														
19														
20														
21														
22														
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30														
31														

file MYLCLL.DD

CLINICAL TRIAL

PAGE 2

SWAB 1

	1 Phase 2		2		3		4		5		6	7
	30"	1min	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0		
7/12/86 1	1	1	1	1	1	1	1	1	1	1		
7/13 2	1	1	1	1	1	1	1	1	1	1		
7/14 3	1-	1-	1-	1-	1-	1-	1	1+	1+	2-		
7/15 4	1	1	1	1	1	1	1+	1+	1.5	2-		
7/16 5	1	1	1	1	1	1	1	1	1	1		
7/17 6	1	1	1	1	1	1	1	1	1	1		
R ROTTING ONLY 7/18 7	1	1	1	1	1	1	1	1	1	1		
7/19 8	1+	2	3-	3.5	3.5	3.5	4-	4-	4	4		
7/20 9	3+	4-	4-	4	4	4	4	4	4	4		
7/21 10	2	3+	3.5	4-	4-	4	4	4+	4.5	4.5		
7/22 11	2	3	3+	4-	4-	4	4	4+	4.5	4.5		
7/23 12	2	3	3+	4-	4-	4	4+	4+	4.5	4.5		
7/24 13	3-	3.5	4-	4	4.5	5-	5-	5-	5-	5-		
7/25 14	2	3	3+	3.5	4-	4	4	4+	4.5	5-		
7/26 15	2	3-	3	3	3.5	3.5	3.5	3.5	4-	4-		
7/27 16	1+	1+	2	2.5	3	3	3+	3.5	3.5	4-		
7/28 17	2-	2	3	3+	3+	3+	3.5	4-	4-	4-		
18												
19												
20												
21												
22												
23												
24												
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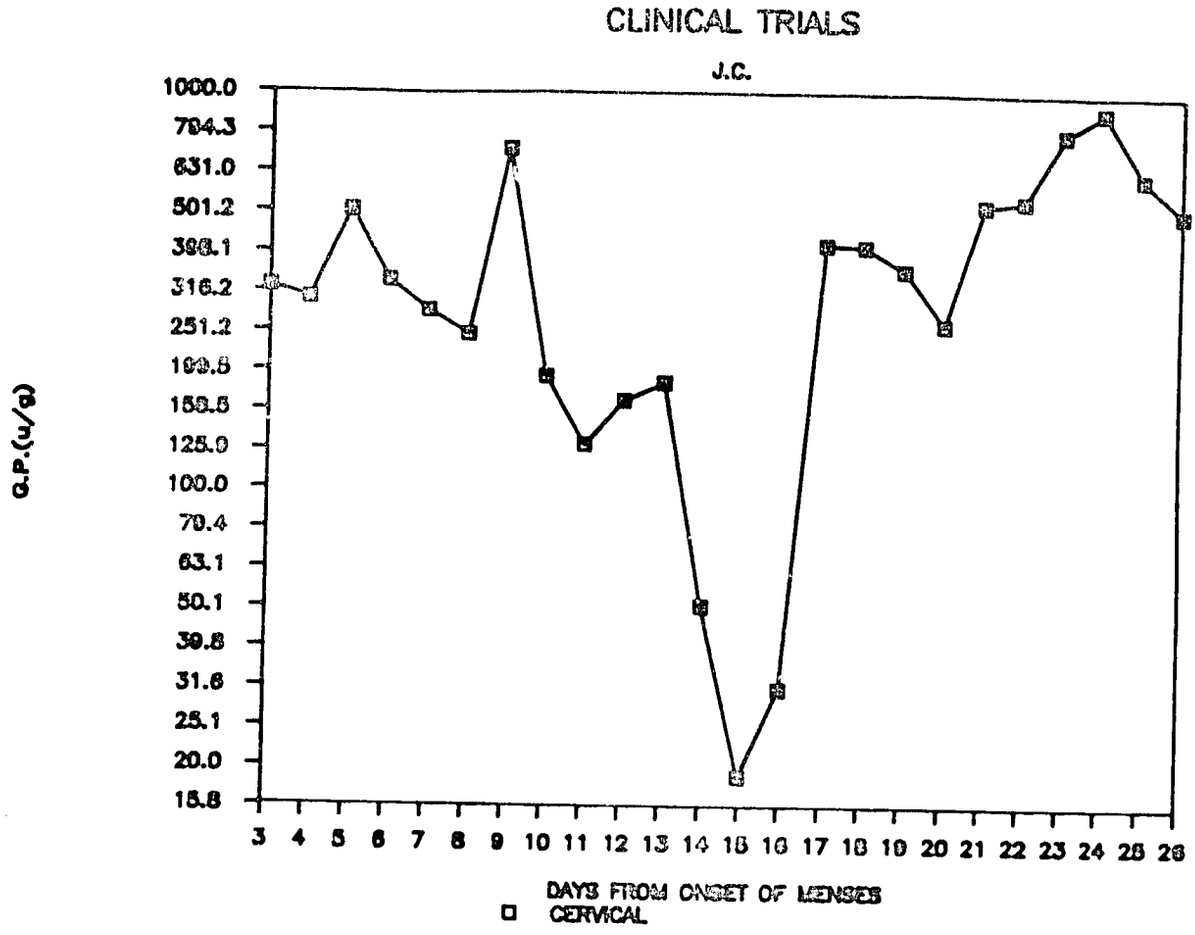
26												
27												
28												
29												
30												
31												


CLINICAL TRIALS
J.C.
1000.0
794.3
631.0
501.2
398.1
316.2
251.2
199.5
158.5
125.9
100.0
79.4
63.1
50.1
39.8
31.6
25.1
20.0
15.8
G.P.(u/g)
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
DAYS FROM ONSET OF MENSES
CERVICAL

SWAB 1

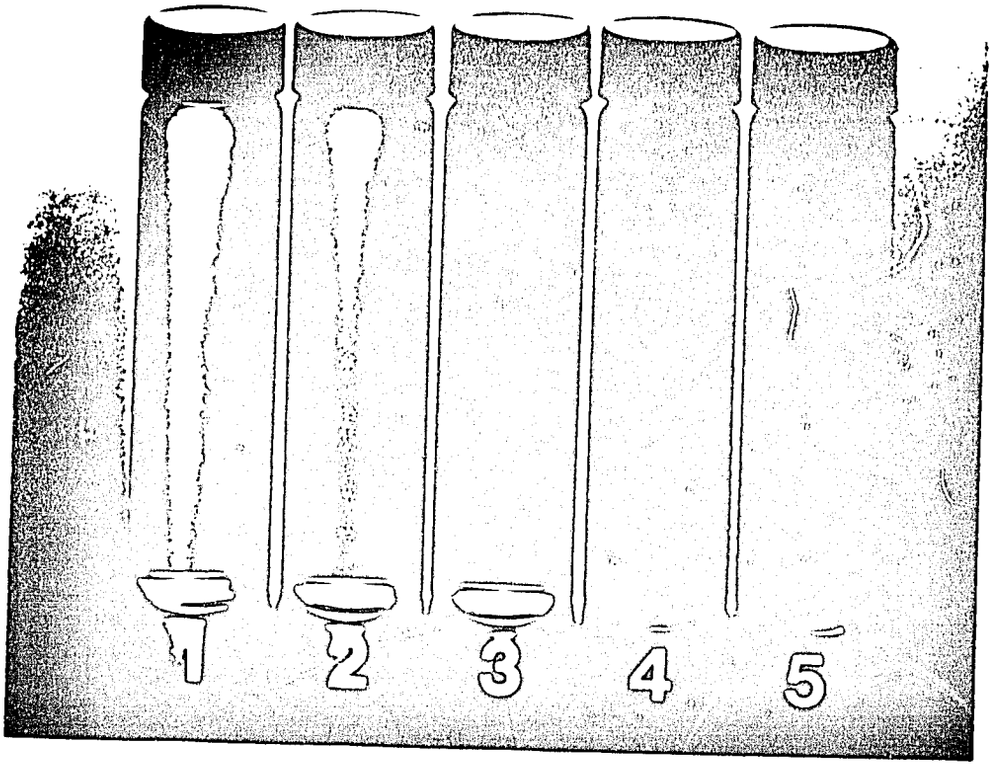
PAGE

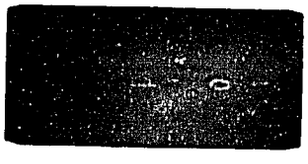
SWAB 1 WEIGHT (g)		1 DATE	2 DFOM	3 CX u/g	4 PHASE 1 30	1	5 1.5	2	6 2.5	3	7 3.5	4	4.	
.038	1	9/8/86	3	325.6	4	5-	5-	5-	5-	4.5	4.5	4.5	4.	7.
.041	2	9/9	4	303.5										
.051	3	9/10	5	505.0										
.035	4	9/11	6	337.0										
.053	5	9/12	7	283.1	3.5	4+	5-	5-	5-	5-	5-	5-	5-	5
.043	6	9/13	8	246.0	4-	5-	5-	5-	5-	5-	5-	5-	5-	5
.033	7	9/14	9	720.0	3+	4	4.5	5-	5-	5-	5-	5-	5-	5
.044	8	9/15	10	192.8	3+	4	4.5	5-	5	5	5	5	5	
.041	9	9/16	11	129.5	4	5-	5-	5-	5-	5-	5-	5-	5-	5
.054	10	9/17	12	167.7	4-	5-	5+	5+	5+	5+	5+	5+	5	5
.067	11	9/18	13	186.3	4.5	5-	5-	5-	5-	5-	5-	5-	4.5	4.
.053	12	9/19	14	50.6	4	5-	5-	5-	5-	5-	5-	5-	5-	5
.067	13	9/20	15	18.8	3+	4-	4.5	5-	5-	5	5	5	5	5
.073	14	9/21	16	31.3	3-	3+	4-	4.5	5-	5-	5	5-	5-	5
.047	15	9/22	17	416.5	4	5	5	5-	5-	5-	5-	5-	5	5
.049	16	9/23	18	412.5	4	5-	5	5	5	5-	5-	5-	5-	5
.047	17	9/24	19	360.0	4.5	5	5-	5-	5-	5-	5-	5-	5-	5
.024	18	9/25	20	262.0	4-	4.5	5-	5-	5-	5-	5-	4.5	4.5	4.
.027	19	9/26	21	525.6	3.5	4.5	5	5	5	5+	5+	5+	5+	5
.040	20	9/27	22	540.9	3+	4	5-	5	5+	5+	5+	5+	5+	5
.044	21	9/28	23	796.7	3.5	4.5	5	5+	5+	5+	5+	5+	5	5
.048	22	9/29	24	906.7	3+	4.5	5-	5	5+	5+	5	5	5	5
.062	23	9/30	25	617.0	3.5	4.5	5-	5-	5	5+	5	5	5-	5
.057	24	10/1	26	503.2	3	3.5	4	4.5	5-	5-	5-	5	5	5
	25													
	26													
	27													
	28													
	29													
	30													
	31													

SWAB 1

	1 PHASE 2		2		3		4		5		6	7
DATE	30	1	1.5	2	2.5	3	3.5	4	4.5	5		
9/8/86 1	4	5-	5	5-	5-	5-	5-	5-	5-	5-		
9/9 2												
9/10 3												
9/11 4												
9/12 5	3+	4-	4.5	5-	5-	5-	5-	5-	5-	5-		
9/13 6	3	3.5	4-	4	4.5	5-	5-	5-	5-	5-		
9/14 7	2	3	3+	3.5	4-	4-	4	4.5	5-	5-		
9/15 8	3	3.5	4	4	4.5	5-	5-	5-	5-	5-		
9/16 9	3	3+	4-	4-	4+	4.5	5-	5-	5-	5-		
9/17 10	3	3.5	4-	4.5	5-	5-	5-	5	5	5		
9/18 11	3+	4-	4.5	5-	5-	5-	5-	5-	5-	5-		
9/19 12	3-	3+	3.5	4-	4	4+	4.5	5-	5-	5-		
9/20 13	2	3-	3+	3.5	4-	4-	4	4.5	5-	5-		
9/21 14	2.5	3	3.5	4-	4+	5-	5-	5	5	5-		
9/22 15	3.5	4-	4.5	5-	5-	5-	5-	5-	5-	5-		
9/23 16	3+	4-	4.5	5-	5-	5	5	5-	5-	5-		
9/24 17	3+	4-	4+	4.5	5-	5-	5-	5-	5-	5-		
9/25 18	2.5	3+	3.5	4-	4	4.5	4.5	5-	5-	5-		
9/26 19	2+	3	3+	3.5	4-	4-	4	4	4+	4.5		
9/27 20	3	3.5	4-	4+	4.5	5-	5-	5	5	5+		
9/28 21	3+	4-	4+	4.5	5-	5-	5	5	5-	5-		
9/29 22	3	3.5	4-	4.5	5-	5-	5	5	5	5-		
9/30 23	3+	4-	4+	5-	5-	5-	5-	5-	5-	5-		
10/1 24	2.5	3+	3.5	4-	4+	4.5	5-	5-	5-	5		
25												
26												
27												
28												
29												
30												
31												

COLOR STANDARDS





SCHEMA 1

POST-IODINATION TREATMENT OF MUCUS

1. CENTRIFUGATION : SOLUBLE PROTEINS
2. 0.5 M CALCIUM EXTRACTION : GP , etc.
3. REDUCTION OF -S-S- WITH DTT+SDS : " LINKER
4. REMOVAL OF CARBOHYDRATES (N-GLYCANASE)
5. COMPLETE HYDROLYSIS , HPLC :

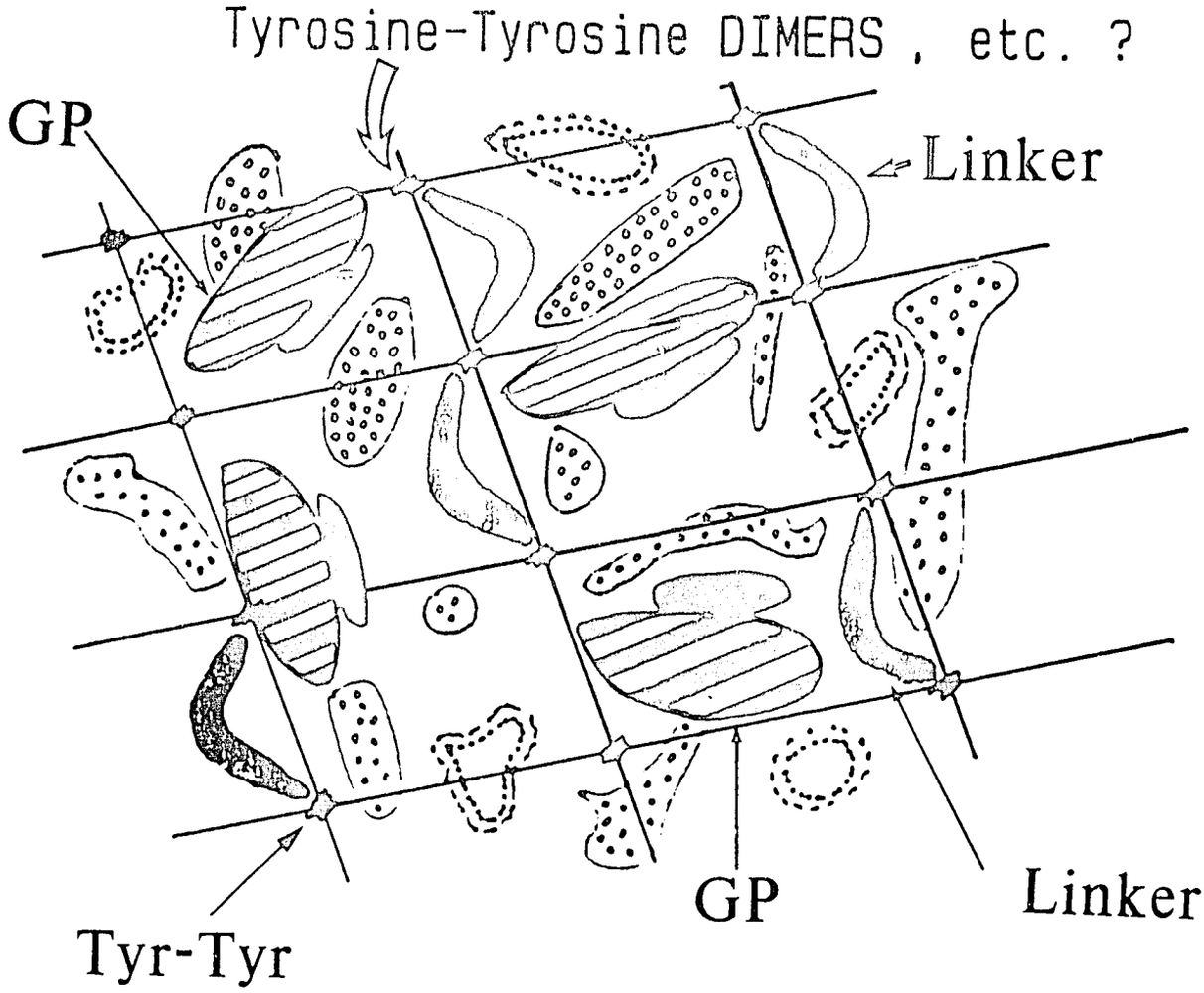


FIGURE 4

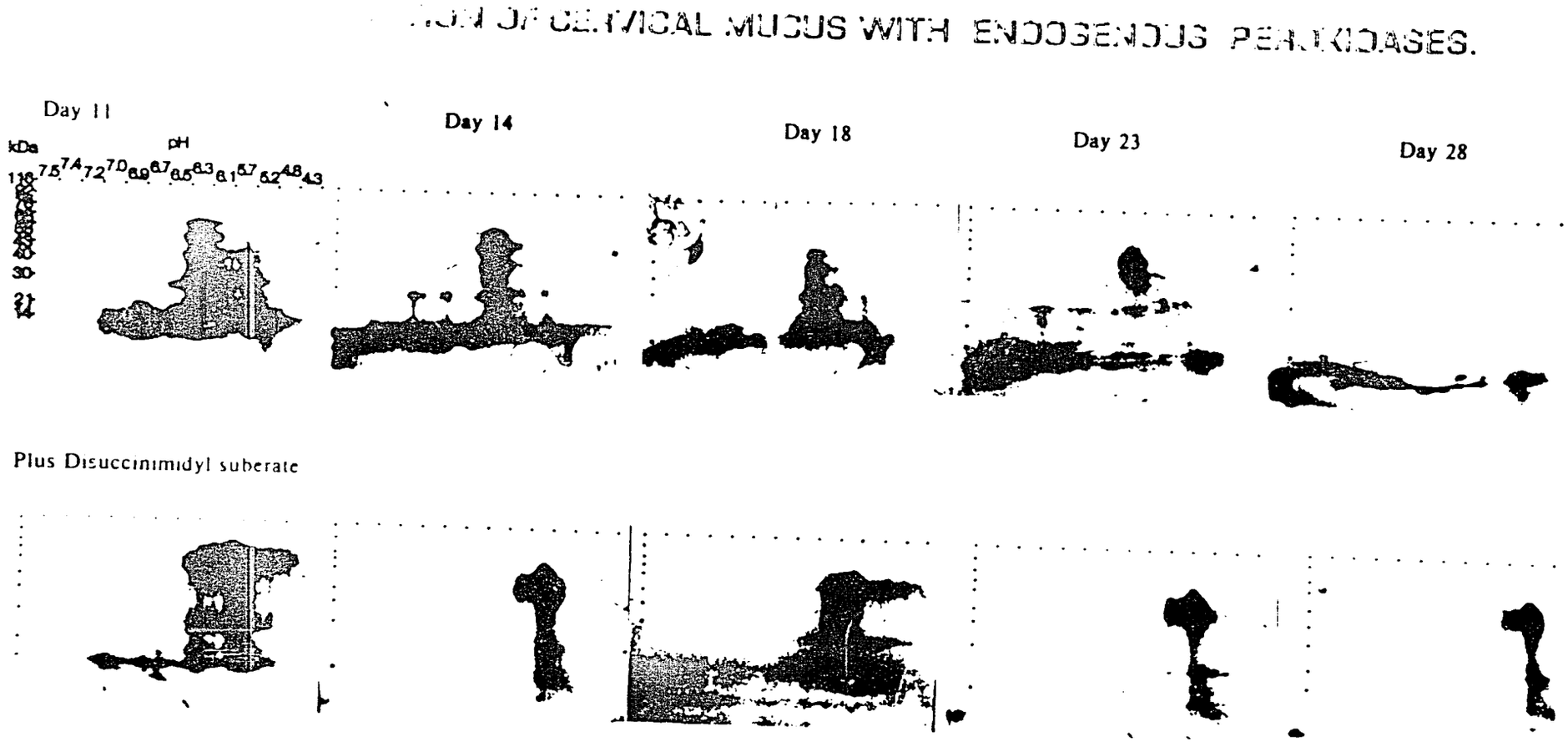

...ION OF CERVICAL MUCUS WITH ENDOGENOUS PEROXIDASES.
Day 11
Day 14
Day 18
Day 23
Day 28
kDa
pH
7.5 7.4 7.2 7.0 6.9 6.7 6.5 6.3 6.1 5.7 5.2 4.8 4.3
30
21
Plus Disuccinimidyl suberate

FIGURE 4A

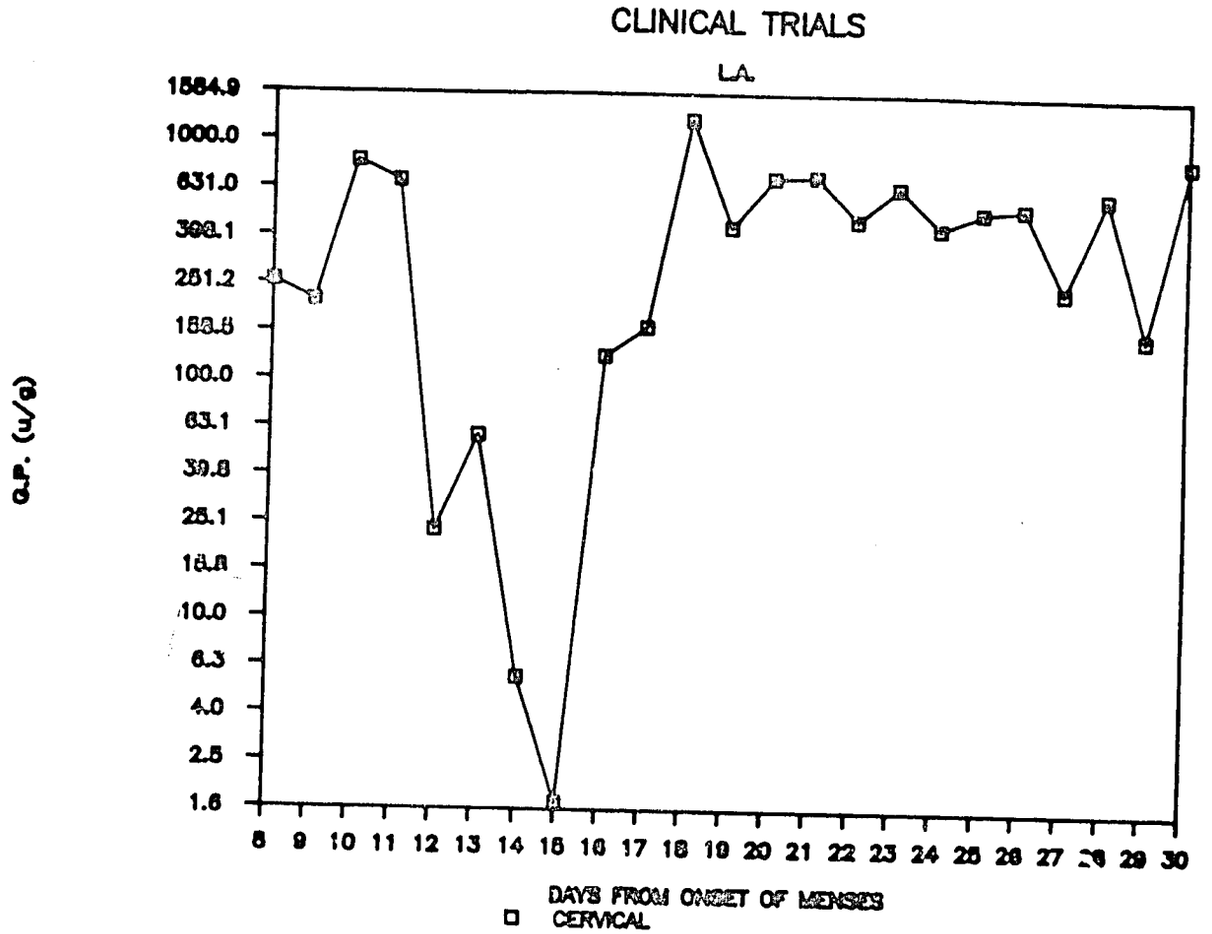

CLINICAL TRIALS
L.A.
1584.9
1000.0
631.0
398.1
251.2
158.5
100.0
63.1
39.8
25.1
15.8
10.0
6.3
4.0
2.5
1.6
G.P. (u/g)
8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
DAYS FROM ONSET OF MENSES
CERVICAL

FIGURE 5

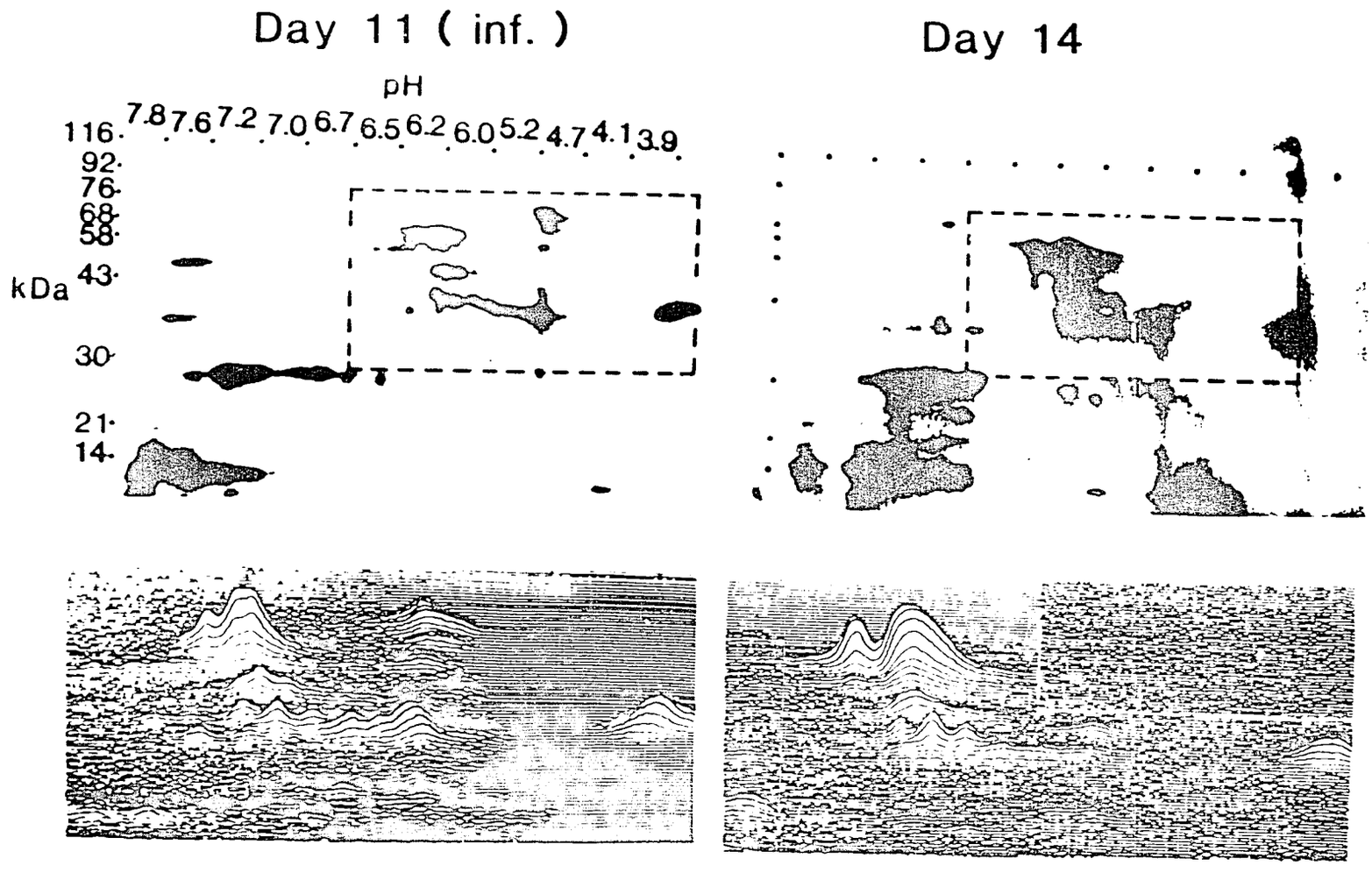
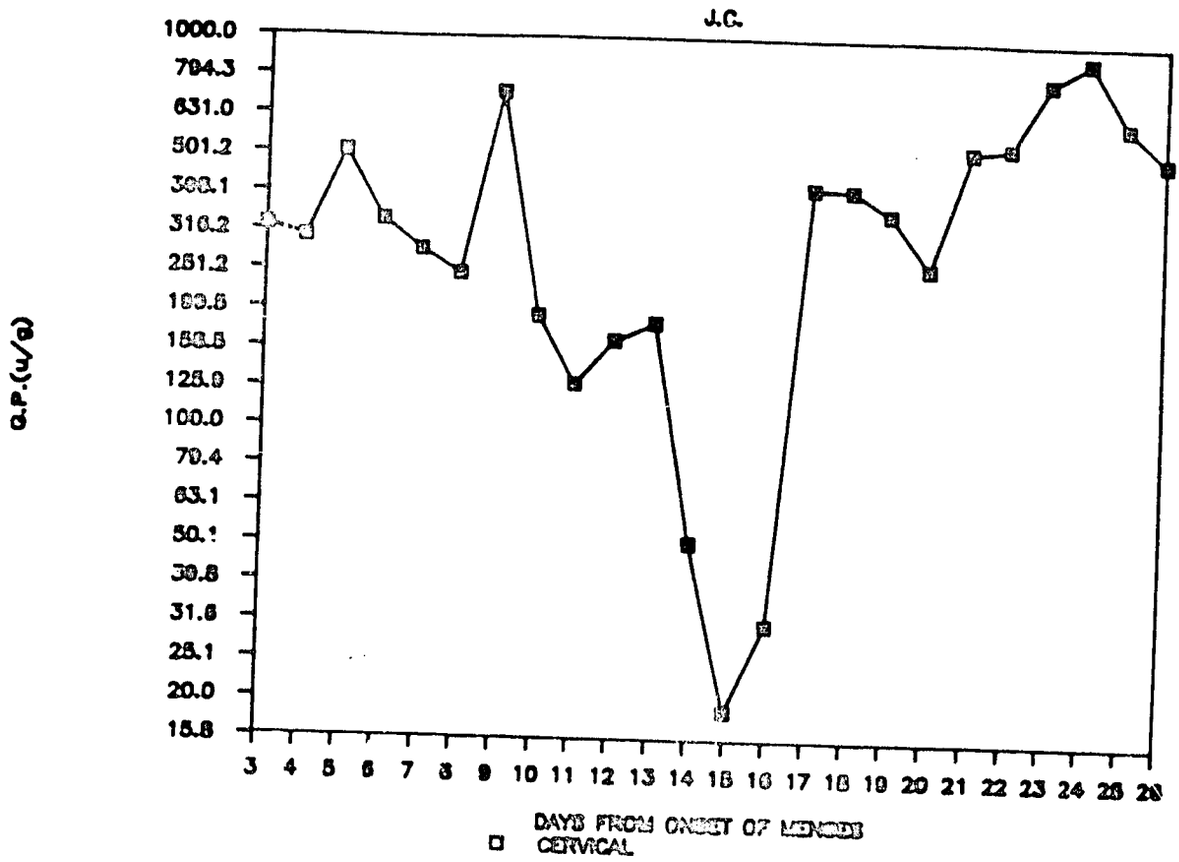

Day 11 (inf.)
Day 14
pH
7.8 7.6 7.2 7.0 6.7 6.5 6.2 6.0 5.2 4.7 4.1 3.9
116
92
76
68
58
43
kDa
30
21
14

FIGURE 6

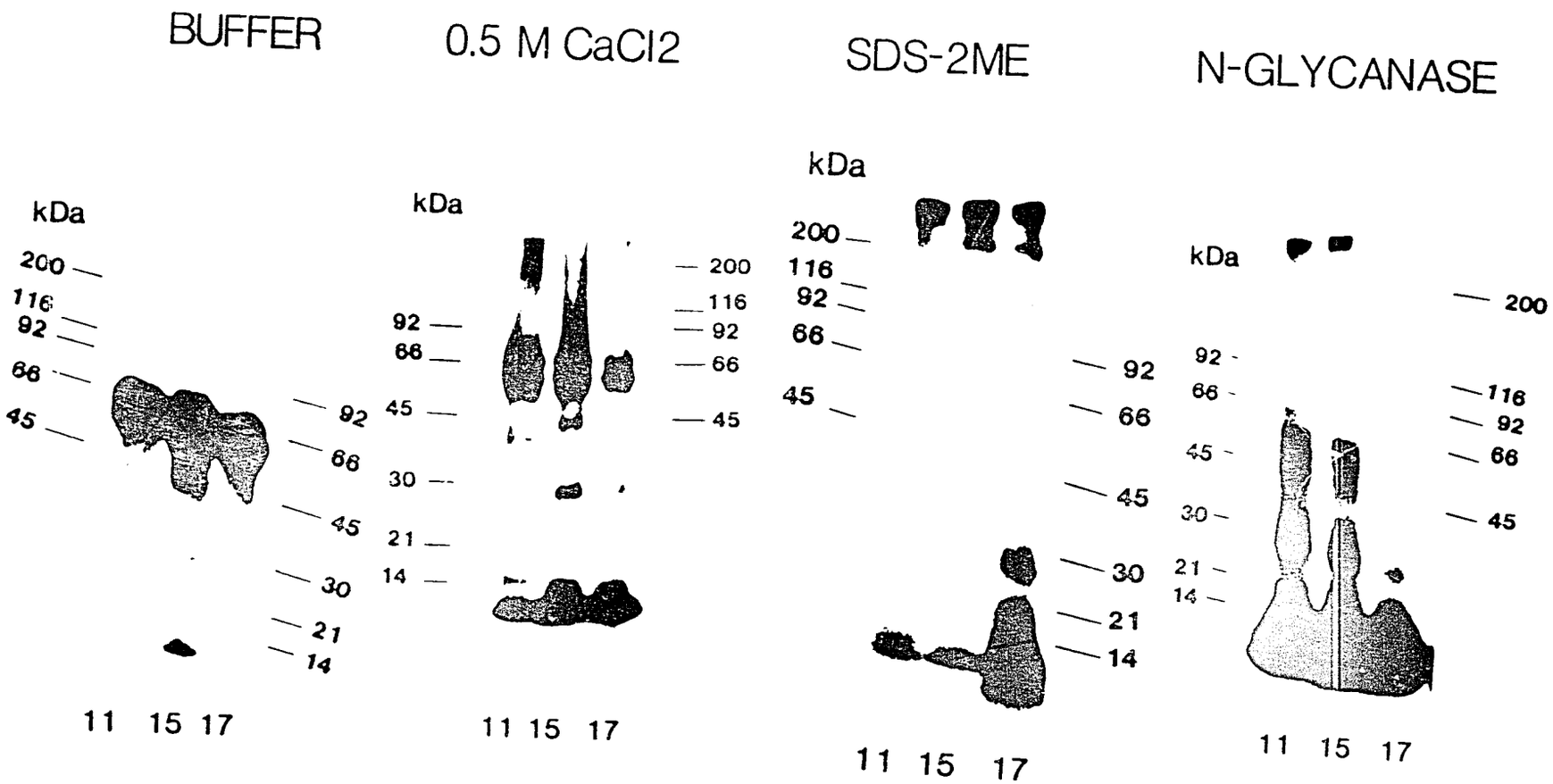


Mucus from Days 11,15 and17 was used in Intra-iodination studies.

FIGURE 7

INTRA-IODINATION OF CERVICAL MUCUS PROTEINS

EXTRACTION PROCEDURE



DAYS FROM ONSET OF MENSES

PARFR-362

"COMBINATION INJECTABLE STEROIDAL MICROSPHERE - CONTINUATION OF PARFR-332"

DANNY H. LEWIS, PH.D.

10/1/84-11/30/85

FINAL TECHNICAL REPORT: SEE IND #28,263

FINAL TECHNICAL REPORT ON PROJECT NO. PARFR-363, NOVEMBER, 1986

I. PROJECT REPORT SUMMARY

A. General Information

Title: **Laboratory Studies on the Antispermatogenic Agent, THP, for Control of Male Fertility.** PARFR-363.

Principal Investigator: Dr. John P. Wiebe
Hormonal Regulatory Mechanisms Laboratory
Department of Zoology
The University of Western Ontario
London, Ontario, CANADA N6A 5B7
(519) 679-6451

Institution: THE UNIVERSITY OF WESTERN ONTARIO

Subagreement Period. Starting date: October 1, 1984.

Ending date: November, 1986.

B. Narrative Summary

The overall objective of the project was to determine the antifertility effects of intratesticular injections of 1,2,3-trihydroxypropane (THP; glycerol) solution in small mammals and subhuman primates. During this period of the PARFR subagreement progress was made in several areas. It was determined that THP exerts a dose-response on spermatogenic arrest and that the volume of THP injected must be adequate for dispersion of the substance throughout the testis. In adult rats 200 ul per testis of a 35-70% THP solution resulted in sustained infertility for at least 16 months. Histological evidence indicates that the treatment may be partially reversible in some instances and further work is required to establish the appropriate treatment regimens. Experiments with ^{14}C-THP have shown that the substance is 99.8% removed from the testes by 24 hours after an injection. An increase up to about 13% of the injected radioactivity is observed in the blood at 30 minutes, followed by a rapid decline. Some of the THP is metabolized to CO_2 and about 10% of the radioactivity appears in the urine and feces. Relatively small amounts (< 2%) appear in the epididymides, prostate, seminal vesicles, kidney, liver, and body fat during the first 30 minutes after an injection and then rapidly decline to mere traces. Experiments to explore the possible mechanism of action of THP have shown that the tight junctions between Sertoli cells do not appear to be disrupted by the THP treatment and that the effect may be due to osmotic shock. Five separate experiments examined the effects of THP on gonadotropin receptors and serum gonadotropin levels from 1 to 120 days after the injection. The results indicate increases in serum LH and FSH by 4-8 days after the treatment and concomitant reduction in LH and FSH receptors in the testes; by 15-32 days after the treatment, the gonadotropins and the receptors have returned to control values and remain the same as the controls thereafter. Experiment with rabbits have shown that THP significantly reduces sperm formation and results in complete infertility in this species but that several injections appear to be necessary. Levels of androgen binding proteins in testicular tissues of rats and rabbits were not altered by the THP treatment. Experiments in progress with Squirrel monkeys show that a single injection abolishes sperm in ejaculates for at least 7 months (longest sampling to date) and that the serum hormone levels are not altered. The studies in our laboratory are the first to consider the antispermatogenic action of THP. Our investigations to date have shown that THP has the potential of acting as an antifertility agent, not only in rats, but also in rabbits and monkeys and that the endocrine systems, libido, and accessory structures remain unaffected and no untoward side-effects are noted. It is the first chemical contraceptive method for males which does not employ hormones or toxic substances. Patents are pending in the U.S.A. and Canada and trials should be conducted on humans.

Support for these studies via PARFR subagreement No. PARFR-363 is gratefully acknowledged.

II. FINAL SUBSTANTIVE TECHNICAL RESEARCH REPORT

A. Statement of Objectives

The general objective of the research project is to determine the antifertility effects of intratesticular injections of 1,2,3-trihydroxypropane (THP; glycerol) solution in small animals and subhuman primates. More specifically, the objectives are as follows.

(1) To determine whether rats treated intratesticularly with THP are permanently sterilized.

(2) If fertility returns after any dose, to examine sperm morphology and motility, conception rate and development of offspring (fetal and postnatal) during the recovery phase.

(3) To determine the effectiveness of THP treatment in rabbits and monkeys.

(4) To examine early responses, including tissue distribution of radiolabelled THP and histological changes in testes and epididymides.

(5) To initiate morphological and biochemical assessment of organs which may be exposed to significant concentrations of THP after intratesticular administration of effective doses. This includes attempts to determine the mechanism of action of THP viz. effects on cell membranes and tight junctions, effects on androgen binding protein (ABP) and possible production of auto-antibodies.

(6) To establish the intratesticular distribution of THP and the biochemical and morphological changes in Sertoli and germinal cells.

B. Summary of Progress

OBJECTIVE 1. Before exploring whether rats treated intratesticularly with THP are permanently sterilized it was necessary to determine the most effective dose and volume. Several experiments were performed, on a total of 222 rats, to assess these parameters. In one experiment, 5 mature male rats were used per treatment group and received either no injection, an injection of distilled water, or 50, 100, or 200 ul of THP (70%) solution. The results showed a progressive decrease in weights of testes and epididymides with increasing volume of THP; the number of epididymal sperm was decreased by 37%, 67%, and 94% by the 50, 100, and 200 ul injections, respectively. The distribution of THP throughout the testis following injection appears to depend primarily on diffusion and consequently treatment with the larger volume indicates the most pronounced effect. In other experiments, young adult rats were injected (150-200 uL) intratesticularly with either 0, 10, 20, 30, 35, 40, 50, 60, or 70% THP solution. Each rat was allowed to cohabit with a virgin female for five days, approximately every 60 days; matings were confirmed by the appearance of a vaginal plug and the number of pups per litter were recorded. The matings showed that the 30-70% THP solutions resulted in infertility which was still in effect at 7, 9, 12, 14 and 16 months

post-injection. Some rats were sacrificed at various times after the injection and the weights of testes and epididymides and the number of sperm in the epididymides were determined. The treatments resulted in approximately progressive decreases in testis and epididymis weights and sperm numbers, the maximum effect occurring with solutions containing 40-70% THP. In general, treatment with 40-60% THP resulted in permanent infertility. However, two males which had been infertile during 4-5 successive mating periods (2-10 months after the treatment) sired a litter of 2 and 3 normal offspring each at 10 and 12 months, respectively, following the injection. In the longest experiment, 6 out of 8 treated (60% THP) rats had essentially no sperm (99.9994% reduction re controls) in the epididymides at 19.5 months post-injection; two treated rats contained sperm (about 10% of controls) in the epididymides while the controls had an average of 3.4×10^8 sperm each. Not all the histological studies have been completed but some results from long-term experiments indicate regions with resumption of spermatogenesis in testes treated 9 months previously. It appears from the studies completed that a single injection of 40-70% THP solution generally results in permanent or near-permanent infertility in the rat. Whether reversible infertility might be routinely achieved with several injections of a lower concentration or volume of THP remains to be explored.

OBJECTIVE 2. The experiments set up under Objective 1 were also used for Objective 2. As indicated above, the question of reversibility of the treatment has not been resolved and more studies are required. In two instances where previously-infertile males sired small litters, these offspring appeared to develop normally.

OBJECTIVE 3. **RABBITS.** Two experiments have been conducted with rabbits (n=7-10); one experiment was completed and one is still in progress. Each testis received injections of 0.2-1.0 ml water (Control) or THP solution, and semen collections were made regularly by artificial vaginae. The results indicate that in the rabbit, multiple injections appear to be more effective than a single treatment. The results of the first experiment in which rabbits received 5 injections are shown in the Table below.

Treatment	Ejaculations per Rabbit	Sperm No./ Ejaculate ($\times 10^{-6}$)	Sperm Motility (0-4)	Sperm Viability (%)	Fertility (litters/ mating)
Control	11.3 ±1.3	500 ±187	3.50 ±0.22	69 ±2	6/6
THP	14.8 ±1.9	0.06 ±0.04	0.36 ±0.28	0.02 ±0.02	0/6

(From Wiebe et al., In Male Contraception: Advances and Future Prospects, edited by G.I. Zatuchni, A. Goldsmith, J.M. Spieler, & J.J. Sciarra, 1986, pp.252-270.)

The THP treatment did not affect the volume of fluid per ejaculation but resulted in 99.9% reduction in sperm number per ejaculate, 100% infertility upon matings, without significant changes in libido, serum androgen and estrogen, testicular steroidogenesis, gonadotropin-receptor binding and accessory sex structures. The second experiment is still underway and will include more detailed gonadal steroidogenesis studies, histology, and the long term effects, in addition to the above parameters.

PRIMATES. We encountered some constraints related to the primate experiments. At the time of the proposal, Dr. J. Wong of the Animal Resources Division of Health and Welfare Canada, supplied a letter which stated that we would be able to carry out the monkey experiments in the holding facilities of H & W, Ottawa. A pilot experiment was set up with 8 males (4 controls; 4 THP treated). These were males that could not be used for the H & W mating program because of excessive aggression. They were available to us and we used them to explore injection and electroejaculation techniques. They received two injections of 0.4ml/testis. Because of serious cutbacks in the H & W program and resulting space limitations, it was necessary to abruptly halt the trials.

After numerous unsuccessful attempts to obtain holding facilities at various institutions, experiments on 20 Squirrel monkeys were begun November, 1985, at the facility of Dr. R. Dukelow (Michigan State University, East Lansing) and these are still in progress. Ten monkeys were injected with saline (C) and 10 with THP and ejaculates and blood samples were obtained every 2-3 weeks. By the 7th week after injections the number of sperm was 330 and 7 ($x10^6$) for C and THP, respectively, and only 2 out of 10 THP monkeys had sperm. Seven months after the injection (the latest samples analized) the number of sperm in ejaculates was still zero in 9 out of the 10 monkeys; ejaculates from one of the THP monkeys have consistently contained sperm and the size of the testes indicate that the THP solution had not been properly injected into the testes. Analyses of 70 serum samples from the control and THP treated monkeys indicate no differences due to treatment in serum testosterone, FSH, and LH levels. From the results to date it is apparent that THP treatment of primates also results in cessation of spermatogenesis without any adverse side effects. It is planned to terminate half of the treated monkeys within the next few months and to maintain the rest for a period of approximately 1-2 years to determine whether the effect is permanent. At the termination of the experiment, the tissues will be processed for histology, testicular steroidogenesis, hormones and hormone receptors, epididymal sperm morphology and numbers, and accessory sex structures.

OBJECTIVE 4. To determine the extent to which THP is distributed to the rest of the body tissues, ^{14}C-THP (2.5×10^5 dpm/ 150 ul/ testis) was injected intratesticularly into 49 rats. At time 0, 5, 15, 30, 45, 60 minutes, and 2, 3, 4, and 24 hours, and 7 days, 3-5 rats were killed and gonads, epididymides, prostate, seminal vesicles, kidneys, and samples of blood, liver, and fat were removed. The tissues were homogenized (using a Polytron) and extracted in 80% ethanol and 1.0 N NaOH and the radioactivity was determined by scintillation spectrometry (Philips Model PW4700), using dioxane or Triton X-100 scintillation fluid. The results show that the radioactivity within the testes decreases rapidly to about 25% (of initial) by 1 hour, 13% by 2 hours, and 0.2% by 24 hours after the injection. About 1.5% of the radioactivity is transferred to the epididymides, 0.15% to the prostate, and 0.4% to the seminal vesicles within 5 minutes of an injection and the radioactivity in these tissues then gradually declines to less than 0.1% by 24 hours. An increase up to about 13% of the injected radioactivity was observed in the blood during the first 30 minutes, followed by a rapid decline to about 2.6% at 1 hour and a gradual decrease to less than 0.9% at 24 hours and less than 0.07% at 7 days. Small percentages of radioactivity

were noted in other tissues, the peaks occurring at 30 minutes in the kidneys (0.6%), 45 minutes in the fat (1.9%), and at 60 minutes in the liver (1.65%). That some of the THP injected intratesticularly may be metabolized is suggested by the radioactivity recovered from the expired air, the urine, and the feces. About 1.2% of the initial radioactivity was present in the CO_2 trapped in KOH during the first hour after an injection, and the total radioactivity recovered in the KOH in a 24-hour period following an injection amounted to approximately 5%. In a 24-hour period, about 10% and 1.3% of the initial radioactivity was recovered in the urine and feces, respectively.

(In order to measure ^{14}C-THP conversion to $^{14}CO_2$ a special apparatus was designed, employing a dessicator, which permitted controlled flow of incoming air and CO_2 trap for all outgoing air. The apparatus was tested using ^{14}C-labelled bicarbonate with controlled acidification.)

OBJECTIVE 5. The testes are exposed to the highest concentrations of THP under the treatment regimens which we employ and as indicated under Objective 4, above, THP is rapidly metabolized so that only very small amounts are present in any tissue 24 hours after an injection. It is well documented that THP is normally present in the serum of mammals and is readily utilized by nearly all tissues. Because of the normal ubiquitous distribution of THP in animal tissues, and its involvement in cellular structures and in lipid and carbohydrate metabolism, and the lack of appreciable concentration of THP in any tissues following an injection, we do not anticipate any toxic effects from a single intratesticular treatment. THP has been employed in humans and animals, without any deleterious effects, at levels which are several thousand fold higher than those employed in our treatment regimens.

We examined the possible mechanism of action of the THP treatment at the level of testicular ultrastructure, gonadotropin receptors, testicular steroidogenesis, and androgen binding proteins (ABP). In a series of experiments, rats were injected with either the THP or the control solution and 24 hours, 9 days, 14 days, or 60 days later the testes were fixed by perfusion with glutaraldehyde/formalin mixture. Examination of tissues at the light and electron microscope level indicate that the limiting membranes around the seminiferous tubules may be affected by the treatment but the myoid cells, Leydig cells, Sertoli cells, and primary germ cells appear normal, and Sertoli cell tight junctions retain the capacity to exclude lanthanum. A physico-chemical change occurs in some seminiferous tubules which results in an obstruction that appears to block the tubules. In order to test whether the changes result from osmotic shock, testes were treated with sucrose, glucose, or propylene glycol. Histological examination of the testes 2 weeks later indicated that these substances are able to induce similar obstructions in the tubules. In one experiment rats were treated with either saline (C), THP (50%), propylene glycol (50%), or glucose (6.85 M) and various parameters were examined 4, 14, 60, and 120 days later. Although THP, propylene glycol, and glucose treatments were all effective, the THP effect was greatest with respect to reduction in testis and epididymis size, and sperm numbers.

The early effects of THP treatment on gonadotropin receptors in the

testes and on serum gonadotropin levels have been examined in five separate experiments. Rats were injected with 150-200 ul (per testis) of either the control or the THP solution and then were sampled at 1, 4, 8, 15, 32, 55-60, and 120 days after the injection. The THP treatment appears to result in increases in serum LH and FSH by 4 days after the treatment and after 15 days the levels appear to be the same as in the controls. On the other hand, the hCG-receptor and FSH-receptor binding are significantly reduced ($p < 0.05$) between 1 and 8 days after the THP treatment. By 15-32 days after the treatment, the gonadotropin-receptor binding appears to have returned to the control values and no differences between control and treated tissues were observed thereafter. These results suggest that there may be an initial effect on the pituitary gonadotropins which is reversed within 2 weeks after the treatment. The decreased gonadotropin-receptor binding may be a reflection of higher receptor occupancy during the first 2 weeks when serum levels are high.

The ABP content of rat and rabbit testicular tissue was determined and no differences were found between the controls and the THP treated testes. Tests on rabbit serum samples indicate no differences in agglutination due to autoantibodis between control and THP treated animals.

OBJECTIVE 6. In vivo and in vitro experiments suggest that THP is distributed more or less throughout the testis if the injected volume is adequate. Biochemical and morphological changes are being examined (see objective 5) and indicate an osmotic effect on the seminiferous tubules which results in a permanet or semi-permanent alteration of the milieu preventing spermatogenesis. The volume of THP solution employed appears to be important. If not enough is injected, it appears that areas may remain in the testis where spermatogenesis is continued. It had been our assumption that most of the injected THP remained in the testis and that the action might be due to a prolonged osmotic effect or a disruption of the tight junctions between Sertoli cells. The studies with the radiolabelled THP indicate that it is metabolized and/or removed from the testis very rapidly and that the antispermatogenic effect may be induced during the first 24 hours after treatment. The effect does not appear to be due to a disruption of the junctional complexes.

Dissemination and Utilization of Research Results

Some of the results were presented at the IARFR International Workshop on Male Contraception, held in Geneva, May, 1985, and appeared as Chapter 24 of the proceedings, MALE CONTRACEPTION: ADVANCES AND FUTURE PROSPECTS, Edited by G.I. Zatuchni, A. Goldsmith, J.M. Spieler, and J.J. Sciarra, Harper & Row, Philadelphia, pp.252-270, 1986.

Results have been presented at the 1984, 1985, and 1986 annual meetings of The Society for the Study of Reproduction. Several papers are in preparation.

Several invited lectures have been presented at other institutions and societies in Canada and Europe and on national television and radio networks.

FINAL TECHNICAL REPORT

Submitted to the:

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

I. GENERAL INFORMATION:

1. Title and PARFR Subagreement Number:

"Antifertility Effects of Microencapsulated LH-RH Agonist"

Project Number: PARFR-364

2. Principal Investigator:

Francisco J. Rojas, Ph.D.

3. Present Institution:

University of California, Irvine
California College of Medicine
University of California, Irvine Medical Center
Department of Obstetrics and Gynecology
101 The City Drive
Orange, California 92668

4. Subagreement Period:

a) Original Starting Date: September 1, 1984

b) Ending Date: June 30, 1986

5. Date of Last Technical Report:

July 1986

II. GENERAL OBJECTIVES:

The objective of the present study was to determine whether the administration of microencapsulated D-Trp-6-LH-RH once a month, for a period of eight (8) months, can consistently inhibit ovulation in the regularly cycling cynomolgus monkeys.

III. PROJECT SCHEDULE AND METHODS

Microcapsules of poly[DL-Lactide-Co-Glycolide] containing D-Trp-6-LH-RH at a release rate of 20 ug/day for 30 days, or vehicle were used. The microcapsules were supplied by Dr. Andrew V. Schally.

For injection, the microcapsules were suspended in sterile vehicle containing 2% of carboxymethylcellulose (CMC) and 1% of Tween 20 (by weight) in saline. Each animal received a single 1 ml S.C. injection of about 20 mg of microcapsules in vehicle or vehicle only.

The animals were individually caged and exposed to centrally controlled temperature (23 +1 C), humidity (50%) and light-dark photoperiod (0600-2000 h.).

Twenty regularly cycling cynomolgus monkeys, divided into groups of 5 animals each, were used according to the following treatment protocol (Table 1):

TABLE 1. TREATMENT PROTOCOL

Group	Vehicles	D-Trp-6-LH-RH microcapsules	Progesterone in oil	Oil vehicle
1	-	+	-	+
2	-	+	+	-
3	+	-	+	-
4	+	-	-	+

Each animal received:

1. A single s.c. injection of microcapsules, or vehicle, every 30 days for 8 consecutive months, starting on week 1 of the first menstrual cycle.

2. A Progesterone challenge (50 mg i.m. in oil), or oil vehicle, on day 25 of each cycle.

Before the first injection of D-Trp-6-LH-RH microcapsules, daily i.m. injectons of 100 ug D-Trp-6-LH-RH, starting on day 1 of the first menstrual cycle and for 7 days, were administered to ensure a faster and better inhibition of pituitary-gonadal axis. In subsequent months, the monkeys received only the microcapsules every 30 days, as indicated in the above design.

Laparoscopies were performed at periodic intervals to determine the degree of foliculogenesis and to time the ovulation date for each month.

Blood was drawn every week. Circulatory concentration of FSH, LH, PRL, estradiol, and progesterone was determined by RIA. Also, the levels of D-Trp-6-LH-RH in peripheral plasma was monitored throughout the study.

The following was determined in animals of all groups:

1. Ovulatory versus anovulatory rate monitored by serial laparoscopies and determinations of circulatory concentrations of FSH, LH, prolactin, estradiol, and progesterone.

2. Menstrual bleeding pattern.

3. Return to normal menstrual cyclicity after discontinuation of D-Trp-6-LH-RH microcapsules for 3 months.

4. Endometrial histology in animals treated with D-Trp-6-LH-RH microcapsules compared to those with D-Trp-6-LH-RH microcapsules and progesterone.

IV. RESULTS

1. Menstrual cycle changes

In accordance with the project protocol, we first analyzed the regularity of the menstrual cycles in each of the twenty cynomolgus monkeys involved in the study. To accomplish this, all the animals were periodically checked for regular cycles by vaginal swabbing, serial laparoscopies, and serum estrogen and progesterone determinations. The present study was carried out with monkeys that underwent at least two consecutive normal, ovulatory menstrual cycles.

A single s.c. injection of D-Trp-6-LH-RH microcapsules every 30 days for 8 months resulted in inhibition of normal ovulation during treatment in all animals (Groups 1 and 2), as determined by laparoscopy and hormone analysis (Table 2).

Three of the five monkeys receiving D-Trp-6-LH-RH microcapsules only (Group 1) were amenorrheic. One monkey had uterine bleeding for two days on one occasion, another had one-day bleeding, twice, during the 8 months of treatment (Table 2).

In the group receiving D-Trp-6-LH-RH microcapsules plus a progesterone challenge on day 25 of each month (Group 2), four of the five monkeys had either cyclical or irregular uterine bleedings during treatment. One monkey had uterine bleeding only once (Table 2).

All control animals (Group 2 and 3) presented monthly ovulatory cycles.

Table 2.

OVULATION AND UTERINE BLEEDING CHANGES DURING 8 MONTHS TREATMENT

Group	Animal	Ovulations	Uterine bleedings
1. D-Trp-6-LH-RH microcapsules	N-25	Anovulatory	2 days bleeding, once
	N-41	Anovulatory	Amenorrhea
	N-33	Anovulatory	Amenorrhea
	N-11	Anovulatory	Amenorrhea
	M-59	Anovulatory	1 day bleeding, twice
2. D-Trp-6-LH-RH microcapsules plus progesterone challenge	N-29	Anovulatory	2 days bleeding, once
	N-39	Anovulatory	cyclical (monthly)
	N-27	Anovulatory	irregular
	N-45	Anovulatory	irregular
	M-97	Anovulatory	irregular

2. Endocrine changes

In contrast to control groups (Group 2 and 3), no specific variations in hormone patterns were observed during treatment with D-Trp-6-LH-RH microcapsules (Group 1). This was also the case for Group 2 receiving D-Trp-6-LH-RH microcapsules plus a progesterone challenge, except that serum progesterone levels changed cyclically in accordance to the injections of progesterone.

Mean values of serum estradiol and progesterone concentrations during the first 4 months of microcapsule injections in Group 1 are shown in Fig. 1. Estradiol levels remained about 50 pg/ml and progesterone fluctuated as a mean within anovulatory range throughout the treatment.

Corresponding patterns of serum FSH, LH, and PRL concentrations from one monkey (N-25) are shown in Fig. 2.

Similar hormone results were observed during the entire length of treatment.

We also monitored concentrations of D-Trp-6-LH-RH in peripheral serum throughout the study in the monkeys receiving microcapsules. Immunoreactive serum D-Trp-6-LH-RH levels were measured by RIA using anti-D-Trp-6-LH-RH antiserum as described by Mason-Garcia et. al. (Proc. Natl. Acad Sci. 82:1547-1551, 1985). A typical standard curve is shown in Fig. 3. Intra-assay coefficient of error was less than 10% at 30% maximum binding, and the sensitivity was 50 pg/ml serum.

We found that a single injection of D-Trp-6-LH-RH microcapsules every 30 days provided a sustained concentration of immunoreactive D-Trp-6-LH-RH in the serum for 8 consecutive months. Mean values of D-Trp-6-LH-RH measured one week after the first injection of microcapsules averaged 750 pg/ml. Serum D-Trp-6-LH-RH levels declined thereafter and remained relatively stable after 6 weeks, for the remaining months of treatment. Fig. 4 shows the mean values of D-Trp-6-LH-RH during the first 4 months of microcapsule injections in Group 1.

FIGURE 1:

SERUM CONCENTRATIONS OF ESTRADIOL AND PROGESTERONE IN CYNOMOLGUS MONKEYS THAT RECEIVED A SINGLE INJECTION EVERY 30 DAYS OF THE D-TRP-6-LH-RH MICROCAPSULES.

(MEANS ± SE.)

FIGURE 1:

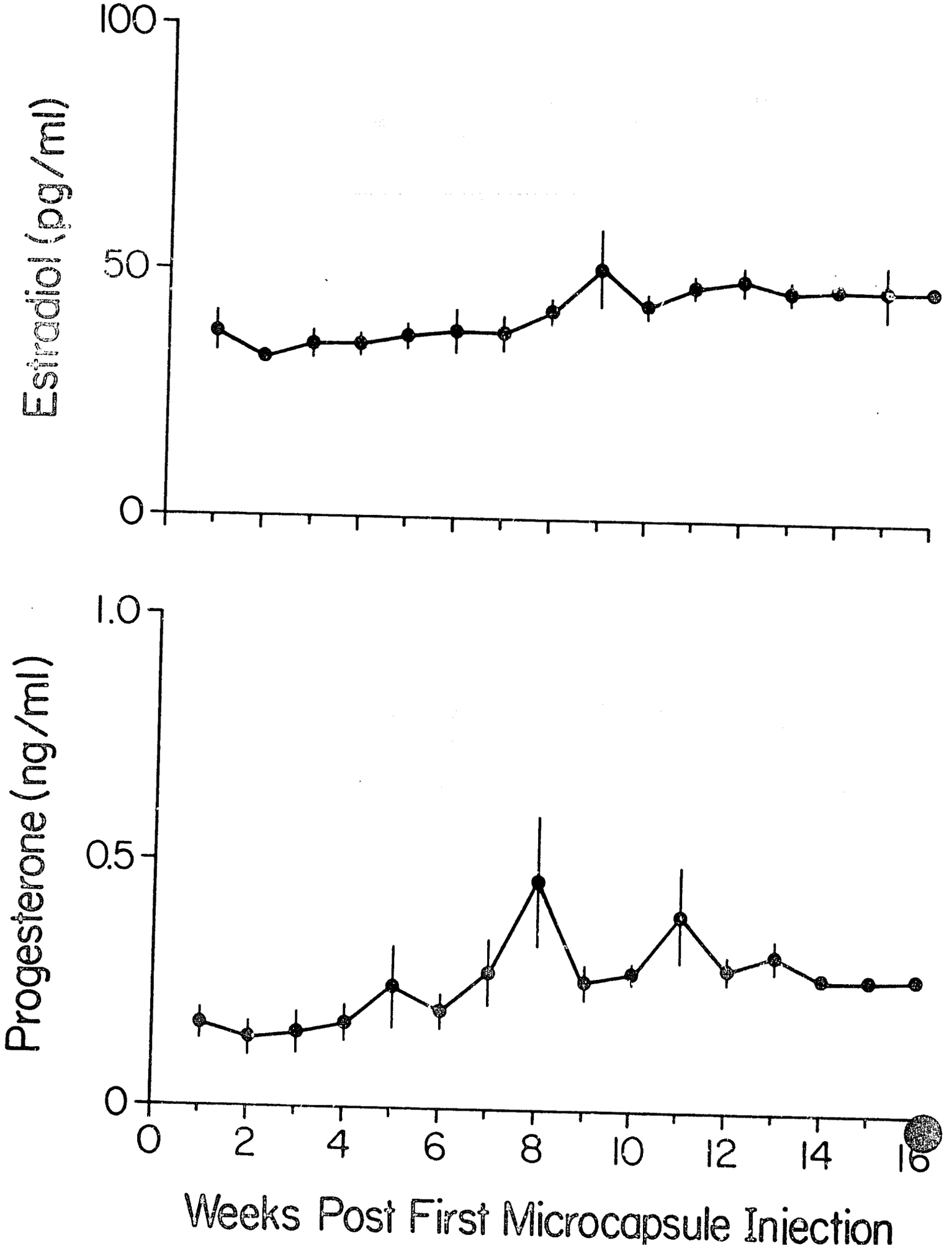

100
50
0
Estradiol (pg/ml)
1.0
0.5
0
Progesterone (ng/ml)
0
2
4
6
8
10
12
14
16
Weeks Post First Microcapsule Injection

FIGURE 2:

SERUM CONCENTRATIONS OF LH, FSH AND PRL IN A REPRESENTATIVE CYNOMOLGUS MONKEYS THAT RECEIVED A SINGLE INJECTION EVERY 30 DAYS OF THE D-TRP-6-LH-RH MICROCAPSULES

FIGURE 2

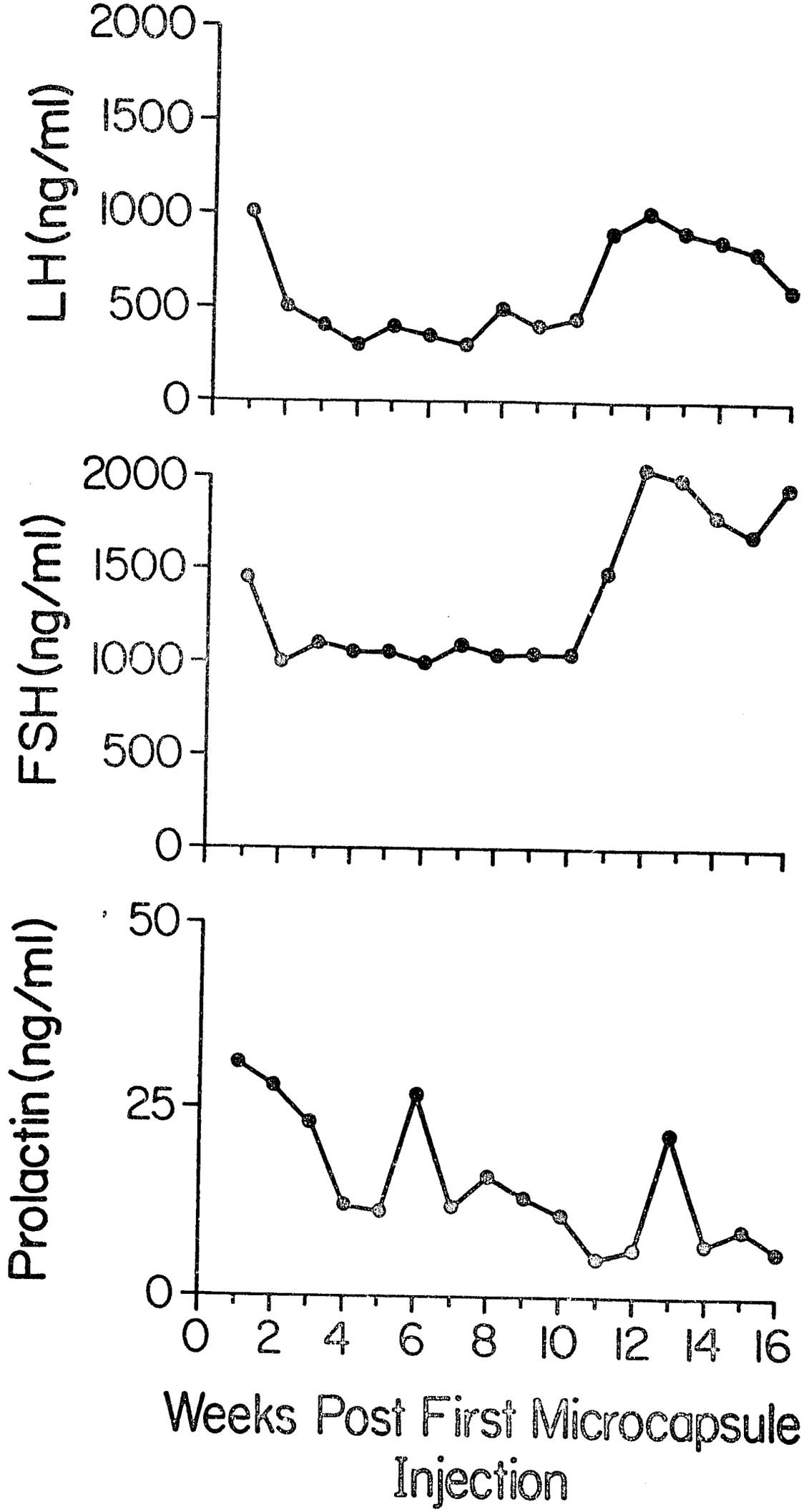

LH (ng/ml)
2000
1500
1000
500
0
FSH (ng/ml)
2000
1500
1000
500
0
Prolactin (ng/ml)
50
25
0
0 2 4 6 8 10 12 14 16
Weeks Post First Microcapsule Injection

FIGURE 3:

REPRESENTATIVE STANDARD CURVE FOR THE AGONIST D-TRP-6-LH-RH RADIOIMMUNOASSAY. ASSAYS WERE PERFORMED USING RABBIT ANTI-D-TRP-6-LH-RH ANTISERUM. IODINATED D-TRP-6-LH-RH WAS ADDED IN ALIQUOTS OF 100 ul CONTAINING 25,000 cpm OF WHICH ABOUT 30% OF THE LABELED LIGAND WAS BOUND. DATA ARE PRESENTED BY PLOTTING LOGIT B/B AGAINST LOG DOSE. EACH DATA POINT REPRESENTS THE MEAN OF TRIPLICATE DETERMINATIONS.

FIGURE 3

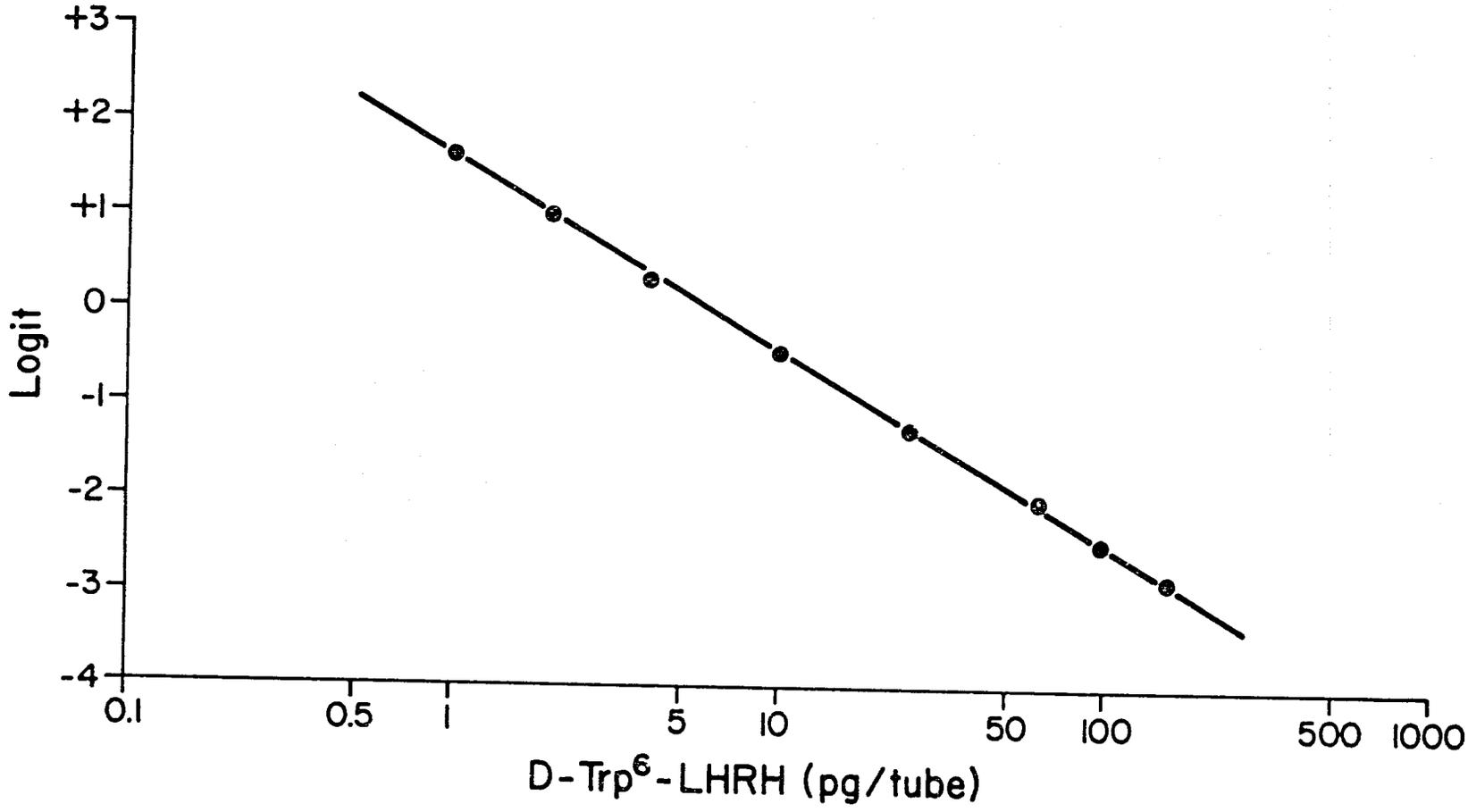
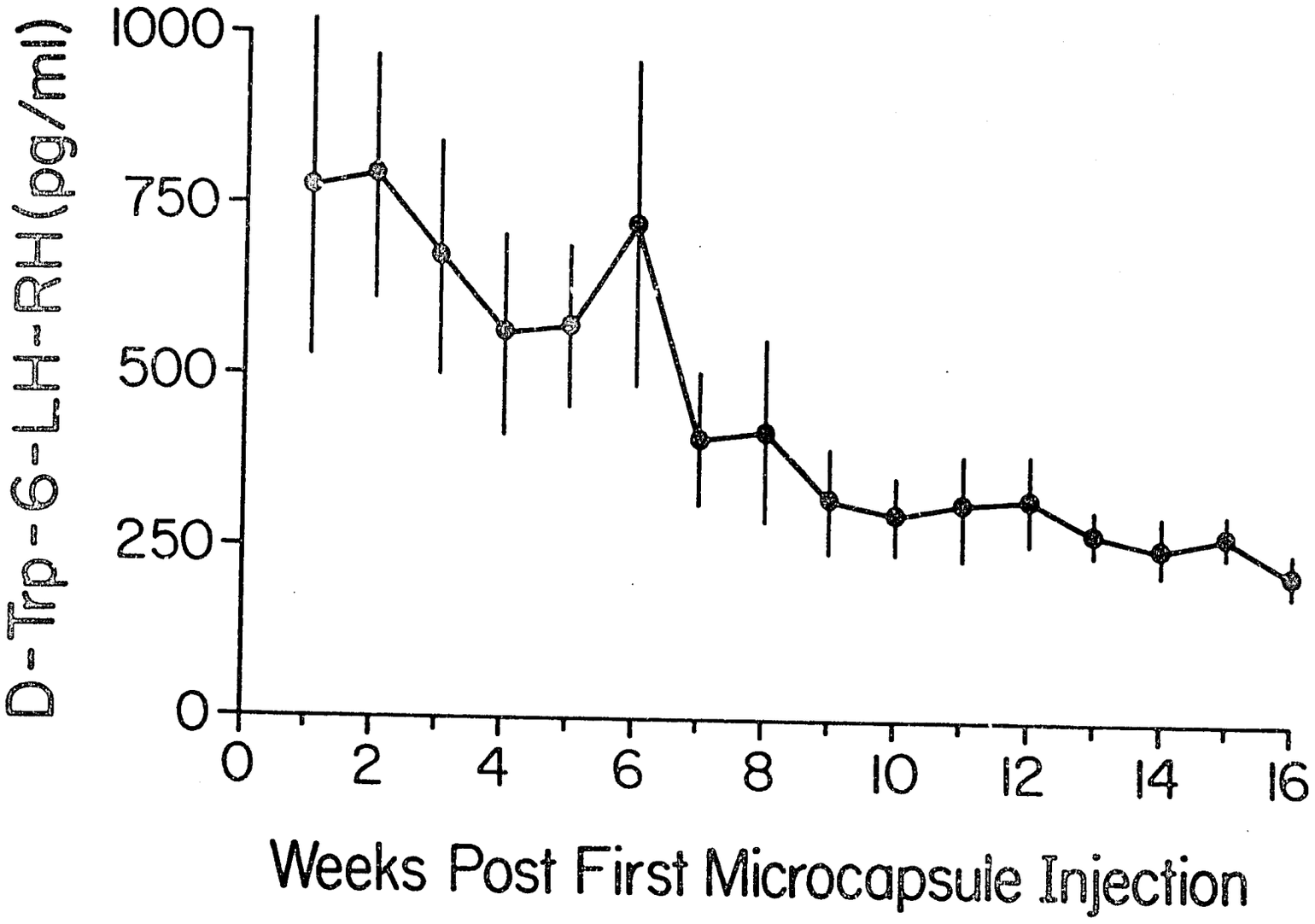


FIGURE 4

SERUM CONCENTRATIONS OF D-TRP-6-LH-RH IN CYNOMOLGUS MONKEYS AFTER A SINGLE INJECTION EVERY 30 DAYS OF THE D-TRP-6-LH-RH MICROCAPSULES.

(MEANS ± SE.)

FIGURE 4


D-Trp-6-LH-RH(pg/ml)
1000
750
500
250
0
0
2
4
6
8
10
12
14
16
Weeks Post First Microcapsule Injection

3. Results of uterine biopsies

All specimens examined by light microscopy in Group 1 showed a thin endometrium, with an inactive proliferative pattern. Marked proliferation or hyperplasma was never observed. Thus, there were no signs of hyperestrogenic stimulation of the endometrium, in the animals receiving D-Trp-6-LH-RH microcapsules. Similar conclusions were drawn from biopsies of Group 2.

4. Recovery

All treated animals returned to normal uterine bleeding after discontinuation of D-Trp-6-LH-RH microcapsules. Table 3 shows the interval to the next menstrual bleeding after the last injection of microcapsules.

Regularity of two consecutive cycles following discontinuation of treatment was checked by vaginal swabbing, laparoscopies, and serum estrogen and progesterone determinations. In all animals of experimental groups (Groups 1 and 2), ovulation and subsequent luteal phase had normal characteristics and were similar to those of both vehicle-injected controls and non-treated animals in our colony.

Table 3

MENSTRUAL BLEEDING AFTER LAST INJECTION OF D-TRP-6-LH-RH MICROCAPSULES

Group	Animal	Interval to next menstrual bleeding (days)
1. D-Trp-6-LH-RH microcapsules	N-25	97
	N-41	83
	N-33	73
	N-11	83
	M-59	84
		Average = 84 days
2. D-Trp-6-LH-RH microcapsules plus progesterone challenge	N-29	87
	N-39	74
	N-27	83
	N-45	83
	M-97	88
		Average = 83 days

V. CONCLUSIONS AND SIGNIFICANCE OF THE STUDY

The present results indicate that a single subcutaneous injection of microencapsulated D-Trp-6-LH-RH once a month, can supress ovulation in regularly cycling cynomolgus monkeys as determined by laparoscopy and hormone analysis. The inhibition of ovulation was consistent for a period of eight (8) months among the animals injected with the potent LH-RH agonistic analogue, as compared to the control (vehicle-treated) animals.

There were no specific variations in serum FSH, LH, and PRL patterns during treatment. Serum estradiol concentrations remained low, at about 50 pg/ml, and serum progesterone levels fluctuated as a mean within anovulatory range. Determinations of immunoreactive D-Trp-6-LH-RH demonstrated that microcapsule injections provided sustained concentrations of the agonist in the peripheral serum for 8 consecutive months.

Light microscopic studies performed on uterine biopsies revealed that an inactive proliferative endometrial pattern, with no signs of hyperplasia. These observations did not agree with a previous contention that prolonged treatment with an LH-RH agonist may cause proliferative and hyperplastic changes in the endometrium due to an unopposed endogenous estrogen secretion.

We also found that all treated animals returned to normal menstrual cyclicity within three months after discontinuation of D-Trp-6-LH-RH microcapsules. This indicates that the prolonged treatment with the microcapsule formulation does not result in long-term detrimental effects on the menstrual cycle.

In conclusion, the results of the present study provide strong support to the concept that once a month injections of LH-RH agonists formulated in microcapsule preparations could have considerable clinical value as a long-term female contraceptive.

Final Progress Report

to

The Program For Applied Research
on Fertility Regulation

Investigator and Organization

Danny H. Lewis, Ph.D.
Vice President, New Product Development

Stolle Research and Development Corporation
P.O. Box 5310
Decatur, Alabama 35601

Title of Project

Development of a 90-Day Contraceptive
Based on Levonorgestrel
Microspheres
Subagreement PARFR-373

October 3, 1986

I. PROJECT REPORT SUMMARY

A. General Information

1. Development of a 90-day contraceptive based on levonorgestrel microspheres. Subagreement PARFR-373.

2. Danny H. Lewis, Ph.D. Stolle Research and Development Corporation, P.O. Box 5310, Decatur, Alabama 35601. (205)353-3814

3. Stolle Research and Development Corporation 2964 St. Rt. 42 South, Lebanon, Ohio 45036.

4. Contract Period: April 1, 1985 - March 31, 1986.

5. Inclusive Dates of this Technical Report: April 1, 1985 - September 1, 1986.

B. Narrative Summary

The objective of this project is the development of a 90-day injectable microsphere system for the delivery of levonorgestrel. The availability of such a product would allow a direct comparison of levonorgestrel, norgestimate, and norethisterone to be made from basically the same controlled-release systems. The same general microencapsulation process and the same biodegradable polymeric excipient is involved in all three of the injectable contraceptive products. The microencapsulation process is the property of Ortho Pharmaceutical Corporation. Stolle R&D received permission from Ortho to produce a levonorgestrel formulation in order that a clinical comparison could be made of the three steroids. It is important to state that any further development of a levonorgestrel system by Stolle R&D would require authorization from Ortho.

We synthesized the appropriate 85:15 copolymer excipient, developed a satisfactory microencapsulation procedure for levonorgestrel, characterized the prototype product in vitro, and conducted a pharmacokinetic study in the baboon model. We found that levonorgestrel can be formulated according to the Stolle/Ortho process to afford good controlled-release characteristics in vivo. The prototype formulation is quite similar to the 90-day norethisterone product in regard to microsphere size distribution, visual appearance, and drug content. The baboon studies confirmed that the test material offers potential as a 90-day injectable contraceptive product. It appears that the next step should be a Phase I clinical trial to establish the pharmacokinetics in humans.

II. Substantive Technical Research Report

A. Statement of Objectives

The objectives of this research project were as follows:

Task 1. Synthesis of research quantities of poly(lactide-co-glycolide) as the biodegradable excipient.

Task 2. Optimization of the Stolle microencapsulation procedure for levonorgestrel.

Task 3. In vitro characterization of levonorgestrel microspheres including SEM, size distribution, and release profiles.

Task 4. Pharmacokinetic studies in baboons to determine the in vivo performance of the most promising formulations.

B. Technical Progress and Accomplishments

1. Synthesis of Biodegradable Excipient

We synthesized an 85:15 lactide:glycolide copolymer as the excipient for the 90-day levonorgestrel formulation. Our experience in developing and testing the 90-day norethisterone and progesterone systems indicated that the 85:15 polymer would be appropriate for the levonorgestrel product. This copolymer biodegrades in about 6 months. We utilized a polymer with an inherent viscosity of about 0.5 to 0.7 dl/g in chloroform at 30°C. The weight average molecular weight was about 50,000. The polymer used in this research program was non-GMP material not for use in humans.

2. Microencapsulation of Levonorgestrel

A solvent-evaporation microencapsulation procedure was used to formulate the levonorgestrel delivery system. This process involves evaporation of an organic solvent such as methylene chloride or chloroform from an oil-in-water emulsion under controlled stirring conditions. The drug and polymer are both present in the oil phase and are combined as solid microspheres as the solvent is removed. This process is particularily useful for the microencapsulation of water-insoluble drugs.

Our experiments with levonorgestrel indicate that this material behaves quite similarly to norgestimate and norethisterone in regard to its microencapsulation. We were able to achieve rather high drug content levels in the final product (45 to 50 % by weight). This high drug content is important in regard to reducing the mass of material needed for a single 90-day treatment.

Our findings indicate that the microencapsulation process can be scaled up in the same manner as for the 90-day norethisterone product. Present work at Stolle R&D on norethisterone should also provide useful scale up information

for the levonorgestrel system if a decision is made to further develop this product.

3. In Vitro Characterizations of Levonorgestrel Microspheres

We characterized all batches of the levonorgestrel product according to scanning electron micrographs, microsphere size distribution, and in vitro release profile.

A representative in vitro release study on Batch SR018-1 is shown in the appendix. Those microspheres were 25 to 90 microns in diameter and were sterilized with 2.0 Mrad of gamma radiation. Note that the in vitro release profile demonstrates very little burst of drug during the initial time period. The in vitro experiments were conducted at 37°C in a receiving fluid of 30% by volume ethanol in water.

The microsphere size distribution for all batches was determined by seiving the product through stainless steel seives of the appropriate size and also by analysis on a HIAC/Royco Instrument. We determined by in vitro release studies and in vivo studies in the baboon that microspheres between 25 and 90 microns in diameter were suitable for the 90-day in vivo release of the steroid. HIAC/Royco particle size data are shown below:

Size, Microns	No. Particles	%
5-25	1280	14.3
25-45	4159	46.5
45-63	1721	19.2
63-90	1346	15.0
90-125	436	4.9

We determined the actual drug content of the microspheres to be 48.0. The theoretical loading was 50% by weight.

The quality of the microsphere formulation was determined by SEM. The Figure shows the surface characteristics of the batch used for the primary pharmacokinetic study in the baboon.

4. Evaluations in Baboons

Preclinical pharmacokinetics and pharmacodynamics of the long-acting formulation of levonorgestrel were evaluated in nine baboons The poly (d,l-lactide-coglycolide) microspheres contained 48% LN by weight, and ranged in size from 25 to 90 microns. Three groups of three normally cycling female baboons were each injected intramuscularly with microspheres containing 12, 25, or 50 mg LN on day 3, 4 or 5 of the perineal cycle. Serum levels of LN were measured by radioimmunoassay, and the effect of treatment on ovarian function was monitored by

observing changes in sex skin turgescence and by measuring serum levels of progesterone. By these criteria treatment inhibited normal ovarian function for 3 months in all groups. Vaginal bleeding occurred for 1 to 15 days during the first 2 months posttreatment in baboons treated with the low dose (12 mg LN), and the three baboons in this group resumed perineal cyclicity on days 92, 93 and 95 posttreatment (PT). Vaginal bleeding occurred on only 2 days in the six baboons in the other two groups (25 and 50 mg LN) during the first 90 days PT. Baboons in the 25 mg dose group resumed normal cyclicity 103, 103 and 105 days PT, and perineal cycles of the baboons treated with 50 mg LN were suppressed for 101, 110 and 126 days follwing treatment. The interval from treatment to resumption of perineal cyclicity in all groups was 103 ± 10.4 days. The results of this study demonstrate the feasibility of controlled release formulations for achieving finite menstrual cycle control among several treatment doses. The individual data are shown in the appendix.

C. Unanticipated Results

The project proceeded as expected with no unanticipated results.

D. Research Design

The original plan of work was followed.

E. Operational Significance

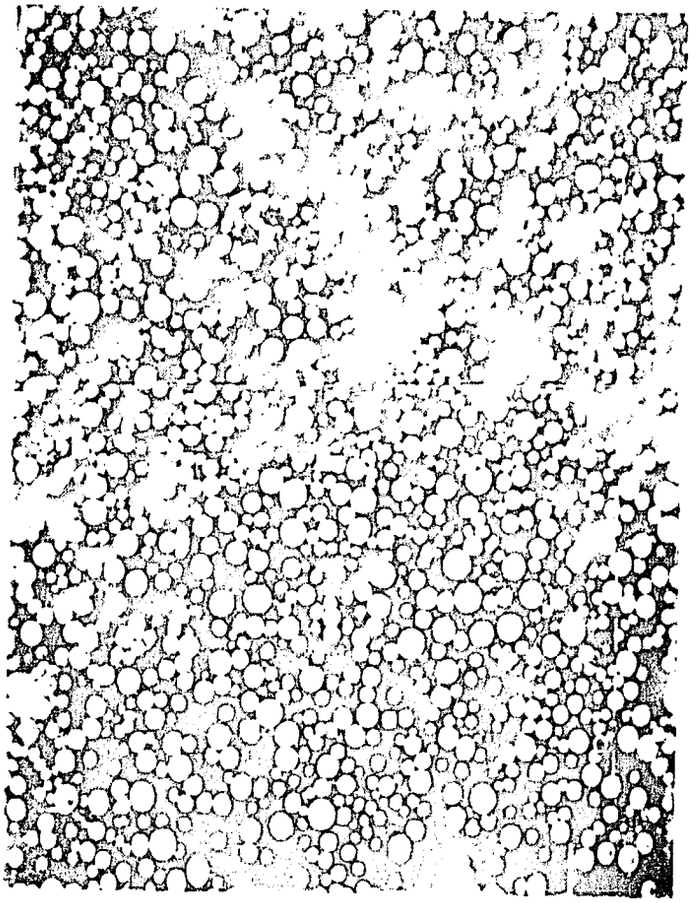
This project resulted in a prototype levonorgestrel formulation suitable for further testing in a Phase I clinical trial.

F. Dissemination of Research Results

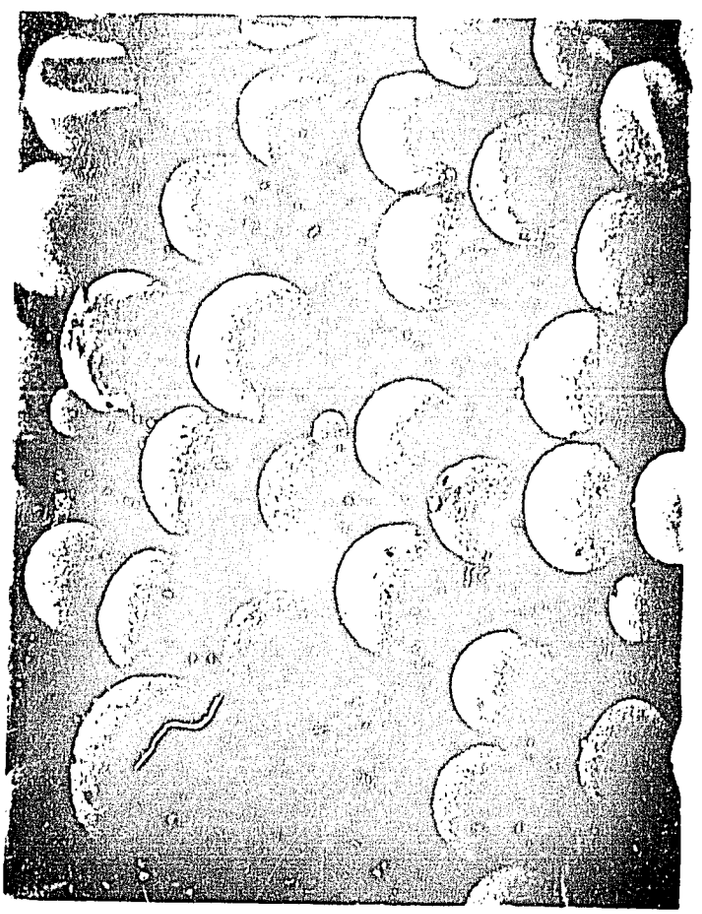
A paper by Beck, Pope, and Lewis was presented at the Society for Advancement of Contraception meeting in Chicago, September 25, 1986.

Submitted by:

Danny H. Lewis, Ph.D.

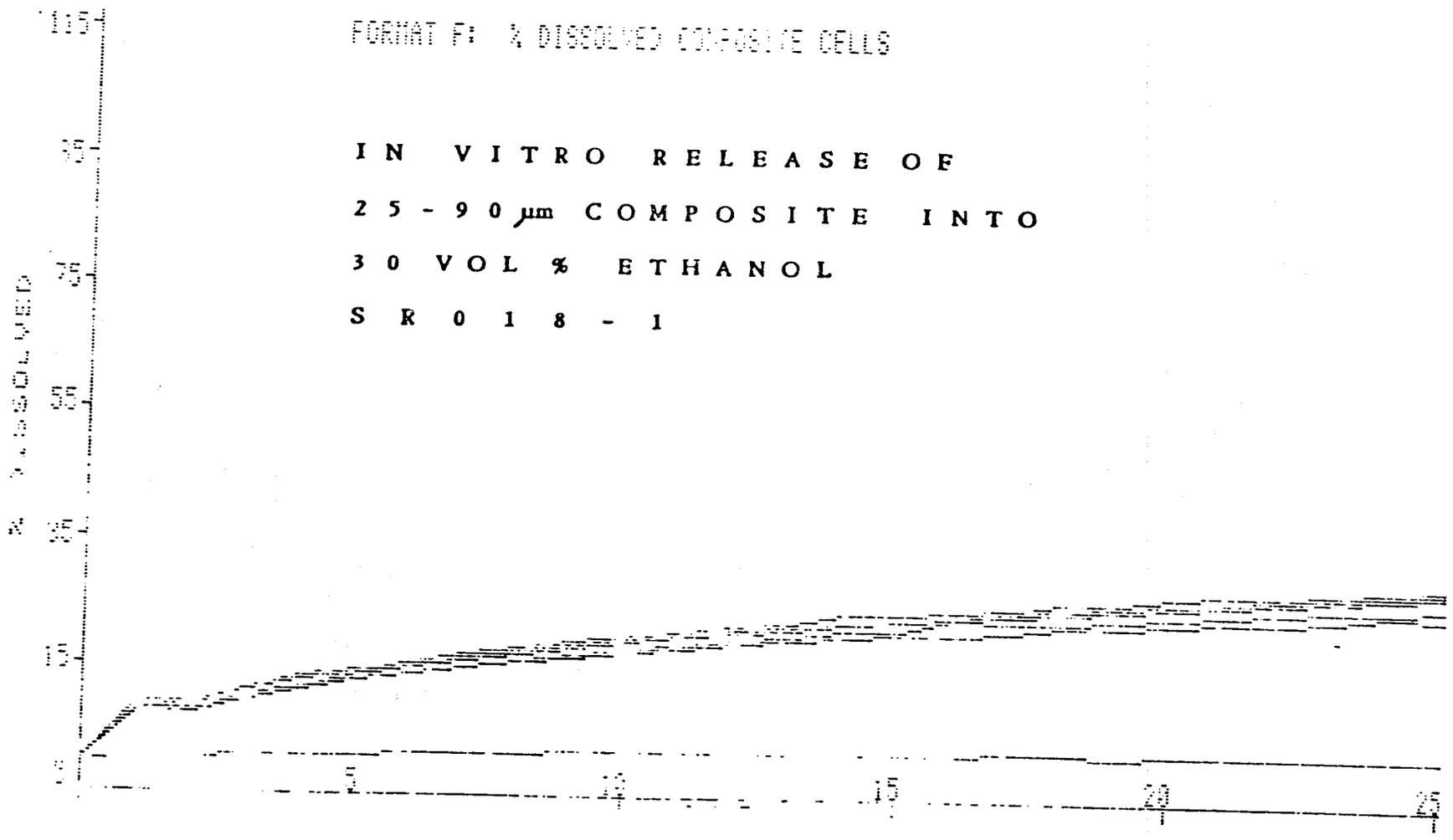


M1071



M1174

Electron micrographs of 48 wt% levonorgestrel microspheres


FORMAT F: % DISSOLVED COMPOSITE CELLS
IN VITRO RELEASE OF
25-90 µm COMPOSITE INTO
30 VOL % ETHANOL
SR018-1
% DISSOLVED
115
95
75
55
35
15
5
0
5
10
15
20
25

METHOD NAME: LG1

1. PRODUCT NAME: LEVONORGESTREL MICROCAPSULES
2. INFORMATION: SR018-1-1.15,25-9UM,2.0 MRAD

3. METHODOLOGY:

METHOD:	PADDLE USP#2	FORMATS:	C,D,E,F,G
SOLVENT:	ETHANOL/WFI	VOLUME:	900 ML
CONC:	30 V/V%	STIR RPM:	50

4. WAVELENGTH (190.0-900 NM): 247

5. % DISSOLVED TEST POINTS (1-100%): 20,40
6. TIME TEST POINTS (1-999 MIN):

7. CYCLE TIME (1-1440 MIN): 60 CYCLES (1-3200): 24
8. EXECUTE BASELINE (.1-1 MIN DWELL TIME): .1
9. EXECUTE STANDARD (.1-1 MIN DWELL TIME): .1 STANDARD: 100 %

FORMAT C: % DISSOLVED DATA

METHOD NAME: LG1 PRODUCT NAME: LEVONORGESTREL RUN: LG1X
INFORMATION: SP018-1-1.15,25-9UM,2.0 MRAD USER: MR
METHOD: PADDLE USP#2 SOLVENT: ETHANOL/WFI VOL: 500 ML
STIR: 50 WAVELENGTH: 247 CONC: 30 V/V%

% DISSOLVED

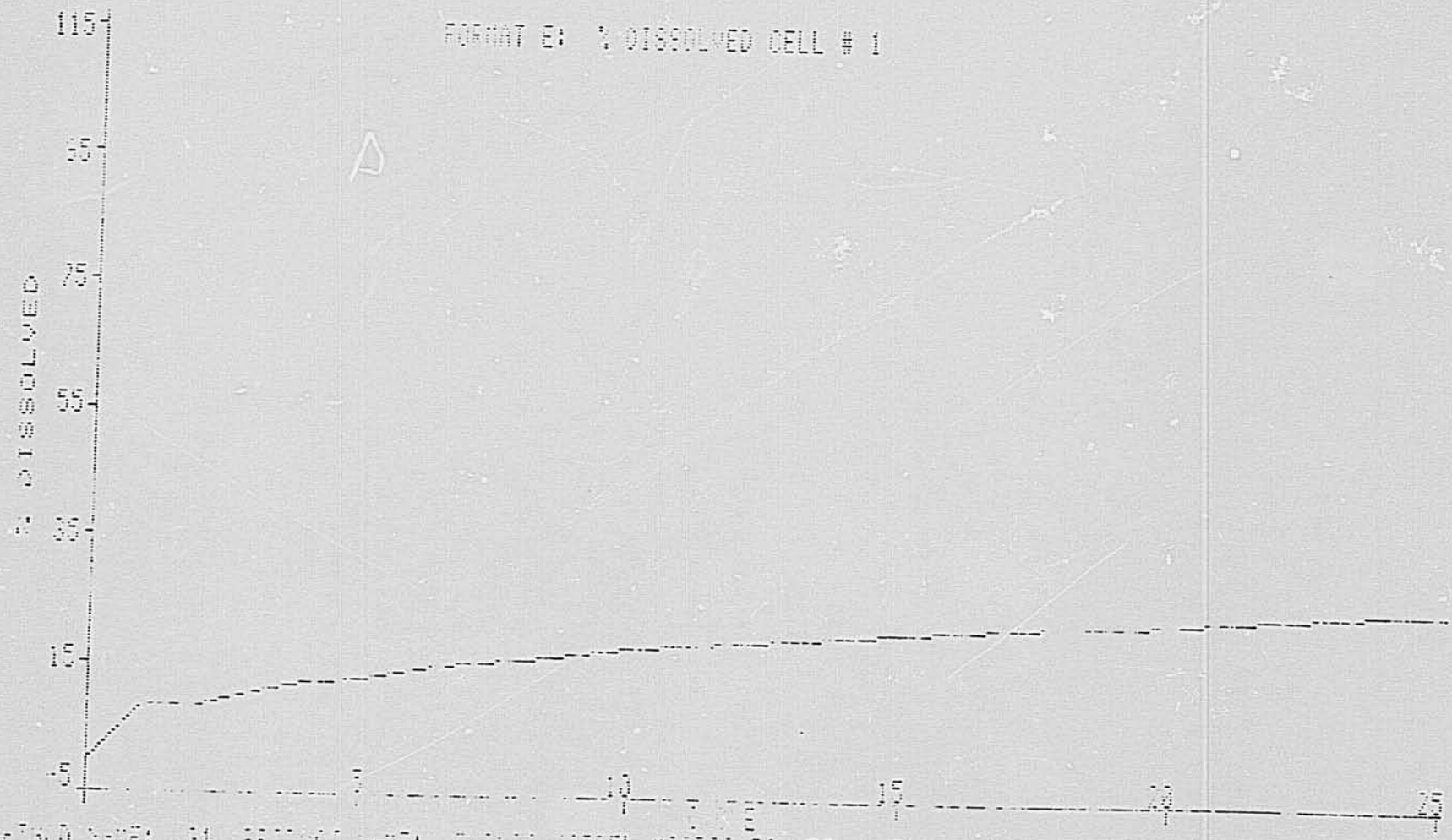
CELL:		1	2	3	4	5	6
BASE:	ABS	-.003	-.007	-.003	-.007	0.006	-.000
STD:	ABS	0.384	0.376	0.384	0.377	0.391	0.383

CYCLE/TIME

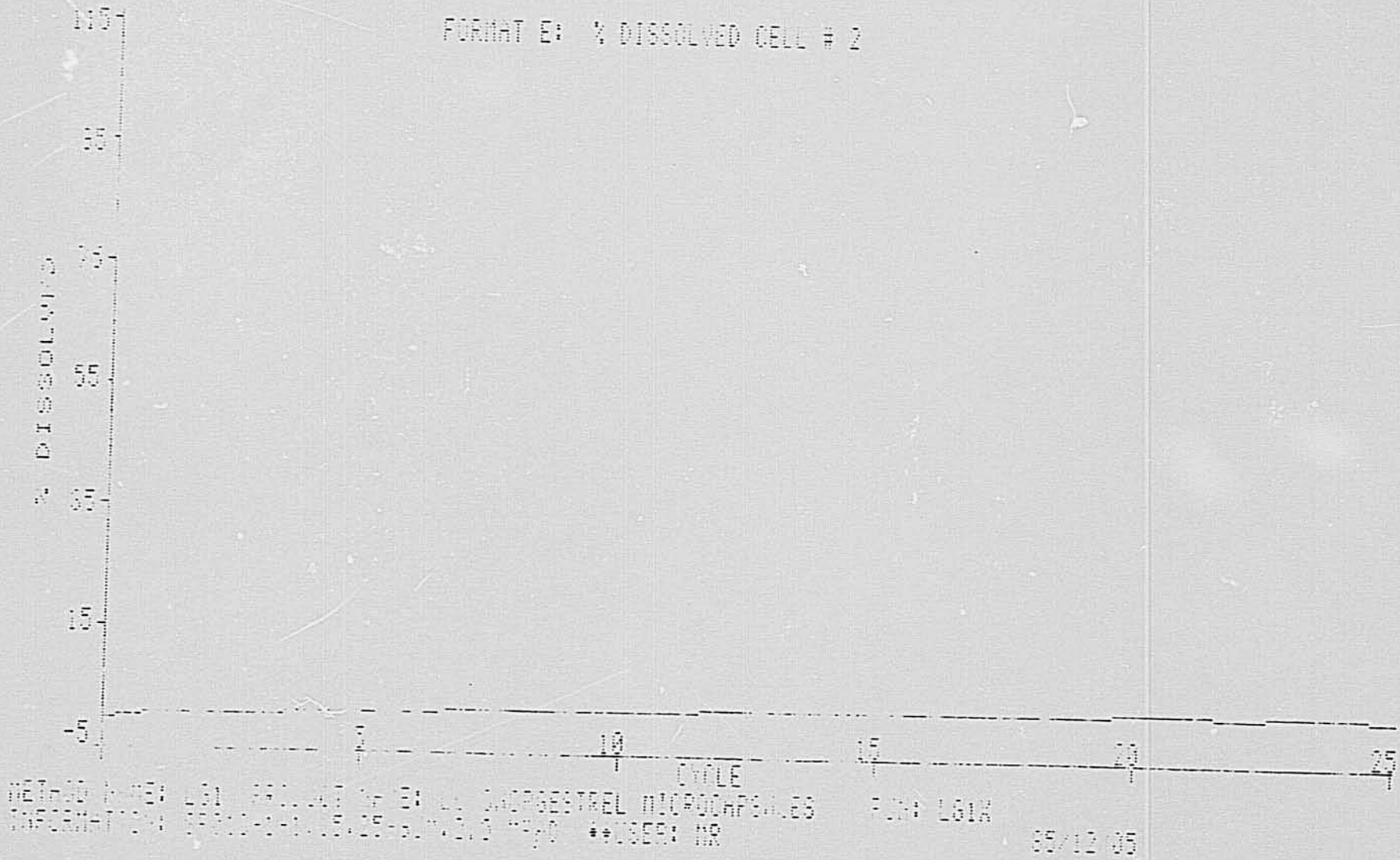
1/ 0	ABS	0.030	-.004	0.028	0.028	0.036	0.034
	%D	8.46	0.65	7.89	9.19	7.70	8.97
2/ 60	ABS	0.030	-.004	0.028	0.028	0.036	0.034
	%D	8.46	0.65	7.89	9.19	7.70	8.97
3/ 120	ABS	0.037	-.003	0.037	0.037	0.044	0.042
	%D	10.27	0.91	10.22	11.53	9.78	11.06
4/ 180	ABS	0.043	-.002	0.044	0.042	0.050	0.050
	%D	11.62	1.18	12.03	12.83	11.35	13.14
5/ 240	ABS	0.047	-.002	0.050	0.047	0.055	0.055
	%D	12.06	1.19	13.58	14.13	12.65	14.45
6/ 300	ABS	0.052	-.001	0.055	0.052	0.060	0.060
	%D	14.15	1.14	14.88	15.43	13.95	15.75

7/ 360	ABS	0.056	0.000	0.060	0.056	0.065	0.065
	%D	15.18	1.70	16.17	16.47	15.25	17.05
8/ 420	ABS	0.059	0.000	0.064	0.061	0.069	0.069
	%D	15.96	1.70	17.21	17.77	16.29	18.10
9/ 480	ABS	0.062	0.001	0.068	0.064	0.072	0.073
	%D	16.74	1.96	18.24	18.55	17.07	19.14
10/ 540	ABS	0.065	0.001	0.072	0.068	0.076	0.077
	%D	17.77	1.96	19.28	19.59	18.11	20.18
11/ 600	ABS	0.068	0.001	0.076	0.071	0.079	0.080
	%D	18.29	1.96	20.31	20.37	18.89	20.96
12/ 660	ABS	0.071	0.002	0.078	0.074	0.082	0.083
	%D	19.06	2.22	20.83	21.16	19.67	21.75
13/ 720	ABS	0.073	0.002	0.081	0.077	0.085	0.086
	%D	19.58	2.22	21.60	21.94	20.45	22.53
14/ 780	ABS	0.075	0.002	0.084	0.080	0.088	0.091
	%D	20.10	2.22	22.38	22.72	21.23	23.83
15/ 840	ABS	0.077	0.002	0.086	0.082	0.089	0.092
	%D	20.62	2.22	22.90	23.24	21.49	24.09
16/ 900	ABS	0.079	0.002	0.088	0.084	0.097	0.094
	%D	21.13	2.22	23.42	23.76	23.58	24.62
17/ 960	ABS	0.081	0.003	0.091	0.087	0.094	0.097
	%D	21.65	2.48	24.19	24.54	22.79	25.40
18/ 1020	ABS	0.084	0.003	0.093	0.089	0.095	0.100
	%D	22.43	2.49	24.71	25.06	23.05	26.18

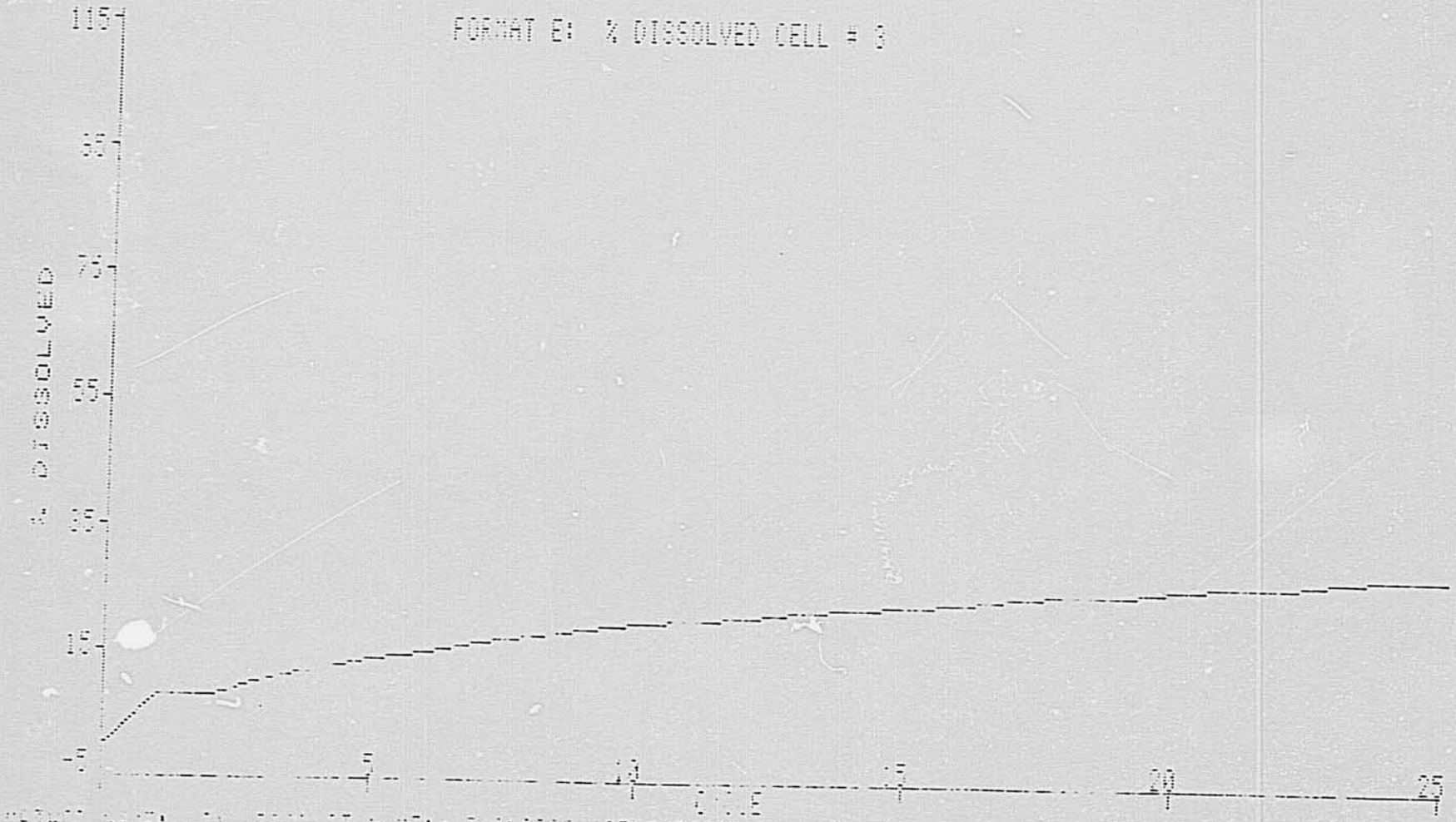
19/1080	ABS	0.094	0.002	0.094	0.091	0.096	0.101
	%D	22.43	2.22	24.97	25.58	23.32	26.44
20/1140	ABS	0.086	0.004	0.097	0.094	0.099	0.104
	%D	22.94	2.74	25.74	26.36	24.10	27.22
21/1200	ABS	0.088	0.004	0.099	0.096	0.101	0.107
	%D	23.46	2.74	26.26	26.88	24.62	28.01
22/1260	ABS	0.089	0.003	0.100	0.097	0.102	0.107
	%D	23.72	2.48	26.52	27.14	24.88	28.01
23/1320	ABS	0.091	0.004	0.103	0.100	0.105	0.109
	%D	24.24	2.74	27.30	27.92	25.66	28.53
24/1380	ABS	0.094	0.004	0.105	0.102	0.106	0.110
	%D	25.01	2.74	27.81	28.44	25.92	28.79
25/1440	ABS	0.093	0.003	0.106	0.103	0.107	0.113
	%D	24.75	2.48	28.07	28.70	26.18	29.57


FORMAT E: % DISSOLVED CELL # 1
% DISSOLVED
115
95
75
55
35
15
-5
5
10
15
20
25
TIME
METHOD NAME: L01
RUN: L01K
05-12-85

870


FORMAT E: % DISSOLVED CELL # 2
% DISSOLVED
115
95
75
55
35
15
-5
5
10
15
20
25
CYCLE
METHOD NAME: 31
INFORMATION
MICROBEITREL MICROCAPSULES
FILM: L81X
**USER: MR
85/12/05

871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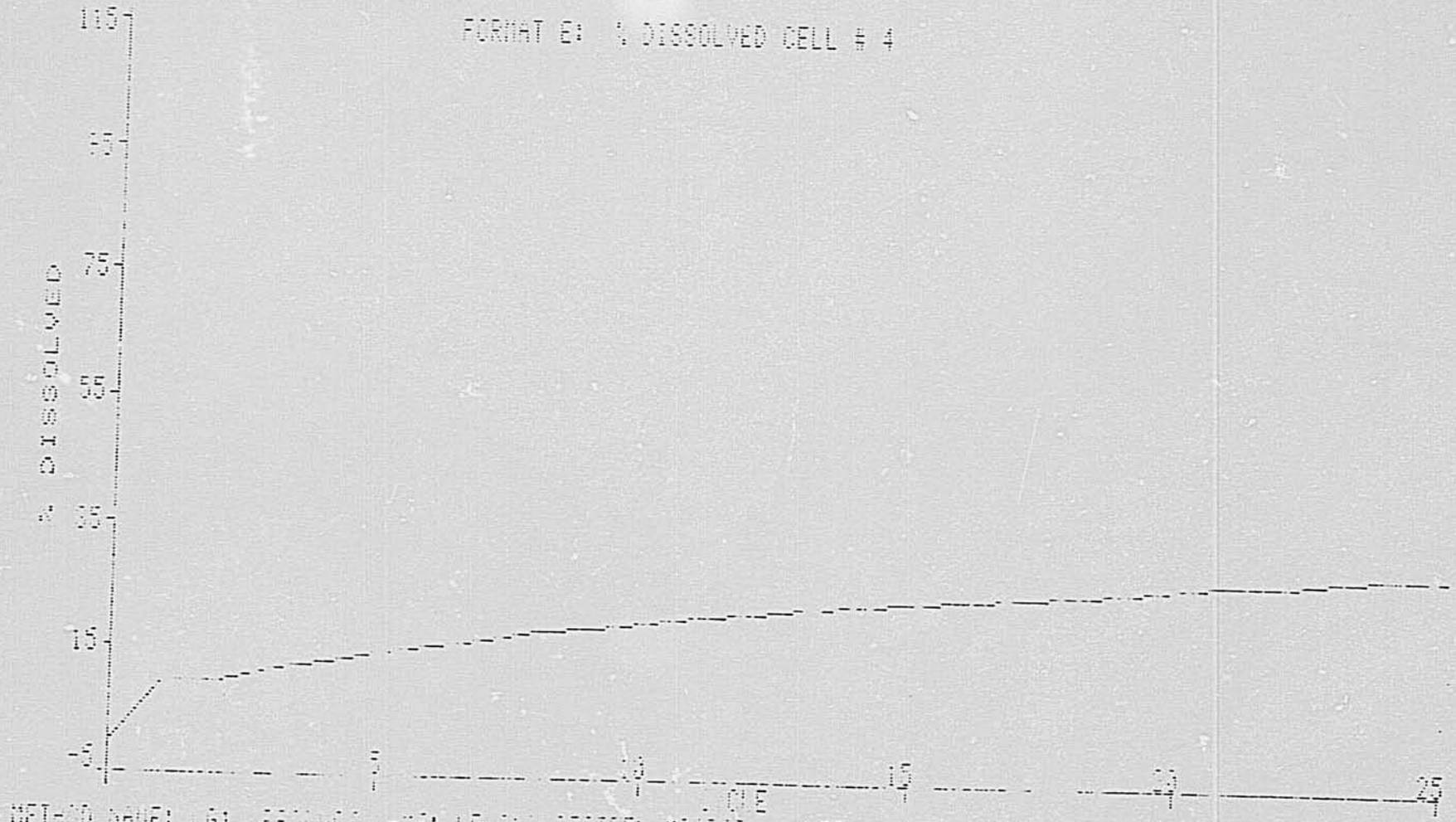
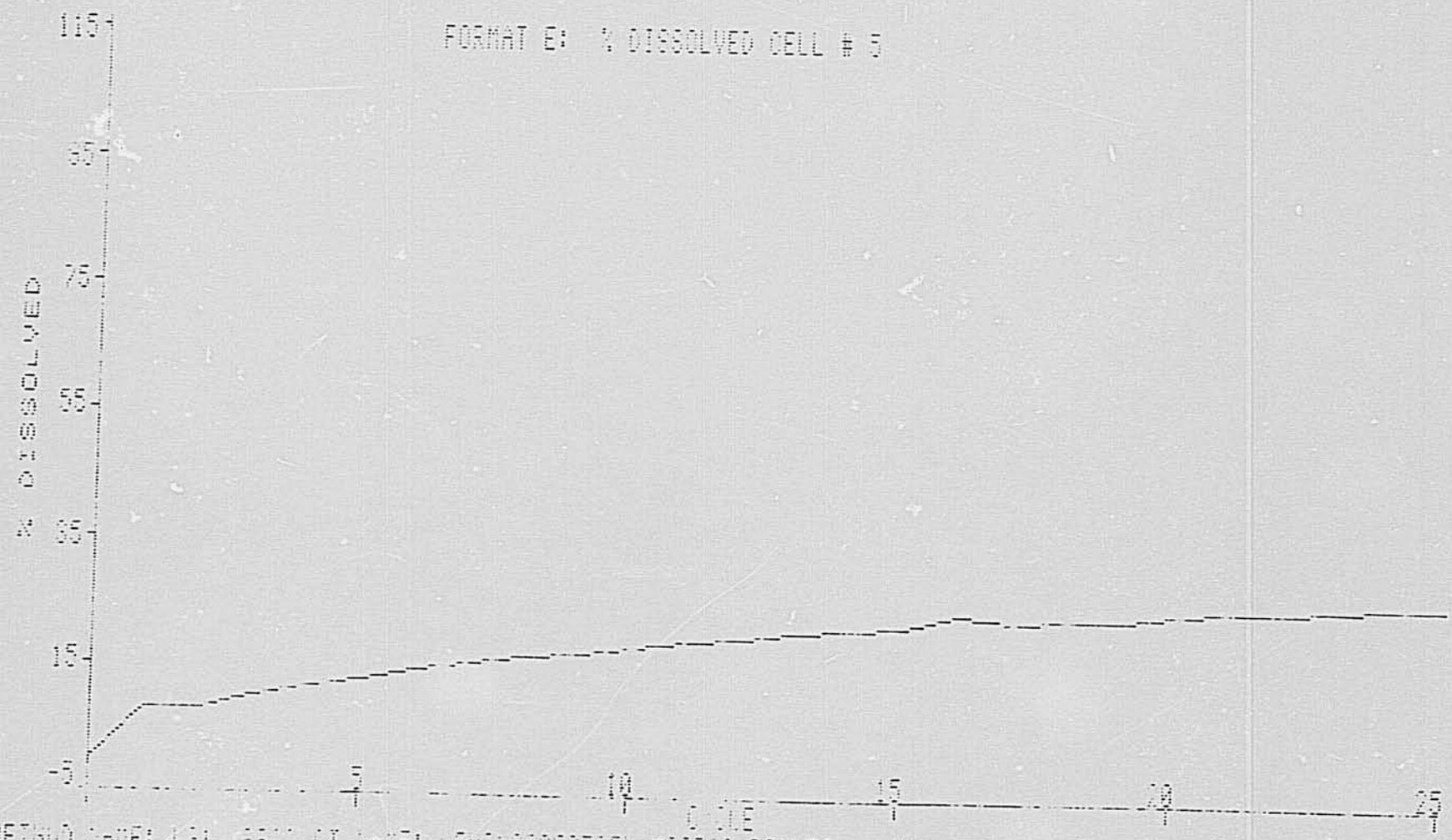
% DISSOLVED
115
95
75
55
35
15
-5
5
10
15
20
25
TIME



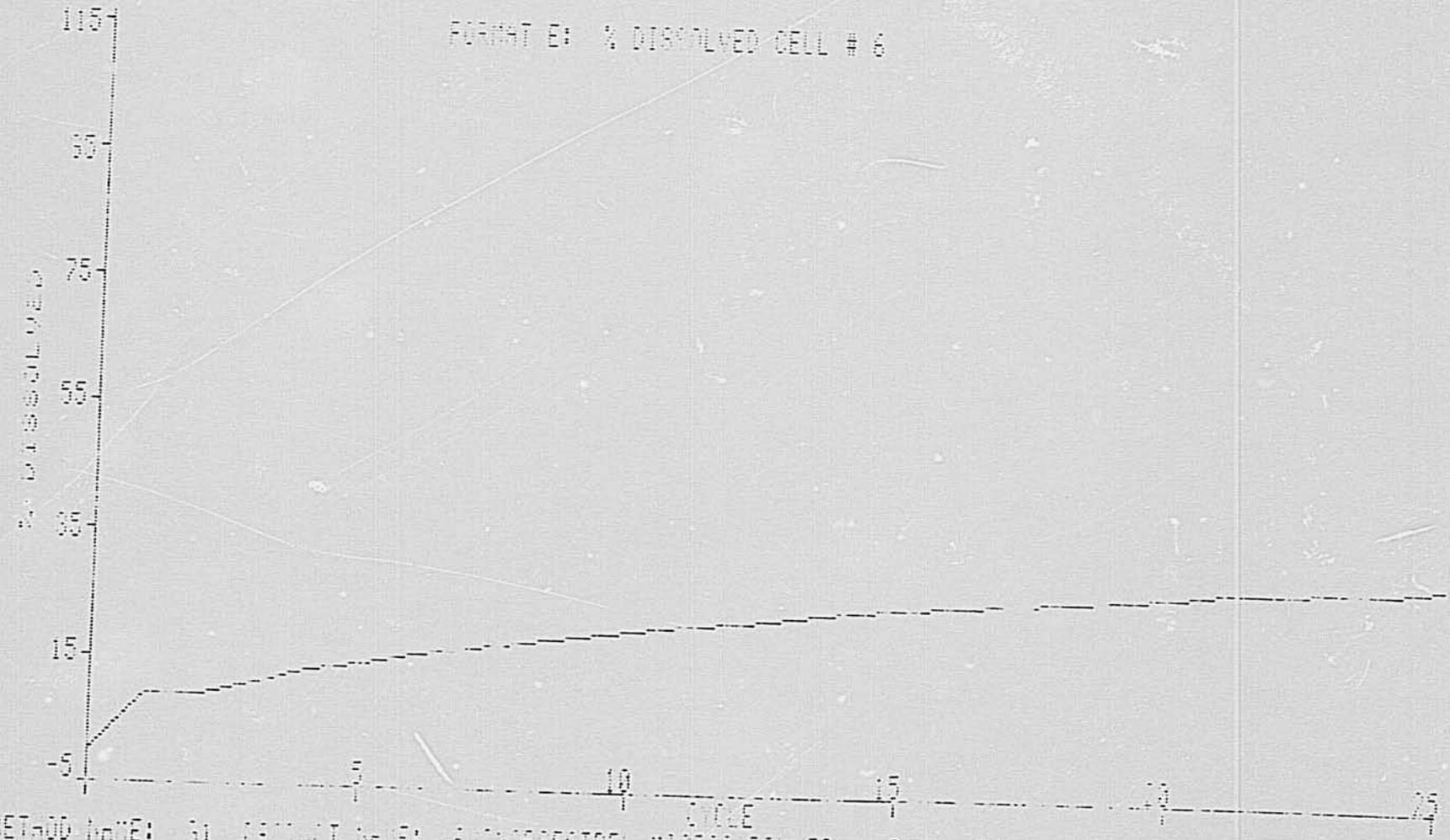
METHOD NAME: L61 PRODUCT TYPE: LEVO-NESTREL MICROSPHERES RUN: L61X
INSTRUMENTION SR318-1-1,15,19 [illegible] **USER' 9 85/12/05

813



METHOD NAME: L61 PRODUCT NAME: LEVONORGESTREL MICROCAPSULES RUN: L61X
INFORMATION: 5/2/95-1-1,15,25-5,+1,3 MPHO WASHER: R 05/12/95

874



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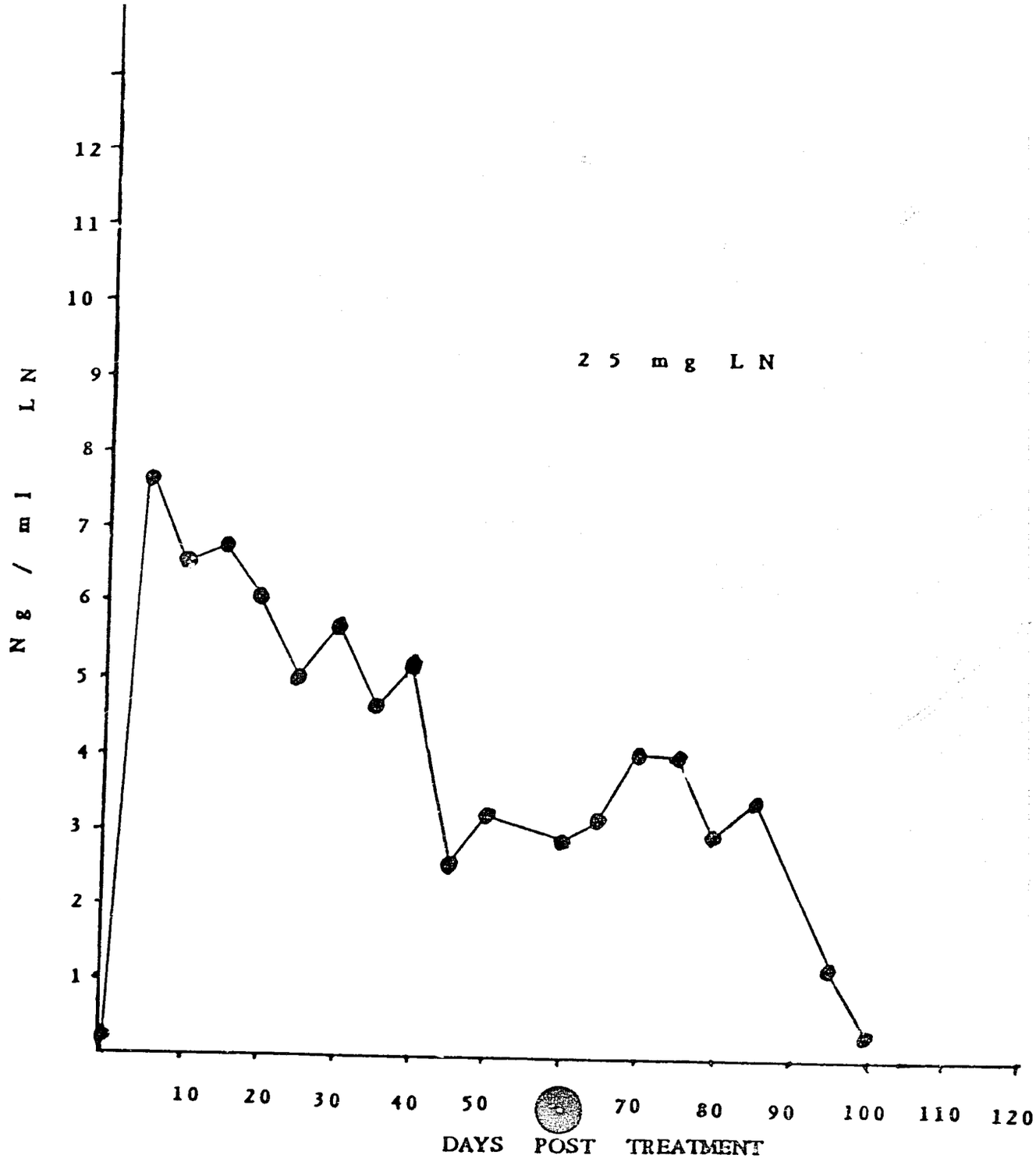
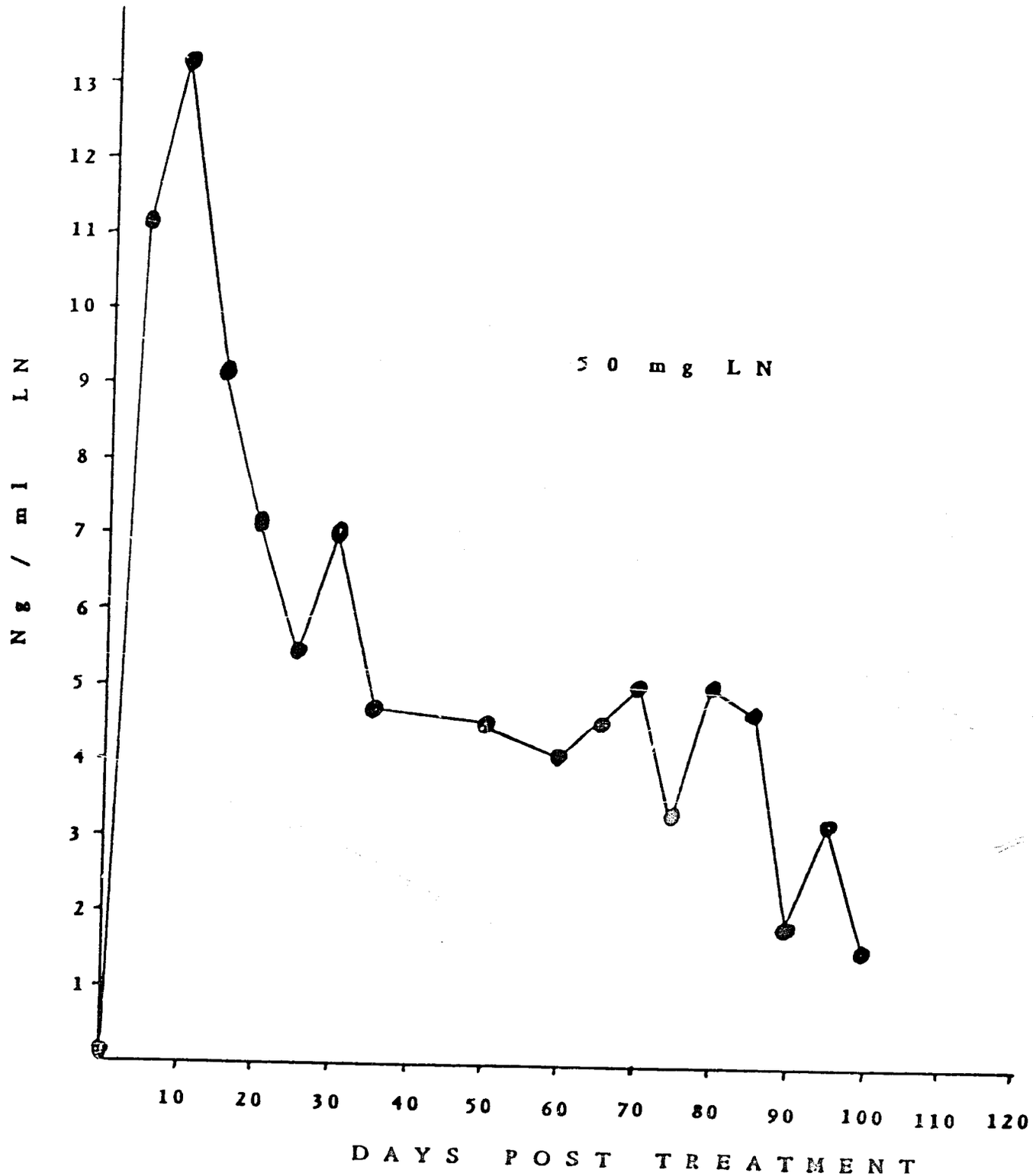
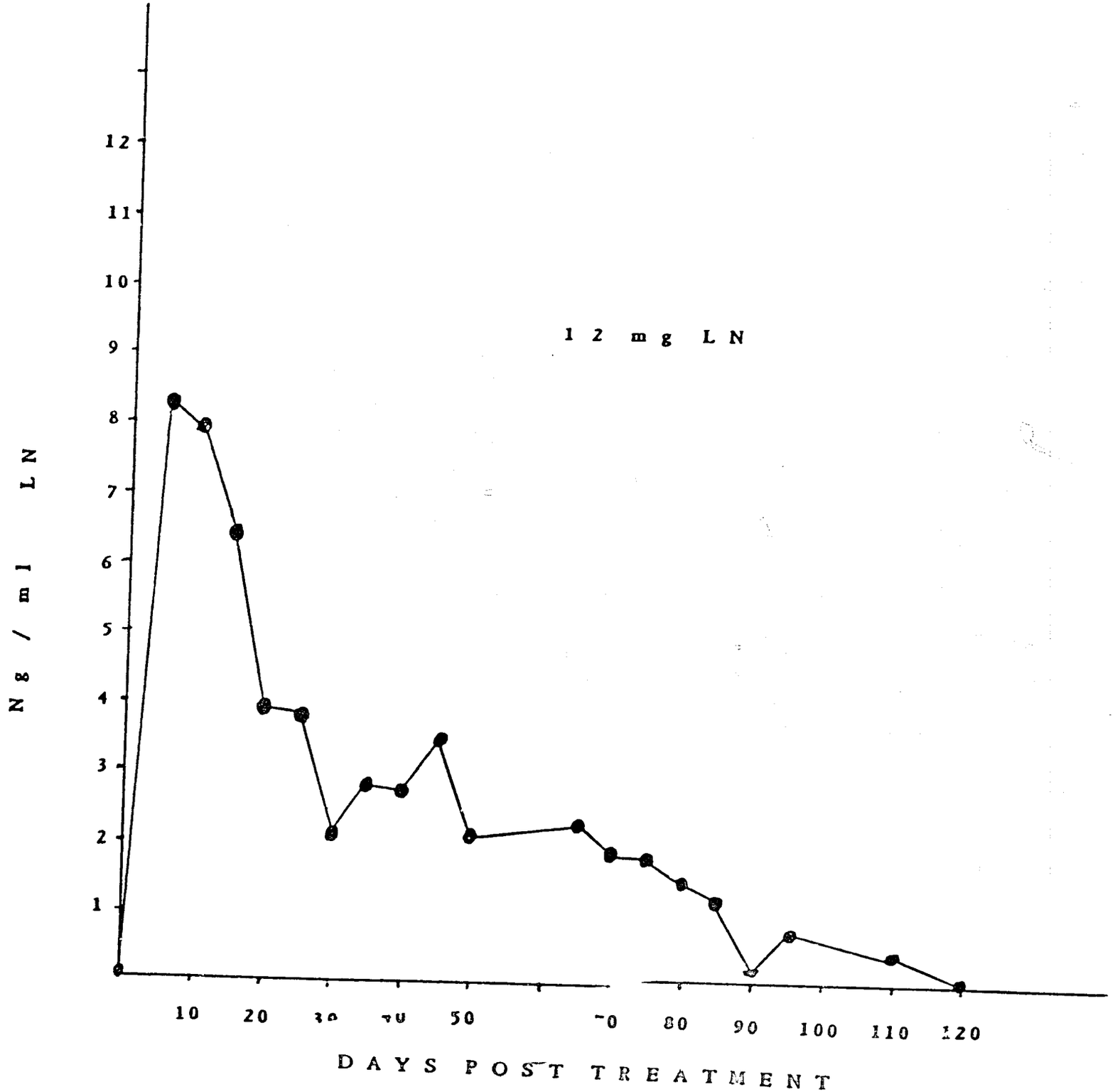
85/12/05

8-5


25 mg LN
Ng / ml LN
12
11
10
9
8
7
6
5
4
3
2
1
10
20
30
40
50
70
80
90
100
110
120
DAYS POST TREATMENT


50 mg LN
Ng / ml LN
13
12
11
10
9
8
7
6
5
4
3
2
1
10
20
30
40
50
60
70
80
90
100
110
120
DAYS POST TREATMENT


12 mg LN
Ng / ml LN
12
11
10
9
8
7
6
5
4
3
2
1
10
20
50
80
90
100
110
120
DAYS POST TREATMENT

Ac #S8 - Group A - 12 mg LN

Days PT	BABCCN: CB	DB	IB	Ave
	NG/ML Norgestrel (IN)			
0	.06			.06
1-5	7.79		10.22*	8.33
			6.99	
6-10	6.69		8.74	7.99
			8.54	
11-15	5.67		8.34	6.45
	5.21		6.57	
16-20	3.78	5.00	4.39	3.95
		2.62		
21-25	2.91*	2.44	5.40	3.84
			4.59*	
26-30	2.29	2.00		2.15
	2.15*			
31-35	3.46	1.72	3.88	2.86
		2.06	3.20	
36-40	2.11		3.38	2.75
41-45			3.56	3.56
46-50		1.61	2.66	2.14
51-55				
56-60				
61-65	2.54	2.18		2.36
66-7[illegible]	1.94	2.23	1.78*	1.98
71-7[illegible]			1.84	1.84
76-8[illegible]	1.61	1.19	1.85	1.55
81-85		1.44	1.06	1.25
86-90		.23		.23
91-95	.76		.92*	.84
96-100				
101-105				
106-110	.85		.13	.49
111-115				
116-120			.13	.13

*Sample value CV 10-15%

Ac #S8 - Group B - 25 mg LN

Days PT	BABOON: IA	LA	XA	Ave
	NG/ML Norgestrel (LN)			
0	.36		.11	.24
1-5	7.76	7.32	9.55	7.63
	4.81	7.58	8.62	
	7.18 *		8.32	
	6.34		8.82	
6-10	6.69	7.28	6.64 *	6.48
	6.23 *	5.55		
11-15	5.67	8.82	5.79	6.76
16-20	6.34	6.79	5.34	6.16
21-25	4.93	7.14	4.24	5.03
			3.79	
26-30		5.74		5.74
31-35		5.32		4.66
		3.99		
36-40		5.22		5.22
41-45	2.08	2.78	2.58	2.57
		2.84		
46-50	2.54	3.93		3.24
51-55				
56-60	1.71	3.08	3.88	2.89
61-65	3.18	3.65	2.70	3.18
66-70		4.09		4.09
71-75	4.07			4.07
76-80	3.30	4.87	.78	2.98
81-85		5.51	1.34 *	3.43
86-90				
91-95	2.26 *		.13	1.20
96-100	.76		.01	.39
101-105			.01	.01
106-110				
111-115				
116-120				

*Sample CV 10-15%

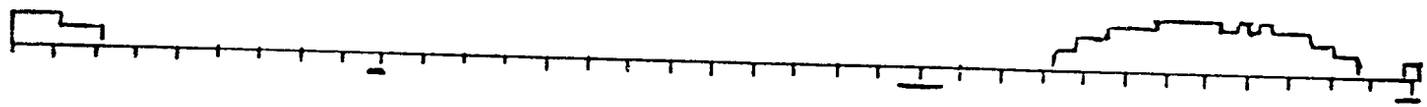
Ac #S8 - Group C - 50 mg LN

Days PT	BABOON: AB	JA	MB	Ave
	NG/ML Norgestrel (IN)			
0			.19	.19
1-5	13.09	21.58	5.00	11.20
	8.25	16.80	6.81	
	8.98	14.68 *	5.60	
6-10	8.96	23.36	7.52	13.36
	4.41	21.11	14.77	
11-15	2.83 *	14.75	8.16 *	9.19
		11.03 *		
16-20	6.30	9.98	6.08	7.19
			6.38	
21-25	5.93	6.34	4.59 *	5.47
	5.03			
26-30	4.31	10.52	4.07	7.12
		9.58		
31-35	4.71			4.71
36-40				
41-45				
46-50	3.05	8.86	1.90	4.60
51-55				
56-60	3.30	5.94	3.02	4.09
61-65	4.04	6.04	3.68	4.59
66-70		7.02 *	3.09	5.06
71-75	3.34			3.34
76-80	3.41	7.61	4.20	5.07
81-85		4.74		4.74
86-90	1.84			1.84
91-95	2.21	3.59	3.91	3.24
96-100	1.72	1.61	1.45	1.59
101-105				
106-110				
111-115				
116-120				

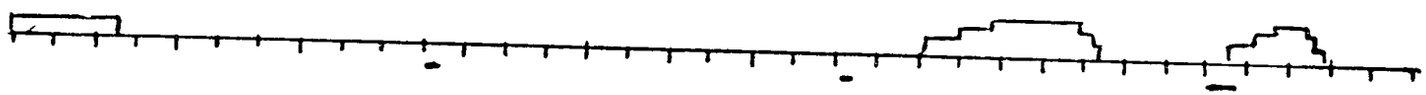
*Sample CV 10-15%

50 mg LN

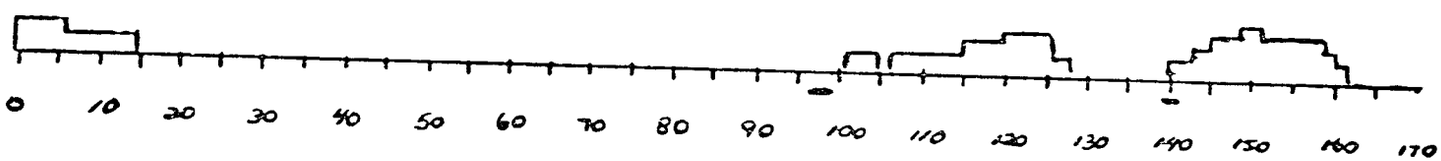
Mary Bell



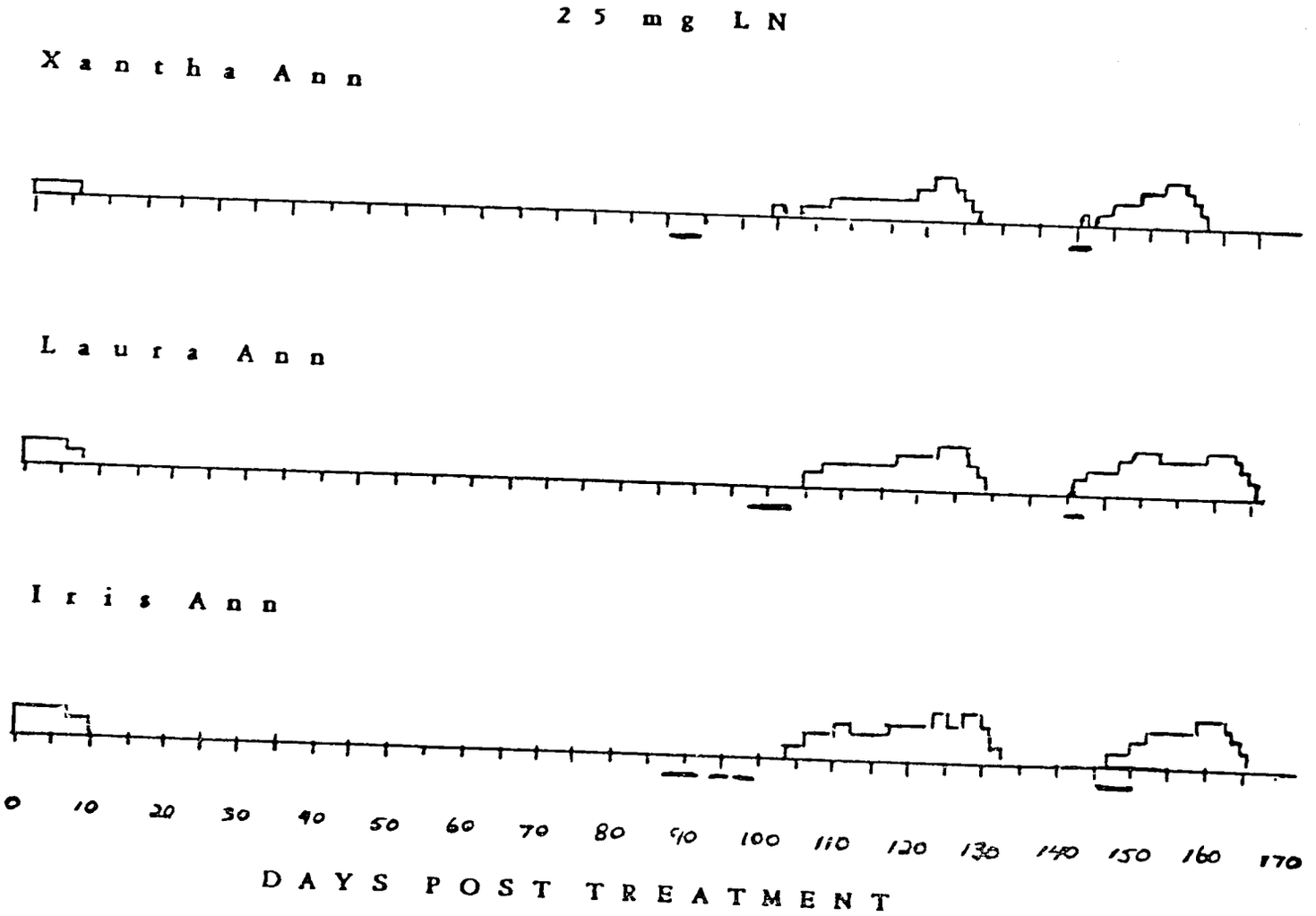
Arlene Bell



Janice Ann

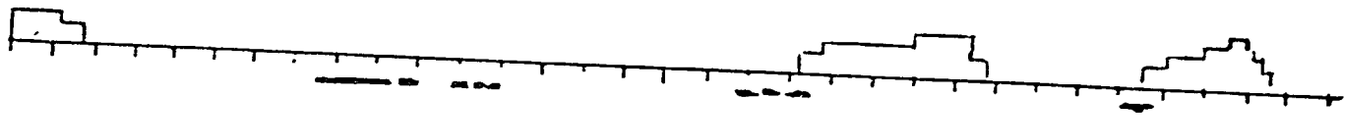


DAYS POST TREATMENT

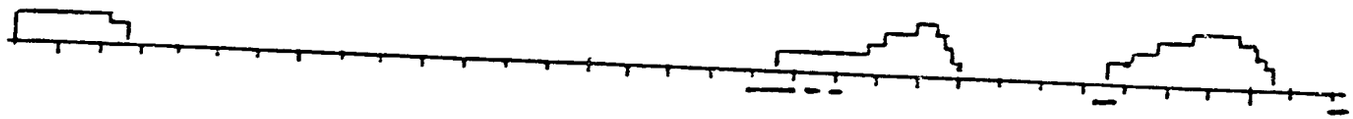

25 mg LN
Xantha Ann
Laura Ann
Iris Ann
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
DAYS POST TREATMENT

1 2 mg LN

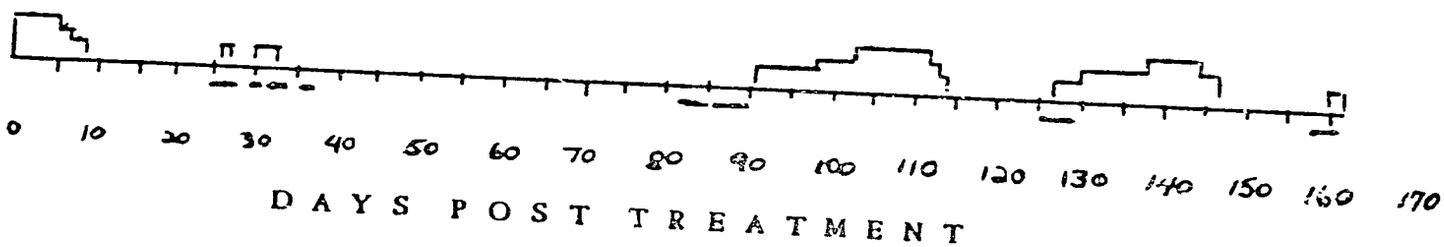
Ida Bell



Candy Bell



Dottie Bell



OVULATION INHIBITION BY ANORDRIN

PARFR 309

Principal Investigator
Robert T. Chatterton, Ph.D.
Department of Obstetrics and Gynecology
Northwestern University Medical School and
Northwestern Memorial Hospital
333 East Superior Street
Chicago, Illinois 60611
Tel. (312) 908-2374

Original Starting Date 1/1/82
Ending Date of Current Contract 12/31/85
Date of Last Technical Report 6/85
Inclusive Dates of This Report 6/85-11/85

Narrative Summary

In this report we summarize the studies on the mechanism of action of anordrin and its efficacy for continuously suppressing ovulation when given once a month as an intramuscular injection. Also described is our progress in preclinical studies of its toxicity, teratogenicity, pharmacokinetics, and metabolism.

Anordrin was not found to prevent follicular development by suppressing gonadotropin secretion. Nor does it cause a pseudo-menopausal condition by continuously elevating gonadotropins. During the time that anordrin is exerting its effect, gonadotropins apparently are released in normal preovulatory amounts in periods of between 10 to 14 days.

Despite the surges of gonadotropins, the ovary does not respond. Anordrin prevents follicular development at an early stage; it seems to block recruitment of follicles. This is supported by the fact that by day 6 of the cycle anordrin is effective in only about 50% of monkeys. After day 6 it is ineffective. Estrogen excretion during the early follicular phase of the menstrual cycle is also suppressed in proportion to the dose of anordrin given.

Monthly intramuscular injections of anordrin were effective in continuously suppressing ovulation without preventing normal recovery of ovulatory function thereafter according to hormonal indices. Postmenopausal changes did not occur according to gonadotropin excretion and vaginal epithelial cytology.

Pure anordrin has been prepared according to GLP guidelines, and treatment of monkeys, dogs, and rats has begun in toxicity studies at the University of Illinois. Pre-teratology dose finding studies are underway at Northwestern University. 14C-Anordrin has been prepared by Amersham and has been administered to seven monkeys. Sampling is about 2/3 completed and the major metabolites have begun to be identified. Preliminary results reveal very strong binding of anordrin to a serum component and a half-life of over 20 days.

SUBSTANTIVE TECHNICAL RESEARCH REPORT

A. Statement of Objective

Conduct preclinical studies that are required to assess the safety and efficacy of anordrin before clinical trials are conducted.

B. Progress to Date

1. Gonadotropin response to anordrin.
2. Estrogen production after anordrin treatment.
3. Studies on monthly administration of anordrin.
   a. vaginal bleeding patterns.
   b. vaginal cytology.
   c. pattern of serum estradiol and progesterone.
   d. recovery of menstrual cycles.
4. Toxicity testing
5. Pharmacokinetics and metabolism
6. Teratology

Gonadotropin response to anordrin

_____Serum obtained from monkeys daily for 5 days after an intramuscular injection of anordrin was assayed for monkey LH and FSH. The mean and +\- SE values for the 5-day period for control monkeys and monkeys given different doses of anordrin are shown in Fig 1. It is clear that suppression of gonadotropins is not the reason for the failure of follicular recruitment in anordrin-treated monkeys. In fact, the highest dose studied, 0.4 mg/kg body weight, resulted in a significant elevation of LH over that in controls. The daily pattern of serum LH levels is shown in Fig 2. The elevation which is about half of the normal preovulatory LH peak occurs on days 3 and 4 after anordrin.

Urinary immunoreactive LH excretion has also been measured. The normal pattern is shown in Fig 3. Even though the daily excretion is quite variable, there is clearly a higher level at midcycle than at 12 days before or afterward. The pattern after injection of 0.2 mg/kg anordrin characteristically included bursts of LH release that were in the range of the preovulatory LH surge. Generally, these occurred with a period of between 10 and 14 days. Fig 4 shows the pattern in one monkey (#38). The average urinary LH excretion in the preovulatory period of normal menstrual cycles was 28.8+\-5.6 SE ng/day, and the mean peak value of the LH surges during treatment cycles was 27.6+\-2.7 SE. Thus, anordrin does not create a pseudomenopausal condition even though ovarian function and steroid secretion is suppressed. The reason that LH levels are not more chronically elevated may be because of the weak intrinsic estrogenic activity of anordrin itself.

Estrogen production after anordrin treatment.

Urinary immunoreactive estrone was measured in the period 3 to 8 days after administration of different doses of anordrin. As shown in Fig 5 it is clear that estrogen formation and excretion are suppressed in a dose-related manner. This confirms that anordrin brings about its effect by a direct action on the ovary. Subsequent to the suppression of follicular growth and function, follicles do develop in an apparently normal manner. Fig 6 shows the pattern of immunoreactive estrone and pregnanediol excretion in a monkey that received a 0.2 mg/kg dose of anordrin on day 1 of the menstrual cycle. Note that although the follicular phase has been extended, there is a relatively normal pattern of excretion of estrogen and progesterone metabolites thereafter. For comparison, the pattern of immunoreactive estrone and pregnanediol in normal ovulatory cycles is shown in Fig 7. One aspect of the ongoing studies that has not been completed is the histological evaluation of ovaries that were removed from anordrin-treated monkeys 3 days after the estrone peak. This study must be completed before the effect of anordrin on ovulation can be ascertained. That is, we have not yet determined whether the delay in follicular maturation is associated with corpus luteum formation or only luteinized unruptured follicles.

Suppression of estradiol and progesterone during monthly administration of anordrin.

Serum concentrations of estradiol and progesterone remained at low levels during monthly treatment of monkeys with anordrin. The results are shown in Figs 8a,b,c,and d. Anordrin was administered initially to each monkey as a single i.m. injection in sesame oil within the first 4 days of the menstrual cycle. The dose was based on the amount of anordrin for each monkey that had extended the follicular phase of the menstrual cycle by 25 to 30 days where this information was known. In most monkeys that we have studied this is 0.2 mg/kg. However, in 1 of the 4 monkeys available for this study a dose of 0.4 mg/kg was required. The two additional monkeys (#65 and#67) that had not been studied previously were given the 0.2 mg/kg dose. Table 1 summarizes the menstrual patterns of these monkeys.

TABLE 1

Monkey	Dose mg/kg	First/Last Injection	Duration of Amenorrhea	Menses Dates
#27	0.4	4/27-8/17/84	6.5 mo.	11/7-9,12/5-7,1/2-4
#32	0.2	4/27-8/17/84	6.0 mo.	11/1-3,12/1-4,1/2-6
#39	0.2	4/27-8/17/84	7.0 mo.	11/23 ,12/20-22,1/17-18
#40	0.2	4/27-8/17/84	6.5 mo.	11/7-9,12/14-20,1/15-19
#65	0.2	4/27-8/17/84	8.0 mo.	12/28 ,2/15-16
#67	0.2	5/1-8/24/84	8.0 mo.	12/28-31,2/15-17
		mean	7.0+\-0.8 mo.	

The return to normal menstrual cycles is also indicated in Figs 8a-d as a rise in estradiol or progsterone approximately two months after the last injection of anordrin to the range of the preovulatory or midluteal phases, respectively, of the normal menstrua cycle. Since blood was drawn only weekly, the peak concentrations were not always observed.

Pregnanediol was also measured during selected periods of the monthly anordrin regimen. The highest values attained in 4 of the monkeys was 1.2 ug/day; these monkeys had no incidences of vaginal bleeding during the 7 months of amenorrhea. Two monkeys (#40 and #67) each had one day of spotting; both incidences occurred at a little over a month from the date of the first injection of anordrin and were preceeded within the prior month by pregnanediol excretion of between 2.0 and 3.0 ug/day. This is close to the mean midluteal pregnanediol excretion in normal cycles of 4.0 ug/day. Thus the initial dose of anordrin probably should have been higher in these two monkeys. Nevertheless, the efficacy of anordrin in a monthly regimen is adequately proven by the experiments.

Toxicity testing

The starting date for dosing of monkeys, dogs, and rats at the College of Pharmacy, University of Illinois, was October 23, 1985. Blood samples have been collected for hormone studies, for total cholesterol, LDL, and HDL, for blood coagulation studies, and for blood enzymes and electrolytes prior to administration of the first dose of anordrin and after the first month on treatment according to the protocol. Anordrin for these studies was prepared by Dr. Chettur at IITRI under GLP standards. At the present time we have no indication of any gross pathology in anordrin-treated animals. The concentration of anordrin in the sesame oil dose was confirmed by gas chromatography. The stability of anordrin that had been stored in sesame oil at room temperature for one year and five years was also determined. No significant deterioration was found at either time interval. Hormone and other assays have not yet begun on the blood samples that have been drawn. For greatest precision and accuracy these analyses will not be done until a complete set of samples have been collected.

Pharmacokinetics and metabolism of anordrin.

Uniformly labeled 3H-anordrin and 2-14C-anordrin were prepared by Amersham Corporation. Samples of both compounds have been recrystallized with pure anordrin synthesized by Dr. Chettur. In each case approximately 30,000 cpm of the labeled compound was added to 20 mg of the pure anordrin. In both cases the specific activity remained constant at close to 1500 cpm/mg upon repeated crystallization, proving that the labeled compounds are chemically identical to the pure anordrin and essentially pure.

In the pilot studies with 3H-anordrin (before the 14C-anordrin was available) 2 monkeys were given the dose intravenously and then intramuscularly. Kinetics of distribution and elimination were calculated. Although it was clear from this study that anordrin has a long half-life and strong binding to a serum protein, accurate estimates of metabolic clearance could not be calculated because we found in the binding studies that some exchange of tritium had occurred. This points out very clearly the value in spending the additional money to obtain the compound with 14C in the ring structure. Subsequent descriptions of the kinetics and metabolism will, therefore, be limited to work with the 14C-anordrin.

In vitro binding. When 14C-anordrin is to be added to serum in vitro, it must first be dissolved in a solvent that is also miscible with water. The steroid was therefore added to test tubes as a solution in 0.05 ml of methanol. Within 5 min at room temperature adequate equilibration with 1.0 ml of serum had occurred. However, the steroid at this time was was found to be only about 5% extractable with diethyl ether. Many procedures have been tried to attempt to extract the bound steroid including incubation of the serum with acid or alkali prior to mixing with

organic solvent. These procedures did not release the bound anordrin. Precipitation of the serum proteins with ethanol or heat was similarly unsuccessful. el filtration of the serum-anordrin complex revealed that anordrin was bound to a substance of low molecular weight, probably less than 5000 daltons. This work was then temporarily discontinued while we attempted to determine the form of 14C-compounds in serum of animals that had been given the labeled anordrin intravenously. Since most of the labeled material in these samples was also not ether extractable, we saturated the serum with NaCl, acidified to pH 2, and added acetone to extract any glucuronide, sulfates, or other conjugates of anordrin into the organic solvent. In this case we found that 90% of the material that was unextractable into ether was extracted into the organic solvent. After thin-layer chromatography of the acetone extractable material in a solvent system that separates glucuronides, sulfates, and unconjugated steroids, we discovered that what had been extracted into acetone was an unconjugated steroid with a chromatographic mobility similar to that of anordrin.

Therefore, we went back to the problem of the unextractable 14C-labeled compound(s) in serum after the in vitro incubation and attempted to extract them using the acid-salt-acetone procedure. This was successful in extracting a labeled compound which, after chromatography, appears to be anordrin and some of a partial hydrolysis product.

Thus, anordrin binds rapidly and strongly to a serum component. This binding is not broken by the addition of organic solvents in which anordrin in soluble such as diethyl ether. The binding of anordrin to a serum component is different in this respect than the binding of progesterone or cortisol to CBG or of testosterone to SHBG, and it appears to be a much stronger bond.

<u>In vivo binding and metabolism of anordrin.</u>

_____As described above under in vitro binding of anordrin it was discovered that little of the 14C activity is extractable with ether from serum of animals that have received the labeled compound intravenously, and that anordrin and its hydrolysis products are extractable with a combination of salt, acid and acetone. This was further evaluated to determine what the critical components for extraction of anordrin from serum of animals receiving the drug are. It was found that diethyl ether could not be substituted for acetone as the solvent after salt and acid addition, but that acidifying the serum to pH 2 was not required.

Using the procedure of extracting the unbound anordrin and/or metabolites from serum with diethyl ether and then saturating the serum with NaCl and extracting with acetone to obtain the bound fraction has now been decided upon as the procedure to be followed in analyzing all serum samples in the pharmacokinetic studies. Although only a relatively few samples have been analyzed to date, it appears that anordrin is rapidly bound. Thereafter, within a few minutes in vivo the propionate

esters are cleaved, presumably by pseudocholinesterases in blood, to the diol. The latter compound will hereafter be referred to as anordiol. Other metabolites, if present, are not more than a few percent of the total extractable material. However, we have not analyzed samples from monkeys more than a few hours from the time of administration of 14C-anordrin.

Kinetics of anordrin after an intravenous dose of anordrin.

In the final protocol three monkeys were to receive a 0.2 mg/kg dose of 14C-anordrin. After they menstruated, they were to be given the same dose intramuscularly. Another 3 monkeys were to receive a 1.0 mg/kg dose of the 14C-anordrin intramuscularly. This will allow us to determine the availability and the dose response of the i.m. route of administration. Urine was collected daily from all monkeys for at least 2 months before they were selected for the pharmacokinetic study. These urine specimens were assayed for pregnanediol and estrone to ascertain the occurrence of ovulation and luteal function. It is necessary to establish the hormone pattern for each monkey in order to relate the kinetic parameters to the duration of effect. At the present time only one of the 4 monkeys that received the i.v. dose has menstruated. The duration of effect of the i.v. anordrin in this monkey was 53 days, almost exactly the average for all monkeys that had previously received the 0.2 mg/kg dose intramuscularly. This suggests that a large part of the long duration of the effect of anordrin must be attributed to its half life rather than to a very slow uptake from the injection site.

Since we have only recently solved the problem of extracting anordrin and its hydrolysis products from serum, we have not analyzed many of the numerous serum samples that we have accumulated. On the assumption that after the first 5 minutes all of the circulating 14C-material is anordiol, the data fits a three compartment, open mammillary model quite well. The volume of distribution was found to be 5.9 L or 1.37 L/kg with a clearance of 0.625 ml/min (0.145 ml/min.kg). This gives a half-life of 23 days.

After an intramuscular injection of 14C-anordrin (1.0 mg/kg), there was a rise in the concentration of 14C to a plateau at about 2 days after the injection. There was little discernable change in the level of the plateau for the next 12 days. The results from monkey 100 are shown in Fig 9.

Because of the long half-life and slow absorption, we have had to draw blood for over two months in monkeys receiving the i.m. dose in order to ascertain the kinetic parameters.

Teratology.

Based on previous studies in mice and rats, we know that these animals are most sensitive to the abortifacient effects of anordrin within the first week of pregnancy. In order to find the dose which will not abort all animals for study of potential teratogenic effects, we have administered anordrin only on day 8

in rabbits and day 6 of pregnancy in rats. That is, the day after implantation in each species. Five rabbits and 5 rats have been injected with 0.8 mg/kg of anordrin; the same number of each species have been injected with the vehicle sesame oil. All 5 pregnant rabbits that received anordrin had resorbing fetuses on day 14. Pregnant rabbits that had received only the vehicle had an average of 8.5 live fetuses. Two of 6 vehicle treated controls autopsied to date had one non-viable fetus each. Therefore, the next group of pregnant rabbits will be treated with 0.4 mg/kg anordrin. If this dose is tolerated, we will test them with the schedule for teratology, i.e., every 4 days throughout pregnancy. Rats receiving 0.8 mg/kg have not been autopsied yet.

FIGURE LEGENDS

Fig. 1. Mean serum immunoreactive LH and FSH during 5 consecutive days after administration of different doses of anordrin or vehicle. Values are +/- the standard error.

Fig. 2. Daily concentrations of serum immunoreactive LH in the 5 consecutive days after administration of anordrin on day one of the menstrual cycle in the dose range of 0.2 to 0.4 mg/kg body weight. The mean concentration of LH during the preovulatory peak of normal menstrual cycles is shown for comparison.

Fig. 3. Daily excretion of immunoreactive LH in urine of 4 normal, regularly ovulating monkeys. Values were plotted from the day of the peak concentration in serum.

Fig. 4. Daily urinary immunoreactive LH in one monkey (#38) after a dose of 0.2 mg/kg on day one of the cycle. For comparison, the mean preovulatory peak of LH in normal cycles is shown .

Fig. 5. Mean urinary concentration of immunoreactive estrone during days 3 through 8 after different doses of anordrin or vehicle. Values are +/- the standard error.

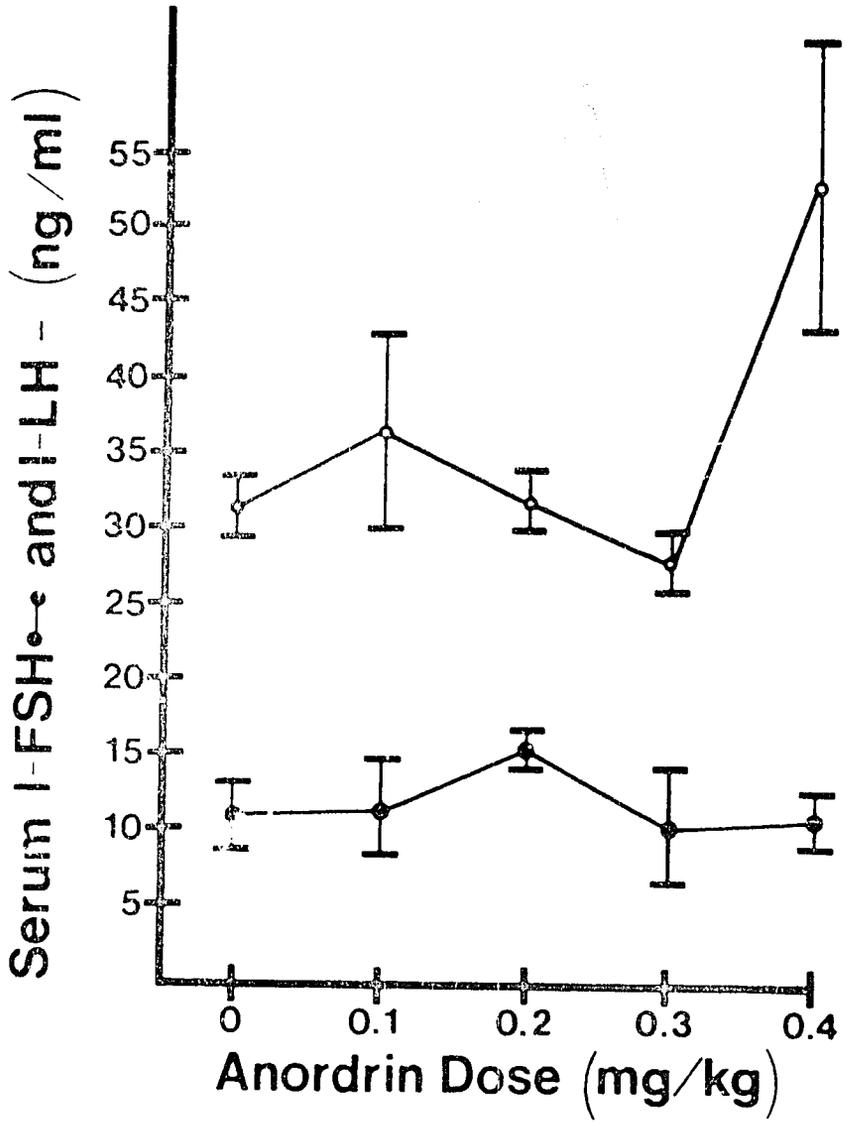
Fig. 6. Daily urinary immunoreactive pregnanediol and estrone in one monkey after treatment with 0.2 mg/kg anordrin on day one of the cycle.

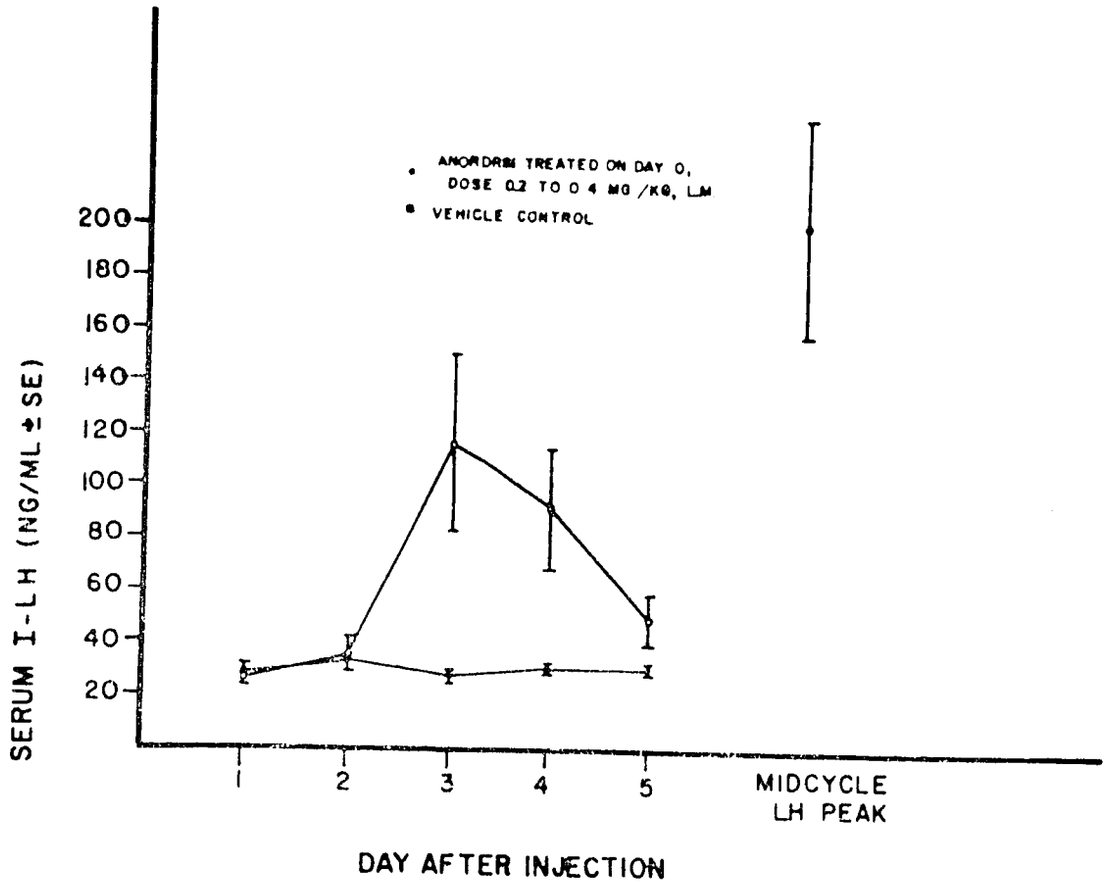
Fig. 7. Pattern of urinary immunoreactive pregnanediol and estrone excretion in normally cycling, apparently ovulating monkeys. Values are +/- the standard error.

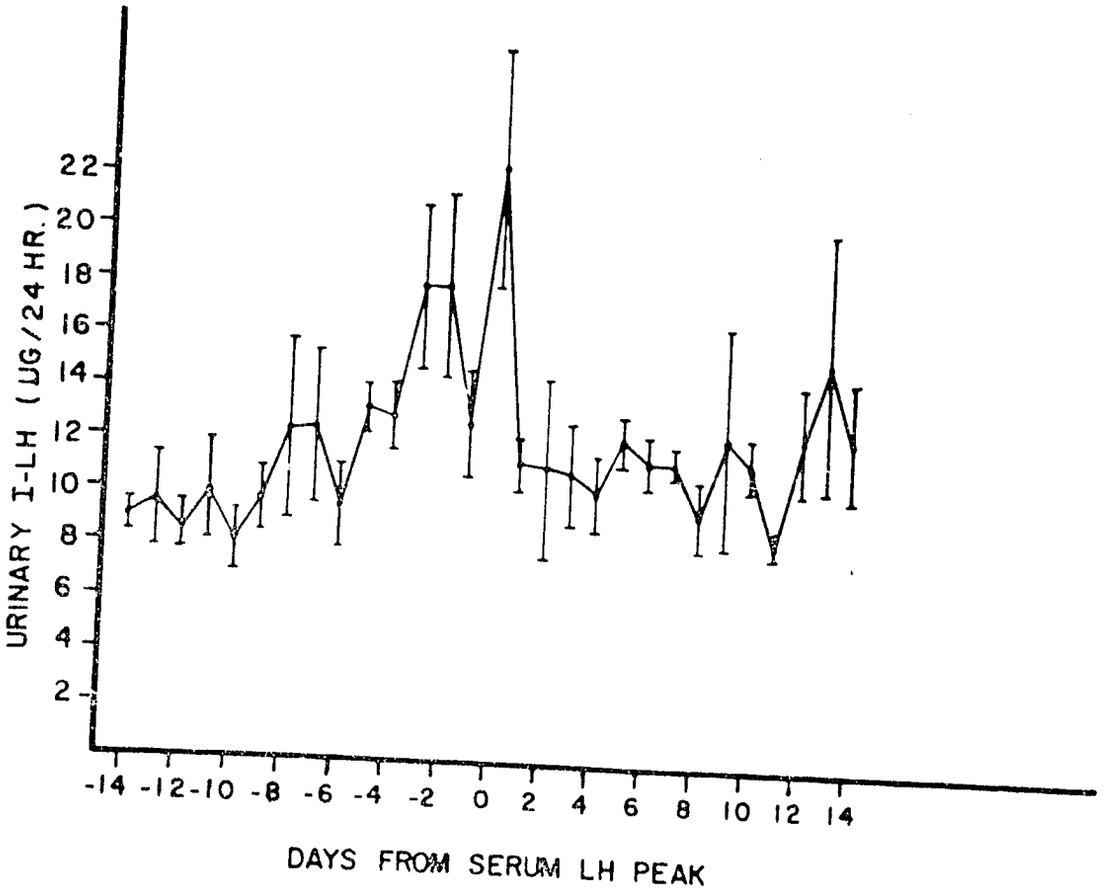
Fig. 8a and 8b. Weekly serum concentrations of estradiol in monkeys treated on day one of the menstrual cycle and monthly thereafter for a total of 5 months. The times of anordrin administration are shown as arrows. Monkey #27 (Fig 8a) received a dose of 0.4 mg/kg and monkey #39 (Fig 8b) received a dose of 0.2 mg/kg of anordrin. The concentration of estradiol in the preovulatory peak of normal, untreated monkeys is also shown with its standard error.

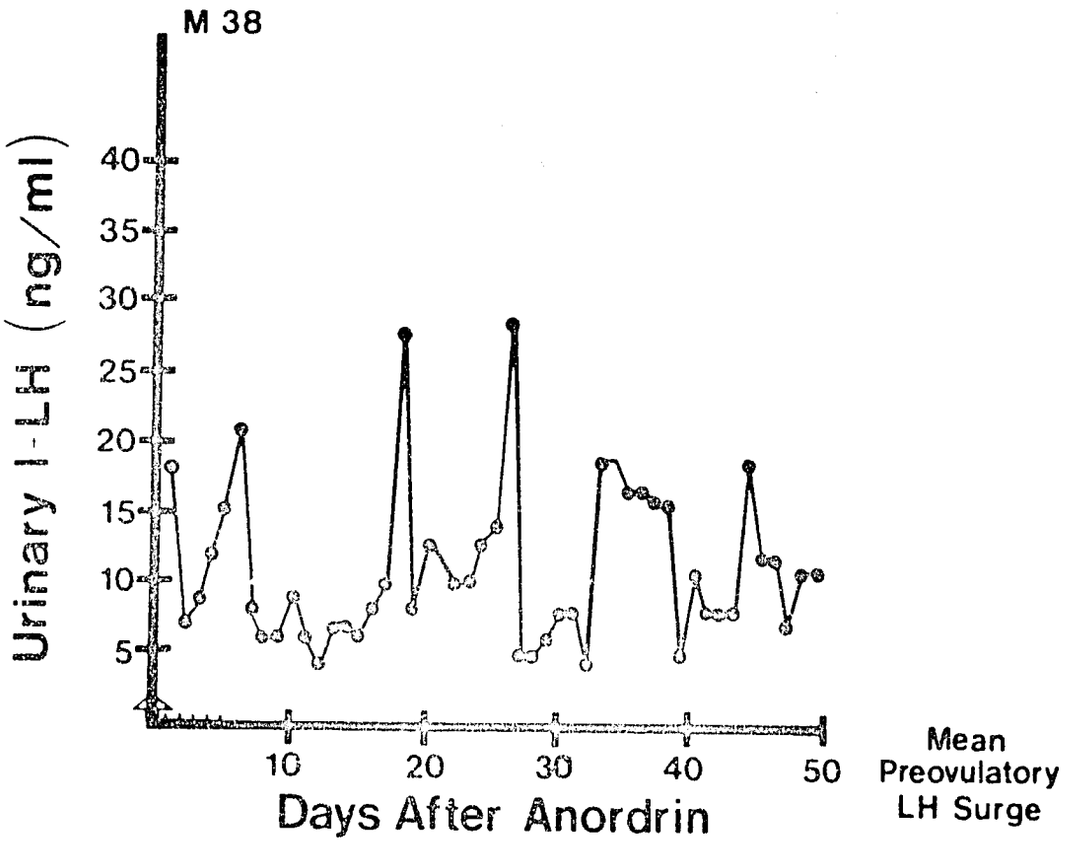
Fig. 8c and 8d. Weekly serum concentrations of progesterone in monkeys treated on day one of the menstrual cycle and monthly thereafter for a total of 5 months. Treatment of the monkeys #27 and #39 was as described above. The concentration of progesterone in the preovulatory peak of normal, untreated monkeys is also shown with its standard error.

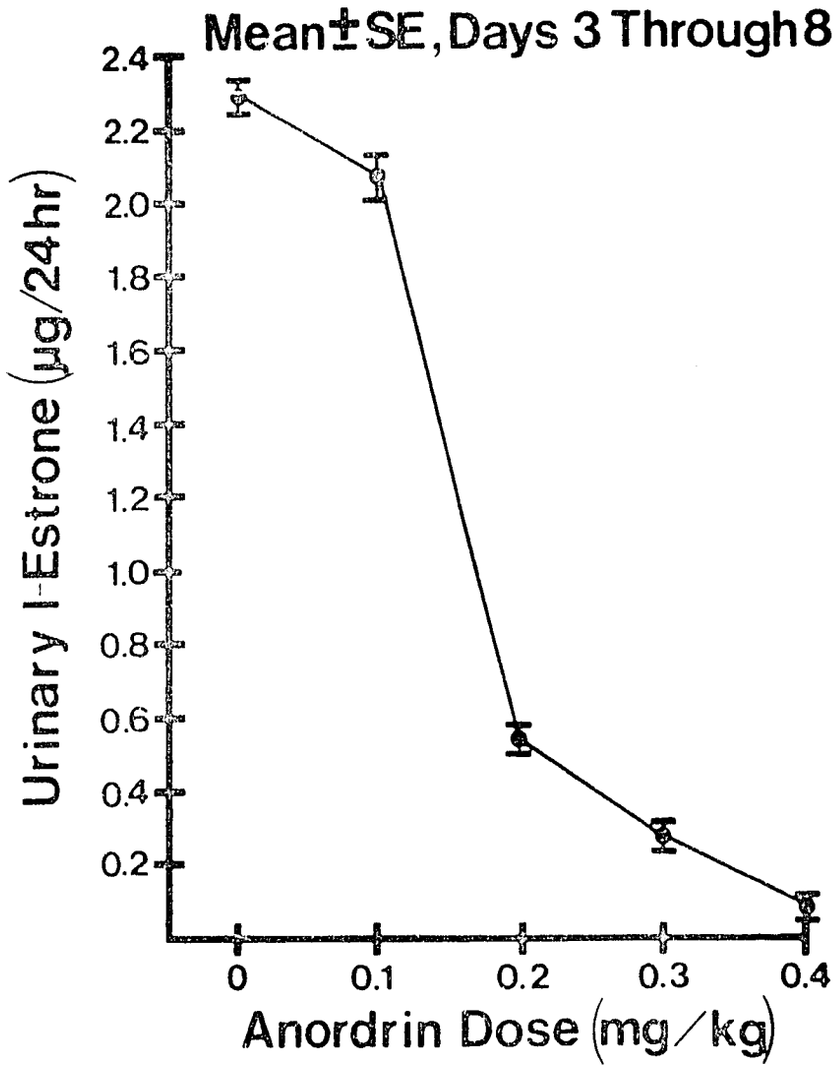
Fig. 9. Pattern of 14C-radioactivity in serum of one monkey after an intramuscular injection of 14C-anordrin (1.0 mg/kg body weight).

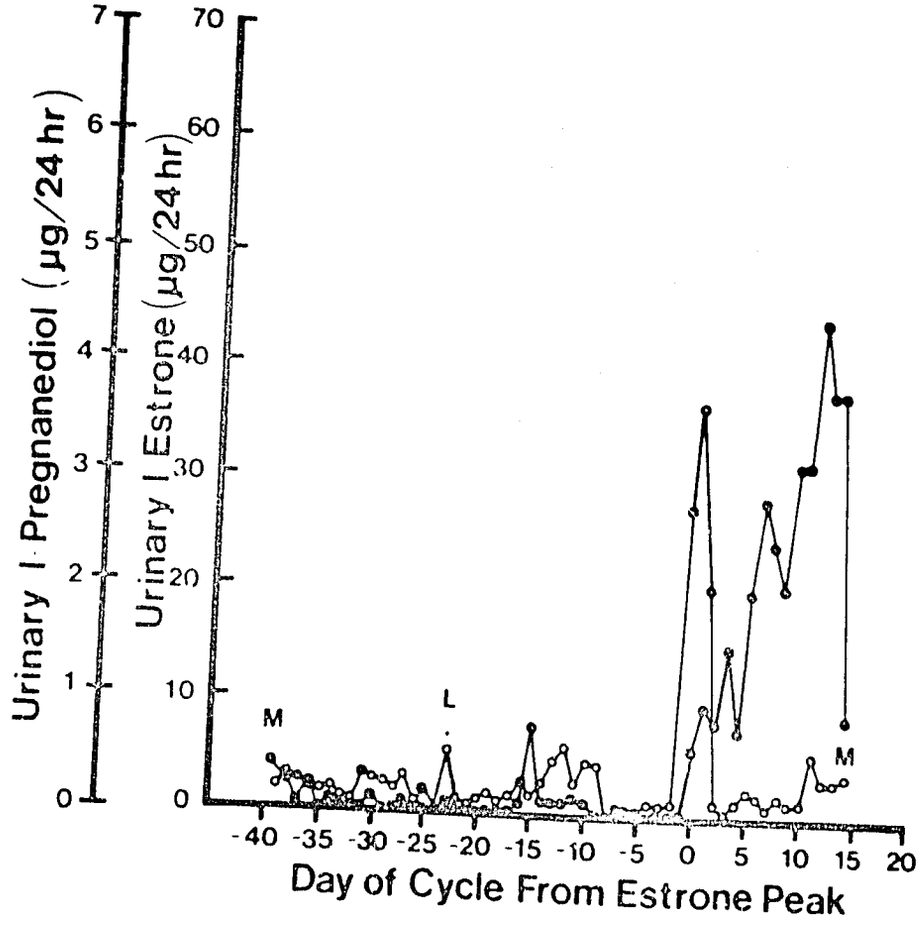

Serum I-FSH and I-LH (ng/ml)
55
50
45
40
35
30
25
20
15
10
5
0
0.1
0.2
0.3
0.4
Anordrin Dose (mg/kg)

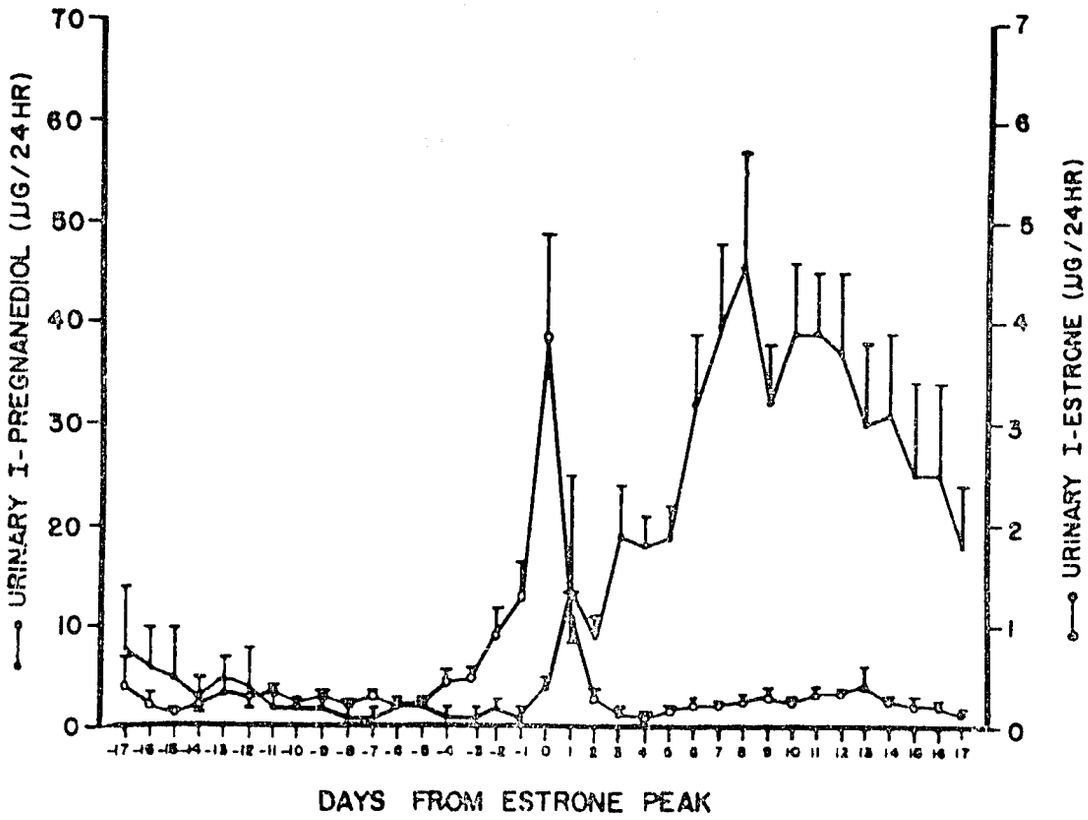

ANORDRIN TREATED ON DAY 0,
DOSE 0.2 TO 0.4 MG/KG, I.M.
VEHICLE CONTROL
200
180
160
140
120
100
80
60
40
20
SERUM I-LH (NG/ML ± SE)
1
2
3
4
5
MIDCYCLE
LH PEAK
DAY AFTER INJECTION

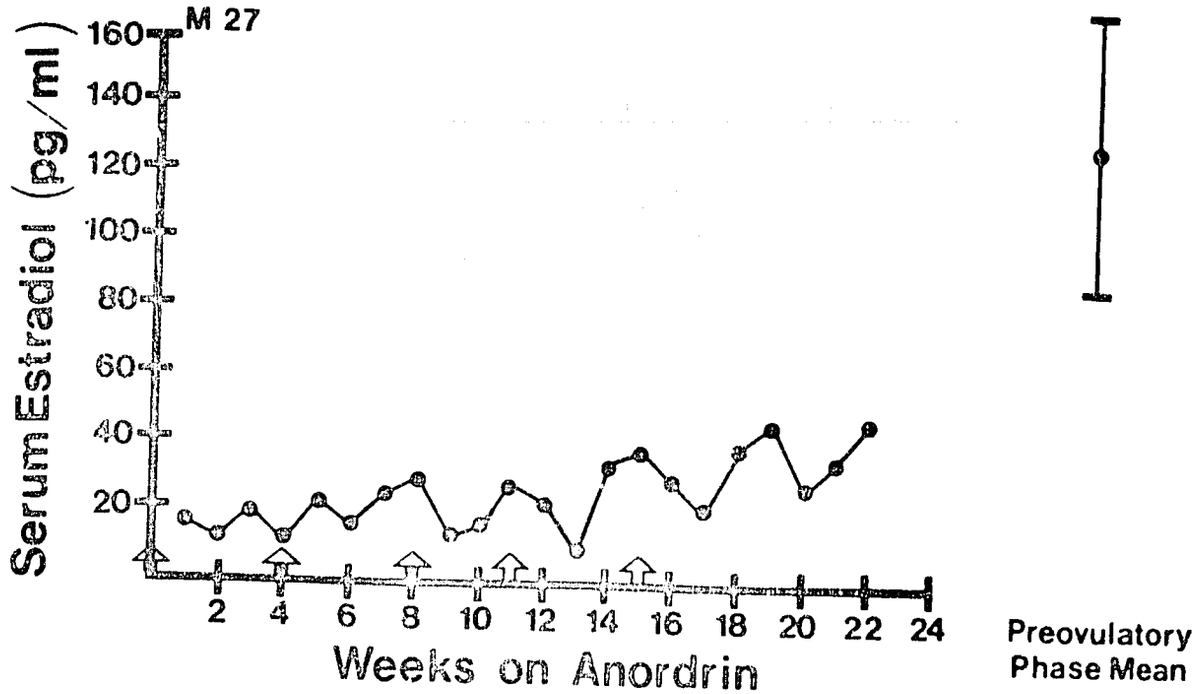

URINARY I-LH (UG/24 HR.)
22
20
18
16
14
12
10
8
6
4
2
-14 -12 -10 -8 -6 -4 -2 0 2 4 6 8 10 12 14
DAYS FROM SERUM LH PEAK

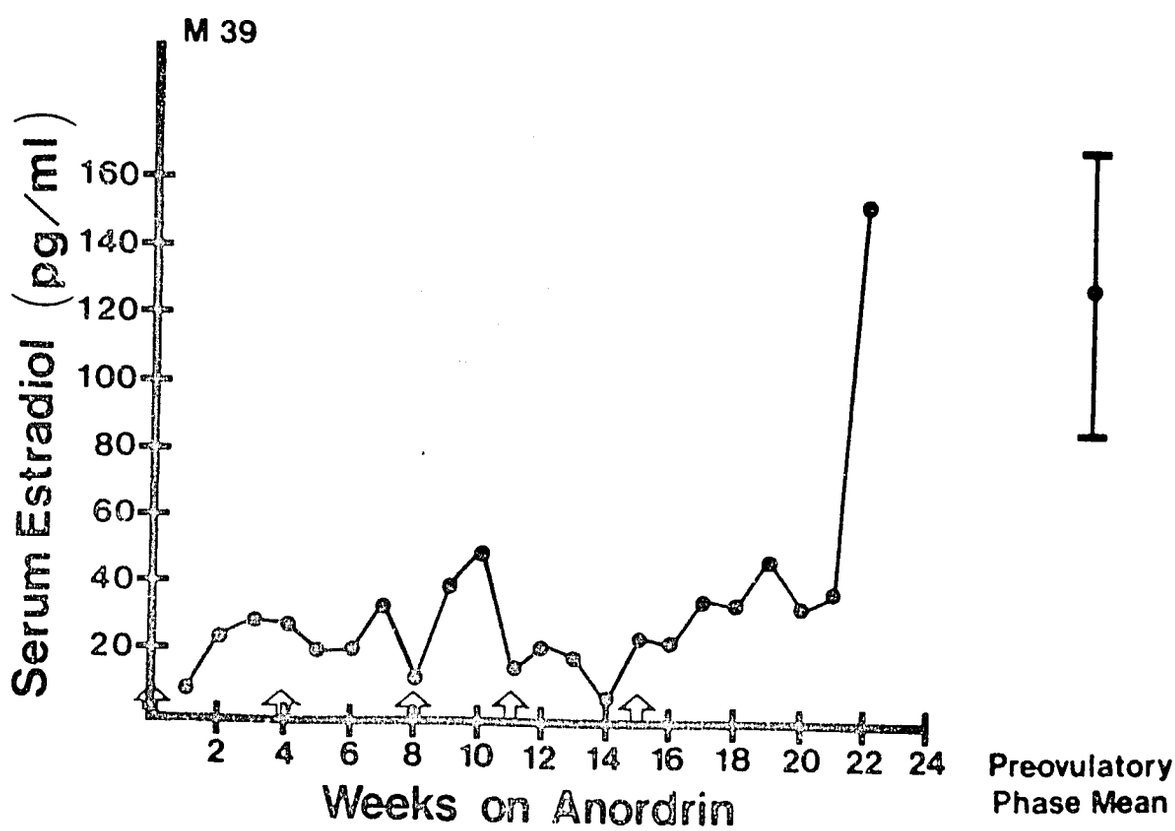

M 38
Urinary I-LH (ng/ml)
40
35
30
25
20
15
10
5
10
20
30
40
50
Days After Anordrin
Mean
Preovulatory
LH Surge

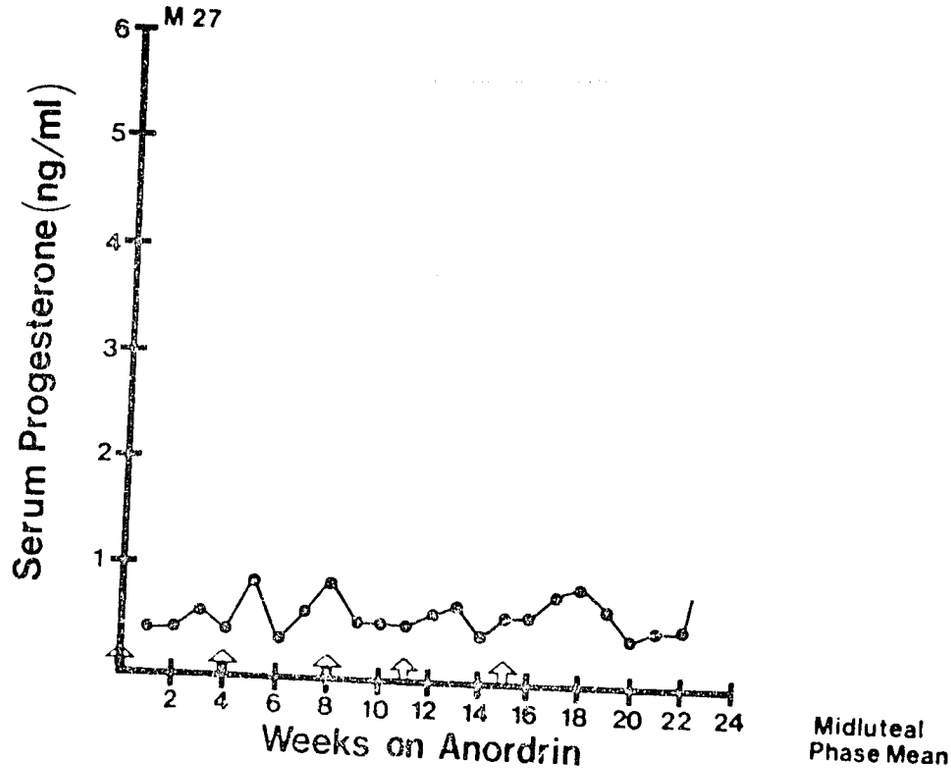

Mean±SE, Days 3 Through 8
Urinary I-Estrone (µg/24hr)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0.1
0.2
0.3
0.4
Anordrin Dose (mg/kg)

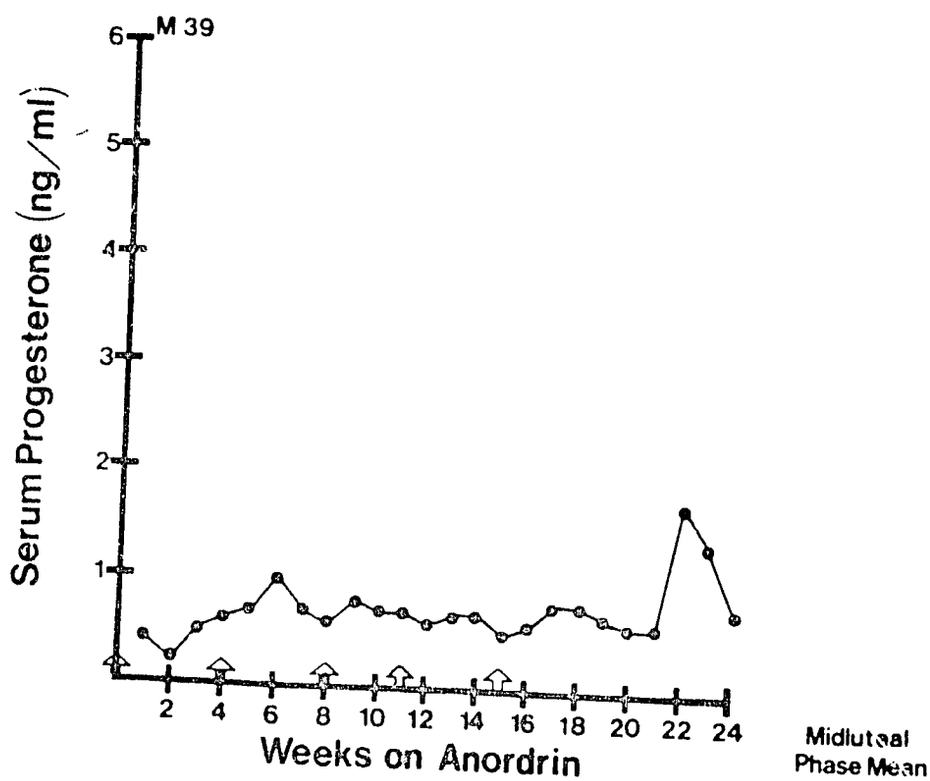

Urinary I Pregnanediol (µg/24 hr)
Urinary I Estrone (µg/24 hr)
7
6
5
4
3
2
1
0
70
60
50
40
30
20
10
0
M
L
M
-40 -35 -30 -25 -20 -15 -10 -5 0 5 10 15 20
Day of Cycle From Estrone Peak

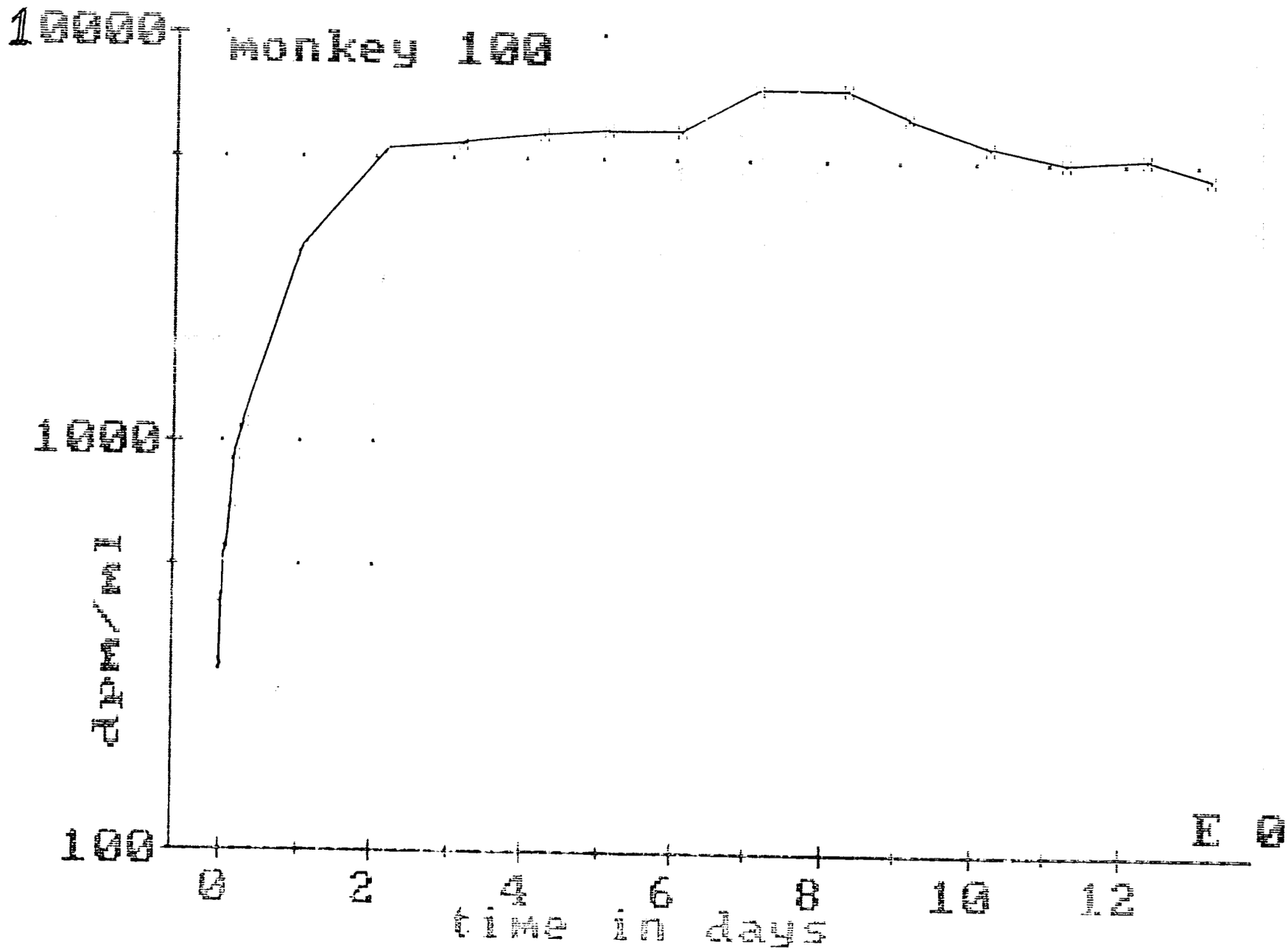

URINARY I-PREGNANEDIOL (µG/24HR)
URINARY I-ESTRONE (µG/24HR)
DAYS FROM ESTRONE PEAK


M 27
Serum Estradiol (pg/ml)
160
140
120
100
80
60
40
20
2
4
6
8
10
12
14
16
18
20
22
24
Weeks on Anordrin
Preovulatory
Phase Mean


M 39
Serum Estradiol (pg/ml)
160
140
120
100
80
60
40
20
2
4
6
8
10
12
14
16
18
20
22
24
Weeks on Anordrin
Preovulatory
Phase Mean


M 27
Serum Progesterone (ng/ml)
6
5
4
3
2
1
2 4 6 8 10 12 14 16 18 20 22 24
Weeks on Anordrin
Midluteal
Phase Mean


M 39
Serum Progesterone (ng/ml)
6
5
4
3
2
1
2 4 6 8 10 12 14 16 18 20 22 24
Weeks on Anordrin
Midluteal
Phase Mean


monkey 100
10000
1000
100
IE/ml
0
2
4
6
8
10
12
time in days
E 0

PROGRAM FOR
APPLIED RESEARCH ON
FERTILITY REGULATION

Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085
Cable: PARFR Chicago, Illinois

October 7, 1986 + November 5, 1986
Second Request - Please send as soon as possible. Thank you.
Mary

Danny H. Lewis, Ph.D.
Vice President, New Product Development
Stolle Research and Development
Corporation
P.O. Box 5310
Decatur, Alabama 35601

Dear Dr. Lewis:

PARFR has received the final technical report on PARFR-373, "90-Day Levonorgestral Microspheres." In order to complete our files, please send me a copy of the paper written by Beck, Pope, and yourself which you presented at the Society for Advancement of Contraception in Chicago, September 25, 1986.

Thank you for your prompt attention to this matter.

Sincerely,

Mary Nemeth

Mary Nemeth
Administrator

MN/aw

DEAR MARY.

ENCLOSED IS the Abstract of. The PAPER. PRESENTED AT the. SAC. MEETING IN CHICAGO Sept. 25, 1986. We. HAVE NOT written. A full. PAPER.

— ABSTRACT —

Preclinical Pharmacokinetic and Pharmacodynamic Evaluation of an Improved Polymer Microsphere System for the Parenteral Administration of Levonorgestrel

Preclinical pharmacokinetics and pharmacodynamics of an improved long-acting formulation of levonorgestrel (LN) were evaluated in nine baboons. The poly (d,l-lactide-coglycolide) microspheres contained 50% LN by weight, and ranged in size from 25 to 90 um. Three groups of three normally cycling female baboons each were injected intramuscularly with microspheres containing 12, 25 or 50 mg LN on day 3, 4 or 5 of the perineal cycle. Serum levels of LN were measured by radioimmunoassay, and the effect of treatment on ovarian function was monitored by observing changes in sex skin turgescence and by measuring serum levels of progesterone. By these criteria treatment inhibited normal ovarian function for 3 months in all groups. Vaginal bleeding occurred for 1 to 15 days during the first 2 months posttreatment in baboons treated with the low dose (12 mg LN), and the three baboons in this group resumed perineal cyclicity on days 92, 93 and 95 posttreatment (PT). Vaginal bleeding occurred on only 2 days in the six baboons in the other two groups (25 and 50 mg LN) during the first 90 days PT. Baboons in the 25 mg dose group resumed normal cyclicity 103, 103 and 105 days PT, and perineal cycles of the baboons treated with 50 mg LN were suppressed for 101, 110 and 126 days following treatment. The interval from treatment to resumption of perineal cyclicity in all groups was 103 +/-10.4 days. The results of this study demonstrate the feasibility of controlled release formulations for achieving finite menstrual cycle control among several treatment doses.

PLEASE: COMPLETE FORM AND MAIL TO SAC OFFICE
ATTACH PHOTOGRAPHS IF APPLICABLE

Note: All participants must register for the conference, and complete registration forms must be received by the abstract deadline.

THE DEADLINE FOR ALL APPLICATIONS IS 15 JUNE, 1986.

FINAL TECHNICAL REPORT ON PROPOSAL 375

I. PROJECT REPORT SUMMARY

TITLE: Baboon immunologic evaluation with LDH-C4 and *in vitro* fertilization studies in rodents.

PARFR Subagreement No. 375

Principle Investigator: Kenneth S.K. Tung, MD

Address: Department of Pathology
Biomedical Research Facility, Rm. 337
University of New Mexico
Albuquerque, NM 87131

Telephone: (505) 277-5874

Signature: _____
Kenneth S. K. Tung, M.D.

Subagreement period: Starting date: May 1, 1985
Ending date: September 30, 1986

Date of last technical report submitted to PARFR: July 1, 1986

Inclusive dates of this technical report: 5/1/85 to 9/30/86

Narrative Summary: From detailed immunopathologic and clinical laboratory investigations on female baboons immunized with LDH-C4 paptides 5-15, we did not detect any difference between LDH-X immunized baboons and untreated controls. All baboons had some evidence of hepatic dysfunction (hypergammaglobulinemia, anti-smooth muscle antibody, elevated LDH) and IgM deposits in the glomerular mesangia and Kupfer cells, suggestive of hepatic parasitic infection. Some of the anti-LDH-C4 monoclonal antibodies from Dr. Goldberg were found to inhibit in vitro fertilization in mice. An experimental model for evaluating effects of antibody on fertility in vivo in mice was developed. Finally, injection of interleukin 2 (IL-2) was found to have profound adjuvant effect on anti-LDH-C4 antibody response. Ongoing studies include detection of cell-mediated immune response to LDH-C4, evaluation of in vivo effect of anti-LDH-C4 antibody on mouse fertility, and ultrastructural localization of LDH-C4 on mouse sperm.

II. SUBSTANTIVE TECHNICAL REPORT

A. Objectives:

1. To study the immunopathology of normal female baboons and baboons immunized with the murine LDH-C4 peptide, 5-15; specifically: to study kidneys and ovaries for immune deposits and ultrastructural changes and to evaluate laboratory profiles of vital organ functions.

2. To study effects of polyclonal and monoclonal antibodies to LDH-C4 and to specific LDH-C4 peptides on mouse *in vitro* fertilization rate.

3. To develop a reproducible model for analysis of anti-LDH-C4 and other antisperm antibodies on fertility *in vivo* in mice.

4. To study the effect of recombinant interleukin 2 (IL2) on the magnitude of murine anti-LDH-C4 antibody responses. The rationale of this study will be described below.

B. Progress to Date:

1. Baboon immunopathology. The laboratory findings show increase in serum LDH isoenzyme, and serum anti-smooth muscle antibodies in all animals studied, regardless of whether they were normal or immunized with LDH-C4. In some baboons, slight elevation of total serum proteins were also detected. These findings suggest abnormal liver function. In addition, immunofluorecence studies revealed deposits of granular IgM in renal glomeruli mainly in the mesangial region, and in sinusoidal lining cells of the liver, most likely Kupffer cells. These are likely immune complexes in monocyte/macrophage cells. Histologic evaluation and electron microscopic studies on selected immunized baboons failed to reveal significant pathology. In view of the frequent finding of hepatic parasitic infections in the laboratory baboons, the immunopathologic findings on the baboons most likely reflect the appropriate response to an infectious state. In summary, detailed analysis of immunized baboons did not reveal significant laboratory abnormalities when compared with normal untreated baboons.

   Interestingly, Burek et al. recently reported serum anti-smooth and skeletal muscle autoantibodies in baboons immunized with hCG-diphtheria toxoid (Fed. Proc. 45: abst. 618, 1986), and the autoantibodies were absorbed by the carrier protein, diptheria toxoid. This report supports in part our findings, and suggests that the autoantibody to smooth muscle antigen may arise as a result of at least two mechanisms: crossreactive antigenic stimulation by diphtheria toxoid and parasitic infection.

2. Effect of anti-LDH-C4 antibodies on murine IVF rate. Three kinds of antibodies were investigated: polyclonal rabbit antiserum or antibody to defined LDH-C4 peptides, polyclonal mouse antibody to defined LDH-C4 peptides, and monoclonal antibodies to LDH-C4. In all instances, IgG was isolated from the sera, culture supernatants or ascites fluids by protein-A affinity chromatography, and each reagent tested three times, each at 3-7 antiserum doses. The results can be summarized as follows: 1) we confirmed that antibody to peptide 5-15 indeed inhibited IVF in a dose-dependent manner; 2) purified rabbit anti-LDH-C4 also inhibited IVF in a dose-dependent manner; 3) two monoclonal antibodies to LDH-C4 (of unknown peptides) inhibited IVF, and these antigens were localized to the sperm tail surface - interestingly, the distribution of one was diffuse and the other granular; 4) the other antibodies to LDH-C4 had no effect on IVF.

3. Development of an in vivo model to study antibody inhibition of fertility. Female mice were injected with pregnant mare serum and 48 hr later hCG. Antiserum or IgG was injected i.p. with the hormones. Natural mating was monitored by examination of vaginal plague. Between 24 and 48 hr later, the ova from each Fallopian tube were flushed into medium, fixed, and the percentage of ova with sperm tail, male pronuclei, or definite 2-4 cell cleavage recorded. We have shown that A/J mouse autoantiserum to sperm completely suppressed fertility, whereas the injection of the ascites from one of the monoclonal antibody to LDH-C4 had no effect. The same monoclonal antibody also had no effect on IVF. This technique is rapid, quantitative, and permits rapid screening of many antibodies on murine fertility in vivo.

4. Attempt to modulate murine antibody response to LDH-C4. A major concern in the design of any anti-fertility vaccine is the fact that the immune response to any antigen is under Ir gene control. This means that the magnitude of the antibody response among individuals in an outbred population are variable, hence the efficacy of vaccine-induced infertility is expected to be variable. In order to drive such a population to 100% responders, a number of strategies has been considered. One of these is the novel finding that the T cell lymphokine IL2 can convert a non-responder to a high responder in the immune response to some antigens. We have developed a reproducible enzyme-linked antibody assay in our laboratory for anti-LDH-C4 antibodies, and confirmed the report of Wheat and Goldberg that anti-LDH-C4 antibody response among inbred mice is variable. By incorporating recombinant human IL2 generously provided by the Hoffman La Roche Laboratory in the injection materials, antibody response to LDH-X in C3H and C57BL/6 mice were markedly elevated, by a magnitude of 2 $\log^{10}$. The influence of this highly elevated anti-LDH-X antibody response on fertilization will be investigated.

C. Unanticipated Results: None

D. Research Design. During this research period, we developed a number of new techniques for our laboratory, including in vitro and in vivo evaluation of anti-sperm antibodies on mouse fertilization and fertility and quantitation of anti-LDH-C4 antibodies. Initially, Tere Yule was involved as the major technical assistant in the project. In January, she returned to graduate school and was replaced by Mary Raymond. Raymond later left on maternity leave and was replaced by Evelyn Heaphy. The PI and co-PI, Tung and Kasai were unchanged.

E. Operational Significance. Results from our study have provided considerable support for the validity of LDH-C4 as a candidate antigen in contraceptive vaccine development. We showed that the female baboons that were immunized with the LDH-C4 peptide 5-15 did not have overt immunopathologic or pathologic complications attendant to the immunization procedure, supporting the possibility that this is a safe vaccine. We showed that monoclonal and polyclonal antibodies to murine LDH-C4 and its peptides inhibited murine fertilization in vitro. However, our data also found that antibodies to some antigenic peptides of LDH-C4 had no biologic effects. The development of an in vivo assay for mouse fertility to study antibody effect and the discovery of adjuvant effect of recombinant IL2 on anti-LDH-C4 antibody response should help to design future experiments that will further investigate LDH-C4 as a contraceptive vaccine.

F. Dissemination and Utilization of Research Results.

Project Output:

1. Goldberg, E., Shelton, J.A., Kasai, K., and Tung, K.S.K. (1986) Inhibition of in vitro fertilization of mouse ova with mouse sperm by monoclonal antibodies to LDH-C4. J. Andrology. in press.

2. Tung, K.S.K. (1985) Immunopathologic consideration of antifertility vaccine. In Male contraception. Advances and future prospects. Eds. G.I. Zatuchni, A. Goldsmith, J.M. Spieler and J.J. Sciarra. Harper and Row, N.Y. p. 382.

3. Tung, K.S.K. (1986) Immunopathologic aspects of anti-sperm immunity. J. Reprod. Immunol. In press.

4. Kasai, K., Teuscher, C., Matzner, P. and Tung, K.S.K. (1986) Strain variations in anti-sperm antibody response and antifertility effects in inbred mice. Biol. Reprod. In press.

5. Kasai, K., Shelton, J.A., Goldberg, E. and Tung, K.S.K. (1986) Effects of polyclonal antibodies to LDH-C4 peptides and monoclonal anti-LDH-C4 antibodies on fertility: in vitro and in vivo studies. In preparation.

Dissemination: None

G. Work Plan for Coming Report Period.

Anticipated accomplishments:

1. Evaluate additional polyclonal or monoclonal antibodies to known peptides of murine LDH-C4 for their ability to inhibit fertilization *in vitro*.

2. Determine the effect of those antibodies known to inhihit IVF on murine fertility *in vivo*.

3. Determine the mechanism of inhibition of fertilitzation by any functional antibodies identified above by studying the effect of antibodies on IVF using cumulus intact ova, cumulus free ova and zona free ova and by antibody treatment of sperm during capacitation.

4. Extend our study on the effect of recombinant IL2 on the magnitude relative affinity, kinetic and anti-fertility effect of anti-LDH-C4 antibody responses in inbred mice.

Procedures and activities:

Rationale. Although the LDH-C4 peptide 5-15 has been shown to have *in vitro* and *in vivo* effects on murine fertility, the effect was evident when high concentrations of antibody were used. Studies on monoclonal antibodies B3A3 and B2A8 indicated that antibodies to other determinants were more efficient in fertility inhibition. It is therefore important to select LDH-C4 peptides to which antibodies should have the greatest antifertility effect. Later, when antibody to human LDH-C4 peptides become available, they will also be evaluated.

1. To study and to quantitate the effects of anti-LDH-C4 antibodies on IVF. IgG will be isolated from antisera, ascites and tissue culture supernants by protein A affinity chromatography or by preparative high performance liquid chromatography techniques, and adjusted to 5 mg/ml. Serial dilutions of antiserum or antibody IgG will be studied for their effect on murine IVF. Outbred Swiss Webster mice will be used in these studies. Controls will include culture medium alone or with addition of normal sera, culture supernatants and ascites of NS1 myeloma.

2. To study and to quantitate the effects of anti-LDH-C4 antibodies on fertility *in vivo*. This will be carried out according to the method we have developed, as described above. For this study, sufficient volumns of antisera or ascites fluids of monoclonal antibodies must be available. Outbred Swiss Webster mice of known fertility will be used. Groups of 6 mice will be injected with different volumns of antibody mixture, and their fertility rate quantitated. Controls will be injection of sera from normal mice or ascites of NS1 myeloma.

3. To study mechanism of inhibition of fertility. We will determine the mechanisms by which the antibody inhibits ferilization. Study based on cumulus intact ova will determine effect of antibody on sperm's ability to disperse cumulus. Study based on cumulus free ova will determine the effect of antibody on sperm's ability to attach and penetrate the zona pellucida. Study on zona free ova will determine the effect of antibody on sperm's abilty to fuse with ova. These studies will also provide indirect information on the effect of antibodies on sperm capacitation/acrosome reaction.

   In murine fertility study in vivo, we will further determine the effect of antibody on development of fertilized ova by studying mice at different time intervals beyond 48 hrs. Any embryotoxic effect of antibody will be determined by studying implantation sites on day 10 gestation and compare the results with the number of corpus luteum on the ovaries. We will also begin to develop in vitro embryo culture system in order to determine the effect of antibody and complement effect on embryo development.

4. To further characterize the booster effect of recombinant IL2 on anti-LDH-C4 antibody response. This exciting obervation will now be followed up with additional experiments that will determine (1) the effect of antibodies on fertilization in vitro and in vivo, (2) the optimum number and dose of IL2 injections required for maximum adjuvant effect, (3) the duration of the elevated antibody response, and (4) effect on cell-mediated immune response to LDH-X.

Anticipated results: The experimental design and methods are straight forward and all the immunologic and reproductive biologic expertise are available in this laboratory, thus we anticipate no difficulties in carry out all the studies described. However, the studies are only as good as the reagents that will be supplied to us by Dr. Goldberg's laboratory. So far, they have been excellent, and the two laboratories have kept constant communications throughout the study period, and we expect the same to continue.

Potential factors that may promote or impede accomplishments: Mice may not be suitable for in vivo evaluation of antibody effect on fertility since they inseminate intra-uterally and antibody effect on sperm transit may not be affected.

Proposals for additional personnel and institutions: None

Plans for dissemination and utilization of findings: To report data in scientific meetings and publish data in scientific journals.

H. Budget Forcast:

The formulation of the budget is based on that of the last submission plus inflationary adjustments.

I. Personel

A. Kenkiche Kasai, PhD	(100%)	Salary:	$ 4,249
		Fringe benefit:	935
B. Evelyn Heaphy (50%)		Salary:	2,475
		Fringe benefit:	544
C. Kenneth Tung (10%)		Salary:	none

II. Supplies

A. Mice for in vitro and in vivo fertility studies:	4,125
B. Other supplies (culture medium, serum proteins, protein A sepharose, hormones, chemicals and enzyme-linked antibodies, xeroxing, telephone):	1,375

III. Travel

One trip for Kenkiche Kasai or Kenneth Tung to travel to Chicago to discuss experimental design and results with Dr. Goldberg:	200
TOTAL DIRECT COST:	13,903
INDIRECT COST (15% of direct cost):	2,085
TOTAL BUDGET:	$15,988

I. APPENDIX.

A. Data on effects of recombinant IL2 on anti-LDH-X antibody responses.

B. Data on effects of antibodies to LDH-C4 on fertilization in vitro.

ANTI-LDHX ANTIBODY LEVEL BY EIA

C3H FEMALE MICE

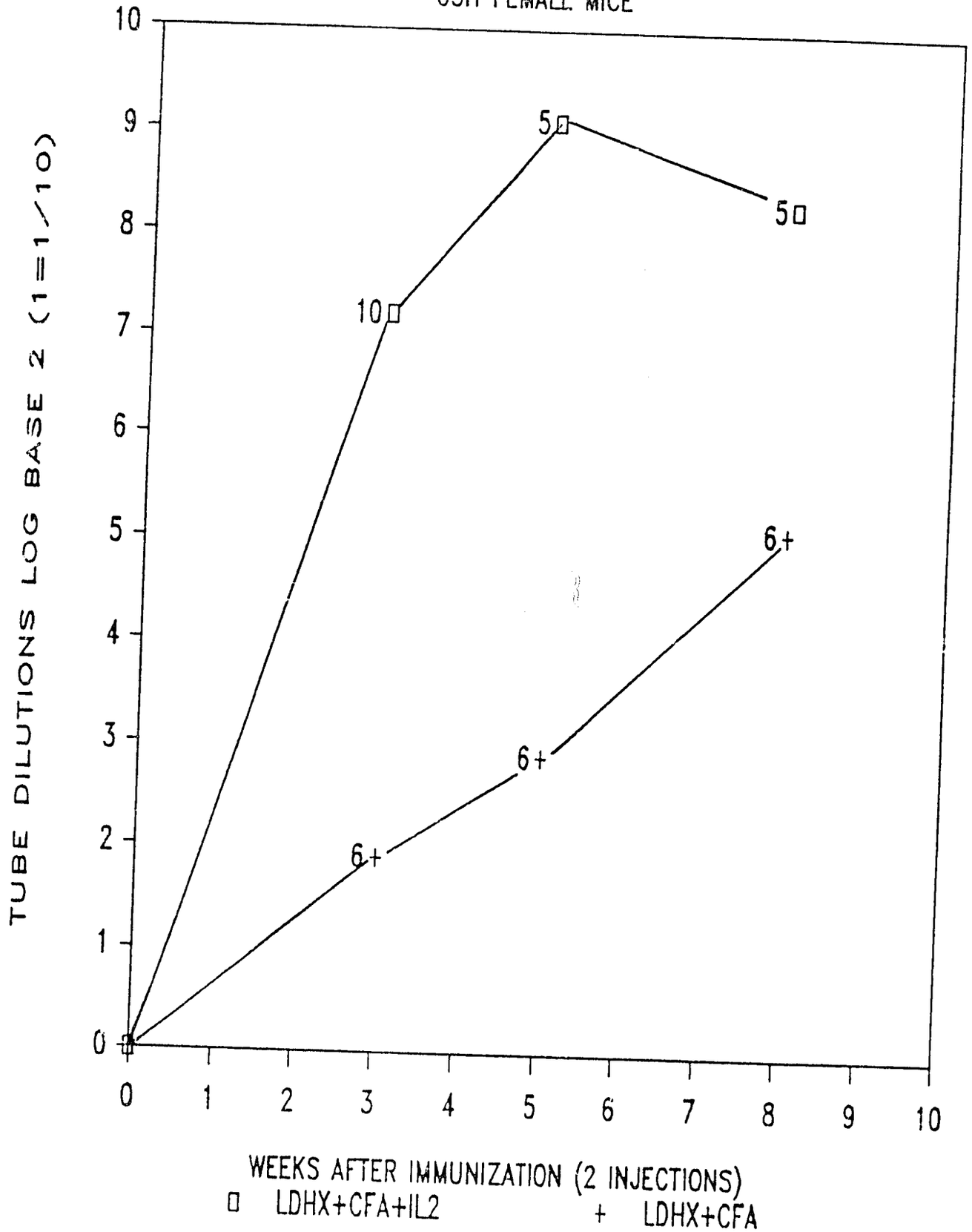


TABLE I. SUMMARY OF IVF DATA BASED ON ANTI-LDHX ANTISERA FROM DR. GOLDBERG'S LABORATORY.

REAGENTS	ANTIBODY	# EXP	% IVF AT TUBE DILUTIONS^{-2}							
			m-KRB	7	6	5	4	3	2	1[a]
Monoclonal ascites (? conc)	F11.F4	2	93	73	94	70	92	94	5	0
			82	100	60	100	93	54	47	0
		x	88	87	77	85	92	74	26	0
	A8.H10	3	88	-	96	-	86	96	9	7
			100	98	93	98	98	96	9	4
			90	94	98	95	94	78	13	7
		x	93	96	96	96	92	90	10	6
	B6.G1	3	74	-	77	74	75	96	94	25
			90	77	94	65	96	100	81	19
			98	88	92	76	88	87	93	-
		x	87	82	88	72	86	94	87	22

[a] 1:10

TABLE I (Continued)

REAGENT	ANTIBODY	# EXP	% IVF AT IgG CONCENTRATIONS (mg/ml) OF							
			m-KRB	.003-.005	.007-.01	.02-.05	.06-.10	.11-.40	1.3	2.7
Monoclonal IgG	B3A3	3	93	-	-	-	-	21	4	0
			91	100	81	71	-	13	0	0
			97	89	83	86	-	46	0	0
		x	94	94	82	78	-	26	2	0
	B2A8	3	77	-	-	-	-	13	0	0
			79	71	65	86	-	24	30	0
			96	61	98	63	-	40	13	0
		x	84	66	81	75	-	26	14	0
	C1F1	1	92	-	-	-	-	96	96	61
	B3F7	2	76	98	100	95	-	93	91	-
			94	-	97	82	-	64	55	56
		x	85	98	98	88	-	82	73	56
	C1010G (5-15)	1	70	-	-	-	95	93	88	-
	C117D (5-15)	1	75	-	-	-	95	94	98	-
	C119D (5-15)	1	100	-	-	-	-	-	97	-
	C115E (5-15)	1	100	-	-	-	-	-	96	-
	C112B (5-15)	1	100	-	-	-	-	-	44	-

TABLE I (Continued)

REAGENT	ANTIBODY	# EXP	% IVF AT IgG CONCENTRATIONS (mg/ml) OF						
		m-KRB	.003-.005	.007-.01	.02-.05	.06-.10	.11-.40	1.3	2.7
	F6F12C1	2	72	71	97	90	69	61	-	-
			96	79	94	75	24	-	-	-
		x	83	88	92	72	42	-	-	-
	G10G6	2	80	-	-	56	0	-	-	-
			100	-	100	67	0	-	-	-
		x	90	-	100	61	0	-	-	-
	G4D9	2	96	-	86	67	-	-	-	-
			94	73	66	39	29	-	-	-
		x	95	73	76	53	29	-	-	-

TABLE I (Continued)

REAGENT	ANTIBODY	#EXP	% IVF AT TUBE DILUTIONS OF							
			m-KRB	7	6	5	4	3	2	1
Purified rabbit antibody	Anti-LDHX	3	100	67	-	98	74	0	0	0
			100	57	90	80	100	0	0	0
			95	68	93	91	100	0	0	0
		x	98	68	93	91	91	0	0	0
Mouse polyclonal antiserum	Anti-DTM 5-15 (BALB/c)	4	90	94	23	84	14	91	58	-
			97	91	97	45	46	23	30	-
			81	57	57	63	57	18	53	-
			93	100	95	95	63	76	100	97
		x	90	86	68	72	45	52	60	97
	Anti-CFA (BALB/c)	2	50	-	-	-	-	-	74	94
			85	-	-	-	-	-	75	97
		x	67	-	-	-	-	-	74	95
	Anti-DT (SJL/J)	2	50	-	-	-	-	-	41	58
			85	-	-	-	-	-	86	68
		x	67	-	-	-	-	-	63	63
	Anti-LDHX	2	83	-	96	66	85	94	96	-
			85	-	-	100	92	90	79	-
		x	84	-	96	83	89	92	88	-

Tube dilution of 1 = 1:10.

TABLE I (Continued)

REAGENT	ANTIBODY	#EXP	% IVF AT IgG CONCENTRATIONS (mg/ml) OF					
			m-KRB	0.1	0.3	1.1	3.3	5.0
Rabbit IgG	LDHX	2	90	-	89	68	86	-
			100	-	97	97	74	-
		x	95	-	93	83	80	-
	5-15	2	97	-	75	87	100	-
			79	-	87	79	100	-
		x	88	-	81	83	100	-
	97-110	1	50	-	78	60	80	-
	211-226	1	91	-	96	73	54	-
	304-316	4	80	66	86	57	93	0
			85	79	94	42	4	0
			96	98	95	63	61	0
			63	69	53	69	-	-
		x	81	78	82	58	53	0
	318-330	1	50	-	80	80	73	-
Mouse IgG	304-316	1	89	-	100	85	67	-

PARFR-378

"PREPARATION OF TESTOSTERONE MICROSPHERES"

DANNY H. LEWIS, PH.D.

7/1/85-9/30/86

FINAL TECHNICAL REPORT: SEE IND #27,826

PARFR-379

FINAL TECHNICAL REPORT

Submitted to the

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

Project Title: "Idiotope Vaccine for Sperm-Targetted Immunocontraception"

Principal Investigator: Christopher P. Carron

I. PROJECT REPORT SUMMARY

A. General Information

1. Project Title: Idiotope Vaccine for Sperm-Targetted Immunocontraception

2. Project Number: PARFR-379

3. Recipient Institution: Duke University Medical Center

4. subagreement Period:

   a) Effective Date: August 1, 1985

   b) Termination Date: September 30, 1986

5. Last Technical Report Submitted: July 15, 1986

6. Inclusive Dates of this Technical Report: 8/1/85-9/30/86

B. Narrative Summary

During the past 13 months we have examined the feasibility of inducing antigen-specific anti-sperm antibodies in mice by active immunization with rabbit antibodies prepared against the idiotype of murine monoclonal anti-sperm antibodies. While we originally selected 4 different antibodies upon which to concentrate, we ultimately narrowed this to 2 mAbs, M29.6 and M42.15, because of practical considerations and because these 2 mAbs inhibit fertilization *in vivo* and *in vitro*. Sera from rabbits inoculated with purified mAbs were absorbed with normal mouse and isotype-specific immunoglobulin (Ig) and the anti-idiotype Ig fraction (Ab2) isolated by Protein A-chromatography. Binding specificity of Ab2 M42 for M42 was confirmed by measuring the reactivity of Ab2 with homologous and heterologous Mabs in an ELISA. Ab2 M42 competitively inhibited ^{125}I-labeled M42 binding to mouse sperm. Sperm extracts containing soluble M42 antigen inhibited binding of Ab2 to Ab1 by 70-75% suggesting that the Ab2 possessed subpopulations directed against sites similar or adjacent to the antigen-binding site of M42. Ab2 reactive with the antigen-combining site of M42 may contain subpopulations which mimic the mouse sperm epitope recognized by M42. Immunization with Ab2 induced Ab3 which react with native antigen: mice immunized with Ab2 M42 develop antibodies (Ab3) which competitively inhibit binding of ^{125}I-labeled M42 to immobilized Ab2 M42. Similar results have been obtained with M29 Mab. A more complete account of our work in the area of monoclonal anti-sperm antibodies and anti-idiotype reagents can be found below.

These results are consistent with the idea that immunization of mice with idiotype-specific Ab2 antibodies generated from sperm-specific monoclonal antibodies can induce antibodies to a specific sperm antigen.

II. SUBSTANTIVE TECHNICAL RESEARCH REPORT

A. Statement of Objectives

To demonstrate the feasibility of an idiotope vaccine for immunocontraception against sperm by immunization with anti-idiotype antibodies mad against sperm monoclonal antibodies.

B. Progress to Date

The concept of an immunocontraceptive vaccine composed of sperm components arises from two related observations: a) the antigenicity of spermatozoa and b) the strong association between infertility and anti-sperm antibodies (1,2). Recently, a variety of sperm antigens have been identified and partially characterized using antibodies which interfere with specific steps of gamete interaction.

One problem with the various sperm antigens identified to date is that useful quantities necessary for precise structural and immunochemical characterization are not immediately available. Morover, the potential for the utilization of native sperm components, once identified and characterized, as components of an immunocontraceptive vaccine is at the very least unlikely, because of substantial practical considerations. Therefore the development of methodologies for generation of native sperm antigen substitutes is both desirable and necessary.

A new approach to vaccine production utilizing immunological reagents as antigen substitutes may provide both an additional and/or complementary method for the generation of native antigen substitutes. The network hypothesis of Jerne (3) defines the immune system as a network of idiotypes and anti-idiotypes intimately involved in regulation of the immune system. A postulate of the network theory is that the production of antibody in response to foreign antigen subsequently elicits the production of a second group of antibodies against the first. Some of the second group of antibodies, called anti-idiotype antibodies (Ab2), are directed against the variable region of the first antibody (Ab1) and carry an internal image of the foreign antigen. Ab2 which mimic the antigen can be exploited as antigen substitutes: injection of the anti-idiotype antibody is in many ways equivalent to injecting the antigen itself. Although research aimed at the development of vaccines based upon internal image bearing Ab2 is still in the early stage of development, several groups have shown that it is possible to produce anti-idiotype antibodies that evoke the formation of protective antibodies in animals (4-10).

Work we have done over the past 12 months supports our original hypothesis that that immunization of mice with rabbit polyclonal Ab2 made against murine monoclonal anti-sperm antibodies (Ab1) results in the induction of antigen-specific anti-sperm antibody titers. Active immunization with Ab2, made against mouse sperm monoclonal antibodies, may induce contraceptive anti-sperm antibodies and prevent fertilization.

RESULTS AND DISCUSSION

A. Monoclonal Antibodies

Sperm specific monoclonal antibodies utilized in this study were generated using syngeneic mouse testis as immunogen and have been described elsewhere (see Appendices 2-8). The monoclonal antibodies (mAb) designated M29, M42, and W108 were purified by methods described below and used either as immunogen or in some instances in the construction of immunoaffinity columns.

Antibodies of the IgM class (M29 and W108) were purified according to the followir protocol: Ascites fluids is harvested by peritioneal aspiration, allowed to clot and centrifuged. The supernatant is brought to 50% ammonium sulfate, centrifuged at 20,000 rpm for 10 minutes, the pellet washed with 43% ammonium sulfate and centrifuged. The resultant pellet is dissolved in a small volume of PBS, dialysed and chromatographed on a Sepharose-6B column. Column fractions are analysed by SDS gel electrophoresis and the IgM containing fractions pooled. The IgM pool prepared in this manner appears to be 80-90% pure. The M42 mAb (IgG_1) was purified from ascites by Protein A-chromatography using the Bio-Rad MAPS buffer system. Fractions eluted with 0.1 M sodium citrate, pH 3.0 were pooled and characterized by SDS gel electrophoresis.

B. Rabbit Anti-idictype Antibody Preparation

B.1 Immunization of Rabbits with Monoclonal Antibodies

Ab2 were generated in New Zealand white rabbits by well established methods using monoclonal antibodies as immunogen (5,6,10). Rabbits were pre-screened prior to immunization with mouse monclonal for the presence of anti-mouse Ig antibodies were injected subcutaneously at multiple sites with 300 ug purified monoclonal antibody emulsified in Freund's complete adjuvant on day 0 and boosted intramuscularly with 100 ug of purified antibody in PBS on days 7 and 30. Seven to 10 days following the 2nd booster, the rabbits were anesthetized and bled by cardiac puncture. Rabbits were maintained on a 30 day boost/bleed schedule thereafter.

B.2 Binding Assay for the Detection of Rabbit Anti-mouse Ig

A solid phase radioimmunoassay was developed and used to detect the presence of rabbit anti-mouse Ig in control and pre-immune rabbits and to monitor the development of rabbit anti-mouse Ig in the sera of rabbits immunized with mouse mAb.

Individual monoclonal antibody or normal mouse immunoglobulin (10 ug/ml) diluted in PBS/10 mM N_3 was passively adsorbed to wells of 96-well microtiter plates by overnight incubation at 4°C and remaining free non-specific protein binding sites blocked by incubation of wells containing bound Ig with PBS/1.0% gelatin. Plates were washed with PBS/0.5% Tween 20 and 50 ul aliquots of test sera, serially diluted (1:400-409,600) in PBS/Tween 20 were added and incubated for 1 hr at 37°C. The plates were washed x4 with PBS/0.05% Tween to remove non-bound rabbit Ig and incubated with 50,000 cpm/well ^{125}I-goat anti-rabbit Ig to detect bound rabbit Ig. After 1 hr at RT, the plates were washed with PBS/Tween 20 bound radioactivity measured in a gamma counter. Figure 1. shows a typical response to repeated inoculation with purified mouse monoclonal antibody. All animals immunized according to this protocol developed high levels of anti-mouse Ig by day 35-40.

B.3 Purification and Characterization of Anti-idiotype Antibody

Immunoabsorbant columns were prepared by coupling purified mAb or normal mouse Ig to CNBr-activated Sepharose 4B following the procedures outlined by Phamacia (Pharmacia Fine Chemicals). Antibodies were dialysed exhaustively against coupling buffer (CB) 0.5M NaCl, 0.15 M $NaHCO_3$, pH 8.5, combined with CNBr-Sepharose (5 mg/ml swollen gel, previously hydrated in 1mM HCl and rapidly rinsed with CB), at a gel:buffer ratio of 1:2 and allowed to react overnight at 4°C with gently agitation. The supernatant was rem by centrifugation and the gel pellet resuspended in 0.2 M glycine, pH 8.5 and reacte for 2 hrs at 20°C. The antibody-Sepharose conjugate was packed in a column and washed sequentially with 0.5 M NaCl, 0.1 M sodium acetate, pH 4.0 and CB and equilibrated and

stored in PBS/10mM NaN_3. We estimate the coupling efficiency to be greater than 90%. Similar procedures were used in the construction of all immunoaffinity columns.

Each rabbit antiserum was made specific for the idiotype of the respective monoclonal antibody by absorption with myeloma protein having the same isotype as immunogen but unrelated specificity. For example, antisera to M29 (IgM) was absorbed with immobilized W108, an anti-sperm IgM mAb unrelated to M29. Anti-sera M42 mAb was absorbed with immobilized MOPC-21 a commercially available mouse monoclonal of the IgG_1 subclass. All antisera were repeatedly absorbed with immobilized normal mouse immunoglobulin prior to passage over isotype-specific immunoabsorbants.

Typical procedures and results for the preparation of Ab2 M42 are presented below and in Figure 2. 2 mls M42 mAb antiserum (rabbit #110, bleed date 12-12-85; day 42 immune serum) was centrifuged at high speed and applied (0.2 ml/min) to a 5 ml column of immobilized normal mouse Ig (NMIg column) connected in series to a 5 ml column of immoblized MOPC21, cycled for 2-4 hrs, washed and the flow-through pooled. Anti-isotype and xenotypic antibodies were retained by these columns, whereas the NMIg and the MOPC21 flow through contained antibodies reactive with idiotypic determinants of M42 mAb. The flow through-pool was applied to a 5 ml column of Protein A, washed with PBS and the total IgG fraction eluted with 0.1M sodium citrate, pH 3.0, pooled and dialysed against PBS/10mM NaN_3. Antibodies adsorbed to the NMIg and MOPC21 immunoabsorbants were eluted with 0.1M diethylamine, pH 11.5, pooled and dialysed against PBS/10mM NaN_3.

Before absorption, rabbit antiserum to M42 mAb reacted with both M42 and M41 mAb suggesting a strong reaction to determinants common to M42 and M41. M41 is an IgG_1 anti-sperm monoclonal antibody unrelated to M42. Xenotypic and anti-isotype Ig eluted from the NMIg and MOPC21 immunoabsorbant columns, reacted strongly with both M42 and M41. The flow through material, eluted from Protein A, reacted only with M42 and therefore contained Ab2 specific for M42.

B.4. Preparation of Idiotype Affinity-Purified Anti-idiotype mAb

The IgG fraction recovered by Protein A chromatography contains Ab2 as well as irrelevant rabbit Ig. To eliminate or reduce the contamination of Ab2 with irrelevant rabbit Ig, Ab2 antibodies were purified from the absorbed flow through-pool by affinity purification on the respective idiotype column e.g., M29 or M42. In the example presented below, the flow through from the NMIg and W108 column was applied to an M29 column, washed with PBS and specific anti-idiotype antibody, free of other contaminating irrelevant antibodies, eluted with 4 M guanidine/10mM Tris-HCl, pH 7.0 and dialysed against PBS/10 mM NaN_3 prior to use.

TABLE 1. shows the relative yield of Ab2 M29 purified from rabbit sera by Protein A or M29 affinity chromatography. In this case, 20 mls rabbit (#103) sera was exhaustively absorbed with NMIg and W108 and 10 mls of sera applied to either the M29 or Protein A column, washed with PBS and bound antibody eluted as described above. The process was repeated until residual Ab2 binding activity in the flow through fraction was reduced to insignificant levels. Ab2 binding-activity was completely removed by 2 passages over Protein A or the M29 affinity-column (data not presented). Eluted antibodies were pooled, dialysed and the protein concentration determined. Total protein recovered by the Protein A vs idiotype affinity-chromatography was 1.74 and 0.035 mg/ml sera respectively.

Figure 3. shows that the idiotype affinity-purified Ab2 preparation exhibited similar titration characteristics when compared with either Protein A-purified Ab2 or untreated sera suggesting almost complete recovery of Ab2 binding activity on the idiotype column.

Assuming almost 100% recovery of Ab2 on both the Protein A and idiotype columns and both preparations exhibit similar binding acitivities then together these data sugge that idiotype affinity-chromatography generates a 50 fold (1.74/0.035) increase in t specific binding activity of the Ab2 preparation. Similar results were obtained for anti-idiotype M42 except that the serum concentration of anti-idiotype M42 was 3 fold greater (0.09 mg/ml rabbit sera #110). These serum values are in agreement with the anti-idiotype antibody concentrations reported by others in response to immunization with mAb (11,12).

The anti-idiotypic nature of Protein A purified rabbit Ab2 is apparent from the data presented in TABLE 2. which shows that anti-idiotype M29 and anti-idiotype M42 are specific for their homologous mAb. Monoclonal anti-sperm antibodies, MOPC21 or normal mouse Ig was passively adsorbed to wells of 96-well plates by overnight incubation at 4°C and free protein binding sites blocked with 1% gelatin/PBS. Protein A-purified rabbit anti-idiotype M42 or M29, diluted 1:10,000 in 0.1% gelatin/PBS, was added to wells in triplicate, incubated 1 hr at 37°C and washed extensively to remove free anti-Id. Bound rabbit Ab2 was detected by incubation with HRP-labeled goat anti-rabbit IgG. Wells were washed extensively with PBS/0.05% Tween 20, developed with ABTS/H_2O_2 for 20 min. and the OD_{414} recorded. Idiotype affinity-purified Ab2 exhibit comparable if not greater specificty for homologous mAb (compare Table 2A and 2B)

The data presented above show that both Protein A and idiotype affinity-purified Ab2 M42 and M29 reacted only with the immunizing Ab1. Idiotype affinity purified and Protein A purified Ab2 are pure rabbit Ig as determined by SDS gel electrophoresis (data not presented). However, we estimate that only 2% (0.35/17.4 X 100) of the Protein A Ab2 M29 (and 6% of the anti-idiotype M42) preparation are Ab2 with the remainder being normal (irrelevant) rabbit serum antibodies. In contrast, by definition the idioty affinity-purified preparation is homogenous: 100% of the antibodies in this preparation are Ab2. Therefore idiotype affinity-chromatography would seem the method of choice for the preparation of a polyclonal Ab2 for use in immunization protocols.

B.5 Combining-Site Specific Ab2

Clearly Ab2 prepared by either Protein A or idiotype affinity-chromatography react specifically with the mAb (Ab1) immunogen. However, there are at least two types of anti-idiotype antibodies: Ab2 specific for framework determinants of the Ab1 idiotype and Ab2 with Ab1 antigen combining-site specificity. Ab2 carrying idiotopes which mimic the sperm antigen epitope recognized by Ab1 (internal image antibodies) have potential to induce anti-Ab2 antibodies which resemble Ab1 in terms of antigen specificity (13,14,15). Therefore we developed two different assays designed to detect and estimate the proportion of the Ab2 response to the framework vs the antigen-combining site of Ab1.

Inhibition of the binding of ^{125}-labeled mAb to mouse sperm by Ab2 was demonstrated in an assay that determines whether Ab2 binds idiotopes within or near Ab1 combining site. The results presented in Figure 5. show that anti-idiotype M42 and M29 inhibited binding of homologous Ab1 to sperm by greater than 95%. In control experiments, Ab2 did not significantly inhibit heterologous Ab1 binding to sperm. These results suggest that Ab2 is directed to one or more epitopes within or near the combining site of Ab1 (16,17).

A second assay was developed to test the ability of sperm antigen to prevent the interaction between Ab1 and Ab2. The basis for this assay is that the sperm antigen recognized by Ab1 will inhibit Ab2 binding to Ab1 if Ab2 and sperm antigen compete f the same binding site on Ab1. Antigen inhibition of Ab2 binding to Ab1 is an indication of the presence of antigen combining-site specificities in Ab2 (for example see ref.6).

Sperm extract and not purified antigen was used in these experiments. Sperm were extracted in 50 mM NaCl/10mM Tris, pH 7.2/2mM EDTA/20mM CHAPS/1mM PMSF/1 mg/ml SBTI on ice and centrifuged for 90 min at 30,000 rpm in a Ti55 rotor. The supernatant of the high speed spin contains the M42 antigen (determined by immunoblotting). M29 antigen remains in the pellet under these conditions.

M29 and M42 mAb (2 ug/ml) were adsorbed to Removawell strips and free protein binding sites subsequently blocked with 1% gelatin/PBS/N_3. Increasing amounts of sperm extract, adjusted to contain 0.1% gelatin, was added to wells and preincubated for 1 hr at 4°C. Iodinated Ab2 M42 or M29 was added and the incubation continued for an additional 1 hr. Figure 5 shows that sperm extract inhibited anti-idiotype M42 (Ab2) binding to M42 mAb in a dose dependent fashion but failed to inhibit anti-idiotype M29 binding to M29 mAb. Sperm extract inhibited anti-idiotype M42 binding to Ab1 by 70-75%. The inability to inhibit 100% of Ab2-Ab1 binding suggests that both framework and antigen combining-site specificities were present in the anti-idiotype M42 population. The Ab2 population with combining site-related specificity represented a maximum of 70-75% of the Ab2. These results suggested that a major population of Ab2 might contain the internal image of the M42 sperm antigen.

In contrast, anti-idiotype M29 binding to M29 was not inhibited by the same sperm extract. The failure of supernatant sperm extract to inhibit anti-idiotype M29 binding to M29 mAb is not surprising since the M29 antigen is not solubilized under these conditions and suggests that inhibition of Ab2 M42 binding to Ab1 is not likely to be a non-specfic e.g., proteolytic/glycolytic, effect of sperm extract on antibody interaction. Future experimentation of this type will be conducted using idiotype affinity-purified antigen in place of whole sperm extract.

C. Active Immunization of Mice with Anti-Idiotype Antibody: Induction of anti-(Ab2) antibodies (Ab3).

C.1 Ab3 Inhibition of Ab1 Binding to Ab2

Taken together the results presented above suggest that a portion of the Ab2 recognize idiotopes within or near the combining site of Ab1 and consequently may bear the internal image of the sperm antigen recognized by the mAb (Ab1). Immunization of mice with Ab2 bearing the internal image of the sperm antigen recognized by Ab1 may induce antibodies which react with native sperm antigen. Induction of Ab1-like Ab3 in mice was monitored by a solid phase RIA in which inhibition of ^{125}I-labeled mAb binding to immobilized Ab2 by Ab3 was measured.

Groups of 8 animals were immunized subcutaneously with Protein A-purified rabbit anti-idiotype M42, anti-idiotype M29, a cocktail of anti-idiotype M42 **and** anti-idiotype M29 or rabbit Ig over a period of 90 days. 7 days after the last booster injection, the animals were bled and 1 ml of sera of each group pooled, repeatedly absorbed with immobilized rabbit Ig to remove mouse anti-rabbit Ig and the mouse Ig purified on Protein A. Figure 6. shows that sera from mice immunized with Ab2 M29 or Ab2 M29 and Ab2 M42 inhibited binding of ^{125}I-labeled M29 to Ab2 M29 in a dose dependent manner. Sera from mice immunized with Ab2 M42 alone had no effect. Similarly, only sera from mice immunized with Ab2 M42 or Ab2 M42 and Ab2 M29 inhibited M42 binding to Ab2 M42. Sera from mice immunized with Ab2 M29 alone had no effect. Similar results were obtained with the sera of mice immunized with idiotype purified Ab2.

C.2 Ab3 Inhibition of Ab1 Binding to Sperm Target Cells

Our initial immunization protocol used Ab2 purified by Protein A-chromatography. In a recent and ongoing experiment, we immunized mice with Ab2 purified by idiotype affinity-chromatography; Ab2 prepared by this procedure show a substantial increase in specific activity and purity when compared with Ab2 isolated on Protein A. We are currently analysing sera of these mice for the induction of Ab-1 like Ab3 using the competitive binding assay described in C.1 and the sperm binding-assay described in B.2.

A competitive sperm binding-assay was developed based upon the concept that Ab1-like antibodies induced in mice by immunization with Ab2 would inhibit Ab1 binding to target cells (see ref. 16). Briefly, mouse epidydimal sperm, lightly fixed with formaldehyde and treated with glycine/PBS to quench unreacted aldehyde, are suspended in PBS/1% BSA to block free protein binding sites and reduce non-specific binding in subsequent steps. Fixed sperm (5×10^5) are combined with Ab3 test sera in 400 ul microfuge tubes, incubated 12 hrs (overnight) at 4°C with end-over-end rotation and combined with 5×10^5 cpm Ab1. Sperm bound ^{125}I-Ab1 is separated from free antibody by filtration and bound ^{125}I-labeled Ab1 determined by gamma counting. Non-specific binding was defined as counts bound in the presence of 100-fold excess cold Ab1. Inhibition of Ab1 binding to sperm by Ab3, when compared with control mouse sera, provides evidence for the presence of antibodies which compete for Ab1 binding sites on the sperm. Results to date are preliminary but nevertheless provide strong support for the hypothesis that immunization of mice with Ab2 induces Ab-1 like anti-sperm antibodies.

FIGURE 1

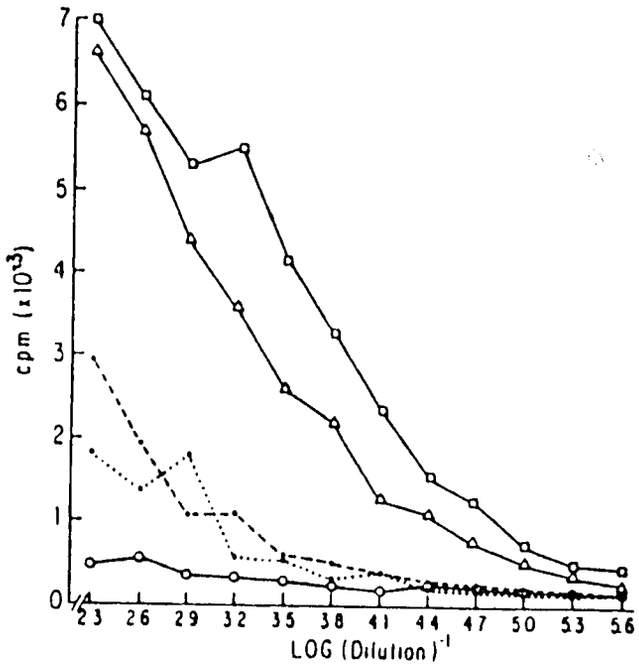


FIG. 1. Detection of anti-mouse M42 Ig in rabbit serum by RIA. Rabbit #110 was immunized with 300 ug M42 in Freund's complete adjuvant on day 0 and with 100 ug M42 in PBS on days 7 and 30. The animals were bled for immune serum on days 7, 14, 30 and 40. Purified M42 monoclonal antibody (10 ug/ml) was passively adsorbed to wells of flat-bottom 96-well microtiter plates and excess protein-binding sites blocked. Twofold serial dilutions of sera (preimmune o , day 7 -o-, day 14--o--, day 30 Δ and day 40 □) were added in duplicate to wells. The plates were washed extensively, and the amount of bound antisera determined using ^{125}I-labeled goat anti-rabbit antibody.

FIGURE 2

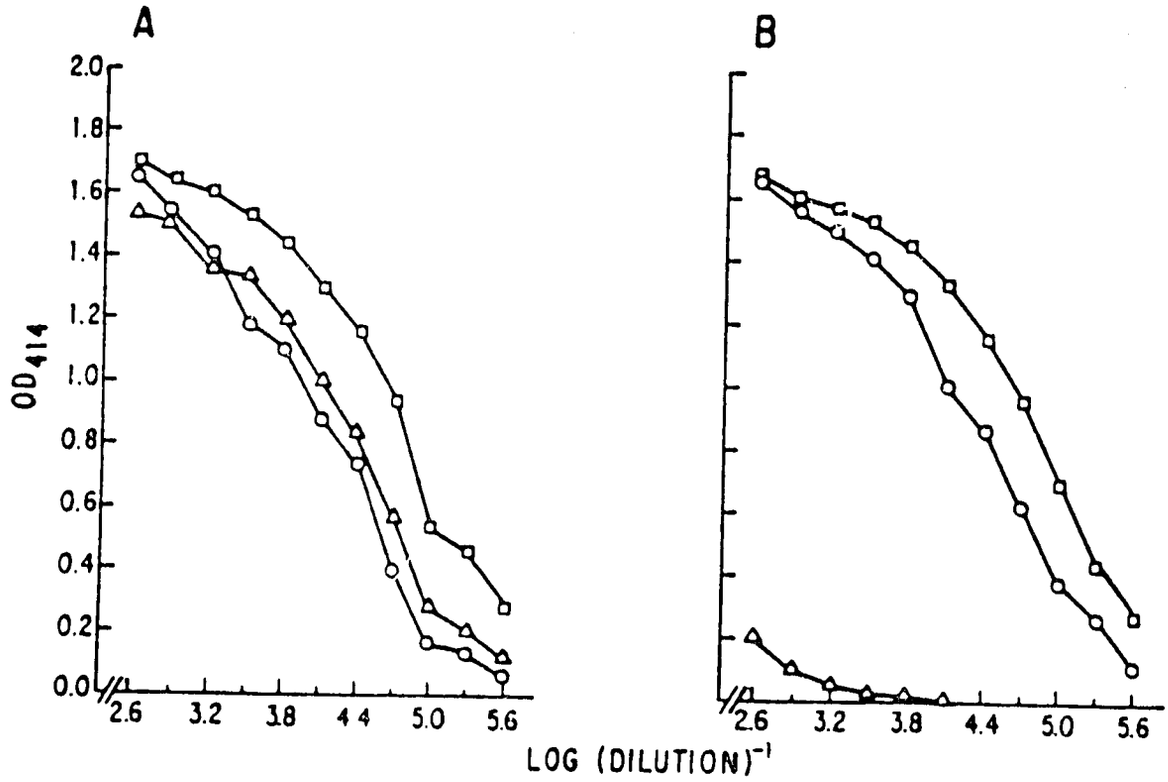


FIG. 2. Characterization of Ab2 activity by ELISA. Typical results for the preparation of Ab2 from rabbit immune sera are shown for Ab2 M42. M42 mAb (A) or M41 mAb (b) at a concentration of 10 ug/ml PBS was passively adsorbed to wells of 96-well plates by overnight incubation. M42, M41 mAb and the myeloma protein protein MOPC21 are IgG_1s. Two fold dilutions of hyperimmune sera □ , pooled Ig eluted from the NMIg and MOPC-21 columns o , and the anti-idiotype fraction eluted from Protein A Δ , were added to wells in duplicate and incubated for 1.5 hrs. The plates were washed extensively and bound rabbit Ig detected using HRP-labeled goat anti-rabbit IgG and ABTS/H_2O_2 as substrate. OD_{414} was determineed with a automatic ELISA plate reader.

FIGURE 3

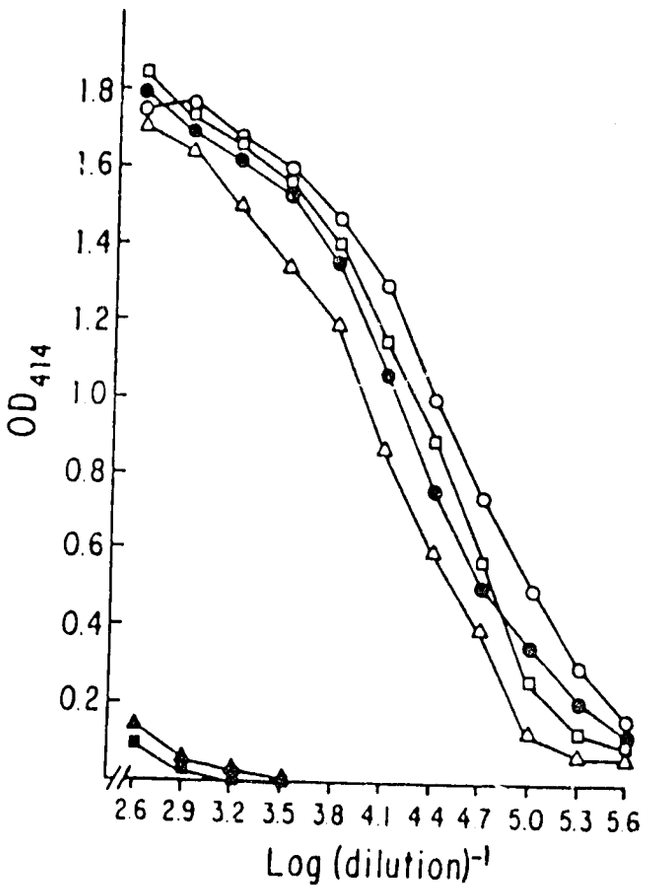


Fig. 3. Titration of Protein A and Idiotype AffinityPurified Ab2. Typical results are shown for the titration of M29 Ab2 on M29 or M2 mAb. Untreated sera binds equally well toM29(o) and M2 (□) Ab1. Protein A (o) and Idiotype (Δ) purified Ab2 M29 exhibit similar titration curves for binding to M29. Neither preparation shows appreciable reactivity with M2 (Protein A, □; Idiotype purified, Δ).

TABLE 1. Fractionation of Rabbit Anti-M29 mAb Serum

	Whole Serum	NMIg & W108.38 Pool	Protein A Purified Ab2	Idiotype Affinity Purified Ab2
Volume¶ (mls)	10.0	20.0	9.5	5.0
Protein Concentration (mg/ml)*	95.1	0.40	1.83	0.07
Total Protein	951	8.0	17.39	0.35

*Protein was measured with the Bio-Rad Protein Assay using bovine Ig as standard.
¶Volume of pooled eluate from respective affinity columns after dialysis. Starting material was 10 mls rabbit (#103) sera.

TABLE 2A & 2B. Binding of **Protein A** (Table 2A) and **Idiotype Affinity** (Table 2B) Purified-Rabbit Anti-Id M42 AND M29 Antibodies To Anti-Sperm Monoclonal Antibodies, MOPC-21 And Mouse Ig.

TABLE 2A.

BINDING OF PROTEIN A-PURIFIED RABBIT ANTI-IDIOTYPE M42 AND M29 ANTIBODIES TO ANTI-SPERM MONOCLONAL ANTIBODIES, MOPC-21 AND MOUSE Ig.

	W108	P220	M42	M41	M29	M2	NMIg	MOPC 21
Anti-Id M29	0.00	0.05	0.09	0.02	0.70	0.04	0.06	0.04
Anti-Id M42	0.00	0.05	1.07	0.09	0.09	0.04	0.02	0.01

TABLE 2B.
BINDING OF IDIOTYPE AFFINITY-PURIFIED RABBIT ANTI-IDIOTYPE M42 AND M29 ANTIBODIES TO ANTI-SPERM MONOCLONAL ANTIBODIES, MOPC-21 AND MOUSE Ig.

	W108	P220	M42	M41	M29	M2	NMIg	MOPC 21
^{125}I-Anti-Id M29	182	53	0	79	9840	275	37	21
^{125}I-Anti-Id M42	78	159	7868	98	0	0	69	0

Note: Binding of protein A-purified Ab2 to Ig was determined in a ELISA and the $OD_{414\ nm}$ determined on an ELISA plate reader. The specificty of binding of idiotype affinitypurified Ab2 to Ig was determined in a radioimmune bindingassay employing ^{125}I-labeled Ab2 M29 or M42. The results are presented as (specific) counts per minute of labeled antibody bound.

FIGURE 4

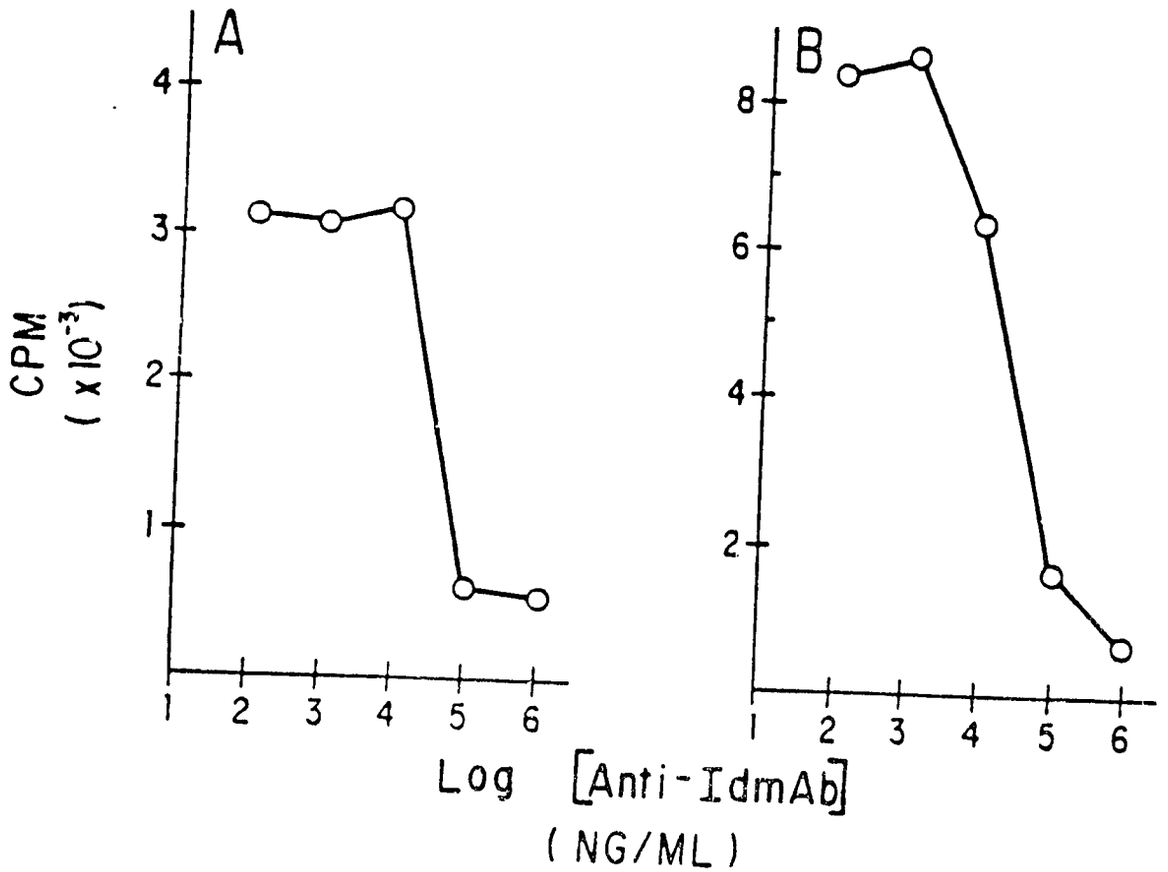


Fig. 4. Inhibition of M29 mAb and M42 mAb binding to sperm by anti-Id M29 and anti-Id M42. Competitive inhibition studies showed that purified rabbit anti-Id mAb contain antibodies directed against the antigen-binding site of the Ab1. Mouse sperm, fixed with 2% formadehyde/PBS, washed and resuspended in 2% normal mouse serum/ 5% rabbit serum/PBS, were combined with ^{125}I-labeled M29 or M42. 10 ul of various concentrations of rabbit anti-Id mAb M29 or anti-Id M42 (1 mg/ml to 1 ug/ml) were added, the total volume adjusted to 200 ul and the complete mixture incubated for 2 hrs. Sperm and bound mAb were separated from free mAb by filtration. The results indicate that Ab2 M29 (Panel A) and Ab2 M42 inhibited binding of their respective mAb to sperm in a dose-dependent manner. Anti-idiotype antibodies reactive with the antigen-combining sites of the anti-sperm monoclonal antibodies M29 and M42 may contain subpopulations which mimic the sperm-antigen epitopes recognized by these antibodies.

FIGURE 5

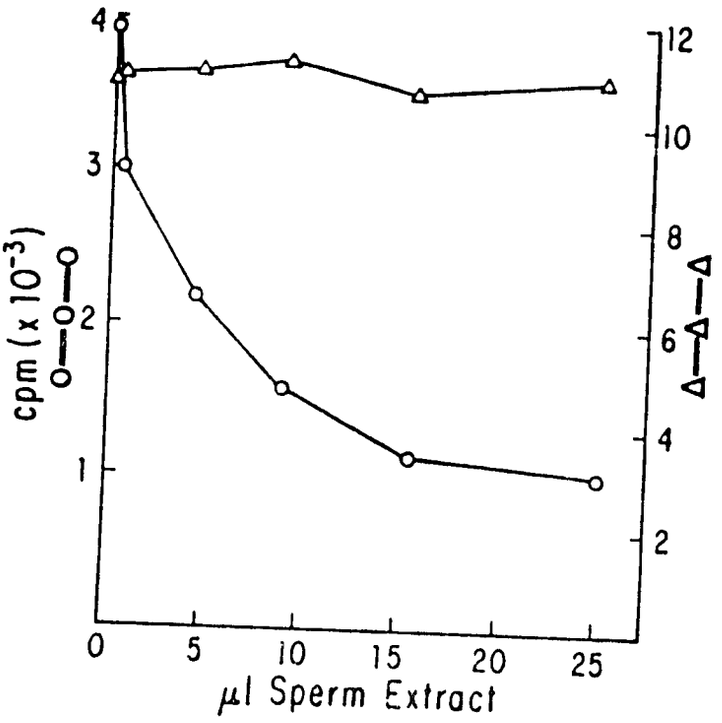


Figure 5. Competition by sperm antigencontainingextract for interaction of Ab2 M42 and M29 with homologous mAb. Mouse sperm (3×10^8) were extracted in 1 ml Extraction Bufffer (see text for details) on ice and centrifuged for 90 min at 30,000 rpm in a Ti55 rotor. The supernatant of the high speed spin contains the M42 antigen but not the M29 antigen (determined by immunoblotting). M29 and M42 mAb (2 ug/ml) were adsorbed to Removawell strips and free protein binding sites subsequently blocked with 1% gelatin. Increasing amounts of sperm extract, adjusted to contain 0.1% gelatin, was added to wells and incubated for 1 hr at 4°C. ^{125}-labeled Ab2 M42 or M29 was added and the incubation continued for an additional 1 hr. Bound Ab2 was determined in a gamma counter. Antigen inhibition of Ab2 binding to Ab1 is an indication of the presence of antigen combining-site specificities in Ab2. Sperm extract inhibited Ab2 M42 binding (O) in a dose dependent fashion but failed to inhibit Ab2 M29 binding to M29 mAb (Δ). The inability to inhibit 100% of Ab2-Ab1 binding suggests that both framework and antigen combining-site specificities were present in the Ab2 M42 population. The Ab2 population with combining site-related specificity represented a maximum of 70-75% of the Ab2. These results suggested that a major population of Ab2 might contain the internal image of the M42 sperm antigen. That anti-Id M29 binding to M29 was not inhibited by the same sperm extract is not surprising since the M29 antigen is not solubilized under these conditions and suggests that inhibition of Ab2 M42 binding to Ab1 is not likely to be a non-specfic e.g., proteolytic/glycolytic, effect of sperm extract on antibody interaction.

FIGURE 6.

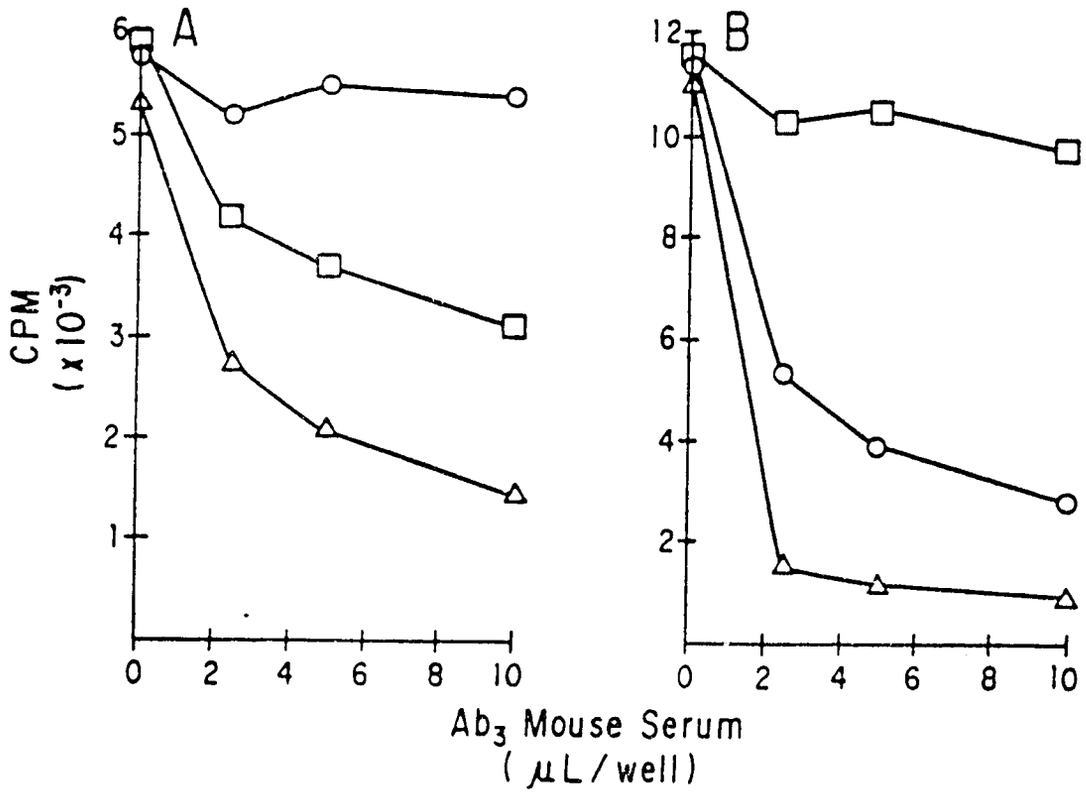


FIG. 6. Induction of Ab3 antibodies (Ab3). Immunization of mice with anti-Id mAb may induce antibodies which react with native sperm antigen. Induction of Ab1-like Ab3 in mice was monitored by in a solid-phase RIA in which inhibition of ^{125}I-labeled mAb binding to immobilized anti-Id mAb by Ab3 was measured. Groups of 8 animals were immunized subcutaneously with Protein A-purified rabbit Ab2 M42, Ab2 M29, a cocktail of Ab2 M42 and Ab2 M29 or rabbit Ig over a period of 90 days. 7 days after the last booster injection, the animals were bled and 1 ml of sera of each group pooled, absorbed with immobilized rabbit Ig and the mouse Ig purified on Protein A. Results are presented in terms of CPM of labeled mAb bound as a function of ul of Ab3 mouse serum equivalents/assay well. **Panel A** shows that sera from mice immunized with Ab2 M29 (□) or Ab2 M29 and Ab2 M42 (Δ) inhibited binding of ^{125}I-labeled M29 to Ab2 M29 in a dose dependent manner. Sera from mice immunized with Ab2 M42 (O) alone had no effect. Similarly, **Panel B** shows that only sera from mice immunized with Ab2 M42 (O) or Ab2 M42 and Ab2 M29 (Δ) inhibited M42 binding to Ab2 M42. Sera from mice immunized with Ab2 M29 (□) alone had no effect.

These results provide preliminary evidence for the induction of Ab1-like Ab3 by immunization with Ab2.

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III. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. Project Output:

Carron, C.P., H.W. Jarvis, L.S. Rippy and P.M. Saling. (1986). Anti-idiotype antibodies: Vaccine for sperm-targetted immunocontraception. J. Reprod. Immuno. Suppl:82A.

Carron, C.P., H.W. Jarvis, and P.M. Saling. Induction of antigen-specific sperm antibodies: Immunization with anti-idiotype monoclonal sperm antibodies. J. Cell Biol. In press.

Carron, C.P., H.W. Jarvis and P.M. Saling. Induction sperm antigen-specific antibodies: Immunization with xenotypic antibodies to the idotype of monoclonal anti-sperm antibodies. In preparation.

The PI attended the symposium entitled "Idiotype Networks and Immune Regulation: Potential Uses in Vaccines and In Understanding Human Diseases" in December, 1985. The subject of this symposium was directly related to the research supported by PARFR-379 on the development of an idiotpe vaccine for sperm-targetted immunocontraception and was a valuable opportunity for interaction and input from a variety of experts in this field.

FINAL REPORT

PRELIMINARY EVALUATION OF 3-M IMPRESSION MATERIAL AS AN INJECTABLE VAS OCCLUSION DEVICE

PARFR 380

Principal Investigator: L.J.D. Zaneveld

Institution: Rush-Presbyterian-St. Luke's Medical Center
Chicago, IL 60612

Funding Period: 8/1/85 - 4/30/86

Amount Funded: $10,832

SUMMARY AND CONCLUSION

3-M Impression Material was injected into the vasa of 4 dogs resulting in the obstruction of sperm transport. Five to six months after implantation, the dogs were euthanized. No gross pathological lesions were seen on autopsy. It can be concluded that 3-M Impression Material can prevent sperm transport through the vas without causing excessive pathology, at least for a 5-6 month period.

OBJECTIVE

The objective of this work was to perform prelminary experiments in dogs to determine if the injection of 3-M Impression Material (vinyl polysiloxane impression material, light body-regular set, type 1, low viscosity; Dental Products, 3M, St. Paul, MN) into the vas deferens results in the obstruction of sperm transport.

PROTOCOL

Four beagle dogs were purchased and ejaculation was attempted by manual manipulation. One of the dogs never ejaculated and was replaced by another (no. 3658). With the exception of this last dog which was obtained later, the dogs were ejaculated 3 times over a 2.5 month period to get them used to the procedure and to obtain baseline semen analyses. Subsequently, the dogs were anesthesized, the scrotal vas was exposed and 3-M Impression Material was injected under sterile condition. Since no needle could be attached to the 3-M injection apparatus, some experimentation was necessary to allow

introduction of the material. One vas from one of the dogs (no. 3658) was used for this purpose. This side was subsequently vasectomized. The final technique consisted of injecting the mixed 3-M material first into a 10 ml syringe. The syringe was subsequently attached to a 22 gauge angiocath needle which had been threaded into the lumen of the vas. The vas was clamped off approximatley 2.5 cm from the injection site. Initially, a metal, bent Allison tissue forceps was used for this purpose but later the vas was clamped manually. The material was injected. Speed was essential because the material began to harden with 1-2 min. after mixing which caused th eneedle to clog up. A luer lock syringe was very helpful so that more pressure could be applied without the syringe being separated from the needle (this occurred due to the thickness of the material). The rapid hardening also made it impossible to measure exactly how much material was injected into the vas. Roughly, enough material was injected to cause the vas to become rigid (probably a 20-30% increase in its normal diameter). Injection was always towards the seminal vesicles.

After injection, the needle was removed. The vas was kept clamped for another 5 min. In two of the dogs, 6-0 ethicon suture was passed through the vas and the hardened material, and tied loosely around the vas. No other sutures were applied to the vas. The scrotal incision was closed with 3-0 Dexon.

After recovery from the surgery, the dogs were ejaculated approximately every two weeks for a 3 month period after which the dogs were euthanized with Euthobarb. The vasa were exposed, observed for gross lesions, excised and fixed in Bouin's solutions. Sections were also taken of the testis and the epididymis. The tissues were sent to Dr. Ralph Richart. The abdomen and

chest cavity were opened and the organs observed for gross lesions.

RESULTS

The data obtained for each dog are summarized in the attached Tables. Before implantation, all the dogs produced semen samples that contained spermatozoa although ejaculation was somewhat erratic, particularly in dog no. 3656. After implantation of the 3-M Impression Material, several of the dogs were hesitant to ejaculate and one (no. 3657) never ejaculated again no matter what was tried. Thus, postimplantation semen analyses are only available on three dogs.

After implantation, either no spermatozoa were found in the ejaculate, or only a few immotile, mostly decapitated sperm. On autopsy, all vasa appeared intact although some changes due to the implantation technique were occasionally noted. The material remained in place whether or not a suture had been passed through the vas and the material. No lesions were seen in the testis and epididymis, or in any of the abdominal and thoracic organs.

DOG No: 3655

Semen Data Before Implantation

Date	Volume (ml)	Conc. ($x10^6$/ml)	Motility (%)	Forward Progression (%)
8/25/85	2.0	88	80	84
9/1/85	2.3	283	87	89
9/8/85	0.6	361	95	98
9/13/85	1.5	399	91	96
9/22/85	0.3	55	52	36
10/13/85	0.7	786	89	95
10/27/85	0.4	141	89	78
11/10/85	1.5	326	87	71

DOG No: 3655 (cont.)

Comments During Surgery

Date: 12/9/85

Left side: Manually clamped vas. Injection went well. Aproximately 2.5 cr of vas was filled with material. Somewhat more material was injected than in right side (about 3 cm in length). Ethicon suture was placed through vas and material.

Right side: Metal vas clamp was used. Needle became clogged. Second injection was needed. Approximately 2.5 cm of vas was filled with material. Ethicon suture was placed through vas and material.

DOG No: 3655 (cont.)

Semen Data After Implantation

Date	Volume (ml)	Conc. ($x10^6$/ml)*	Motility (%)	Progression (%)
1/5/86	Would not ejaculate			
1/19/86	0.8	Few	0	0
2/2/86	0.6	Few	0	0
2/16/86	1.7	Few	0	0
3/2/86	0.5	Few	0	0
3/9/86	1.7	Few	0	0
4/6/86	1.2	Few	0	0

* Few means a total of 1-20 sperm per ejaculate. Most sperm were decapitated.

DOG No: 3655 (cont.)

Comments During Autopsy

Date: 4/17/86

General appearance:

Dog in good health. Shiny coat. Incision completely healed. Testicles normal size.

Left side: Everything looked perfectly normal except some slight swelling in vas at injection site and a few very small adhesions. Ethicon suture in place. 3 cm device. Whitish fluid appeared on incising the epididymis.

Right side: Vas looked normal. Ethicon suture in place. Length of implant 1.5 cm. Epididymis and testis looked normal. On cutting epididymis - whitish fluid came out (probably epididymal fluid and sperm).

Fixed vasa with material in place.

All abdominal organs, heart and lungs appeared grossly normal.

DOG No: 3656

Semen Data Before Implantation

Date	Volume (ml)	Conc. ($x10^6$/ml)	Motility (%)	Forward Progression (%)
8/25/85	0.5	314	78	82
9/1/85	0.5	73	25	2
9/8/85	0.3	0	-	-
9/13/85	0.2	53	32	0
9/22/85	0.5	98	57	0
10/13/85	0.3	155	69	61
10/27/85	0.2	4	42	3
11/10/85	0.6	48	42	0

DOG No: 3656 (cont.)

Comments During Surgery

Date: 11/20/85

Left side: Good implantation. Manually clamped vas. Approximately 2.5 cm of vas was filled with material.

Right side: Needle was pushed out during injection of material. Reinserted needle and completed injection. Approximately 2.5 cm of vas was filled with material. Metal vas clamp was used.

DOG No: 3656 (cont.)

Semen Data After Implantation

Date	Volume (ml)	Conc. ($x10^6$/ml)*	Motility (%)	Forward Progression (%)
12/22/85	0.1	Few	0	0
1/5/86	Trace	0	-	-
1/19/86	Would not ejaculate			
2/2/86	0.4	Few	0	0
2/16/86	Would not ejaculate			
3/2/86	0.2	0	-	-
3/9/86	0.3	Few	0	0
4/6/86	0.6	0	-	-

* Few means a total of 1-20 sperm per ejaculate. Most sperm were decapitated.

DOG No: 3656 (cont.)

Comments During Autopsy

Date: 4/17/86

General Appearance:

Dog in good health. Shiny coat. Incision completely healed. Testicles normal size.

Left side: Everything appeared normal. No ethicon suture. Device 2.5 cm long (remained in vas upon fixation). Some white fluid extruded from the epididymis when cut. Epididymis not swollen.

Right side: Epididymis swollen. At least a portion of the device had migrated. Some corrugated, swollen areas in vas were present where the material had been injected. While working with vas, part of the material fell out (2.2 cm in length). This may be all of the material or only some that passed by the clamp on injection. No ethicon suture. Testis appeared normal.

All abdominal organs, heart and lungs appear grossly normal.

DOG No: 3657

Semen Data Before Implantation

Date	Volume (ml)	Conc. ($x10^6$/ml)	Motility (%)	Forward Progression (%)
8/25/85	1.5	29	0	0
9/1/85	11.0	91	32	2
9/8/85	2.4	314	96	99
9/13/85	6.2	148	52	2
9/22/85	3.1	419	72	90
10/13/85	1.4	658	86	94
10/27/85	2.6	210	88	88
11/10/85	3.2	227	72	81

DOG No: 3657 (cont.)

Comments During Surgery

Date: 12/9/85

Left side: Manually clamped vas. No ethicon suture. Approximately 2.5 cm of vas was filled with material. Everything went well.

Right side: Same as left side.

Complication: Dog almost died due to anesthesia overdose. Veterinary staff thought that the dog had left ventricular hypertrophy.

DOG No: 3657 (cont.)

Semen Data After Implantation

Date	Volume (ml)	Conc. ($x10^6$/ml)	Motility (%)	Forward Progression (%)
1/5/86	Would not ejaculate			
1/19/86	Would not ejaculate			
2/2/86	Would not ejaculate			
2/16/86	Would not ejaculate			
3/2/86	Would not ejaculate			
3/9/86	Would not ejaculate			
4/6/86	Would not ejaculate			

DOG No: 3657 (cont.)

Comments During Autopsy

Date: 4/17/86

General Appearance:

Dog in good health. Shiny coat. Incision completed healed. Testicles normal size.

Left side: Vas somewhat swollen and corrugated over 3 cm area. No ethicon suture. Material inside corrugated area. Small piece of material extruded out of the vas at the injection site and at end of corrugated area. Small diameter material fell out of distal end of vas when cut. Most material remained in the vas so that the material that fell out was probably some that passed beyond the clamped site. Testis and epididymis normal. Some whitish fluid exuded from epididymis when cut.

Right side: No ethicon suture. Vas somewhat enlarged and harder over a 3.5 cm area. Vas otherwise normal. Testis and epididymis normal.

Fixed vasa with devices in place.

All abdominal organs, heart and lungs appeared grossly normal.

DOG No: 3658

Semen Data Before Implantation

Date	Volume (ml)	Conc. ($x10^6$/ml)	Motility (%)	Forward Progression (%)
9/13/85	0.5	12	0	0
9/22/85	trace	N.D.	71	36
10/13/85	0.2	0	-	-
10/27/85	1.4	149	64	52
11/10/85	0.4	137	69	75

DOG No: 3658 (cont.)

Comments During Surgery

Date: 11/20/85

Left side: Left vas was experimental to practice insertion of the material. Various holes were made in vas to decide appropriate size of needle. Insertion of material practiced. Left side was vasectomized.

Right side: Metal clamp applied about 3 cm from entry of needle in vas. 6-0 Ethicon sutured through vas and material. While injecting, the needle was pushed out of the vas. Reinserted needle.

DOG No: 3658 (cont.)

Semen Data After Implantation

Date	Volume (ml)	Conc. ($x10^6$/ml)*	Motility (%)	Forward Progression (%)
12/22/85	Would not ejaculate			
1/5/86	Would not ejaculate			
1/19/86	0.5	Few	0	0
2/2/86	0.5	100 total	2	0
2/16/86	Would not ejaculate			
3/2/86	0.3	0	-	-
3/9/86	0.2	0	-	-
4/6/86	0.8	0	-	-

* Few means a total of 1-20 sperm per ejaculate. Most sperm were decapitated.

DOG No: 3658 (cont.)

Comments During Autopsy

Date: 4/17/86

General Appearance:

Dog in good health. Shiny coat. Incision completely healed. Testicles normal size.

Left side: Vasectomized. Everything looked normal otherwise. Whitish material came out of epididymis when cut. Epididymis, proximal vas and testis looked the same as sides where material had been implanted, i.e., normal. Fixed distal portion of vas.

Right side: Ethicon suture present. 2.3 cm device. Vas, epididymis and testis appeared normal. Vas not swollen and no corrugated areas. On cutting epididymis, some whitish fluid appeared.

Fixed right vas with device in place.

All abdominal organs, heart and lungs appeared grossly normal.

PARFR-382

FINAL REPORT: POSTCOITAL EFFECTIVENESS OF THE VAGINAL SPERMICIDAL BARRIER

Gerald S. Bernstein, Ph.D.,M.D.
Principal Investigator

November 12, 1986

The Vaginal Spermicidal Barrier (VSB) is a medicated, solid mechanical barrier designed to inhibit sperm transport by both a mechanical and spermicidal effect. It is an elliptically-shaped device formulated from ethylene vinyl acetate copolymer and coated with nonoxynal-9 (N-9) in polyethylene glycol. The objective of this study was to determine whether the VSB interfered with sperm transport in the human as determined by post-coital testing(PCT).

1. Subjects: Volunteers were recruited according to the following criteria:

   - Age 18-45
   - Regular menses
   - Post-tubal ligation or using a plastic or copper-bearing IUD
   - Normal baseline PCT
   - Partner has normal semen analysis

2. Procedures

   PCT's were done according to the method of Davajan and Kunitake (Fertil Steril 20:197, 1969) approximately 16 days prior to the next expected menses (i.e. on the 12th or 13th day of an expected 28 day cycle) and approximately two hours after intercourse.

   The test is conducted as follows: a speculum is inserted into the vagina and material is aspirated from the vaginal pool to determine whether spermatozoa are present and whether they are motile. The exocervix is cleansed with a dry cotton ball and a catheter made from intravenous connecting tubing is placed at the external cervical os and advanced into the endocervical canal while gentle suction is applied with a 12ml syringe. The tubing is held with a long Allisclamp that partially occludes the lumen. When the catheter reaches the level of the internal cervical os, the clamp is closed and the tubing is slowly withdrawn from the endocervical canal. As the tip of the catheter cleans the external cervical os the trailing cervical mucus is cut with a long Metzenbaum scissors to prevent the mucus from being withdrawn from the catheter.

The clinician records the amount of mucus obtained and its appearance. Three 0.5 cm sections of the tubing are cut off, one from the catheter tip, one from the "syringe end" and one from the midportion of the mucus column. The mucus is expressed from each segment onto a slide, the spinnbarkeit of the mucus is determined, and a cover slip (22 x 22mm) is placed over the blob of mucus. Five fields in each of the three samples are examined in detail at 400x magnification. The fields are located at approximately the middle of the drop and at 3, 6, 9 and 12 o'clock around the periphery about three mm from the edge. The fields are selected by placing the selected portion of the mucus drop in the light path before examining the areas to be evaluated. The number of sperm in each field is determined and sperm motility is noted. Motility is evaluated according to the scheme shown in Table 1. The total number of sperm in each category is determined and divided by 15 to obtain the average number of sperm per HPF in each category of motility. The test is considered to be normal if there is a mean of at least 5 sperm with progressive motility (grade 3+ or 2b) per HPF.

The test was performed in a similar manner when the VSB was used, but other, ancillary procedures were also performed. These are described below.

3. Baseline PCT's

We obtained adequate baseline PCT's on ten subjects. Eight of these subjects also had satisfactory PCT's with the VSB in place (i.e. sperm were present in the vagina and the mucus was of good quality). A number of other baseline and VSB tests were done but were unsatisfactory.

Although we usually perform the PCT approximately two hours after intercourse it was not feasible to adhere to this schedule in all cases. Conditions varied according to the male partners' work schedule, traffic conditions, etc. Table 2 summarizes the intervals between intercourse and the PCT's for the baseline studies. The results of the baseline studies are shown in Table 3.

4. PCT's After Intercourse with VSB in Place

a. Procedures - the following protocol was followed when PCT's were done after intercourse with a VSB in place.

- a careful digital examination was done to determine the position of the VSB.

- a short-bladed speculum was inserted far enough into the vagina to allow aspiration of the vaginal pool, if one was present, in front of the device without disturbing the position of the device. The speculum was then removed.

- The VSB was removed and washings were taken from both sides of the device.

- the speculum was reinserted and the PCT was performed as described for the baseline PCT.

b. Results - Subjects were permitted to insert the VSB at their convenience prior to intercourse. In most cases, this was one hour or less (Table 2). The longest time interval was slightly over 22 hours. This subject (008) was unable to insert the VSB properly. The device tended to rotate to the side as it was inserted so that the cervical os was not covered when the insertion was completed. Despite a long time spent in instructing this participant, she was unable to master the technique. To resolve this problem, we inserted the VSB ourselves the day prior to the test.

Although the tests were not done in every case at two hours following intercourse for each subject, the time interval between coitus and testing was similar for the baseline and VSB studies.

In each case, the VSB was covering the cervical os at the time of the PCT's. No motile sperm were found in the vagina when the VSB was in the vagina. When there was insufficient material in the vagina for observation, sperm were found in washings of the front side of the VSB. In addition to intact, non motile sperm, the vagina and/or VSB contained many spermatozoa whose heads had become detached from their tails.

Table 4 shows the results of testing with a VSB in place. In five instances, there were no sperm seen in the mucus. One subject (P008) had a rare sperm with non-progressive motion, while

two women (P001, P003) had sperm with progressive motility. Both tests were abnormal, however. Comparison with the baseline PCT's shows a marked inhibition of sperm transport by the VSB.

Subject P001 is of interest. She has participated in a number of previous sperm transport studies with other vaginal contraceptives. She had a normal PC in the presence of vehicle barrier such as aerosol foam, foaming suppositories, and C-film. The VSB had a marked inhibitory effect, however, as did a cervical cap in another study. In this case, these solid mechanical barriers showed an advantage over the vehicle barriers we tested previously.

5. Discussion

The data demonstrate that the VSB had a marked inhibitory effect on sperm transport in the subjects studied. Every subject except one, was able to insert the device properly after a brief instruction session, and the device was in place over the cervix at the time of the PCT. These women who had used a diaphragm previously had less difficulty in learning the proper technique for inserting the VSB.

The results of this study suggest that further studies of the VSB are warranted in order to determine tolerance to clinical use and its efficacy in preventing pregnancy.

TABLE 1: CATEGORIES OF MOTILITY

3+	Rapid, progressive motility
2b	Sluggish, progressive motility
2a	Rapid tail movements without forward movement of sperm
1+	Sluggish tail movements without forward movement of sperm
0	No motility

TABLE 2. TIME INTERVALS BETWEEN INTERCOURSE (IC) AND PCT AND BETWEEN INSERTION OF DEVICE AND INTERCOURSE

SUBJECT	BASELINE IC TO PCT		VSB INSERTION TO IC		IC TO PCT	
	HRS	MIN	HRS	MIN	HRS	MIN
001	5	25	1	-	4	50
002	4	35	7	15	4	10
003	2	35	-	20	2	10
005	1	-	-	10	2	20
006	1	-	-	25	1	25
007	3	15	-	30	4	-
008	2	-	22	10	1	20
010	3	-	5	45	1	45

TABLE 3

POST COITAL TESTING: BASELINE STUDIES

	MEAN NUMBER OF SPERM PER HPF		
	QUALITY OF MOTILITY		
SUBJECT	PROGRESSIVE (3,2b)	NON-PROGRESSIVE (2a, 1)	NONMOTILE (0)
P001	49.6	24.2	14.2
P002	24.0	4.2	1.2
P003	101.2	31.2	46.0
P005	5.2	0.7	0.2
P006	75.0	25.0	17.0
P007	7.8	6.7	6.2
P008	15.7	2.0	2.5
P010	34.3	8.7	18.7

TABLE 4

POST COITAL TESTING WITH VSB

	MEAN NUMBER OF SPERM PER HPF		
	QUALITY OF MOTILITY		
SUBJECT	PROGRESSIVE (3,2b)	NON-PROGRESSIVE (2a, 1)	NONMOTILE (0)
P001	0.2	0.2	0.7
P002	0	0	0
P003	1.7	1.7	0.4
P005	0	0	0
P006	0	0	0
P007	0	0	0
P008	0	0.1	2.9
P010	0	0	0

PARFR-384

FINAL TECHNICAL REPORT

TO

Program for Applied Research on Fertility Regulation

Northwestern University

Project:	Evaluation of Effects of Immunization of Female Baboons with Zona Pellucida Antigens on Ovarian Function
Principal Investigator:	Vernon C. Stevens Professor Department of Obstetrics & Gynecology The Ohio State University 410 W. 10th Avenue Columbus, Ohio 43210
Period Covered by Report:	July 1, 1984 - May 31, 1986

OBJECTIVES

The specific objectives of this project are:

1. To establish control menstrual cycle data on 10 female baboons (cycle length, sex skin changes, ovulatory status, etc.) during three consecutive cycles. During one of these cycles, frequent serum samples will be collected and stored.
2. To perform serum estradiol and progesterone assays on samples collected during a control cycle on each animal. To perform, if directed by Project PI, assays for FSH and LH serum concentrations.
3. To immunize five animals with the zona pellucida antigen ZP-1 and five with ZP-3 and to collect serum samples for antibody titration.
4. To monitor events of menstrual cycles following immunization and, when the existence of significant antibody levels is confirmed, collect frequent serum samples for hormonal analysis.
5. To continue monitoring menstrual cycles of the animals for alterations of cycle events (including ovulation).
6. After nine menstrual cycles from the last booster immunization, to surgically remove ovaries from all animals and to fix tissues and/or preserve as directed by the PI.

RESULTS

Studies of ZP-1 Immunized Animals

Study Design

Five adult female baboons exhibiting ovulatory menstrual cycles were selected for study. Three control menstrual cycles were monitored for cycle length, sex skin changes and ovulation evidence by progesterone measurements. During the second of these three cycles, frequent serum samples were collected for estradiol and progesterone level assessment. During menstrual cycle days 1-5 of the fourth cycle, females were immunized with 0.5 mg of ZP-1, together with 0.25 mg of a synthetic adjuvant compound (CGP-11637) by emulsifying these materials with squalene and injecting the emulsion intramuscularly at two sites in a total volume of 1.0 ml. Two additional immunizations were given at 28 day intervals and a fourth injection was given at approximately six months after the initial immunization. Serum samples collected at frequent intervals were shipped to the project PI for antibody level determinations. Frequent serum samples for estradiol and progesterone assessment were collected during the third cycle from the first immunization. Menstrual cycle parameters were monitored for nine cycles (or time equivalents in those with disrupted cycles) after "high" antibody levels were detected. At the end of this period (August 1985), ovaries from all the females were surgically removed, preserved according to instructions from the PI and shipped to her laboratory for histological evaluation.

Effects of Immunization on Menstrual Cycle Parameters

Data regarding cycle lengths and ovulations in control and post-immunization cycles are shown in Tables 1-5. Two of the five females exhibited few effects of treatment on cycle length or ovulation, however, the remaining three had at least

TABLE 1

BABOON: Donna NUMBER: 082412

STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	31	16	15	yes	
2	34	19	15	yes	
3	30	15	15	yes	
Immunized					
1	32	18	14	yes	
2	34	17	17	yes	
3	32	16	16	yes	
4	29	13	16	yes	short follicular
5	37	19	18	yes	? long luteal pha...
6	35	16	19	yes	? long luteal phase
7	32	15	17	yes	
8	33	16	17	yes	
9	> 92	51	> 41	no	

TABLE 2

BABOON: Xandra NUMBER: 082416

STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	36	21	15	yes	
2	35	20	15	yes	
3	32	17	15	yes	
Immunized					
1	33	20	13	yes	
2	38	23	15	yes	
3	40	22	18	yes	long luteal phase
4	42	23	19	no	long luteal phase
5	47	32	15	no	long follicular phase
6	>149	15		no	ovaries out day 149

TABLE 3

BABOON: Zerna NUMBER: 082419

STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	30	18	12	yes	
2	31	16	15	yes	
3	31	17	14	yes	
Immunized					
1	33	18	15	yes	
2	32	17	15	yes	
3	33	16	17	yes	
4	30	14	16	yes	
5	33	15	18	yes	
6	28	13	15	yes	
7	30	14	16	yes	
8	34	14	20	yes	last booster
9	63	48	15	yes	long follicular phase
10	28	14	14	yes	

TABLE 4

BABOON: Ariel NUMBER: 051256

STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	38	20	18	yes	
2	37	21	16	yes	
3	35	17	18	yes	
Immunized					
1	35	20	15	yes	
2	39	21	18	yes	
3	30	16	14	yes	short follicular/lutea phases
4	31	14	17	yes	short follicular phase
5	35	19	16	yes	
6	32	14	18	yes	short follicular phase
7	38	19	19	yes	last booster injection
8	36	19	17	yes	
9	37	18	19	yes	
10	31	15	16	yes	short follicular phase

TABLE 5

BABOON: Winnie NUMBER: 123048

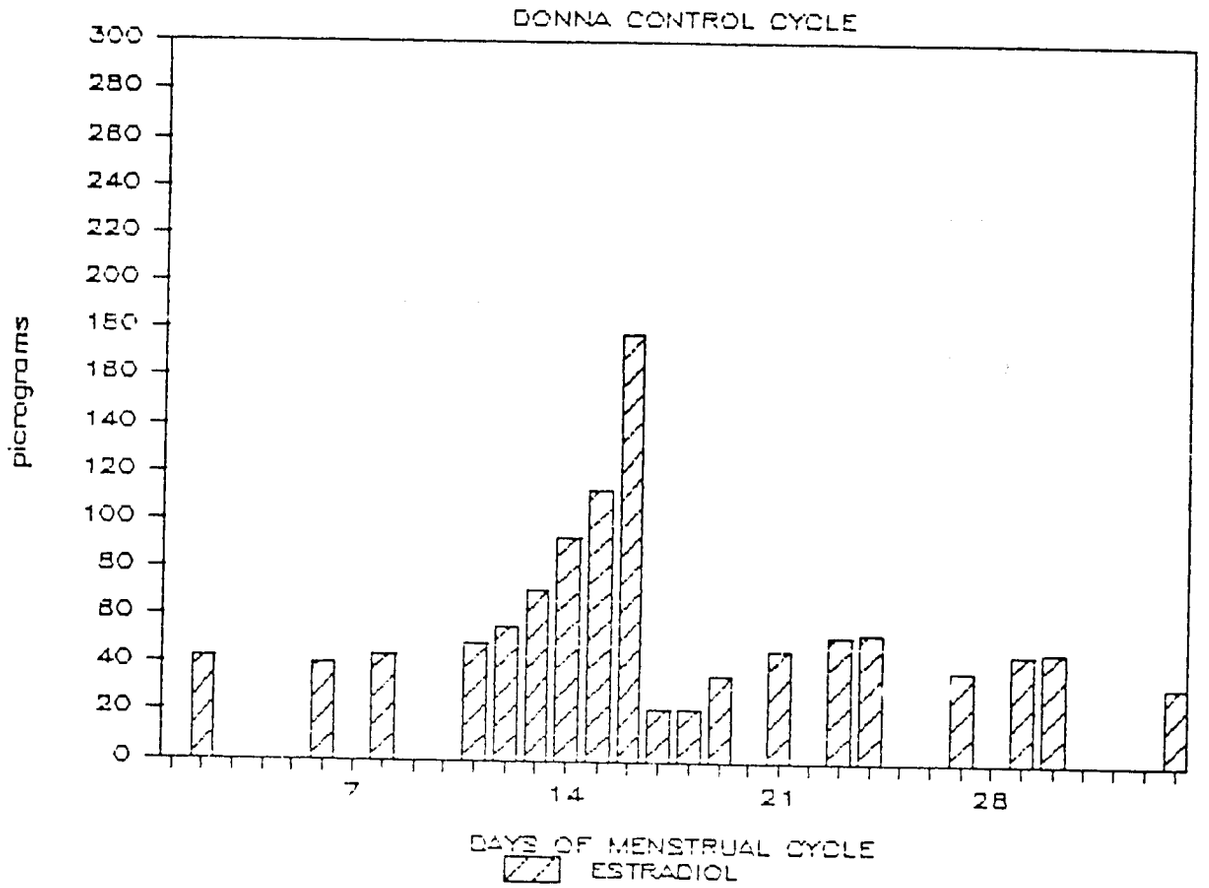
STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	28	15	13	yes	
2	34	20	14	yes	
3	30	16	14	yes	
Immunized					
1	31	18	13	yes	
2	29	18	11	yes	? short luteal phase
3	30	17	13	yes	
4	31	17	14	yes	
5	28	15	13	yes	
6	29	17	12	yes	
7	28	15	13	yes	
8	31	17	14	yes	
9	30	18	12	yes	
10	29	17	12	yes	
11	29	16	13	yes	
12	> 31	17		yes	ovaries out day 31

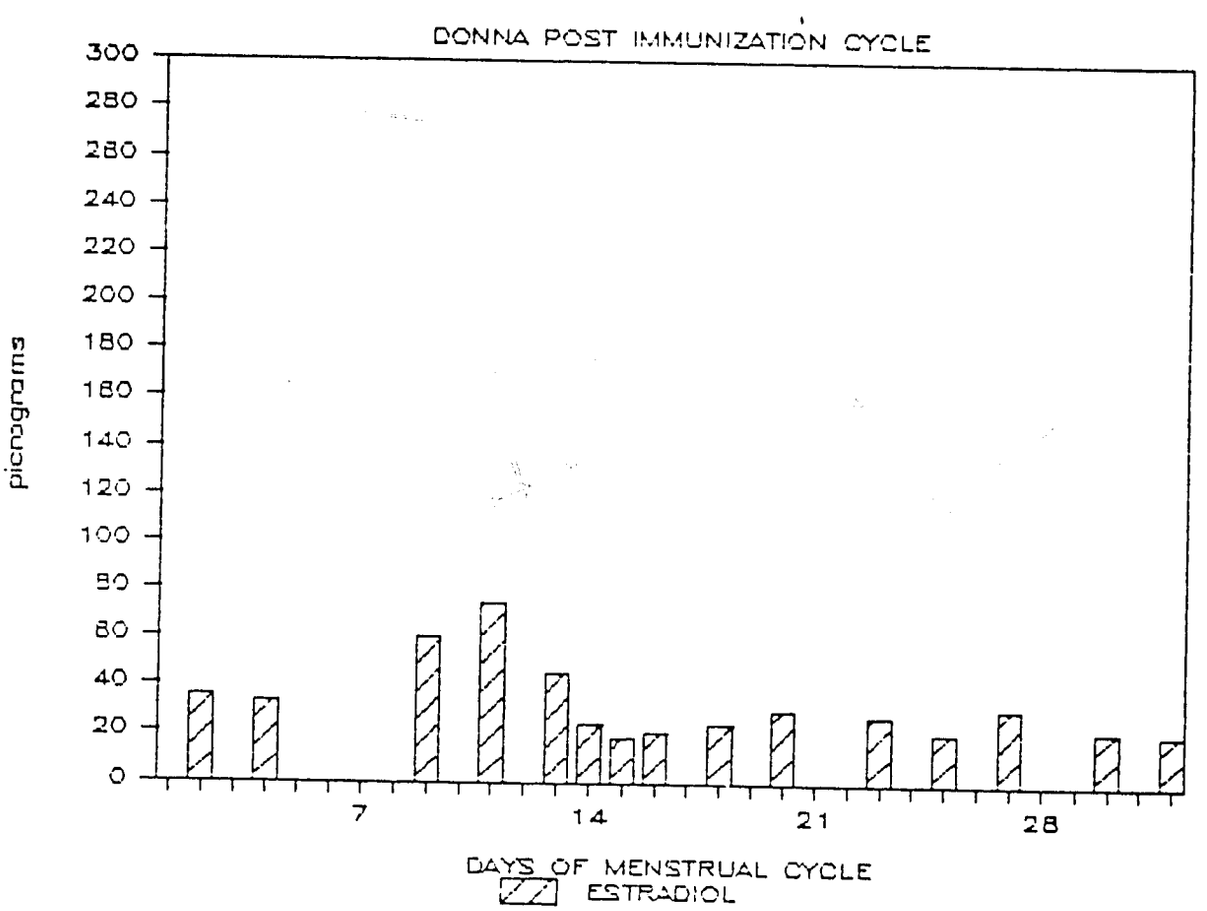
one cycle of extended duration and/or anovulation. Two of these three became amenorrhic at the end of the study period with no evidence of ovulation.

Effects of Immunization on Hormone Profiles

Data showing levels of serum estradiol and progesterone levels in a control and a post-immunization cycle for each female are presented in Figures 1-10. These data clearly demonstrate reduced estradiol levels in all animals following immunization while progesterone levels were little effected by this procedure. Progesterone levels were reduced in only one (Xandra) although the post-immunization cycle levels were compatible with normal luteal function. (This female became anovulatory in later cycles.)

FIGURE 1


DONNA CONTROL CYCLE
picograms
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL


DONNA POST IMMUNIZATION CYCLE
picograms
300
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280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
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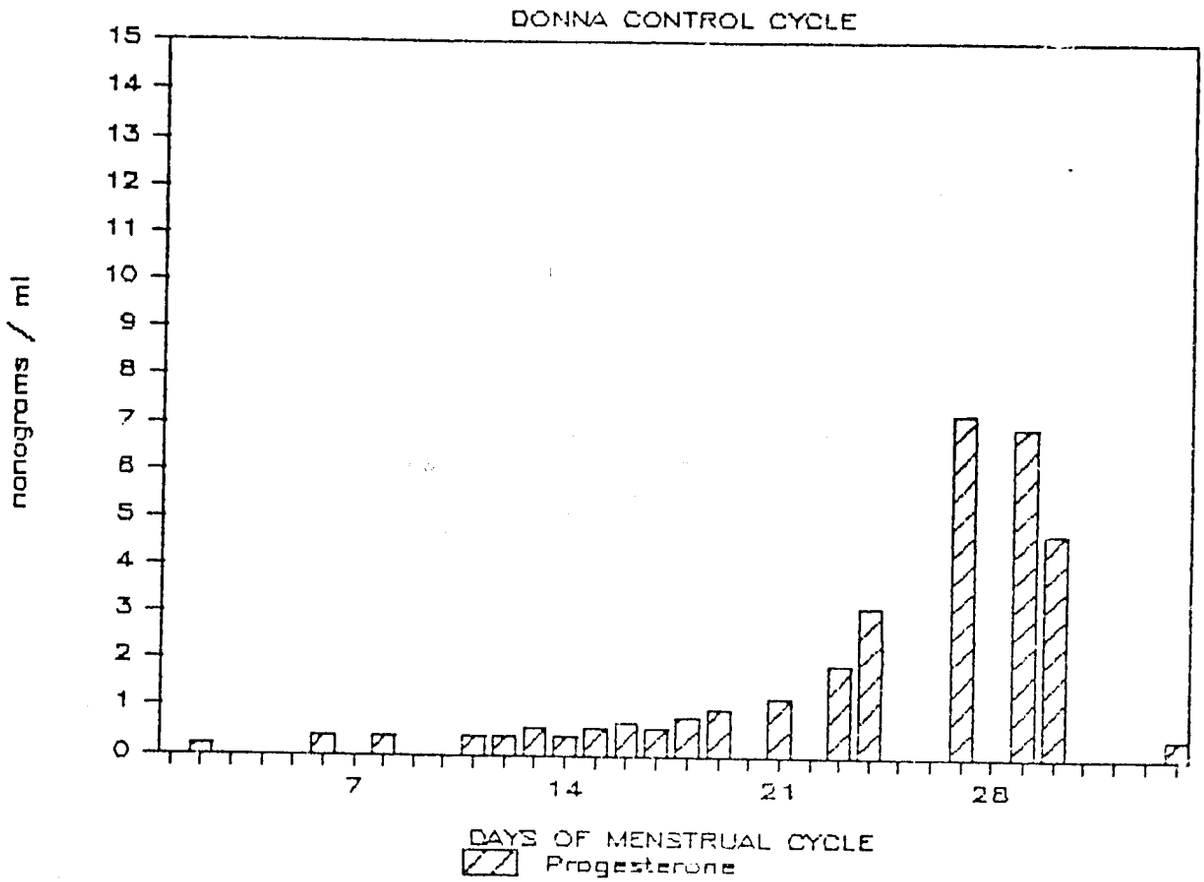
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 2


DONNA CONTROL CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
Progesterone

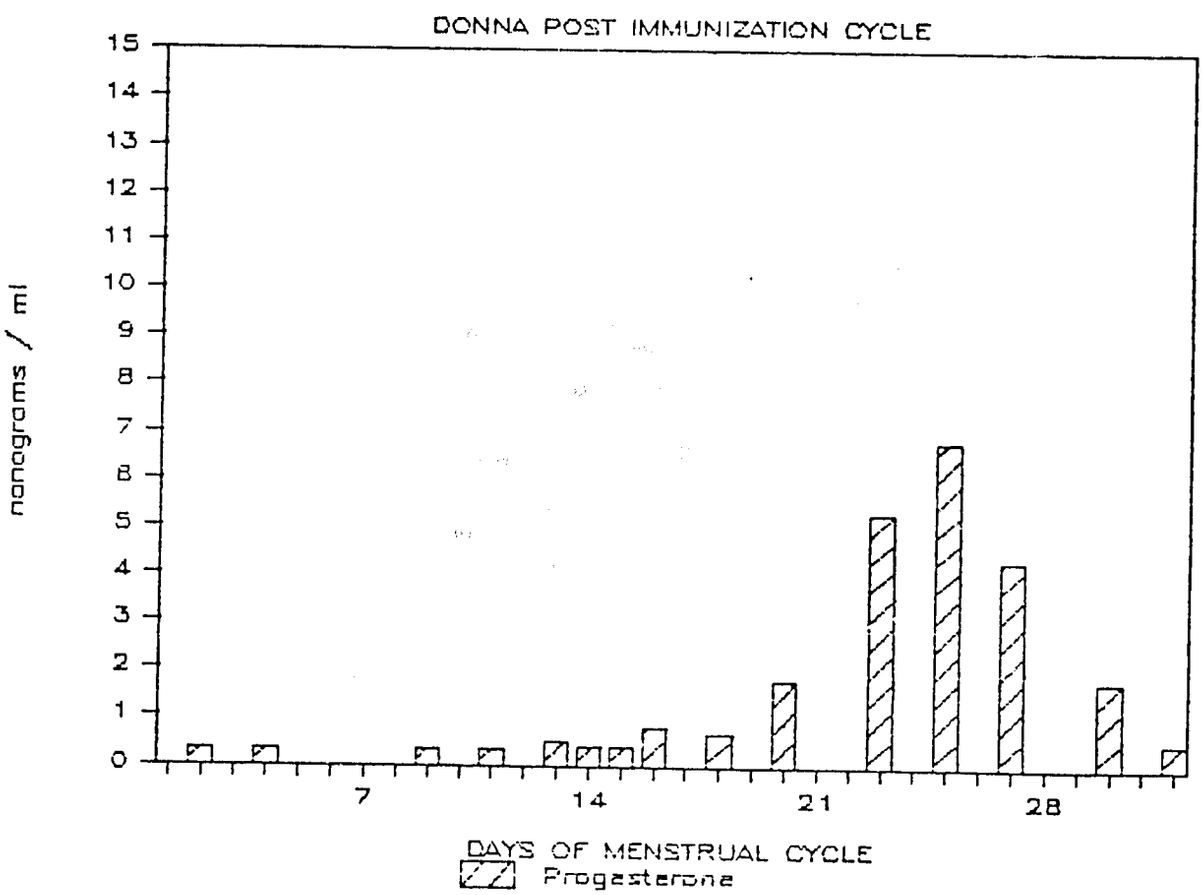
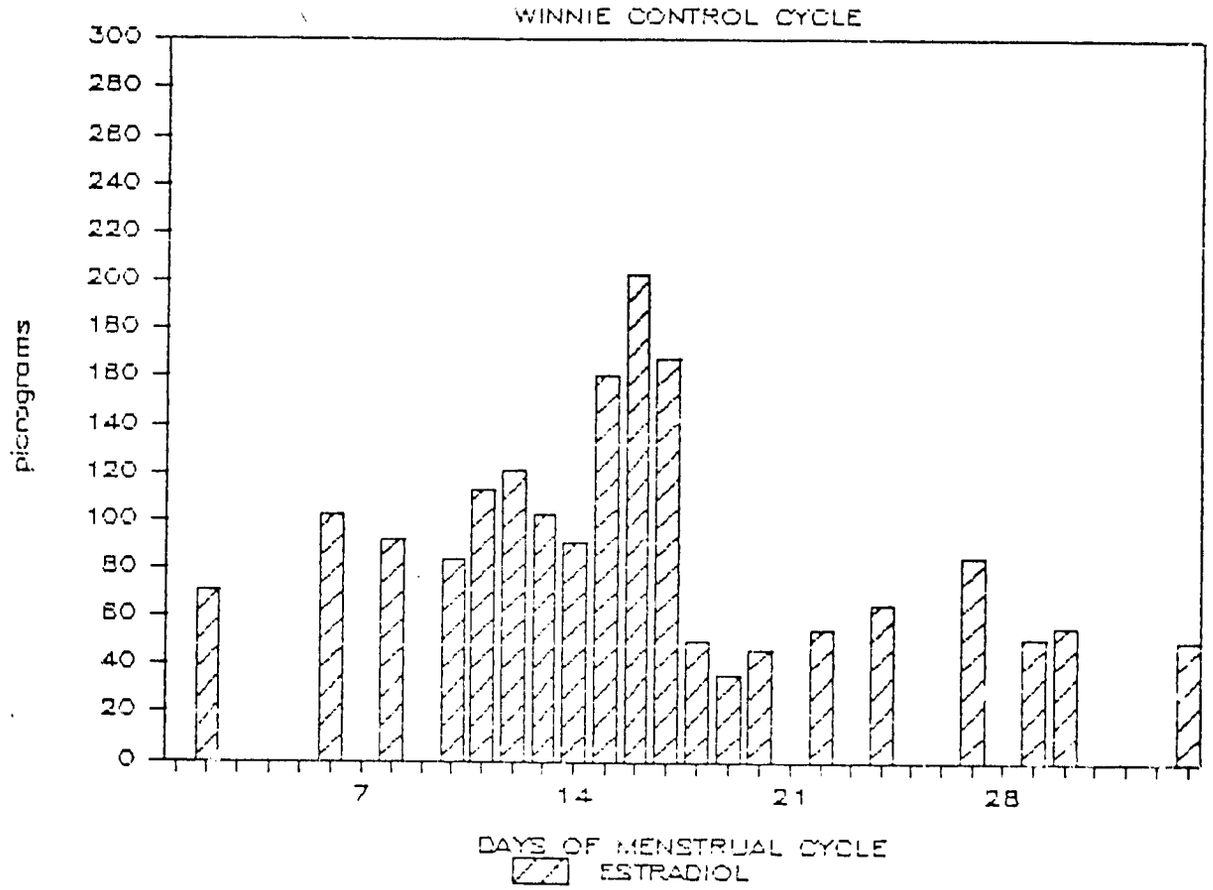

DONNA POST IMMUNIZATION CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
Progesterone

FIGURE 3


WINNIE CONTROL CYCLE
picograms
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280
240
220
200
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180
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120
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80
60
40
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0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

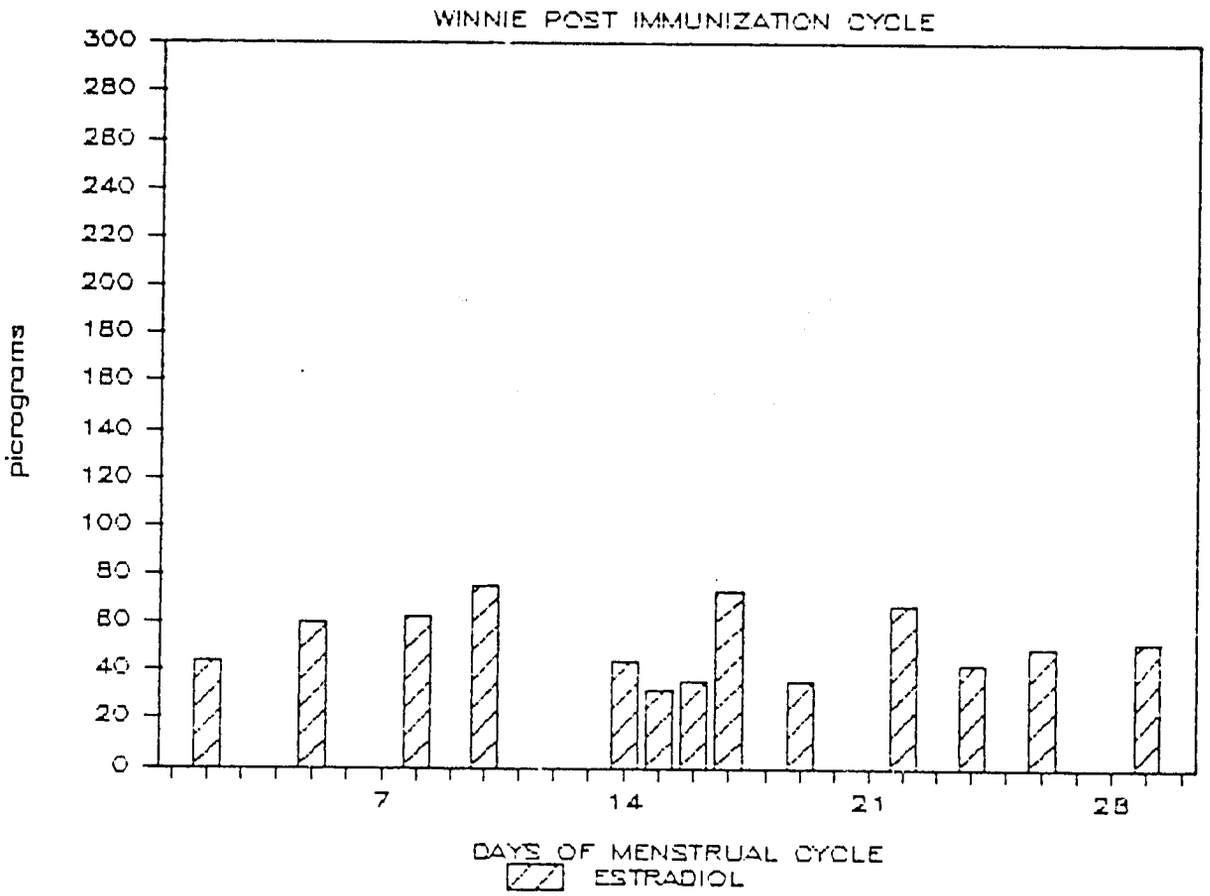
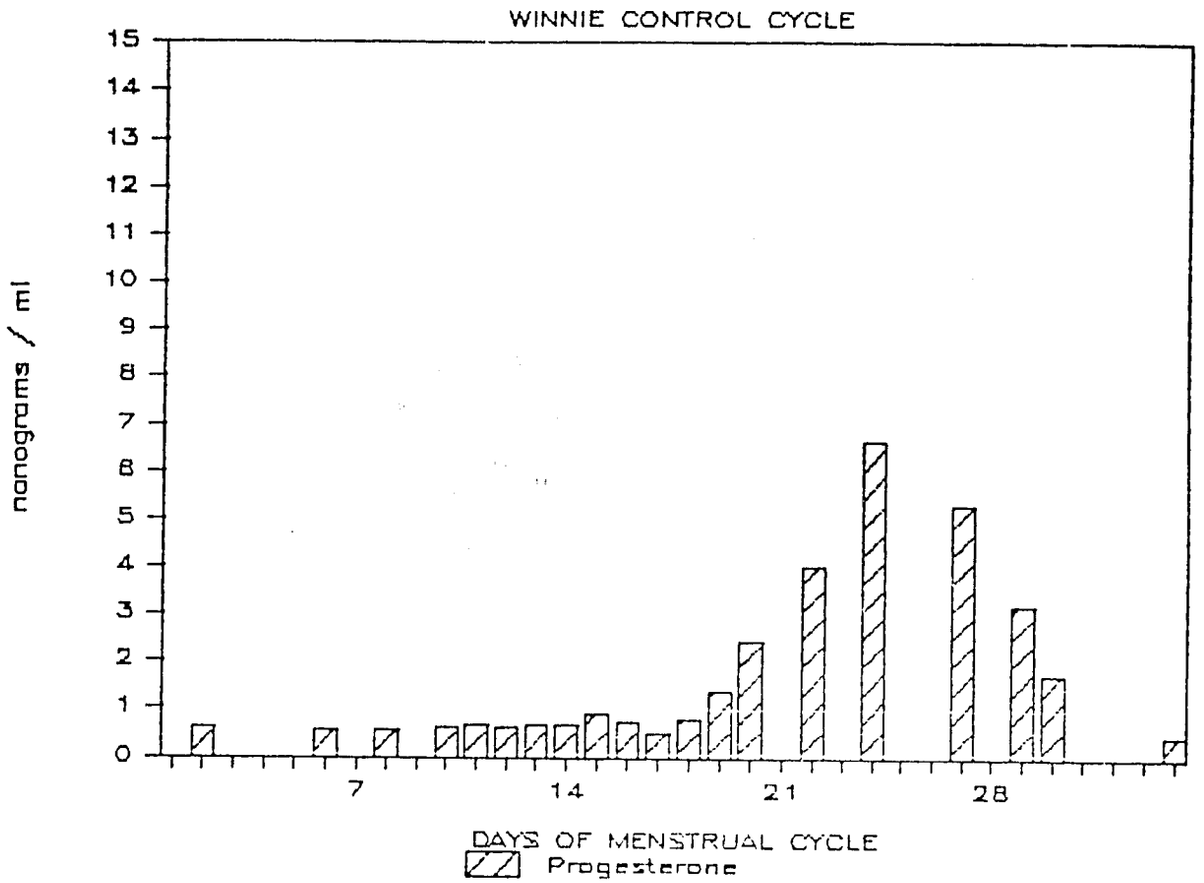

WINNIE POST IMMUNIZATION CYCLE
picograms
300
280
280
240
220
200
180
180
140
120
100
80
60
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 4


WINNIE CONTROL CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
Progesterone

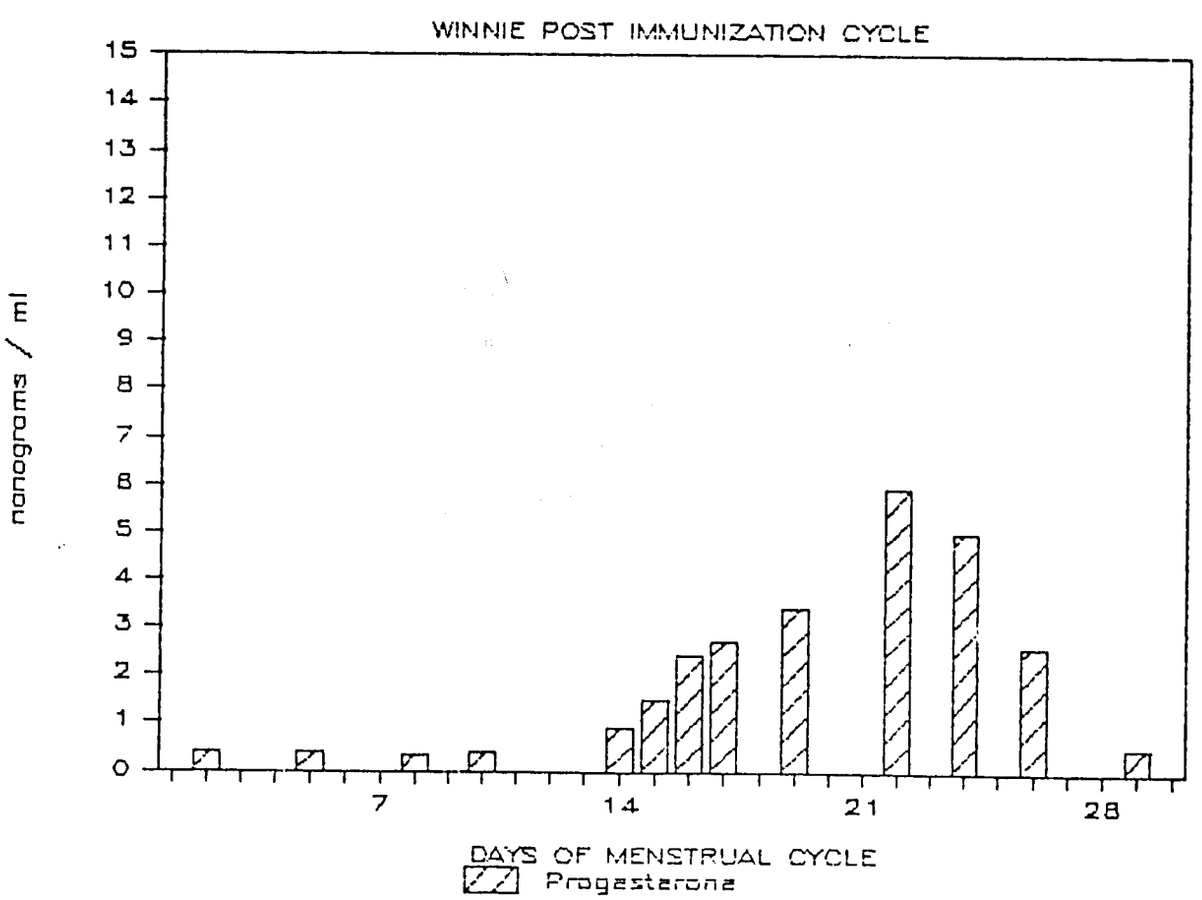
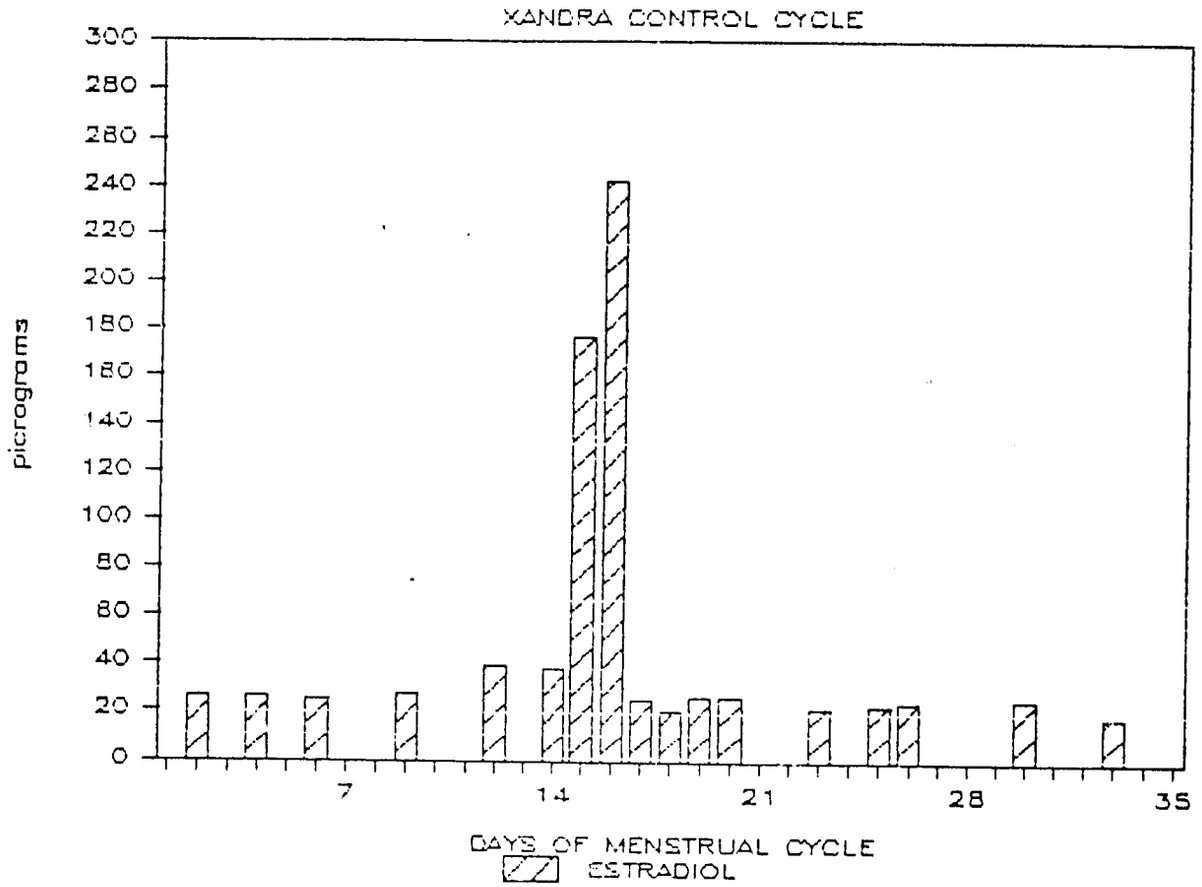

WINNIE POST IMMUNIZATION CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
Progesterone

FIGURE 5


XANDRA CONTROL CYCLE
picrograms
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
35
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

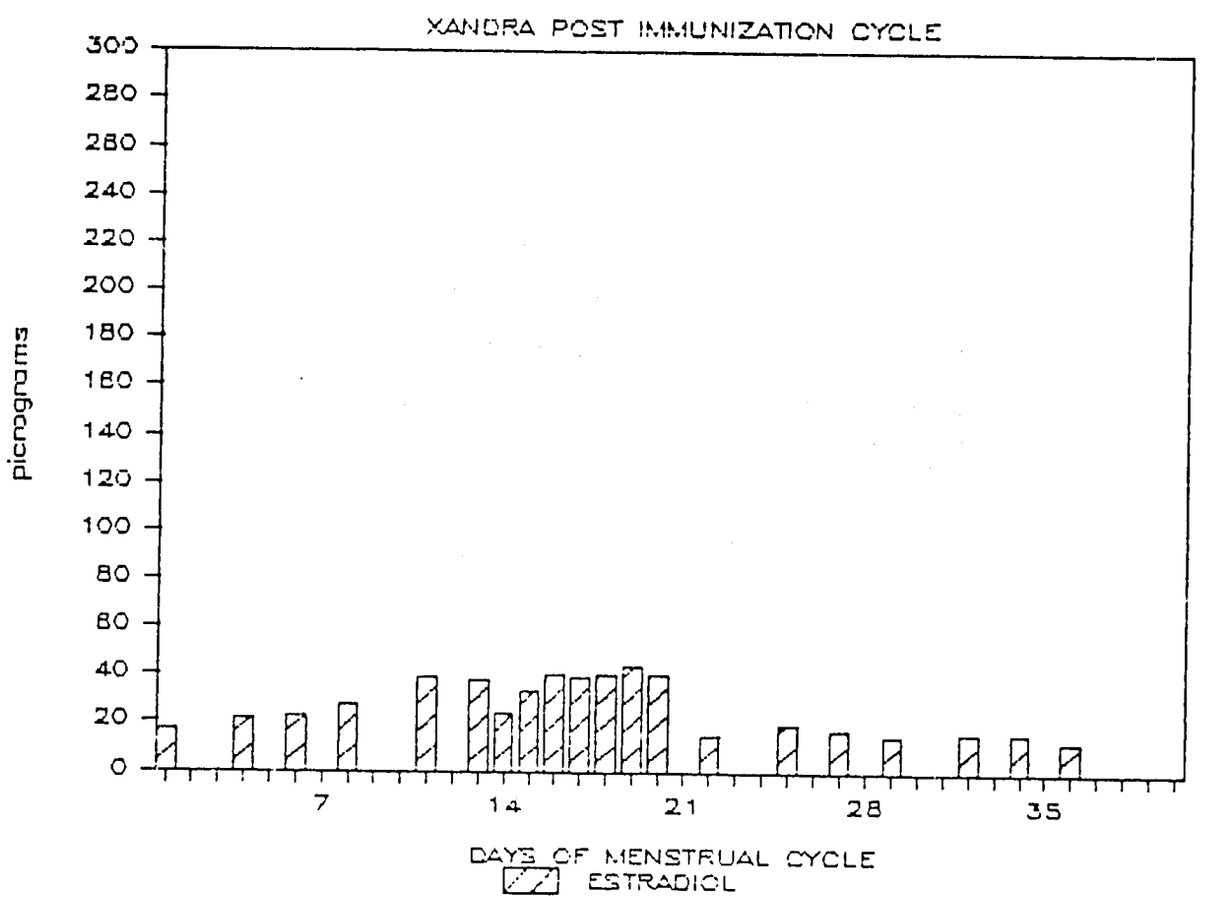
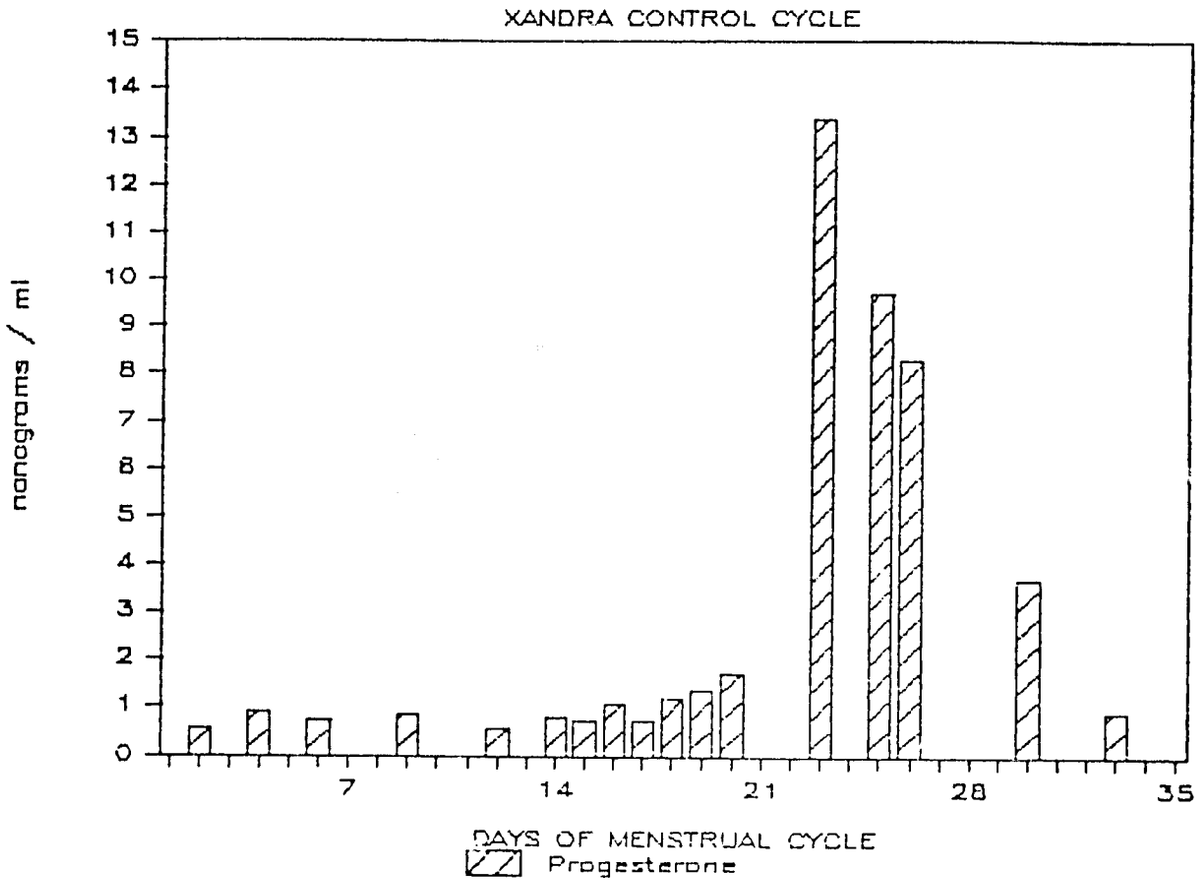

XANDRA POST IMMUNIZATION CYCLE
picrograms
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
35
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 6


XANDRA CONTROL CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
8
5
4
3
2
1
0
7
14
21
28
35
DAYS OF MENSTRUAL CYCLE
Progesterone

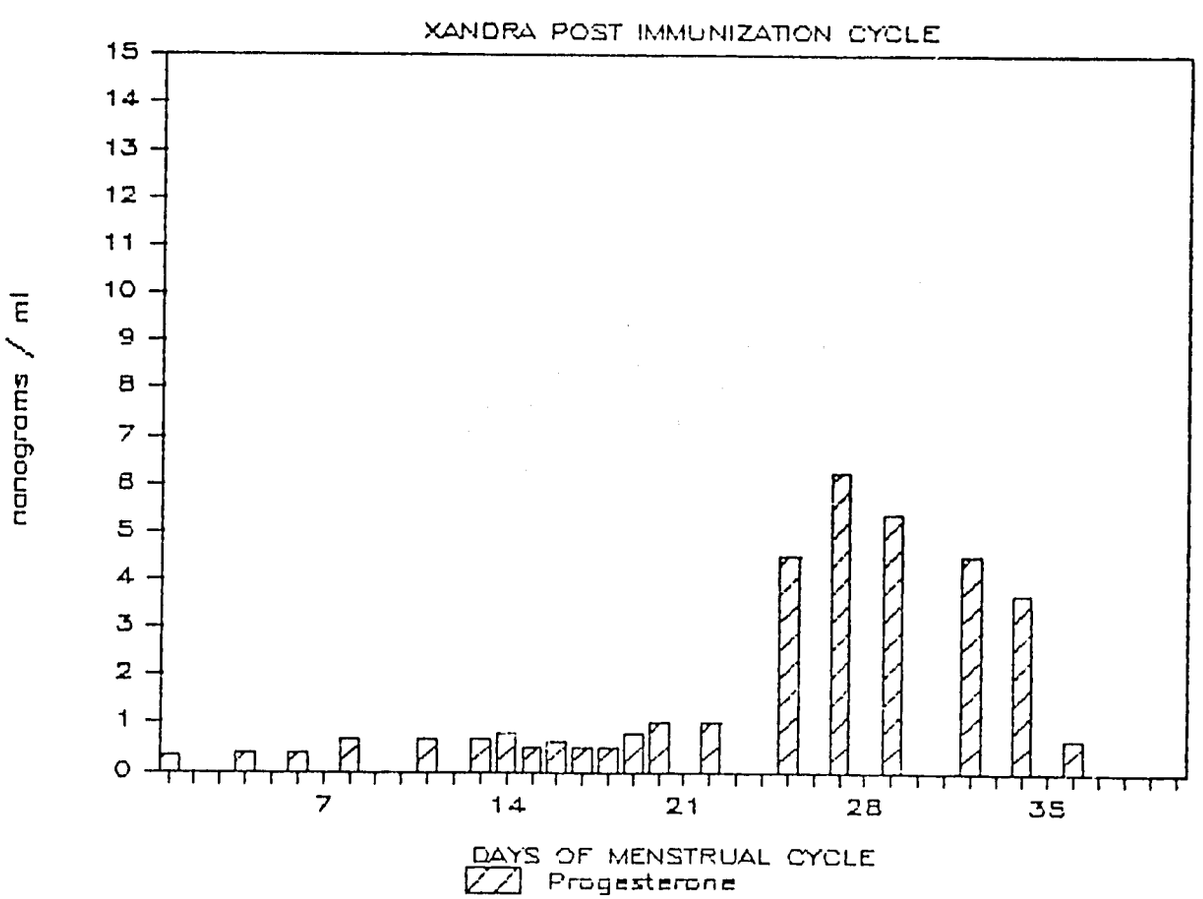
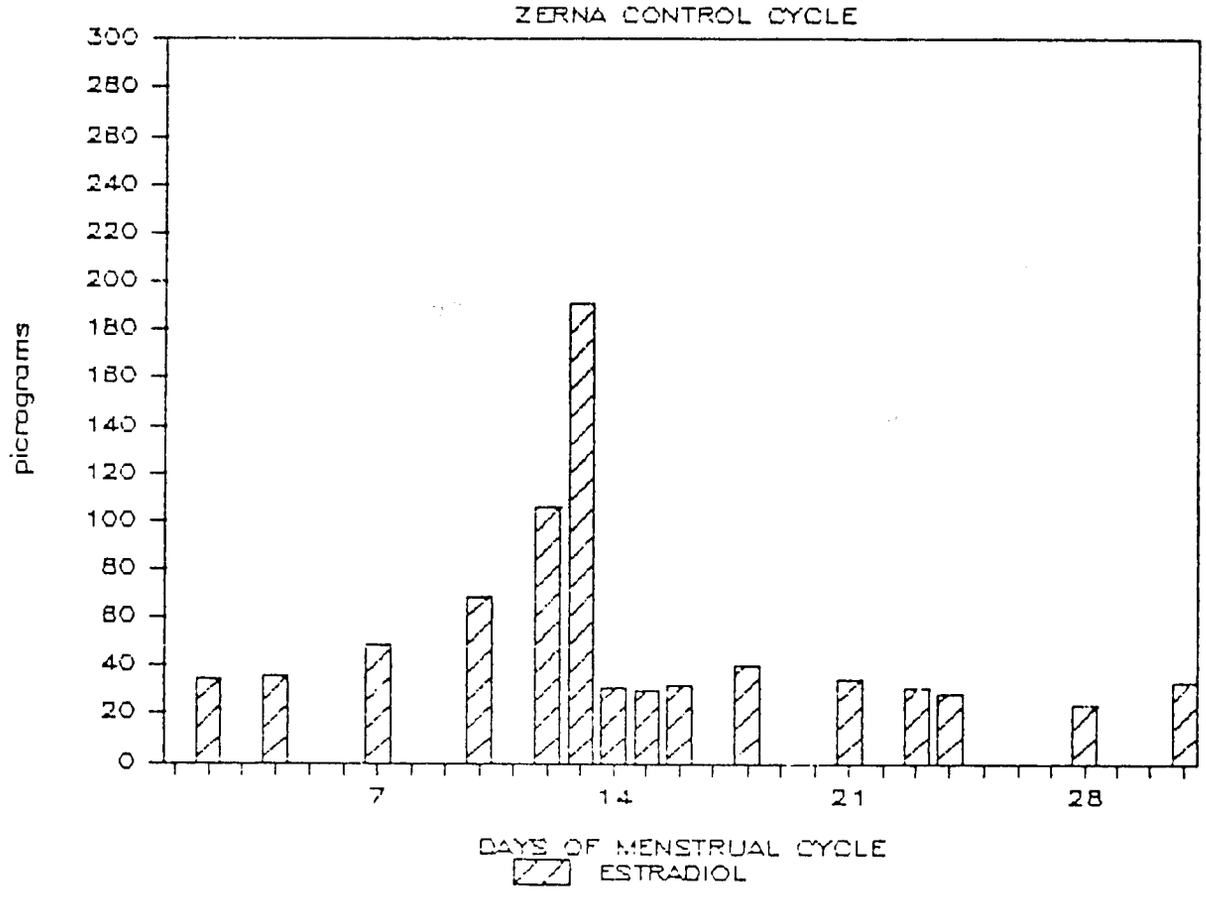

XANDRA POST IMMUNIZATION CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
8
5
4
3
2
1
0
7
14
21
28
35
DAYS OF MENSTRUAL CYCLE
Progesterone

FIGURE 7


ZERNA CONTROL CYCLE
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
picrograms
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

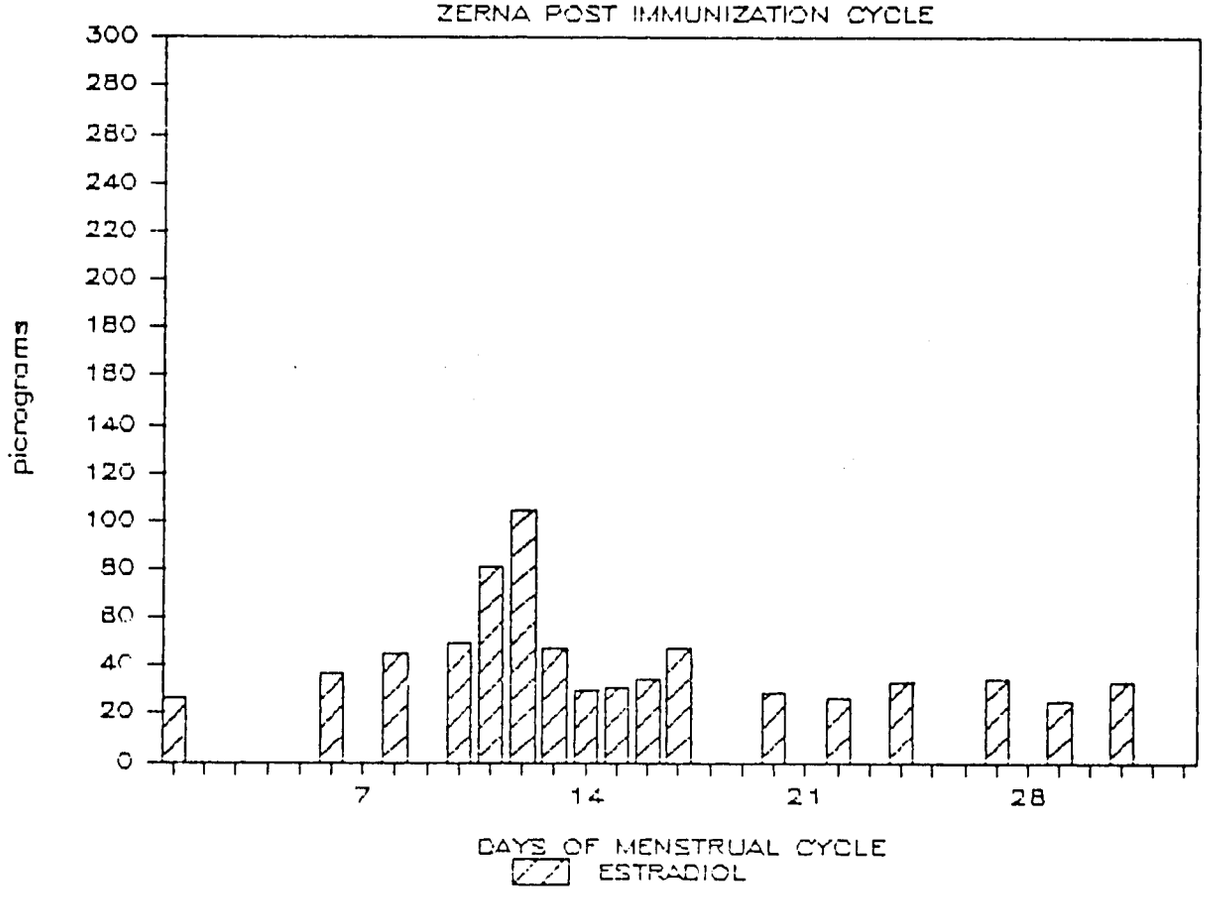
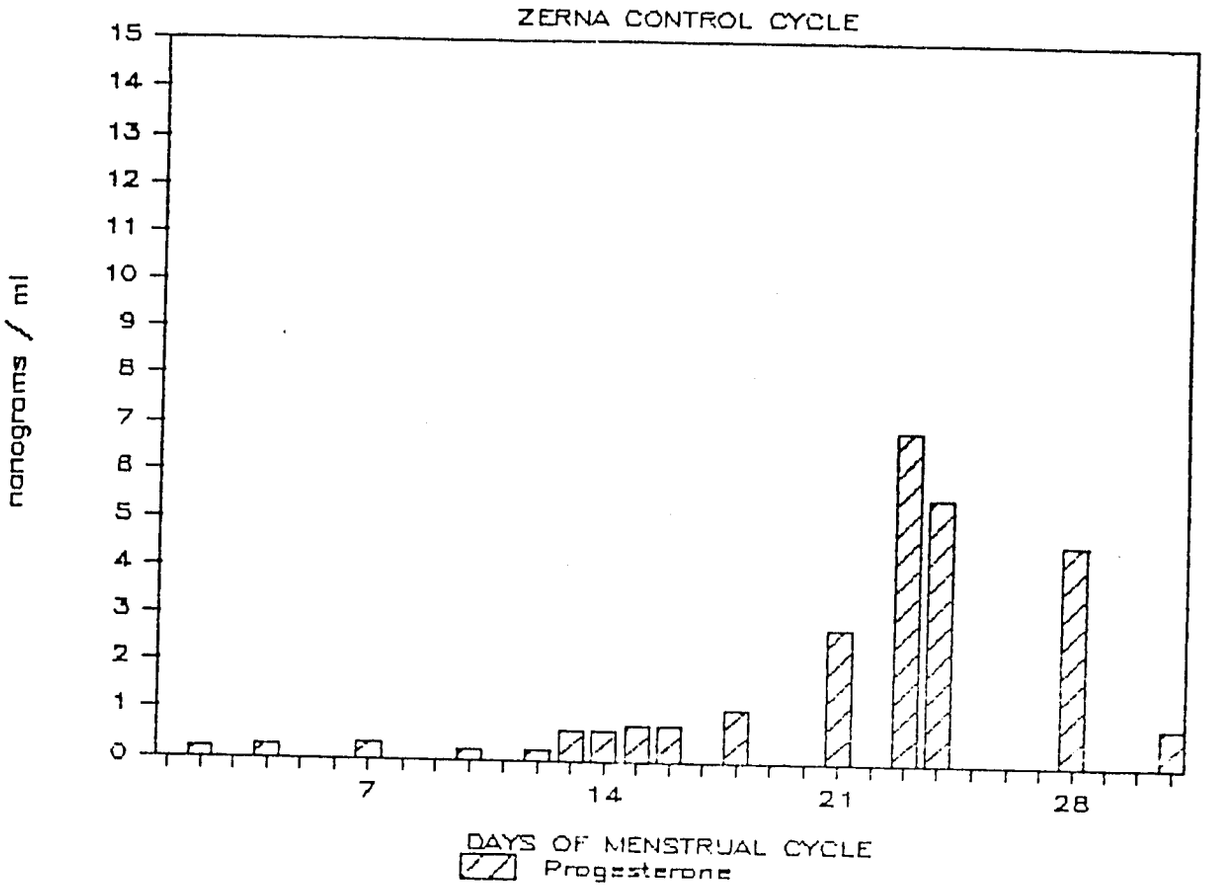

ZERNA POST IMMUNIZATION CYCLE
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
picrograms
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 8


ZERNA CONTROL CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
8
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
Progesterone

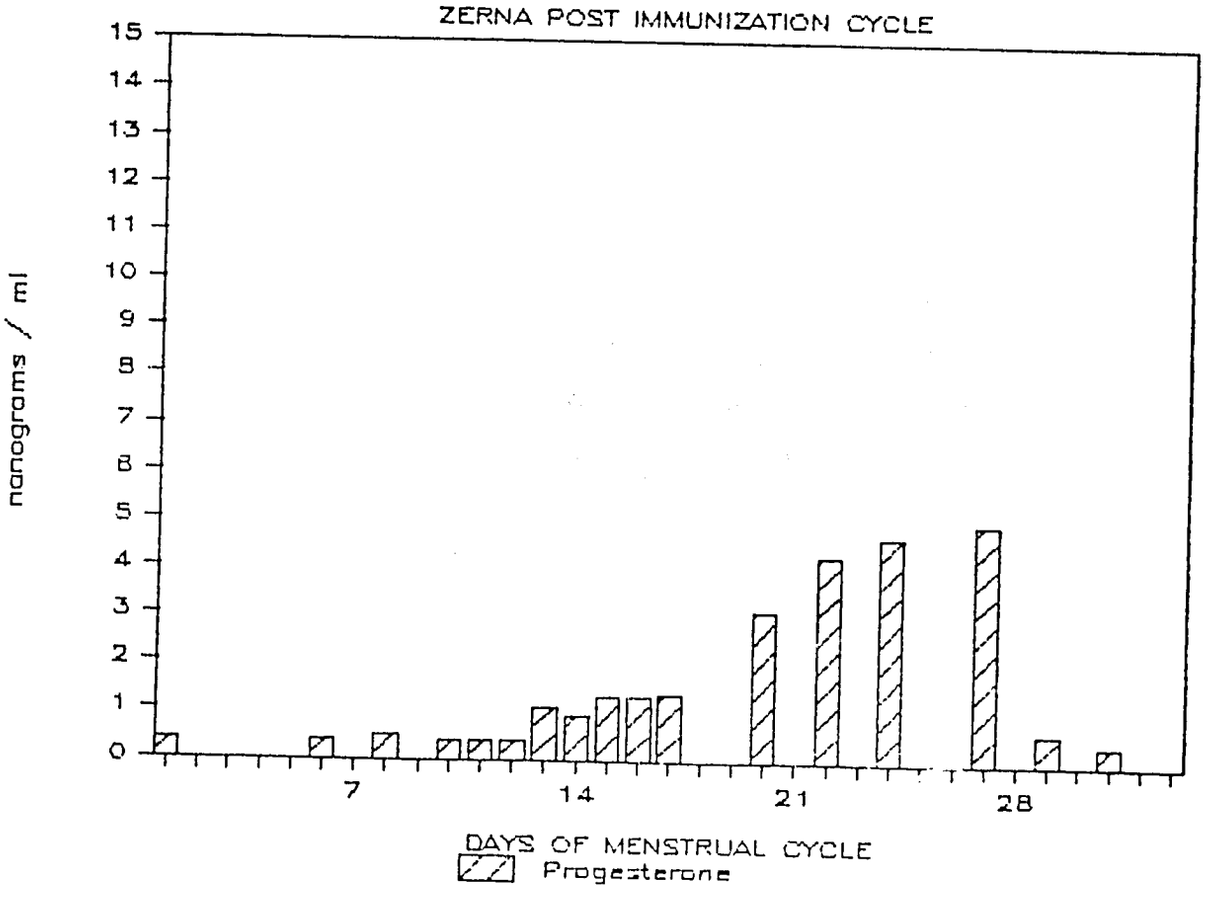
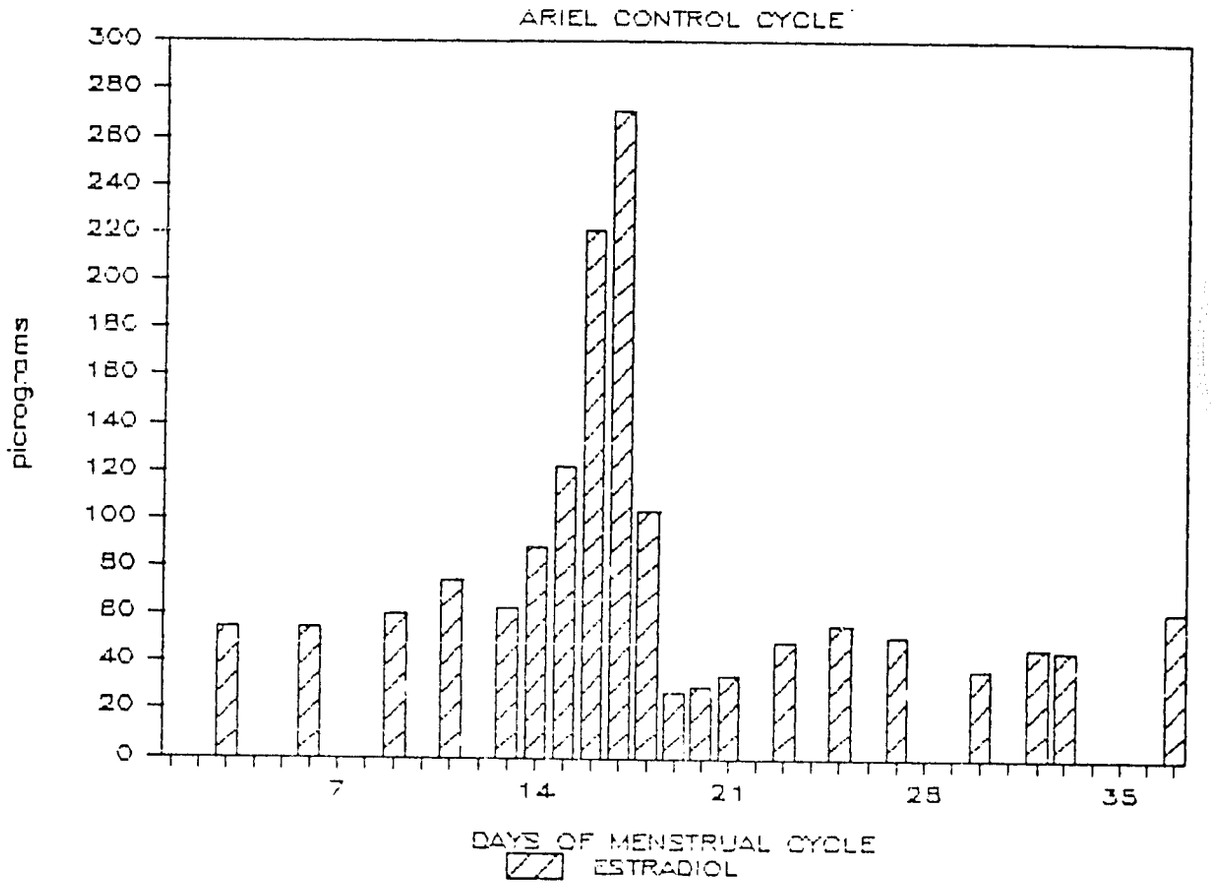

ZERNA POST IMMUNIZATION CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
8
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
Progesterone

FIGURE 9


ARIEL CONTROL CYCLE
picrograms
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
35
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

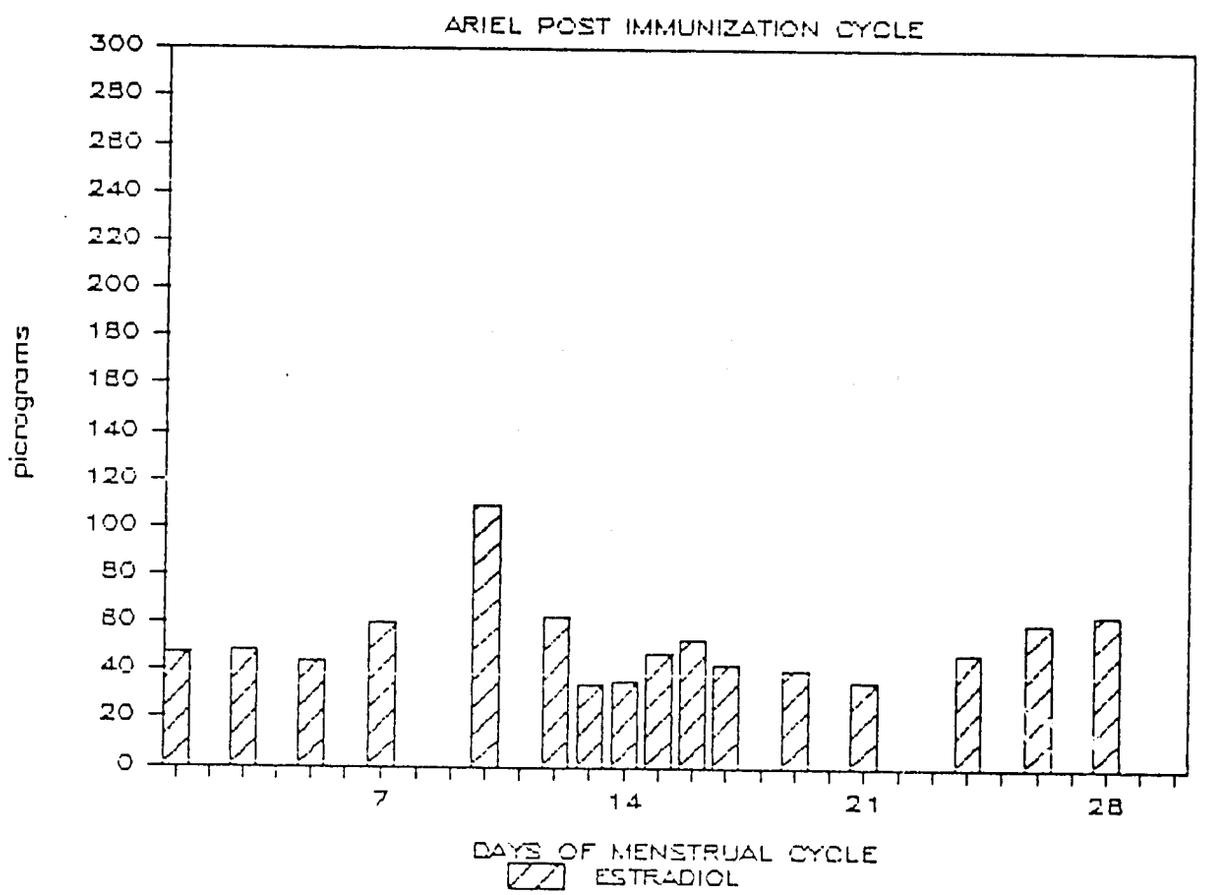
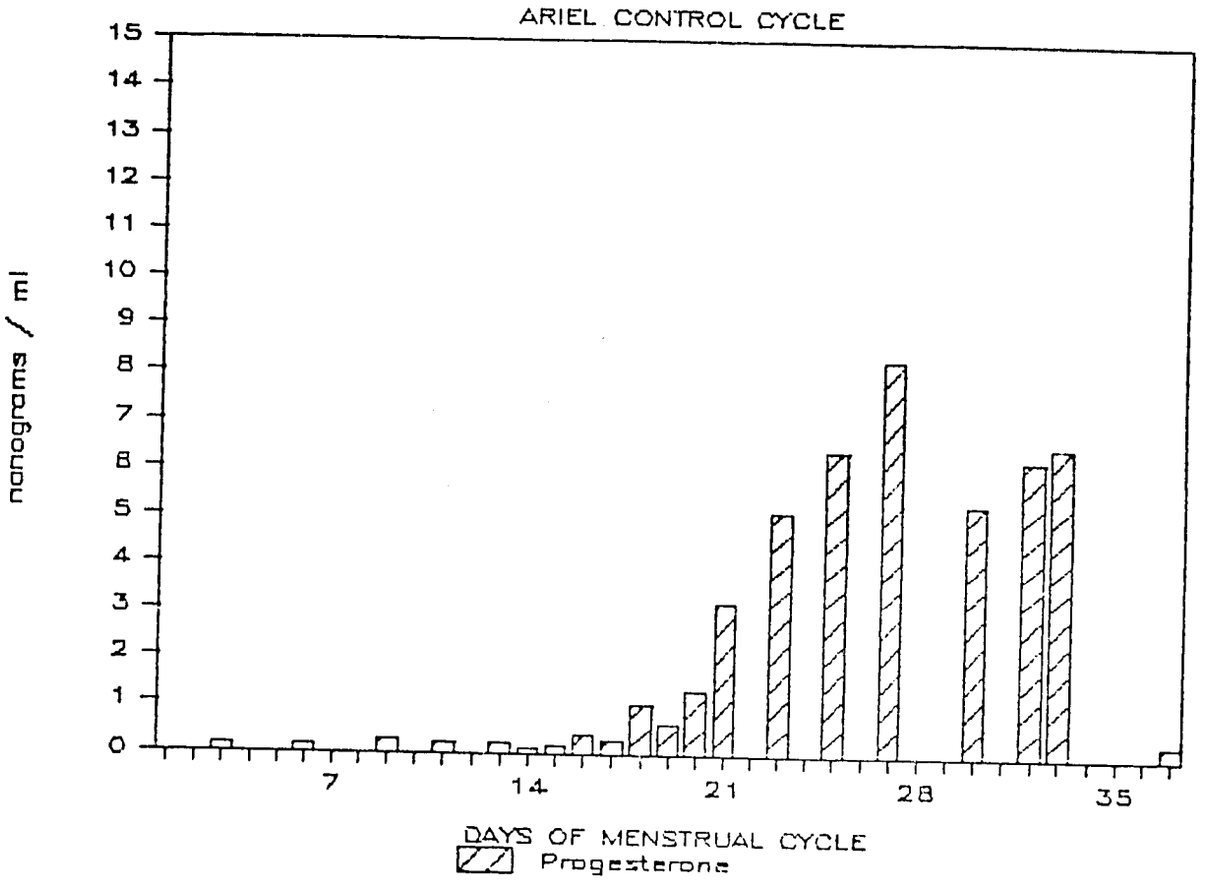
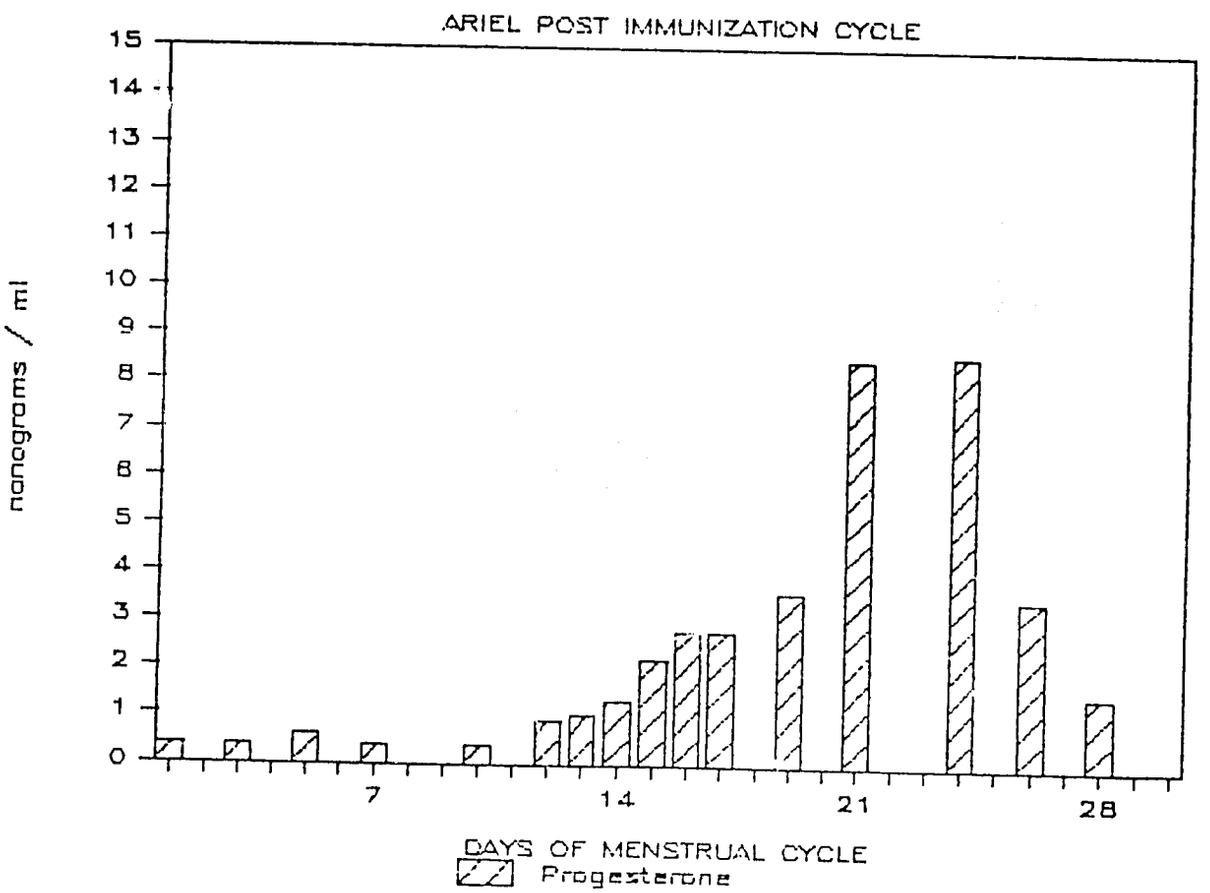

ARIEL POST IMMUNIZATION CYCLE
picrograms
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 10


ARIEL CONTROL CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
35
DAYS OF MENSTRUAL CYCLE
Progesterone


ARIEL POST IMMUNIZATION CYCLE
nanograms / ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
Progesterone

Studies of ZP-3 Immunized Animals

Study Design

The design of these studies was similar to those for ZP-1 antigen excepting a longer control period was used (antigen not available for immunizations) and the immunization period extended due to a weak response to immunizations (low antibody levels). Hormone levels were assessed in control cycle 2 but post-immunization levels were not measured until cycles 7-8 from the first immunization. A total of four immunizations were given but these were spaced farther apart than those using ZP-1 due to unavailability of sufficient antigen at a given time. Therefore, the time required to monitor nine menstrual cycles after the last booster injection extended the total study period for these animals.

Effects of Immunizations on Menstrual Cycle Parameters

Menstrual cycle data in ZP-3 immunized animals are shown in Tables 6-10. Fewer effects on cycle length and ovulation were found in these females than those receiving ZP-1 immunizations. Two animals had two and one other had one anovulatory cycle but no significant cycle length extensions were observed. Whilst these alterations may have been related to immunizations, one or two anovulatory cycles during a period of 16-18 months is not uncommon in baboons receiving no treatment.

Effects of Immunizations on Hormone Profiles

Quantities of ZP-3 antigen available for immunization were lower than for ZP-1 and perhaps for this reason, lower antibody levels were found following the initial immunizations. A final booster injection was made during cycle 6 or 7. Beginning with the onset of menses in the next cycle, frequent blood samples were

TABLE 6

BABOON: Effie NUMBER: 082410

STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	29	14	15	yes	
2	34	18	16	yes	
3	33	16	17	yes	
4	33	15	18	yes	
5	32	17	15	yes	
Immunized					
1	34	17	17	yes	
2	33	15	18	yes	
3	34	17	17	yes	
4	33	15	18	yes	
5	33	16	17	yes	
6	32	15	17	yes	
7	34	17	17	yes	last booster injection
8	34	18	16	yes	
9	33	17	16	yes	
10	31	14	17	yes	
11	35	17	18	yes	
12	31	15	16	yes	
13	30	13	17	yes	
14	32	17	15	yes	
15	34	18	16	yes	
16	33	16	17	yes	

TABLE 7

BABOON: Ina NUMBER: 051259

STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	30	13	17	yes	
2	36	17	19	yes	
3	36	20	16	yes	
4	36	18	18	yes	
Immunized					
1	37	21	16	yes	
2	38	21	17	yes	
3	36	20	16	yes	
4	35	18	17	yes	
5	39	22	17	yes	
6	40	21	19	yes	last booster injection
7	33	17	16	yes	
8	37	19	18	yes	
9	39	20	19	yes	
10	35	19	16	yes	
11	35	18	17	yes	
12	35	20	15	yes	short luteal phase
13	37	19	18	yes	
14	34	18	16	yes	
15	35	15	20	yes	

TABLE 8

BABOON: Laurel NUMBER: 082414

STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	32	16	16	yes	
2	32	17	15	yes	
3	36	20	16	yes	
4	34	17	17	yes	
	31	14	17	yes	
	36	14	22	yes	long luteal phase
	31	13	18	yes	
3	29	14	15	yes	
4	29	15	14	yes	
5	34	16	18	yes	
6	27	12	15	yes	last booster injection short follicular
7	28	14	14	no	
8	32	17	15	yes	
9	31	14	17	yes	
10	28	14	14	yes	
11	36	21	15	yes	long follicular
12	34	19	15	no	
13	30	15	15	yes	
14	34	18	16	yes	
15	33	18	15	yes	

TABLE 9

BABOON: Ukena NUMBER: 082418

STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	30	15	15	yes	
2	32	17	15	yes	
3	36	21	15	yes	
4	31	20	11	yes	
5	31	19	12	yes	
Immunized					
1	35	23	12	yes	
2	35	19	16	yes	
3	29	15	14	yes	
4	34	20	14	yes	
5	36	21	15	yes	
6	30	15	15	yes	last booster injection
7	32	20	12	yes	
8	37	21	16	yes	
9	33	18	15	yes	
10	29	16	13	yes	
11	34	20	14	yes	
12	31	16	15	yes	
13	29	16	13	no	short follicular/luteal
14	33	19	14	yes	
15	33	19	14	yes	

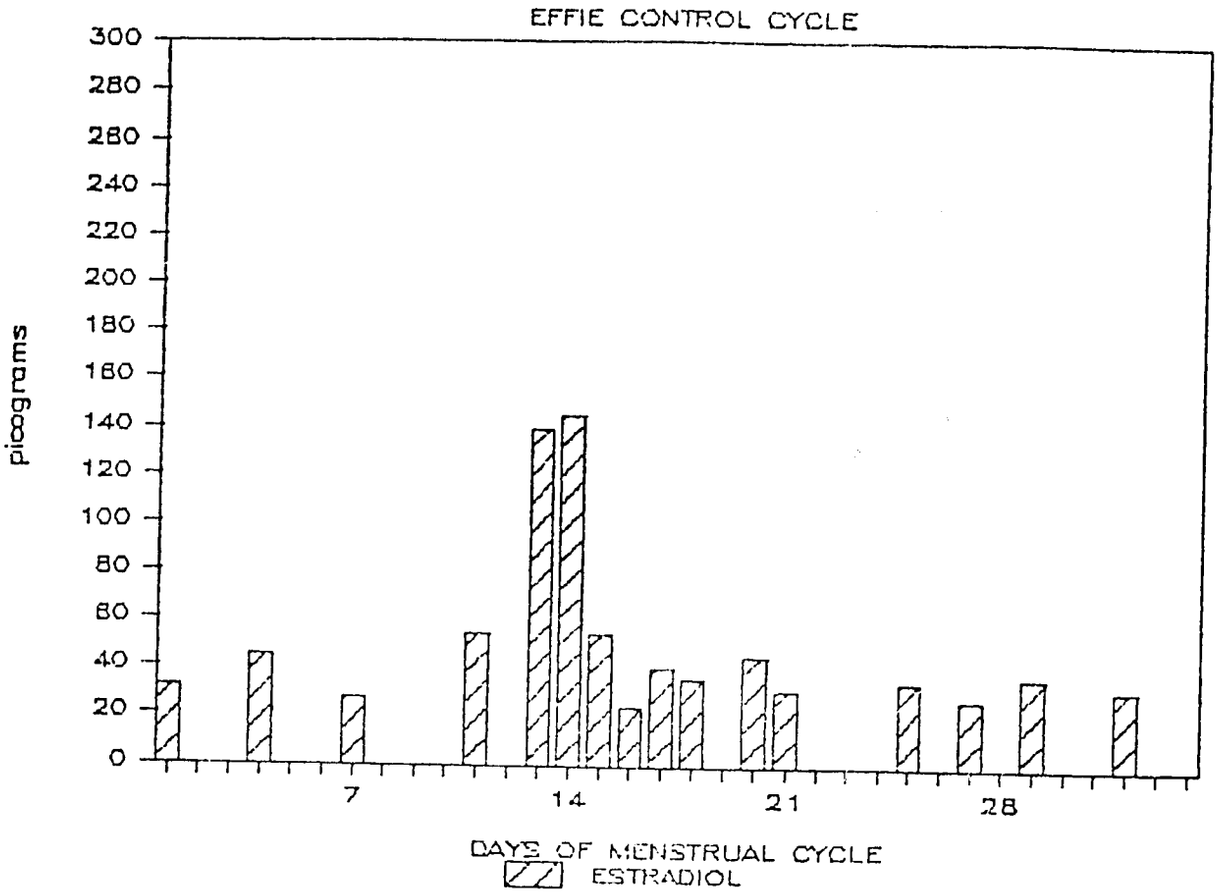
TABLE 10

BABOON: Whitney NUMBER: 082420

STUDY CYCLE	CYCLE LENGTH (days)	FOLLICULAR PHASE (days)	LUTEAL PHASE (days)	? OVULATION (yes/no)	COMMENTS
Control					
1	33	19	14	yes	
2	30	15	15	yes	
3	30	15	15	yes	
4	29	19	10	yes	long follicular/ short luteal phases
5	28	15	13	yes	
Immunized					
1	31	17	14	yes	
2	30	17	13	yes	
3	29	15	14	yes	
4	30	15	15	yes	
5	31	16	15	yes	
6	29	14	15	yes	
7	31	14	17	yes	last booster injection
8	30	16	14	yes	
9	29	16	13	yes	
10	29	16	13	yes	
11	30	16	14	no	
12	31	16	15	yes	
13	29	15	14	no	
14	30	16	14	yes	
15	32	15	17	yes	
16	27	13	14	yes	

collected to assess effects of immunizations on serum estradiol and progesterone levels. Hormone levels in the control and post-immunization cycle on each animal are shown in Figures 11-20. The only observation of altered ovarian function made was an anovulatory pattern of progesterone in one female (Figure 16). However, this female was ovulatory in subsequent cycles.

FIGURE 11


EFFIE CONTROL CYCLE
picograms
300
280
280
240
220
200
180
160
140
120
100
80
60
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

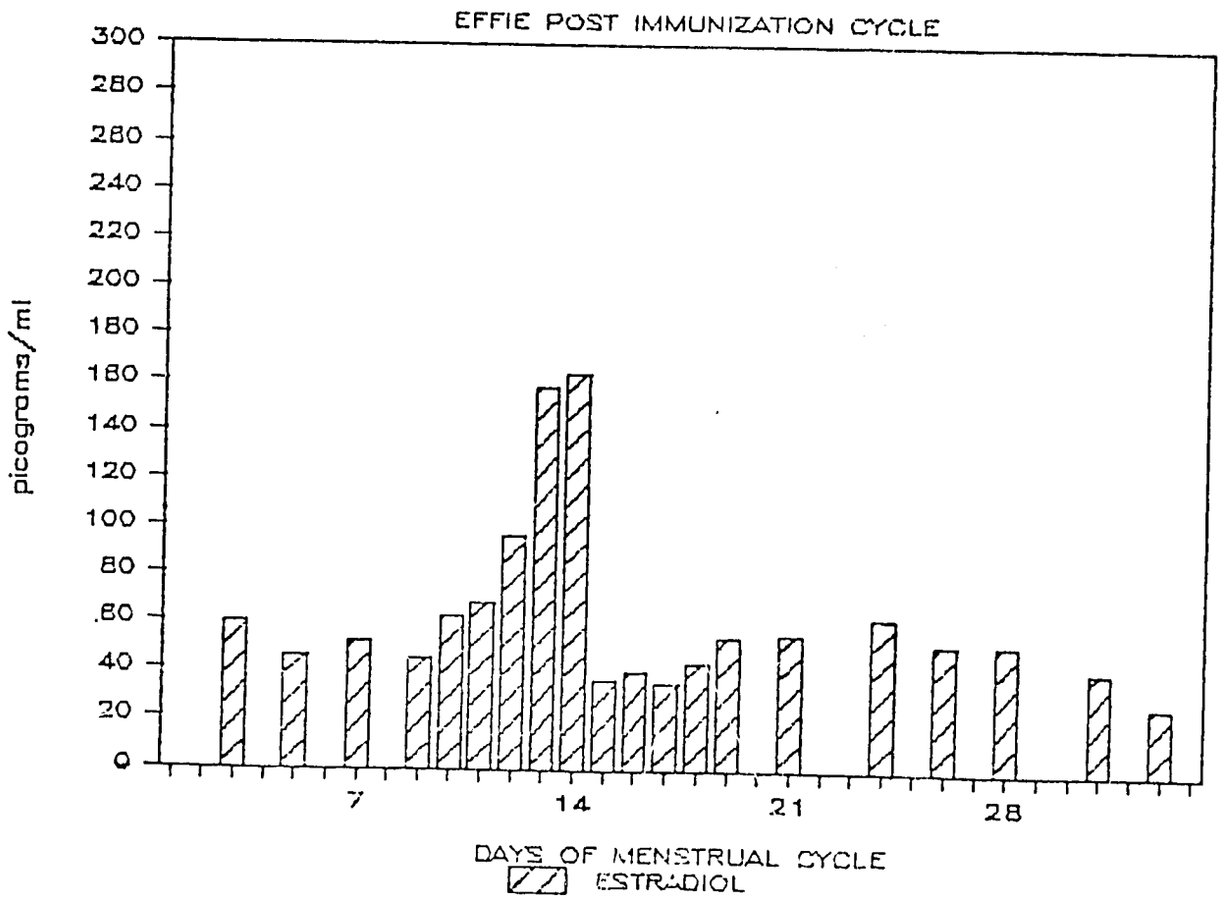
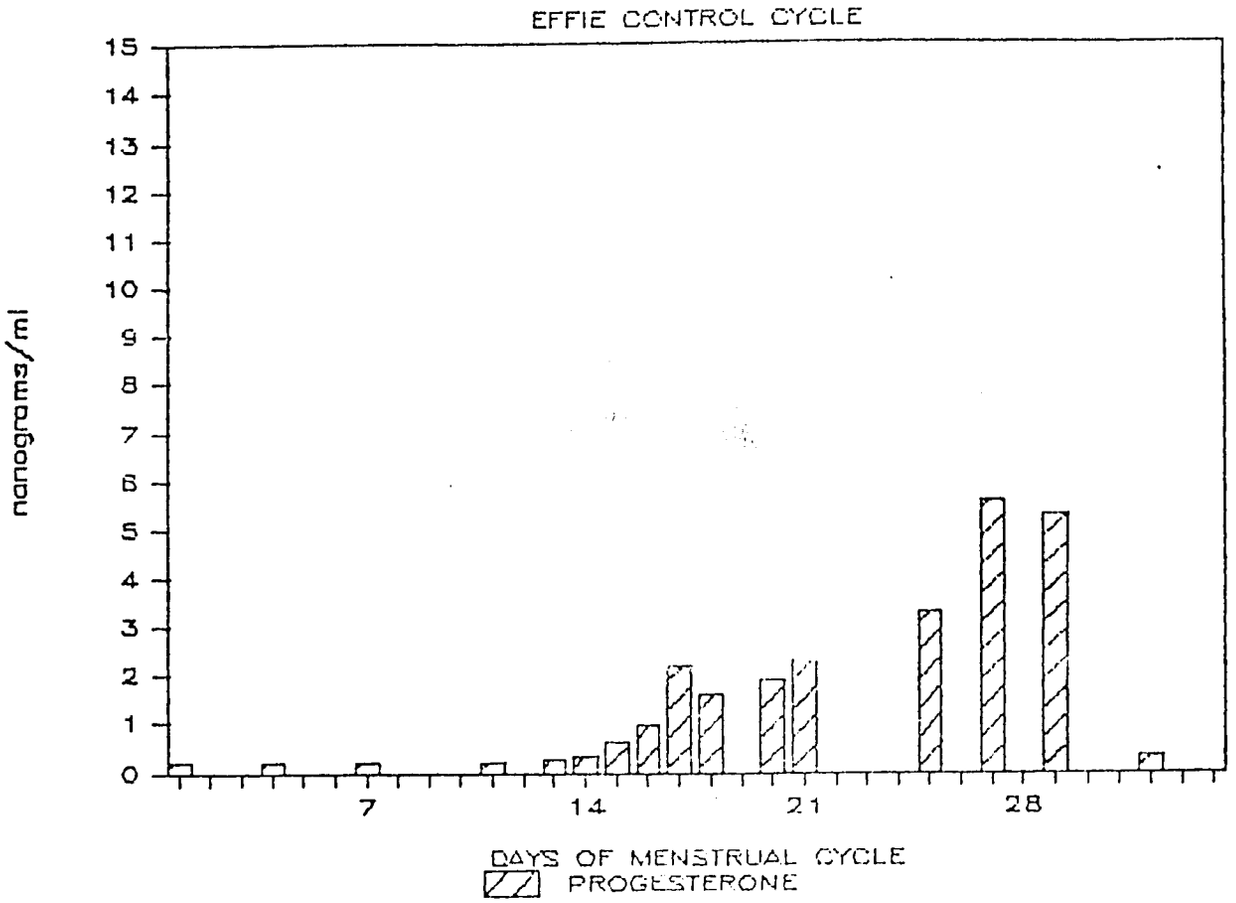

EFFIE POST IMMUNIZATION CYCLE
picograms/ml
300
280
280
240
220
200
180
160
140
120
100
80
60
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 12


EFFIE CONTROL CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
PROGESTERONE

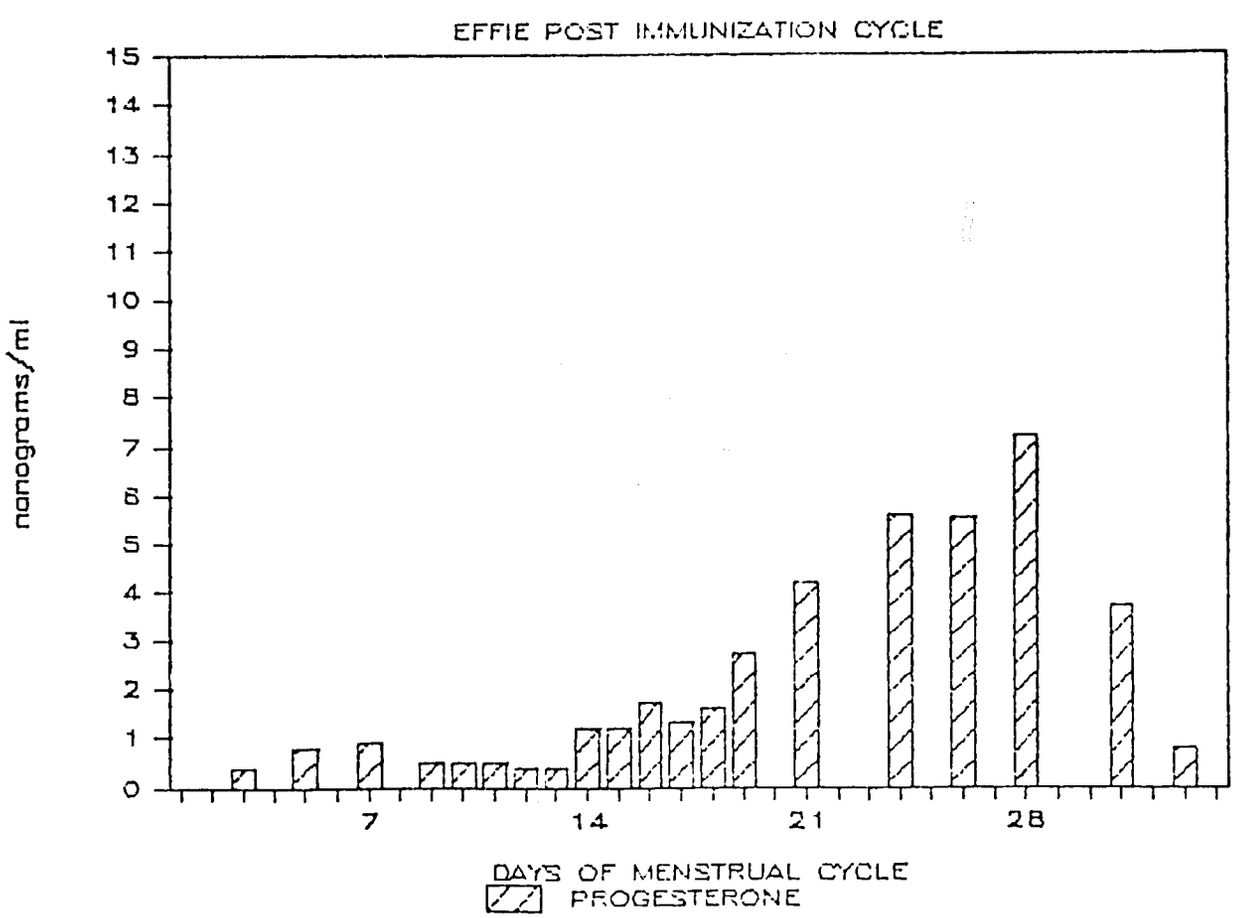
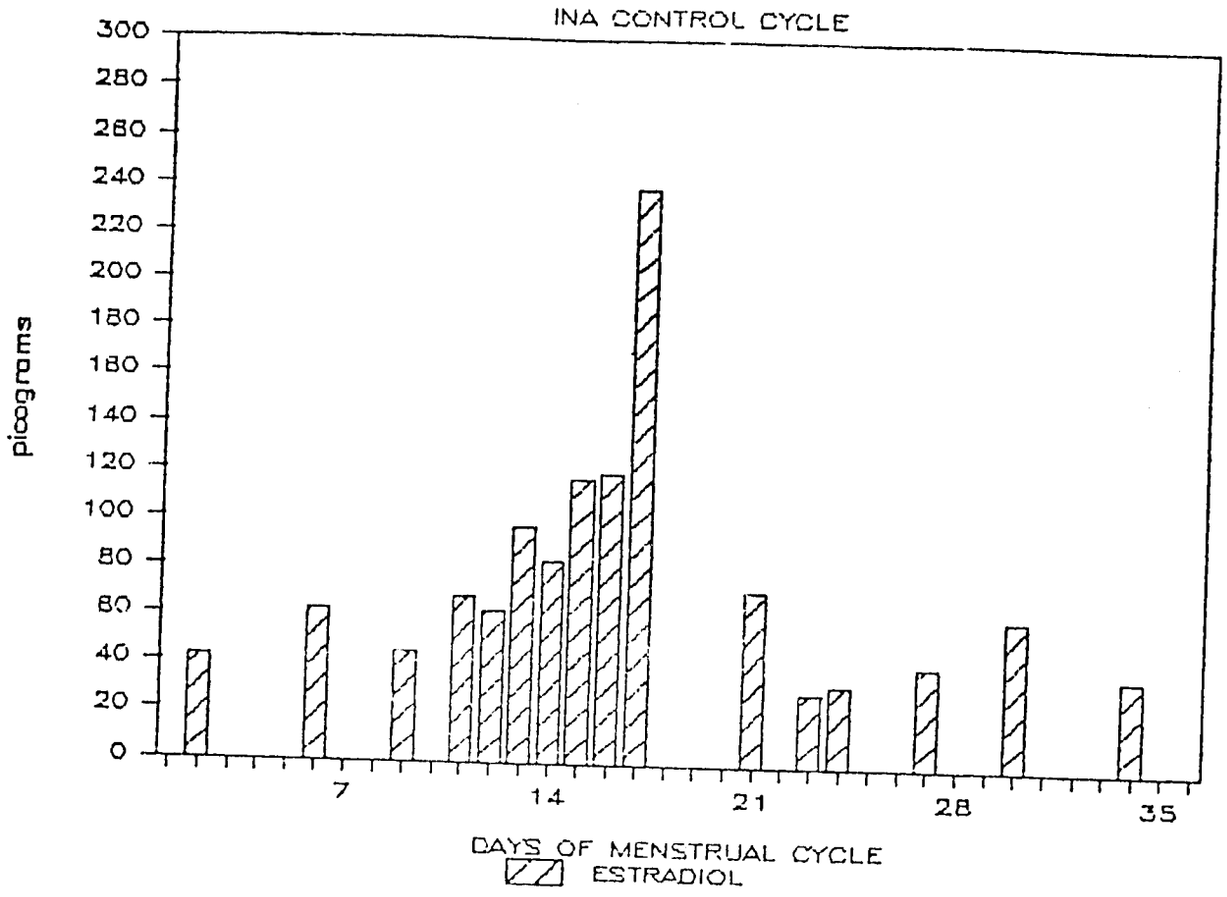

EFFIE POST IMMUNIZATION CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
PROGESTERONE

FIGURE 13


INA CONTROL CYCLE
picograms
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
35
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

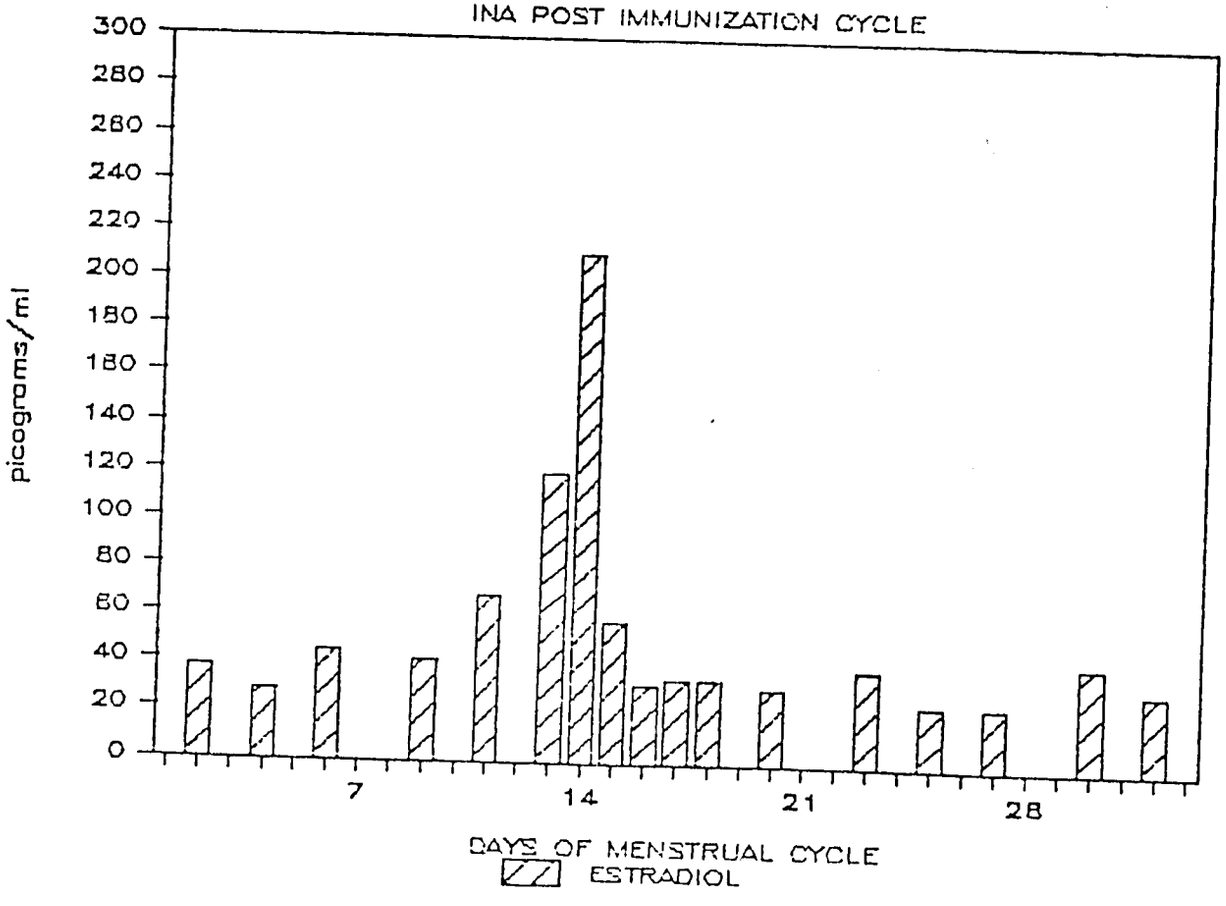
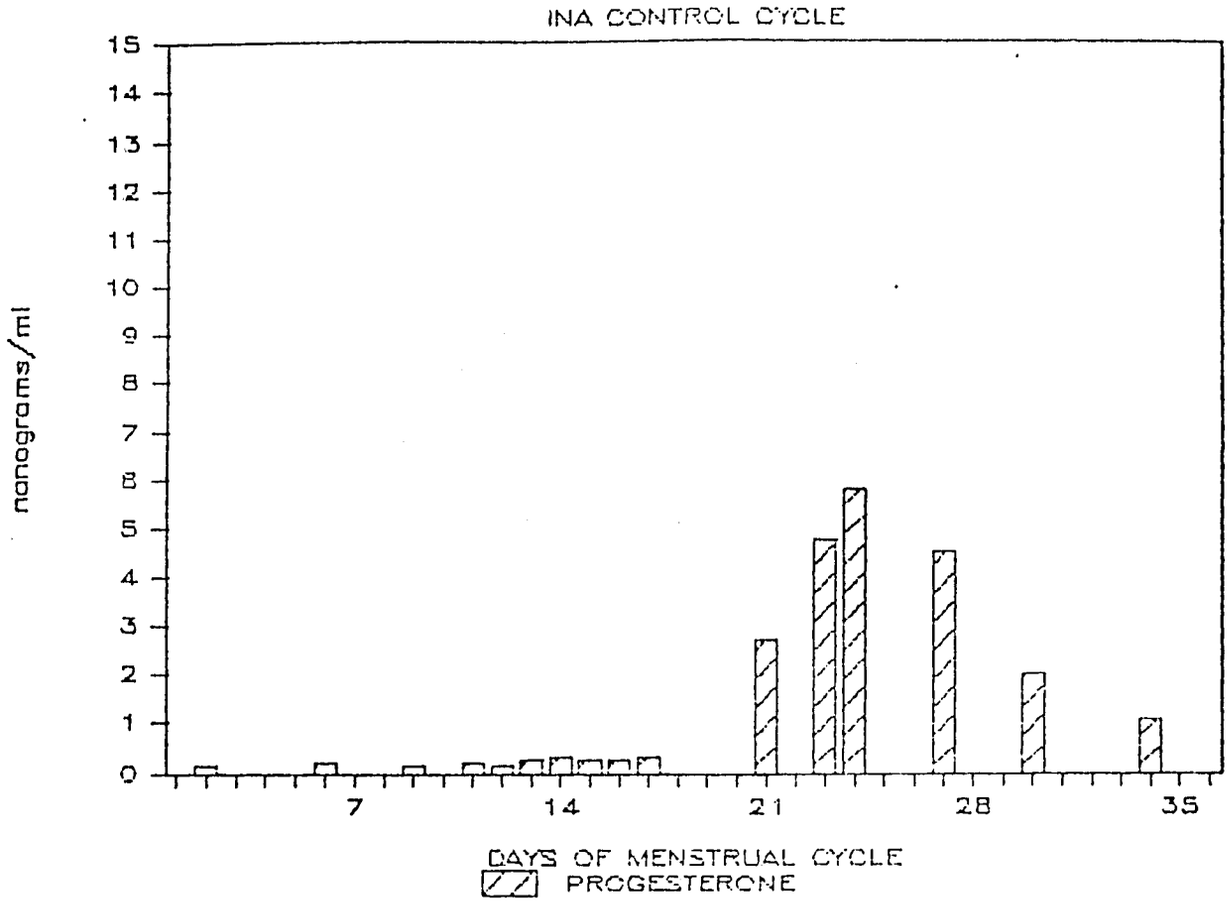

INA POST IMMUNIZATION CYCLE
picograms/ml
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 14


INA CONTROL CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
35
DAYS OF MENSTRUAL CYCLE
PROGESTERONE

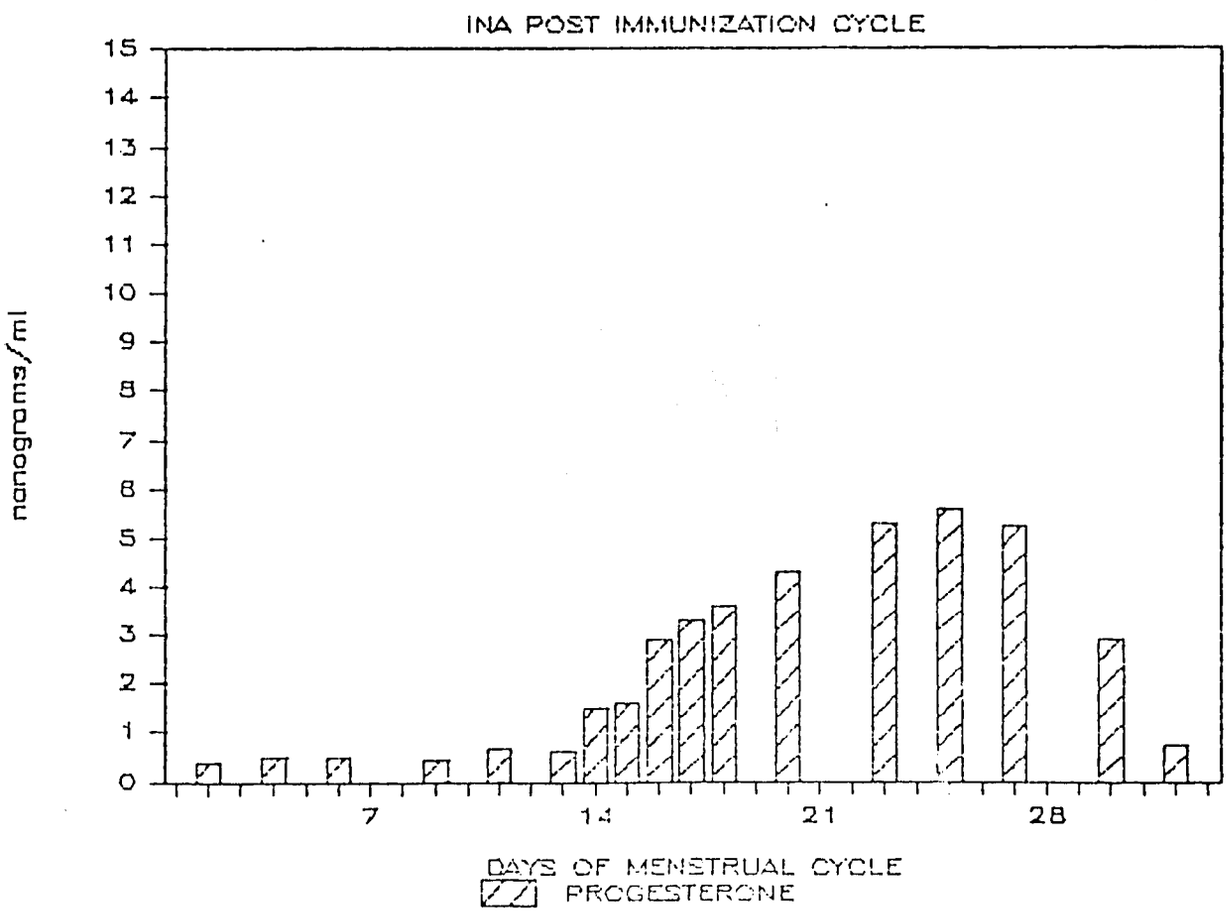
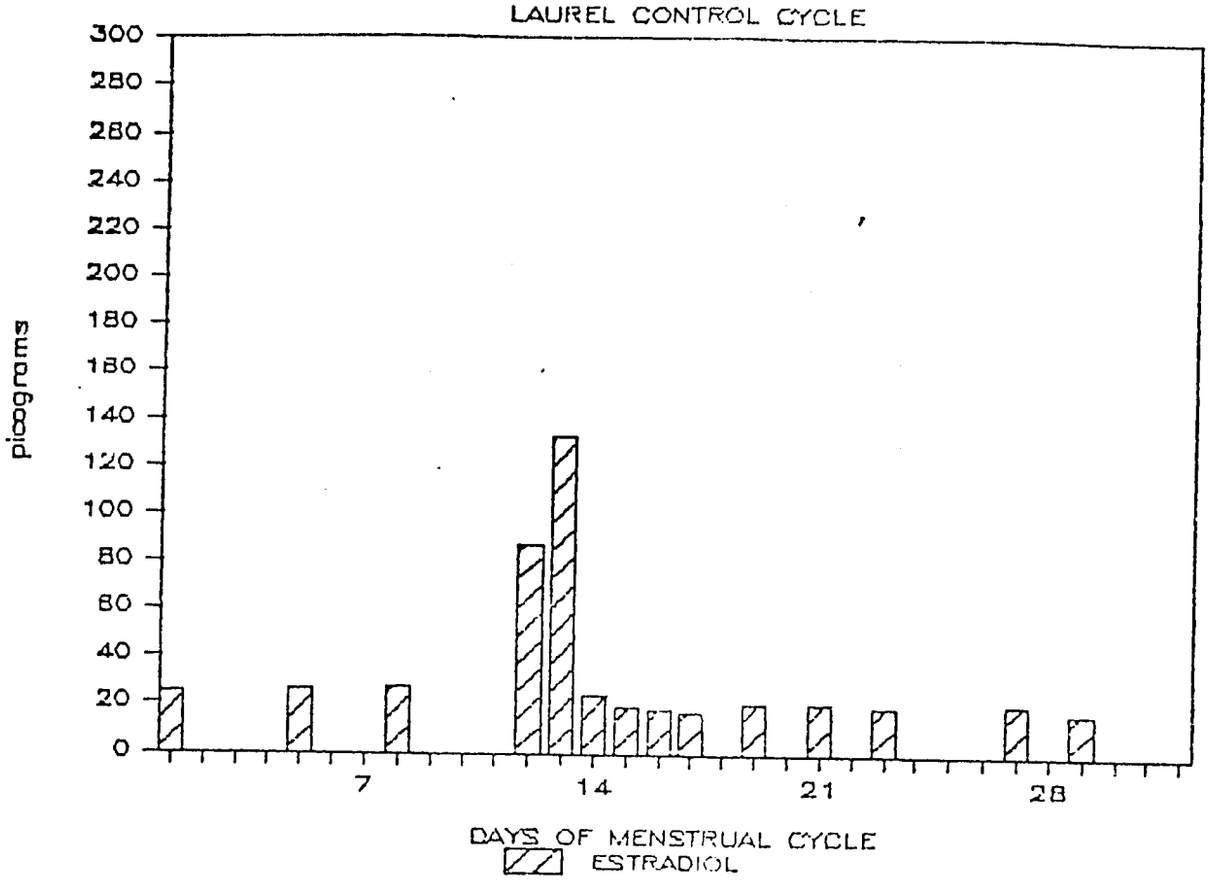

INA POST IMMUNIZATION CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
PROGESTERONE

FIGURE 15


LAUREL CONTROL CYCLE
picograms
300
280
280
240
220
200
180
180
140
120
100
80
60
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

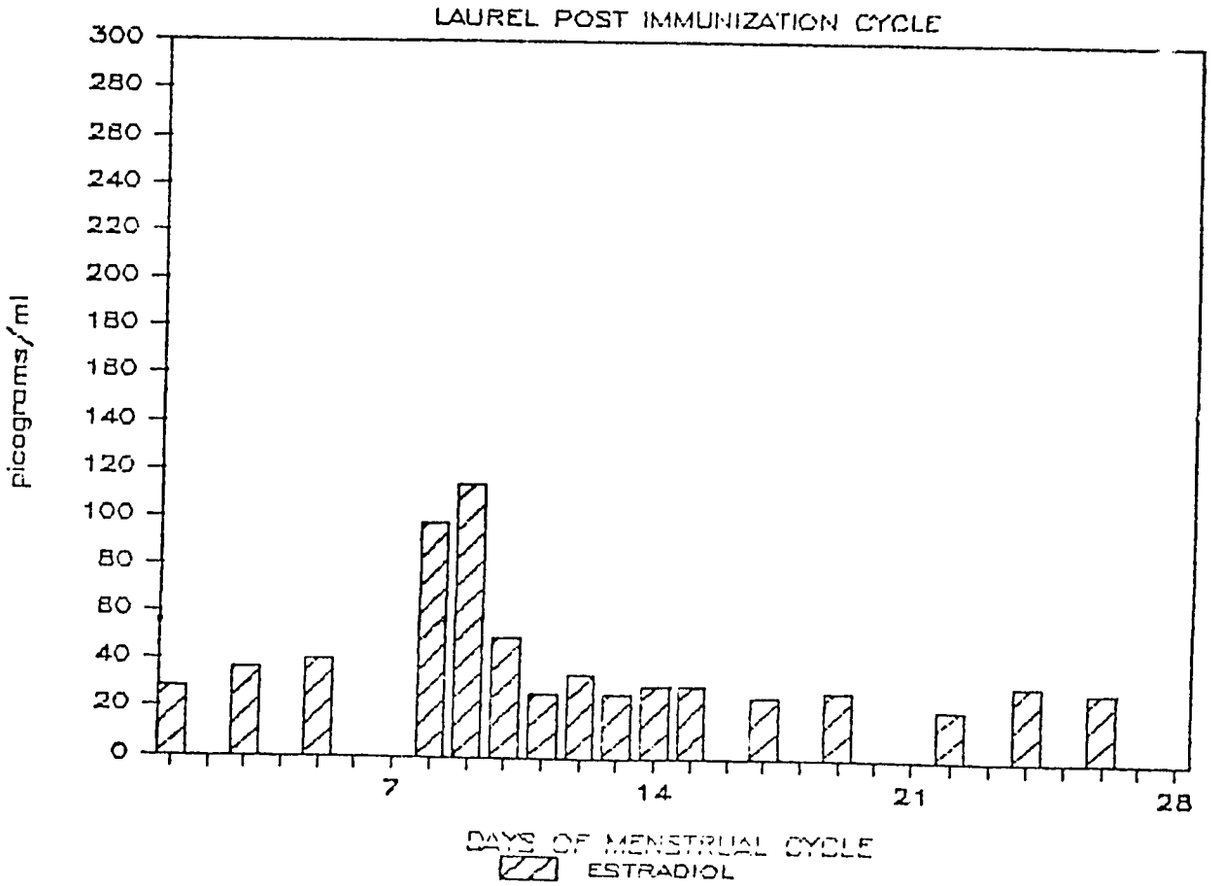
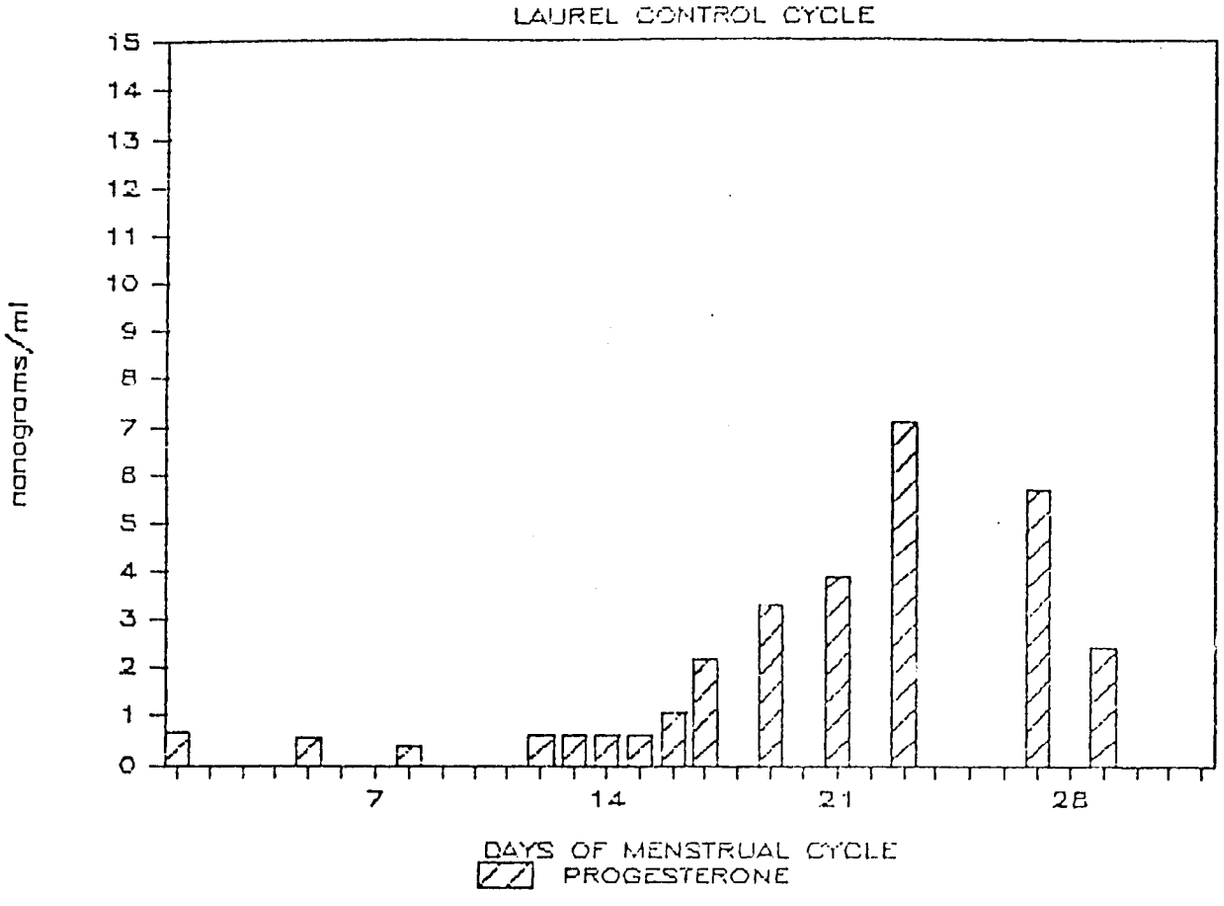

LAUREL POST IMMUNIZATION CYCLE
picograms/ml
300
280
280
240
220
200
180
180
140
120
100
80
60
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 16


LAUREL CONTROL CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
PROGESTERONE

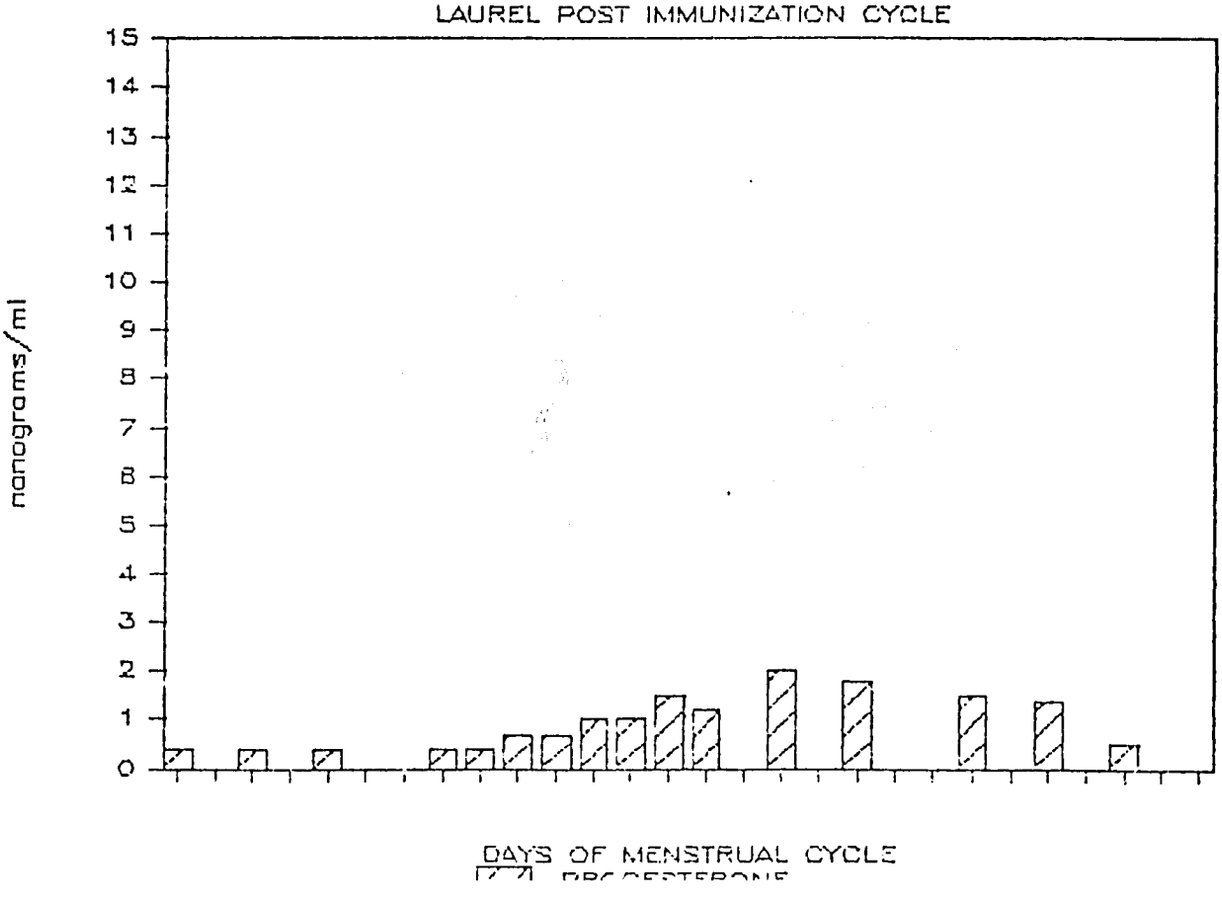
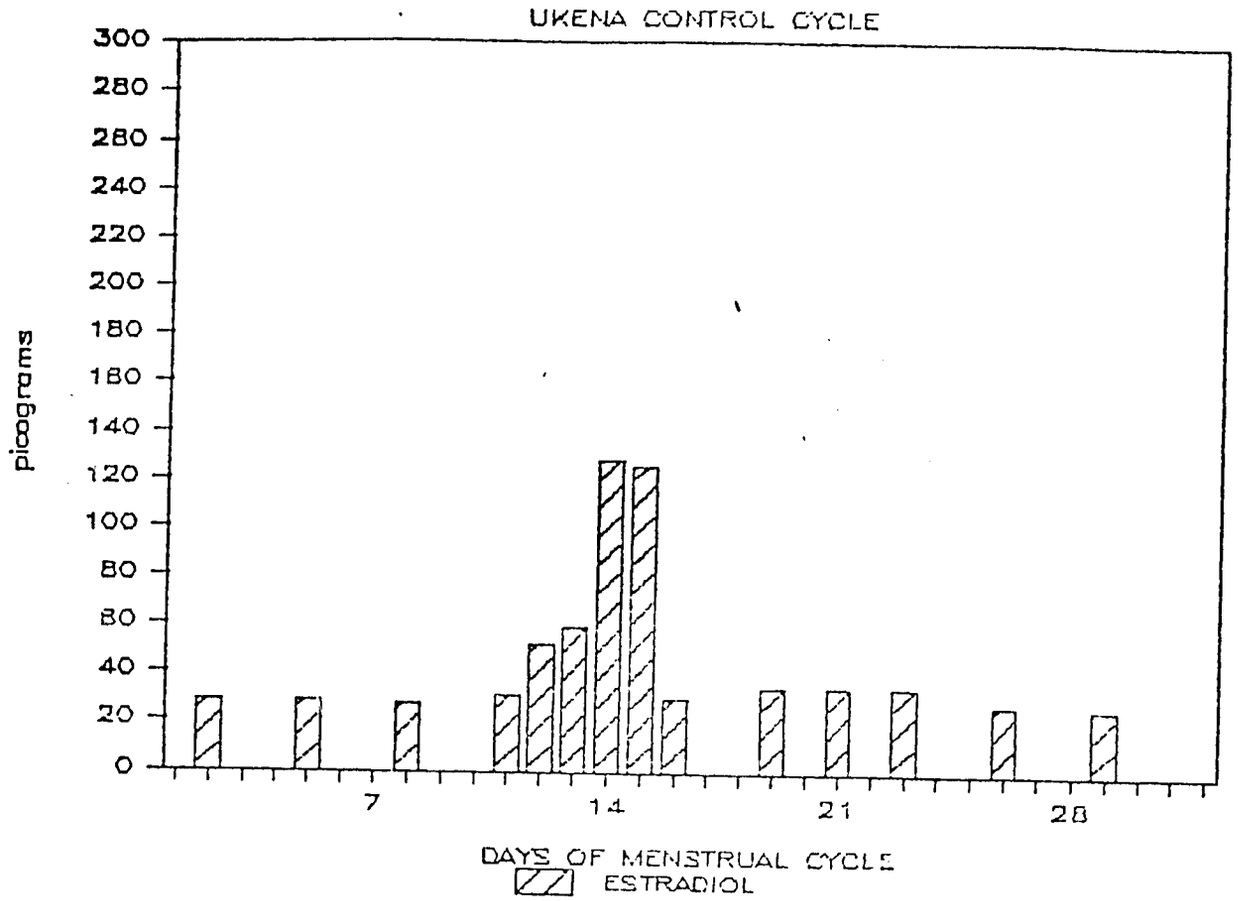

LAUREL POST IMMUNIZATION CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
DAYS OF MENSTRUAL CYCLE

FIGURE 17


UKENA CONTROL CYCLE
picograms
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

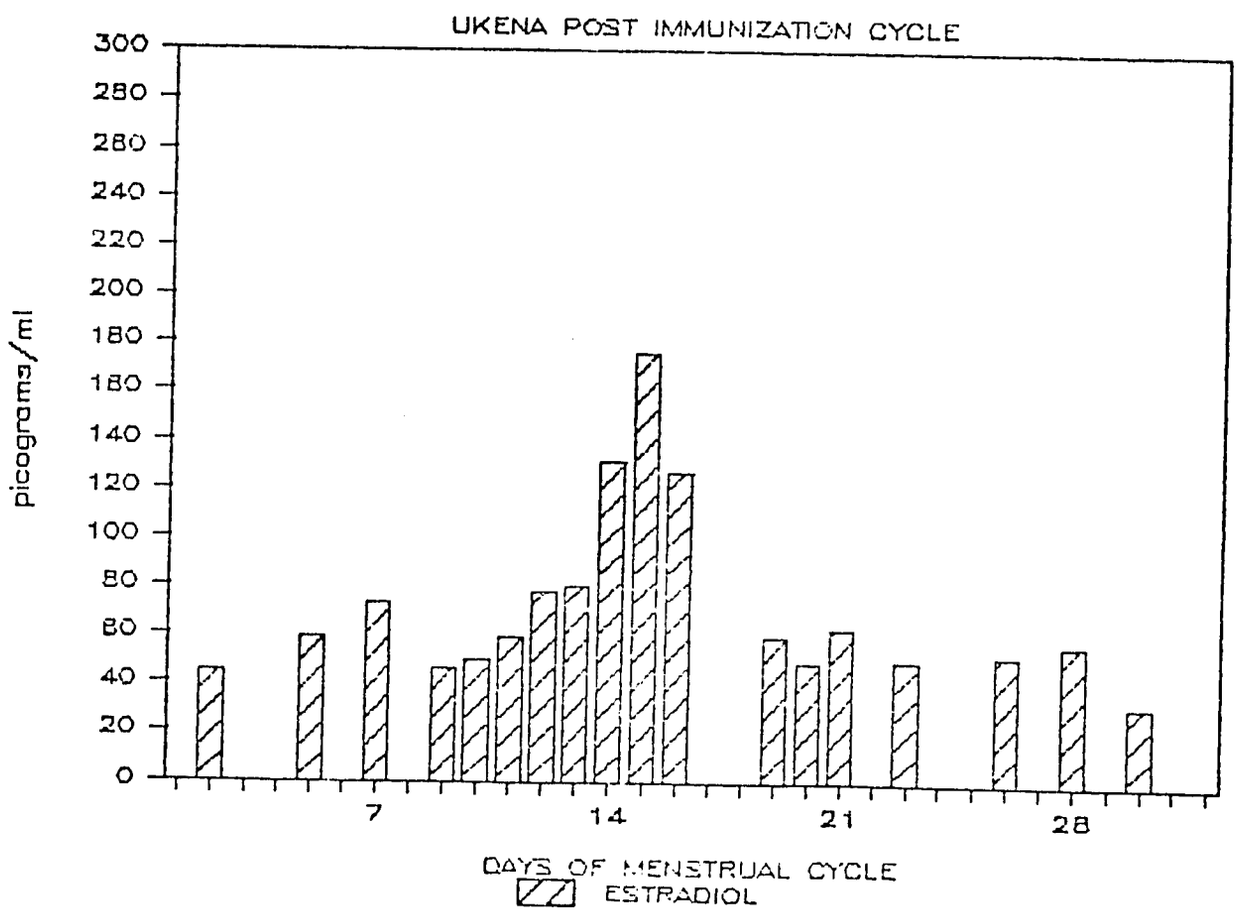
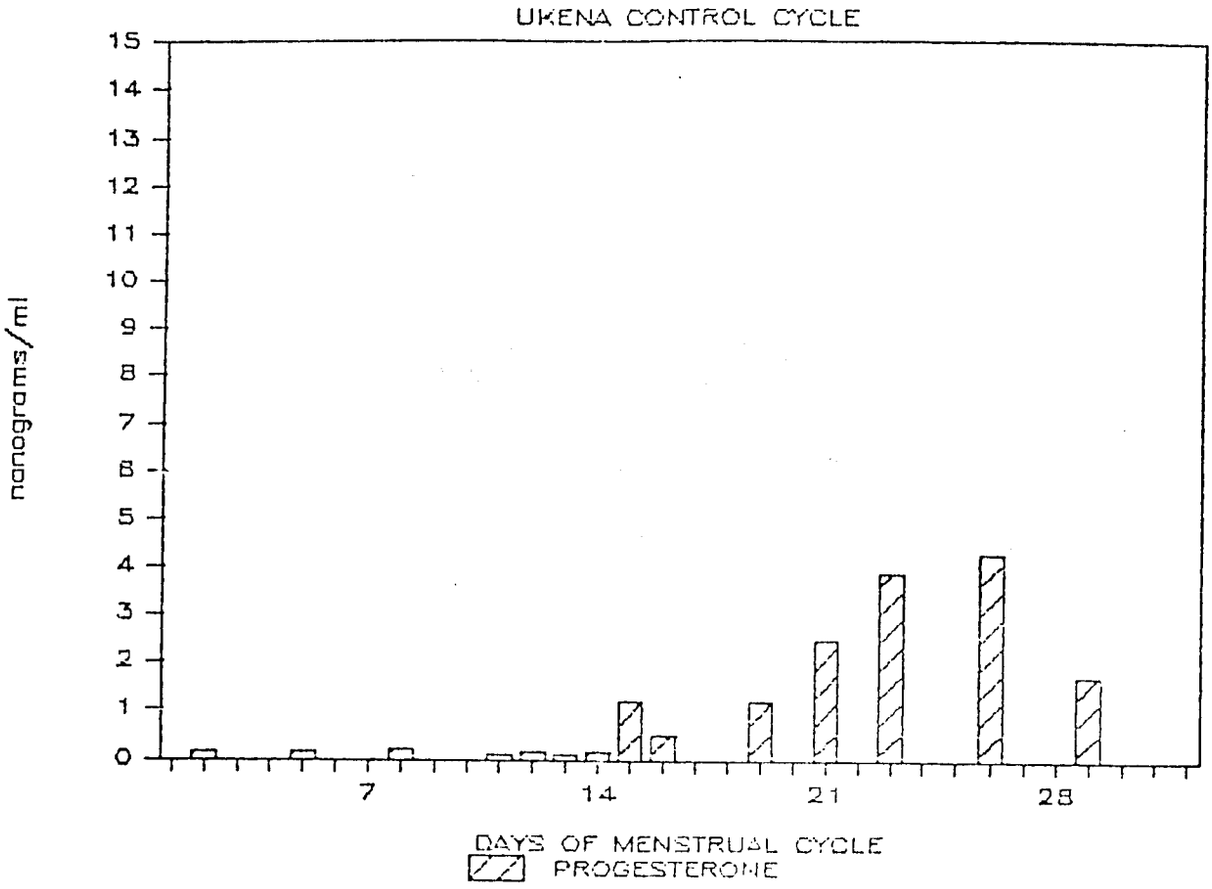

UKENA POST IMMUNIZATION CYCLE
picograms/ml
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 18


UKENA CONTROL CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
PROGESTERONE

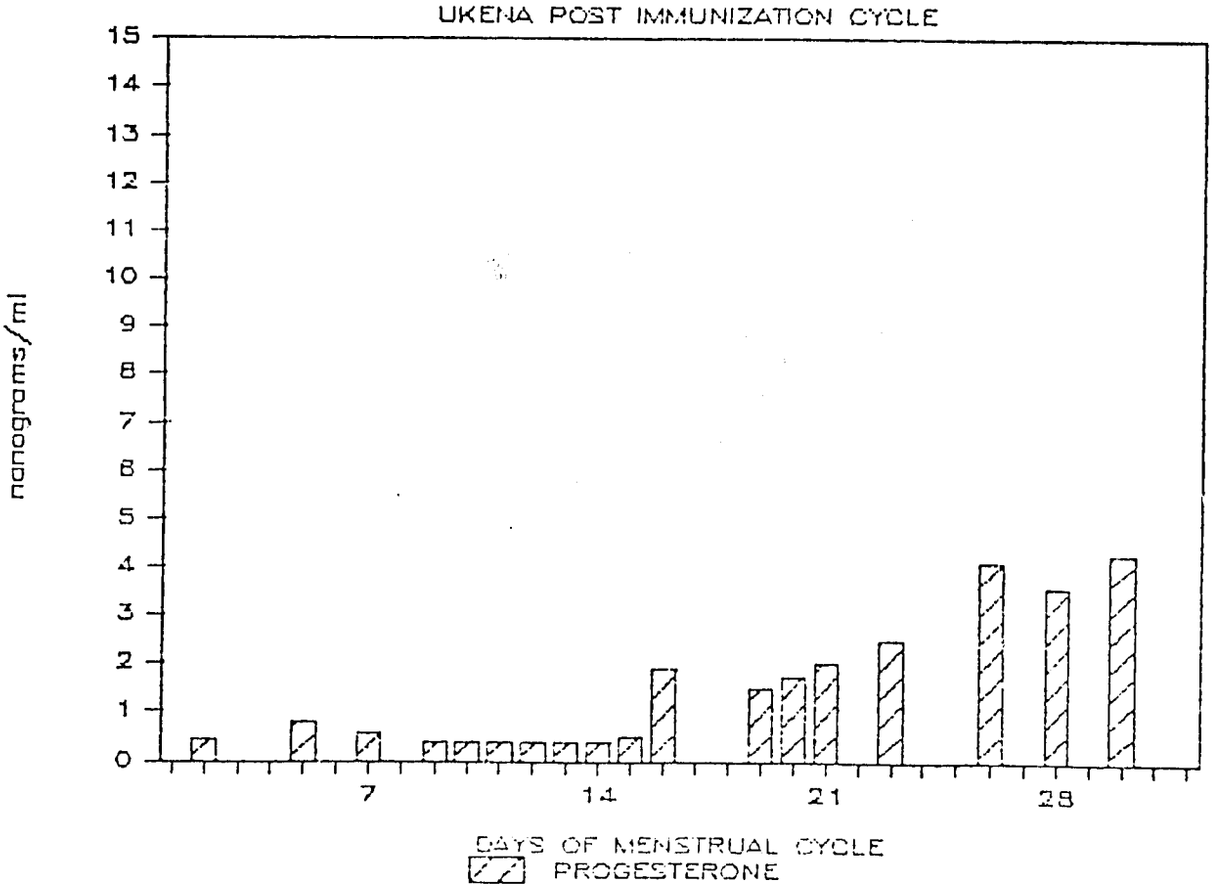
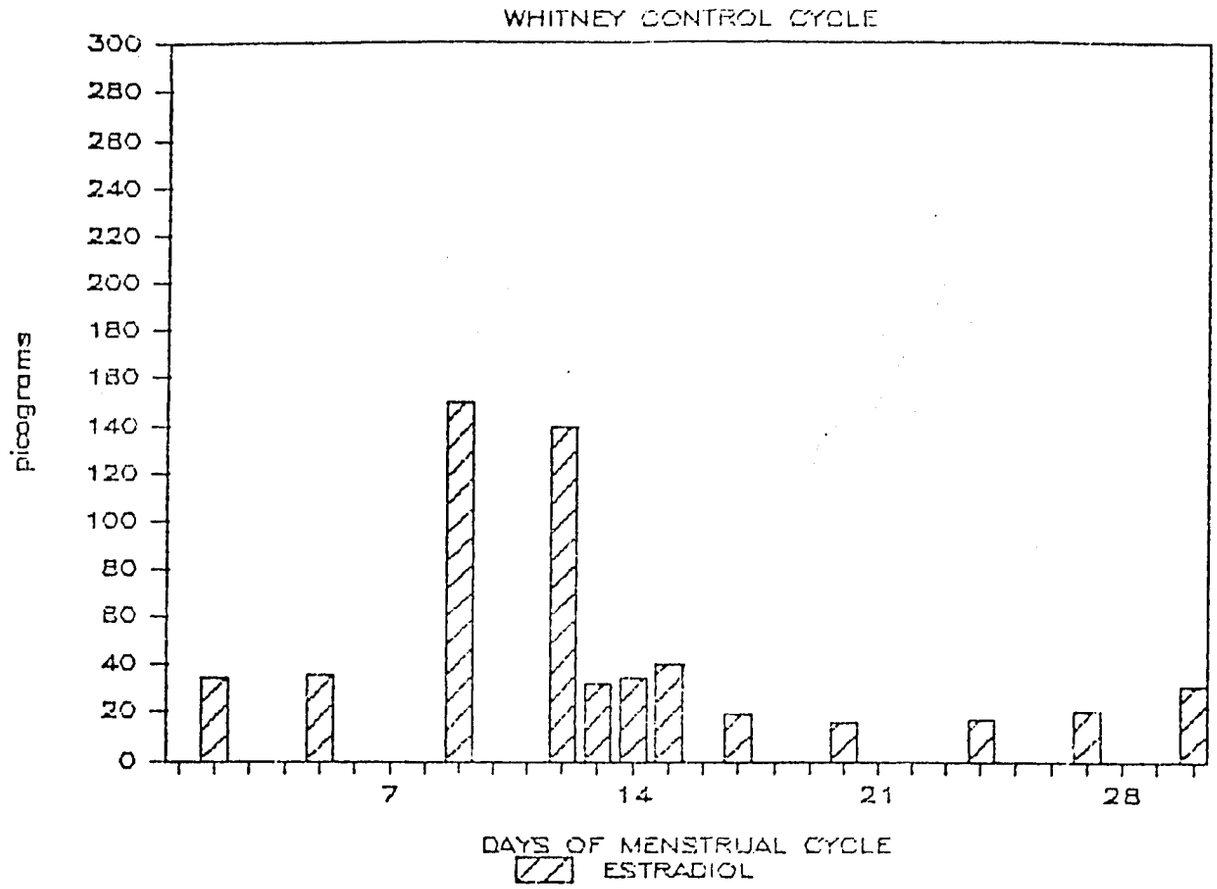

UKENA POST IMMUNIZATION CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
PROGESTERONE

FIGURE 19


WHITNEY CONTROL CYCLE
picograms
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

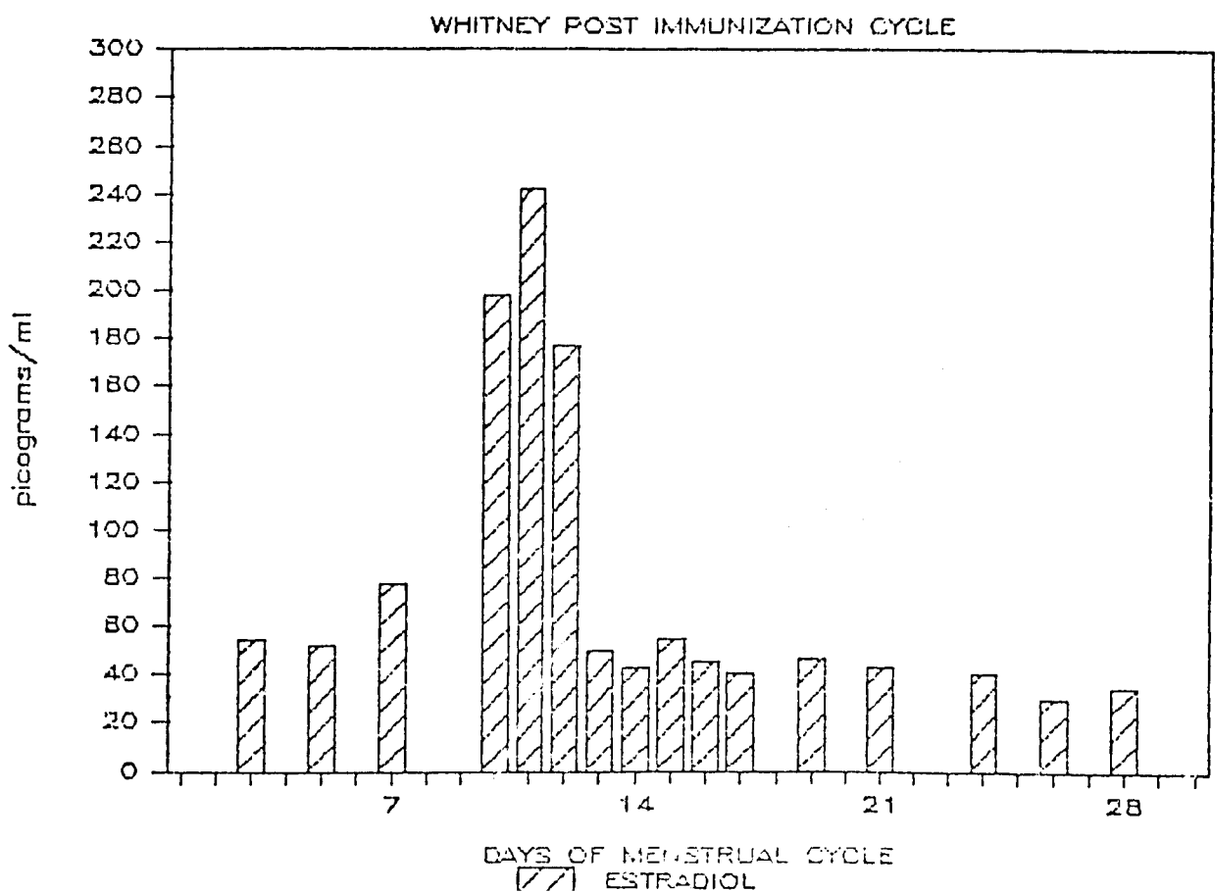
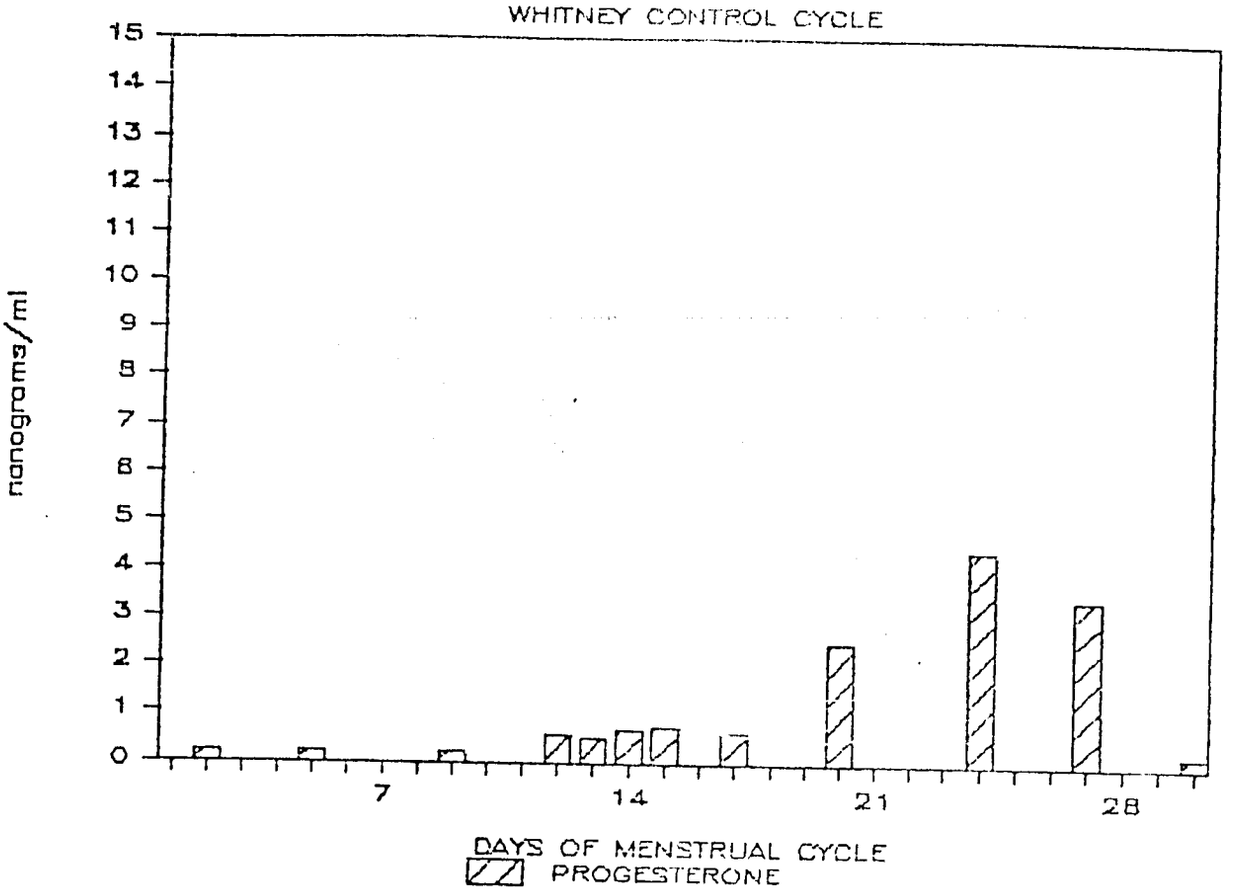
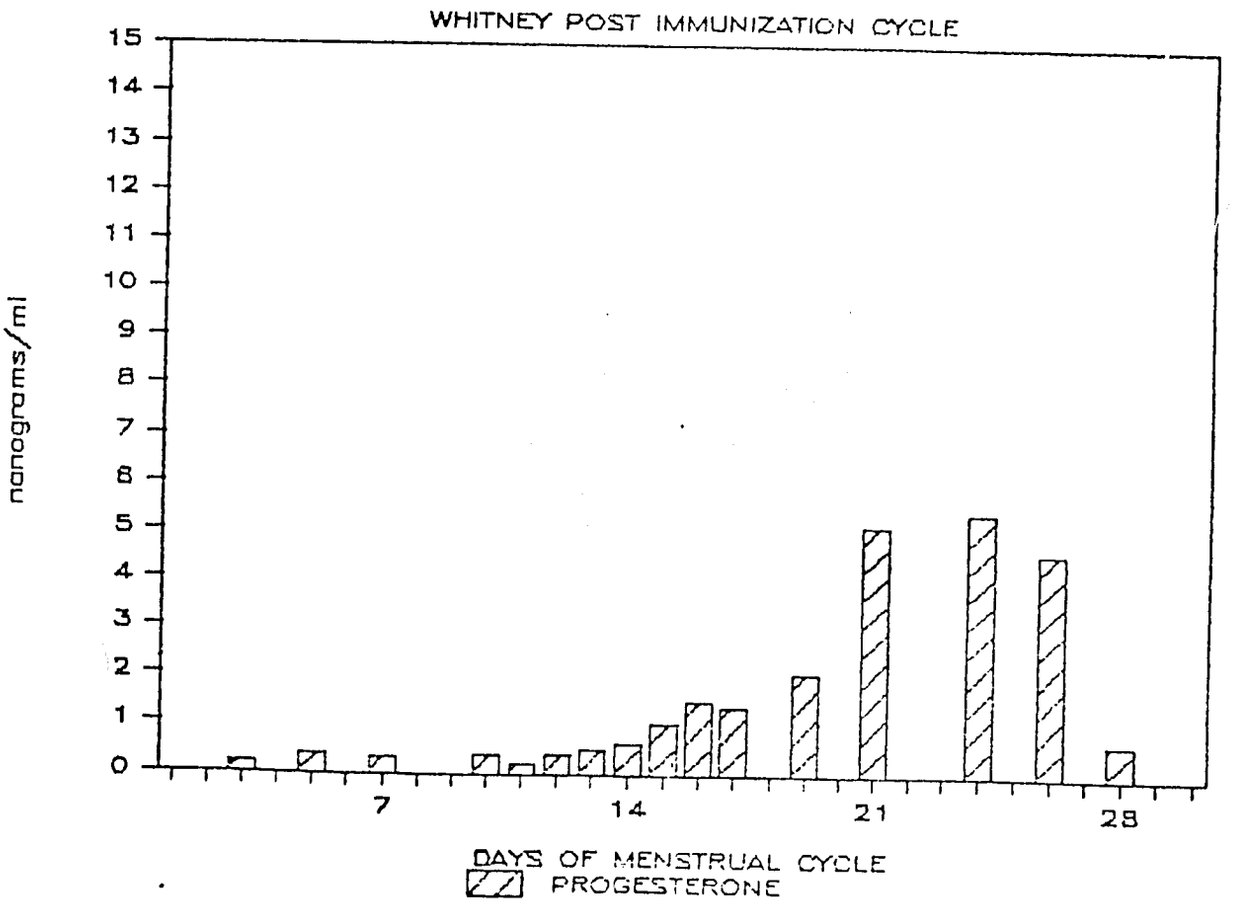

WHITNEY POST IMMUNIZATION CYCLE
picograms/ml
300
280
280
240
220
200
180
180
140
120
100
80
80
40
20
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
ESTRADIOL

FIGURE 20


WHITNEY CONTROL CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
PROGESTERONE


WHITNEY POST IMMUNIZATION CYCLE
nanograms/ml
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
DAYS OF MENSTRUAL CYCLE
PROGESTERONE

CONCLUSIONS

In the absence of data on the quantitative and qualitative characteristics of the antigens provided and the levels of antibodies attained at given times of the study, the observations made by this investigator are not readily interpretable. However, the PI has all the data from the study and her report will be more comprehensive in these details.

Despite these uncertainties, it is apparent that immunizations with the ZP-1 antigen provided clearly interfered with ovarian function in the baboon. Effects found with ZP-3 immunizations were minor, if any, and a careful correlation of antibody levels and the few anovulatory cycles will need to be made before it can be determined whether this antigen holds promise for human antifertility vaccine development. Should it be revealed that significant titers existed throughout the period of study in which menstrual cycle parameters were normal, antifertility effects from immunization could be assessed in other females immunized with ZP-3.

FINAL REPORT

TOXICITY STUDY OF AN IODINE TRANSCERVICAL STERILIZATION MEDIUM

Program for Applied Research on Fertility Regulation (PARFR)
Northwestern University Subagreement Number 385

Principal Investigator: D.P. Waller Ph.D.
Associate Professor
Department of Pharmacodynamics
College of Pharmacy

Institution: University of Illinois at Chicago
Health Sciences Center
P.O. Box 6998
Chicago, Illinois 60680

Subagreement Period:

Starting Date - 1 November 1985
Ending Date - 30 June 1986

A. SUMMARY

A new iodine containing formulation to be administered using the Femcept^R delivery system is being developed for transcervical sterilization of women. This study was performed to evaluate the potential toxicity of the iodine/iodide sterilization medium in direct contact with the uterus and the peritoneal cavity. The Femcept^R delivery system minimizes contact of the formulation with the uterus, but some direct contact may occur upon withdrawal of the administration device. There is also a potential for the formulation to spill over into the peritoneal cavity. This is also unlikely due to the small amount of formulation administered relative to the volume of the fallopian tubes. This study was not intended to evaluate the efficacy of the sterilization medium.

Preclinical studies were conducted to determine if toxicity will occur following administration of the agent due to direct contact of the material within the reproductive tract or in the peritoneal cavity should leakage occur via the fallopian tubes.

The Cynomolgus monkey was the animal model. The test formulation was administered using the transcervical and intraperitoneal routes. The transcervical route of administration is the proposed route of clinical administration. The intraperitoneal route was used to mimic an accidental leakage of the sterilization medium out of the reproductive tract into the peritoneal cavity.

The animals were monitored on a regular basis for clinical behavior and blood obtained for clinical chemistry just prior to treatment, 15 days after treatment and just prior to sacrifice(day 45). Forty five days after administration of ICF the animals were sacrificed and necropsied.

No adverse effects of the iodine/iodide containing medium were observed by either gross or microscopic histopathological examination of tissues from IP or transcervically treated animals.

B. BACKGROUND

Recent studies by Richert & Li demonstrated that a mixture of iodine, potassium iodide, VASCORAY^R, glycerol and gum tragacanth may be a safe and effective tubal closure agent which could be administered transcervically with the FEMCEPT^R system.

However, prior to the initiation of the clinical trials preclinical toxicology must be performed.

Dr. J. Shelton(AID) discussed with the FDA what the requirements would be for preclinical toxicity testing. It was agreed that a study which investigated target tissue contact and intraperitoneal contact with the proposed sterilizing agent be performed in sub-human primates.

C. STATEMENT OF OBJECTIVES

General

The primary purpose of this project is to evaluate the toxicity of a new iodine containing formulation(ICF) to be used for transcervical sterilization of women.

Prior to the initiation of clinical trials, preclinical toxicity testing must be performed on the iodine/iodide formulation. Both direct reproductive tissue contact and possible leakage of the ICF to local tissue sites must be considered.

Specific Objectives of the Study

The test material will be prepared according to GMP by BioNexus, Inc. Because of the expertise required for the transcervical administration of agents to the cynomolgus monkey, it is necessary for Dr. T. Parmley, Johns Hopkins, to perform the actual surgical procedures.

The techniques to be used are described below.

Peritoneal Administration

The animal will be anesthetized and a small midline abdominal incision made and using a 0.5 ml syringe with a blunt needle, 0.25 ml of the test material will be placed within the peritoneal cavity between the uterus and colon. The incision is closed and the animal placed in an upright position with the pelvis down for fifteen minutes to allow good distribution of the test substance within the peritoneal cavity.

Transcervical Administration

The monkeys are anesthetized and placed in a prone position. A nasal speculum is used to open the vagina and visualize the cervix. An 18 guage, blunt tip needle, approximately 12 cm long and with a slightly bent tip is placed into the endocervical canal and manipulated so that it passes into the endometrial cavity.

After placement of the needle, the animal will be positioned under a fluoroscope. A volume of 0.1 ml of the test compound will be injected and monitored. Repeated doses of 0.1 ml will be administered until two thirds of the fallopian tubes are filled with the test substance.

The monkeys will be divided into four test groups.

Group 1(Intraperitoneal vehicle control): Three monkeys treated intraperitoneally with 0.25 ml of the vehicle.

Group 2(Intraperitoneal test compound): Five monkeys treated intraperitoneally with 0.25 ml of the test material.

Group 3(Transcervical vehicle control): Three monkeys treated transcervically with vehicle to fill 2/3 of the fallopian tubes.

Group 4(Transcervical test compound): Five monkeys treated transcervically with vehicle to fill 2/3 of the fallopian tubes

All animals will be identified,weighed and placed in quarantine. At the start of the study all monkeys will be weighed and randomized into groups.

The animals will be dosed as described in the previous section and observed daily. All animals that die prior to scheduled sacrifice will be necropsied by one of the veterinary staff of the BRL to determine the cause of death.

Blood will be drawn from the monkeys just prior to the administration of the test material. Samples will also be drawn after surgery and on days 1,2,15 and 44 of the study. Blood workup will include routine electrolyte and enzymes as well as hemogram with differential. In addition, T_3, T_4 and TBI will be determined.

D. PROGRESS TO DATE

The test material was prepared according to GMP by BioNexus, Inc.(Raleigh, NC) as preloaded 10 ml syringes containing 5 ml of the test or vehicle control formulation. Each 0.15 ml of the test material contained 8.25 mg I_2, 19.8 mg KI, 0.015 ml glycerol, 0.1 ml VASCORAY and 4.5 mg gum tragacanth. The test preparation was shipped and stored in light-tight containers at room temperature. Vehicle (Each 0.15 ml contained 0.1 VASCORAY and 4.5 mg gum tragacanth) was also prepared for vehicle treated control groups.

Because of the expertise required for the transcervical administration of agents to the Cynomolgus monkey, Dr. T. Parmley, Johns Hopkins performed the actual surgical procedures.

A group of 19 Cynomolgus macaques were quarantined for at least 60 days prior to surgery. Upon arrival each animal was weighed and a complete physical examination performed. Each animal was randomly assigned to a treatment group after the quarantine period.

Prior to treatment all animals were anesthetized with Ketamine, weighed and blood obtained for a hemogram with differential(1 ml in EDTA tube) and complete blood chemistry analysis(24 parameters) including a thyroid profile(at least 4 ml in untreated tube).

For transcervical administration, each animal was placed in a prone position on an inclined support with the hips elevated and the hind limbs hung over the end of the table. This provided ready access to the vaginal orifice. A nasal speculum was inserted into the vagina, the cervix visualized and the posterior lip of the cervix grasped with a mucosaclip to stabilize the cervix. An 18-guage, blunt tip needle, approximately 12 cm long and with a slightly bent tip was placed into the endocervical canal. The needle was inserted into the cervical opening with the curve directed ventrally. When resistance was encountered, the needle was withdrawn several millimeters and rotated so that the curve of the needle was in a dorsal position. Gentle probing with the needle was required until the main canal was engaged and entered.

After placement of the needle, the animal was positioned under a fluoroscope. A 10 ml syringe preloaded with a minimum of 5 ml of either vehicle or iodine formulation was attached to the needle and injected into the uterine cavity while being monitored by the fluoroscope. The formulation was injected until the entire uterus was visualized. In all successful cases one or both of the fallopian tubes were visualized during the injection of the formulation. It should be noted that the fallopian tube of the Cynomolgus monkey is much smaller than the human. In some cases, the ovarian end of the fallopian tube was visualized without observing the proximal end. This was probably due to the small amount of formulation within the small diameter portions of the fallopian tube. During injection of formulation into the uterus of the last five animals (Two controls and three iodine treated animals), a picture of the fluoroscope visualization was obtained. Three of the pictures clearly show the penetration of the formulation throughout one or both of the fallopian tubes(prints of the flat plate for these animals are attached as ADDENDUM 1).

A clear outline of the uterus was not obtained during the injection of one animal being treated with vehicle and a second with iodine formulation. Injection of the medium resulted in a dispersion of the formulation as observed under fluoroscopic examination. It was assumed that the formulation was being dispersed into the fluids of the peritoneal cavity. Perforation of the cervix/uterus was assumed for both of these animals. Neither of these animals were included in the study groups. Both animals later exhibited signs of abdominal infections. The animal administered the vehicle formulation was sacrificed due to its deteriorating health. A massive peritonitis was observed upon sacrifice. The other animal administered the iodine/iodide formulation appeared to recover and was sacrificed at the end of the experiment. These observations provide some evidence that the iodine/iodide formulation may be bacteriocidal. It is possible that the bacteriocidal activity of

the test formulation inhibited the growth of organisms introduce into the peritoneal cavity following perforation of the cervix.

The time required for successful penetration into the uterine cavity varied greatly between animals(approx 3-30 min). Transcervical administration of a formulation was successful in seven of the fifteen animals attempted. Three animals were treated with vehicle formulation, three with iodine formulation and one animal was unintentionally treated with both vehicle and the iodine formulation.

Intraperitoneal injections were begun upon completion of the transcervical administrations. There were no problems with the IP administration. The formulations were transferred from the ten ml syringes to 1.0 ml syringes just before surgery by Dr. Parmley using sterile techniques. Ketamine was used (15-20 mg/kg I.M) for induction and an I.V. drip used for the administration of ketamine as required during surgery. The animal was prepped with prepodyne and a sterile drape applied. A small midline incision (1-2 cm long) 2 cm below the umbilicus was made. The omentum was gently lifted out of the peritoneal cavity and the formulation placed directly onto the small bowel. The omentum was repositioned within the peritoneal cavity and the incision was then closed using 3-0 Dexon [with a 19 mm cutting needle(CE-4)] using a figure of 8 stitch.

Three animals were administered vehicle and five animals were administered the iodine/iodide formulation. Attempts to visualize the intraperitoneal injection site using fluoroscopy were unsuccessful. The formulation is apparently readily distributed within the peritoneal cavity and too dilute to be observed using normal visualization methods.

Blood was drawn from the monkeys just prior to the administration of the test material and on days 1, 2, 14 and 44 of the study. Blood workup included routine electrolyte and enzymes as well as a hemogram with differential(a list of the parameters measured is attached as ADDENDUM 2). In addition T_3, T_4 and free T_3, T_4 were determined. No major changes were observed in either the treated or control groups. The mean values of T_3, T_4 and free T_3, T_4 for the treatment groups are found in ADDENDUM 3.

All animals were weighed and clinically observed every three days for 2 weeks, then weekly thereafter. On day 45 the animals were sacrificed and necropsied. Tissue sections for histopathological evaluation were obtained as follows: 1) All gross lesions/masses, 2) reproductive organs including ovaries, fallopian tubes, uterus, cervix and upper vagina, 3) large and small intestine, 4) kidney, 5) lung, 6) heart, 7) spleen, 8) thyroid and 9) liver.

The tissue specimens were sent to Pathology Associates for histopathological examination.

The histopathological examination revealed that the iodine sterilization medium resulted in fibrotic lesions in the lamina propria of the oviducts of three of four monkeys administered the material transcervically. Similar changes were not present in any monkey given a placebo via the same route. During the administration of the formulation there was clear spillover into the peritoneal cavity in most animals due to the large amount of formulation administered. There were no manifestations of toxicity in the peritoneal cavities of these animals.

There was no evidence of pathology following intraperitoneal administration of the formulation. Mild multifocal inflammation on the serosal surface of the uterus was present in only one of five monkeys given the iodine sterilization medium via the intraperitoneal route and in no monkeys given placebo via the same route.

These studies provide evidence that the iodine/iodide sterilization medium is not toxic to organs which may accidentally come into contact with the sterilization medium.

The complete histopathological report is attached as ADDENDUM 4.

E. UNANTICIPATED RESULTS

During the attempts to administer the iodine/iodide formulation transcervically, the cervix/uterus of two animals was perforated. This was followed by the administration of the control formulation in one animal and the iodine/iodide formulation in the second animal. Both animals were excluded from the treatment groups but were followed clinically and necropsied. The animal treated with the control formulation developed a severe peritonitis and was autopsied prior to the end of the exposure period. The animal treated with the iodine/iodide formulation developed some signs of a peritoneal infection but its health did not deteriorate such that an early sacrifice was required.

It was surmised that the animal treated with the control formulation became ill because of bacterial contamination following perforation of the cervix/uterus, while the iodine/iodide formulation acted as a local sterilization medium following perforation of the uterus and the administration of the formulation.

F. RESEARCH DESIGN

The random assignment of the animals to groups was not followed in the actual treatment of the animals. The transcervical administration of the formulations could not be performed in some animals(See interim report by Dr. T. Parmley). It was thus necessary to attempt transcervical administration to all animals in the group until an adequate number of animals had been treated with both the control and iodine/iodide formulations.

The number of animals in the transcervical treated group had to be decreased to three animals instead of the five proposed due to the difficulties associated with the transcervical administration and the perforation of the uterus in two animals.

The volume of the formulations administered by the transcervical route was greater than the amount anticipated. The viscosity of the medium and the amount filling the uterine cavity was larger than anticipated resulting in larger volumes of formulations required to force the medium into the fallopian tubes.

G. OPERATIONAL SIGNIFICANCE

The project has demonstrated the nontoxic nature of an iodine/iodide formulation intended to be administered by the transcervical route of administration.

H. BUDGET REPORT

Attached as ADDENDUM 5

The budget report sent to PARFR on 22 October, 1986 requests a payment of $36,304.07. This amount exceeds the budgeted amount by $5,913.97. These costs were inappropriately charged to the PARFR-385 account and a transfer of funds has been submitted for this amount to reduce the total cost of the project to the budgeted amount. PARFR should receive from the University of Illinois notification of the reduced charges.

BLOOD PARAMETERS MEASURED

HEMATOCRIT	ALK P (U/L)
HEMOGLOBIN	BUN (mg/dL)
WBC	GOT (U/L)
RBC	GPT (U/L)
STAB. NEUT.	CHOL (mg/dL)
SEG. NEUT.	GLU (mg/dL)
EOSINOPHILS	PHOS (mg/dL)
BASOPHILS	ALB (g/dL)
LYMPHOCYTES	BIL T (mg/dL)
MONOCYTES	TP (g/dL)
MCV	CAL (mg/dL)
MCH	CREAT (mg/dL)
MCHC	LDH-L (U/L)
	CPK (U/L)
	TRIG (mg/dL)
	K
	Na
	Cl

ADDENDUM-3

TABLE OF THYROID VALUES

TRANS-PLACEBO

	T4	T3	FT4	FT3
PRIOR TO TREATMENT				
MEAN	6.8	180.0	1.1	0.6
SD	48.9	27.3	0.6	0.1
DAY AFTER				
MEAN	7.8	145.0	1.1	0.5
SD	1.0	10.0	0.2	0.1
2 DAYS AFTER				
MEAN	7.6	138.3	0.8	0.6
SD	2.0	20.1	0.2	0.1
14 DAYS AFTER DOSING				
MEAN	7.1	181.7	0.7	0.6
SD	1.5	19.3	0.2	0.1
AT AUTOPSY				
MEAN	5.9	178.3	0.8	0.3
SD	1.5	25.6	0.3	0.1

TRANS-TREATED

PRIOR TO TREATMENT				
MEAN	6.3	215.0	0.9	0.7
SD	0.9	0.0	0.2	0.1
DAY AFTER				
MEAN	9.0	127.7	2.3	0.5
SD	2.9	54.1	1.3	0.2
2 DAYS AFTER				
MEAN	7.5	106.7	2.1	0.4
SD	1.0	12.5	0.9	.0
14 DAYS AFTER DOSING				
MEAN	5.7	180.0	0.6	0.6
SD	0.5	25.5	0.2	0.1
AT AUTOPSY				
MEAN	4.6	193.3	0.7	0.4
SD	0.7	27.2	.0	0.1

IP PLACEBO

PRIOR TO TREATMENT				
MEAN	9.3	246.7	1.5	1.0
SD	1.9	54.4	0.5	0.2
DAY AFTER				
MEAN	9.7	176.7	0.9	0.7
SD	0.2	27.2	0.4	0.1
2 DAYS AFTER				
MEAN	9.4	216.7	1.0	0.7
SD	1.5	34.7	0.3	0.1
14 DAYS AFTER DOSING				
MEAN	7.5	205.0	0.9	0.8
SD	0.8	22.7	0.3	0.1
AT AUTOPSY				
MEAN	5.6	200.0	0.7	0.4
SD	0.4	16.3	0.1	.0

IP TREATED

PRIOR TO TREATMENT				
MEAN	7.5	158.0	1.0	0.6
SD	1.0	26.8	0.2	0.1
DAY AFTER				
MEAN	6.5	111.0	1.5	0.4
SD	0.3	20.1	1.0	0.1
2 DAYS AFTER				
MEAN	7.5	144.2	1.1	0.6
SD	1.3	45.9	0.2	0.2
14 DAYS AFTER DOSING				
MEAN	5.4	212.0	0.6	0.7
SD	0.5	59.8	0.1	0.2
AT AUTOPSY				
MEAN	5.2	175.0	0.7	0.4
SD	1.2	27.2	0.3	0.1

Addendum 4

TOXICOLOGY RESEARCH LABORATORY
UNIVERSITY OF ILLINOIS AT CHICAGO
HEALTH SCIENCES CENTER
TOXICITY STUDY OF AN IODINE
TRANSCERVICAL STERILIZATION MEDIUM
IN CYNOMOLGUS MONKEYS

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I. PATHOLOGY NARRATIVE

TOXICITY STUDY OF AN IODINE TRANSCERVICAL STERILIZATION MEDIUM

INTRODUCTION

This is the final report by Pathology Associates, Inc. (PAI) to the Toxicology Research Laboratory, University of Illinois at Chicago, Health Sciences Center representing the results of pathology support required to conclude the study designated "Toxicity Study of an Iodine Transcervical Sterilization Medium."

EXPERIMENTAL DESIGN AND METHODS

Sixteen female cynomolgus monkeys were dosed with either an iodine formulation (test) or vehicle (placebo) via transcervical or intraperitoneal (IP) routes of administration. Attempts to pass the dosing needle through the cervix resulted in perforation of the uterus/cervix in two additional test animals. The test groups are shown below.

IP Placebo	IP Test	Transcervical Placebo	Transcervical Test
5226	5227	5224	5228
5229	5238	5230	5231
5243	5239	5247	5235
	5241	5248	5244[1]
	5246		

Perforated cervix/uterus (Placebo Injected) 5245
Perforated cervix/uterus (Iodine medium injected) 5236

[1]Animal 5244 was initially given placebo, then test medium two days later

Forty-five days after treatment, the animals were sacrificed and necropsied in accordance with the protocol. Selected tissues and all gross lesions were fixed in 10% neutral buffered formalin and sent to PAI for processing and histopathologic evaluation. Required tissues and gross lesions were sectioned at five microns, stained with hematoxylin and eosin, and examined microscopically.

RESULTS AND DISCUSSION

Gross Findings

At necropsy, gross lesions were found in the peritoneal cavity of ten monkeys and in the thoracic cavity of one monkey. Most of the peritoneal lesions consisted of adhesions between organs in the lower abdomen while the thoracic lesion consisted of pleural adhesions. One additional animal was found to have pleural adhesions at trim. A list of gross lesions by test group is found in the table below. A comprehensive listing of gross lesions and corresponding microscopic findings is found in the Gross and Microscopic Correlation Table.

Gross Lesion	IP Placebo	IP Test	Transcervical Placebo	Transcervical Test	Other
Adhesion, peritoneal	5229		5223, 5247	5231, 5235, 5244	5236, 5245
Cyst(s) Ovary		5238	5228		
Abscess, retroperitoneal			5230		
Parasite, l. Intestine			5230		
Perforation, l. Intestine			5230		
Discoloration, liver			5230		
Hemorrhage, vagina					5245
Hemorhage, rectum					5245
Adhesion, lung	5226		5224 (trim)		
Purulent exudate, abdomen					5245

Histopathology

All available required tissues and gross lesions were examined microscopically. Findings are presented in three tables. The Histopathology Incidence Table (II), presents diagnoses for all animals in the following manner. Along the left-hand margin, diagnoses are listed by organ/tissue. Corresponding to these diagnoses are numbers in blocks beneath the animal numbers (by test group). Numbers indicate severity of the lesion (1=minimal, 2=mild, 3=moderate, 4=marked). The letters "N" or "M" may also be present signifying that the organ/tissue was NORMAL or MISSING respectively.

A summary of diagnoses by test group is presented in the Project Summary Table (III). Corresponding to the organ/tissue along the left-hand margin are the number of animals in that group for which the organ/tissue was examined.

Corresponding to the diagnoses listed below the organ/tissue are the number of animals in each group for which that diagnosis was made.

The Gross and Microscopic Correlation Table (IV) lists all gross lesions found at necropsy with corresponding microscopic diagnoses. Additional microscopic comments regarding selected animals are found in Section V.

Oviducts - As specified by the study director, sections of each oviduct at the isthmus and ampulla were made. No oviduct lesions were present in any animal in the Intraperitoneal Vehicle Formulation (IP Placebo), Intraperitoneal Iodine Formulation (IP Test), or Transcervical Vehicle Formulation (Transcervical Placebo) groups, nor in Monkey No. 5236 in which the cervix/uterus was perforated and test media was injected (Perforation/ Test). In the Transcervical Iodine Formulation (Transcervical Test) group, two monkeys (5228 and 5244) had lesions in the sections of the isthmus, but not the ampulla. In both animals, changes consisted of thickening and fibrosis of the lamina propria. Monkey 5228 had moderate fibrosis in the lamina propria of the right isthmus, but no lesion in the left oviduct. Monkey 5244 had marked fiborsis in the lamina propria of the left isthmus and moderate fibrosis in the lamina propria of the right isthmus. In monkey 5245 in which the cervix/uterus was perforated and vehicle formulation was subsequently injected (perforation/placebo), a marked active-chronic inflammation with profound fibrous connective deposition was present on the serosal surface of both oviducts as well as most other abdominal tissues. Because of the extensive fibrosis, the isthmus of the left oviduct was not recovered from the wet tissues. The isthmus of the right oviduct, however, had diffuse, moderate, fibrosis in the lamina propria similar to the two monkeys in the Transcervical Test group.

Though not required by protocol, sections of one or both oviducts at the pars interstitialis or at the junction of the isthmus and pars interstitialis were present in the cranial section of uterus of all but one of the monkeys (5230) (data not present on tables, but comments in Section V). Changes were present in only the Transcervical Test group and Monkey 5245 (perforation/placebo). In 5228, both sides had markedly reduced luminal diameters and increased fibrous connective tissue in the lamina propria. In 5244, the changes are bilaterally more severe and are accompanied by marked attenuation of the mucosa. In 5231, only one segment is present (side not identified) and shows similar changes. No lesions were present in the pars

interstitialis of the other monkey in the Transcervical Test group (5235), but Monkey 5245 (perforation/placebo) had lesions in the pars interstitialis identical to the other three Transcervical Test group animals.

Uterus - Microscopic lesions were relatively few and showed no correlation to test group. Hyaline degeneration of uterine blood vessels characterized by marked medial thickening with a lightly eosinophilic acellular material and occassional mineralization was present in two monkeys in the IP Placebo group, one monkey in the IP Test group, one in the Transcervical placebo group, and both monkeys having perforation of the cervix/uterus. Such changes are common in multiparous animals of many species and warrant no further discussion. Other lesions included mild inflammation in the myometrium of two monkeys (5229, 5235), marked inflammation of the serosa of one monkey (5245), mild or moderate multifocal inflammation of the serosa of two monkeys (5231, 5239), mild or moderate endometrial hemorrhage in two monkeys (5230, 5245), and mild endometrial gland dilatation in one monkey (5230). The uterine changes do not appear related to the test article.

Cervix, Vagina - The only lesion considered to be experiment-related is the mild serosal fibrosis in monkey 5245 (perforation/placebo).

Ovary - Follicular cysts were present in four monkeys. The inflammation accompanying the pentonitis in other tissues in monkey 5245 was present along the serosa of the ovaries. No test medium-associated changes were present.

Lung - Brown, granular, anisotropc pigment was present in the lungs of all monkeys indicative of infestation with lung mites, Pneumonyssus simicola. The inflammation and hemorrhage and other changes seen in several lungs are probably associated with the presence of these parasites. These parasites and associated changes are ubiquitous in this species of primates.

Liver - Liver flukes (Opisthorchis sinensis) were present in the bile ducts of three monkeys. With the exception of the serosal inflammation in monkey 5245, other findings were considered incidental.

Large Intestine-Nematode parasites (Oesophagostomum sp.) were present in the large intestine of one monkey (5230). It is likely that these parasites resulted in bowel perforation and the subsequent retroperitoneal

inflammation seen in that animal. The only other lesions noted in the large intestine were serosal inflammation (peritonitis) seen in monkey 5245 and a focal abscess in monkey 5236 which was probably associated with

perforation of the cervix/uterus. All other lesions noted are considered incidental findings and will not be further addressed.

CONCLUSION

The iodine sterilization medium resulted in fibrotic lesions in the lamina propria of the oviducts of three of four monkeys administered the material transcervically. Similar changes were not present in any monkeys given a placebo via the same route and in only one other monkey in which marked peritonitis was the likely result of experimental perforation of the cervix/uterus.

Mild multifocal inflammation on the serosal surface of the uterus was present in only one of five monkeys given the iodine sterilization medium via the intraperitoneal route and in no monkeys given placebo via the same route. Under the conditions of this study, the iodine Transcervical Medium is not considered toxic in organs other than the oviducts.

Lucas H. Brennecke

Lucas H. Brennecke, D.V.M.
Diplomate, ACVP
2 April 1986

II. HISTOPATHOLOGY INCIDENCE TABLES

HISTOPATHOLOGY INCIDENCE TABLE

Study No. 0013
Toxicity Study of Iodine Transcervical Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo			Intraperitoneal Test Medium					Transcervical Placebo				Transcervical Test Medium				Perforation (Placebo)	Perforation (Test Medium)
	522 6	522 9	524 3	522 7	523 8	523 9	524 1	524 6	522 4	523 0	524 7	524 8	522 8	523 1	523 5	524 4	524 5	523 6
LEFT OVIDUCT (AMPULLA)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N
Inflammation, acute, multifocal																	2	
SEROSA-Inflammation, active-chronic, diffuse																	4	
LEFT OVIDUCT (ISTHMUS)	N	N	N	N	N	N	N	N	N	N	N	N	N	M	N		M	N
LAMINA PROPRIA-Fibrosis, diffuse																4		
RIGHT OVIDUCT (AMPULLA)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N
SEROSA-Inflammation, active-chronic, diffuse																	4	
RIGHT OVIDUCT (ISTHMUS)	N	N	N	N	N	N	N	N	N	N	N	N		N	N			N
SEROSA-Inflammation, active-chronic, diffuse																	4	
LAMINA PROPRIA-Fibrosis, diffuse													3			3	3	

N = Normal M = Missing P = Present

1: Minimal 2: Mild 3: Moderate 4: Marked

HISTOPATHOLOGY INCIDENCE TABLE

Study No. 0013
Toxicity Study of Iodine Transcervical Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo			Intraperitoneal Test Medium					Transcervical Placebo				Transcervical Test Medium				Perforation (Placebo)	Perforation (Test Medium)
	52266	52299	52443	52227	52238	52239	52241	52246	52224	52230	52247	52248	52228	52231	52235	52244	52245	52236
UTERUS (UPPER)	N			N		N	N	N	N		N	N	N	N		N		
BLOOD VESSELS-Hyaline degeneration, multifocal		2	3		4												2	3
BLOOD VESSELS-Hyaline degeneration, focal										2								
BLOOD VESSELS-Mineralization, multifocal					3													
ENDOMETRIUM-Hemorrhage, multifocal										2							3	
MYOMETRIUM-Inflammation, subacute, multifocal															2			
SEROSA-Inflammation, active-chronic, multifocal																	4	
UTERUS (LOWER)	N		N	N	N		N	N	N		N	N	N		N	N	M	N
MYOMETRIUM-Inflammation, subacute, multifocal		2																
GLANDS-Dilatation, multifocal										2								
SEROSA-Inflammation, pyogranulomatous, multifocal														3				
SEROSA-Inflammation active-chronic, multifocal						2												

N = Normal M = Missing P = Present

1: Minimal 2: Mild 3: Moderate 4: Marked

HISTOPATHOLOGY INCIDENCE TABLE

Study No. 0013
Toxicity Study of Iodine Transcervical Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo			Intraperitoneal Test Medium					Transcervical Placebo				Transcervical Test Medium				Perforation (Placebo)	Perforation (Test Medium)
	5226	5229	5243	5227	5238	5239	5241	5246	5224	5230	5247	5248	5228	5231	5235	5244	5245	5236
CERVIX	N		N	N	N		N	N	N	N	N	N	N	N	N	N		
Inflammation, acute, multifocal						2												2
SEROSA-Fibrosis, multifocal																	2	
SEROSA-Hemorrhage, multifocal		2																
VAGINA	N	N	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N
Lymphocytic infiltrate, multifocal													2					
OVARY, LEFT	N	N	N	N		N	N	N	N	N	N	N			N	N		N
Follicular cyst, focal													3	2				
Follicular cyst, multifocal					3													
SEROSA-Inflammation, active-chronic, diffuse																	4	
OVARY, RIGHT	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N
Follicular cyst, focal					3	2												
SEROSA-Inflammation, active-chronic, diffuse																	4	

N = Nor M = Missing P = Present

1: Mini... 2: Mild 3: Moderate 4: Marked

HISTOPATHOLOGY INCIDENCE TABLE

Study No. 0013
Toxicity Study of Iodine Transcervical Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo			Intraperitoneal Test Medium					Transcervical Placebo				Transcervical Test Medium				Perforation (Placebo)	Perforation (Test Medium)
	522 6	522 9	524 3	522 7	523 8	523 9	524 1	524 6	522 4	523 0	524 7	524 8	522 8	523 1	523 5	524 4	524 5	523 6
LUNG																		
Pigment, multifocal	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hemorrhage, focal		3																2
Edema, diffuse										3							3	
Congestion, multifocal																	3	
PERIBRONCHIAL-Inflammation, active-chronic, mf									2									
INTERSTITIAL-Inflammation, acute, focal				1										2				
INTERSTITIAL-Inflammation, active-chronic, multifocal							2											
PLEURA-Hemorrhage, focal									3									
PLEURA-Fibrosis, focal									2									
HEART	N	N		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
BLOOD VESSEL-Hyalin degeneration, focal			2															
BLOOD VESSEL-Mineralization, focal			2															

N = Normal M = Missing P = Present

1: Minimal 2: Mild 3: Moderate 4: Marked

HISTOPATHOLOGY INCIDENCE TABLE

Study No. 0013
Toxicity Study of Iodine Transcervical Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo			Intraperitoneal Test Medium					Transcervical Placebo				Transcervical Test Medium				Perforation (Placebo)	Perforation (Test Medium)
	5226	5229	5243	5227	5238	5239	5241	5246	5224	5230	5247	5248	5228	5231	5235	5244	5245	5236
LIVER																		
Lymphocytic infiltrate, focal	N		N		N		N	N					N					N
Lymphocytic infiltrate, multifocal																2		
Inflammation, subacute, multifocal									1									
Inflammation, necrotizing, focal		2		2							2						2	
Cytoplasmic vacuolization, multifocal		2																
Hepatocellular, degeneration, centrilobular, multifocal										4								
SEROSA-Inflammation, active-chronic, multifocal															2			
BILE DUCT-Cyst, focal																	3	
BILE DUCT-Metazoan parasite, focal										3								
BILE DUCT-Metazoan parasite, multifocal						2												
												2		3				
SMALL INTESTINE																		
SEROSA-Inflammation, active-chronic, multifocal	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N
																	2	

N = Nor I = Missing P = Present

1: Mini Mild 3: Moderate 4: Marked

HISTOPATHOLOGY INCIDENCE TABLE

Study No. 001
Toxicity Study of Iodine Transcervical Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo			Intraperitoneal Test Medium					Transcervical Placebo				Transcervical Test Medium				Perforation (Placebo)	Perforation (Test Medium)
	522 6	522 9	524 3	522 7	522 8	523 9	524 1	524 6	522 4	522 0	524 7	524 8	522 8	523 1	523 5	524 4	524 5	523 6
LARGE INTESTINE	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N		
Abscess, parasitic, focal										3								
Abscess, focal																		3
SEROSA-Inflammation, active-chronic, diffuse																	3	
Inflammation, active-chronic, multifocal										4								
KIDNEY, LEFT		N	N	N	N	N	N	N				N	N		N	N	N	N
INTERSTITIAL-Inflammation, chronic, multifocal	2								2									
TUBULES-Mineralization, multifocal									2	2	2							
Lymphocytic infiltrate, multifocal														2				
KIDNEY, RIGHT	N		N	N		N		N	N		M	N	N		N	N	N	N
Lymphocytic infiltrate, multifocal		2					1			2				2				
INTERSTITIAL-Inflammation, chronic, focal					2													

N = Normal M = Missing P = Present

1: Minimal 2: Mild 3: Moderate 4: Marked

HISTOPATHOLOGY INCIDENCE TABLE

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo			Intraperitoneal Test Medium					Transcervical Placebo				Transcervical Test Medium				Perforation (Placebo)	Perforation (Test Medium)
	522 6	522 9	524 3	522 7	523 8	523 9	524 1	524 6	522 4	523 0	524 7	524 8	522 8	523 1	523 5	524 4	524 5	523 6
SPLEEN	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N		N
Congestion										2							2	
Lymphoid depletion																	2	
SEROSA-Inflammation, active-chronic, multifocal																	3	
THYROID	N	N	N			N	N				N	N	N	N	N	N	N	N
Lymphocytic infiltrate, focal									2									
Hyperplasia, focal				2				1										
FOLLICLE-Dilatation, focal										2								
FOLLICLE-Dilatation, multifocal					2													
ABDOMEN / SKELETAL MUSCLE																		
Inflammation, active-chronic, multifocal																	3	
Sarcocyst																	P	

N = Normal H = Missing P = Present

1: Minimal 2: Mild 3: Moderate 4: Marked

HISTOPATHOLOGY INCIDENCE TABLE

Study No. 0013
Toxicity Study of Iodine Transcervical Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo			Intraperitoneal Test Medium					Transcervical Placebo				Transcervical Test Medium				Perforation (Placebo)	Perforation (Test Medium)
	522 6	522 9	524 3	522 7	523 8	523 9	524 1	524 6	522 4	523 0	524 7	524 8	522 8	523 1	523 5	524 4	524 5	523 6
SKELETAL MUSCLE, NOS																		
Inflammation, active-chronic, multifocal										4								
Sarcocyst										P								
URINARY BLADDER																		
SEROSA-Inflammation, active-chronic, diffuse																	4	
BROAD LIGAMENT																		
Inflammation, active-chronic, multifocal																	2	
TISSUE NOS																		
Necrogranuloma, focal															3			

N = Normal M = Missing P = Present

1: Minimal 2: Mild 3: Moderate 4: Marked

III. SUMMARY INCIDENCE TABLES

Summary Incidence Table

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo	Intraperitoneal Test medium	Transcervical Placebo	Transcervical Test Medium	Perforation (Placebo)	Perforation (Test Medium)
LEFT OVIDUCT (AMPULLA)	3	5	4	4	1	1
Inflammation, acute, multifocal					1	
SEROSA-Inflammation, active-chronic, diffuse					1	
LEFT OVIDUCT (ISTHMUS)	3	5	4	3	0	1
LAMINA PROPRIA-Fibrosis, diffuse				1		
RIGHT OVIDUCT (AMPULLA)	3	5	4	3	1	1
SEROSA-Inflammation, active-chronic, diffuse					1	
RIGHT OVIDUCT (ISTHMUS)	3	5	4	4	1	1
SEROSA-Inflammation, active-chronic, diffuse					1	
LAMINA PROPRIA-Fibrosis, diffuse				2	1	

Summary Incidence Table

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo	Intraperitoneal Test medium	Transcervical Placebo	Transcervical Test Medium	Perforation (Placebo)	Perforation (Test Medium)
UTERUS (UPPER)	3	5	4	4	1	1
BLOOD VESSELS-Hyaline degeneration, multifocal	2	1			1	1
BLOOD VESSELS-Hyaline degeneration, focal			1			
BLOOD VESSELS-Mineralization, multifocal		1				
ENDOMETRIUM-Hemorrhage, multifocal			1		1	
MYOMETRIUM-Inflammation, subacute, multifocal				1		
SEROSA-Inflammation, active-chronic, multifocal					1	
UTERUS (LOWER)	3	5	4	4	0	1
MYOMETRIUM-Inflammation, subacute, multifocal	1					
GLANDS-Dilatation, multifocal			1			
SEROSA-Inflammtion, pyogranulomatous, multifocal				1		
SEROSA-Inflammation, active-chronic, multifocal		1				

Summary Incidence Table

Study No. 0013
Toxicity Study of Iodine Transcervical Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo	Intraperitoneal Test medium	Transcervical Placebo	Transcervical Test Medium	Perforation (Placebo)	Perforation (Test Medium)
CERVIX	3	5	4	4	1	1
Inflammation, acute, multifocal		1				1
SEROSA-Fibrosis, multifocal					1	
SEROSA-Hemorrhage, multifocal	1					
VAGINA	3	5	4	4	1	1
Lymphocytic infiltrate, multifocal				1		
OVARY, LEFT	3	5	4	4	1	1
Follicular cyst, focal				2		
Follicular cyst, multifocal		1				
SEROSA-Inflammation, active-chronic, diffuse					1	
OVARY, RIGHT	3	5	4	4	1	1
Follicular cyst, focal		2				
SEROSA-Inflammation, active-chronic, diffuse					1	

Summary Incidence Table

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo	Intraperitoneal Test medium	Transcervical Placebo	Transcervical Test Medium	Perforation (Placebo)	Perforation (Test Medium)
LUNG	3	5	4	4	1	1
Pigment, multifocal	3	5	4	4	1	1
Hemorrhage, focal	1					1
Edema, diffuse			1		1	
Congestion, multifocal					1	
PERIBRONCHIAL-Inflammation, active-chronic, mf			1			
INTERSTITIAL-Inflammation, acute, focal		1		1		
INTERSTITIAL-Inflammation, active-chronic, multifocal		1				
PLEURA-Hemorrhage, focal			1			
PLEURA-Fibrosis, focal			1			
HEART	3	5	4	4	1	1
BLOOD VESSEL-Hyalin degeneration, focal	1					
BLOOD VESSEL-Mineralization, focal	1					

Summary Incidence Table

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo	Intraperitoneal Test medium	Transcervical Placebo	Transcervical Test Medium	Perforation (Placebo)	Perforation (Test Medium)
LIVER	3	5	4	4	1	1
Lymphocytic infiltrate, focal				1		
Lymphocytic infiltrate, multifocal			1			
Inflammation, subacute, multifocal	1	1	1		1	
Inflammation, necrotizing, focal	1					
Cytoplasmic vacuolization, multifocal			1			
Hepatocellular, degeneration, centrilobular, mf				1		
SEROSA-Inflammation, active-chronic, multifocal					1	
BILE DUCT-Cyst, focal			1			
BILE DUCT-Metazoan parasite, focal		1				
BILE DUCT-Metazoan parasite, multifocal			1	1		
SMALL INTESTINE	3	5	4	4	1	1
SEROSA-Inflammation, active-chronic, multifocal					1	

Summary Incidence Table

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo	Intraperitoneal Test medium	Transcervical Placebo	Transcervical Test Medium	Perforation (Placebo)	Perforation (Test Medium)
LARGE INTESTINE	3	5	4	4	1	1
Abscess, parasitic, focal			1			
Abscess, focal						1
SEROSA, Inflammation, active-chronic, diffuse					1	
Inflammation, active-chronic, multifocal			1			
KIDNEY, LEFT	3	5	4	4	1	1
INTERSTITIAL-inflammation, chronic, multifocal	1		1			
TUBULES-Mineralization, multifocal			3			
Lymphocytic infiltrate, multifocal				1		
KIDNEY, RIGHT	3	5	3	4	1	1
Lymphocytic infiltrate, multifocal	1	1	1	1		
INTERSTITIAL-Inflammation, chronic, focal		1				

Summary Incidence Table

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo	Intraperitoneal Test medium	Transcervical Placebo	Transcervical Test Medium	Perforation (Placebo)	Perforation (Test Medium)
SPLEEN	3	5	4	4	1	1
Congestion			1		1	
Lymphoid depletion					1	
SEROSA-Inflammation, active-chronic, multifocal					1	
THYROID	3	5	4	4	1	1
Lymphocytic infiltrate, focal			1			
Hyperplasia, focal		2				
FOLLICLE-Dilatation, focal			1			
FOLLICLE-Dilatation, multifocal		1				
ABDOMEN /SKELETAL MUSCLE					1	
Inflammation, active-chronic, multifocal					1	
Sarcocyst					1	

Summary Incidence Table

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

	Intraperitoneal Placebo	Intraperitoneal Test medium	Transcervical Placebo	Transcervical Test Medium	Perforation (Placebo)	Perforation (Test Medium)
SKELETAL MUSCLE, NOS			1			
Inflammation, active-chronic, multifocal			1			
Sarcocyst			1			
URINARY BLADDER					1	
SEROSA-Inflammation, active-chronic, diffuse					1	
BROAD LIGAMENT					1	
Inflammation, active-chronic, multifocal					1	
TISSUE, NOS				1		
Necrogranuloma, focal				1		

IV. CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLES

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Group/Animal Number	Tissue	Gross Observations	Microscopic Observations
IP Placebo 5226	Lungs	Adhered to diaphragm	No corresponding lesion
IP Placebo 5229	Multiple abdominal organs	Multiple intra-abdominal adhesions and innoculations of old blood consistent with endometriosis	No microscopic findings consistant with endometriosis were found
IP Test 5238	Ovaries	Both have large follicle cysts	L. Ovary-Follicular cyst, multifocal, moderate R. Ovary- Follicular cyst, focal, moderate
Trans Placebo 5224	Lung/Pleura	Adhesion-left diaphragmatic lobe detected at trim	Hemorrhage, focal, moderate Fibrosis, focal, mild
Trans Placebo 5230	Liver	Yellowish discoloration, 8 mm diameter	Cytoplasmic vacuolization, multifocal marked
	Liver	Possible encased parasite	/Bile duct - Cyst, focal moderate
	L. Intestine/Colon	Possible parasite	Abscess, parasitic, focal, moderate
	Skeletal Muscle/ Retroperitoneal	Abscess 3 cm x 5 cm, walled of abscess on dorsal RT retroperitoneal	Inflammation, active-chronic, multi-focal, marked
	L. Intestine/Cecum	Perforated and adhered to above abscess	Inflammation, active-chronic, multi-focal, marked
	Urinary Bladder	Gelatinous fluid on either side (about 1cc)	No Section Taken
Trans Placebo 5247	Urinary Bladder	Omental adhesion (minimal)	No corresponding lesion

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Group/Animal Number	Tissue	Gross Observations	Microscopic Observations
Trans Test 5228	Left Oviduct	Solitary ômental adhesion to the left tube	No corresponding lesion
	Ovary, left	Preovulatory-large follicle cyst	Follicular cyst, focal, moderate
Trans Test 5231	Uterus	Omentum adhesion to top of the uterus and to the pelvic peritoneum. Rectal	Serosa-Inflammation, pyogranulomatous multifocal, moderate
		sigmoid adherent to the posterior uterus and broad ligament. Multiple	
		omental adhesions to all surfaces.	
Trans Test 5235	Tissue, NOS	Possible area of old hemorrhage and adhesion	Necrogranuloma, focal, moderate
	Thyroid	Dark tan color	No corresponding lesion
Trans Test 5244	Uterus	Intraperitoneal adhesions omentum densely adherent to top of uterus; to both adnexa	No corresponding lesion
Perf Placebo 5245	Peritoneum	Omentum adhered to peritoneum and intestines-thickened purulent exudate	Ovaries, oviducts, uterus, urinary bladder, L. Intestine--Serosa-
		filling cavity	Inflammation act.-chronic, diffuse marked
	Uterus	Green-fibrous adhesions	Serosa-Inflammation, active-chronic, marked
	Urinary Bladder	Reddish area 5mm in diameter	Serosa-Inflammation, acitve-chronic marked
	Vagina	Hemorrhage of ventral vaginal wall adjacent to bladder	No corresponding lesion

Study No. 0013
Toxicity Study of Iodine Transcervical
Sterilization Medium in CYNO Monkeys

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Group/Animal Number	Tissue	Gross Observations	Microscopic Observations
Perf Placebo 5245	L. intestine	Petechial hemorrhages on rectum	Serosa-Inflammation, active-chronic, diffuse, moderate
Perf Test 5236	Abdomen, NOS	Large area of adhesions on left lower abdomen region typical of pelvic abscess	No corresponding lesion
	Uterus	Omentum and rectosigmoid were fixed to post. surface of uterus, the adhesions	No corresponding lesion
		incorp. the left adnexa and obliterated the posterior surface of uterus [coldfac]	

V. MICROSCOPIC COMMENTS

MICROSCOPIC COMMENTS

Intraperitoneal Placebo

5226

Both foci of inflammation in the left kidney are consistent with small chronic infarcts.

5229

No microscopic findings consistant with endometriosis were found.

5243

There is a prominent hydatid of Morgagni on the right ovary.

Intraperitoneal Test Medium

5227

One small sarcocyst is present in the peritracheal muscle adjacent to the thyroid gland.

The epithelial cells lining the oviducts appear poorly fixed in that the cells have indistinct borders and a very smudgy morphologic appearance.

Numerous granulocytes are present along the superficial vaginal epithelium, but none are present within the tissue.

5239

The parasite in the liver is morphologically consistant with a fluke, most likely *Opisthorchis sinensis*.

The moderate number of neutrophils in the vaginal lumen are assumed to have originated in the cervix, as very few are present within the vaginal wall.

5246

The secretory mucosal epithelial cells in the oviducts are very active.

Transcervical Placebo

5224

One of the foci of inflammation in the L. Kidney is a small chronic infarct.

5230

The right ventricle contains a fibrin clot which is most likely a post-mortem event.

The relatively localized cytoplasmic vacuolization on slide 16 (TGL 1) (fatty change) has a periportal distribution involving approximately 10 lobules. The bile duct cyst is surrounded by a moderate amount of fibrosis.

The parasites in the L. intestine are morphologically consistant with Oesophagostomum sp.

It is likely that the parasitic infestation in the large intestine resulted in bowel perforation and the subsequent retroperitoneal inflammation.

A few granulocytic inflammatory cells were seen in the lumen of the ampulla of both oviducts, but none were found in the walls of the oviducts.

5247

Most of the mucosa is missing from the isthmus of the right oviduct. This appears to be an artifactual alteration.

5248

The parasites in the bile ducts are morphologically consistant with flukes, most likely Opisthorchis sinensis.

The isthmus of each oviduct is immediately adjacent to the uterus on slide 11. The separate cross sections of oviduct on slides 11 and 12 are of ampulla, not isthmus.

Transcervical Test Medium

5228

The pars interstitialis segments of both oviducts have markedly reduced luminal diameters which correspond to the increased fibrous tissue in the lamina propria. The right isthmus is similarly affected, while the isthmus of the left oviduct is normal.

5231

The liver parasites are morphologically consistant with flukes; most likely *Opisthorchis sinensis*.

A modest number of neutrophils are present along the superficial surface of the vagina.

One pars interstitialis segment of the oviduct (side not determined) has a markedly decreased luminal diameter corresponding to a markedly thickened lamina propria due to fibrosis. The other is not present.

5235

The necrogranuloma consisted of a necrotic core surrounded by many hemosiderin-laden macrophages, plasma cells, and granulocytes. It probably represents an old focus of hemorrhage.

5244

The lumina of both pars interstitialis segments are nearly totally occluded. There is marked thickening and fibrosis of the lamina propria and attenuation of the mucosa in both segments as well.

Perforation (Placebo)

5245

The extreme peritonitis made recovery of all segments of the oviduct very difficult.

Both pars interstitialis segments of the oviduct have markedly reduced luminal diameters corresponding to increased fibrosis with subsequent thickening of the lamina propria. The mucosa is also bilaterally attenuated.

Perforation (Test Medium)

5236

Numerous neutraphils are present in the lumen of the cervix, but only a few are found in the stroma.

Modest numbers of neutrophils are present in the vaginal lumen along the epithelial surface, but very few are in the vaginal wall.

No pelvic abscess or inflammation was detected.

VI. QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE STATEMENT

This histopathology project has been conducted in compliance with Good Laboratory Practice regulations promulgated by the U. S. Food and Drug Administration and the U. S. Environmental Protection Agency. Pathology Associates, Incorporated has a functioning and responsive quality assurance unit which has conducted appropriate inspections and reported all findings to management. The following is a record of those inspections and reports:

Date of Inspection[1]	Phase Inspected	Date Findings Reported to Management and Study Pathologist
** 03-21-86	Tissue Trimming	03-21-86
** 03-24-86	Processing	03-25-86
** 03-24-86	Embedding	03-25-86
** 03-31-86	Microtomy	03-31-86
* 03-17-86	Staining	03-17-86
* 03-17-86	Coverslipping	03-17-86
* 03-17-86	Labeling	03-17-86
** 04-04-86	Individual Animal Data	04-04-86
** 04-04-86	Final Pathology Report	04-04-86

* General monthly phase inspection
** Inspection specific for Study Number

[1]In accordance with the PAI Quality Assurance Division's Standard Operating Procedures, phase inspections are routinely conducted on a random basis at a minimum of monthly. Dates of inspection are reported for each study according to the most recent phase inspection conducted during that period.

for: Carol C. Hoffman — April 07, 1986

Director, Quality Assurance Unit — Date

Study Number 0013: "Toxicity Study of an Iodine Transcervical Sterilization Medium".

IND 18,592

A Comparative Evaluation of 30 mg and 50 mg of 90-Day Norethindrone Microspheres for Controlled Release Steroid Contraception

PHASE I STUDY

January 1987

Family Health International
Research Triangle Park
North Carolina 27709

and

Program for Applied Research on Fertility Regulation
Northwestern University
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A Comparative Evaluation of 30 mg and 50 mg of 90-Day Norethindrone Microspheres for Controlled Release Steroid Contraception: Phase I Study

I. INTRODUCTION

Recent research has focused on the development of improved delivery systems for steroidal contraceptives that permit a relatively constant release of hormone through a biodegradable system over an extended period of time. A microsphere formulation which is intended to provide a 90-day delivery of the contraceptive steroid norethindrone (NET) has been developed. This formulation is injected intramuscularly.

A Phase I study of 50 mg, 75 mg and 100 mg of NET microspheres was conducted in 1985 to evaluate safety and to determine the test doses for the Phase II clinical trial. No adverse experiences were reported for the women. Pharmacokinetic data revealed consistent release of NET throughout the 90-day treatment interval, with successful ovulation inhibition in eight of the nine women. One woman, who received the 50 mg dose, had progesterone levels suggestive of ovulation in the first three weeks postinjection.

II. OBJECTIVES

The objectives of this 20-case Phase I study were (1) to evaluate the safety and effect on ovarian function of 30 mg and 50 mg doses of the injectable NET formulation and (2) to assess the serum concentrations of NET over the intended treatment intervals after each dose.

Safety is assessed by documenting and monitoring all reported or observed adverse experiences throughout the study period, including serial clinical laboratory evaluations; in addition, low and high density lipoproteins (LDL/HDL), sex hormone binding globulin (SHBG), and menstrual pattern disruptions in terms of bleeding and spotting episodes were documented over time. Effects on ovarian function were estimated over the course of the study by measuring serum levels of estradiol (E_2) and progesterone (P), and by scoring cervical mucus characteristics.

III. PATIENT SELECTION

Women in good physical health who were readily available for follow-up evaluation were asked to participate in the study. The following patient selection criteria were used to enroll women:

Inclusion criteria:

1. from 18 to 35 years of age

2. history of regular menstrual cycles from 23-35 days in length

3. normal physical, pelvic and breast examination

4. hemoglobin (11.0 g/dl or greater)

5. negative pregnancy test prior to the NET injection

6. sterilized at least 90 days before NET injection

Exclusion criteria:

1. history or evidence of impaired liver function, renal disease, clotting disorders, diabetes, cardiovascular dysfunction, infertility, ectopic pregnancy, or other contraindications of steroidal contraceptives

2. less than 102 lbs (46 Kg) or greater than 173 lbs (74 Kg)

3. breastfeeding

4. use of an injectable contraceptive in the past six months

5. smoking

6. use of barbiturates or antibiotics in the last 30 days

IV. STUDY PROCEDURES

This was a single center, randomly allocated study of single injections of two different doses of the injectable formulation. The center was located in San Antonio, Texas. During the preadmission cycle, baseline data were collected for each woman. Women were scheduled for injection between days 3 and 6 of the next menstrual cycle. Women returned for follow-up visits twice a week throughout the 90-day postinjection interval and until resumption of ovulation (approximately 120 days postinjection or at start of next menses).

The schedule of various tests and procedures is shown in Chart 1.

V. RESULTS

A. Status of Study

The study is complete. Women were enrolled from April 2, 1986 through May 16, 1986. An initial error in assigning study product at the center made it necessary to enroll patients in reverse of the preassigned numerical order; Patient 20 entered first and Patient 01 entered last. Random allocation of the products was followed correctly through the study. A summary of clinic visits completed is given in Table 1.

B. Demographics

Table 2 shows age and total live births for women participating in the study. The mean age of women receiving injections of 30 mg of NET microspheres was 30.9 years; the mean age for the 50 mg group was 32.4 years. Mean total live births was 2.5 for the 30 mg group and 2.8 for the 50 mg group.

C. Efficacy

1. Clinical

Since all women entering the study had been previously sterilized, pregnancy was not used as a measure of efficacy for this study.

2. Laboratory

Serum levels of estrogen and progesterone were evaluated to determine efficacy of ovulation suppression of the two doses. Cervical mucus scores and SHBG also were used to monitor ovulatory activity.

Norethindrone

Figure 1 and Table 3 show the mean serum concentrations of norethindrone for the 30 mg and 50 mg groups. Norethindrone levels show a bimodal curve. There was an initial rise of NET values shortly after injection which decreased until about the third week when values slowly rose and remained constant through week eleven.

NET values for individuals are given in Appendix A.

Progesterone and Estrogen

Figures 2 and 3 and tables 4 and 5 show the mean serum concentrations of progesterone and estrogen for the two dosage groups. Progesterone and estrogen values for individuals are given in Appendix A. The study was not designed to collect data on progesterone and estrogen after the first normal menses.

Of the 10 women in the 30 mg treatment group, five patients (07,11,15,17,19) had progesterone values suggestive of ovulation (> 3 ng/ml) during the period of treatment. Three of the five patients who did not ovulate during the 90 days that the drug was active (01,03,13) recovered ovulatory function (progesterone > 3 ng/ml) by Day 128. The remaining two did not show progesterone levels that suggested ovulation before their next normal menses. All women had had a normal menses by Day 157.

In the 50 mg group, two patients (08,16) had progesterone values suggestive of ovulation dduring the period of treatment. Five of the eight women who did not ovulate during the 90-day injection period returned to ovulation (progesterone > 3 ng/ml) by Day 116. The remaining three did not show progesterone levels that suggested ovulation before their mext normal menses. All women had had a normal menses by Day 154.

Cervical Mucus and SHBG

Cervical mucus scores over 10 are predictive of ovulation. The cervical mucus data obtained in this study did not appear to correspond with rises in estrogen levels and were not followed by elevated progesterone levels (Appendix A).

SHBG is not analyzed at this time because results from all blood samples are not available.

D. Safety

1. Clinical

Adverse Experiences

Table 6 shows medical conditions at admission for women entering the study. One woman in the 30 mg group and three women in the 50 mg group reported breast discharge preinjection.

Table 7 presents events occurring postinjection. Five women reported breast discharge during the study; four of these women had this condition at the time of injection. Eight women in the 30 mg group and seven women in the 50 mg group reported bleeding/spotting problems during the study. See Appendix B for individual patient listings.

Bleeding records

Table 8 shows daily bleeding and spotting reported by women during the study. Mean days of bleeding and spotting are presented for 30-day reference periods. Women reported an increase in the number of days of spotting during the first 90 days after drug administration. Spotting returned to baseline levels for the 91 to 120-day reference period. The increased spotting is similar to that reported for progestogen-only oral contraceptives.

Blood pressure

Table 9 shows elevated blood pressure values. In the 30 mg group, five patients (03,07,09,11,19) had a blood pressure (systolic or diastolic) elevation of 10% or more above baseline during the study period. Only Patient 11 reached a diastolic pressure of 90 mm Hg and this was on Day 119 in the postdrug period; she had returned to within the normal range by the next measurement on Day 147.

In the 50 mg group, Patient 08 had a diastolic pressure of 98 mm Hg at baseline. Throughout the rest of the study, her blood pressure was within the normal range. Three patients (06,12,14) had blood pressure elevations of 10% or more above baseline during the study period. Only Patient 14 reached a diastolic pressure of 90 on Day 63, and she returned to within the normal range by the next measurement on Day 94.

2. Laboratory Values

Laboratory data for individual patients are given in Appendix C.

Hemoglobin

Table 10 shows out-of-range hemoglobin values for women in the study. There were no abnormalities in the 30 mg group.

In the 50 mg group, Patient 05 had a significant reduction (17%) in hemoglobin at Days 28 and 56 postinjection. Although the patient was not treated, hemoglobin returned to the baseline value by Day 80.

Serum Liver Enzymes and Lipids

Table 11 shows out-of-range values of liver function tests (triglycerides, cholesterol, LDH-340, SGOT, CPK, alkaline phosphatase and total bilirubin) for women in the study. There were no abnormalities in cholesterol and alkaline phosphatase values in either treatment group.

In the 30 mg group, two patients (03 and 07) had elevated triglyceride levels but neither were clinically significant; Patient 03 had one elevated value on Day 28 which returned to normal for the rest of the study and Patient 07 had elevated values at entry and on Day 126 which was during the postdrug period. Patient 19 had one clinically insignificant elevated SGOT value on Day 91; her SGOT value had returned to normal by Day 148. The 30 mg group did not have elevated LDH-340, CPK and total bilirubin values during the postinjection period.

For the 50 mg group, four patients (05,08,12,20) had elevated triglyceride values. Patient 05 had an elevated value on Day 83 and Patient 08 had elevated values on Days 35 and 63. The triglyceride levels of both patients returned to normal levels for the rest of the study. Patient 12 had elevated values from entry throughout the study; the investigator reported that the woman refused to fast before blood sampling. Patient 20 had one elevated value at postdrug Day 154. Three patients (05,14,18) had elevated LDH-340 values during the course of the study. Patient 05 had elevated values at Days 28, 83 and 134. Patients 14 and 18 each had one clinically insignificant elevation. The 50 mg group did not have elevated SGOT, CPK and total bilirubin values during the postinjection period.

Table 12 shows LDL and HDL out-of-range values for women in the study. Only Patient 12 in the 50 mg group had an elevated value; at Day 91 her LDL value was very elevated. Since she had low values before and after, this may represent a laboratory error.

Renal Function

There were no abnormal values found for creatinine for either the 30 mg or the 50 mg group (Table 13).

Serum Electrolytes, Serum Protein and Glucose

Table 14 shows out-of-range values of glucose and serum electrolytes (sodium, potassium, chloride, calcium, and phosphorous), total protein and albumin for women in the study. No abnormalities in sodium, chloride, total protein or albumin values were reported in either treatment group.

For the 30 mg group, four patients (01,03,13,17) had elevated glucose values at scattered and irregular times postinjection, but none were considered clinically significant. Four patients (09,11,13,15) had elevated potassium levels. Only Patient 11 had a significant elevation (26%) which occurred at Day 150 postinjection. This treatment group did

not have elevated calcium or phosphorus levels during the postinjection period.

For the 50 mg group, Patient 12 had elevated glucose values postinjection, but none were considered clinically significant. Patient 05 had two slightly elevated potassium values on Days 83 and 134. Patient 18 had an insignificantly elevated phosphorus value on Day 63 which returned to normal thereafter. This treatment group did not have elevated calcium values during the postinjection period.

Urinalysis

Table 15 shows abnormal urinalysis (specific gravity, total protein, pH, WBC, RBC, ketones, glucose, bilirubin) values for women in the study. There were no abnormalities in ketones, glucose or bilirubin in either treatment group.

In the 30 mg group, two patients (11 and 13) were found to have pyuria before the study began and again at Day 91 when a few red blood cells were also found. The specimens obtained on Day 91 were reported by the investigator to be contaminated.

For the 50 mg group, Patients 05 and 06 had pyuria at entry and at their last visits and Patient 04 had a few white blood cells at Day 91. Patient 10 had a contaminated specimen with a few red blood cells on Day 91. Patient 16 had pyuria and a high red blood cell count on Day 91 which may have indicated a urinary tract infection.

VI. SUMMARY AND CONCLUSION

Twenty women were enrolled in a Phase I study to evaluate the safety and effect on ovarian function of 30 mg and 50 mg doses of 90-day NET microspheres.

There was an initial rise of NET values shortly after injection which decreased until the third week when values slowly rose and remained constant for at least 11 weeks.

Five women in the 30 mg treatment group and two women in the 50 mg treatment group had progesterone values suggestive of ovulation (> 3 ng/ml) during the 90-day postinjection period.

No disturbing side effects have been observed. Women reported an increase in the number of days of spotting during the 90 days of drug activity; spotting returned to baseline afer the first 90 days. Similar increased spotting has been reported for progestogen-only oral contraceptives.

Out-of-range laboratory values observed for women in the study occurred at scattered, irregular times postinjection and were clinically insignificant.

One woman (Patient 10) had pyruria and a high red blood count on Day 91 which may have indicated a urinary tract infection.

This study was conducted to assess the safety and potential efficacy of two low doses of the NET microspheres. After the 30 mg dose, 50% of the women had serum progesterone levels suggestive of ovulation, whereas only 20% had ovulatory levels after the 50 mg dose.

IND 18,592

INTERIM REPORT

A Comparative Evaluation of 65 mg and 100 mg of 90-Day Norethindrone Microspheres for Controlled Release Steroid Contraception

PHASE II STUDY

January 1987

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INTERIM REPORT

A Comparative Evaluation of 65 mg and 100 mg of 90-Day Norethindrone Microspheres for Controlled Release Steroid Contraception: Phase II Study

I. INTRODUCTION

Recent research has focused on the development of improved delivery systems for steroidal contraceptives that permit a relatively constant release of hormone through a biodegradable system over an extended period of time. A microsphere formulation which is intended to provide a 90-day delivery of the contraceptive steroid norethindrone (NET) has been developed. This formulation is injected intramuscularly.

A Phase I study of 50 mg, 75 mg and 100 mg of NET microspheres was conducted in 1985 to evaluate safety and to determine the test doses for the Phase II clinical trial. No adverse experiences were reported for the women. Pharmacokinetic data revealed consistent release of NET throughout the 90-day treatment interval, with successful ovulation inhibition in eight of the nine women. One woman, who received the 50 mg dose, had progesterone levels suggestive of ovulation in the first three weeks postinjection.

II. OBJECTIVES

The objectives of this 40-case Phase II study are (1) to evaluate the safety, contraceptive effectiveness and effect on ovarian function of 65 mg and 100 mg doses of the injectable NET formulation and (2) to assess the serum concentrations of NET over the intended treatment intervals after each dose.

Safety is assessed by documenting and monitoring all reported or observed adverse experiences throughout the study period, including serial clinical laboratory evaluations; in addition, low and high density lipoproteins (LDL/HDL), sex hormone binding globulin (SHBG) and menstrual pattern disruptions in terms of bleeding and spotting episodes are documented over time. Contraceptive effectiveness is determined by pregnancy rates. Effects on ovarian function are estimated over the course of the study by measuring serum levels of estradiol (E_2) and progesterone (P) and by scoring cervical mucus characteristics.

III. PATIENT SELECTION

Women in good physical health who were readily available for follow-up evaluation were asked to participate in the study. The following patient selection criteria were used to enroll women:

Inclusion criteria:

1. From 18 to 35 years of age

2. History of regular menstrual cycles from 23-35 days in length

3. Sexually active and at risk of pregnancy

4. Normal physical, pelvic and breast examination

5. Hemoglobin (11.0 g/dl or greater)

6. Negative pregnancy test prior to the NET injection

Exclusion criteria:

1. History or evidence of impaired liver function, renal disease, clotting disorders, diabetes, cardiovascular dysfunction, infertility, ectopic pregnancy, or other contraindications for steroidal contraceptives

2. Less than 102 lbs (46 Kg) or greater than 173 lbs (74 Kg)

3. Breastfeeding

4. Use of an IUD in the last 30 days

5. Use of oral contraceptives within past month if no spontaneous menstruation occurred between 25 and 35 days after discontinuation of oral contraception

6. Use of an injectable contraceptive in the past six months

7. Smoking

8. Use of barbiturates or antibiotics in the last 30 days

IV. STUDY PROCEDURES

This is a two-center, randomly allocated study of two 90-day injections of two different doses of the NET injectable formulation. Centers are located in New York, New York and Pittsburgh, Pennsylvania. During the preadmission cycle, baseline data were collected for each woman. Women were scheduled for their first injection between days 3 and 6 of the next menstrual cycle. Women are scheduled to return for follow-up visits twice a week throughout the 180-day postinjection interval and until resumption of ovulation (approximately 210 days after the first injection or at start of next menses).

The schedule of various tests and procedures is shown in Chart 1.

V. RESULTS

A. Status of Study

The study is still active. Thirty-seven women were enrolled from February 2, 1986 through August 15, 1986. Random allocation of the products has been followed correctly through the study. A summary of clinic visits completed is given in Table 1. Fourteen women in the 65 mg group and 14 women in the 100 mg group have received the second injection.

B. Demographics

Table 2 shows age and total live births for women participating in the study. The mean age of women receiving injections of 65 mg of NET microspheres was 26.0 years; the mean age for the 100 mg group was 23.2 years. Mean total live births was 0.5 for the 65 mg group and 0.5 for the 100 mg group.

C. Efficacy

1. Clinical

No pregnancies have occurred in the study. There have been 77 women-months of use for the 65 mg group and 80 women-months of use for the 100 mg group.

2. Laboratory

Serum levels of estrogen and progesterone were evaluated to determine efficacy of ovulation suppression of the two doses. Cervical mucus scores and SHBG also were used to monitor ovulatory activity.

Norethindrone

Figure 1 and Table 3 show the mean serum concentrations of norethindrone for the 65 mg and 100 mg groups. Norethindrone levels were similar for the two doses and effectively prevented pregnancy. There was an initial rise of NET values shortly after injection. These values then decreased rapidly within two weeks and thereafter fell gradually over the remainder of the 90-day period.

Serum NET values for individuals are given in Appendix A.

Progesterone and Estrogen

Figures 2 and 3 and Tables 4 and 5 show the mean serum concentrations of progesterone and estrogen for the two dosage groups. Progesterone and estrogen values for each individual are given in Appendix A.

Of the 19 women in the 65 mg treatment group, two patients (Center 952, Patients 09,11) have had progesterone values suggestive of ovulation (> 3 ng/ml) during the period of treatment. Patient 09 had progesterone values > 3 ng/ml on Days 70-74 of the first injection and on Days 3-6 of the second injection. Patient 11 had one value > 3 ng/ml on Day 3 of the first injection. Neither patient became pregnant.

In the 100 mg group, none of the 18 patients have had progesterone values suggestive of ovulation during the period of treatment.

Cervical Mucus and SHBG

Cervical mucus scores over 10 are predictive of ovulation. The cervical mucus data obtained in this study did not appear to correspond with rises in estrogen levels and were not consistently followed by elevated progesterone levels (Appendix A). In the 65 mg group, two patients from

center 952 (07,09) had cervical mucus scores > 10. Patient 07 did not show a progesterone value > 3 ng/ml. Patient 09 showed a rise in progesterone indicative of ovulation. In the 100 mg group, two patients from center 952 (01,02) and one from center 9021 (04) had cervical mucus scores > 10. Patient 01 had a cervical mucus score of 11 on Day 220, during the postinjection period. Patient 02 had a score of 13 on Day 28 of the first injection but no rise in progesterone occurred. Patient 04 had a score of 12 on Day 28 of the first injection without a rise in progesterone.

SHBG is not analyzed at this time because results from all blood samples are not available.

D. Safety

1. Clinical

Subject Discontinuation

Two women from center 952 have discontinued the study, one in each dose group. The woman in the 65 mg group (Patient 07) discontinued on Day 106 after the first injection for mood changes and personal reasons related to a change of job. The woman in the 100 mg group (Patient 10) complained of severe leg pain on Day 19 after the first injection. Extensive testing was performed and no other cause for the pain was determined. (See Appendix B for medical case history). The investigator decided to discontinue the patient from the study although she improved and requested to be able to continue.

One woman from Center 9021 (Patient 16) discontinued the study because she was moving from the area. A second woman (Patient 11) was unable to keep appointments for blood drawings.

Adverse Experiences

Table 6 shows medical conditions at admission for women entering the study. One woman in the 65 mg group and two women in the 100 mg group had Pap smears showing atypia.

Table 7 presents events occurring postinjection.

At center 952, two adverse experiences considered serious or unanticipated have been reported by the investigator. One woman (Patient 10) who received the 100 mg dose reported severe leg pain 19 days after the first injection (see Study Discontinuation section). Extensive testing was performed and results are reported in Appendix B. The pain could not be related to a cause, and the patient was not given a second injection. The second woman (Patient 09) who received the 65 mg dose had a functional cyst of the right ovary. Symptoms and cyst disappeared with onset of menstruation.

At center 9021, five adverse experiences considered serious or unanticipated have been reported by the investigator, four in the 65 mg group and one in the 100 mg group. Two women in the 65 mg group had skin reactions after the first injection but continued in the study. One woman (Patient 03) had a papular rash with pruritis on her cheeks and chin one

week after the first injection. The second woman (Patient 05) had a history of allergies and reported intermittant hives two months after the first injection. There was no reaction following the second injection for either woman which indicated that the skin irritations were not drug related.

A third woman (Patient 07) reported daily headaches for several weeks 60-90 days after the first injection. The relationship to the study drug is unknown. Two other women reported headaches but this was not considered an adverse experience by the investigator.

Patient 10 had a drop in hemoglobin of 2.3 gm/dl from baseline (17.7%) at Day 28 after the first injection. There was no excessive bleeding and no obvious source of hemoglobin loss, although the patient had a history of anemia. The hemoglobin returned to baseline level with iron supplementation at the next testing and remained there throughout the study.

The fifth woman (Patient 02) who received the 100 mg dose had her uterus perforated while undergoing an endometrial biopsy.

Other events reported in Table 7 show the usual problems reported by women in this age group attending an ob/gyn clinic. At center 952, one woman, (Patient 11) entered the study with a mitral valve prolapse. A cardiologist made an evaluation before study admission and recommended that the patient be allowed to enter the study. At Center 9021, four women had heart conditions reported at follow-up visits. The investigator confirmed that these conditions were present at study admission. Two women had Pap smears showing atypia during the study; one of these women had this condition at study admission. There were fewer complaints of dysmenorrhea during the postinjection period than at study admission.

See Appendix C for individual patient listings of adverse experiences.

Bleeding Records

Table 8 shows daily bleeding and spotting reported by women during the study. Mean days of bleeding and spotting are presented for 30-day reference periods. In the 65 mg group, women reported an increase in the number of days of spotting during the first 60 days of drug activity. Spotting returned to baseline levels for later reference periods. In the 100 mg group women reported a slight increase in the number of days of spotting during the 31 to 90-day period of drug activity which returned to baseline levels for later reference periods.

Blood Pressure

Table 9 profiles women with blood pressure values more than 10% above baseline. There were no elevated systolic (> 149 mm Hg) and diastolic (> 89 mm Hg) values reported for either dose in either center.

For eight women in the 65 mg group and 10 women in the 100 mg group, there were rises of systolic or diastolic values of 10% over baseline but all were still within normal limits.

2. Laboratory Values

Laboratory data for individual patients are given in Appendix D.

Hemoglobin

Table 10 shows out-of-range hemoglobin values for women in the study. In the 65 mg group, patient 15 from center 9021 showed a decline of 2.0 gm/dl (16.7%) from baseline at the time of the second injection; she was having daily bleeding and was given an iron supplement.

Patient 10 from center 9021 had a drop in hemoglobin of 2.3 gm/dl (17.7%) from baseline at Day 28 after the first injection. Although there was not excessive bleeding, the patient had a history of anemia and was given an iron supplement. This patient does not appear in the out-of-range table because by protocol her hemoglobin value was rounded up to 11 gm/dl.

There were no abnormalities in the 100 mg group.

Serum Liver Enzymes and Lipids

Table 11 shows out-of-range values of liver function tests (triglycerides, cholesterol, LDH-340, SGOT, CPK, alkaline phosphatase and total bilirubin) for women in the study.

In the 65 mg group, two patients (09 and 17) from Center 952 had elevated, but clinically insignificant, cholesterol levels after the first injection. Patient 09 had had an equally elevated value at baseline.

Patient 16 from Center 9021 had an elevated LDH-340 value on Day 65 after the first injection, the last reported visit. Patient 07 from Center 952 had a clinically insignificant elevation of SGOT on the last reported visit, Day 106.

In Center 952 two patients (03 and 05) had elevated CPK values on Day 64 and 28 respectively. The values returned to normal thereafter. In Center 9021 three patients (10,11 and 19) had elevated CPK values post-injection. For Patient 11 the rise was clinically insignificant. The investigator reports that in tests subsequent to the last data available for this report, these patients had normal values.

The 65 mg group did not have elevated triglycerides, alkaline phosphatase and total bilirubin values during the postinjection period.

In the 100 mg group, Patient 14 from Center 9021 had elevated cholesterol values at baseline and on Days 28,62 and 89 postinjection. The patient has been referred for evaluation.

Patient 16 from Center 952 had an elevated LDH-340 value on Day 31 postinjection but had returned to normal by Day 64.

Patient 08 from Center 952 had elevated CPK values on Days 24 and 89 postinjection. Neither value was high enough to be clinically significant. Patients 06 and 13 from Center 9021 had elevated CPK values postinjection.

Patient 13 returned to normal at the next testing. Later test results for Patient 06 have not been received.

Patient 02 from Center 9021 had an elevated total bilirubin value at baseline which rose from Day 31 to the time of the second injection. Later tests show that total bilirubin values had returned to predrug levels.

The 100 mg group did not have elevated triglycerides, SGOT and alkaline phosphatase values during the postinjection period.

Table 12 shows LDL and HDL out-of-range values for women in the study.

For the 65 mg group, there were no elevated LDL values postinjection. Depressed HDL values occurred for four patients (03,05,06,11) from Center 952 postiniection. All of these patients had depressed values of HDL at baseline.

For the 100 mg group, one Patient (14) from Center 9021 had elevated LDL values at baseline and throughout the study period. Seven patients (01,02,04,08,10,14 and 16) had depressed HDL values postiniection. Patients 01,02 and 16 had depressed levels at baseline. The other patients had low normal baseline values.

Renal Function

Table 13 presents out-of-range values found for creatinine.

In the 65 mg group, Patient 05 from Center 9021 had a clinically insignificent rise on Day 134 after the second injection.

In the 100 mg group, two patients (01 and 08) from Center 952 had elevated creatinine values which were clinically insignificant.

Serum Electrolytes, Serum Protein and Glucose

Table 14 shows out-of-range values of glucose and serum electrolytes (sodium, potassium, chloride, calcium and phosphorous), total protein and albumin for women in the study.

For the 65 mg group, Patient 05 from Center 9021 had a low sodium value at baseline and on Day 134 after the second injection. It was normal during the period of drug activity.

Patients 05 and 17 from Centers 952 and Patients 01 and 07 from Center 9021 had elevated chloride values once during the postiniection period. For Patients 17, 01 and 07, the values returned to normal at the next testing. Patient 05 had an elevated value at her last reported test, Day 84 after the second injection; the elevation was clinically insignificant.

Patient 05 from Center 952 had one low calcium value at the time of the second injection. The value returned to normal at the next test.

Patient 05 from Center 9021 had one clinically insignificant elevation of phosphorus on Day 87 after the second injection. The value had returned to normal by Day 134.

There were no abnormalities in glucose, potassium, total protein and albumin for the 65 mg group.

For the 100 mg group, Patient 09 from Center 9021 had a low glucose value on Day 87 after the first injection; the depressed value was clinically insignificant.

Patients 01 and 04 from Center 952 had out-of-range sodium values post-injection. Patient 01 had an elevated value on the day of the second injection which returned to normal thereafter. Patient 04 had a low value on the day of the second injection which returned to normal at the next testing. The two abnormalities were clinically insignificant.

Patient 16 from Center 952 had an elevated potassium value on Day 31 after the first injection which returned to normal by Day 64. Patient 02 from Center 9021 had an elevated value on Day 86 after the first injection which returned to normal by Day 31 after the second injection.

Patients 01,02,08 and 10 from Center 952 had elevated chloride values during the study; the elevations were clinically insignificant.

Patients 01 and 04 from Center 952 had out-of-range calcium values during the study. Patient 01 had a clinically insignificant low value on Day 31 after the second injection which returned to normal by Day 62. Patient 04 had an elevated calcium level at the time of the second injection; the value returned to normal and was not clinically significant. The patient also had a rise in sodium at the same visit.

Patient 01 from Center 952 had an elevated albumin value at the time of the second injection. The value returned to normal at the next testing.

There were no abnormalities for phosphorous and total protein for the 100 mg group.

Urinalysis

Table 15 shows abnormal urinalysis (specific gravity, total protein, pH, WBC, RBC, ketones, glucose, bilirubin) values for women in the study. There were no abnormalities in specific gravity, glucose or bilirubin in either treatment group.

In the 65 mg group, Patient 13 from Center 952 had proteinuria on Day 3 after the first injection. The investigator retested for protein and the results were negative. Patient 16 from Center 9021 had proteinuria and hematuria on Day 65 after the first injection. The patient was bleeding at the time the specimen was taken. Patient 01 from Center 9021 had hematuria at the time of her second injection. The patient's daily bleeding record shows that the patient was bleeding at the time the specimen was taken.

In the 100 mg group, Patient 08 at Center 952 had proteinuria at the time of the second injection; the investigator indicated that there was a trace of protein present. Patients 01 from Center 952 and Patients 02,04 and 08 from Center 9021 had hematuria postinjection. Patients 01,02 and 04 were bleeding at the time the specimen was taken. Patient 08 was not

bleeding. The investigator reported that the specimen was contaminated with bacteria.

Patient 12 from Center 9021 had 2+ ketonuria at the time of the second injection.

VI. SUMMARY AND CONCLUSION

Thirty-seven women were enrolled in a Phase II study to evaluate the effectiveness, safety and effect on ovarian function of 65 mg and 100 mg doses of 90-day NET microspheres.

Norethindrone levels were similar for the two doses and effectively prevented pregnancy for the period covered by this interim report.

Two women in the 65 mg treatment group and no women in the 100 mg treatment group had progesterone values suggestive of ovulation (> 3 ng/ml) during the 90-day period following the injection.

Four women have discontinued the study; one for mood changes and personal reasons, the second for severe leg pain, the third because she was moving from the area and the fourth because she was unable to return for scheduled testing.

Seven adverse experiences considered serious or unanticipated were reported--two cases of skin irritation and one each of severe leg pain, functional cyst, daily headaches, a decrease in hemoglobin and a perforated uterus (see pages 4 and 5 for exact details).

No disturbing side effects have been observed. Women reported an initial increase in the number of days of spotting after the first injection which return to baseline after the first 90 days.

Out-of-range laboratory values observed for women in the study occurred at scattered, irregular times postinjection and in most cases were clinically insignificant. From an overall laboratory parameter standpoint, both dose levels were safe, with no indication that the higher dose produced more abnormalities than the lower dose.

This study was conducted to determine the optimal Phase III dose of the NET microspheres. On the basis of this study, the 65 mg NET microsphere dose would be the choice. It is the lower dose and is contraceptively effective and safe.

IND 18,592

INTERIM REPORT

A Comparative Evaluation of the Safety and Contraceptive Effectiveness of 65 mg and 100 mg of 90-Day Norethindrone Microspheres for Controlled Release Steroid Contraception

PHASE II STUDY

January 1987

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INTERIM REPORT

A Comparative Evaluation of the Safety and Contraceptive Effectiveness of 65 mg and 100 mg of 90-Day Norethindrone Microspheres for Controlled Release Steroid Contraception: Phase II Study

I. INTRODUCTION

Recent research has focused on the development of improved delivery systems for steroidal contraceptives that permit a relatively constant release of hormone through a biodegradable system over an extended period of time. A microsphere formulation which is intended to provide a 90-day delivery of the contraceptive steroid norethindrone (NET) has been developed. This formulation is injected intramuscularly.

A Phase I study of 50 mg, 75 mg and 100 mg of NET microspheres was conducted in 1985 to evaluate safety and to determine the test doses for the Phase II clinical trial. No adverse experiences were reported for the women. Pharmacokinetic data revealed consistent release of NET throughout the 90-day treatment interval, with successful ovulation inhibition in eight of the nine women. One woman, who received the 50 mg dose, had progesterone levels suggestive of ovulation in the first three weeks postinjection.

II. OBJECTIVES

The objectives of this 120-case Phase II study are to evaluate the safety and contraceptive effectiveness of 65 mg and 100 mg doses of the injectable NET formulation.

Safety is assessed by documenting and monitoring all reported or observed adverse experiences throughout the study period, including serial clinical laboratory evaluations. Menstrual pattern disruptions in terms of bleeding and spotting episodes are determined. Contraceptive effectiveness is determined by pregnancy rates.

III. PATIENT SELECTION

Women in good physical health who were readily available for follow-up evaluation were asked to participate in the study. The following patient selection criteria were used to enroll women:

Inclusion criteria:

1. From 18 to 35 years of age

2. History of regular menstrual cycles from 23-35 days in length

3. Sexually active and at risk of pregnancy

4. Normal physical, pelvic and breast examination

5. Hemoglobin (11.0 g/dl or greater)

6. Negative pregnancy test prior to the NET injection

Exclusion criteria:

1. History or evidence of impaired liver function, renal disease, clotting disorders, diabetes, cardiovascular dysfunction, infertility, ectopic pregnancy, or other contraindications for steroidal contraceptives

2. Less than 102 lbs (46 Kg) or greater than 173 lbs (74 Kg)

3. Breastfeeding

4. Use of an injectable contraceptive in the past six months

5. Use of barbiturates or antibiotics in the last 30 days

IV. STUDY PROCEDURES

This is a six-center, randomly allocated study of four 90-day injections of two different doses of the NET injectable formulation. Centers are located in Atlanta, Georgia, Jacksonville, Florida, Miami, Florida, Rome, Italy, Durango, Mexico and Santiago, Chile. During the preadmission cycle, baseline data were collected for each woman. Women were scheduled for their first injection between days 3 and 6 of the next menstrual cycle. Women are scheduled to return for follow-up visits once a month throughout the 360-day postinjection interval and until resumption of ovulation (approximately 390 days after the first injection or at start of next menses).

The schedule of various tests and procedures is shown in Chart 1.

V. RESULTS

A. Status of Study

The study is still active. Ninety-four women were enrolled from January 6, 1986 through August 11, 1986. Random allocation of the products has been followed correctly through the study. A summary of clinic visits completed is given in Table 1. Seventy-one women have received the second injection and 38 women have received the third injection.

At Center 242, 10 of the first 13 patients (Patients 02,03,04,06,07,08 09,10,12,13) were injected outside protocol time limits of 3 to 6 days of the menstrual cycle. These women were excluded from the study. None became pregnant.

B. Demographics

Table 2 shows age and total live births for women participating in the study. The mean age of women receiving injections of 65 mg of NET

microspheres was 26.1 years; the mean age for the 100 mg group was 26.8 years. Mean total live births was 1.6 for the 65 mg group and 1.6 for the 100 mg group.

C. Efficacy

1. Clinical

No pregnancies have occurred in the study. There have been 236 women-months of use for the 65 mg group and 224 women-months of use for the 100 mg group.

2. Laboratory

Serum levels of norethindrone were evaluated to determine release profiles of the two doses.

Norethindrone

Figure 1 and Table 3 show the mean serum concentrations of norethindrone for the 65 mg and 100 mg groups. Although average norethindrone levels were generally lower for the 65 mg dose, this dose effectively prevented pregnancy.

Serum NET values for individuals are given in Appendix A.

D. Subject

1. Clinical

Subject Discontinuation

Eleven women have discontinued the study, five in the 65 mg group and six in the 100 mg group. In the 65 mg group, four women discontinued after the first injection; two moved from the area (Center 243, Patient 07 and Center 8035 Patient 05); one planned to become pregnant (Center 8035, Patient 17) and the fourth developed a rash after the first injection (Center 242, Patient 15). The fifth woman was lost-to-follow-up at the time she was scheduled for her third injection (Center 243, Patient 03).

In the 100 mg group, one woman (Center 242, Patient 16) discontinued on Day 90 after the first injection because of migraine headaches. The second woman (Center 242, Patient 11) discontinued on Day 90 because of amenorrhea. The third woman (Center 242, Patient 01) was discontinued at the time of the third injection by the investigator because she did not comply with scheduled protocol visits. The fourth woman (Center 10, Patient 02) accepted a new job and refused to come for follow-up. The fifth woman (Center 10, Patient 04) moved from the area. A sixth woman (Center 243, Patient 18) discontinued before the third injection because of headaches and insomnia.

Adverse Experiences

Table 4 shows medical conditions at admission for women entering the study. Six women in the 65 mg group and 10 women in the 100 mg group had pap smears showing atypia. All of the cases in the 65 mg group and seven of the cases in the 100 mg group were from Center 273. About half of the women in the two groups reported dysmenorrhea.

Table 5 presents events occurring postinjection.

Seven adverse experiences considered serous or unanticipated have been reported by investigators, five in the 65 mg group and two in the 100 mg group. In the 65 mg group, two women had skin rashes (Center 242, Patient 15 and Center 825, Patient 08). Patient 15 reported the rash on Day 15 after the first injection. She had no history of allergies. The rash had disappeared by Day 30. The investigator decided not to reinject the patient. Patient 08 reported a rash on her legs two months after her first injection. She had no history of allergies. There was no reaction after the second injection, which indicated that the skin rash was not drug related.

Patient 07 from Center 243 reported migraine headaches two months after the first injection; she discontinued study product because she moved from the area. Patient 14 from Center 242 reported ringing in the ears which was related to taking a concomitant medication. Patient 17 from Center 242 experienced a decrease in hemoglobin after the first injection. She had a history of iron deficiency anemia. There was no excessive bleeding and no obvious source of blood loss. She was given an iron supplement and her hemoglobin returned to within normal limits.

In the 100 mg group, Patient 11 from Center 242 reported a skin rash one month after the first injection. The rash lasted one day. The patient discontinued the study product because of amenorrhea. Patient 16 from Center 242 reported migraine headaches on Days 15-20 after the first injection. The patient had a history of migraines.

Other events reported in Table 5 show the usual problems reported by women in this age group attending an ob/gyn clinic. A second woman (Center 242, Patient 05) in the 65 mg group reported migraine headaches but this was not considered an adverse experience by the investigator. Patient 11 from Center 243 had a Pap smear that showed atypia during the study; she did not have this condition at admission. Two women in the 65 mg group had ovarian cysts. Sixteen women in each treatment group complained of heavy or prolonged bleeding or spotting associated with the method. Reports of dysmenorrhea decreased postinjection.

See Appendix B for individual patient listings.

Bleeding Records

Table 6 shows daily bleeding and spotting reported by women during the study. Mean days of bleeding and spotting are presented for 30-day reference periods.

In the 65 mg group, women reported an increase in the number of days of spotting in months two and five after drug injection. The number of days of bleeding decreased throughout the study period. In the 100 mg group women reported a slight increase in the number of days of spotting during the 31 to 60-day period after drug injection. Spotting returned to baseline levels for later reference periods. The number of days of bleeding decreased from baseline.

Blood Pressure

Table 7 profiles women with blood pressure values more than 10% above baseline. There were no elevated systolic (> 149 mm Hg) and diastolic (> 89 mm Hg) values reported for either dose in either center.

For 17 women in the 65 mg group and 15 women in the 100 mg group, there were rises of 10% over baseline systolic and diastolic values, but all were still within normal limits.

2. Laboratory values

Laboratory data for individual patients are given in Appendix B.

Hemoglobin

Hemoglobin was to be determined at admission and termination from the study. Occasionally investigators did hemoglobin tests at other times. In the 65 mg group, Patient 17 from Center 242 experienced a decrease in hemoglobin below 11.0 after the first injection. She had a history of anemia and was given an iron supplement. Her hemoglobin returned to within normal limits. This was a nonscheduled hemoglobin test.

There were no abnormalities in the 100 mg group.

Serum Liver Enzymes

Table 8 shows out-of-range values of liver function tests (triglycerides, cholesterol, LDH-340, SGOT, CPK, alkaline phosphatase and total bilirubin) for women in the study.

In the 65 mg group, three patients had elevated triglycerides postinjection. Patient 07 from Center 243 had a clinically insignificant elevated value on Day 28 after the first injection which returned to normal on Day 88. Patient 01 from Center 273 had a clinically insignificant elevation on Day 34. Later test results have not been received. Patient 05 from Center 825 had elevated triglyceride values at baseline and at Day 30.

Patient 15 from Center 242 had an elevated cholesterol value on Day 97 after the first injection.

Two patients had clinically insignificant elevated LDH-340 values postinjection. Patient 02 from Center 273 and Patient 19 from Center 8035 also had elevated LDH-340 values at baseline and at the one month visit.

Patient 07 had one clinically insignificant SGOT elevation at Day 28 after the first injection. She had returned to normal by Day 88.

Three patients from Center 825 (08,11 and 17) had elevated alkaline phosphatase values postinjection. Patients 08 and 11 had values that were clinically insignificant. Patient 17 had a significant increase on Day 31, almost three times her baseline value. Her SGOT and total bilirubin values were normal.

Patient 15 from Center 242 had a clinically insignificant total bilirubin elevation on Day 97 after the first injection.

The 65 mg group did not have elevated CPK during the postiniection period.

In the 100 mg group, two patients from Center 8035 had elevated LDH-340 values postinjection. Patient 08 also had an elevated value at baseline. Patient 10 had an elevated value on Day 32 which returned to normal by Day 92.

Two patients had elevated SGOT values postinjection. Patient 20 from Center 825 had a significantly elevated value on Day 30. Her alkaline phosphatase value was also elevated. Patient 08 from Center 8035 had an elevated value on Day 30 which had returned to normal by Day 90.

Six patients had elevated alkaline phosphatase values postinjection, one from Center 242 and five from Center 825. Patient 11 from Center 242 had a clinically insignificant elevation on Day 29 postinjection. Patients 06 and 10 from Center 825 had clinically insignificant elevations at the one month testing. Patients 07 and 20 had significant elevations at one month postinjection. For Patient 07 all other liver function tests were normal. For Patient 20, a rise in SGOT also occurred. Patient 16 had a significant rise over her below normal baseline (more than 4 times) although her value was only slightly above the normal range. She also had a rise in total bilirubin.

Two patients had elevated total bilirubin values. One of these patients, Patient 16 from Center 825, also had a significantly elevated alkaline phosphatase value. The second patient, Patient 04 from Center 10, had an elevated value on Day 28 which returned to normal by Day 49.

The 100 mg group did not have elevated triglycerides, cholesterol and CPK during the postinjection period.

Renal Function

Table 9 presents out-of-range values for creatinine. There were no elevated creatinine values postinjection for either treatment group.

Serum Electrolytes, Serum Protein and Glucose

Table 10 shows out-of-range values of glucose and serum electrolytes (sodium, potassium, chloride, calcium and phosphorous), total protein and albumin for women in the study.

In the 65 mg group, 14 patients had low glucose values postinjection. Nine of these patients also had low glucose values at baseline. Four of the five remaining patients (Patient 05 from Center 242, Patient 10 from Center 243 and Patients 14 and 15 from Center 825) had clinically insignificant depressions one month postinjection. Patient 02 from Center 825 had a significant decrease in glucose on Day 30. Results from her next tests are not yet available.

Patients 05 and 10 from Center 243 had elevated potassium values postinjection. The elevations were clinically insignificant. Patient 02 from Center 273 had a clinically insignificant depressed potassium value one month postinjection.

Six patients had elevated chloride values postinjection, five from Center 242 and one from Center 273. Three of the patients from Center 242 (05,14, and 19) had clinically insignificant elevated values at baseline and one month postinjection. Elevations for Patients 15 and 17 were also clinically insignificant. Patient 14 from Center 273 had a clinically insignificent elevated chloride value on Day 32.

Patients 03,11 and 13 from Center 8035 had low calcium values one month postinjection. The depressed values were clinically insignificent.

Two patients had out-of-range phosphorous values postinjection. Patient 05 from Center 10 had an elevated phosphorous level at baseline and on Day 29 postinjection. Patient 13 from Center 8035 had a low phosphorous value on day 32 which was clinically insignificant.

Two patients (09 and 17) from Center 825 had low total protein values postinjection. Both were clinically insignificent and Patient 17 also had a depressed value at baseline.

Patient 05 from Center 825 had an elevated albumin value on Day 30 postinjection. The elevation was clinically insignificant.

There were no abnormalities for sodium postinjection for the 65 mg group.

In the 100 mg group, 13 patients had low glucose values postiniection. Six of these patients also had low glucose values at baseline. Three of the seven remaining patients (Patient 18 from Center 242 and Patients 16 and 18 from Center 243) had clinically insignificant depressions one month postinjection. Patients 06 and 12 from Center 243 and Patients 04 and 16 from Center 825 had depressions more than 10% below the lower limit of normal at one month postiniection. Later test results have not been received for these patients. Lowered values may reflect the amount of time the patient fasted before the test. Patient 10 from Center 825 had a clinically insignificant elevation in glucose on Day 31 postinjection.

Patient 11 from Center 242 had a clinically insignificant elevation in sodium on Day 29 postinjection.

Three patients had elevated potassium values postinjection. Patients 09 and 18 from Center 243 also had elevated potassium values at baseline. Patient 01 from Center 243 had a clinically insignificant elevation on

Day 35 postinjection. Patient 16 from Center 273 had a clinically insignificant depressed potassium value on Day 31 postinjection.

Six patients had elevated chloride levels postinjection. All were clinically insignificant and two of the patients (Patients 11 and 18 from Center 242) had elevated values at baseline. Patients 01 and 20 from Center 242 and Patient 16 from Center 273 had elevated values at one month postinjection. Patient 04 from Center 10 had an elevated chloride value on Day 49 postinjection.

Patient 08 from Center 10 had a clinically insignificant elevated calcium value on Day 28 postinjection. Patients 04 and 10 from Center 8035 had depressed calcium values postinjection. Both were clinically insignificant and Patient 04 also had a low value at baseline.

Two patients (Patient 04 from Center 243 and Patient 10 from Center 8035) had clinically insignificant depressed phosphorous values postinjection.

Three patients had depressed total protein values postinjection. Patients 06 and 07 from Center 825 had clinically insignificant depressed values one month postinjection. Patient 07 also had a low total protein value at baseline. Patient 10 from Center 825 had a clinically insignificant elevated total protein value on Day 31 postinjection.

Three patients had out-of-range albumin values postinjection. Patient 01 from Center 242 and Patient 04 from Center 825 had clinically insignificant elevated values one month postinjection. Patient 04 had had a depressed albumin value at baseline. Patient 07 had clinically insignificant low albumin values at baseline and one month postinjection.

Urinalysis

Table 11 shows abnormal urinalysis (specific gravity, total protein, pH, WBC, RBC, ketones, glucose, bilirubin) values for women in the study. There were no abnormalities in bilirubin in either treatment group.

Patient 05 from Center 243 had proteinuria at baseline at the time of the second injection.

In the 65 mg group, Patient 17 from Center 242 and Patient 08 from Center 825 had pyuria at the time of the second injection. Patient 08 had vaginitis at the time the sample was taken.

Patient 05 from Center 242 and Patient 05 from Center 243 had hematuria. The patients' daily bleeding records showed that the patients were bleeding at the time the specimens were taken.

Patient 03 from Center 825 had a urine glucose of 1+ at the time of the second injection. No blood glucose test was available.

In the 100 mg group, Patient 12 from Center 243 had proteinuria at Day 30 postinjection. The patient had returned to normal by Day 92.

Patients 01 and 18 from Center 242 and Patient 17 from Center 243 had pyuria one month postinjection. Patient 17 also had hematuria, a result of

bleeding at the time the specimen was taken. Patient 16 from Center 242, Patient 12 from Center 243 and Patient 04 from Center 825 also had hematuria postinjection; no bleeding was indicated on the daily bleeding records.

In the 65 mg group at Center 273, there were eight women with elevated WBC and six with hematuria at baseline; in the 100 mg group, eight had elevated WBC and eight had hematuria. No follow-up testing has been reported.

VI. SUMMARY AND CONCLUSION

Ninety-four women were enrolled in a Phase II study to evaluate the safety and effectiveness of 65 mg and 100 mg doses of 90-day NET microspheres.

Norethindrone levels were consistently lower for the 65 mg dose and this dose effectively prevented pregnancy for the period covered by this interim report.

Eleven women have discontinued the study, seven for reasons unrelated to the study product. Three moved from the area, three did not return for follow-up visits as scheduled and one discontinued to become pregnant. Four women discontinued because of method-related problems, one because of skin rash (65 mg), one because of migraine headaches (100 mg), one because of amenorrhea (100 mg) and one because of headaches and insomnia (100 mg).

Seven adverse experiences have been reported--three cases of skin rash (two in the 65 mg group and one in the 100 mg group), two cases of migraine headaches (one in each treatment group) and one each of ringing in the ears (65 mg) and a drop in hemoglobin (65 mg) (see page 4 for exact details).

Women reported an increase in the number of days of spotting which had returned to baseline after a few months. The number of days of bleeding decreased from baseline.

In general, out-of-range laboratory values observed for women in the study occurred at scattered, irregular times postinjection and in most cases were clinically insignificant. Four patients from Center 825 had elevated alkaline phosphatase levels that could not be attributed to specific patient complaints. Although Patients 07 (100 mg) and 17 (65 mg) had elevated alkaline phosphatase values, all other liver function tests were within normal ranges. Patient 16 (100 mg) had an elevated total bilirubin as well as an elevated alkaline phosphatase and Patient 20 (100 mg) had a slightly elevated SGOT as well as an elevated alkaline phosphatase. The patients had no complaints. No other center reported similar elevations.

Patients 01 (100 mg) and 17 (65 mg) from Center 242 and patient 17 (100 mg) from Center 243 had pyuria postinjection. Patient 04 (100 mg) from Center 825, Patient 16 (100 mg) from Center 242 and Patient 12 (100 mg) from Center 243 had hematuria postinjection. No explanation for these test results is available.

From an overall laboratory parameter standpoint, both dose levels were safe, with no obvious indication that the higher dose produced more abnormalities than the lower dose.

This study was conducted to determine the optimal Phase III dose of the NET microspheres. On the basis of this study, the 65 mg NET microsphere dose would be the choice. It is the lower dose and is contraceptively effective and safe.

LPC083/FDA3

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DEPARTMENT OF GYNECOLOGY
AND OBSTETRICS

FINAL REPORT SUMMARY FOR PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

PARFR - 386A

TITLE: "Phase II Poly NET 90 Injectable Study"

INSTITUTION: Emory University School of Medicine

INVESTIGATOR: Howard J. Tatum, M.D., Ph.D.

FUNDING PERIOD: December 1, 1985 through March 31, 1987

Below includes a project summary for the January 1, 1987 to March 31, 1987 period

OBJECTIVE OF THE PROJECT

To evaluate the safety and contraceptive effectiveness of 65mg and 100mg of injectable norethindrone (NET) formulations.

SUMMARY OF THE PROJECT

Recruitment of subjects for the study was initiated January 8, 1986, but was halted temporarily due to administrative delays. These problems were resolved and subject recruitment was resumed on May 19, 1986.

Eight subjects were admitted to the study prior to the August 5, 1986 deadline for admission. Two subjects were dropped from the study, both for non-medical reasons.

Of the six subjects currently in the study, one has received her third and final NET injection. The other five subjects are scheduled to receive their final NET injection by April 20, 1987. All six subjects are scheduled to make their 12-month follow-up visits by July 13, 1987. All six subjects have elected to participate in the Phase III NET study as soon as is allowed by protocol.

No unanticipated side effects or serious adverse experiences have been observed. Out-of-range laboratory values for subjects have occurred at irregular times. Only two out-of-range values required treatment. One subject, who had a hematocrit of 33.9% on admission to the study, was treated with oral iron tablets. Five months later, her hematocrit was 37%. Another subject had an initial Pap

smear which indicated atypia and a repeat Pap smear two months later which indicated atypia and chlamydia. After treatment with tetracycline, a Pap smear performed four months later indicated no atypia and no chlamydia.

Completed data collection forms and bleeding calendars are being forwarded on a regular basis to Family Health International (FHI). Serum samples for NET assay and used syringes and vials have been sent to Stolle regularly.

EXPECTED OUTCOME

The safety and contraceptive effectiveness of the injectable NET formulation will continue to be studied. The anticipated date for the last subject to exit the study is not known, but this is scheduled to occur at the start of a spontaneous menses following the twelve month follow-up visit. We anticipate that this may occur August or September, 1987.

A Phase III study on the safety and contraceptive effectiveness of 65mg of 90-day injectable norethindrone, funded by FHI, is scheduled to begin in May, 1987.

PARFR funding for the current Phase II study terminated on March 31, 1987. Family Health International (FHI) is expected to fund the current study to its expected completion.

Howard J. Tatum, M.D., Ph.D.

April 3, 1987
Date

DEPARTMENT of RESEARCH

Mount Sinai
MEDICAL CENTER
OF GREATER MIAMI

PHASE II POLY NET 90 INJECTABLE STUDY

Report as of March 31, 1987:

A total of 20 volunteers agreed to participate in the study as outlined by the informed consent forms and the protocol. Seven patients have dropped out of the study for reasons having nothing to do with side effects. One failed to report in a timely fashion and had to be dropped; three moved to another city; and the remainder failed to appear and/or respond to letters and telephone calls. The first patient was injected on January 24, 1986 and the last patient will be injected on April 7, 1987. The first interview was undertaken in December 1986 and the last examination will be performed in July 1987. There were no pregnancies. Side effects were few and were mild. About 50% of the subjects reported some degree of oligomenorrhea. Two reported periods of amenorrhea and 10% reported some staining in mid-cycle. All patients were and will be instructed in family planning techniques at the final injuries. There were a few small elevations in cholesterol levels but, until the study is completed we will not know whether these have statistical significance .

Respectfully submitted,

Jerome J. Hoffman, M.D., F.A.C.S.

4300 Alton Road • Miami Beach, Florida 33140 • Telephone (305) 674-2197

PROJECT NET-90 - UNIVERSITY OF ROME

FINAL REPORT

March 31, 1987

At the time when PARFR support for this project ended there were 17 subjects in the study with no drop-out. Eight volunteers are under treatment with NET-90 at the dose of 100 mg every 90 days.

Nine volunteers are being treated with NET-90 at the dose of 65 mg every 90 days.

Total experience with these two dosages comprises 152 women-month exposure, i.e. approximately 9 months per subject, on the average.

The major problem faced in the study was the lack of compliance of women for the monthly appointment for blood withdrawal. Six women missed one appointment; 7 missed two appointments and 4 more than two appointments. On the other hand all subjects came for the injections indicating that their lack of compliance was not due to problems related to the medication itself.

The injection procedure did not cause any specific problem: save for a transient itching sensation at the injection site, no other complaint was recorded.

The major side effects are bleeding-related complaints: both amenorrhea and intermenstrual spotting. Because of these complications 13 extra pregnancy tests were performed as well as 9 unscheduled visits during which the main problem was that of reassuring women that the observed "bleeding irregularities" were not harmful to their health.

Other side-effects were of minor importance: three subjects complained of breast tenderness and 4 of a slight weight increase.

To date no pregnancy has been observed.

TECHNICAL REPORT

Project Title

Phase II Poly NET 90. Injectable Study. PARFR-386M.

Principal Investigator

Roberto Rivera, M. D.
Instituto de Investigacion Cientifica
Universidad Juarez del Estado de Durango
Apartado Postal B-7
34000 Durango, Durango.
Mexico.

Telephone number: (181) 1-62-26

Roberto Rivera, M. D.

Institution

Instituto de Investigacion Cientifica
Universidad Juarez del Estado de Durango.

Subcontract Period

December 1, 1985-May 31, 1987.

Dates of this Report

December 1, 1985-March 31, 1987.

Subject characteristics

The age, number of live births and living children of the 20 participating subjects are presented in Table 1. All were healthy subjects who volunteered to participate in the study; duly signed consent froms are kept for each subject. All of them were subjected to the risk of pregnancy, and did not use any other form of contraception.

Injection and Termination Dates

The dates of the first and last injection for each subject are presented in Table 1. The last subject admitted to the study received the last injection on 06/03/87, with a 90 day injection-duration period, and the subsequent observation of a control cycle, this subject will terminate on July, 1987.

Injection Procedure

Eighteen of the 20 subjects who initiated the study received the 4 injections. Two subjects did not terminate the study, one after receiving two injections, and the other after receiving three injections. Therefore, a total of 77 injections were applied. A good suspension of the microcapsules was obtained with only shaking the syringe. However, a 3 ml syringe was received, and the suspension media almost filled the syringe, limiting the space for shaking and mixing, a 5 ml syringe should be more suitable.

On one injection there was complain of light pain at the time of the injection. On 5 injections the pain was mild, and on 3 injections the pain was severe. Two subjects, each one in association with one of the injections complained of numbness or cramps on the limb of the site of the injection. In summary, in 11 of the 75 injections there was one form of complain associated to the injection. One same subject, in her 4 injec-- tions, complained of mild pain with one injection, and of severe pain with two of the injections. On three injections the syringe got clogged with the suspension, and the shaking had to be repeated.

Continuation Rates

20 subjects were admitted to the study. So far, only two subjects have discontinued. Case Number 9, got pregnant, approximately 70-75 days after receiving her third injection. Case Number 14 discontinued for personal reasons after receiving her second injection. The reminding 18 subjects are still active in the study. 16 of them have already received their 4 injections and are in the follow-up period that follows the last injection. The other two subjects have received their 4 injections and have terminated their participation in the study.

Side Effects

Seven of the 20 subjects did not present any side

effects suring their respective observation periods. Four subjects complained of mild headache. Four subjects presented leucorrhea. Two subjects in each category complained of pains in the limbs, pelvic pain, skin rash, amenorrhea, and prolonged bleeding. One subject in each category complained of nausea and dizziness, nervousness, a watery breast secretion, and mastalgia. One subject presented asymptomatic bacteriuria. Table 2. Only one subject considered withdrawing of the study because of the side effects; she presented a limb pain, most likely of muscular origin, that she attributed to the injection; upon explanation she continued in the study.

Effects on the Menstrual Cycle

The effects on the menstrual cycle are presented as number of days of bleeding and spotting per 30-day reference periods in Table 3. Five subjects have presented fairly normal menstrual cycles (Subjects 3, 8, 9, 16 and 18). Seven subjects have presented amenorrhea, defined as nonbleeding for 90 or more days, (Subjects 2, 4, 6, 7, 10, 17 and 19). Five subjects have presented lon nonbleeding intervals, without reaching amenorrhea, so far (Subjects 1, 5, 12, 14 and 15). Six subjects have presented prolonged bleeding or spotting (Subjects 1, 2, 10, 11, 13 and 20). Three subjects initiated with prolonged bleeding or spotting and then fell in prolonged nonbleeding (Subject 1) or in amenorrhea (Subjects 2 and 10). Subject 11 has been bleeding or spotting

practically all the time since her first injection; however, she has never spontaneously complained of any menstrual side effect. Subject 13 has also been bleeding or spotting most of the time since the first injection, but she has complained of this problem.

The main characteristics of the menstrual cycle are presented in Table 4. The menstrual records for each patient are included in this report.

Contraceptive Efficacy

As previously mentioned, in all the 20 subjects received 77 injections. One subject got pregnant after receiving her third injection, subject 9. She received her third injection on 06/10/86. She had a normal menstrual cycle on 01/12/86. She was again seen on 06/01/87 for her fourth injection, at this time a pregnancy was detected by a pregnancy test and by suggestive changes in the cervix.

TABLE 1. DATA ON SUBJECTS PARTICIPATING IN PHASE II POLY NET 90, PARFR-386M.

CASE	AGE	LIVE BIRTHS	LIVING CHILDREN	DATE OF FIRST INJECTION	DATE OF LAST INJECTION.
1	31	3	3	14-02-86	10-11-86
2	30	3	3	17-02-86	17-11-86
3	33	5	4	03-03-86	01-12-86
4	27	4	3	04-03-86	01-12-86
5	23	2	2	10-03-86	08-12-86
6	29	2	2	11-03-86	08-12-86
7	29	4	4	18-03-86	15-12-86
8	20	1	1	04-04-86	30-12-86
9	31	3	3	09-04-86	discontinued
10	30	5	5	15-04-86	12-01-87
11	25	1	1	21-04-86	19-01-87
12	26	3	3	21-04-86	19-01-87
13	26	3	3	21-04-86	19-01-87
14	23	1	1	21-04-86	discontinued
15	30	3	3	23-04-86	19-01-87
16	25	2	2	23-04-86	19-01-87
17	25	2	2	02-05-86	28-01-87
18	25	3	3	07-05-86	06-02-87
19	24	4	4	04-06-86	02-03-87
20	33	4	3	09-06-86	06-03-87
MEAN	27.25	2.9	2.75		
S.D.	3.59	1.2	1.0		

TABLE 2. SIDE EFFECTS. PHASE II POLY NET 90. PARFR-386M.

SIDE EFFECTS	No. SUBJECTS
No side effects.	7
Headache.	4
Leucorrhea.	4
Limb pain.	2
Pelvic pain.	2
Skin rash.	2
Amenorrhea.	2
Prolonged bleeding.	2
Nausea and dizziness.	1
Nervousness.	1
Breast discharge.	1
Mastalgia.	1
Asymptomatic bacteriuria.	1

TABLE 3. PHASE II POLY NET 90, PARFR-386M. NUMBER OF BLEEDING OR SPOTTING DAYS 30-DAY REFERENCE PERIODS.

SUBJECT	1-30	31-60	61-90	91 120	121 150	151 180	181 210	211 240	241 270	271 300	301 330	331 360	361 390	391 420
1	3	12	30	12	6	17	6	4	1	0	2	0	0	
2	7	12	3	7	15	0	7	14	0	0	0	0		
3	3	4	3	7	3	0	3	5	0	1	4	2		
4	3	4	1	3	6	0	0	3	0	0	0	0		
5	5	4	2	5	9	5	0	0	5	0	3			
6	4	3	2	4	1	6	0	0	0	3	0			
7	6	2	0	0	0	0	0	2	0	0	0			
8	5	4	8	3	1	3	6	4	2	15	1			
9	7	1	7	7	3	11	6	0	14	2	0	Discontinued		
10	5	13	0	21	13	15	0	0	0	0				
11	5	10	25	30	7	25	30	28	30	30				
12	3	4	11	0	5	0	3	4	3	1				
13	3	7	20	16	11	17	30	21	15	4				
14	7	3	0	3	7	17	0	Discontinued						
15	7	8	8	0	18	22	0	0	9	11				
16	6	8	0	16	0	9	6	0	8	7				
17	7	0	1	0	0	0	5	0	0	1				
18	3	4	13	4	1	16	3	6	4	9				
19	3	2	0	0	0	3	0	1	6					
20	6	15	22	12	10	17	11	8						
MEAN	4.9	6	7.8	7.5	5.8	9.15	5.8	5.2	5.3	4.9				
S.D.	1.6	4.3	9.4	8.10	5.4	8.4	8.8	7.7	7.7	7.8				

TABLE 4. MAIN CHARACTERISTICS OF THE MENSTRUAL CYCLE. PHASE II POLY NET 90. PARFR-386M.

MENSTRUAL CHARACTERISTICS	No. SUBJECTS
Normal cycles.	5
Amenorrhea.	7
Long non-bleeding interval.	5
Prolonged bleeding/spotting.	6

INSTITUTO DE INVESTIGACION CIENTIFICA

UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre [illegible]

Registro 001

AÑO { 19 [illegible]

REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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REGISTRO DEL CICLO MENSTRUAL

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Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma:

G = Goteo

S = Sangrado

INSTITUTO DE INVESTIGACION CIENTIFICA

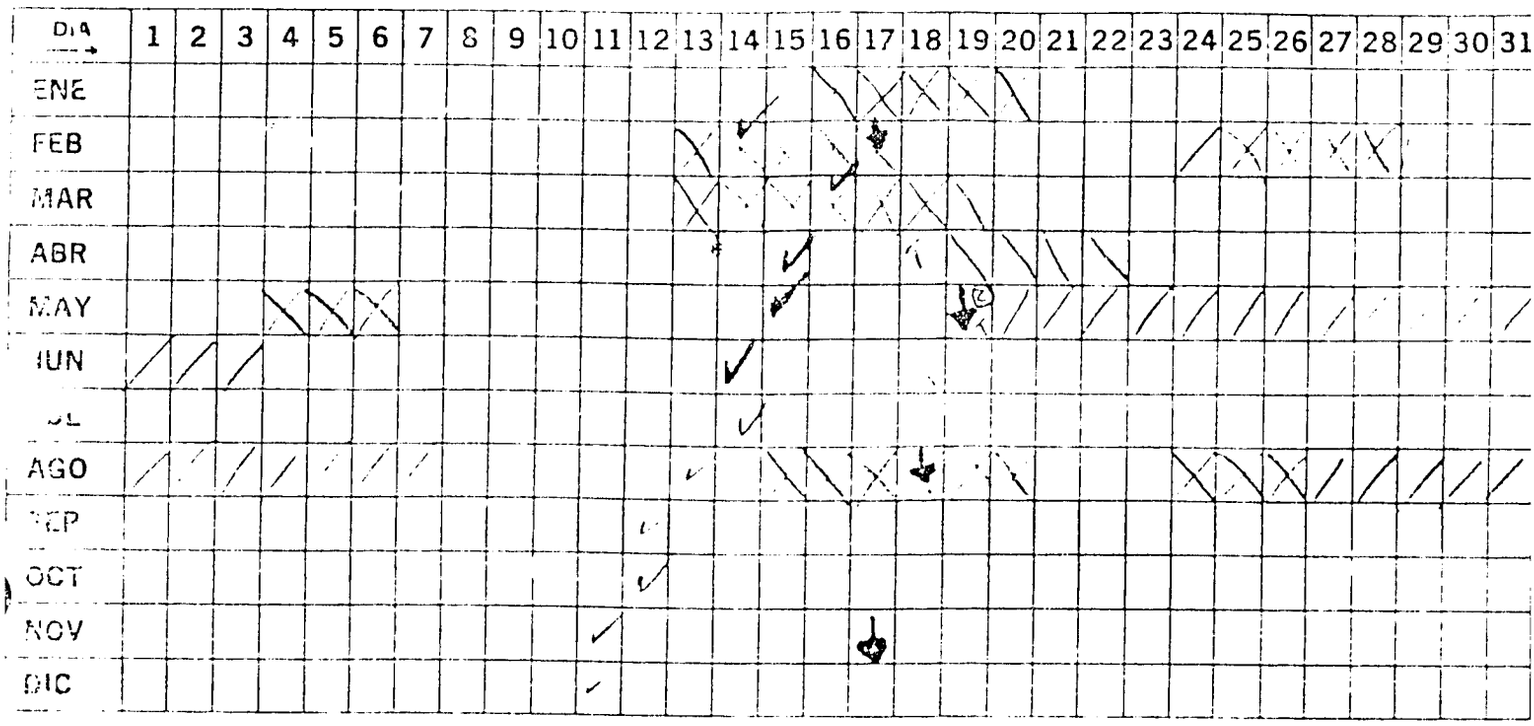
UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre [illegible] Rosa Cisneros.

Registro 007

AÑO 19 [illegible]

REGISTRO DEL CICLO MENSTRUAL



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REGISTRO DEL CICLO MENSTRUAL

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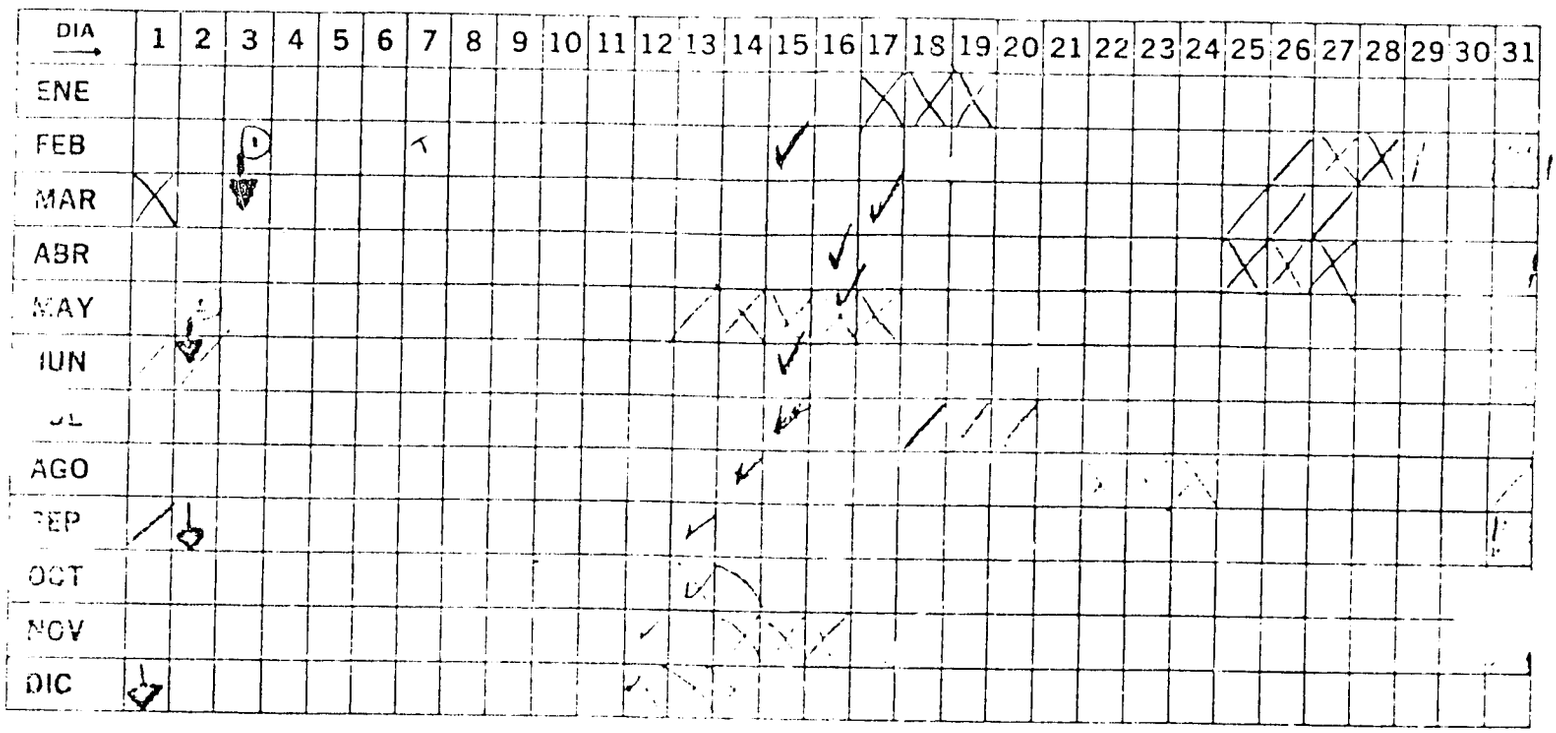
UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre Paula Ubaldo Ramirez

Registro 003

AÑO 19 56

REGISTRO DEL CICLO MENSTRUAL



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Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma: → G = Goteo; S = Sangrado

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UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre Enisa Mayorga Torres

Registro 004

AÑO 19 / 20

REGISTRO DEL CICLO MENSTRUAL

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Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma. →

G = Goteo

S = Sangrado

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UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre Irene Ríos Luna

Registro 005

AÑO { 19 / 20

REGISTRO DEL CICLO MENSTRUAL

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UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre Eudolina Rodriguez.

Registro 006

AÑO 19 / 80

REGISTRO DEL CICLO MENSTRUAL

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INSTITUTO DE INVESTIGACION CIENTIFICA

UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre Rosario Cocom Gonzalez.

Registro 007

AÑO { 1 / 2

REGISTRO DEL CICLO MENSTRUAL

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INSTITUTO DE INVESTIGACION CIENTIFICA

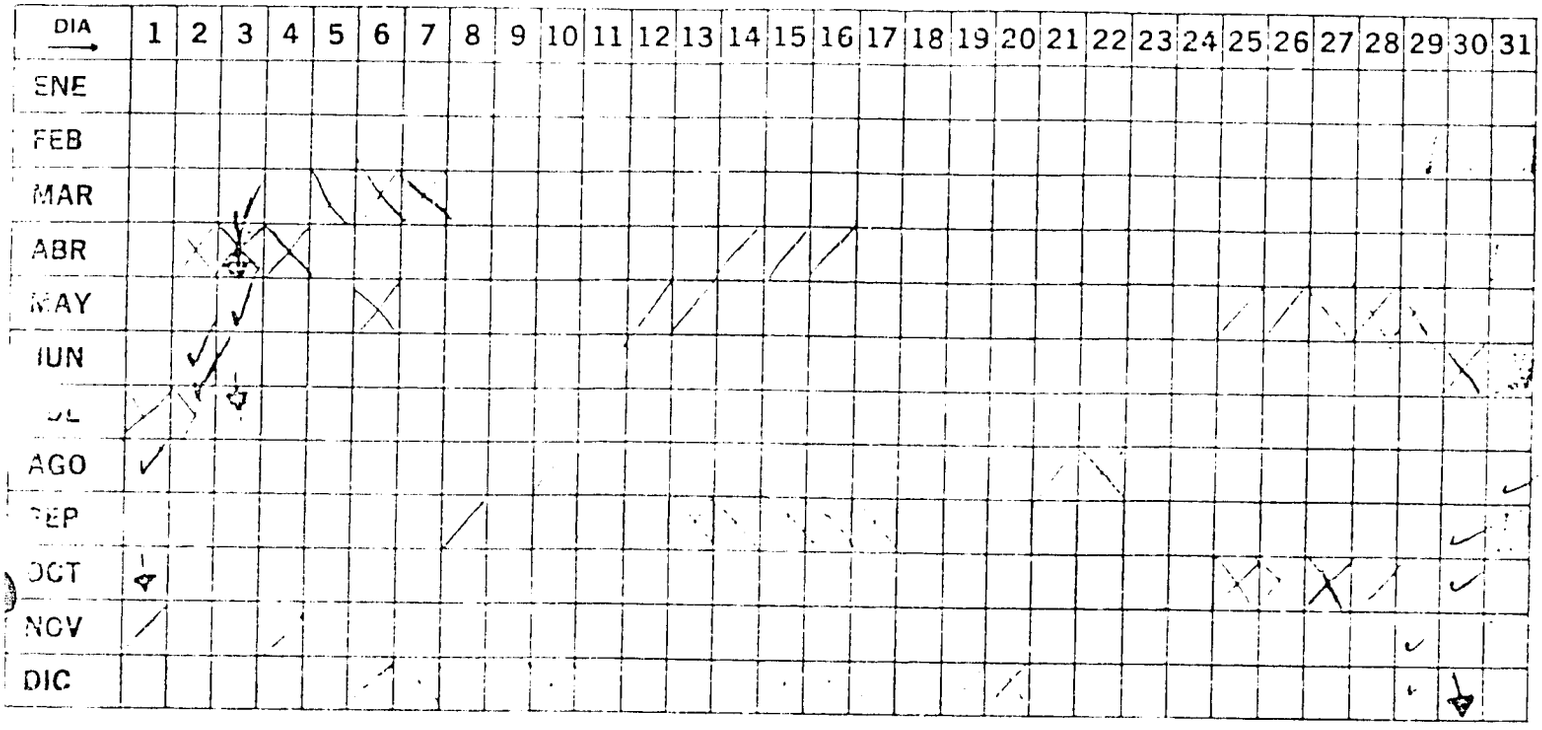
UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre Patricia Cisneros Gonzalez

Registro 008

AÑO 19 / 20

REGISTRO DEL CICLO MENSTRUAL



AÑO 19 / 20

REGISTRO DEL CICLO MENSTRUAL

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INSTITUTO DE INVESTIGACION CIENTIFICA

UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre ADELAIDA TAVIZON LUIZ.

Registro 009

AÑO { 19 / 22

REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma →

G = Goteo

S = Sangrado

INSTITUTO DE INVESTIGACION CIENTIFICA

UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre [illegible]

Registro 010

AÑO [illegible]

REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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AÑO [illegible]

REGISTRO DEL CICLO MENSTRUAL

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INSTITUTO DE INVESTIGACION CIENTIFICA

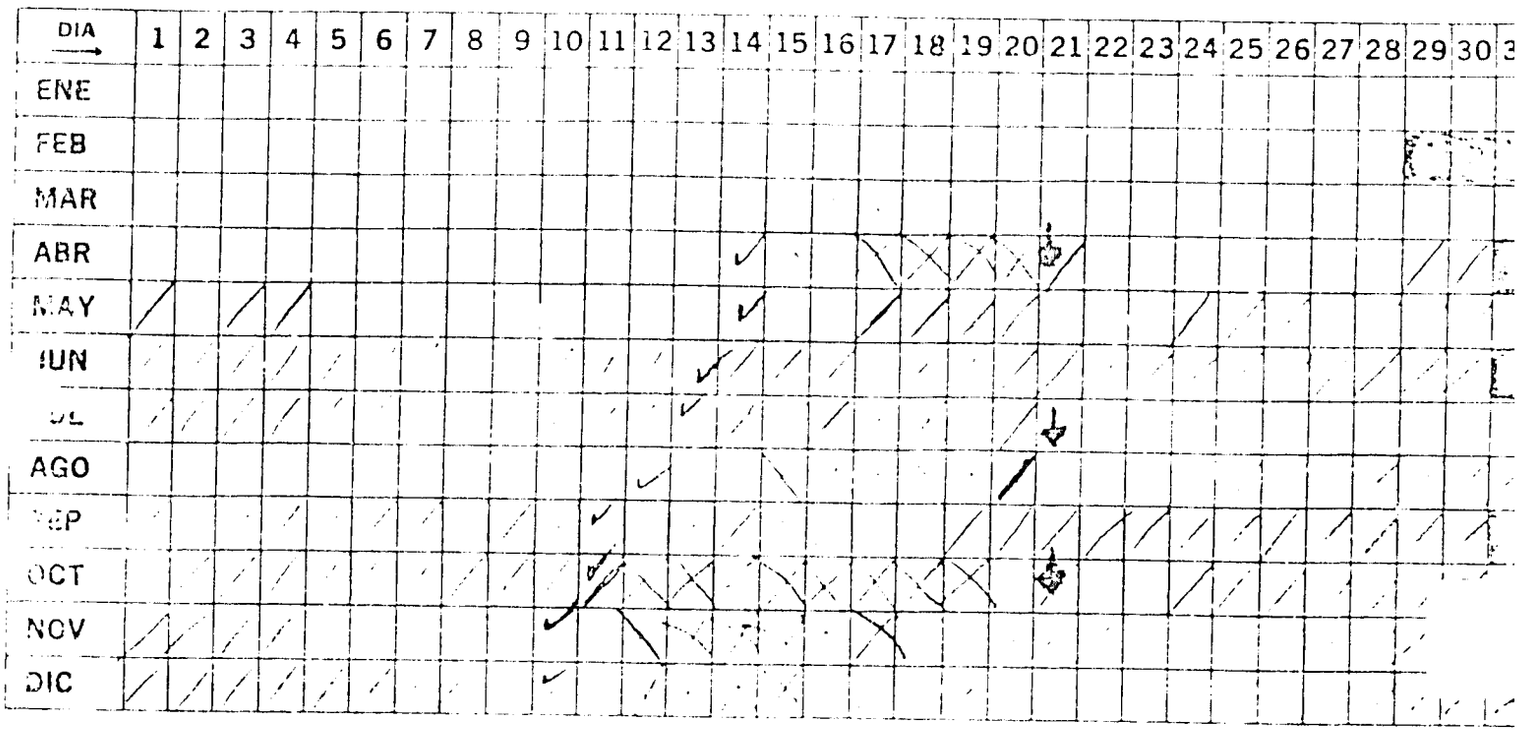
UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre MARIA DEL [illegible]

Registro 011

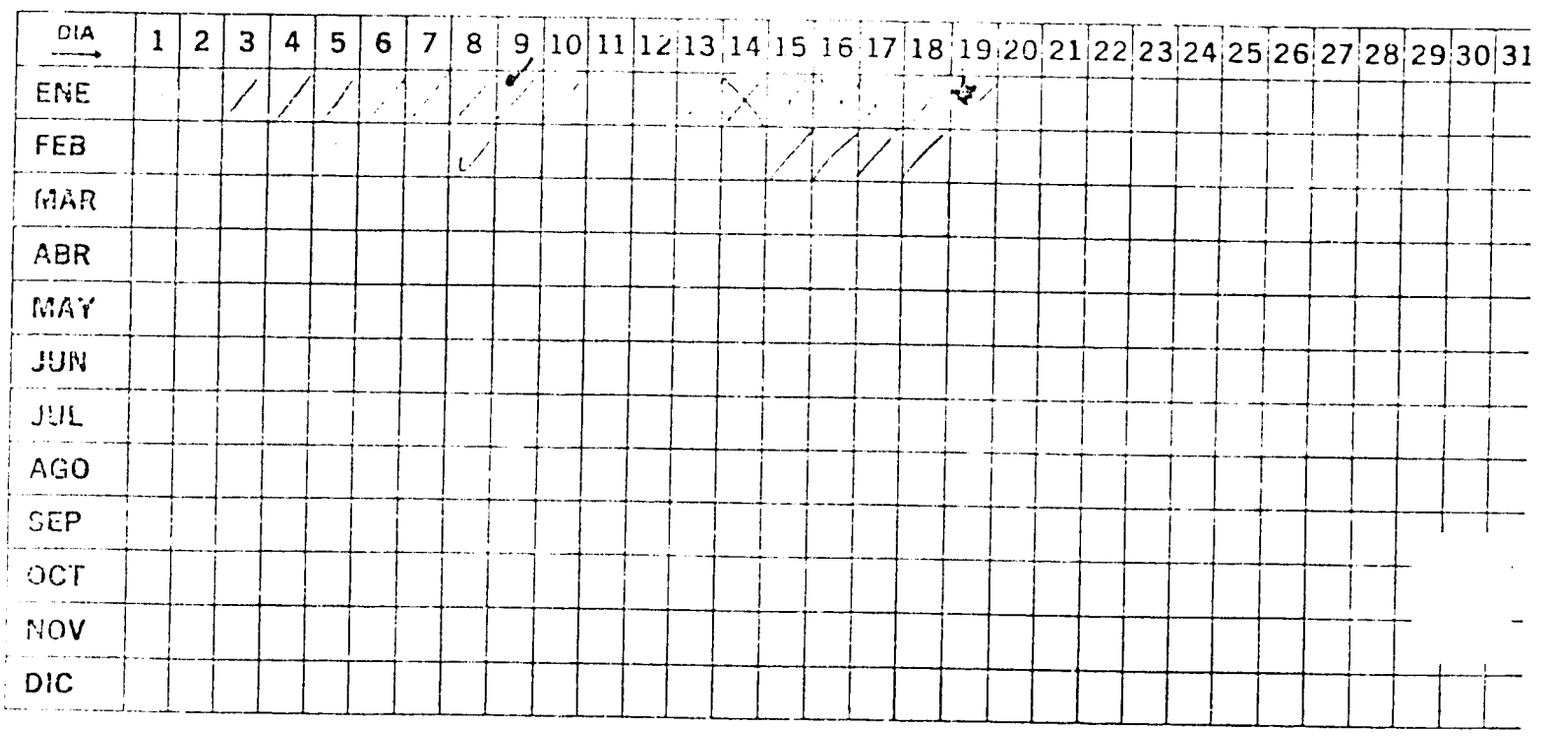
AÑO 19 [illegible]

REGISTRO DEL CICLO MENSTRUAL



AÑO 19 [illegible]

REGISTRO DEL CICLO MENSTRUAL



Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma: → G = Goteo; S = Sangrado

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UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre ISABEL [illegible] MENDOZA

Registro 012

AÑO 19 / 76

REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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AÑO 19 / 77

REGISTRO DEL CICLO MENSTRUAL

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INSTITUTO DE INVESTIGACION CIENTIFICA

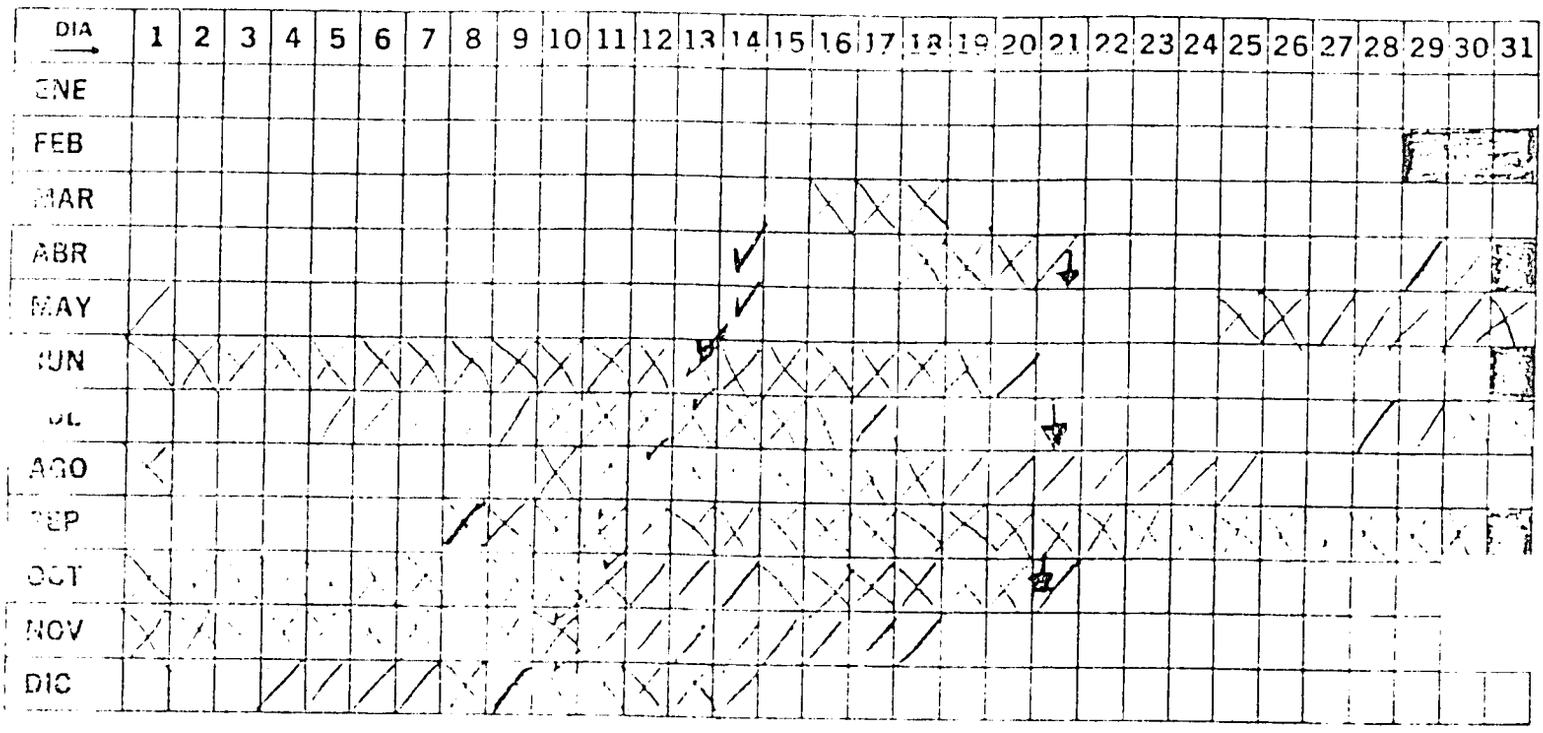
UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre ROSALIA TORRES HERNANDEZ.

Registro 013

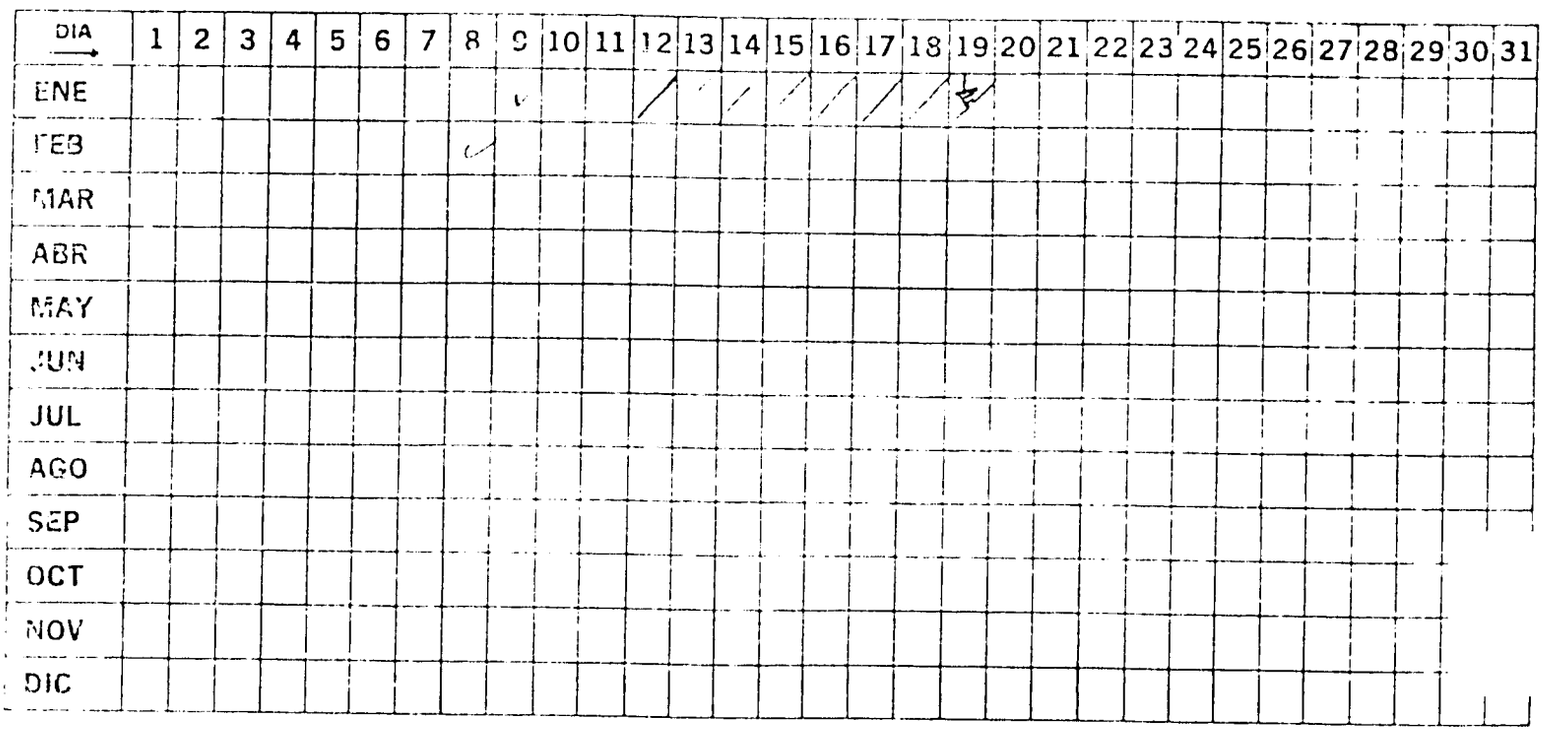
AÑO 19 / 86

REGISTRO DEL CICLO MENSTRUAL



AÑO 19 / 87

REGISTRO DEL CICLO MENSTRUAL



Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma: → G = Goteo; S = Sangrado

INSTITUTO DE INVESTIGACION CIENTIFICA

UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre Delia Flores [illegible]

Registro 014

AÑO 19 86

REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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REGISTRO DEL CICLO MENSTRUAL

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Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo mens. (G = Goteo

INSTITUTO DE INVESTIGACION CIENTIFICA

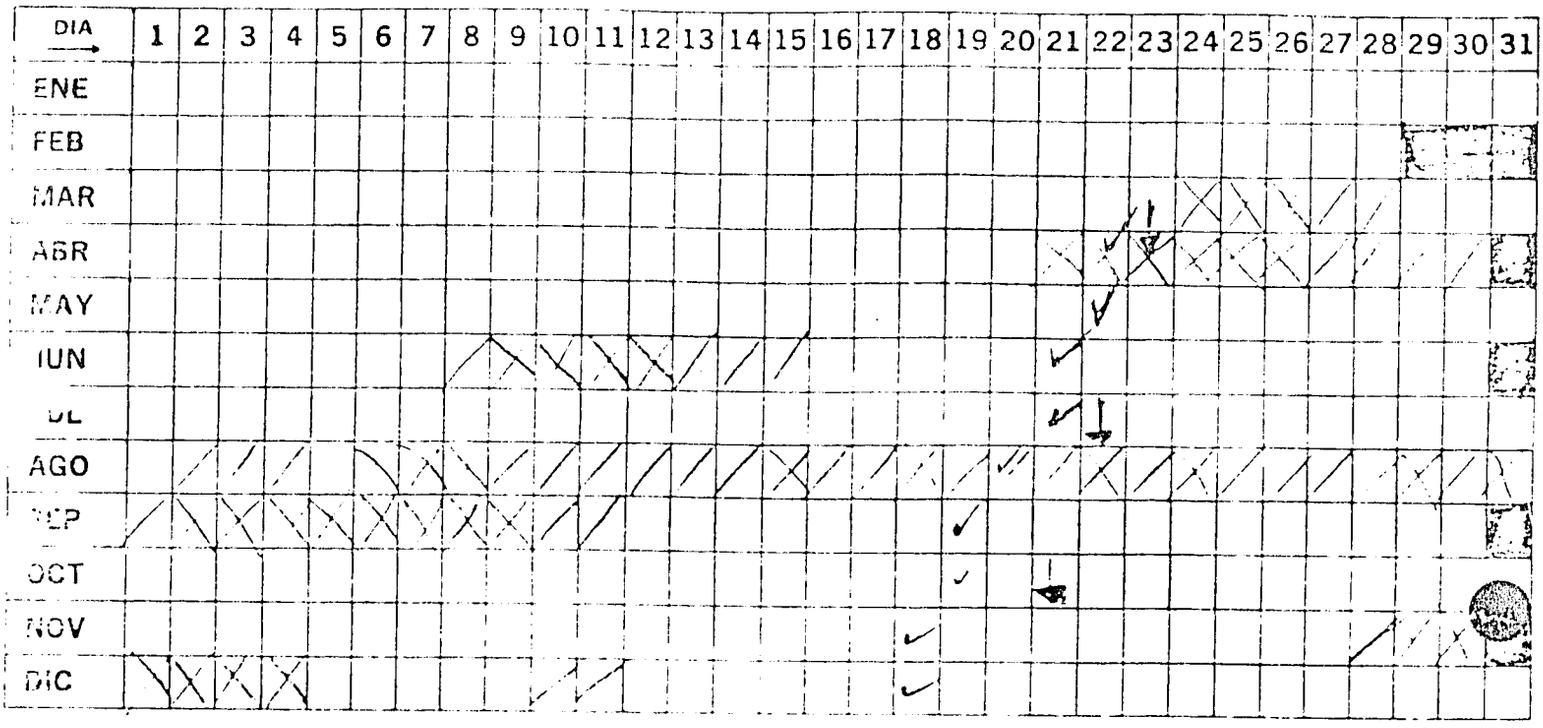
UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre Reyes Luna Perla

Registro 015

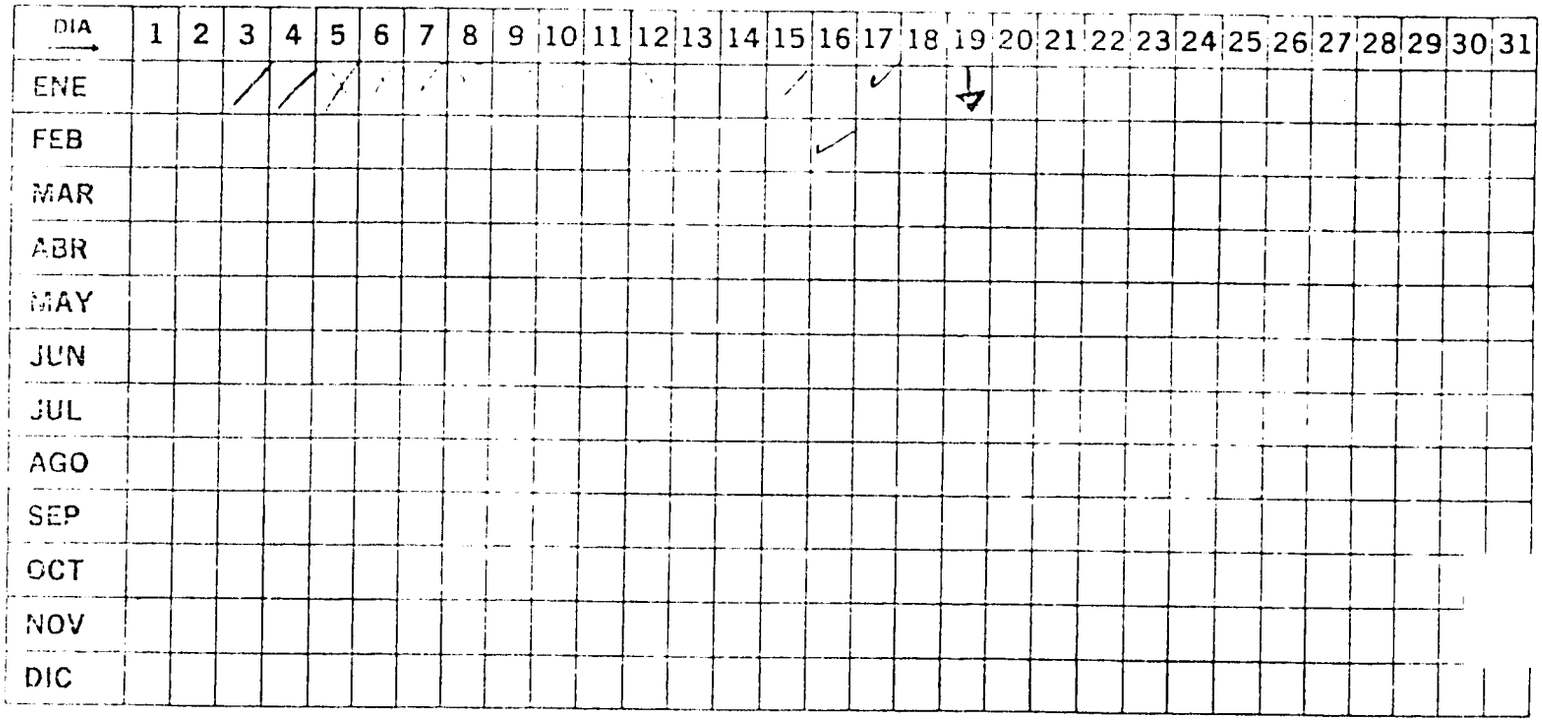
AÑO 19 26

REGISTRO DEL CICLO MENSTRUAL



AÑO 19 27

REGISTRO DEL CICLO MENSTRUAL



Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma. →

G = Goteo

S = Sangrado

INSTITUTO DE INVESTIGACION CIENTIFICA

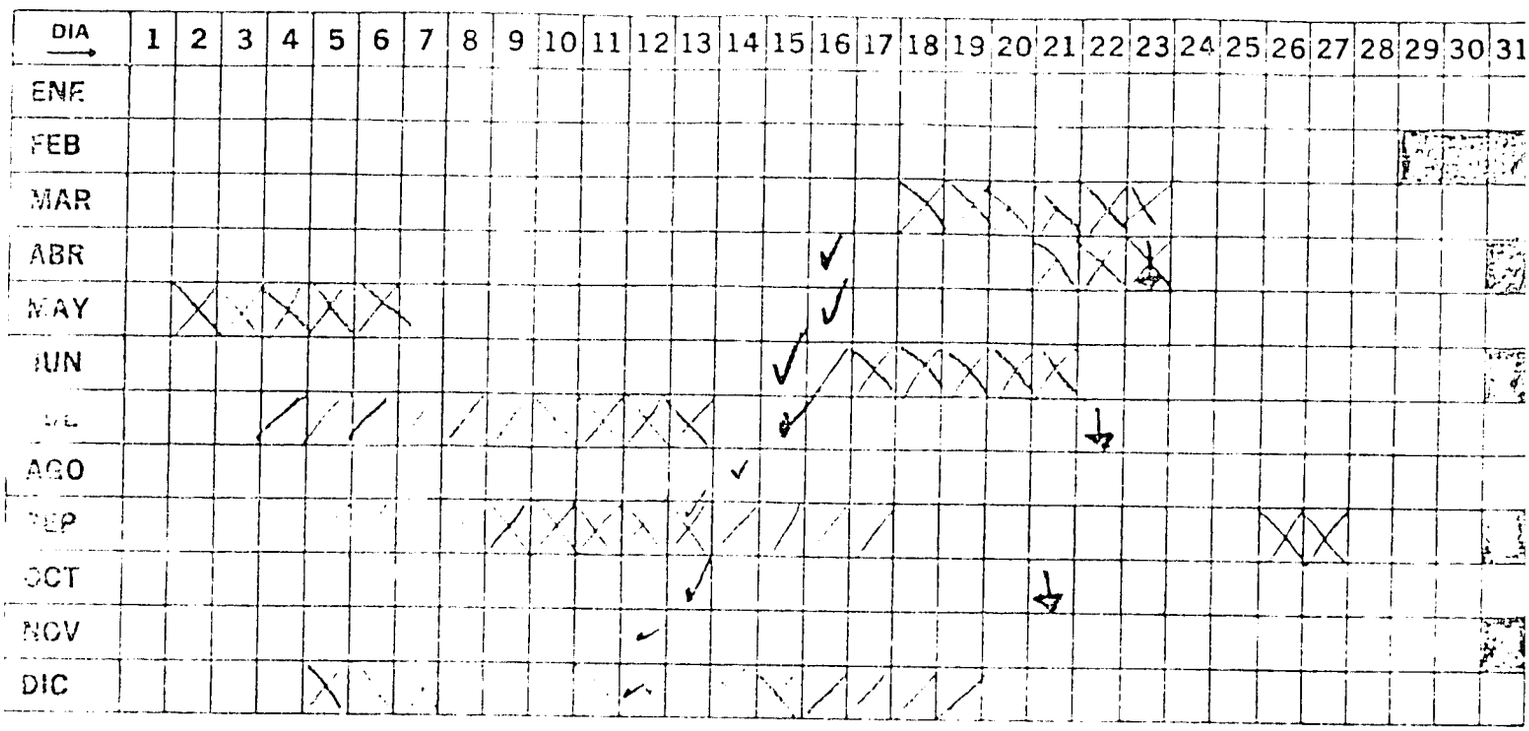
UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre ARREOLA CORRAL MARIA [illegible]

Registro 016

AÑO 19 / 20

REGISTRO DEL CICLO MENSTRUAL



AÑO 19 / 19

REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma: →

G = Goteo

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INSTITUTO DE INVESTIGACION CIENTIFICA

UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre [illegible]

Registro D17

AÑO { 19 / 76

REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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AÑO { 19 / 77

REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma: →

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INSTITUTO DE INVESTIGACION CIENTIFICA

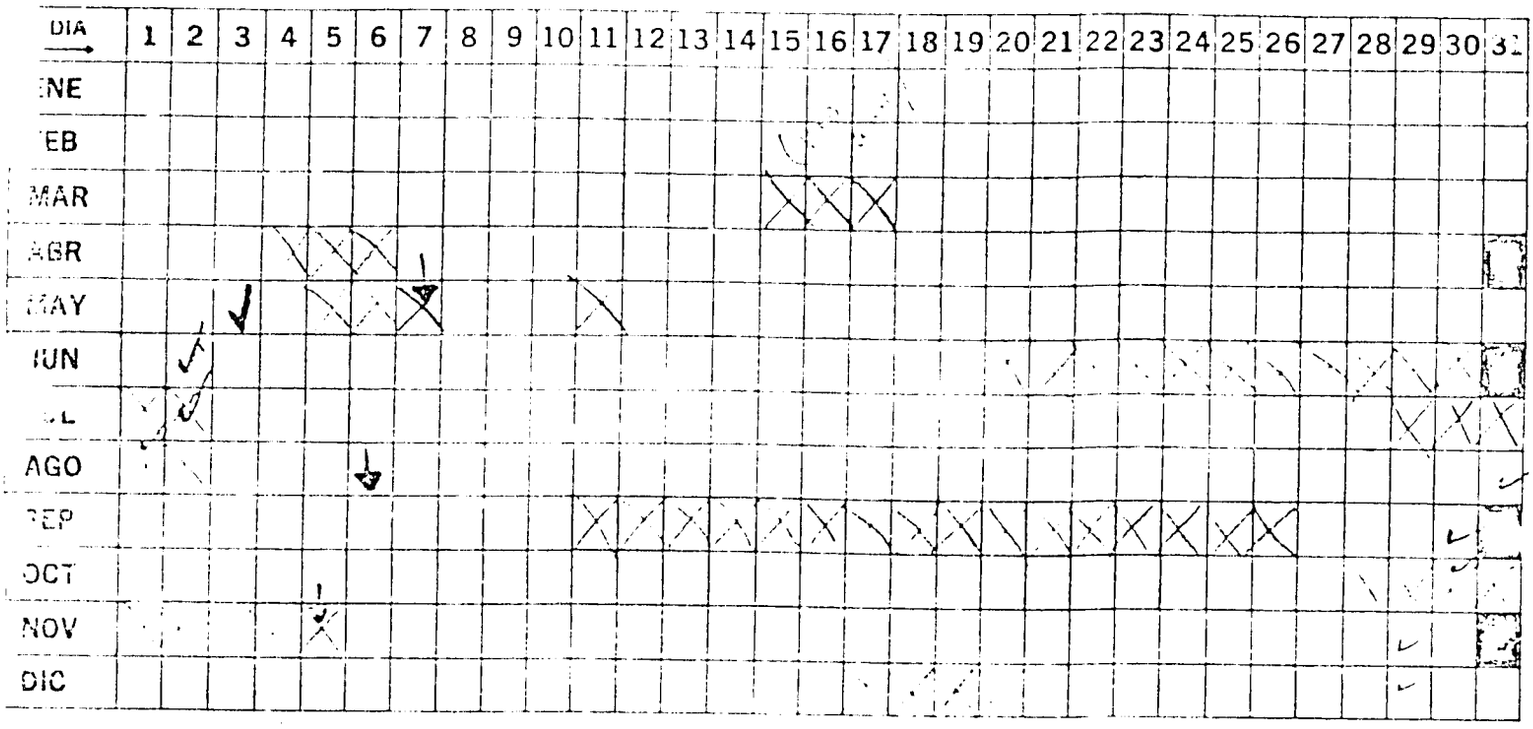
UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre OLGA MARQUEZ RIVAS.

Registro 018

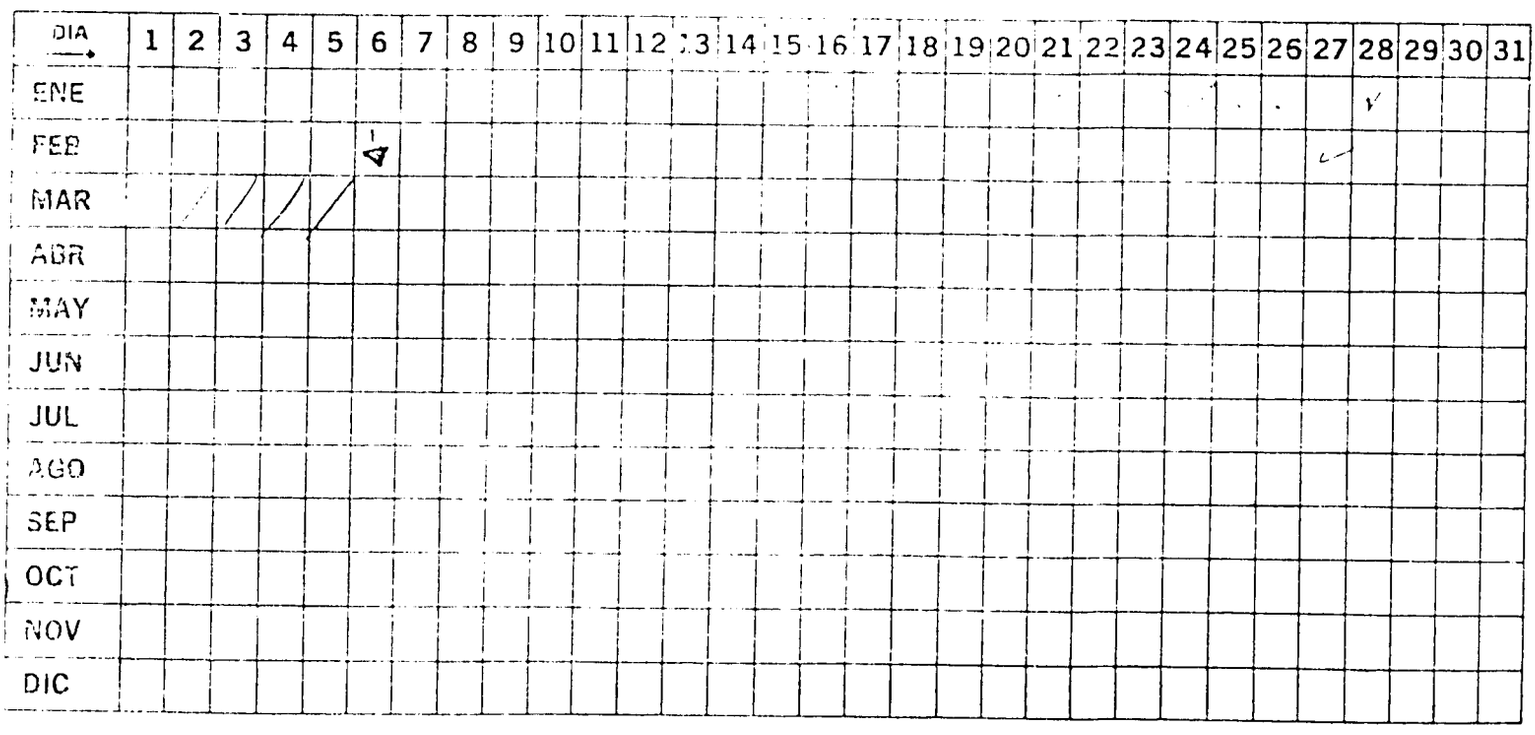
AÑO 19

REGISTRO DEL CICLO MENSTRUAL



AÑO 19

REGISTRO DEL CICLO MENSTRUAL



Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo mens-

G = Goteo

INSTITUTO DE INVESTIGACION CIENTIFICA

UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre MARIA LUISA SOSA HERNANDEZ.

Registro 619

AÑO { [illegible] / [illegible] }

REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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REGISTRO DEL CICLO MENSTRUAL

DIA →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
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INSTITUTO DE INVESTIGACION CIENTIFICA

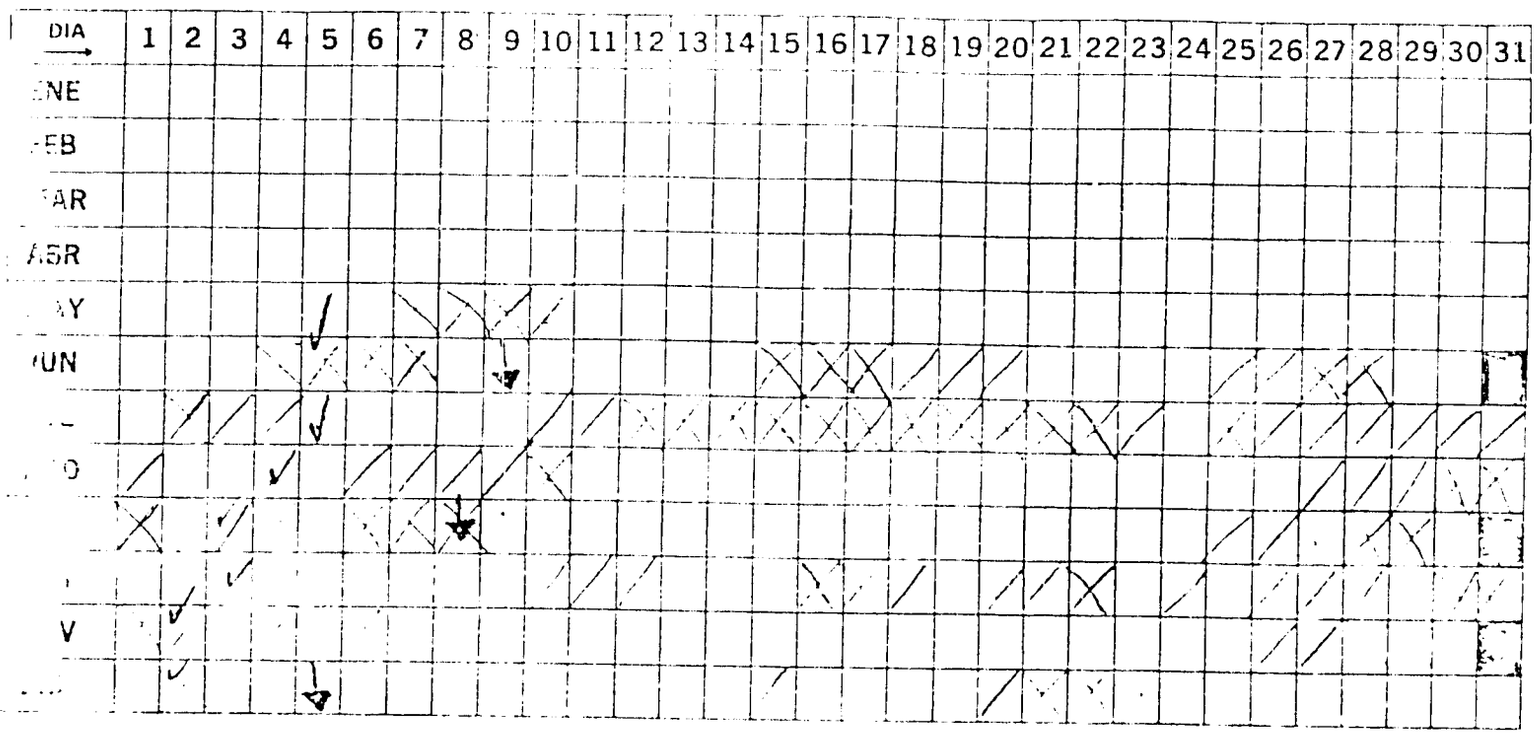
UNIVERSIDAD JUAREZ DEL ESTADO DE DURANGO

Nombre EVANGELINA [illegible] TARACENA

Registro 020

AÑO { 19 / [illegible] }

REGISTRO DEL CICLO MENSTRUAL



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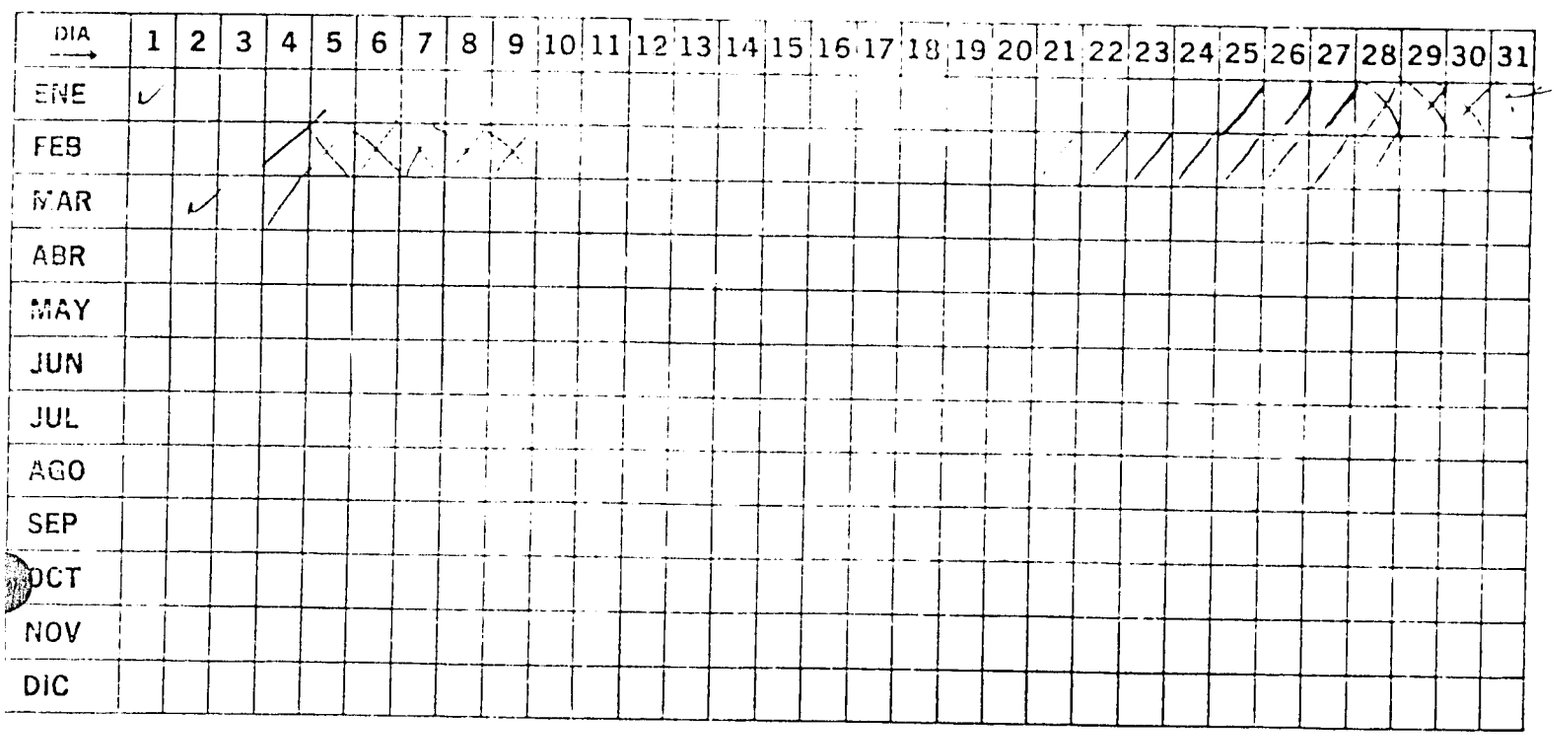
REGISTRO DEL CICLO MENSTRUAL



Favor de anotar en el cuadro correspondiente la letra apropiada para los días del calendario del ciclo menstrual en la siguiente forma: → { G = Goteo; S = Sangrado }

FINAL TECHNICAL REPORT

A Comparative Evaluation of 30 mg and 50 mg of 90-day Norethindrone Microspheres for Controlled release steroid Contraception:

Phase I Study

I- GENERAL INFORMATION

1. PARFR - 387 T -- " Phase I Poly NET 90 Injectable study - Pharmacokinetics "

2. Principal Investigator : Jose P. Balmaceda *
   Assistant Professor
   Department of Obstetrics and Gynecology
   University of Texas
   Health Science Center
   7703 Floyd Curl Drive
   San Antonio,Texas 78284

3. Institution : University of Texas
   Health Science Center at San Antonio
   7703 Floyd Curl Drive
   San Antonio, TX 78284

4. Technical Report Date : October 10, 1986

* Address correspondence to:

Jose P. Balmaceda
Associate Professor
Division of Reproductive Endocrinology and Infertility
University of California, Irvine
Department of Obstetrics and Gynecology
UCI Medical Center
101 The City Drive
Orange, California 92668

II- TECHNICAL REPORT

A- Statement of Objectives

The objectives of this study are to evaluate the safety and effect on ovarian function of 30 mg and 50 mg dose of the injectable NET biodegradable microcapsules. In addition pharmacokinetics will be studied at each dose. Services performed include:

1- Selection of 20 human subjects between 18 and 35 years

2- Treatment of 20 patients by intramuscular injection with two different doses of the microcapsule (10 each)

3- Collection of biological samples and patient records

4- Analysis of results

B- Patient Selection Criteria

Sterilized healthy women who could be follow up for 9 months, were asked to be volunteers for the study. Inclusion criteria were:

1- From 18 to 35 years of age

2- History of regular menstrual cycles from 23-35 days in lenght

3- Normal physical, pelvic and breast examination

4- Hemoglobin: 11 mg/dl or greater

5- Negative pregnancy test prior to the NET injection

C- Study Procedure

This is a single center randomly-allocated study of two dosages of the injectable formulation. The two dosages were randomly assigned to participants who do not know the dose given.

D- Results From Phase I Clinical Study

Two different doses of Norethindrone microcapsules were used to treat 20 volunteers at the University of Texas Health Science Center at San Antonio. Ten subjects received the 50 mg dose of microcapsulated Norethindrone, and ten subjects received a 30 mg dose. The microcapsules were injected on day 3 to 6 of the menstrual cycle. Following treatment blood samples were obtained biweekly. Serum levels of estrogen, progesterone and NET were mesured by radioimmunoassay. Endometrial biopsies were obtained from each subject before and during the treatment, in order to evaluate the effects of continuous norethindrone on the endometrium. High-density lipoproteins in the serum, were mesured before and after the treatment to evaluate the effects of continuous Norethindrone on the serum lipid profile. Daily records on menstrual bleeding were kept by each patient in a dairy.

Tables 1 - 20 lists the serum levels of estrogen, progesterone and norethindrone for the 20 volunteers. Of the patients that received 30 mg of Norethindrone, 2 had anovulatory cycles for the 3 months post-treatment, 7 had anovulatory cycles for the first two months post-treatment and one was anovulatory only one month. After receiving 50 mg of Norethindrone five subjects were anovulatory for three months post-treatment, four for the first two months and one for the first month post-treatment. All 7 patients that were anovulatory for 3 consecutive months after the NET injection (2 injected with 30 mg and 5 injected with 50 mg),recovered ovulatory function within 120 days from the drug administration.

Endometrial biopsies obtained during the luteal phase of the control cycle were all secretory. Endometrial biopsies obtained day 63 after the injection, showed a variable progestational effect. (See table 21).

Irregular vaginal bleeding was associated whith the anovulatory cycles. Number of bleeding and spotting days per 30 day period before and after NET administration are presented in table 22.

No significant changes were observed in SMAC 24, LDL, HDL, Urine analysis values. Control mean hemoglobin concentration values were not significantly different from mean values obtained after the injections.

E- ANALYSIS OF RESULTS

Norethindrone blood levels followed a biphasic shaped curve observed before with injections of microcapsules containing high dosages. There was an initial rise with values peaking whithin 4 days from injection. A continuous descending slope was initiated then, interrupted by a second rise occurring 25 to 30 days after drug administration in patients that received 50 mg and 35-40 days in patients that received 30 mg. Individual serum NET values varied widely for both dosages. Values became not significant between 110-130 days post-injection.

Ovulation as assessed by P_4 levels of more than 3 ng/ml and the existance of a time related menstrual period occurred in a significant proportion of the analysed cycles. If we divide the 90 days

following the injection in three 30 day periods or cycles; in 30% of those time periods we can encounter progesterone values suggestive of ovulation when patients were injected whith 30 mg. This percentage dropped to 20% in patients injected whith 50 mg.

Although NET as a contraceptive agent act at different levels of the genital tract the ovulatory rates observed in this phase I may be incompatible whith an acceptable pregnancy risk.

Bleeding irregularities followed the same pattern observed in previous phase I trials,using NET microcapsules; and consisted mainly of spotting associated whith the anovulatory periods. This was not associated whith significant changes on hemoglobin.

Cervical mucus checks performed as per protocol design did not contribute significant information. The exams were performed at fixed dates and not in relation to possible ovarian activity (i.e. presence of a follicle). For this reason we can not say if in the ovulatory cycles the mucus was not estrogenic around the time of ovulation.

Table 1

Patient LV (# 20)

66 Kg

50 mg NET

Date of Injection
2-4-86

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-7-86	0.014	0.40	-
1-10-86	0.026	0.51	-
1-14-86	0.062	0.27	-
1-17-86	0.324	0.77	-
1-21-86	0.051	6.61	-
1-24-86	0.117	10.31	-
1-28-86	0.091	4.21	-
1-31-86	0.018	1.12	0
2-4-86	0.013	0.64	0
2-5-86	0.019	0.45	5.74
2-6-86	0.017	0.44	4.05
2-7-86	0.043	0.73	2.68
2-11-86	0.028	0.44	1.98
2-14-86	0.015	0.45	1.85
2-18-86	0.015	0.35	1.42
2-21-86	0.028	0.42	1.47
2-25-86	0.014*	0.26	1.16
2-28-86	0.037	0.40	1.15
3-4-86	0.079	0.24	1.62
3-7-86	0.046	0.34	1.60
3-11-86	0.039	0.21	2.09
3-14-86	0.011	0.19*	1.77
3-18-86	0.022	0.20*	1.54
3-21-86	0.021	0.23*	1.24
3-25-86	0.024	0.30*	1.14
3-28-86	0.019	0.27*	1.40
4-1-86	0.028	0.27*	0.90
4-4-86	0.024	0.34*	0.85
4-8-86	0.049	0.49*	1.12
4-11-86	0.113	0.29*	1.12
4-15-86	0.032	0.27*	1.50
4-18-86	0.156	0.27*	1.30
4-22-86	0.224	0.95	1.47
4-29-86	0.197	0.28*	1.63
5-2-86	0.048	0.28*	1.99
5-6-86	0.052	0.29*	0.87
5-9-86	0.054	0.30*	0.96
5-16-86	0.034*	0.26*	0.50
5-20-86	0.090	0.30	0.39

Table 1 (cont.)

Patient LV (# 20)

66 Kg

50 mg NET

Date of Injection
2-4-86

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
5-23-86	0.158	0.29	0.38
5-27-86	0.120	1.31	0.30
5-30-86	0.171	6.86	0.40
6-3-86	0.088	6.57	0.19
6-6-86	0.025	1.37	0.17
6-10-86	0.048	0.47	0.15
6-13-86	0.058	0.40	0.15
7-8-86	0.056*	0.62	0.00

* equal to 0

Table 2

Patient CM (# 19)

48 Kg

30 mg NET

Date of Injection
2-4-86

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-6-85	0.017	0.22	-
1-10-86	0.035	0.06	-
1-14-86	0.111	0.00	-
1-17-86	0.082	0.54	-
1-21-86	0.052	10.29	-
1-24-86	0.118	18.12	-
1-28-86	0.063	3.84	-
1-31-86	0.015	0.65	0.00
2-4-86	0.049	0.23	2.06
2-5-86	0.019	0.87	>8.21
2-6-86	0	0.87	>8.12
2-14-86	0.023	0.01	2.74
2-18-86	0.052	0.02	2.18
2-21-86	0.150	0.05	1.35
2-25-86	0.205	0.04	1.49
2-28-86	0.157	0.01	1.32
3-4-86	0.084	0.09	1.02
3-7-86	0.168	0.25	0.82
3-11-86	0.135	0.54	0.90
3-14-86	0.157	0.71	0.66
3-18-86	0.115	0.25	0.60
3-21-86	0.057	0.19*	0.64
3-25-86	0.167	0.26	0.60
3-28-86	0.145	0.71	0.42
4-1-86	0.160	0.30	0.31
4-4-86	0.074	0.27*	0.56
4-8-86	0.066	0.27*	0.27
4-11-86	0.082	0.27*	0.37
4-15-86	0.090	0.27*	0.34
4-18-86	0.156	0.31*	0.65
4-22-86	0.224	0.27*	0.38
4-29-86	0.197	4.86	0.48
5-2-86	0.112	4.24	0.25
5-6-86	0.029	0.29*	0.28
5-9-86	0.041	0.29*	0.23
5-13-86	0.195	0.29*	0.13
5-16-86	0.265	0.35*	0.12
5-20-86	0.098	6.2	0.25
5-23-86	0.101	11.7	0.19
5-27-86	0.040	1.3	0.10
5-30-86	0.031*	0.16*	0.09
6-3-86	0.074	0.14*	0.12
7-1-86	0.122	0.14*	0.17

* equal to 0

Table 3

Patient CMK (# 18)

65 Kg | 50 mg NET | Date of Injection 2-4-86

Date	E_2	P_4	NET
1-10-86	0.015	0.15	-
1-14-86	0.012	0	-
1-17-86	0.061	0	-
1-21-86	0.022	0.70	-
1-23-86	0.095	4.91	-
1-27-86	0.128	8.88	-
1-31-86	0.032	2.14	0
2-4-86	0.012	0.18	0
2-5-86	0.012	0.03	3.38
2-6-86	0.003	0	3.29
2-7-86	0.015	0	2.32
2-10-86	0.013	0	1.51
2-13-86	0.014	0	1.35
2-17-86	0.161	0.03	1.09
2-20-86	0.336	0	0.94
2-24-86	0.776	0.12	1.04
2-27-86	0.470	0	1.52
3-4-86	0.059	0	1.70
3-11-86	0.012*	0	3.40
3-7-86	0.012*	0.03	2.01
3-14-86	0.005*	0.09*	2.13
3-19-86	0.005*	0.09*	2.32
3-24-86	0.005*	0.19*	2.10
3-28-86	0.019*	0.27*	1.41
3-31-86	0.019*	0.27*	1.68
4-3-86	0.019*	0.31*	2.49
4-8-86	0.019*	0.40	2.44
4-10-86	0.019*	0.27*	2.50
4-14-86	0.019*	0.27*	2.87
4-17-86	0.019	0.27*	2.55
4-22-86	0.015	0.27*	1.71
4-24-86	0.036	0.56	1.35
4-28-86	0.115	0.27*	0.98
5-2-86	0.198	0.29*	0.64
5-6-86	0.432	0.29*	0.49
5-9-86	0.628	0.29*	0.44
5-13-86	0.191	0.29*	0.34
5-15-86	0.062	0.16*	0.35
5-19-86	0.087	0.19*	0.24
5-22-86	0.123	0.16*	0.18

Table 3 (Cont.)

Patient CMK (# 18)

65Kg 50 mg Date of Injection 2-4-86

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
5-27-86	0.089	0.16*	0.29
5-30-86	0.031	0.16*	0.12
6-2-86	0.062	0.22*	0.40
6-5-86	0.196	0.14*	0.11
6-27-86	0.048	0.14*	0

* equal to 0

Table 4

Patient TS (# 17)

68 Kg 30 mg Date of Injection 2-10-86

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-14-86	0.012	0.44	-
1-17-86	0	0.09	-
1-21-86	0.025	0.09	-
1-23-86	0.119	0	-
1-27-86	0.109	1.00	-
1-30-86	0.078	9.76	-
2-5-86	0.071	6.85	-
2-7-86	0.046	4.44	0
2-10-86	0.022	0.31	0
2-11-86	0.017	0.28	3.30
2-12-86	0.019	0.22	1.59
2-13-86	0.018	0.15	0.87
2-18-86	0.067	0.05	0.50
2-20-86	0.138	0.03	0.39
2-24-86	0.196	0.96	0.68
2-27-86	0.075	5.05	0.62
3-4-86	0.045	5.93	0.66
3-6-86	0.035	8.56	0.60
3-12-86	0.018*	0.25*	0.74
3-14-86	0.023	0.22	0.96
3-17-86	0.091	0.19*	0.87
3-20-86	0.130	0.19*	0.83
3-25-86	0.185	0.37*	0.70
3-27-86	0.130	0.28*	0.95
3-31-86	0.117	0.34*	3.17
4-3-86	0.027	0.27*	0.78
4-7-86	0.032	0.27*	0.86
4-10-86	0.030	0.28*	1.23
4-16-86	0.039	0.27*	0.91
4-17-86	0.026	0.27*	0.72
4-22-86	0.029	0.27*	0.71
4-24-86	0.092	0.27*	0.55
4-28-86	0.258	0.34	0.52
5-1-86	0.289	0.55	0.36
5-8-86	0.063	0.29*	0.23
5-12-86	0.076	0.29*	0.20
5-15-86	0.038	0.21*	0.15
5-19-86	0.065	0.34	0.13
5-27-86	0.076	0.27*	0.11
5-30-86	0.031*	0.17*	0.11

Table 4

Patient TS (# 17)

68 Kg

30 mg NET

Date of Injection
2-10-86

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
6-2-86	0.145	0.17*	0.07*
6-5-86	0.124	0.26	0.11
6-9-86	0.039	2.30	0.10
6-25-86	0.031	0.21*	0

* equal to 0

Table 5

Patient DJ (# 16)

67 Kg

50 mg NET

Date of Injection
2-11-86

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-21-86	0.194	0.51	-
1-24-86	0.102	0.39	-
1-28-86	0.076	0.32	-
1-31-86	0.053	0.43	-
2-4-86	0.036	0.47	-
2-7-86	0.076	0.30	-
2-11-86	0.056	1.37	0
2-12-86	0.026	2.59	4.98
2-13-86	0.062	5.19	4.59
2-14-86	0.062	9.65	4.15
2-18-86	0.095	20.97	1.33
2-21-86	0.108	12.78	1.30
2-25-86	0.020*	0.74	2.69
2-28-86	0.028	0.46	2.79
3-4-86	0.031	0.17	1.92
3-7-86	0.049	0.35	2.00
3-14-86	0.030	0.35	1.95
3-18-86	0.059	0.59	1.55
3-21-86	0.076	1.07	1.21
3-24-86	0.078	0.50	1.50
4-4-86	0.109	0.89	2.12
4-8-86	0.041	0.59	1.08
4-11-86	0.064	2.91	1.05
4-15-86	0.044	0.87*	1.15
4-18-86	0.034	0.28*	1.33
4-22-86	0.105	0.40*	1.00
5-2-86	0.383	1.41	0.45
5-6-86	0.286	0.55	0.30
5-9-86	0.326	0.60	0.19
5-13-86	0.336	0.47	0.17
5-16-86	0.198	0.99	0.10
5-20-86	0.293	0.54	0.11
5-23-86	0.126	1.20	0.11
5-27-86	0.199	1.32	0.10
5-30-86	0.094	0.71	0.10
6-3-86	0.056	1.06	0.09
6-6-86	0.059	0.86	0
6-10-86	0.058	0.60	0
7-15-86	0.109	15.75	0

* equal to 0

Table 6

Patient LO (#15)

54 Kg

30 mg NET

Date of Injection (2-11-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-10-86	0.010	0.01	-
1-14-86	0.015	0	-
1-17-86	0.021	0	-
1-21-86	0.028	0.01	-
1-24-86	0.112	0	-
1-28-86	0.034	2.67	-
1-31-86	0.089	6.51	-
2-4-86	0.075	5.77	-
2-7-86	0.002	0.81	-
2-10-86	0.005	0.01	0
2-11-86	0.009	0.02	0.15
2-12-86	0.004	0.02	2.40
2-13-86	0.022	0.09	1.93
2-18-86	0.005	0.03	0.62
2-21-86	0.016	0.04	0.34
2-25-86	0.031	0.16	0.19
2-28-86	0.205	0	0.19
3-4-86	0.069	4.00	0.26
3-7-86	0.098	7.96	0.46
3-11-86	0.126	6.65	0.68
3-14-86	0.011	0.90	0.80
3-21-86	0.011	0.20*	1.05
3-25-86	0.005*	0.19*	0.92
3-28-86	0.019*	0.27*	1.13
4-1-86	0.019*	0.27*	0.99
4-4-86	0.019*	0.27*	0.81
4-8-86	0.019*	0.27*	0.86
4-11-86	0.019*	0.27*	1.03
4-15-86	0.024	0.27*	1.48
4-22-86	0.018	0.27*	1.58
4-24-86	0.018	0.27*	1.56
4-28-86	0.028	0.27*	1.06
5-2-86	0.018*	0.29*	0.80
5-6-86	0.034	0.40	0.46
5-9-86	0.017*	0.29	0.38
5-13-86	0.043	0.29*	0.16
5-16-86	0.049	0.16*	0.22
5-19-86	0.155	0.16*	0.19
5-22-86	0.165	0.59	0.24
5-27-86	0.102	10.20	0.12
5-29-86	0.150	8.87	0.15

Table 6 (Cont.)

Patient LO (# 15)

54 Kg

30 mg NET

Date of Injection (2-11-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
6-2-86	0.050*	3.78	0.13
6-5-86	0.032	0.24	0.17
6-10-86	0.030	0.14*	0.09
7-10-86	0.028*	0.14*	0

* equal to 0

Table 7

Patient CA (# 14)

52 Kg

50 mg NET

Date of Injection (2-14-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-14-86	0.012	0.20	-
1-17-86	0.016	0.24	-
1-21-86	0.034	0.14	-
1-23-86	0.159	0.09	-
1-27-86	0.235	1.09	-
1-31-86	0.178	15.38	-
2-3-86	0.157	14.26	-
2-5-86	0.210	17.45	-
2-10-86	0.026	2.13	-
2-14-86	0.015	0.33	0
2-15-86	0.008	0.44	3.92
2-16-86	0.038	0.12	2.47
2-18-86	0.028	0.19	2.56
2-20-86	0.049	0.11	1.33
2-25-86	0.090	0.08	0.65
2-28-86	0.107	0.06	0.75
3-5-86	0.140	0.14	0.47
3-12-86	0.164	0.19*	0.52
3-14-86	0.294	0.21	0.73
3-18-86	0.036	0.18*	0.94
3-21-86	0.039	0.33	1.96
3-24-86	0.031	0.18*	3.01
3-27-86	0.020*	0.34*	3.53
4-1-86	0.019*	0.27*	3.09
4-3-86	0.035	0.27*	4.18
4-7-86	0.043	0.27*	4.65
4-10-86	0.020*	0.27*	5.17
4-15-86	0.025	0.27*	5.15
4-18-86	0.010*	0.32*	6.11
4-24-86	0.034	0.35*	8.09
4-28-86	0.010	0.27*	6.35
5-1-86	0.033	0.29*	4.05
5-5-86	0.039	0.29*	3.18
5-8-86	0.018*	0.29*	1.89
5-12-86	0.027	0.29*	1.19
5-15-86	0.034	0.29*	1.18
5-19-86	0.044	0.34*	0.80
5-22-86	0.073	0.27	0.50
5-27-86	0.191	0.16*	0.26

Table 7 (Cont.)

Patient CA (# 14)

52 Kg 50 mg NET Date of Injection (2-14-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
5-30-86	0.578	0.48	0.22
6-3-86	0.214	12.40	1.33
6-5-86	0.275	15.57	0.21
6-12-86	0.023	2.21	0.19
6-13-86	0.062	1.21	0.23
6-16-86	0.053	0.33	0.19

* equal to 0

Table 8

Patient DA (#13)

70 Kg

30 mg NET

Date of Injection
(2-14-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-21-86	0.014	0.24	-
1-24-86	0.086	0.09	-
1-28-86	0.080	0.28	-
1-31-86	0.092	7.91	-
2-4-86	0.074	5.53	-
2-7-86	0.044	2.77	-
2-11-86	0.014	0.15	0
2-14-86	0.038	0.52	0.11
2-15-86	0.164	0.34	1.14
2-16-86	0.174	0.36	1.19
2-17-86	0.197	0.22	0.83
2-21-86	0.387	0.39	0.54
2-25-86	0.755	0.24	0.70
2-28-86	0.590	0.21	0.73
3-4-86	0.096	0.08	0.75
3-7-86	0.324	0.25	0.75
3-11-86	0.202	0.14	0.65
3-14-86	0.040	0.57	0.67
3-18-86	0.044	0.25*	0.69
3-21-86	0.078	0.31	1.51
3-25-86	0.027	0.19*	1.12
3-28-86	0.037	0.79	1.73
4-1-86	0.026	0.27*	1.11
4-4-86	0.021	0.42	1.26
4-8-86	0.081	0.41	1.38
4-15-86	0.328	0.35*	1.21
4-18-86	0.366	0.39*	1.11
4-22-86	0.063	0.27*	1.00
4-29-86	0.109	0.27*	1.07
5-2-86	0.110	0.37	0.80
5-6-86	0.101	0.29*	0.47
5-9-86	0.135	0.55	0.32
5-13-86	0.140	0.29	0.29
5-16-86	0.063	0.53	0.27
5-20-86	0.094	0.28*	0.29
5-23-86	0.273	0.31	0.34
5-27-86	0.109	3.46	0.16
5-30-86	0.150	8.37	0.21

Table 8 (Cont.)

Patient DA (# 13)

70 Kg | 30 mg NET | Date of Injection (2-14-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
6-3-86	0.072	4.24	0.15
6-6-86	0.034	0.67	0.13
6-10-86	0.048	0.23*	0
6-13-86	0.091	0.32	0.10
7-1-86	0.017	0.58	0.08

* equal to 0

Table 9

Patient MS (# 12)

78 KG

50 mg NET

Date of Injection (2-14-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-6-86	0.011	0.09	-
1-10-86	0.013	0.18	-
1-14-86	0.006	0	-
1-17-86	0.015	0.17	-
1-21-86	0.033	0.22	-
1-24-86	0.291	0.91	-
1-28-86	0.053	5.53	-
1-31-86	0.063	10.20	-
2-4-86	0.066	5.16	-
2-7-86	0.016	0.61	-
2-11-86	0.014	0.76	-
2-14-86	0.017	0.14	0
2-15-86	0.018	0.08	0.65
2-16-86	0.018	0.21	0.51
2-17-86	0.020	0.33	0.52
2-21-86	0.030	0.18	0.56
2-25-86	0.025	0.23	0.56
2-28-86	0.079	0.13	3.19 **
3-7-86	0.127	1.08	0.81
3-11-86	0.137	0.67	0.79
3-14-86	0.047	0.22	0.65
3-18-86	0.009*	0.19*	0.69
3-21-86	0.016	0.19*	0.54
3-25-86	0.006*	0.19*	0.44
3-28-86	0.032	0.27*	0.36
4-1-86	0.020*	0.27*	0.32
4-4-86	0.031	0.27*	0.35
4-8-86	0.032	0.36	0.31
4-11-86	0.021*	0.27*	0.28
4-15-86	0.029	0.27*	0.32
4-18-86	0.026	0.27*	0.47
4-22-86	0.022	0.33*	0.73
4-29-86	0.024	0.27*	0.59
5-2-86	0.044	0.35	0.42
5-6-86	0.021*	0.29*	0.51
5-9-86	0.028	0.28*	0.60
5-13-86	0.039	0.29*	0.38
5-16-86	0.055	0.35*	0.39

Table 9 (Cont.)

Patient MS (# 12)

78 Kg

50 mg NET

Date of Injection (2-14-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
5-20-86	0.053	0.16*	0.33
5-23-86	0.042	0.21*	0.26
5-27-86	0.060	0.16*	0.11
5-30-86	0.071	0.16*	0.19
6-3-86	0.047	0.26	0.13
6-10-86	0.056	0.17	0.18
6-13-86	0.040	0.14*	0
8-5-86	0.259	0.50	0.11

* equal to 0

** original value: 3.19, . Was repeated, new value: 0.72

Table 10

Patient RG (#11)

55 Kg

30 mg NET

Date of Injection (2-14-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-14-86	0.018	0.31	-
1-16-86	0.025	0.49	-
1-21-86	0.032	1.00	-
1-24-86	0.106	0.43	-
1-28-86	0.265	0.79	-
1-31-86	0.101	8.74	-
2-4-86	0.177	20.13	-
2-7-86	0.239	23.07	-
2-11-86	0.055	1.83	0
2-14-86	0.042	0.60	0
2-15-86	0.029	0.58	0.83
2-16-86	0.028	0.81	1.05
2-17-86	0.029	0.87	0.77
2-21-86	0.029	0.97	0.37
2-25-86	0.089	0.55	0.28
2-28-86	0.206	0.30	0.30
3-4-86	0.070	3.77	0.53
3-7-86	0.070	5.72	0.65
3-11-86	0.081	9.54	1.22
3-14-86	0.059	7.08	1.07
3-18-86	0.018	0.59	1.17
3-21-86	0.019	0.60	1.03
3-25-86	0.036	0.69	1.04
3-28-86	0.039	0.41	0.88
4-1-86	0.050	0.59	0.59
4-4-86	0.039	0.72	0.51
4-8-86	0.063	0.79	0.74
4-11-86	0.029	0.53	0.87
4-15-86	0.135	0.65	0.78
4-18-86	0.382	0.83	1.04
4-22-86	0.311	0.35*	1.08
4-29-86	0.114	0.29*	0.61
5-2-86	0.163	0.42	0.58
5-6-86	0.136	0.30*	0.30
5-9-86	0.161	0.70	0.18
5-16-86	0.213	0.86	0.17
5-20-86	0.041	0.93	0.12
5-22-86	0.056	0.20*	0.09
5-23-86	0.085	1.06	0.12
5-27-86	0.140	2.18	0.27
5-30-86	0.075*	0.77	0.16

Table 10 (Cont.)

Patient RG (# 11)

55 Kg

30 mg NET

Date of Injection (2-14-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
6-3-86	0.139	1.44	0.08
6-6-86	0.070	0.80	0.15
6-10-86	0.080	0.91	0.11
6-13-86	0.072	1.04	0.14
7-11-86	0.036	1.08	0.26

* equal to 0

Table 11

Patient LV (# 10)

66 Kg

50 mg NET

Date of Injection (2-14-86)

Date	E_2	P_4	NET
1-21-86	0.044	0.24	-
1-24-86	0.142	0.25	-
1-28-86	0.059	2.00	-
1-31-86	0.095	14.81	-
2-4-86	0.113	14.20	-
2-7-86	0.035	3.97	-
2-11-86	0.022	0.36	0
2-14-86	0.055	0.42	0
2-15-86	0.080	0.18	2.08
2-16-86	0.103	0.06	2.41
2-17-86	0.113	0.15	2.74
2-18-86	0.130	0.19	3.47
2-21-86	0.092	0.26	2.51
2-25-86	0.219	0.25	0.79
2-28-86	0.520	0.39	1.04
3-4-86	0.148	0.43	0.64
3-7-86	0.068	0.21	0.84
3-11-86	0.050	0.09	0.81
3-14-86	0.045	0.24	0.86
3-18-86	0.032	0.19*	1.43
3-21-86	0.044	0.20*	2.60
3-25-86	0.030	0.19*	1.79
3-28-86	0.031	0.55	2.89
4-1-86	0.019*	0.53	2.32
4-8-86	0.019*	0.33	2.81
4-11-86	0.019*	0.27*	1.92
4-15-86	0.019*	0.46	1.80
4-18-86	0.023	0.27*	2.00
4-22-86	0.040	0.32*	1.34
4-29-86	0.145	0.27*	1.51
5-2-86	0.133	0.29*	1.35
5-6-86	0.030	0.29*	1.70
5-13-86	0.032	0.29*	1.39
5-16-86	0.032*	0.30*	1.71
5-20-86	0.075	0.16*	0.99
5-23-86	0.414	0.31	0.90
5-27-86	0.066*	0.22	0.78
6-3-86	0.141	0.33	0.59
6-6-86	0.358	0.71	0.57
6-10-86	0.120	6.43	0.42
6-13-86	0.135	9.45	0.33
6-20-86	0.033	0.45	0.22

* equal to 0

Table 12

Patient RC (# 9)

54 Kg | 30 mg NET | Date of Injection (2-21-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-28-86	0.027	0.07	-
1-31-86	0.060	0.30	-
2-4-86	0.147	0.33	-
2-7-86	0.119	4.45	-
2-11-86	0.096	9.52	-
2-14-86	0.018	0.56	-
2-21-86	0.020	0.48	0
2-22-86	0.022	0.16	1.53
2-23-86	0.028	0.12	1.68
2-24-86	0.029	0.15	1.37
2-28-86	0.119	0.33	1.42
3-4-86	0.085	0.31	0.50
3-11-86	0.142	0.43	0.70
3-14-86	0.156	0.21*	0.62
3-18-86	0.013	0.19*	0.64
3-21-86	0.043	0.25*	1.55
3-25-86	0.016	0.25*	1.28
3-28-86	0.019*	0.75	1.50
4-1-86	0.025	0.32*	1.40
4-4-86	0.027	0.65	1.26
4-8-86	0.047	0.44	1.08
4-11-86	0.053	0.38	1.46
4-15-86	0.049	0.27*	1.05
4-18-86	0.150	0.27*	1.09
4-22-86	0.133	0.31*	1.18
4-29-86	0.050	0.30*	0.98
5-2-86	0.038	0.59	1.30
5-6-86	0.037	0.29*	1.27
5-9-86	0.060	0.39	0.86
5-13-86	0.030	0.29*	0.70
5-16-86	0.051	0.58	0.51
5-20-86	0.059	0.34	0.57
5-23-86	0.031*	0.49	0.30
5-27-86	0.050	0.75	0.44
5-30-86	0.078	0.28	0.29
6-3-86	0.068	0.56	0.15
6-6-86	0.103	1.80	0.15

Table 12

Patient RC (# 9)

54 Kg

30 mg NET

Date of Injection (2-21-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
6-10-86	0.066	1.95	0.24
6-13-86	0.134	1.34	0.27
6-17-86	0.235	0.59	0.32
6-20-86	0.047	2.60	0.19
7-8-86	0.073	0.61	0

* equal to 0

Table 13

Patient MP (#8)

85 Kg 50 mg NET Date of Injection (3-11-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
2-18-86	0.015	0.09	-
2-21-86	0.038	0.17	-
2-25-86	0.024	0.76	-
2-28-86	0.041	9.29	-
3-4-86	0.048	22.31	-
3-7-86	0.030	15.48	0
3-11-86	0.012*	2.00	0
3-12-86	0.005*	0.84	1.52
3-13-86	0.009	0.21*	1.25
3-14-86	0.015	0.95	1.45
3-18-86	0.030	0.18*	0.64
3-21-86	0.138	0.23*	0.77
3-25-86	0.117	1.79	0.73
4-1-86	0.060	10.49	1.08
4-4-86	0.027	4.47	3.68
4-8-86	0.019*	0.60	1.21
4-11-86	0.023	0.27*	1.16
4-15-86	0.034	0.27*	5.59
4-18-86	0.133	0.27*	1.51
4-22-86	0.226	0.27*	1.48
4-29-86	0.055	0.27*	1.03
5-2-86	0.132	0.49	0.97
5-6-86	0.123	0.29*	0.88
5-9-86	0.149	0.29*	0.89
5-13-86	0.120	0.30*	0.70
5-16-86	0.054	0.28	0.60
5-20-86	0.138	0.39	0.96
5-23-86	0.063	0.16*	1.03
5-27-86	0.073	0.70	1.05
5-30-86	0.054	0.19	0.59
6-3-86	0.029	0.24	0.59
6-6-86	0.031	0.17	0.45
6-10-86	0.054	0.25	0.35
6-17-86	0.047	2.44	0.18
6-20-86	0.071	11.54	0
6-24-86	0.095	15.71	0.17
6-27-86	0.060	5.82	0.12
7-1-86	0.025	0.41	0.12

* equal to 0

Table 14

Patient SM (# 7)

71 Kg

30 mg NET

Date of Injection (3-13-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
2-18-86	0.076	0.30	-
2-21-86	0.177	0.77	-
2-24-86	0.080	4.61	-
3-6-86	0.035	6.79	-
3-11-86	0.026	0.26	0
3-13-86	0.078	0.22*	0
3-14-86	0.085	0.52	1.73
3-15-86	0.054	4.73	0.71
3-16-86	0.056	5.09	0.87
3-21-86	0.057	12.39	0.46
3-25-86	0.089	10.72	0.27
3-31-86	0.053	0.61	0.22
4-3-86	0.056	0.31	2.71
4-7-86	0.063	0.57	0.34
4-11-86	0.094	9.55	1.13
4-14-86	0.082	11.96	1.79
4-17-86	0.097	6.87	1.02
4-22-86	0.026	0.43	1.20
4-24-86	0.036	0.41*	0.89
4-28-86	0.092	0.30*	0.73
5-2-86	0.377	0.37	0.83
5-6-86	0.933	0.94	0.95
5-9-86	0.882	0.38	1.40
5-13-86	0.140	0.28*	2.21
5-15-86	0.090	0.25*	1.58
5-19-86	0.162	0.29	1.49
5-22-86	0.129	0.24	1.41
5-27-86	0.050	0.16	0.79
5-30-86	0.036	0.16*	0.61
6-5-86	0.170	1.66	0.22
6-9-86	0.061	8.49	0.21
6-12-86	0.099	15.90	0.16
6-17-86	0.080	6.94	0
6-19-86	0.019	1.33	0.14
6-24-86	0.053	0.29	0.08
6-27-86	0.115	0.28	0.08
6-30-86	0.153	1.48	0.13
7-9-86	0.083	13.33	0.14
7-16-86	0.028*	0.90	0.14

* equal to 0

Table 15

Patient MC (# 6)

61 Kg

50 mg NET

Date of Injection (3-17-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
1-21-86	0.021	0.03	-
1-24-86	0.212	0.24	-
1-28-86	0.075	1.13	-
1-31-86	0.114	10.84	-
2-4-86	0.147	16.05	-
2-7-86	0.114	14.31	-
2-11-86	0.035	1.70	-
2-14-86	0.039	0.29	-
2-18-86	0.063	0.21	-
2-21-86	0.241	0.22	-
2-25-86	0.109	7.58	-
2-28-86	0.142	13.32	-
3-4-86	0.122	17.37	-
3-7-86	0.070	7.89	-
3-11-86	0.022	0.61	-
3-14-86	0.050	0.32	-
3-17-86	0.101	0.19*	0
3-18-86	0.139	0.19*	3.58
3-19-86	0.272	0.19*	3.70
3-20-86	0.258	0.48	3.30
3-21-86	0.413	0.31	3.58
3-24-86	0.514	0.40	1.38
3-28-86	0.452	0.33	1.35
4-1-86	0.355	0.33	1.45
4-4-86	0.093	0.47	0.79
4-8-86	0.110	0.41	1.59
4-11-86	0.093	0.69	1.17
4-15-86	0.029	0.81	1.73
4-18-86	0.068	0.27*	2.04
4-29-86	0.047	0.27*	2.57
5-2-86	0.033	0.29*	2.55
5-6-86	0.047	0.29*	3.23
5-9-86	0.034	0.29*	2.41
5-13-86	0.050	0.29*	1.44
5-16-86	0.096	0.16*	1.50
5-20-86	0.028*	0.16*	1.60
5-23-86	0.099	0.16	2.26
5-27-86	0.073*	0.41	1.82
5-30-86	0.041*	0.53	1.89
6-3-86	0.020*	0.17*	1.99
6-6-86	0.020	0.20	1.69
6-10-86	0.034	0.17*	1.35
6-13-86	0.038	0.17*	0.74
6-17-86	0.180	0.14*	0.46
6-20-86	0.198	0.16*	0.42

Table 15 (Cont.)

Patient MC (# 6)

61 kg

50 mg NET

Date of Injection (3-17-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
6-24-86	0.065	3.46	0.40
6-27-86	0.134	7.18	0.25
7-1-86	0.090	10.10	0.27
7-8-86	0.028*	0.84	0.13
7-11-86	0.028*	0.14*	0.11
7-15-86	0.028*	0.43	0.12
8-12-86	0.119	0.35	0

* equal to 0

Table 16

Patient SG (# 5)

49 Kg 50 mg NET Date of Injection (4-29-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
3-25-86	0.140	19.37	-
3-28-86	0.129	11.29	-
4-1-86	0.034	0.81	-
4-4-86	0.019*	0.37	-
4-8-86	0.115	0.56	-
4-11-86	0.095	0.37	-
4-15-86	0.087	3.40	-
4-18-86	0.192	15.00	-
4-22-86	0.257	20.00	-
4-29-86	0.046	0.34	0
4-30-86	0.017*	0.28*	6.64
5-1-86	0.057	0.29*	2.69
5-2-86	0.082	0.29*	2.28
5-6-86	0.030	0.29*	1.77
5-9-86	0.069	0.29*	1.24
5-13-86	0.048	0.29*	1.31
5-20-86	0.326	0.32	1.43
5-23-86	0.249	0.16*	1.50
5-27-86	0.126	0.16	3.56
5-30-86	0.031*	0.16*	3.87
6-3-86	0.034	0.19	2.60
6-6-86	0.020*	0.20	2.30
6-13-86	0.021	0.14*	1.70
6-17-86	0.031	0.14*	1.58
6-20-86	0.149	0.17	1.63
6-24-86	0.306	0.20*	1.29
6-27-86	0.208	0.14*	1.48
7-1-86	0.223	0.14*	1.48
7-8-86	0.028*	0.14*	0.81
7-11-86	0.028*	0.39	0.98
7-15-86	0.028*	0.62	0.97
7-18-86	0.028*	0.18	0.56
7-22-86	0.028*	0.21	0.54
7-24-86	0.050	0.14*	0.71
7-29-86	0.415	0.73	0.62
8-1-86	0.235	3.35	0.51
8-5-86	0.223	7.58	0.33
8-8-86	0.119	6.69	0.24
8-12-86	0.069	0.47	0.16
9-10-86	0.150	0.71	0.24

* equal to 0

Table 17

Patient ODC (# 4)

53 Kgs

50 mg NET

Date of Injection (5-6-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
4-11-86	0.026	0.27*	-
4-15-86	0.059	0.27*	-
4-18-86	0.073	0.27*	-
4-22-86	0.269	0.27*	-
4-29-86	0.073	11.14	-
5-2-86	0.097	8.16	-
5-6-86	0.145	3.93	0
5-7-86	0.018*	0.29*	5.00
5-8-86	0.035*	0.29*	6.43
5-9-86	0.018*	0.29*	2.87
5-13-86	0.020	0.29*	2.40
5-16-86	0.031*	0.16*	1.36
5-20-86	0.031*	0.16*	1.10
5-23-86	0.042	0.16*	0.93
5-28-86	0.310	0.25	0.89
6-3-86	0.132	0.15*	1.53
6-6-86	0.053	0.14*	2.20
6-10-86	0.020*	0.24*	3.18
6-13-86	0.020*	0.35	3.75
6-17-86	0.020*	0.14*	3.74
6-24-86	0.020*	0.14*	3.96
6-27-86	0.020*	0.14*	3.09
7-1-86	0.033	0.19*	3.02
7-8-86	0.028*	0.15*	1.62
7-11-86	0.028*	0.16	1.52
7-15-86	0.028*	0.16	1.69
7-22-86	0.043	0.14*	1.65
7-25-86	0.028*	0.14*	1.74
7-29-86	0.028*	0.14*	1.41
8-1-86	0.048	0.26	1.49
8-5-86	0.074	0.26	1.07
8-7-86	0.104	0.26	1.05
8-12-86	0.251	0.26	0.51
8-15-86	0.453	0.26	0.57
8-19-86	0.397	0.26	0.39
8-22-86	0.053	0.26	0.40
8-26-86	0.108	0.26	0.32
8-29-86	0.139	0.29	0.28
9-15-86	0.080	0.25	0.18

* equal to 0

Table 18

Patient MO (# 3)

67 Kg

30 mg NET

Date of Injection (5-13-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
4-22-86	0.144	0.69	-
4-29-86	0.109	8.04	-
5-2-86	0.124	20.96	-
5-6-86	0.092	8.36	-
5-9-86	0.047	2.19	-
5-13-86	0.046	0.48	0
5-14-86	0.038*	0.26*	2.82
5-15-86	0.037*	0.31	1.76
5-16-86	0.066	0.35	1.56
5-20-86	0.187	0.31	0.81
5-23-86	0.263	0.24*	0.42
5-27-86	0.423	0.35*	0.41
5-30-86	0.792	1.05	0.42
6-3-86	0.183	0.31	0.27
6-6-86	0.078	0.14*	0.44
6-10-86	0.071	0.24	0.57
6-13-86	0.103	0.18	0.89
6-17-86	0.040	0.14*	1.49
6-20-86	0.020*	0.14*	1.20
6-24-86	0.043	0.42	2.40
6-27-86	0.020	0.14*	2.42
7-1-86	0.028	0.14*	1.92
7-8-86	0.141	0.41	0.79
7-11-86	0.119	0.45	0.43
7-15-86	0.333	0.52	0.79
7-18-86	0.223	0.49	0.63
7-22-86	0.029*	0.17*	0.69
7-29-86	0	0.16	0.91
8-1-86	0.080	0.58	1.19
8-5-86	0.095	0.26	0.82
8-8-86	0.088	0.26	0.90
8-12-86	0.086	0.26	0.42
8-15-86	0.155	0.26	0.39
8-19-86	0.256	0.61	0.38
8-22-86	0.162	4.27	0.27
8-26-86	0.173	5.16	0.14
8-29-86	0.093	1.74	0.17
9-2-86	0.092	0.42	0.20

Table 18

Patient MO (# 3)

67 Kg 30 mg NET Date of Injection (5-13-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
9-18-86	0.186	10.65	0.20

* equal to 0

Table 19

Patient LTS (# 2)

62 Kg 30 mgNET Date of Injection (5-16-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
5-1-86	0.147	0.50	-
5-5-86	0.180	1.27	-
5-8-86	0.188	14.59	-
5-9-86	0.125	4.99	-
5-13-86	0.064	2.71	-
5-15-86	0.021	0.54	0
5-16-86	0.031*	0.29	0.11
5-17-86	0.031*	0.30	1.78
5-18-86	0.031*	0.31	1.62
5-19-86	0.031*	0.16*	0.91
5-23-86	0.040*	0.19*	0.64
5-29-86	0.028*	0.16*	0.46
6-3-86	0.022	0.16*	0.46
6-5-86	0.020	0.54	0.47
6-9-86	0.052	0.14*	0.35
6-12-86	0.026	0.38	0.42
6-16-86	0.033	0.28	0.47
6-19-86	0.078	0.46	0.82
6-23-86	0.077	0.69	0.81
6-26-86	0.020*	0.15*	1.08
6-30-86	0.020*	0.14*	0.94
7-3-86	0.036	0.42	1.09
7-7-86	0.028*	0.25	0.85
7-10-86	0.028*	0.27	0.59
7-14-86	0.028*	0.16*	0.48
7-17-86	0.028*	0.24	0.66
7-21-86	0.028*	0.24	0.71
7-24-86	0.028*	0.14*	0.52
8-1-86	0.070	0.53	0.93
8-4-86	0.045	0.26	0.72
8-7-86	0.058	0.26	0.65
8-12-86	0.054	0.31	0.42
8-19-86	0.182	0.26	0.28
8-22-86	0.189	0.83	0.31
8-27-86	0.094	1.60	0.15
8-29-86	0.058	0.50	0.15
9-2-86	0.060	0.58	0.12
9-5-86	0.064	0.69	0.13
9-30-86	0.022	0.16	0.05

* equal to 0

Table 20

Patient NH (# 1)

62 Kg

30 mg NET

Date of Injection
(5-16-86)

Date	E_2 (ng/ml)	P_4 (ng/ml)	NET (ng/ml)
4-24-86	0.059	0.35	-
4-28-86	0.174	0.29	-
5-1-86	0.151	1.05	-
5-5-86	0.156	14.78	-
5-15-86	0.042	0.39	0.15
5-16-86	0.060	0.49	0.09
5-17-86	0.078	0.47	2.19
5-18-86	0.082	0.39*	1.94
5-19-86	0.049	0.16*	1.61
5-22-86	0.053	0.16*	1.12
5-27-86	0.107	0.16*	0.58
5-30-86	0.305	0.22*	0.41
6-5-86	0.353	1.07	0.49
6-13-86	0.125	0.42	0.90
6-16-86	0.135	0.65	0.93
6-19-86	0.222	0.60	1.47
6-23-86	0.026	0.39	2.09
6-27-86	0.091	0.75	1.72
6-30-86	0.020*	0.27*	1.86
7-3-86	0.020*	0.27	1.23
7-9-86	0.028*	0.17	0.84
7-11-86	0.028*	0.37	0.93
7-21-86	0.141	0.39	0.69
7-24-86	0.170	0.22	0.76
7-28-86	0.194	0.43	0.68
7-31-86	0.116	0.39	0.88
8-4-86	0.091	0.27	0.88
8-7-86	0.128	0.49	1.05
8-11-86	0.069	0.34	0.79
8-14-86	0.074	0.70	0.67
8-18-86	0.089	0.31	0.55
8-21-86	0.065	0.33	0.38
8-25-86	0.124	0.31	0.43
8-28-86	0.224	0.32	0.21
9-2-86	0.094	1.88	0.24
9-5-86	0.095	6.34	0.23
9-29-86	0.181	6.27	0.06

* equal to 0

Table 21

NET microcapsules Endometrial biopsies (Day 63 post-injection)
90 days Reference Period Data

30 mg

02	Early Proliferative Endometrium
03	Weak Progestational effect
07	Secretory Exhaustation Compatible with Progestational effect
11	Suppressed Endometrium Compatible with Progestational effect
13	Suppressed Endometrium, compatible with Progestational effect
15	Suppressed Endometrium compatible with Progestational effect
17	Mild Suppression, suggestive of Progestational effect
19	Secretory exhaustation
09	Proliferative Endometrium

50 mg

04	Basal Endometrium Suggestive of Progestational effect
06	Secretory Exhaustation Compatible with Progestational effect
08	Mild Secretory Exhaustation, compatible with Progestational effect
10	Menstrual Endometrium Featuring Progestational effect
12	Suppressed Endometrium Compatible with Progestational effect
14	Insufficient for diagnosis
16	Suppression Compatible with Progestational effect
18	Secretory Exhaustation Compatible with Progestational Drug effect
20	Secretory Exhaustation Compatible with Progestational Drug effect

Table 22

NET microcapsules - Number of days of bleeding and spotting per 30 - day Reference Periods

Case	1-30	31-60	61-90	91-120
30 mg				
01	16	31	27	2
02	9	16	5	4
03	27	9	6	5
07	5	20	6	6
09	0	7	0	5
11	5	15	16	0
13	9	15	23	14
15	5	11	19	4
17	7	16	15	8
19	0	0	8	6
MEAN	8.3	14	12.5	5.4
50 mg				
04	10	8	17	8
05	6	9	9	5
06	1	16	5	4
08	7	14	31	4
10	17	31	12	3
12	6	14	10	0
14	2	0	7	8

Table 22 (Cont...)

NET microcapsules - Number of days bleeding and spotting per 30 - day Reference Periods

Case	1-30	31-60	61-90	91-120
50 mg				
16	11	20	10	6
18	4	15	0	5
20	17	19	11	6
MEAN	8.1	14.6	11.2	4.9

Final Progress Report

to

The Program For Applied Research
On Fertility Regulation

Investigator and Organization

Danny H. Lewis, Ph.D.
Vice President, New Product Development

Stolle Research and Development Corporation
P.O. Box 5310
Decatur, Alabama 35601

Title of Project

Preparation and Characterization of
Poly NET-30 Microspheres for
Phase I Clinical Study
Subagreement PARFR-388

September 26, 1986

I. PROJECT REPORT SUMMARY

A. General Information

1. Preparation and characterization of Poly NET-30 Microspheres for Phase I clinical study. Subagreement PARFR-388.

2. Danny H. Lewis, Ph.D., Stolle Research and Development Corporation, P.O. Box 5310, Decatur, Alabama 35601 (201)353-3814

3. Stolle Research and Development Corporation 2964 St. Rt. 42 South, Lebanon, Ohio 45036

4. Contract Period: November 1, 1985 - March 31, 1986.

5. Inclusive Dates of this Technical Report: November 1, 1985, September 1, 1986.

B. Narrative Summary

The objective of this project was the preparation and preclinical characterization of Poly NET 30 microspheres for a Phase I clinical study. Clinical supplies were produced in the Stolle Parenteral Drug Facility in Cincinnati, Ohio and delivered to PARFR, Northwestern University on March 24, 1986 for distribution to PARFR clinical centers. The microspheres were manufactured by the patented Stolle microencapsulation process previously used to produce a similar Poly NET 90 system. The clinical materials were completely characterized in vitro and a short-term in vivo experiment was conducted in two baboons. A formal storage stability study was initiated as a task of this Subagreement at Stolle R&D on the Phase I supplies. The subagreement was not structured to cover the actual long-term storage stability study requested by the FDA. The 30-day NET system is essentially developed except for scale up manufacture, and expanded Phase II trials could be initiated at the completion of the Phase I study. Future research and development efforts should be aimed at scale up and stability studies as the basic formulation is now in place.

II. Substantive Technical Research Report

A. Statement of Objectives

The objectives of this research project were as follows:

General objective: to produce the Poly NET 30 microsphere clinical dose.

1. Procurement of adequate quantities of 50:50 lactide:glycolide copolymer for use as the excipient.

2. Production of two batches of norethisterone-loaded microspheres in the Stolle sterile facility under GMP conditions.

3. Full in vitro characterization of the clinical materials to include: SEM, core loading analysis, in vitro release profile, size distribution, and residual solvent.

4. Preclinical evaluation of sterilized clinical material in two baboons for quality control only.

5. Initiate storage stability studies on the clinical materials.

6. Package and deliver to PARFR clinical materials to include:

   A. 60 syringes 15 mg NET
   B. 60 syringes 30 mg NET
   C. 120 vials injection vehicle

B. Progress

1. Procurement of Excipient

Suitable quantities of a 50:50 lactide:glycolide copolymer was synthesized under GLP conditions. The glycolide monomer was provided by Ortho Pharmaceutical Corporation. The polymer (Batch SR005-41-8) had an inherent viscosity of 0.56 dl/g at 30°C in chloroform.

2. Preparation of Clinical Supplies in Stolle Sterile Facility

Two batches of norethisterone-loaded microspheres and one batch of placebo microspheres were produced under sterile conditions in the Stolle facilities. The two-step microencapsulation process licensed to Ortho Pharmaceutical Corporation was utilized. Batches SR022-2 and SR-22-3 were drug-loaded and SR022-4 was the placebo batch. Both drug batches were produced on a scale of 75 g each, while 50 g of placebo product was prepared. The theoretical drug content was 50% by weight and the desired size range was 25 to 45 microns. The 50:50 glycolide:lactide copolymer was used as the excipient. Microsphere Batch SR022-3 was selected as the clinical batch.

3. In Vitro Characterization of the Clinical Materials

A full in vitro characterization was conducted on the clinical materials drug content, size distribution, residual solvent, and surface morphology were determined and the data on file in the master batch record at Stolle. The critical data are summarized below.

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	Batch SR022-2	Batch SR022-3
Batch Size	75 g	75 g
Drug Content, Actual	46.0%	46.5%
Residual Chloroform	---	1.95%
Size Distribution, %		
15-25 microns	6	3.0
25-30 microns	28.8	16.2
30-35 microns	36.3	33.7
35-40 microns	18.1	22.8
40-45 microns	8.8	13.7
Greater than 45 microns	8.0	10.5

Scanning electron micrographs indicated that the microspheres were spherical with little unencapsulated drug present. The wall coating appeared to be smooth with good integrity.

4. Preliminary Evaluation in the Baboon

Two animals were injected i.m. with one syringe each of the same batch (SR022-3) used for the Phase I human study. No adverse effects were observed during the treatment period or thereafter.

5. Storage Stability Study

A formal storage stability study was initiated on the 30-day NET clinical supplies. This study is in progress at the present time and no formal report has been prepared. This study is being funded by Stolle R&D.

6. Delivery of Materials to PARFR

On March 24, 1986, 60 syringes of 15 mg NET and 60 syringes of 30 mg NET was shipped to PARFR. The injection vehicle was shipped on December 6, 1985.

C. Unanticipated Results

The project was completed as expected with no unanticipated results.

D. Research Design

The original plan of work was followed.

E. Operational Significance

This program resulted in a novel 30-day injectable contraceptive produced in a sterile manufacturing facility. The project enabled PARFR to conduct a Phase I clinical trial in Mexico on the prototype system.

F. Dissemination and Utilization of Research Results

No publications have been made. We anticipate a paper at a later date.

Submitted by:

Danny H. Lewis, Ph.D.
Principal Investigator

FINAL TECHNICAL REPORT

Project Title

Phase I Poly NET 30 Injectable Study.

Principal Investigator

Roberto Rivera, M.D.
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Telephone Number: (181) 1-62-26

Roberto Rivera, M.D.

Institution

Instituto de Investigacion Cientifica
Universidad Juarez del Estado de Durango

Subcontract Period

March 1, 1986 - February 28, 1987

Dates of this Report

March 1, 1986 - February 28, 1987

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Subject characteristics

The age, number of live births and living children of the 24 participating subjects are presented in Table 1. All were healthy subjects who volunteered to participate in the study, duly signed consent forms are kept for each subject. All of them had been subjected to tubal ligation for personal reasons.

Injection and Termination Dates

The dates of the first and last injection for each subject are presented in Table 1. There was only one subject who discontinued the study after the second injection. She discontinued because she was afraid of the frequent blood sampling. The remaining 23 subjects complied with 100% of the follow-ups.

Injection Procedure

No problems were encountered with the injection procedure in any of the 24 cases, in any of the 71 injections performed. A good suspension of the microcapsules was always obtained by only shaking the syringe. However, a 3 ml syringe was received and the suspension media almost filled the syringe, limiting the space for shaking and mixing. A 5 ml syringe would be more suitable. In subject 21 during the second injection about half of the material was spilled.

There was not any case complaining that the injection had been a painful procedure. There was not in any case any local adverse reaction following the injection, either immediately or throughout the study period.

Continuation rates

24 subjects were admitted to the study. 23 subjects terminated the study, and complied 100% with the scheduled interviews and blood sampling. One subject discontinued after the second injection because she was afraid of the frequent blood sampling required by the study.

Side Effects

15 mg System

Six of the 12 subjects did not present any kind of side effects. Two subjects complained of nausea, one of them of vomiting. One subject complained of headache. One subject presented a transitory loss of consciousness, with spontaneous recovery, without sequelae; no neurological abnormalcy was found. One subject following the second injection experienced a skin rash, that was identified as allergic; she received oral antihystamines, and the rash disappeared in 4 days, no problems were experienced after the third injection. One subject experienced leucorrhea due to trichomonas. One subject complained of weight gain. One subject complained of pain and induration in the left breast; she saw a personal physician who prescribed an anti-inflammatory drug and the problem disappeared in 3 days.

30 mg System

Five of the 12 subjects did not experience any kind of side effect. Four subjects complained of headache. One subject in each category complained of nervousness, cystitis and muscular pain in the left leg.

Effects on the Menstrual Cycle

The effects on the menstrual cycle are presented as number of days of bleeding and spotting per 30-day reference periods in Tables 2 and 3. Most of the subjects experienced one form or another of menstrual irregularities.

In the following analysis, these definitions were used: 1) amenorrhea, non-bleeding interval of 90 or more days, 2) long non-bleeding interval, an interval of more than 40 days, but less than 90, 3) spotting days are considered as non-bleeding days. According to these criteria, in the 15 mg group, 3 subjects presented normal patterns, 8 of them long non-bleeding intervals, and one prolonged bleeding. In the 30 mg group, 6 of the subjects presented long non-bleeding intervals, 4 presented amenorrhea, and 2 prolonged bleeding or spotting.

In a different analysis of the menstrual patterns, the 30-day intervals for each subject were classified as non-bleeding, bleeding and spotting for 1 to 6 days, and bleeding and spotting for more than 7 days. The results of these analyses are presented in Tables 4 and 5. In the 15 mg group, the most frequent finding was that of subjects with bleeding and spotting for 1 to 6 days, followed by longer days of bleeding and spotting, with a relatively small number of cases of non-bleeding, Table 4. In the 30 mg group, the most frequent finding was that of subjects with bleeding and spotting for more than 7 days in each 30-day reference period, followed by one third of the subjects presenting non-bleeding intervals, Table 5.

Assessment of ovarian function

A cycle was considered as ovulatory when the progesterone values were above 5 ng/ml.

15 mg System

Subject 1

According to the progesterone values, no ovulation occurred in the three cycles that were observed during the injection period. The two control post-injection cycles were ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 2

According to the progesterone values, ovulation occurred in the three cycles that were observed during the injection period. The two control post-injection cycles were also ovulatory. Her menstrual pattern showed prolonged bleeding.

Subject 3

According to the progesterone values, no ovulation occurred in the two cycles that were observed during the injection period. The two control post-injection cycles were ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 4

According to the progesterone values, no ovulation occurred in the two cycles that were observed during the injection period. The two control post-injection cycles were ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 5

According to the progesterone values, no ovulation occurred in the three cycles that were observed during the injection period. One control post injection cycle was ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 6

According to the progesterone values, ovulation occurred in the two cycles observed after the first and third injections, while no ovulation occurred in the cycle after the second injection. One control post injection cycle was ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 7

According to the progesterone values, ovulation occurred in the three cycles that were observed during the injection period. Two control post-injection cycles were ovulatory. Two clear ovulatory rises of progesterone that occurred, one in the injection period, and one in the post-injection period, were not followed by menstruation but were considered ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 8

According to the progesterone values, ovulation occurred in the three cycles that were observed after the second and third injections, while no ovulation occurred in the cycle after the first injection. One control post injection cycle was ovulatory. Her menstrual pattern was classified as normal.

Subject 9

According to the progesterone values, ovulation occurred in one cycle that was observed after the second injection, while no ovulation occurred in the two cycles after the first and the third injections. Two control post-injection cycles were ovulatory. Her menstrual pattern was classified as normal.

Subject 10

According to the progesterone values, ovulation occurred in three of the four cycles that were observed during the injection period. No ovulation occurred in the cycle after the first injection. Two control post-injection cycles were ovulatory. Her menstrual pattern was classified as normal.

Subject 11

According to the progesterone values, ovulation occurred in the cycle after the second injection. While no ovulation occurred in the two cycles observed after the first and third injections. One control post-injection cycle was ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 12

The progesterone values were above 5 ng/ml on one determination after the first injection. There was no rise in the estradiol values or subsequent menstruation. This cycle was considered non-ovulatory. Two cycles were observed in the injection period that were considered non-ovulatory. One control post-injection cycle was ovulatory. Her menstrual pattern

was classified as long non-bleeding interval.

The number of ovulatory and non-ovulatory cycles are presented in Table 6.

30 mg System

Subject 1

According to the progesterone values, ovulation occurred in the first cycle after the first injection. No ovulation occurred in the second cycle, which began after the second injection. No ovulation or menstruation had occurred 84 days after the third injection. Her menstrual pattern was classified as amenorrhea.

Subject 2

According to the progesterone values, ovulation occurred in the cycle that was observed following the first injection. The subject was lost to follow-up after the second injection. Her menstrual pattern was classified as prolonged bleeding.

Subject 3

According to the progesterone values, no ovulation occurred in the four cycles that were observed during the injection period. One control post-injection cycle was ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 4

According to the progesterone values, no ovulation occurred in the three cycles that were observed during the injection period. One control post-injection cycle was ovulatory. Her

menstrual pattern was classified as long non-bleeding interval.

Subject 5

According to the progesterone values, ovulation occurred in one of the two cycles that were observed during the injection period. The two control post-injection cycles were ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 6

According to the progesterone values, no ovulation occurred in the three cycles that were observed during the injection period. Two control post-injection cycles were ovulatory. Her menstrual pattern was classified as prolonged spotting.

Subject 7

According to the progesterone values, no ovualtion occurred in the two cycles that were observed during the injection period. One control post-injection cycle was ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 8

According to the progesterone values, ovulation occurred in the cycle that was observed after the third injection, while no ovulation occurred in the two cycles after the first and the second injections. Two control post-injection cycles were ovulatory. Her menstrual pattern was classified as amenorrhea.

Subject 9

According to the progesterone values, ovulation occurred in the three cycles that were observed during the injection period. One control post-injection cycle was ovulatory. Her menstrual pattern was classified as amenorrhea.

Subject 10

According to the progesterone values, ovulation occurred in the cycle that was observed after the third injection, while no ovulation occurred in the two cycles after the first and the second injections. One control post-injection cycle was ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 11

According to the progesterone values, no ovulation occurred in the two cycles that were observed during the injection period. One control post-injection cycle was non-ovulatory. Her menstrual pattern was classified as long non-bleeding interval.

Subject 12

According to the progesterone values, no ovulation occurred in the two cycles that were observed during the injection period. One control post-injection cycle was ovulatory. Her menstrual pattern was classified as amenorrhea.

The number of ovulatory and non-ovulatory cycles are presented in Table 7.

Laboratory Tests

Determinations of glucose, urea, uric acid, creatinine, cholesterol, transaminase glutamic-piruvic, and glutamic-oxalacetic, alkaline phosphatase, bilirubin total and direct, albumin, globulin, total proteins, triglycerides, urinalysis and hemoglobin were performed at admission, and 17, 47 and 90 days after the date of the first injection.

15 mg System

Four subjects (cases 1, 2, 4 and 10) presented moderate rises, above the normal limits in the levels of alkaline phosphatase. Two subjects (cases 3 and 9) also presented moderate rises, above the normal limits in the levels of triglycerides, in the middle of treatment , the levels had returned to normal levels by the 90th day. One subject (case 9) also presented a rise above normal limits of glutamic-piruvic transaminase at the end of the observation period. One subject (case 11) also presented a light rise, but above the normal limits, in the glucose levels after the first injection, with a subsequent return to normal levels.

30 mg System

One subject (case 11) who already had abnormally high triglycerides before admission, showed a subsequent and important rise in the triglyceride levels following the injections, that persisted at day 90.

No abnormalities in the urinalysis were detected in either group.

The hemoglobin levels were not modified throughout the treatment period.

Identification of Cases

In the admission and follow-up forms the subjects were sequentially classified from 1 to 24, according to the time of admission.

Following the random allocation table received from PARFR, 12 subjects received the 15 mg system, and 12 the 30 mg system.

In this report, the cases numbered at admission as 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23, received the 15 mg system, and in this report are designated as cases 1 to 12, respectively. In the same way, cases numbered at admission as 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24, received the 30 mg system, and in this report are designated as cases 1 to 12, respectively.

Table 1. Data on subjects participating in Poly NET 30, PARFR 388M.

Case	Age	Live Births	Living Children	Date first Injection	Date last Injection
1	34	04	04	03-05-86	02-07-86
2	33	04	04	06-05-86	05-07-86
3	30	06	06	06-05-86	05-07-86
4	30	04	04	07-05-86	06-06-86
5	34	02	02	09-05-86	08-07-86
6	27	03	03	13-05-86	12-07-86
7	28	02	02	15-05-86	14-07-86
8	34	04	04	16-05-86	15-07-86
9	34	04	04	16-05-86	15-07-86
10	29	02	02	19-05-86	18-07-86
11	34	03	03	20-05-86	19-07-86
12	31	03	03	12-06-86	01-08-86
13	27	04	04	06-06-86	15-08-86
14	28	02	02	13-06-86	12-08-86
15	29	04	04	16-06-86	15-08-86
16	30	06	06	24-06-86	23-08-86
17	29	03	03	24-06-86	23-08-86
18	28	04	04	27-06-86	26-08-86
19	33	03	03	12-07-86	10-09-86
20	26	03	03	23-07-86	21-09-86
21	33	04	04	30-07-86	28-09-86
22	33	03	03	19-08-86	18-10-86
23	25	03	03	26-08-86	25-10-86
24	34	04	04	02-09-86	01-11-86
MEAN	30.54	3.5	3.5		
S.D.	2.94	1.06	1.06		

Table 2. Total number of days of bleeding or spotting per 30-day reference periods in 12 women using a 30-day system of lactide - co-glycolide microcapsules containing 15 mg of NET.

Case	1-30	31-60	61-90	91-120	121-150	151-180
1	5	8	13	12	0	5
2	6	10	6	19	3	3
3	8	10	9	0	4	4
4	6	3	3	5	2	2
5	11	3	7	16	6	4
6	6	4	20	3	4	2
7	6	9	6	0	0	7
8	3	3	9	6	6	5
9	9	5	4	6	6	5
10	4	6	3	3	3	4
11	12	8	0	11	4	0
12	4	3	1	0	5	0
MEAN	6.6	6	6.7	6.7	3.5	3.4
S.D.	2.8	2.8	5.5	6.3	2.1	2.1

Table 3. Total number of days of bleeding or spotting per 30-day system of lactide-co-glycolide microcapsules containing 30 mg of NET.

Case	1-30	31-60	61-90	91-120	121-150	161-180
1	9	6	12	4	12	0
2	5	4	13	-	-	-
3	5	11	5	6	4	3
4	6	6	17	7	0	3
5	9	7	28	0	0	6
6	11	0	25	7	7	5
7	7	9	6	8	6	4
8	9	0	4	0	4	3
9	6	7	4	0	0	5
10	8	3	10	11	5	0
11	6	0	5	5	4	0
12	7	0	4	0	0	0
MEAN	7.3	4.4	11.08	4.3	3.8	2.6
S.D.	1.8	3.8	8.3	3.8	3.7	2.2

Table 4. Characteristics of the menstrual patterns of 12 women using a 30-day system of lactide-co-glycolide microcapsules containing 15 mg of NET.

Event	Reference Period					
	1 30	31 60	61 90	91 120	121 150	151 180
NONBLEEDING	0	0	1	3	2	2
BLEEDING AND SPOTTING 1-6 DAYS	8	7	6	5	10	9
BLEEDING AND SPOTTING 7-20 DAYS	4	5	5	4	0	1

Table 5. Characteristics of the menstrual patterns of 12 women using a 30-day system of lactide-co-glycolide microcapsules containing 30 mg of NET.

Event	Reference Period 1–30	31–60	61–90	91–120	121–150	151–180
NONBLEEDING	0	4	0	4	4	4
BLEEDING AND SPOTTING 1-6 DAYS	5	4	6	3	5	7
BLEEDING AND SPOTTING 7-28 DAYS	7	4	6	5	3	1

Table 6. Number of ovulatory and non-ovulatory cycles in 12 women using a 30-day system of lactide-co-glycolide microcapsules containing 15 mg of NET.

Case	Cycles	Non-ovulatory	Ovulatory
1	3	3	0
2	3	0	3
3	2	2	0
4	2	2	0
5	3	3	0
6	3	1	2
7	3	0	3
8	4	1	1
9	3	2	3
10	4	1	1
11	3	2	1
12	2	2	0
TOTAL	35	19	16
PERCENTAGE	100	54.3	45.7

Table 7. Number of ovulatory and non-ovulatory cycles in 12 women using a 30-day system of lactide-co-glicolide microcapsules containing 30 mg of NET.

Case	Cycles	Non-ovulatory	Ovulatory
1	2	1	1
2	1	0	1
3	4	4	0
4	3	3	0
5	2	1	1
6	3	3	0
7	2	2	0
8	3	2	1
9	3	0	3
10	3	2	1
11	2	2	0
12	2	2	0
TOTAL	30	22	8
PERCENTAGE	100	73.3	26.7

NORTHWESTERN UNIVERSITY
THE MEDICAL SCHOOL
CHICAGO, ILLINOIS 60611

DEPARTMENT OF
OBSTETRICS AND GYNECOLOGY
333 EAST SUPERIOR STREET

January 12, 1987

Dr. Gerald Zatuchni
PARFR
875 North Michigan Avenue
Suite 1525
Chicago, IL 60611

RE: Progesterone Microcapsule Study – PARFR-391

Dear Gerry:

Attached are copies of the data available thus far on the progesterone microcapsule study.

We have encountered no difficulties in following the protocol and have administered progesterone microcapsules to seven women on days 1-3 of their menstrual cycle. In the five women for whom we now have hormonal data, serum progesterone levels never increased above 1.4 ng/ml (subject 4) and in three others there was no evidence of any increase in progesterone levels following the injection of the microcapsules. We confirmed increased pregnanediol excretion in the luteal phase compatible with ovulation following microcapsule injection in five of the five women for whom data are now available.

Although collection of data and measurement of hormones is not yet complete, I think you can see that considerable work has been carried out.

We conclude that the preparation of progesterone microcapsules did not raise circulating progesterone levels sufficiently to interfere with ovulation.

We do plan to complete the study and analyze all data for a final report to you.

Sincerely yours,

Bob

Robert W. Rebar, M.D.
Professor and Head
Department of Obstetrics
and Gynecology
Section of Reproductive
Endocrinology and Infertility

RWR:ce

Attachments

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 01 * Foll. Cont. Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
1	C 8153	09/30/86	0840	9.5	13	100	< 0.5	01	0
2	C 8154	09/30/86	0855	8.3	13.7	91	< 0.5	01	15
3	C 8155	09/30/86	0910	6.9	13.4	75	< 0.5	01	30
4	C 8156	09/30/86	0925	8.1	13.5	77	< 0.5	01	45
5	C 8157	09/30/86	0940	9.0	14.4	30	< 0.5	01	60
6	C 8158	09/30/86	0955	8.4	8.2	81	< 0.5	01	75
7	C 8159	09/30/86	1010	8.1	9.0	91	< 0.5	01	90
8	C 8160	09/30/86	1025	8.2	8.1	93	< 0.5	01	105
9	C 8161	09/30/86	1040	7.7	9.6	100	< 0.5	01	120
10	C 8162	09/30/86	1055	6.5	8.7	76	< 0.5	01	135
11	C 8163	09/30/86	1110	10.6	9.6	68	< 0.5	01	150
12	C 8164	09/30/86	1125	8.0	9.3	81	< 0.5	01	165
13	C 8165	09/30/86	1140	6.6	10.1	77	< 0.5	01	180
14	C 8166	09/30/86	1155	6.7	9.4	70	< 0.5	01	195
15	C 8167	09/30/86	1210	4.7	6.0	66	< 0.5	01	210
16	C 8168	09/30/86	1225	4.8	6.0	71	< 0.5	01	225
17	C 8169	09/30/86	1240	5.5	8.8	23	< 0.5	01	240

****** 12:28:22 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

1ICRO_P4* SUBJECT 01 * Luteal Cont Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
69	C 8322	10/17/86	0900	4.7	4.5	130	8.3	02	0
70	C 8323	10/17/86	0915	4.6	3.9	110	7.4	02	15
71	C 8324	10/17/86	0930	3.5	< 3.0	94	7.0	02	30
72	C 8325	10/17/86	0945	4.3	3.7	94	6.4	02	45
73	C 8326	10/17/86	1000	5.0	3.0	86	6.0	02	60
74	C 8327	10/17/86	1015	3.1	< 3.0	81	6.3	02	75
75	C 8328	10/17/86	1030	4.9	< 3.0	25	5.6	02	90
76	C 8329	10/17/86	1045	4.2	< 3.0	74	5.9	02	105
77	C 8330	10/17/86	1100	5.3	< 3.0	66	4.7	02	120
78	C 8331	10/17/86	1115	3.8	< 3.0	65	4.4	02	135
79	C 8332	10/17/86	1130	3.2	< 3.0	64	4.0	02	150
80	C 8333	10/17/86	1145	2.9	< 3.0	36	3.9	02	165
81	C 8334	10/17/86	1200	2.9	< 3.0	39	3.9	02	180
82	C 8335	10/17/86	1215	3.6	< 3.0	33	3.7	02	195
83	C 8336	10/17/86	1230	5.3	< 3.0	36	3.3	02	210
84	C 8337	10/17/86	1245	7.0	< 3.0	43	3.0	02	225
85	C 8338	10/17/86	1300	4.9	< 3.0	46	3.6	02	240

****** 12:31:36 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 01 * Injection Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
103	C 8411	10/22/86	0900	7.0	8.4	< 10	< 0.5	03	-60
104	C 8412	10/22/86	0915	8.2	9.2	33	< 0.5	03	-45
105	C 8413	10/22/86	0930	6.5	8.0	24	< 0.5	03	-30
106	C 8414	10/22/86	0945	8.0	9.8	34	< 0.5	03	-15
107	C 8415	10/22/86	1000	4.6	12	34	< 0.5	03	0
108	C 8416	10/22/86	1015	6.6	9	29	< 0.5	03	15
109	C 8417	10/22/86	1030	5.1	11	< 10	< 0.5	03	30
110	C 8418	10/22/86	1045	5.7	9.8	20	< 0.5	03	45
111	C 8419	10/22/86	1100	6.7	8.4	25	< 0.5	03	60
112	C 8420	10/22/86	1115	7.0	9.2	22	< 0.5	03	75
113	C 8421	10/22/86	1130	8.0	8.8	19	0.6	03	90
114	C 8422	10/22/86	1145	4.7	9.2	20	0.5	03	105
115	C 8423	10/22/86	1200	6.4	9.9	21	0.5	03	120
116	C 8424	10/22/86	1215	6.8	9.7	19	< 0.5	03	135
117	C 8425	10/22/86	1230	7.0	9.3	< 10	0.5	03	150
118	C 8426	10/22/86	1245	4.3	7.6	21	0.5	03	165
119	C 8427	10/22/86	1300	6.3	7.8	18	< 0.5	03	180
120	C 8428	10/22/86	1315	7.0	6.7	19	< 0.5	03	195
121	C 8429	10/22/86	1330	7.3	7.3	17	< 0.5	03	210
122	C 8430	10/22/86	1345	7.0	7.6	17	< 0.5	03	225
123	C 8431	10/22/86	1400	5.5	8.0	11	< 0.5	03	240

****** 12:32:31 ******

* ENDOSYS * Progesterone Microcups. - Serum Response *

MICRO_P4* SUBJECT 01 * Follow Up

* print out from PRINT OUT * 01/09/87 * JNH *

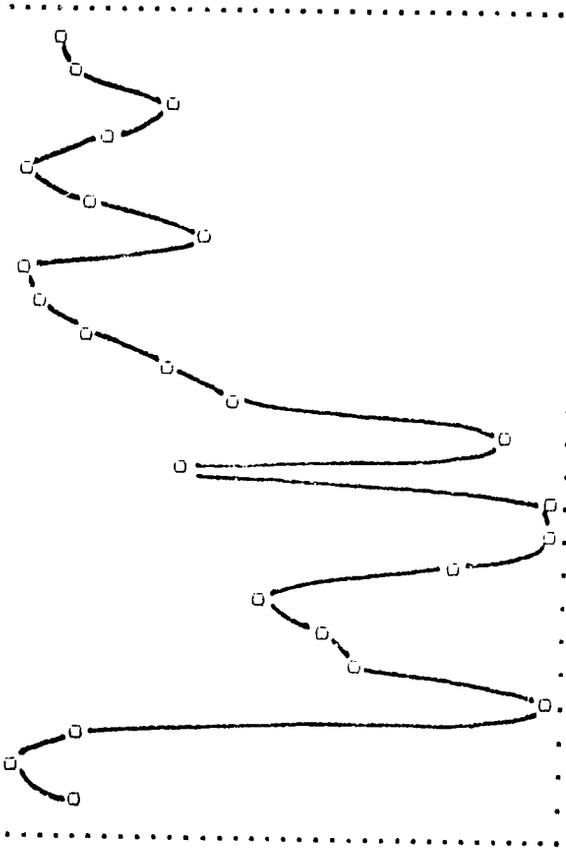
recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
293	C 8432	10/24/86	1100			41	< 0.5	04	1
294	C 8450	10/28/86	1050			130	< 0.5	04	2
295	C 8540	10/31/86	0950			280	< 0.5	04	3
296	C 8564	11/04/86	1115			88	6.1	04	4
298	C 8562	11/07/86	1605			140	12.8	04	5
304	C 8699	11/11/86	1515			110	2.4	04	6
307	C 8701	11/18/86	0920			23	0.6	04	7
320	C 8745	11/25/86	0920			75	1.1	04	8

****** 12:38:00 *******

* ENDOSYS * PARFR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 01 * Month 0

* print out from PRINT OUT * 01/09/87 * JNH *



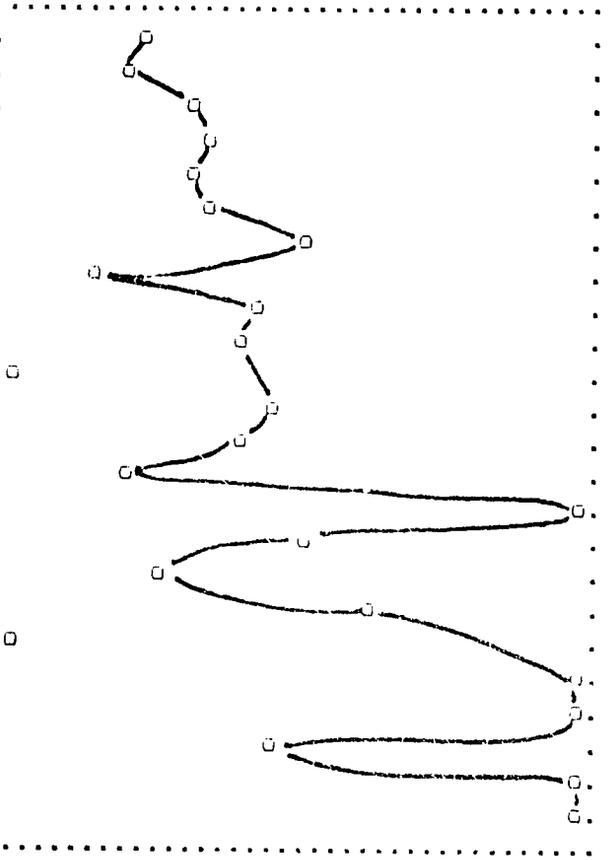
labnum	date	PDIOL(ug/ml)	day	remarks
C20091	09/28/86	1.7	1	
C20092	09/29/86	2.0	2	
C20093	09/30/86	3.6	3	
C20094	10/01/86	2.6	4	
C20095	10/02/86	1.2	5	
C20096	10/03/86	2.3	6	
C20097	10/04/86	4.2	7	
C20098	10/05/86	1.3	8	
C20099	10/06/86	1.5	9	
C20100	10/07/86	2.4	10	
C20101	10/08/86	3.6	11	
C20102	10/09/86	4.6	12	
C20103	10/10/86	9.3	13	
C20104	10/11/86	4.0	14	
C20105	10/12/86	10.0	15	
C20106	10/13/86	10.0	16	
C20107	10/14/86	8.5	17	
C20108	10/15/86	5.2	18	
C20109	10/16/86	6.3	19	
C20110	10/17/86	6.9	20	
C20111	10/18/86	>10.0	21	
C20112	10/19/86	2.4	22	
C20113	10/20/86	1.2	23	
C20114	10/21/86	2.3	24	

****** 11:33:55 *******

* ENDOSYS * PMRPR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 01 * Month 1

* print out from PRINT OUT * 01/09/87 * JNH *



labnum	date	PDIOL(ug/ml)	day	remarks
C20451	10/23/86	2.7	1	
C20452	10/24/86	2.6	2	
C20453	10/25/86	3.5	3	
C20454	10/26/86	4.0	4	
C20455	10/27/86	3.7	5	
C20456	10/28/86	3.8	6	
C20457	10/29/86	5.5	7	
C20458	10/30/86	1.9	8	
C20459	10/31/86	4.8	9	
C20460	11/01/86	4.4	10	
C20461	11/02/86	*	11	
C20462	11/03/86	5.0	12	
C20463	11/04/86	4.4	13	
C20464	11/05/86	2.5	14	
C20465	11/06/86	>10.0	15	
C20466	11/07/86	5.5	16	
C20467	11/08/86	3.2	17	
C20468	11/09/86	6.5	18	
C20469	11/10/86	*	19	
C20470	11/11/86	>10.0	20	
C20471	11/12/86	>10.0	21	
C20472	11/13/86	4.9	22	
C20473	11/14/86	>10.0	23	
C20474	11/15/86	>10.0	24	

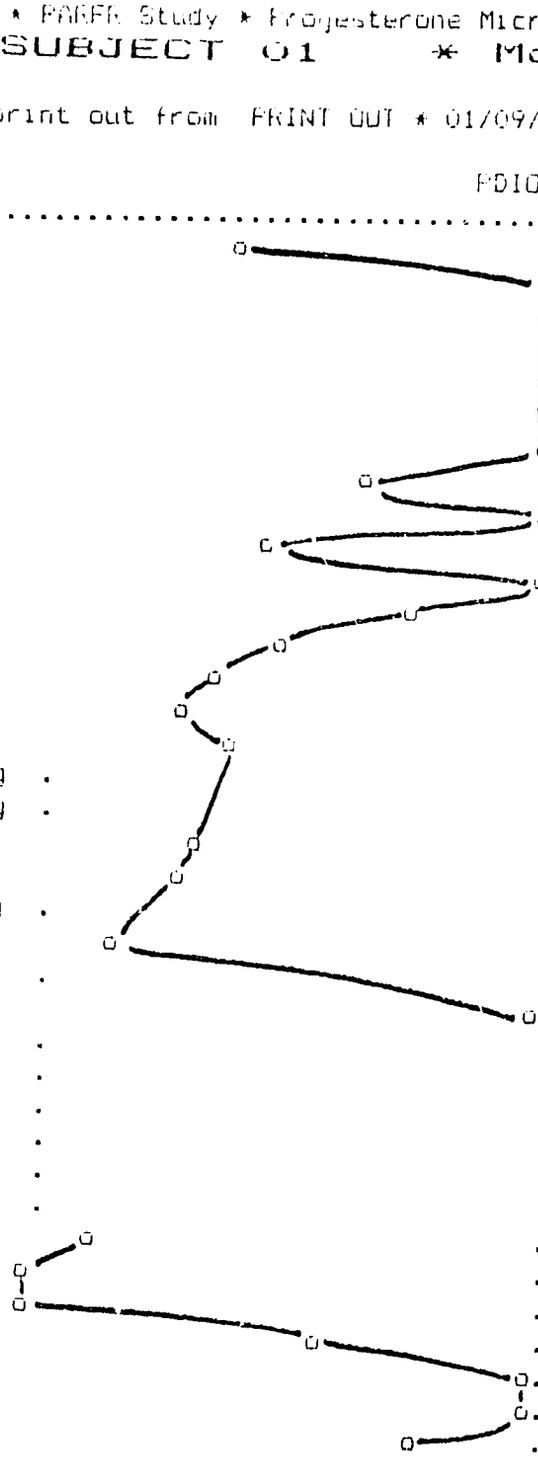
****** 11:40:12 *******

* ENDOSYS * PARFR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 01 * Month 2

* print out from PRINT OUT * 01/09/87 * JNH *

labnum	date	PDIOL(ug/ml)	day	remarks
C20301	11/13/86	4.9	1	Subj over-
C20302	11/14/86	>10.0	2	lapped 13
C20303	11/15/86	>10.0	3	14,15 ??
C20304	11/16/86	>10.0	4	
C20305	11/17/86	>10.0	5	
C20306	11/18/86	>10.0	6	
C20307	11/19/86	>10.0	7	
C20308	11/20/86	7.2	8	
C20309	11/21/86	>10.0	9	
C20310	11/22/86	5.4	10	
C20311	11/23/86	>10.0	11	
C20312	11/24/86	8.1	12	
C20313	11/25/86	5.9	13	
C20314	11/26/86	4.7	14	
C20315	11/27/86	4.3	15	
C20316	11/28/86	5.0	16	
	missing			
	missing			
C20319	12/01/86	4.5	19	Menses
C20320	12/02/86	4.3	20	
	missing			
C20321	12/04/86	3.2	22	
	missing			
C20322	12/06/86	>10.0	24	
	missing			
	missing			
	missing			
	missing			
	missing			
	missing			
C20323	12/13/86	2.8	31	
C20324	12/14/86	1.8	32	
C20325	12/15/86	1.6	33	
C20326	12/16/86	6.7	34	
C20327	12/17/86	>10.0	35	
C20328	12/18/86	>10.0	36	
C20329	12/19/86	8.2	37	



****** 12:47:17 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 02 * Foll. Cont. Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
18	C 8271	10/06/86	1145	7.0	22	92	< 0.5	01	0
19	C 8272	10/06/86	1200	5.8	19.7	73	< 0.5	01	15
20	C 8273	10/06/86	1215	5.6	23.5	65	< 0.5	01	30
21	C 8274	10/06/86	1230	5.9	23.1	64	< 0.5	01	45
22	C 8275	10/06/86	1245	6.9	17.4	64	< 0.5	01	60
23	C 8276	10/06/86	1300	5.5	18.2	66	< 0.5	01	75
24	C 8277	10/06/86	1315	5.4	17.4	49	< 0.5	01	90
25	C 8278	10/06/86	1330	3.7	16.4	42	< 0.5	01	105
26	C 8279	10/06/86	1345	5.2	18.9	44	< 0.5	01	120
27	C 8280	10/06/86	1400	5.2	18.1	46	< 0.5	01	135
28	C 8281	10/06/86	1415	10.2	17.4	40	< 0.5	01	150
29	C 8282	10/06/86	1430	10.4	23.7	50	< 0.5	01	165
30	C 8283	10/06/86	1445	2.2	21.6	53	< 0.5	01	180
31	C 8284	10/06/86	1500	8.5	22.3	56	< 0.5	01	195
32	C 8285	10/06/86	1515	7.9	21.3	50	< 0.5	01	210
33	C 8286	10/06/86	1530	7.5	19.1	62	< 0.5	01	225
34	C 8287	10/06/86	1545	5.4	23.3	58	< 0.5	01	240

****** 12:29:11 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 02 * Luteal Cont Day

* print out from PRINT 001 * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES	day	REMARKS
124	C 8433	10/27/86	1140	4.1	8.6	250	11.3	02	0	
125	C 8434	10/27/86	1155	2.7	7.1	210	8.6	02	15	
126	C 8435	10/27/86	1210	6.1	7.6	200	8.6	02	30	
127	C 8436	10/27/86	1225	5.5	8.4	230	9.4	02	45	
128	C 8437	10/27/86	1240	4.3	8.3	260	10.1	02	60	
129	C 8438	10/27/86	1255	4.2	9.7	260	10.8	02	75	
130	C 8439	10/27/86	1310	3.8	7.9	270	11.2	02	90	
131	C 8440	10/27/86	1325	5.8	8.3	260	10.8	02	105	
132	C 8441	10/27/86	1340	5.6	7.4	260	12.1	02	120	
133	C 8442	10/27/86	1355	3.0	7.1	280	11.7	02	135	
134	C 8443	10/27/86	1410	3.0	9.9	310	13.5	02	150	
135	C 8444	10/27/86	1425	3.9	6.5	310	12.3	02	165	
136	C 8445	10/27/86	1440	3.9	7.3	250	9.6	02	180	
137	C 8446	10/27/86	1455	3.8	7.6	190	9.2	02	195	
138	C 8447	10/27/86	1510	4.1	8.0	150	7.7	02	210	
139	C 8448	10/27/86	1525	3.8	7.7	160	7.3	02	225	
140	C 8449	10/27/86	1540	3.7	6.8	170	6.5	02	240	

****** 12:33:18 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 02 * Injection Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
158	C 8468	11/01/86	1132	5.7	20.0	130	0.7	03	-60
159	C 8469	11/01/86	1147	5.5	18.0	120	< 0.5	03	-45
160	C 8470	11/01/86	1202	8.7	19.1	130	0.5	03	-30
161	C 8471	11/01/86	1217	8.2	21.3	95	< 0.5	03	-15
162	C 8472	11/01/86	1232	8.0	19.7	75	< 0.5	03	0
163	C 8473	11/01/86	1247	6.5	19.5	70	< 0.5	03	15
164	C 8474	11/01/86	1302	5.8	20.5	82	< 0.5	03	30
165	C 8475	11/01/86	1317	5.4	20.6	98	0.5	03	45
166	C 8476	11/01/86	1332	4.3	18.8	96	0.5	03	60
167	C 8477	11/01/86	1347	7.1	19.6	100	0.7	03	75
168	C 8478	11/01/86	1402	6.9	19.9	89	0.6	03	90
169	C 8479	11/01/86	1417	8.6	16.4	83	0.5	03	105
170	C 8480	11/01/86	1432	6.8	18.3	97	0.7	03	120
171	C 8481	11/01/86	1447	9.1	19.4	98	0.6	03	135
172	C 8482	11/01/86	1502	8.9	17.5	100	0.9	03	150
173	C 8483	11/01/86	1517	10.0	17.3	110	0.7	03	165
174	C 8484	11/01/86	1532	11.0	21.5	130	0.8	03	180
175	C 8485	11/01/86	1547	9.2	22.2	140	0.8	03	195
176	C 8486	11/01/86	1602	8.1	19.6	170	0.9	03	210
177	C 8487	11/01/86	1617	7.7	21.1	170	1.0	03	225
178	C 8488	11/01/86	1632	7.4	21.3	160	1.0	03	240

****** 12:34:58 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 02 * Follow Up

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
297	C 8563	11/05/86	0915			83	0.5	04	1
299	C 8670	11/07/86	0905			80	0.7	04	2
302	C 8696	11/11/86	0915			220	0.9	04	3
306	C 8700	11/14/86	0955			410	0.7	04	4
311	C 8705	11/18/86	0910			160	5.4	04	5
314	C 8708	11/21/86	0910			340	19.1	04	6
323	C 8819	11/28/86	1015			84	1.3	04	7

****** 12:38:29 *******

* ENDOSYS * PARFR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 02 * Month 0

* print out from PRINT OUT * 01/09/87 * JNH *

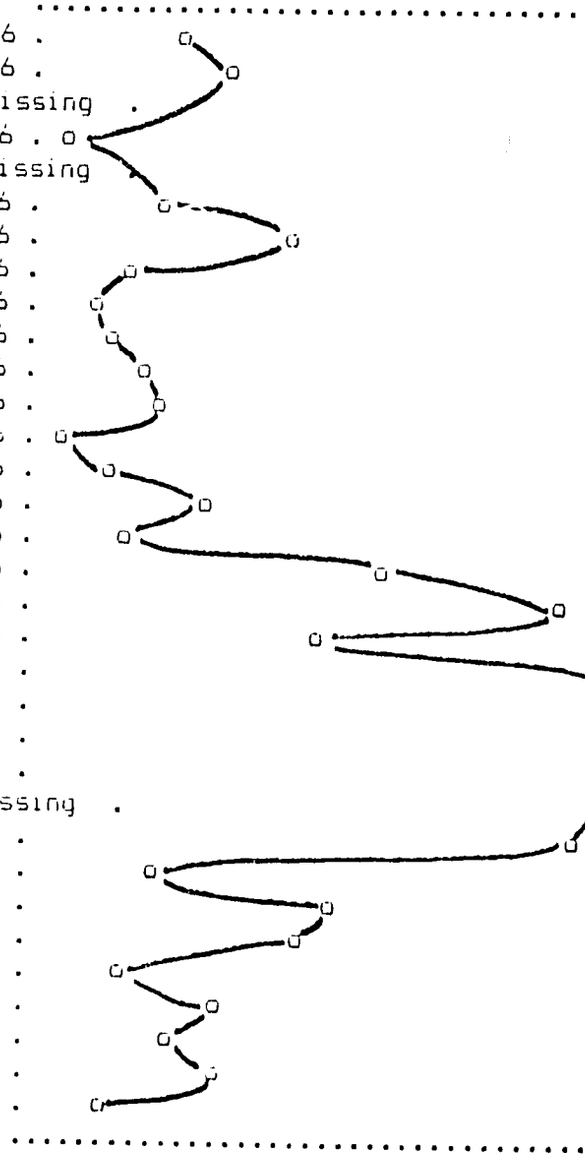
labnum	date	PDIOL(ug/ml)	day	remarks
C20031	10/05/86	< 0.3	1	
C20032	10/06/86	2.7	2	
C20033	10/07/86	1.7	3	
C20034	10/08/86	0.9	4	
C20035	10/09/86	1.3	5	
C20036	10/10/86	1.7	6	
C20037	10/11/86	0.6	7	
C20038	10/12/86	1.0	8	
C20039	10/13/86	1.1	9	
C20040	10/14/86	0.7	10	
C20041	10/15/86	1.3	11	
C20042	10/16/86	0.8	12	
C20043	10/17/86	1.4	13	
C20044	10/18/86	1.9	14	
C20045	10/19/86	2.3	15	
C20046	10/20/86	5.0	16	
C20047	10/21/86	5.8	17	
C20048	10/22/86	>10.0	18	
C20049	10/23/86	>10.0	19	
C20050	10/24/86	>10.0	20	
C20051	10/25/86	7.2	21	
C20052	10/26/86	5.1	22	
C20053	10/27/86	5.9	23	
	missing	.		
C20054	10/29/86	>10.0	25	
C20055	10/30/86	>10.0	26	
C20056	10/31/86	4.9	27	

****** 11:31:12 *******

* ENDOSYS * PARFR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 02 * Month 1

* print out from PRINT OUT * 01/09/87 * JNH *



labnum	date	PDIOL(ug/ml)	day	remarks
C20331	11/01/86	2.7	1	
C20332	11/02/86	3.5	2	
	missing			
C20333	11/04/86	0.9	4	
	missing			
C20334	11/06/86	2.5	6	
C20335	11/07/86	4.8	7	
C20336	11/08/86	1.9	8	
C20337	11/09/86	1.5	9	
C20338	11/10/86	1.8	10	
C20339	11/11/86	2.2	11	
C20340	11/12/86	2.5	12	
C20341	11/13/86	1.0	13	
C20342	11/14/86	1.7	14	
C20343	11/15/86	3.3	15	
C20344	11/16/86	2.0	16	
C20345	11/17/86	6.3	17	
C20346	11/18/86	9.3	18	
C20347	11/19/86	5.3	19	
C20348	11/20/86	>10.0	20	
C20349	11/21/86	>10.0	21	
C20350	11/22/86	>10.0	22	
C20351	11/23/86	>10.0	23	
	missing			
C20352	11/25/86	9.5	25	
C20353	11/26/86	2.6	26	
C20354	11/27/86	5.6	27	
C20355	11/28/86	5.0	28	
C20356	11/29/86	2.1	29	
C20357	11/30/86	3.5	30	
C20358	12/01/86	2.8	31	
C20359	12/02/86	3.5	32	
C20360	12/03/86	1.7	33	

****** 11:37:13 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 03 * Foll. Cont. Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
52	C 8305	10/15/86	0845	10.0	10.6	43	< 0.5	01	0
53	C 8306	10/15/86	0900	7.4	10.6	43	< 0.5	01	15
54	C 8307	10/15/86	0915	8.2	11.6	40	< 0.5	01	30
55	C 8308	10/15/86	0930	7.1	11.0	42	< 0.5	01	45
56	C 8309	10/15/86	0945	6.0	10.8	31	< 0.5	01	60
57	C 8310	10/15/86	1000	5.5	11.0	27	< 0.5	01	75
58	C 8311	10/15/86	1015	6.0	9.6	13	< 0.5	01	90
59	C 8312	10/15/86	1030	4.1	8.8	15	< 0.5	01	105
60	C 8313	10/15/86	1045	8.9	9.5	28	< 0.5	01	120
61	C 8314	10/15/86	1100	8.2	11.0	25	< 0.5	01	135
62	C 8315	10/15/86	1115	8.4	11.0	37	< 0.5	01	150
63	C 8316	10/15/86	1130	4.7	10.8	40	< 0.5	01	165
64	C 8317	10/15/86	1145	6.2	12.3	46	< 0.5	01	180
65	C 8318	10/15/86	1200	5.6	12.0	12	< 0.5	01	195
66	C 8319	10/15/86	1215	5.6	11.7	15	< 0.5	01	210
67	C 8320	10/15/86	1230	4.6	12.0	16	< 0.5	01	225
68	C 8321	10/15/86	1245	4.9	9.3	20	< 0.5	01	240

****** 12:30:48 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 03 * Luteal Cont Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
200	C 8565	11/05/86	0855	4.9	7.6	120	3.2	02	0
201	C 8566	11/05/86	0910	3.9	7.1	100	3.1	02	15
202	C 8567	11/05/86	0925	4.0	6.2	92	2.8	02	30
203	C 8568	11/05/86	0940	3.2	7.3	86	2.5	02	45
204	C 8569	11/05/86	0955	4.0	7.2	75	2.3	02	60
205	C 8570	11/05/86	1010	2.8	6.3	64	1.7	02	75
206	C 8571	11/05/86	1025	4.9	6.4	62	1.7	02	90
207	C 8572	11/05/86	1040	2.5	6.2	57	1.5	02	105
208	C 8573	11/05/86	1055	1.7	7.0	55	1.5	02	120
209	C 8574	11/05/86	1110	1.0	7.0	50	1.6	02	135
210	C 8575	11/05/86	1125	1.7	5.3	50	1.4	02	150
211	C 8576	11/05/86	1140	1.6	5.5	55	1.3	02	165
212	C 8577	11/05/86	1155	2.6	6.9	55	1.3	02	180
213	C 8578	11/05/86	1210	3.2	6.5	56	1.4	02	195
214	C 8579	11/05/86	1225	2.6	5.6	54	1.2	02	210
215	C 8580	11/05/86	1240	1.7	6.3	52	1.3	02	225
216	C 8581	11/05/86	1255	4.9	7.3	52	1.3	02	240

****** 12:36:37 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 03 * Injection Day

* print out from PRINT OUT * 01/09/87 * JNH *

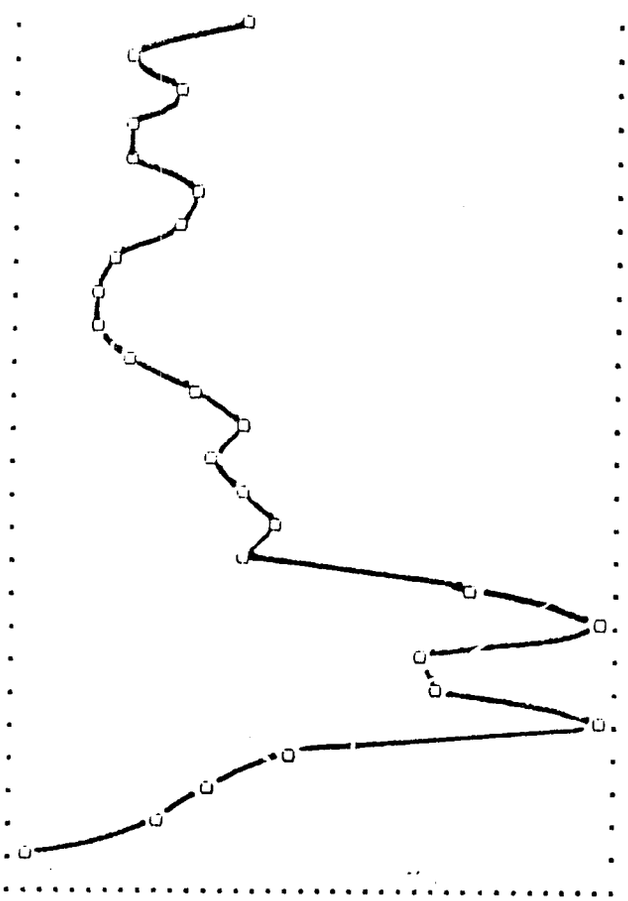
recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
234	C 8649	11/08/86	0737	7.2	14.1	X	X	03 -60	
235	C 8650	11/08/86	0752	6.4	14.0	X	X	03 -45	
236	C 8651	11/08/86	0807	6.5	14.0	X	X	03 -30	
237	C 8652	11/08/86	0822	3.6	13.0	X	X	03 -15	
238	C 8653	11/08/86	0837	5.2	13.0	X	X	03 0	
239	C 8654	11/08/86	0852	1.0	13.0	X	X	03 15	
240	C 8655	11/08/86	0907	2.8	13.4	X	X	03 30	
241	C 8656	11/08/86	0922	3.3	12.4	X	X	03 45	
242	C 8657	11/08/86	0937	7.5	14.1	X	X	03 60	
243	C 8658	11/08/86	0952	6.6	13.0	X	X	03 75	
244	C 8659	11/08/86	1007	5.9	13.0	X	X	03 90	
245	C 8660	11/08/86	1022	3.9	12.7	X	X	03 105	
246	C 8661	11/08/86	1037	6.4	12.6	X	X	03 120	
247	C 8662	11/08/86	1052	7.0	13.0	X	X	03 135	
248	C 8663	11/08/86	1107	6.7	12.2	X	X	03 150	
249	C 8664	11/08/86	1122	4.1	11.6	X	X	03 165	
250	C 8665	11/08/86	1137	5.2	12.7	X	X	03 180	
251	C 8666	11/08/86	1152	2.5	13.2	X	X	03 195	
252	C 8667	11/08/86	1207	6.6	12.8	X	X	03 210	
253	C 8668	11/08/86	1222	6.3	14.0	X	X	03 225	
254	C 8669	11/08/86	1237	6.1	13.0	X	X	03 240	

****** 12:37:30 *******

* ENDOSYS * PARFR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 03 * Month 0

* print out from PRINT OUT * 01/09/87 * JNH *



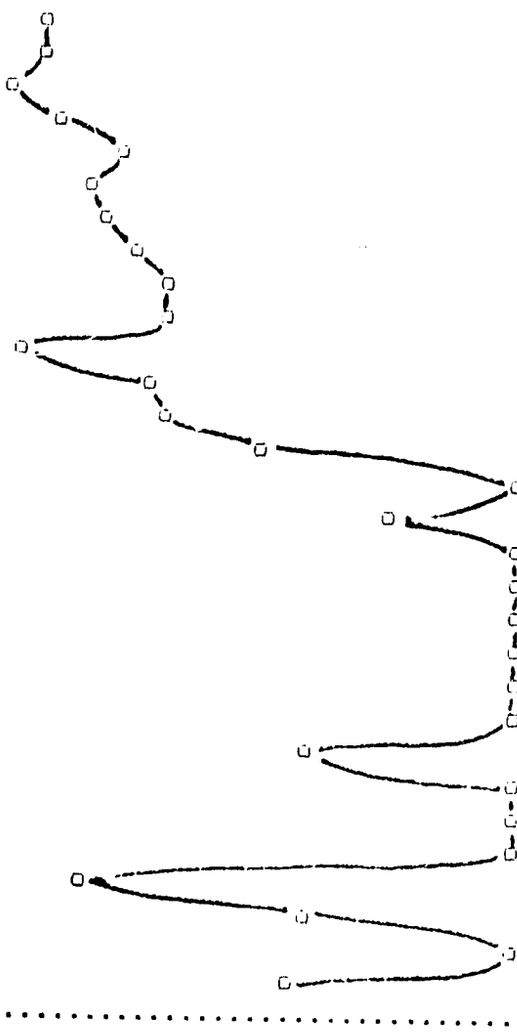
labnum	date	PDIOL(ug/ml)	day	remarks
C20001	10/13/86	4.2	1	
C20002	10/14/86	2.3	2	
C20003	10/15/86	3.1	3	
C20004	10/16/86	2.4	4	
C20005	10/17/86	2.2	5	
C20006	10/18/86	3.3	6	
C20007	10/19/86	3.2	7	
C20008	10/20/86	1.9	8	
C20009	10/21/86	1.8	9	
C20010	10/22/86	1.8	10	
C20011	10/23/86	2.2	11	
C20012	10/24/86	3.3	12	
C20013	10/25/86	4.2	13	
C20014	10/26/86	3.6	14	
C20015	10/27/86	4.1	15	
C20016	10/28/86	4.7	16	
C20017	10/29/86	4.1	17	
C20018	10/30/86	7.9	18	
C20019	10/31/86	>10.0	19	
C20020	11/01/86	7.1	20	
C20021	11/02/86	7.4	21	
C20022	11/03/86	>10.0	22	
C20023	11/04/86	5.0	23	
C20024	11/05/86	3.7	24	
C20025	11/06/86	2.8	25	
C20026	11/07/86	< 0.3	26	Menses 9AM

****** 11:29:40 *******

* ENDOSYS * PARFR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 03 * Month 1

* print out from PRINT OUT * 01/09/87 * JNH *



labnum	date	PDIOL(ug/ml)	day	remarks
C20391	11/08/86	2.1	1	
C20392	11/09/86	2.1	2	
C20393	11/10/86	1.4	3	
C20394	11/11/86	2.4	4	
C20395	11/12/86	3.3	5	
C20396	11/13/86	2.7	6	
C20397	11/14/86	3.0	7	
C20398	11/15/86	3.7	8	
C20399	11/16/86	4.3	9	
C20400	11/17/86	4.2	10	
C20401	11/18/86	1.7	11	
C20402	11/19/86	4.0	12	
C20403	11/20/86	4.1	13	
C20404	11/21/86	5.8	14	
C20405	11/22/86	>10.0	15	
C20406	11/23/86	7.9	16	
C20407	11/24/86	>10.0	17	
C20408	11/25/86	>10.0	18	
C20409	11/26/86	>10.0	19	
C20410	11/27/86	>10.0	20	
C20411	11/28/86	>10.0	21	
C20412	11/29/86	>10.0	22	
C20413	11/30/86	6.5	23	
C20414	12/01/86	>10.0	24	
C20415	12/02/86	>10.0	25	
C20416	12/03/86	>10.0	26	menses
C20417	12/04/86	2.9	27	
C20418	12/05/86	6.5	28	
C20419	12/06/86	>10.0	29	
C20420	12/07/86	6.4	30	

****** 11:41:55 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 04 * Foll. Cont. Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
35	C 8288	10/10/86	1130	10.2	16.2	50	< 0.3	01	0
36	C 8289	10/10/86	1145	11.0	14.1	66	< 0.5	01	15
37	C 8290	10/10/86	1200	9.1	15.5	65	< 0.5	01	30
38	C 8291	10/10/86	1215	10.7	13.5	78	< 0.5	01	45
39	C 8292	10/10/86	1230	13.2	14.3	77	< 0.5	01	60
40	C 8293	10/10/86	1245	11.3	15.9	70	< 0.5	01	75
41	C 8294	10/10/86	1300	11.7	15.0	69	< 0.5	01	90
42	C 8295	10/10/86	1315	9.4	15.6	45	< 0.5	01	105
43	C 8296	10/10/86	1330	13.0	14.0	58	< 0.5	01	120
44	C 8297	10/10/86	1345	13.4	15.2	60	< 0.5	01	135
45	C 8298	10/10/86	1400	11.6	14.3	58	< 0.3	01	150
46	C 8299	10/10/86	1415	9.9	16.0	45	< 0.5	01	165
47	C 8300	10/10/86	1430	9.2	16.9	27	< 0.5	01	180
48	C 8301	10/10/86	1445	9.0	15.2	45	< 0.5	01	195
49	C 8302	10/10/86	1500	9.5	14.5	31	< 0.5	01	210
50	C 8303	10/10/86	1515	12.6	9.8	55	< 0.5	01	225
51	C 8304	10/10/86	1530	12.7	9.4	45	< 0.5	01	240

****** 12:30:00 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 04 * Luteal Cont Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
141	C 8451	10/29/86	1222	7.7	< 3.0	130	8.0	02	0
142	C 8452	10/29/86	1237	7.6	< 3.0	95	6.1	02	15
143	C 8453	10/29/86	1252	6.5	< 3.0	98	6.6	02	30
144	C 8454	10/29/86	1307	6.6	< 3.0	95	6.9	02	45
145	C 8455	10/29/86	1322	6.7	< 3.0	110	7.0	02	60
146	C 8456	10/29/86	1337	5.3	< 3.0	130	7.3	02	75
147	C 8457	10/29/86	1352	5.2	< 3.0	120	7.4	02	90
148	C 8458	10/29/86	1407	5.7	< 3.0	110	6.8	02	105
149	C 8459	10/29/86	1422	4.6	< 3.0	89	5.8	02	120
150	C 8460	10/29/86	1437	5.4	< 3.0	87	4.9	02	135
151	C 8461	10/29/86	1452	4.5	< 3.0	94	5.2	02	150
152	C 8462	10/29/86	1507	6.3	< 3.0	90	4.6	02	165
153	C 8463	10/29/86	1522	4.7	< 3.0	79	4.9	02	180
154	C 8464	10/29/86	1537	4.7	< 3.0	71	4.5	02	195
155	C 8465	10/29/86	1552	5.9	< 3.0	64	4.1	02	210
156	C 8466	10/29/86	1607	7.3	< 3.0	99	4.2	02	225
157	C 8467	10/29/86	1622	9.4	< 3.0	86	4.5	02	240

****** 12:34:05 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 04 * Injection Day

* print out from PRINT OUT * 01/09/87 * JNH *

recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
179	C 8541	11/04/86	1205	10.4	11	35	< 0.3	03 -60	
180	C 8542	11/04/86	1220	11.4	16	33	< 0.3	03 -45	
181	C 8543	11/04/86	1235	11.9	16	37	< 0.3	03 -30	
182	C 8544	11/04/86	1250	8.0	16	27	< 0.3	03 -15	
183	C 8545	11/04/86	1305	8.6	15.6	32	< 0.3	03 0	
184	C 8546	11/04/86	1320	10.2	16.7	35	< 0.3	03 15	
185	C 8547	11/04/86	1335	16.1	16.6	40	0.5	03 30	
186	C 8548	11/04/86	1350	15.8	16.4	43	0.6	03 45	
187	C 8549	11/04/86	1405	15.2	17.3	45	0.7	03 60	
188	C 8550	11/04/86	1420	15.3	16.6	37	< 0.3	03 75	
189	C 8551	11/04/86	1435	13.7	15.1	32	< 0.3	03 90	
190	C 8552	11/04/86	1450	14.6	17.6	32	< 0.3	03 105	
191	C 8553	11/04/86	1505	14.4	15.5	34	< 0.3	03 120	
192	C 8554	11/04/86	1520	14.1	14.5	27	0.5	03 135	
193	C 8555	11/04/86	1535	16.7	16.0	33	< 0.3	03 150	
194	C 8556	11/04/86	1550	15.6	16.4	23	< 0.3	03 165	
195	C 8557	11/04/86	1605	13.9	16.7	36	< 0.3	03 180	
196	C 8558	11/04/86	1620	13.6	15.8	27	< 0.3	03 195	
197	C 8559	11/04/86	1635	15.1	15.0	29	< 0.3	03 210	
198	C 8560	11/04/86	1650	14.0	14.7	30	0.5	03 225	
199	C 8561	11/04/86	1705	17.7	15.0	32	< 0.3	03 240	

****** 12:35:51 *******

* ENDOSYS * Progesterone Microcaps. - Serum Response *

MICRO_P4* SUBJECT 04 * Follow Up

* print out from PRINT OUT * 01/09/87 * JNH *

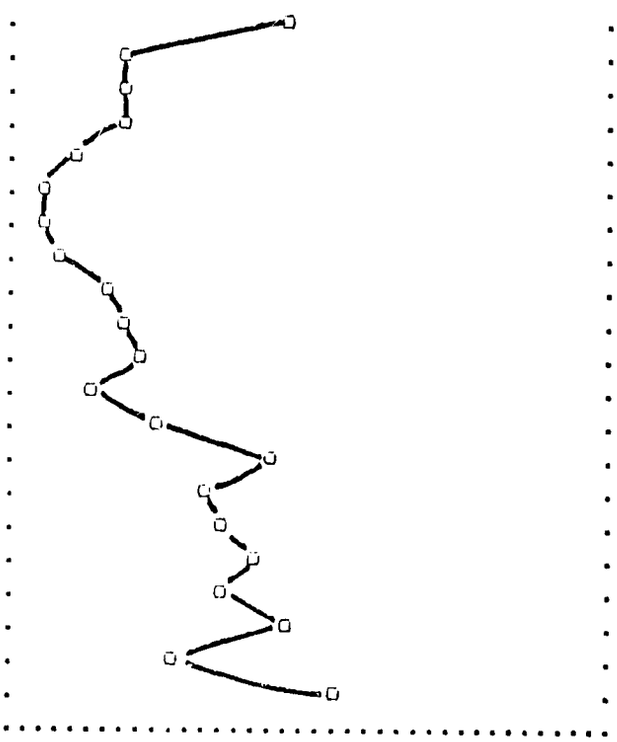
recno	LABNUM	DATE	TIME	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P4 ng/ml	SERIES day	REMARKS
300	C 8671	11/07/86	1014			33	1.4	04	1
301	C 8673	11/10/86	0905			170	0.5	04	2
305	C 8698	11/14/86	0845			46	2.8	04	3
310	C 8704	11/17/86	0900			82	7.2	04	3
316	C 8710	11/21/86	0900			100	10.3	04	4
318	C 8743	11/24/86	0905			100	9.9	04	5
325	C 8621	11/28/86	0920			31	1.5	04	6

****** 12:38:58 *******

* ENDOSYS * PARFR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 04 * Month 0

* print out from PRINT OUT * 01/09/87 * JNH *



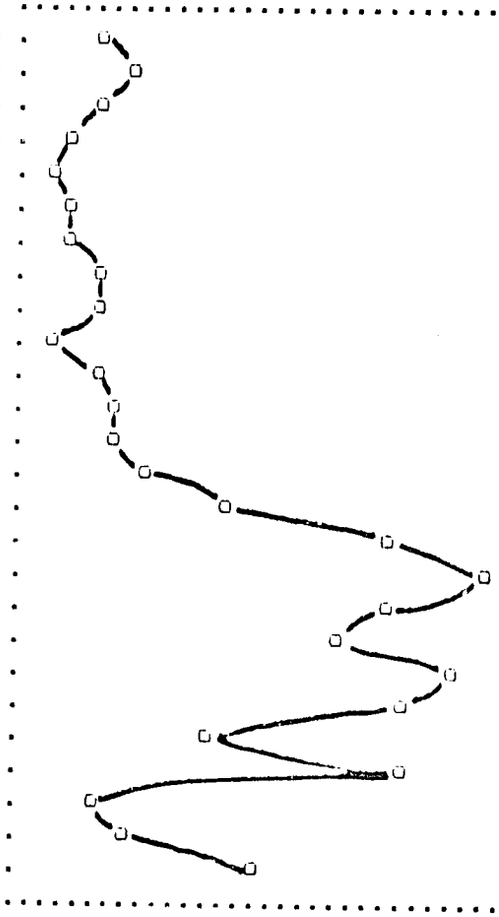
labnum	date	PDIOL(ug/ml)	day	remarks
C20061	10/09/86	4.9	1	
C20062	10/10/86	2.3	2	
C20063	10/11/86	2.2	3	
C20064	10/12/86	2.3	4	
C20065	10/13/86	1.4	5	
C20066	10/14/86	1.0	6	
C20067	10/15/86	0.9	7	
C20068	10/16/86	1.1	8	
C20069	10/17/86	2.1	9	
C20070	10/18/86	2.4	10	
C20071	10/19/86	2.5	11	
C20072	10/20/86	1.7	12	
C20073	10/21/86	2.9	13	
C20074	10/22/86	4.7	14	
C20075	10/23/86	3.5	15	
C20076	10/24/86	4.0	16	
C20077	10/25/86	4.5	17	
C20078	10/26/86	4.0	18	
C20079	10/27/86	5.1	19	
C20080	10/28/86	3.0	20	
C20081	10/29/86	5.7	21	

****** 11:32:29 *******

* ENDOSYS * PARFR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 05 * Month 0

* print out from PRINT OUT * 01/09/87 * JNH *



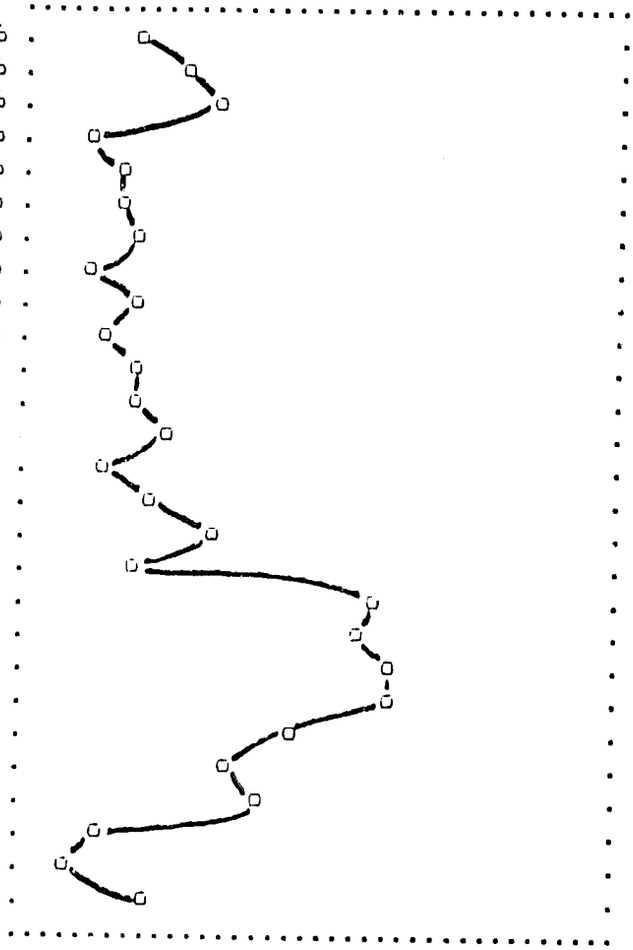
labnum	date	PDIOL(ug/ml)	day	remarks
C20181	10/16/86	1.8	1	
C20182	10/17/86	2.2	2	
C20183	10/18/86	1.6	3	
C20184	10/19/86	1.2	4	
C20185	10/20/86	0.9	5	
C20186	10/21/86	1.1	6	
C20187	10/22/86	1.1	7	
C20188	10/23/86	1.7	8	
C20189	10/24/86	1.7	9	
C20190	10/25/86	1.0	10	
C20191	10/26/86	1.8	11	
C20192	10/27/86	2.0	12	
C20193	10/28/86	2.0	13	
C20194	10/29/86	2.6	14	
C20195	10/30/86	3.8	15	
C20196	10/31/86	6.7	16	
C20197	11/01/86	8.2	17	
C20198	11/02/86	6.6	18	
C20199	11/03/86	5.9	19	
C20200	11/04/86	7.6	20	
C20201	11/05/86	6.9	21	
C20202	11/06/86	3.6	22	
C20203	11/07/86	7.0	23	
C20204	11/08/86	1.8	24	
C20205	11/09/86	2.3	25	
C20206	11/10/86	4.5	26	

****** 11:35:26 *******

* ENDOSYS * PARFR Study * Progesterone Microcapsules *

P4_MICRO* SUBJECT 05 * Month 1

* print out from PRINT OUT * 01/09/87 * JNH *



labnum	date	PDIOL(ug/ml)	day	remarks
C20421	11/11/86	2.2	1	
C20422	11/12/86	3.0	2	
C20423	11/13/86	3.7	3	
C20424	11/14/86	1.5	4	
C20425	11/15/86	2.1	5	
C20426	11/16/86	2.1	6	
C20427	11/17/86	2.2	7	
C20428	11/18/86	1.4	8	
C20429	11/19/86	2.3	9	
C20430	11/20/86	1.6	10	
C20431	11/21/86	2.3	11	
C20432	11/22/86	2.4	12	
C20433	11/23/86	2.9	13	
C20434	11/24/86	1.7	14	
C20435	11/25/86	2.5	15	
C20436	11/26/86	3.5	16	
C20437	11/27/86	2.4	17	
C20438	11/28/86	6.2	18	
C20439	11/29/86	6.0	19	
C20440	11/30/86	6.6	20	
C20441	12/01/86	6.5	21	
C20442	12/02/86	4.9	22	
C20443	12/03/86	3.9	23	
C20444	12/04/86	4.4	24	
C20445	12/05/86	1.6	25	
C20446	12/06/86	1.3	26	
C20447	12/07/86	2.6	27	

****** 11:38:47 *******

FINAL REPORT

Project: Injectable Progesterone Microcapsules for Nursing Women

Protocol: Assessment of Progesterone Plasma Levels after the Injection of Progesterone Microcapsules

Principal Investigator: Horacio B. Croxatto, M.D.

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Santiago, Chile

METHODOLOGY

Characteristics of the subjects: Potencial participants in this study were living in the central area of Santiago at the time of delivery, which occurred at the Hospital Paula Jaraquemada, the public hospital of the area. They were selected from postpartum women who wanted to breastfeed their child for as long as possible. Other criteria for selection were: age 18-35, parity 1-3, normal pregnancy, and vaginal term delivery of a healthy child of normal birth weight. Absence of chronic disease or any other condition requiring continuous drug therapy were also required. The nutritional condition of the mother was established by the weight-height ratio using the ICNND (7) standard as reference. Women with a ponderal index under 9, were not included. Instructions on the adequate technique for breastfeeding on demand were given at the hospital by a member of the research team within the first 2 days after delivery. Women were given an appointment at days 7, 20 and 30 postpartum for clinical evaluation and reinforcement of instructions for breastfeeding on demand. On days 30-35 postpartum, a general physical, pelvic and breast examination and PAP smear and hemoglobin were performed. Hemoglobin values higher than 11 g% were an additional requirement.

Actual volunteers who entered the study protocol were healthy women in exclusive breastfeeding at day 40 after delivery. They were selected for their willingness to participate in the study.

Formulation tested: a) Injectable progesterone microcapsules consisting in microspheres made of biodegradable poly-lactide-co-glycolide in which progesterone was homogenously dispersed. b) Placebo consisting of same materials but containing no progesterone.

Protocol of the Study:

Women were protected by a Copper TCu 200 device which was inserted at day 40 after delivery. At day 45 $\pm$ 1 postpartum they received the progesterone microcapsules injection or the placebo. Seven women entered each group.

Blood samples were drawn before injection, every three days during the first 9 days and weekly thereafter up to day 90 post-treatment.

Progesterone RIA was performed using the methods and reagents of WHO Programme for the Provision of Matched Assay Reagents for the RIA of Hormones in Reproductive Physiology.

Clinic visits were scheduled monthly to check maternal and infant health and the breastfeeding pattern.

RESULTS

There were no problems related to the procedure of injecting the microcapsules, other than mild local pain. No local effects were reported or observed later.

Characteristics of the population enrolled are shown in Table I. No major health problems were diagnosed. All subjects were in exclusive breastfeeding at day 120 postpartum and supplements were prescribed before day 180 after delivery in 2 and 3 cases from the progesterone and placebo group respectively.

Plasma progesterone values obtained in both groups are shown in Fig. 1. Mean levels were distinctly higher from week 3 to week 9 in the group treated with progesterone but they never reached the 3 ng/ml landmark.

The bleeding pattern is shown in Fig. 2. A longer amenorrhea was experienced by the majority of progesterone treated women in comparison with IUD users who reinitiated bleedings at an earlier postpartum interval.

CONCLUSIONS

The data indicate that the preparation is well tolerated, it elevates the circulating levels of progesterone but not enough hormone is delivered for a sufficient length of time to achieve the desired dose. The period of maximum release appears to be between the 3rd and 9th week following the injection.

TABLE I

Progesterone Microcapsules in Nursing Women:
Characteristics of the Study Population

Case Nº	Age y	Weight Kg	Height m	Parity	Hb Admission g %	Weight of Newborn g
Progesterone group						
01	26	68	1.54	2	11.2	3980
03	23	59	1.54	1	14.2	3480
04	20	49	1.52	1	11.8	3320
05	22	54	1.51	3	12.9	3100
06	22	56	1.52	3	13.2	3100
09	26	68	1.55	3	11.8	3490
11	26	58	1.47	1	14.2	3650
n =	7	7		7	7	7
X =	23.6	58.8		2	12.7	3445
D.S =	2.4	7.0		1	1.2	312.3
Placebo group						
02	21	58	1.59	1	13.5	2550
07	31	60	1.63	3	12.9	2960
08	21	76	1.64	1	11.2	3460
10	27	55	1.47	3	12.9	3150
12	26	80	1.58	4	14.5	3160
13	25	58	1.47	2	12.2	2550
14	27	58	1.48	2	13.5	3110
n =	7	7		7	7	7
X =	25.4	63.5		2.3	12.9	2991
D.S =	3.5	10.0		1.1	1.0	336

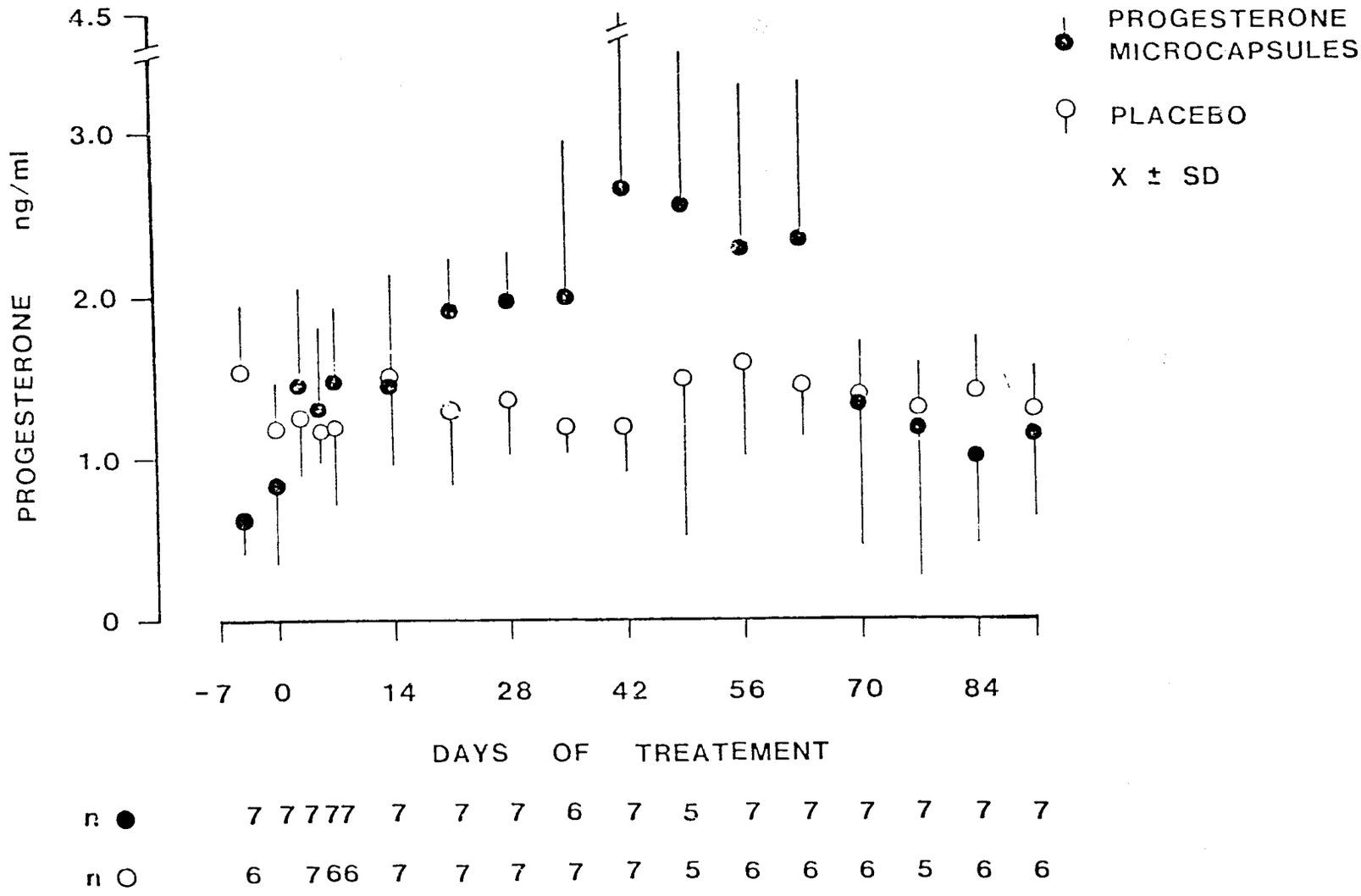

Fig. 1: Plasma progesterone levels in nursing women treated with progesterone microcapsules or placebo.

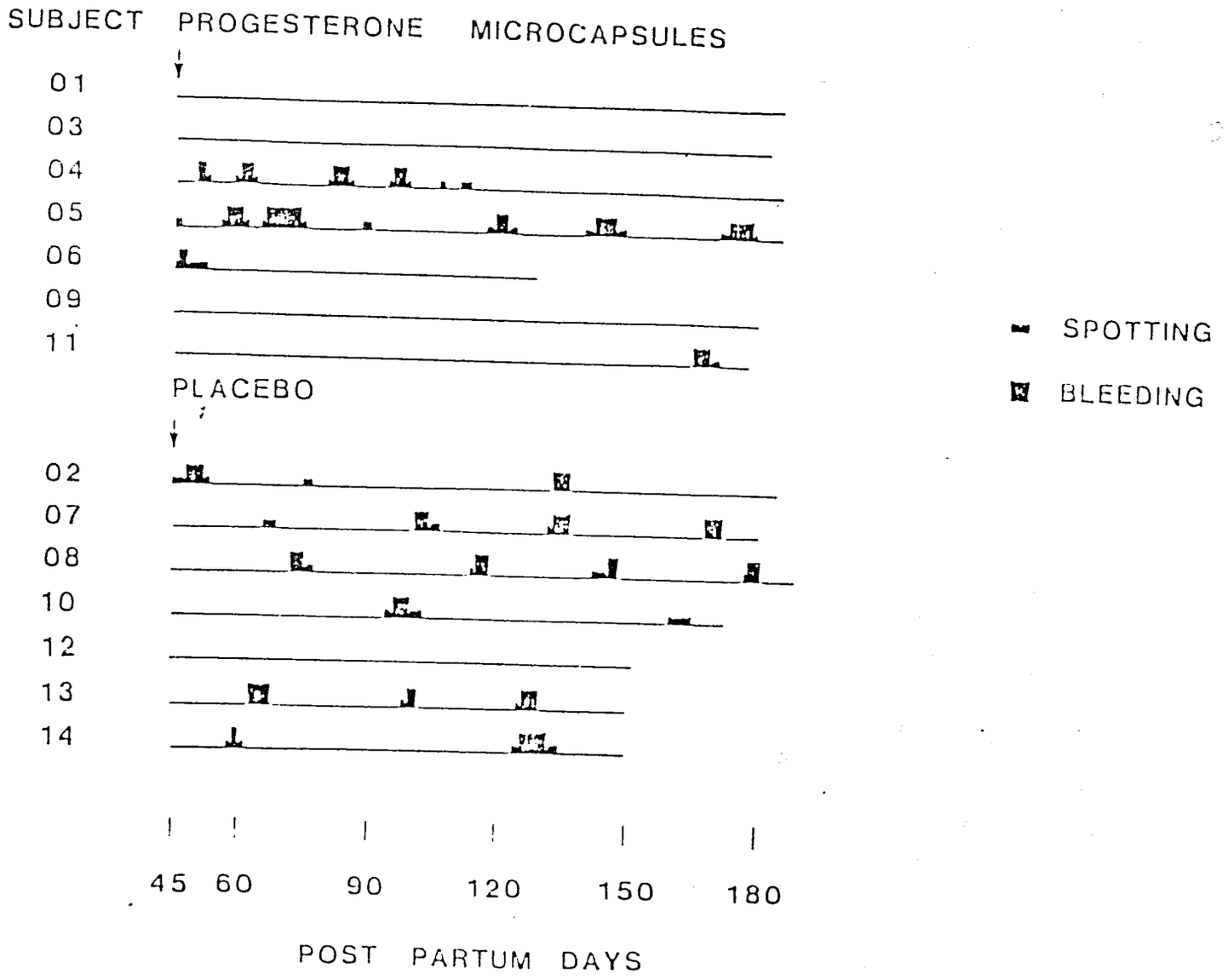

Fig. 2: Bleeding pattern in progesterone and in placebo treated women

FINAL REPORT

AN EVALUATION OF ENDOMETRIAL BIOPSIES OBTAINED JANUARY 1986 THROUGH DECEMBER 1986 AS PART OF THE PARFR PROGRAM FOR TESTING NORETHINDRONE FORMULATIONS TO REGULATE FERTILITY IN WOMEN

RESPONSE OF HUMAN ENDOMETRIUM TO 30 MG, 50 MG, 65 MG, AND 100 MG OF NORETHINDRONE MICROSPHERES DURING SIX MONTHS OF CONTROLLED RELEASE STEROID CONTRACEPTION: AN ANALYSIS BY CORRELATIVE LIGHT MICROSCOPY, HISTOCHEMISTRY, TRANSMISSION ELECTRON MICROSCOPY, AND SCANNING ELECTRON MICROSCOPY

POLY NET-90 STUDY 1986

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(89 pages)

I. SUMMARY

This report describes the endometrial biopsies obtained for the Poly Net-90 Phase I Clinical Investigation. The objective of the Poly NET-90 Study was to identify a safe dose level of microencapsulated norethindrone (NET) that would serve as an effective 90-day contraceptive when injected into human females in their reproductive years. Endometrial biopsies were taken to gain an understanding of the endometrial response to 30, 50, 65, and 100 mg dose levels of NET at 2, 5, and 6 months post-injection (PI).

Based on the results available from animal and human studies when the Poly NET-90 Investigation was designed, it was conceivable that a single injection of any of the dose levels to be tested (30, 50, 65, and 100 mg) could suppress ovulation for 90 days, and that additional doses at periodic intervals could suppress ovulation indefinitely. The longest period of continuous medication tested in this study was about 6 months (180 days). To achieve 6 months of continuous treatment, each subject was given 2 injections of NET (65 mg or 100 mg/injection) separated by an interval of 90 days. Ovarian suppression was judged by records on the bleeding patterns of each subject, estradiol and progesterone serum levels determined by radioimmunoassay (RIA), and anaylses of endometrial biopsies.

Biopsies were obtained at three Centers (Table I, II, and III). A total of 52 subjects were enrolled at the three Centers to participate in this study of a novel method for fertility regulation. Personnel at each Center kept records of bleeding patterns, drew blood samples for RIA, and did the endometrial biopsies.

Eighty-nine biopsies were obtained from the 52 volunteers. A baseline, control, or pre-treatment biopsy was obtained from each potential subject. An unexpected finding from these biopsies was that almost 25% of the candidates had anovulatory cycles, endometritis, or glandular-stromal asynchrony, and were inelgible to be enrolled in the study because they did not meet the criteria of normalcy specified in the protocol. The baseline biopsy was important for selecting subjects with healthy endometrium, for demonstrating precisely the state of the endometrium before treatment, and for attributing endometrial changes following NET injection to the effects of treatment. Treated biopsies were obtained from 27 of the 52 subjects.

All baseline and treated biopsies were evaluated by correlative light microscopy (LM), histochemistry, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). The histochemical methods permitted the detection of exogenous or endogenous estrogen and /or progestin via effect(s) on endometrial carbohydrates, DNA, and RNA. SEM revealed sites of breakthrough bleeding and the estrogen-progestin dependent surface features of epithelial cells, such as cilia, microvilli, and extruded secretory products. TEM depited the cell organelles and inclusions that function in processes important for maintaining a suppressed endometrium and for endometrial recovery upon the return of ovarian function.

Evaluation of biopsies from 10 subjects at 2 months PI 30 mg NET showed that ovulation had probably been suppressed in all 10 subjects, although 2 biopsies contained inadequate endometrium for definitive evaluation, and 2 biopsies were in the proliferative phase undergoing repair. It appeared that the latter two subjects would have ovulated prior to 90 days PI, and that the remaining 8 subjects would not have ovulated before 90 days PI.

Evaluation of biopsies from 10 subjects at 2 months PI 50 mg NET showed that ovulation had probably been suppressed in all 10 subjects. However, adequate endometrium for definitive evaluation could not be obtained from one subject, and another subject had proliferative, but anovulatory, endometrium.

Bleeding occurred with both the 30 mg and the 50 mg injections, bu the amount of bleeding and endometrial damage was far less with the 50 mg dose. The morphological basis for this difference was indicated by the response of the stromal cells. They became hypertrophic with the 5 mg injection and produced numerous reticular fibers. The latter checke endometrial bleeding and shedding by intertwining between the stromal elements and re-enforcing the basement membranes of the blood vessels, glands, and surface epithelium. This study showed that a progestin-onl contraceptive should elicit stromal cell hypertrophy and reticular fibe production to deter endometrial bleeding.

Histochemical results indicated that ovarian estradiol production fluctuated considerably during continuous NET treatment. Based on findings from the present study and on other data that we have obtained from animals, it is evident that endometrium which has been suppressed with progestin-only cannot withstand estrogen fluctuations without considerable bleeding. The microvasculature during continuous NET treatment showed periodic estrogen-elicited growth spurts that resulted in vascular ramifications and bleeding. It can now be speculated that injection of a correct dose level of an estrogen and a progestin will suppress FSH levels and also prevent severe endometrial regression; without FSH, there will be no growth of ovarian follicles, or erratic estradiol production and bleeding.

Biopsies were obtained from 6 subjects who had received 2, 65 mg NET injections separated by a 3 month interval. Proliferative endometrium was present in 3 of the 6 biopsies, and it seemed that the return of ovulatory cycles was eminent. Only 1 of the 3 remaining biopsies contained adequate endometrium for definitive evaluation. The endometrium was suppressed. Material available in the other 2 biopsies indicated that the 2 subjects would not have had ovulatory cycles for at least another month.

Biopsies were obtained from 8 subjects who had received 2, 100 mg NET injections separated by a 3 month interval. Two of 8 biopsies contained proliferative endometrium, and the remaining 6 had suppressed endometrium. It was not possible to conclude when the 8 subjects would have had ovulatory cycles.

Based on the analyses of all biopsies and the correlation of data obtained by all investigative procedures, it was apparent that the 65 mg dose level of NET elicted a better endometrial response than did the 100 mg dose level. Furthermore, there was less shedding with the 65 mg dose level than with any other dose level tested. Biopsies were limited, and it was unfortunate the one Center did not provide the treated biopsies that would have shown the endometrial response to the 65 and 100 mg dose levels at 5 months PI. All dose levels resulted in microscopically recognizable bleeding. Fluctuations in ovarian activity occurred during ontinuous NET treatment, and bleeding and repair took place simultaneously.

II. OBJECTIVES

1. To gain an understanding of the endometrial response at the histological, histochemical, cellular, and subcellular levels to 30, 50, 65, and 100 mg of microencapsulated norethindrone (NET) at 2, 5, and 6 months post-injection.

2. To identify a safe dose level of microencapsulated NET that would serve as an effective 90-day contraceptive when injected into human females in their reproductive years.

3. To determine if any undesirable endometrial alterations of significance are present after 3 months and 6 months of continuous NET treatment.

III. MATERIALS AND METHODS

Three Centers provided endometrial biopsies for this study. The Centers and their Directors were (1) The University of Texas Health Science Center at San Antonio, Dr. Jose Balmaceda; (2) University of Pittsburgh, Magee Women's Hospital, Dr. David Archer; and (3) Cornell University Medical College, Dr. Brij Saxena. The Study Nurses/Study Coordinators for the study were (1) Liz Kivi, Caroline Jarke, and Janell Mariorana with Dr. Balmaceda, (2) Barbara Ross and Nancy Kirimli with Dr. Archer, and (3) Stephanie Schroeder and Leila Macey with Dr. Saxena. Dr. Saxena was assisted by another physician, Dr. Mukhul Singh. Henceforth, each Center will be referred to by the name of its Director.

Personnel who handled the biopsies at the University of South Alabama were Walter Wilborn, Ph.D., Barbara Hyde, M.T.A., Marion Sokol, H.T., and Edward Dowling, M.D. Dr. Dowling is in charge of surgical pathology at the University of South Alabama Medical Center. The other personnel are staff members of the Electron Microscopy Center, University of South Alabama.

The study was designed so that two endometrial biopsies would be taken from each subject who was admitted to the study. A pre-treatment (control or baseline) biopsy was to be obtained in the secretory phase of the cycle preceding treatment. A subject was to be elgible for admission to the study if her biopsy showed normal endometrium and if she met other criteria in the protocol. The second endometrial biopsy was to be a treated biopsy that was to be obtained at one of three times post-injection. Henceforth, the two biopsies will be designated control biopsy and treated biopsy.

A pathology report for each biopsy is included in the appendix. PARFR was sent the original and four carbon copies of each report.

Biopsies were received via overnight Federal Express in polyfoam shipping containers with refrigerant packs (ice packs) to keep the specimens cold during shipment. Each biopsy was inside a 20 ml plastic scintillation vial immersed in fixative. The fixative was 1% glutaraldehyde and 1% paraformaldehyde in Millonig's phosphate buffer, pH 7.3.

Upon receiving a biopsy, the information on the label (e.g., Center No., Study No., Patient No., & Date of Biopsy) was written in the Tissue Record Book, and the biopsy was assigned an accession number which began with the letter W. The biopsy was then removed from the container, placed on a paraffin plate, and inspected while covered with fixative. Using a stereomicroscope (dissecting microscope), the quality of the biopsy was judged and recorded along with the amount of endometrium, mucus, and blood that comprised the biopsy material.

Next, the biopsy was divided for light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM). When a biopsy contained insufficient tissue for all three studies, material was first selected for LM. LM was necessary to date the endometrium and for histochemistry.

Tissue for LM was immersed overnight in 10% neutral buffered formalin before dehydrating the specimen in graded ethyl alcohols, clearing in xylene, and embedding in paraffin. Tissue for TEM was cut into 1 mm cubes, postfixed 2 h in cold 2% osmium tetroxide in Millonig's buffer, dehydrated through graded ethyl alcohols and acetone, and embedded in Spurr's low viscosity epoxy resin.

Strips of tissue that could be recognized under the dissecting microscope to have surface epithelium were selected for SEM. The strips were postfixed in osmium tetroxide as described for TEM specimens. Following postfixation, they were dehydrated through ascending concentrations of ethyl alchol, placed into amyl acetate, and dried by the critical point method using liquid carbon dioxide. Specimens were mounted on aluminum stubs, sputtercoated with gold palladium and viewed in a scanning electron microscope. SEM is unique in that it allows large specimens of unsectioned endometrium to be viewed tridimensionally at magnifications that range from 10X to 30,000X.

Paraffin blocks of tissue for LM were serially sectioned at 5 micrometers. It was important to study numerous sections of the same biopsy since endometrial alterations attributable to contraceptive steroids are sometimes focal, small, and occupy such a minute area of the biopsy that they can go undetected unless the biopsy is serially sectioned and carefully evaluated from beginning to end. Sections were placed on chemically cleaned glass slides and stained by one of the following methods: (1) hematoxylin and eosin (H&E), (2) periodic acid-Schiff (PAS), (3) periodic acid-Schiff diastase (PAS-D), (4) alcian blue (AB) at pH 2.5, (5) AB at pH 0.5, (6) colloidal iron and Van Gieson combination (CI), (7) Pyronin-Y (P-Y), and (8) methyl green-pyronin (MGP). These techniques were used on each biopsy in order to get the most information possible from the material.

H&E revealed the salient features of each biopsy, permitted dating biopsies of control endometrium, and provided the information for routine pathology reports. PAS and PAS-D specifically identified glycogen in the epithelial and stromal cells, showed sites of glycoprotein secretory products and granules in the glands, surface epithelium, and vascular endothelium, and delineated clots of platelet aggregates in the microvasculature. Colloidal iron (CI) demonstrated reticular fibers, sialomucins, and sulfomucins. The two latter components are acid glycosaminoglycans (carboxyl- and sulfate containing, respectively) important for endometrial growth and implantation of the blastocyst. Reticular fibers are produced by stromal cells and their quantity in the endometrium indicates the degree of stromal cell activity and/or the extent the endometrium has shed

due to bleeding. Reticular fibers also help to distinguish polyps from normal endometrium. AB at pH 2.5 stains sialidase-labile sialomucins whose synthesis is stimulated by progestins. AB (pH 0.5), as well as the P-Y technique, demonstrates sulfomucins. The latter are estrogen-dependent.

The various histochemical methods for carbohydrates were used to detect an estrogen and/or a progestin of either exogenous or endogenous origin via the effect(s) that the hormone(s) exerted on endometrial carbohydrates. A final histochemical technique, methyl green-pyronin, was used to demonstrate RNA and DNA. Increased cytoplasmic RNA (basophilia) is an estrogen effect, and increased nuclear RNA (in the nucleoli) is a progestin effect.

One micrometer sections were made of all blocks of tissue that had been embedded in plastic for TEM. The sections were stained with toluidine blue and evaluated by LM. They provided exceptional resolution of endometrial cytology and revealed details, such as discontinuities in the microvasculature, that were not apparent in H&E stained sections. Ultrathin sections were made from at least 3 blocks/biopsy. The sections were mounted on 150 mesh copper grids and double-stained with uranyl acetate and lead citrate before examining by TEM.

All biopsies were first evaluated by accession number only. Slides and micrographs were judged independently by at least three qualified individuals in order to eliminate any possible bias in interpretation.

Forty-five biopsies were received from Dr. Balmaceda. Five were from subjects not admitted to the study because they did not have normal endometrium: 3 had anovulatory endometrium, 1 had proliferative endometrium with stromal hyperplasia, and 1 had glandular-stromal asynchrony. The 40 remaining biopsies, comprised of 20 control biopsies and 20 treated biopsies, were from 20 subjects who were admitted to the study. Table I lists the biopsies from Dr. Balmaceda. They were taken to show the endometrial response to 2 months of continuous NET treatment.

With one exception, control biopsies from all Centers were obtained in the secretory phase of the cycle. One control biopsy from Dr. Balmaceda was taken in the proliferative phase (see Table I, Subject No. 16, DJ, W-56-86). Treated biopsies from Dr. Balmaceda were taken 63 days post-injection with the exception of the biopsy from Subject No. 7. It was obtained 61 days post-injection (Table I).

Forty biopsies were received from Dr. Archer. Six were from subjects who did not have normal endometrium and were not admitted to the study. Five subjects had anovulatory endometrium, and 1 had chronic endometritis. As shown in Table II, 19 subjects were admitted to the study, and a post-injection endometrial biopsy was

obtained from 14 of the subjects. Thus, a total of 33 biopsies came from Dr. Archer. Normal, secretory endometrium was identified in control biopsies from 18 of the subjects. The endometrium of one subject, Subject No. 6 (CLG), showed changes compatible with ovulation superimposed upon an anovulatory cycle. Fourteen biopsies were obtained on days 89 through 95 after the second injection of either 65 mg or 100 mg of NET. The second injection was given approximately 90 days after the first injection. The same dose level was given at each injection. The treated biopsies from Dr. Archer were to show the endometrial response to 6 months of continuous NET treatment.

Seventeen biopsies were received from Dr. Saxena. One was from a subject who was not admitted to the study because of chronic endometritis. As shown in Table III, 13 control biopsies came from the 13 subjects admitted to the study. From the 13 control subjects, only 3 treated biopsies were received. Although the biopsies from Dr. Saxena were to show the endometrial response to 5 months of continuous NET therapy, little could be learned from his material. Biopsies were unsuitable for study, and two different numbers were sometimes assigned to the same subject. Consequentley, Dr. Saxena's biopsy reports in the appendix should be identified by checking the subject's initials with the information provided in Table III.

Despite the failure of one Center to perform as specified in the protocol, results were obtained and conclusions were reached from the study of biopsies provided by two of the Centers.

TABLE I

ENDOMETRIAL BIOPSIES FROM DR. JOSE BALMACEDA, UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER, SAN ANTONIO, TEXAS

CONTROL AND TREATED BIOPSIES FROM SUBJECTS WHO RECEIVED A SINGLE INJECTION OF 30 MG OR 50 MG MICROENCAPSULATED NORETHINDRONE FOR THREE MONTHS OF CONTROLLED RELEASE STEROID CONTRACEPTION

SUBJECT: NO. & INITIALS	CONTROL BX: NO. & DATE	MG NET INJECTED	TREATED BX: NO., DAYS POST-INJECTION, & DATE
1 NH	W-292-86 05-24-86	30	W-502-86; D-63; 07-29-86
2 LTS	W-291-86 05-24-86	50	W-503-86; D-63; 07-29-86
3 MO	W-277-86 05-24-86	30	W-504-86; D-63; 07-29-86
4 ODC	W-255-86 05-05-86	50	W-505-86; D-63; 07-29-86
5 SG	W-236-86 04-25-86	30	W-421-86; D-63; 06-24-86
6 MC	W-071-86 02-18-86	50	W-312-86; D-63; 05-28-86
7 SM	W-136-86 03-13-86	30	W-290-86; D-61; 05-24-86
8 MP	W-111-86 03-11-86	50	W-313-86; D-63; 05-28-86
9 RC	W-086-86 02-20-86	30	W-237-86; D-63; 04-25-86

10 LV	W-070-86 02-18-86	50	W-238-86; D-63; 04-25-86
11 RG	W-057-86 02-13-86	30	W-239-86; D-63; 04-25-86
12 MS	W-043-86 02-03-86	50	W-240-86; D-63; 04-25-86
13 DA	W-069-86 02-18-86	30	W-241-86; D-63; 04-25-86
14 CA	W-067-86 02-18-86	50	W-242-86; D-63; 04-24-86
15 LO	W-054-86 02-13-86	30	W-224-86; D-63; 04-22-86
16 DJ	W-056-86 02-13-86	50	W-225-86; D-63; 04-22-86
17 TS	W-068-86 02-18-86	30	W-226-86; D-63; 04-22-86
18 CK	W-055-86 02-13-86	50	W-204-86; D-63; 04-15-86
19 CM	W-041-86 02-03-86	30	W-202-86; D-63; 04-15-86
20 LV	W-042-86 02-03-86	50	W-203-86; D-63; 04-15-86

Treated Specimens inadequate for complete evaluation: (1) 5, SG, W-421-86 (no LM, SEM, or TEM); (2) 1, NH, W-502-86 (no LM, SEM, or TEM); (3) 14, CA, W-242-86 (no LM, or SEM); (4) 18, CK, W-204-86 (no SEM); (5) 19, CM, W-202-86 (no SEM).

TABLE II

ENDOMETRIAL BIOPSIES FROM DR. DAVID ARCHER, UNIVERSITY OF PITTSBURGH, MAGEE WOMEN'S HOSPITAL, PITTSBURGH, PENNSYLVANIA

CONTROL AND TREATED BIOPSIES FROM SUBJECTS WHO RECEIVED TWO SINGLE INJECTIONS OF 65 MG OR 100 MG OF MICROENCAPSULATED NORETHINDRONE FOR SIX MONTHS OF CONTROLLED RELEASE STEROID CONTRACEPTION. AN INTERVAL OF APPROXIMATELY 90 DAYS SEPARATED FIRST AND SECOND INJECTIONS

SUBJECT: NO. & INITIALS	CONTROL BX: NO. & DATE	MG NET INJECTED/ INJECTION	TREATED BX: NO., DAYS AFTER 2ND INJECTION, & DATE
1 JAP	W-077-86 02-14-86	065	W-556-86; D-93; 08-19-86
2 MAC	W-102-86 02-24-86	100	W-586-86; D-90; 08-28-86
3 RDB	W-074-86 02-10-86	065	W-601-86; D-89; 09-05-86
4 SMK	W-142-86 03-12-86	100	W-636-86; D-94; 09-15-86
5 EJ	W-170-86 03-24-86	065	W-680-86; D-92; 09-26-86
6 CLG	W-209-86 04-09-86	100	W-718-86; D-90; 10-13-86
7 BJS	W-250-86 04-25-86	065	W-761-86; D-90; 10-24-86
8 BEV	W-281-86 05-07-86	100	W-799-86; D-92; 11-04-86

9 VL	W-280-86 05-07-86	100	W-819-86; D-90; 11-14-86
10 JC	W-325-86 05-23-86	065	W-865-86; D-93; 12-01-86
12 RKW	W-347-86 06-02-86	100	W-874-86; D-95; 12-03-86
13 PSR	W-353-86 06-04-86	100	W-897-86; D-94; 12-04-86
14 JMR	W-370-86 06-10-86	100	W-937-86; D-93; 12-16-86
15 JLT	W-371-86 06-11-86	065	W-898-86; D-93; 12-10-86
16 RMT	W-443-86 06-30-86	---	No biopsy received
17 PER	W-422-86 06-25-86	---	No biopsy received
18 MAM	W-446-86 07-03-86	---	No biopsy received
19 CAL	W-521-86 08-01-86	---	No biopsy received
20 MJM	W-530-86 08-05-86	---	No biopsy received

Treated specimens inadequate for complete evaluation: (1) 1, JAP, W-556-86 (no SEM or TEM); (2) 2, RDB, W-601-86 (no LM, SEM, or TEM); (3) 7, BJS, W-761-86 (incomplete LM; no SEM); (4) 8, BEV, W-799-86 (incomplete LM; no SEM or TEM); (5) 9, VL, W-819-86 (incomplete LM); (6) 12, RKW, W-874-86 (no SEM or TEM); (7) 13, PSR, W-897-86 (no SEM); (8) 15, JLT, W-898-86 (no SEM); (9) 14, JMR, W-937-86 (no SEM or TEM).

TABLE III

ENDOMETRIAL BIOPSIES FROM DR. BRIJ SAXENA, CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK, NEW YORK

CONTROL AND TREATED BIOPSIES FROM SUBJECTS WHO RECEIVED TWO SINGLE INJECTIONS OF 65 MG OR 100 MG OF MICROENCAPSULATED NORETHINDRONE FOR SIX MONTHS OF CONTROLLED RELEASE STEROID CONTRACEPTION. AN INTERVAL OF APPROXIMATELY 90 DAYS SEPARATED FIRST AND SECOND INJECTIONS

SUBJECT: NO. & INITIALS	CONTROL BX: NO. & DATE	MG NET INJECTED/ INJECTION	TREATED BX: NO., DAYS AFTER 2ND INJECTION, & DATE
1 HOC	W-132-86 03-06-86	100	W-563-86; D-68; 08-26-86
3 LIS	W-164-86 03-19-86	---	No biopsy received
4 DOT	W-172-86 03-26-86	---	No biopsy received
5 MGB	W-184-86 03-28-86	---	No biopsy received
6 RSB	W-191-86 04-01-86	100	W-600-86; D-64; 09-08-86
7 KYK	W-198-86 04-04-86	---	No biopsy received
8 CHB	W-231-86 04-18-86	---	No biopsy received
9 MHL	W-232-86 04-18-86	---	No biopsy received

10 SRB	W-287-86 05-09-86	---	No biopsy received
11 DEW	W-295-86 05-14-86	---	No biopsy received
12 KRO	W-308-86 05-21-86	---	No biopsy received
13 MGR	W-318-86 05-23-86	---	No biopsy received
14 MDM	W-326-86 05-27-86	065	W-811-86; D-64; 11-17-86

Treated specimens inadequate for complete evaluation: (1) 1, HOC, W-563-86 (no LM, SEM, or TEM); (2) 6, RSB, W-600-86 (no LM, SEM, or TEM); (3) 14, MDM, W-811-86 (no LM, SEM, or TEM).

IV. RESULTS

A. Light Microscopy and Histochemistry

1. Dr. Balmaceda

Biopsies were obtained approximately 2 months (61 days and 63 days) post-injection for both the 30 mg and 50 mg dose levels of NET (Table I). There were 10 biopsies from subjects who received a single 30 mg injection of NET. Of these, 2 were in the proliferative phase and recovering from treatment, 6 were suppressed, and 2 did not contain adequate endometrium for evaluation. The suppressed endometrium from 5 of 6 subjects contained blood and appeared to be bleeding at the time the biopsies were taken. Perhaps the two subjects who did not provide endometrium for evaluation had shed or bled to the basalis and no endometrium could be obtained.

Of the 10 biopsies from subjects who received a single 50 mg injection of NET, 1 was in the proliferative phase and recovering from treatment, 8 were suppressed, and 1 contained inadequate endometrium for evaluation. Blood could be identified in biopsies from 4 of 9 subjects, but less blood was present than in biopsies from subjects who received the 30 mg injection. Again, one can speculate that the subject who did not provide endometrium for evaluation had shed to the basalis.

Suppressed endometrium from subjects injected with 30 mg of NET typically contained small glands lined by low epithelium and embedded in a loose stroma of sparse reticular fibers and small stromal cells that manifested the naked nucleus appearance. In comparison, suppressed endometrium from subjects who received 50 mg of NET characteristically had larger stromal cells and more reticular fibers. Stromal cells, however, were not as markedly as hypertrophic as they become in the latter part of the menstrual as cycle or during pregnancy. Reticular fibers intertwined between the stromal elements and re-enforced the basement membranes of the glands and the vessels of the microvasculature. Bleeding occurred with both dose levels, but there was less with the higher dose level. It was concluded that the amount of bleeding in endometrium from the corpus-fundus uterus was inversely proportional to the number of reticular fibers and the degree of stromal cell hypertrophy.

The colloidal iron (CI) histochemical method illustrated the dose-dependent differences in the quantity and distribution of reticular fibers, and showed that the fibers were sparse in areas of bleeding and in areas where the endometrium had been shed. Intact areas that had remained since treatment began were rich in reticular fibers.

Other histochemical methods showed additional important features of the endometrium during NET treatment. Collectively, the techniques indicated that the endometrium was not totally deprived of estrogen during continuous NET medication and that the endometrium responded focally during treatment. Tissue was lost and repair occurred. Sites of mitotic activity could be recognized. Fluctuations took place in cytoplasmic RNA and nucleolar RNA that indicated the epithelial cells were carrying out limited synthetic functions. Focally, some glycogen, sialomucins and sulfomucins appeared as if their synthesis had been triggered by estrogen and by estrogen-progestin interactions. Growing ovarian follicles were the likely source of the estrogen. All of the changes can be elusive and go unnoticed unless biopsies are serially sectioned and evaluated according to a battery of histochemical methods specifically for information on endometrial histophysiology.

2. Dr. Archer

All treated biopsies from Dr. Archer were from subjects who had received about 6 months of continuous NET treatment. All showed bleeding. As noted earlier, treatment consisted of giving each subject 2 NET injections (65 mg or 100 mg/injection) separated by an interval of 90 days. Dr. Balmaceda's biopsies, in comparison, were taken after 2 months of a single dose of 30 mg or 50 mg of NET.

Six biopsies came from subjects who had received two, 65 mg injections of NET: 1 biopsy/day was obtained on day 89, 90 and 92 following the second injection and 3 biopsies were taken on day 93 following the second injection (Table II). Of these, 2 were in the proliferative phase, 2 were suppressed, and 2 were unsuitable for histological evaluation. One suppressed biopsy also contained sites of proliferative activity.

Eight biopsies were obtained from subjects who had received two, 100 mg injections of NET: 3 were taken on day 90, 1 on day 92, 1 on day 93, 2 on day 94, and 1 on day 95 following the second NET injection (Table II). The endometrium was proliferative in 2 biopsies, and suppressed in 6 biopsies. Two biopsies of suppressed endometrium contained sites of proliferative activity.

Some biopsies contained insufficient endometrium for complete evaluation. It was possible to evaluate reticular fibers by the colloidal iron method in only 4 biopsies from subjects who had received the 65 mg dose level, and in 7 biopsies from subjects who had received the 100 mg dose level. With the 65 mg dose level, only 1 of 4 subjects shed her endometrium during 6 months of treatment; with the 100 mg dose level, 4 of 7 subjects shed their endometrium during treatment. Thus, more shedding was associated with the higher dose level of NET.

Seven of the 14 treated biopsies from Dr. Archer had proliferative activity, an indication that ovarian function was returning. Histochemical results showed some degree of estrogen stimulation as manifested by epithelial growth, mitotic figures, prominent ciliation, scattered aggregates of glycogen, cytoplasmic basophilia, and sulfomucins at the apices of epithelial cells. Bleeding in biopsies from subjects who received the 65 mg dose level seemed to be due to the return of ovarian function and endometrial cyclicity.

3. Dr. Saxena

Three treated biopsies were received from Dr. Saxena's 13 subjects. As noted earlier, they were to show the endometrial effects of 5 months of continuous NET treatment.

One biopsy was from a subject who had received two, 65 mg injections, and 2 biopsies were from subjects who had received two, 100 mg injections. Only 1 of the 3 biopsies contained endometrium for evaluation, and it was from a subject who had received the high level of NET. The specimen provided a minimum representation of proliferative endometrium that showed recovery and stromal cells of considerable size and density. There was no endometrium for histochemistry, SEM or TEM. Unfortunately, the information gained from the treated biopsies from Dr. Saxena was negligible and they need not be mentioned further.

B. Transmission Electron Microscopy

TEM showed the subcellular changes that occur in the endometrium during continuous NET treatment. Specimens from both Dr. Balmaceda and Dr. Archer varied somewhat in appearance and some were recovering from suppression. Thus, changes at the subcellular level were not always identical or of the same magnitude. Micrographs representative of the alternations in the surface epithelium, glands, stroma, and blood vessels are shown in the order named in Figures 1 through 9. At the time the biopsies were obtained, Dr. Balmaceda's subjects who received the 50 mg dose level provided endometrial biopsies that showed the most favorable response to treatment.

Secretory cells and ciliated cells were present in the surface epithelium during treatment. Secretory cells predominated. They were lined by microvilli and could be divided into light cells and dark cells based on the electron density of their cytoplasm (Fig. 1). Cytoplasmic microfilaments accounted for the electron density of dark cells.

Pools of extravascular blood and tissue fluid tended to accuumulate beneath the surface epithelium. Protrusions formed above the pools. Breaks occured on the prptrusions perhaps as a consequence of the action of enzymes in the blood or simply to an overstretching of the surface epithelium. When viewed in profile, a break appeared to involve one or only a few cells, but even such

a small area allowed considerable blood to escape into the uterine cavity (Fig. 2). SEM showed that these sites were numerous. Any correlation between uterine bleeding and vaginal bleeding is unknown since bleeding charts on the subjects were not supplied with the biopsies.

Stratification of the surface epithelium was observed histologically and by TEM in areas of proliferative activity. The stratified cells represented a type of metaplasia in areas undergoing repair, but they had no pathological significance. Dark cells predominated in stratified areas. They cytoplasmic filaments may provide the cells with the migratory ability necessary to reach defective areas for re-epithelialization.

Glands became atrophic with continuous NET treatment (Fig. 4). They contained the same cell types described for the surface epithelium (i.e., ciliated, secretory, light, and dark). Extravascular leukocytes were present between the gland cells.

Biopsies with negligible bleeding had a stroma rich in reticular fibers, vessels with intact endothelium, large stromal cells, and little or no edema (Fig. 5). Stromal cell hypertrophy and the production of reticular fibers are progestin effects.

Biopsies with considerable bleeding had a stroma with few reticular fibers, vessels with discontinuous endothelium, small stromal cells, and appreciable edema (Fig. 6). Distended venules, capillaries, and venules of normal caliber were leaky in biopsies with pronounced bleeding.

Vascular clots of platelet aggregates were infrequent (Fig. 7). They were no more frequent than in control biopsies.

Swollen endothelial cells were observed in arterioles, capillaries, and venules of all treated biopsies. The swollen cells had electron lucents cytoplasm and nuclei that were usually larger than nuclei of normal endothelial cells (Figs. 8 & 9). They increased in number as the length of treatment increased. Vascular lumina were narrowed where swollen cells were present. This alterations needs further study.

C. Scanning Electron Microscopy

To better appreciate the impact of NET on the endometrium, micrographs of control biopsies are shown and described. The control biopsies were obtained in the cycle preceding the first injection of NET. Folds, depressions, and gland openings characteristic of post-ovulatory endometrium near the time of implanation were visible at low magnification (Figs. 10 & 11). Close inspection failed to show any significant defects. Expelled secretory products were the only materials visible on the epithelial surfaces and in the gland openings.

Apices of secretory cells were densely covered with microvilli (Fig. 12). Cilia were longer than microvilli. The ratio of secretory cells to ciliated cells was about 20:1.

Treated biopsies from Dr. Balmaceda and Dr. Archer showed similar alterations. The response with the 50 mg and 65 mg dose levels was more favorable than with the other dose levels.

NET treatment caused great suppression and thinning of the endometrium with the formation of mounds or protuberances of various sizes. Corresponding edematous and blood-filled irregularities of the endometrium were noted by LM and TEM. Apices of the mounds were lined by thin, flat cells (Fig. 14). The cells manifested breaks and discontinuities that allowed blood to pass into the uterine cavity.

Glands decreased in number during endometrial suppression. Exfoliated glands were often seen on the endometrial surface (Fig. 15). Interestingly, most of them had their luminal aspects directed outward.

Focal breaks and blood could be identified throughout the period of suppression (Fig. 15). A break usually involved only a few dissociated cells admixed with erythrocytes (Fig. 16). The cells associated with a break had lost their microappendages and orientation and had porous cells membranes.

As noted earlier, several endometrial biopsies had weakly proliferative activity. This was interpreted as a sign of recovery from NET suppression and the beginning of estrogen production by growing follicles. These biopsies both resembled and differed from biopsies of suppressed endometrium. Like suppressed endometrium, the endometrium during recovery manifested mound-like irregularities of the surface, cellular disruptions, and bleeding (Fig. 17). The irregularities became less prominent as recovery continued. Break-through bleeding was commonly observed on mounds where the epithelium had become thin or had degenerated (Figs. 18 & 19).

A characteristic and distinctive feature of the endometrium of recovery was the presence of bridges or columns of cells which often extended from gland openings for considerable distances across the endometrial surface (Fig. 20). Such columns accounted for the focally stratified areas that were observed by LM and TEM. The cells appeared to elongate and to migrate to defective areas for re-epithelialization (Fig. 21 & 22). They resembled cells present in normal endometrium during recovery from menstruation.

D. Legends

Fig. 1. An intact, suppressed surface epithelium is depicted with light and dark cells. Venules are present in the stroma. 63 d PI 30 mg NET. x5,700.

Fig. 2. A small break appears to involve a single cell in the surface epithelium. Blood in a subepithelial pool can flow into the uterine cavity through the break. 63 d PI 50 mg NET. x4,500.

Fig. 3. The stratified surface epithelium depicted is a result of repair. 63 d PI 50 mg NET. x7,500.

Fig. 4. The atrophic gland is lined by viable cells. Lymphocytes are present at the gland base. 63 d PI 30 mg NET. x4,500.

Fig. 5. Reticular fibers are numerous, stromal cells are of moderate size, and the venule is intact. There is no bleeding. 63 d PI 30 mg NET. x5,700.

Fig. 6. The endothelium of the venule is discontinuous and the lumen is filled with red blood cells that are escaping into the stroma. x5,700.

Fig. 7. Platelet aggregate in venule. Clots were no more frequent than in untreated control biopsies from the same subjects. 63 d PI mg NET. x5,700.

Fig. 8. Capillary with swollen endothelium. 93 d PI 2nd dose 65 mg NET. x10,200.

Fig. 9. Capillary with swollen endothelium. 94 d PI 2nd dose 100 mg NET. x7,500.

Fig. 10. Low magnifications of control secretory endometrium showing highly folded mucosa and gland openings. x150.

Fig. 11. Low magnification of control secretory endometrium showing the large amount of surface available for study, surface folds, and gland openings. x150.

Fig. 12. Control secretory endometrium depicting ciliated cells and secretory cells of the surface epithelium. x375.

Fig. 13. Suppressed endometrium with prominent mounds or protrusions. Correlative LM and TEM showed the mounds were filled with blood, sometimes edematous, and covered by squamous epithelium. 63 d PI 50 mg NET. x75.

Fig. 14. Higher magnification of apex of mound illistrating outlines of squamous epithelial cells that cover the mounds associated with suppressed endometrium. Breaks and loss of the epithelium permit leakage of blood and fluid from the underlying stroma. 63 d PI 50 mg NET. x1,500.

Fig. 15. Suppressed endometrium that shows protuberances and numerous focal breaks and sites where cells have been lost. These are characteristics of the endometrium of subjects with pronounced bleeding. The cylindrical structure at lower left is an expelled and everted gland. 93 d PI 2nd dose 65 mg NET. x300.

Fig. 16. An area of focal breakdown is shown in the suppressed endometrium. A few red blood cells can be seen among the dissociated epithelial cells. 93 d PI 2nd dose 65 mg NET. x3,000.

Fig. 17. Endometrium during recovery showing gaps, disruptions, and cell columns that are characteristic of recovery. 63 d PI 30 mg NET. x750.

Fig. 18. Endometrium during recovery depicting a focal area of breakthrough bleeding. Blood cells and extracellular granules can be seen in the stroma where the epithelium has been denuded. 93 d PI 2nd dose 65 mg NET. x3,000.

Fig. 19. Endometrium during recovery showing a site where the epithelium has been lost, exposing the stroma and blood. 94 d PI 2nd dose 100 mg NET. x3,750.

Fig. 20. Endometrium during recovery. A long column of cells extends from the gland opening across the endometrial surface. The columns may permit cells to reach and repair denuded or injured areas. 63 d PI 30 mg NET. x3,000.

Fig. 21. Endometrium during recovery. Bridges of cells appear to be migrating from the large cell column. 93 d PI 2nd dose 65 mg NET. x750.

Fig. 22. Endometrium during recovery. Elongated cells at base of cell column appear to be migrating to a nearby defective area at right of the micrograph. 92 d PI 2nd dose 65 mg NET. x1,500.

V. CONCLUSIONS

1. All dose levels of NET tested for an effective 90-day injectable resulted in microscopically recognizable bleeding. The extent that this bleeding became apparent clinically was unknown since copies of bleeding charts were not available.

2. NET did not completely suppress ovarian function. It was speculated that FSH periodically reached a level that triggered development of ovarian follicles. The follicles produced estradiol, but ovulation did not occur.

3. To deter bleeding, it was found that a dose level of NET should be administered that stimulates the hypertrophy of stromal cells and the production of reticular fibers.

4. Of the dose levels of NET tested, the 30 mg was too low, the 100 mg was too high, the 50 mg was borderline, and the 65 mg was satisfactory in that ovulation was inhibited and there was less bleeding than with other dose levels. Bleeding and recovery occurred simultaneously.

5. After considerable experience in this and other studies in evaluating the endometrial response to NET, the results of the present study were the first to show convincingly that an injectable can be forthcoming. There were no clots, polyps, or other serious endometrial abnormalities. As additional studies are undertaken, they should include the analysis of biopsies by correlative LM, histochemistry, TEM, and SEM in order to increase the sample size for evaluating the long-term effects of NET on the endometrium.

6. To control bleeding, an estrogen should be injected with NET.

APPENDIX

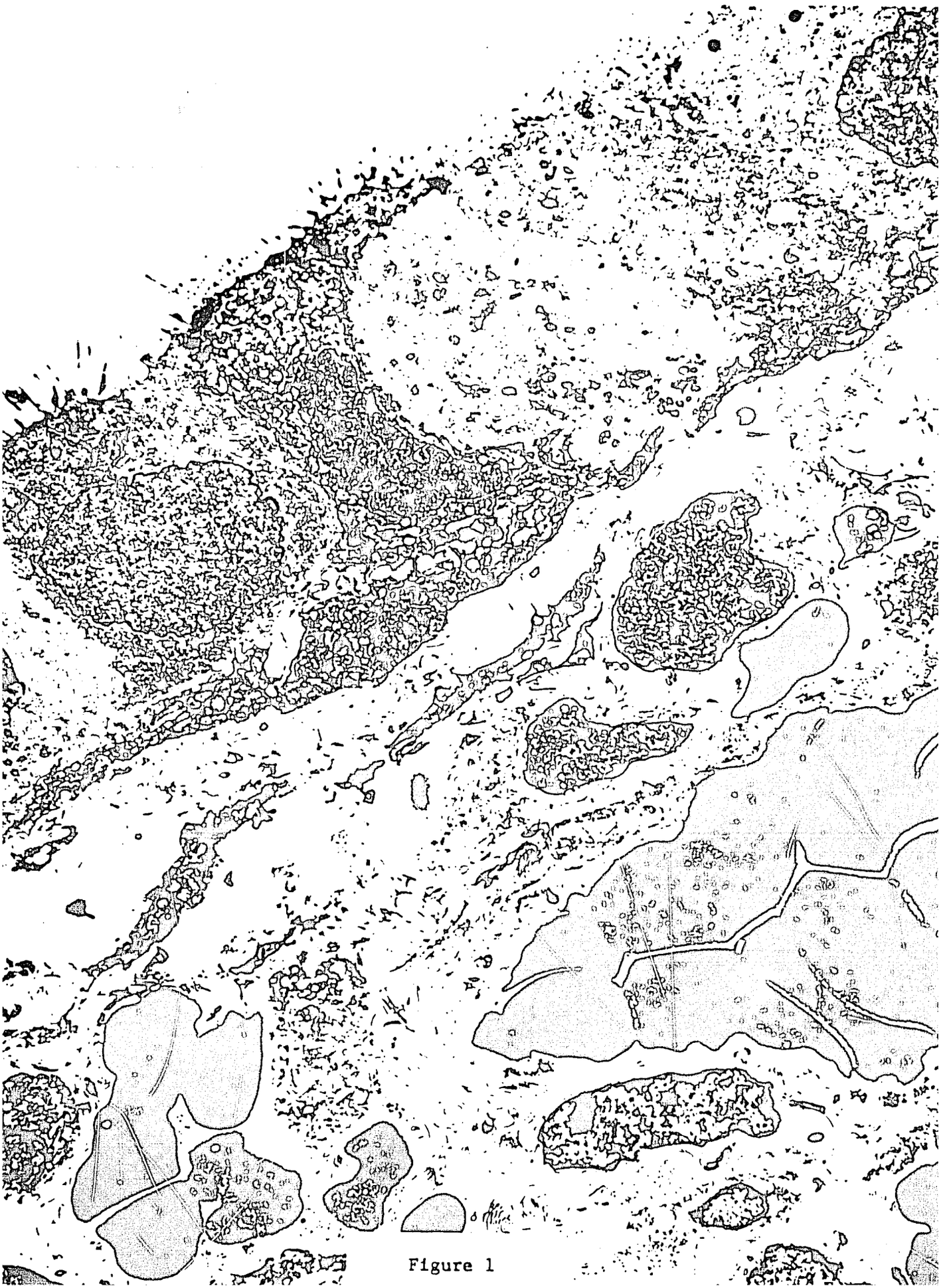
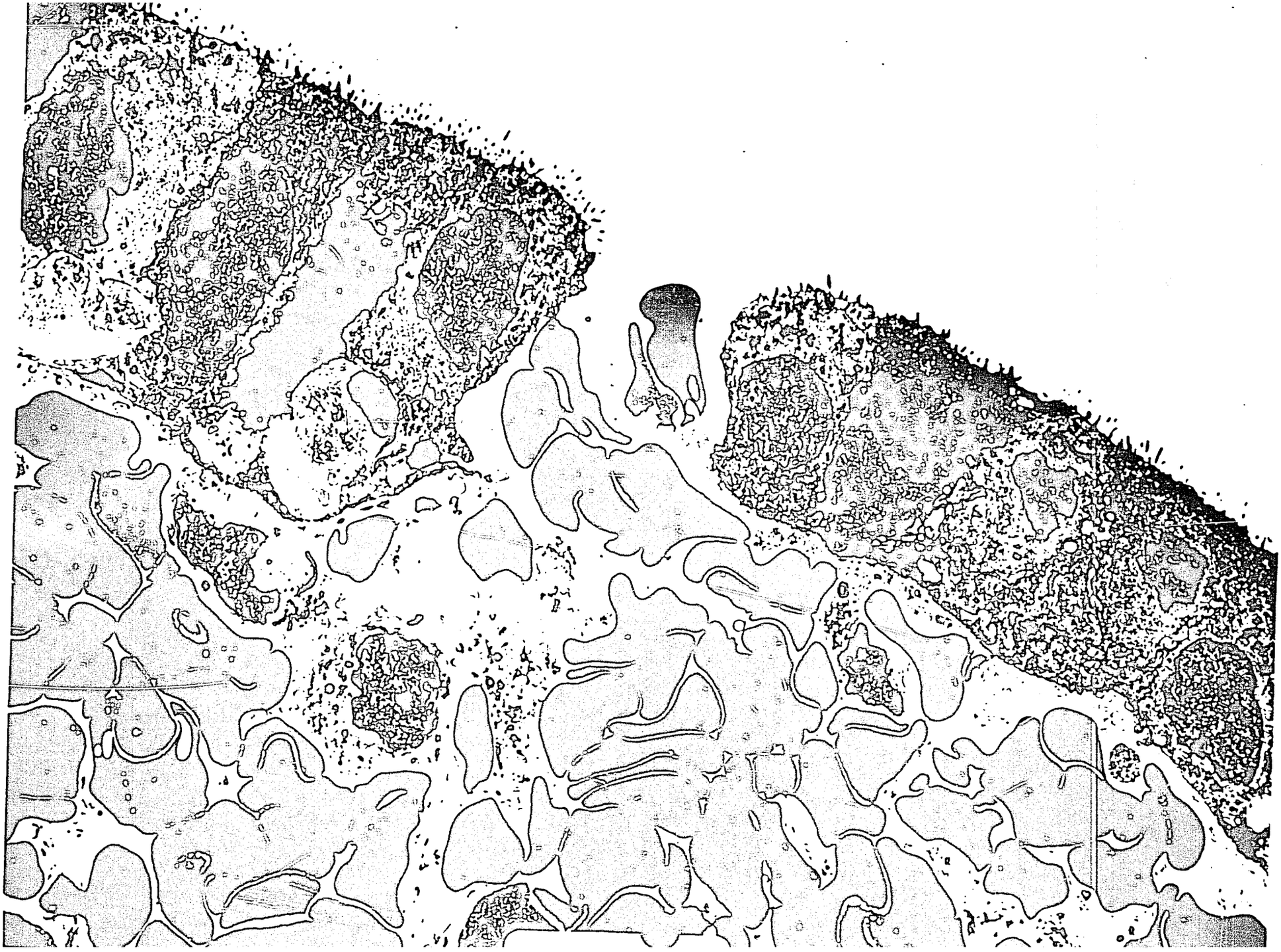
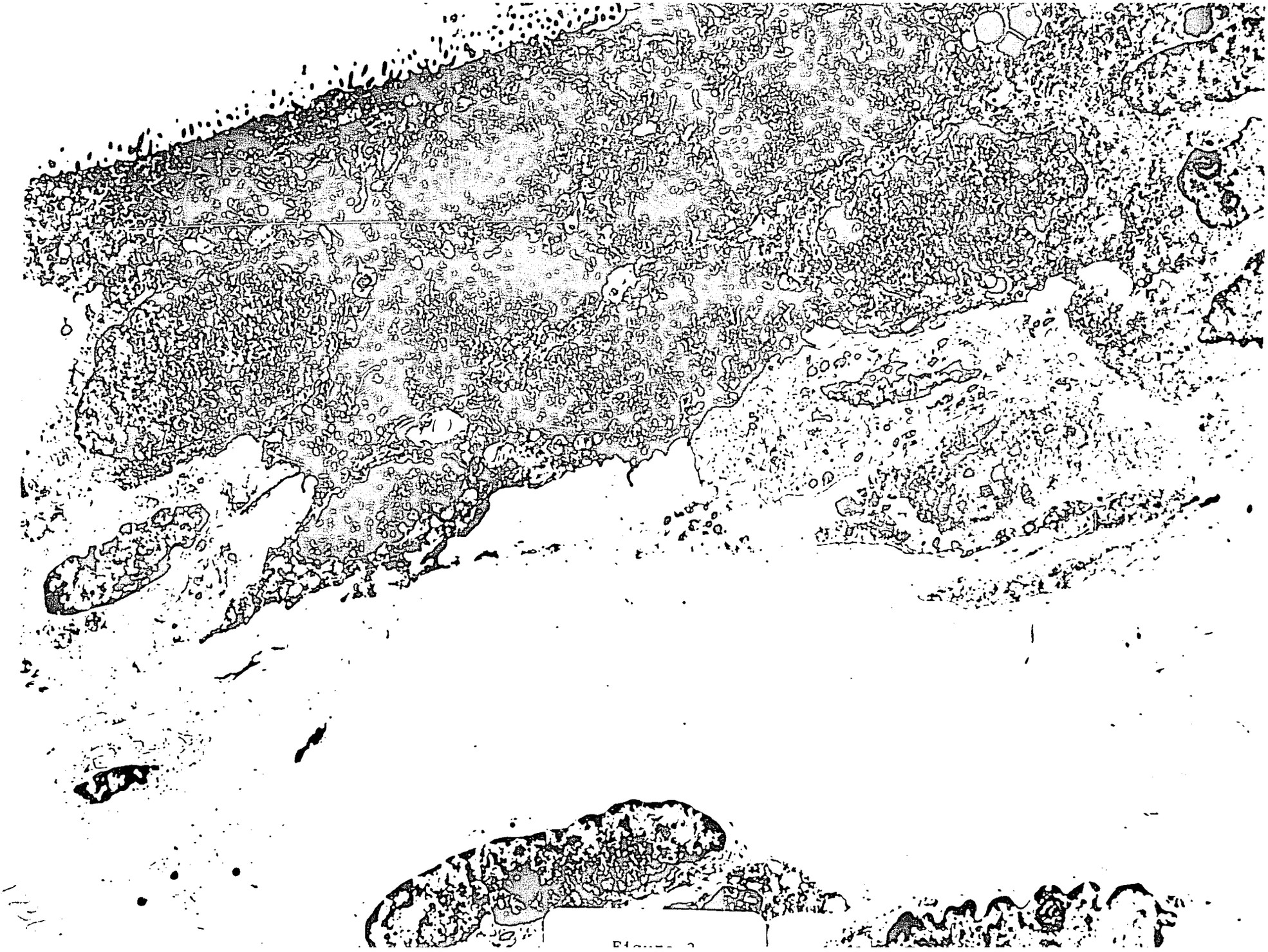


Figure 1





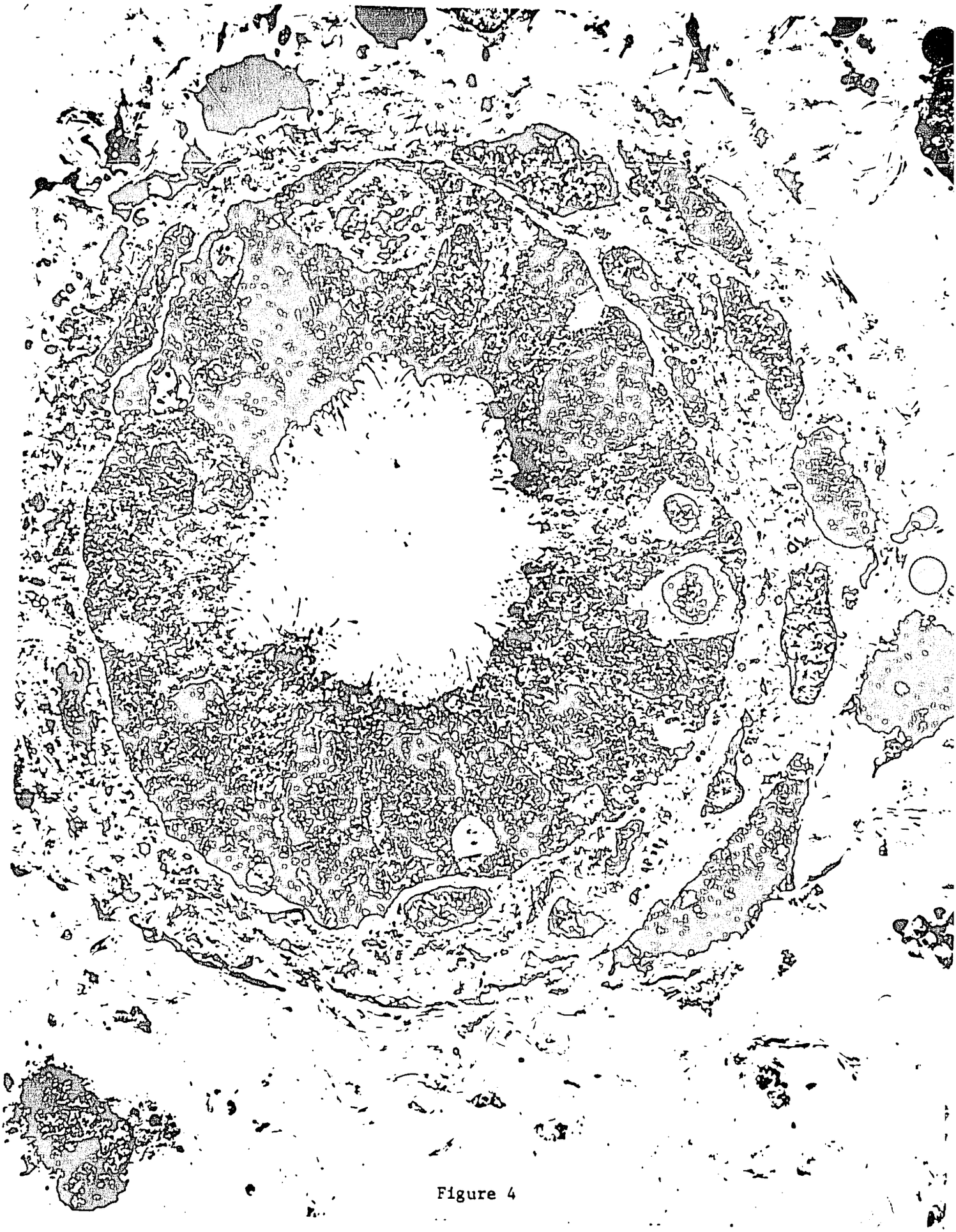


Figure 4

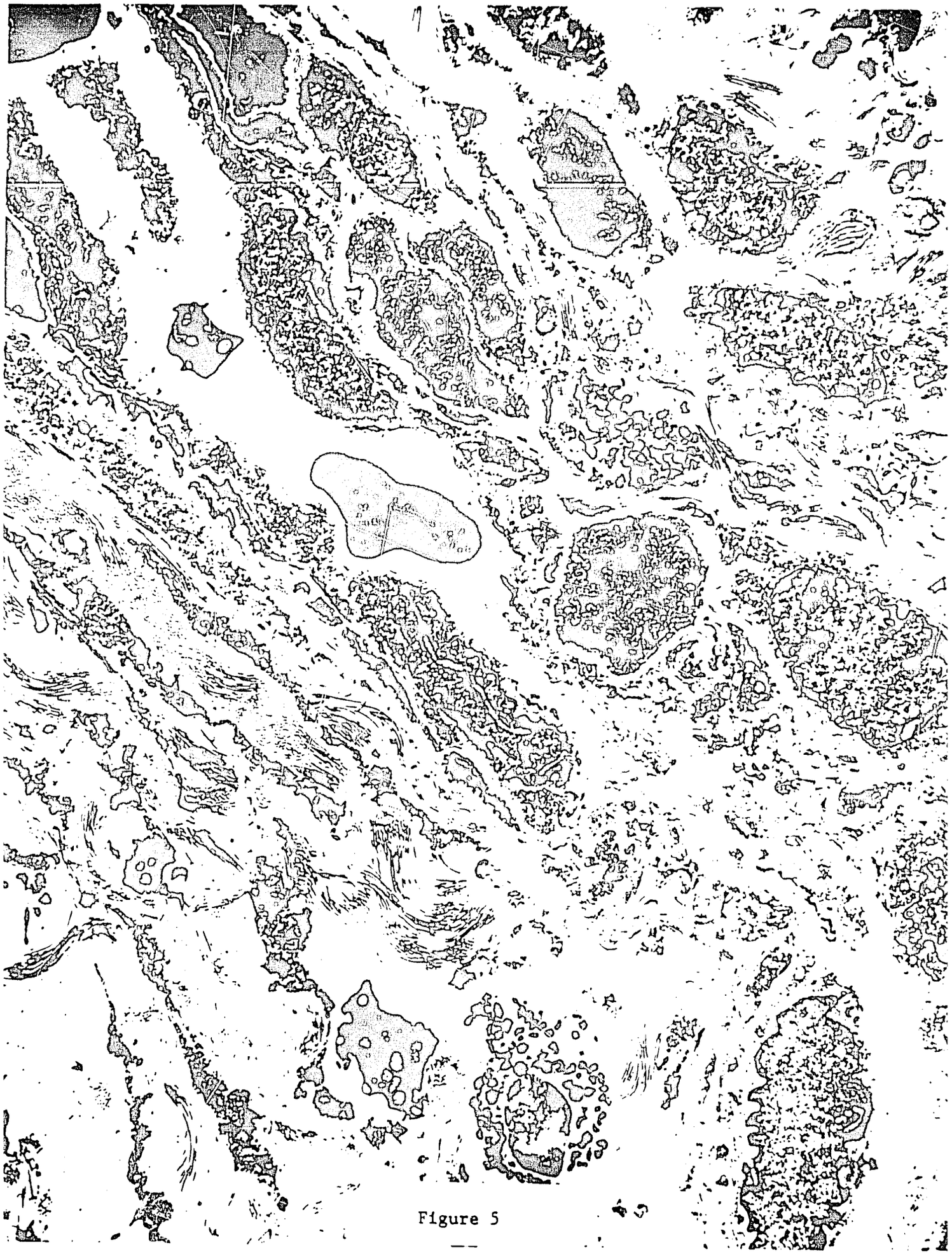


Figure 5

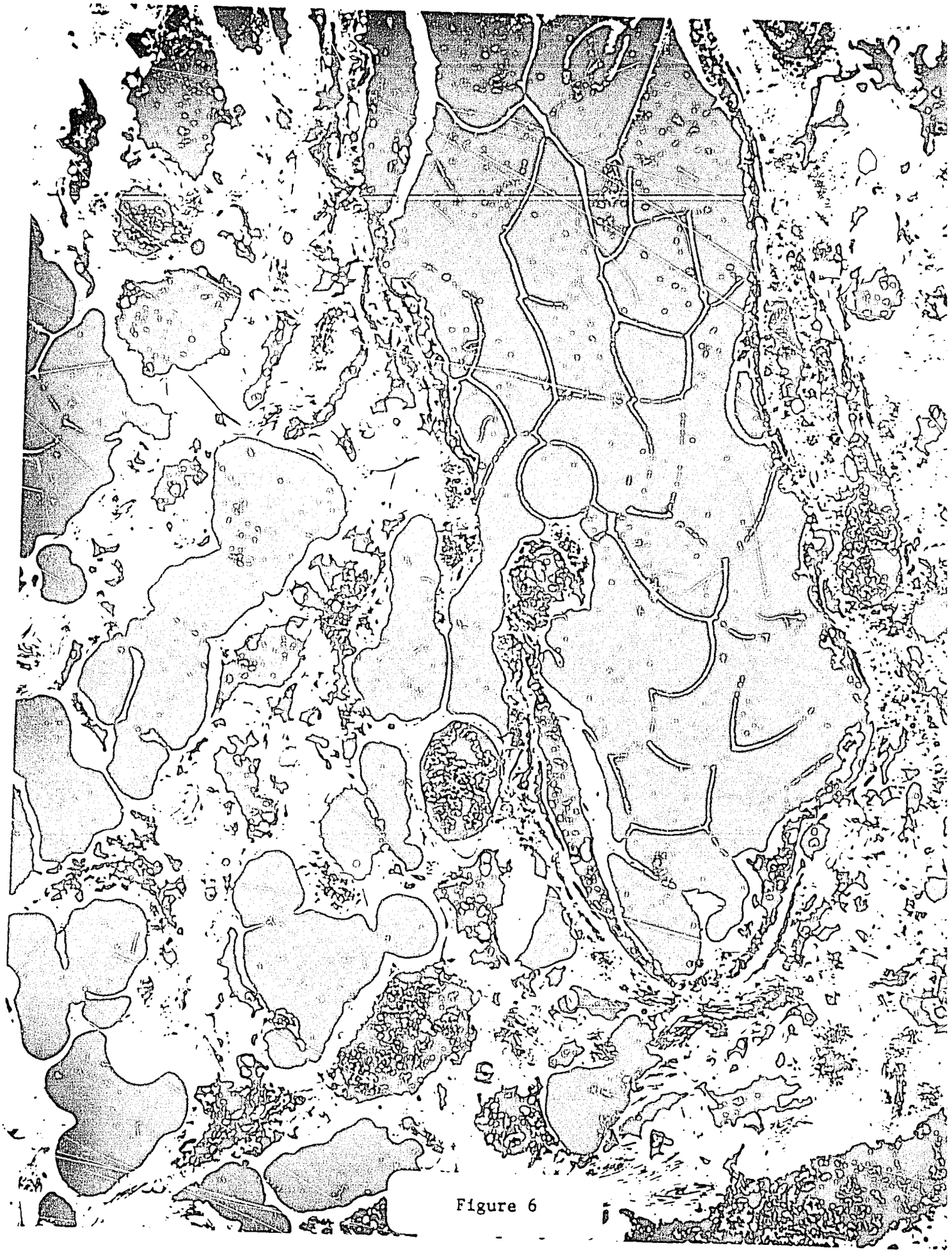


Figure 6

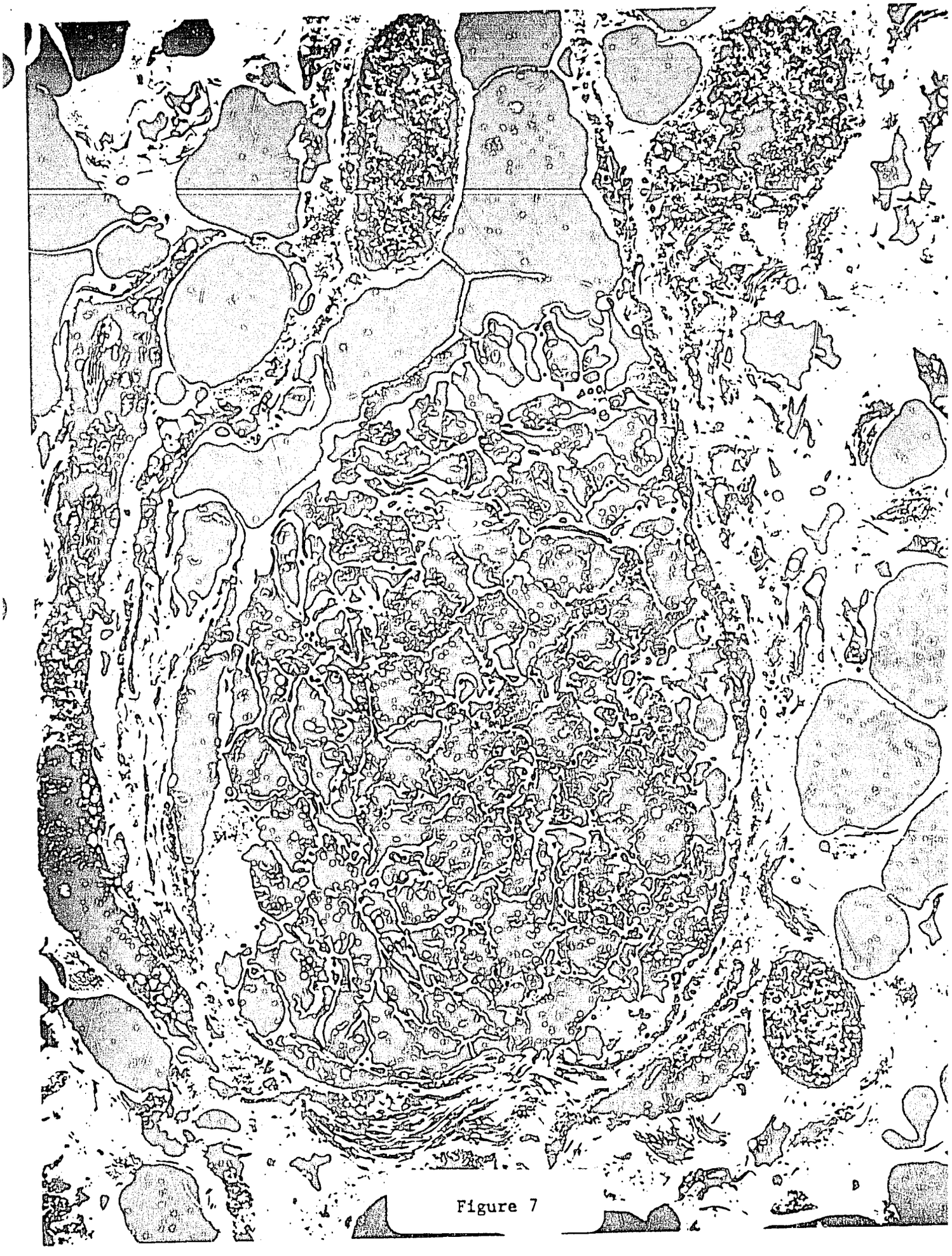


Figure 7

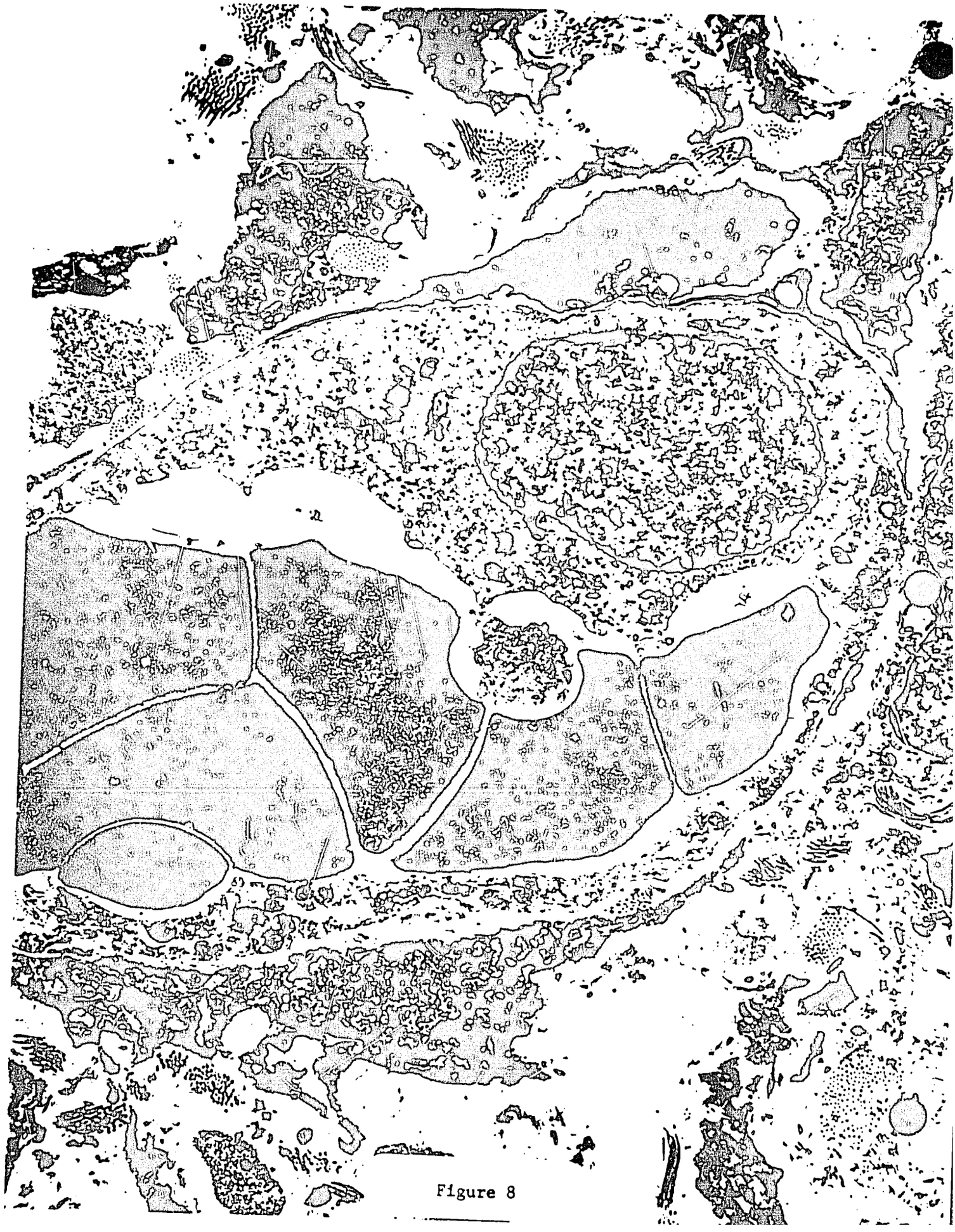


Figure 8

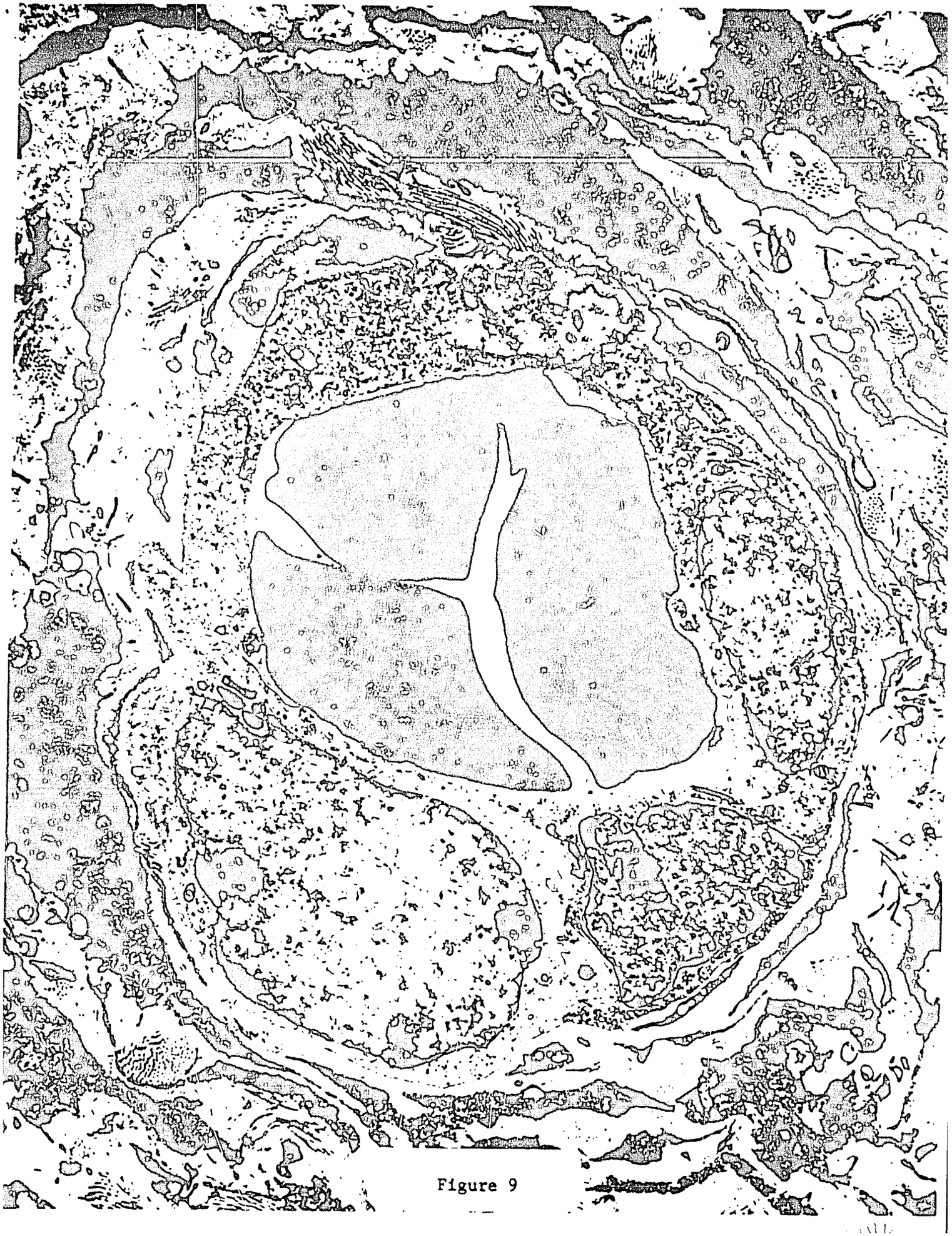


Figure 9

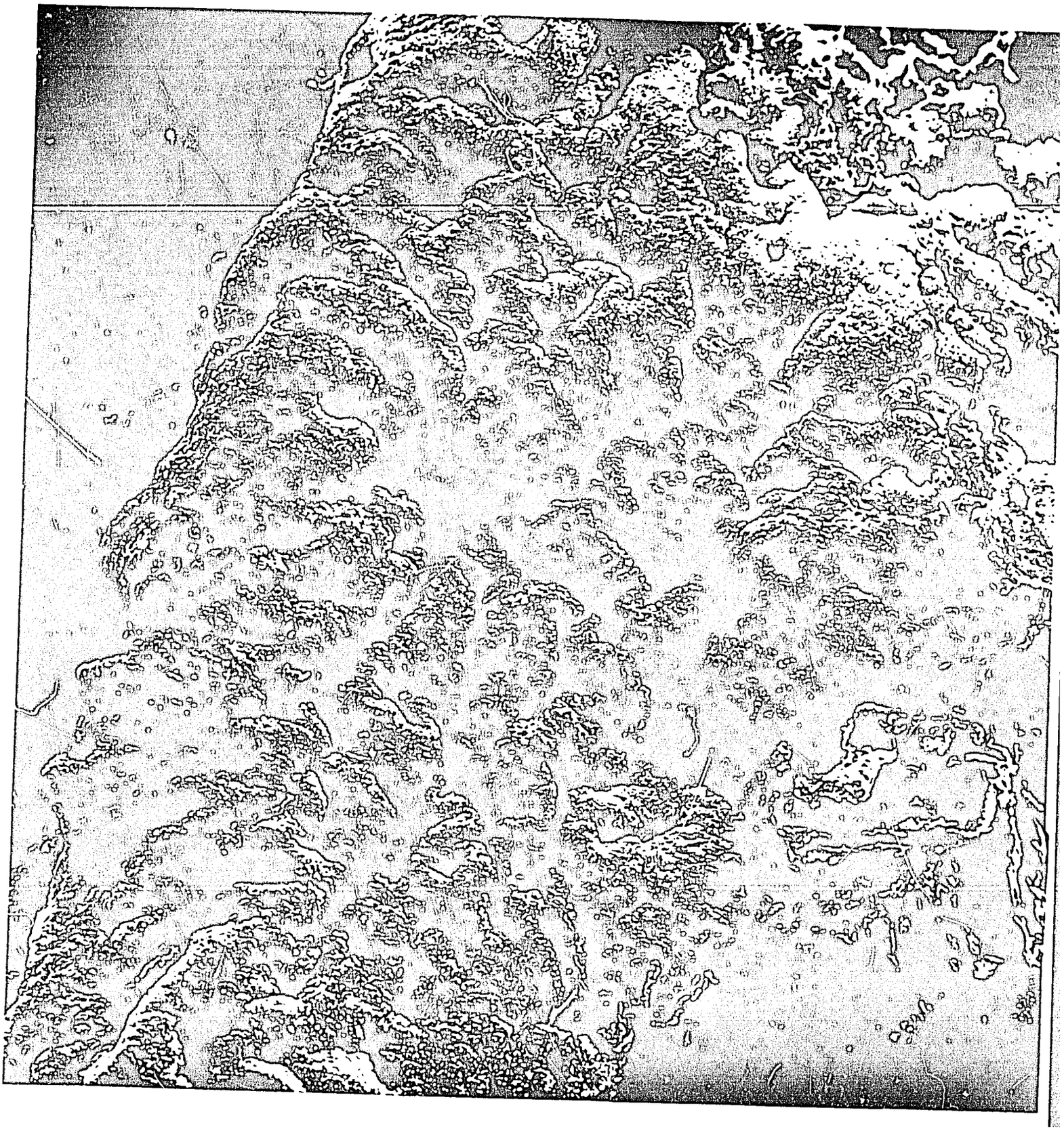


Figure 10

Figure 11

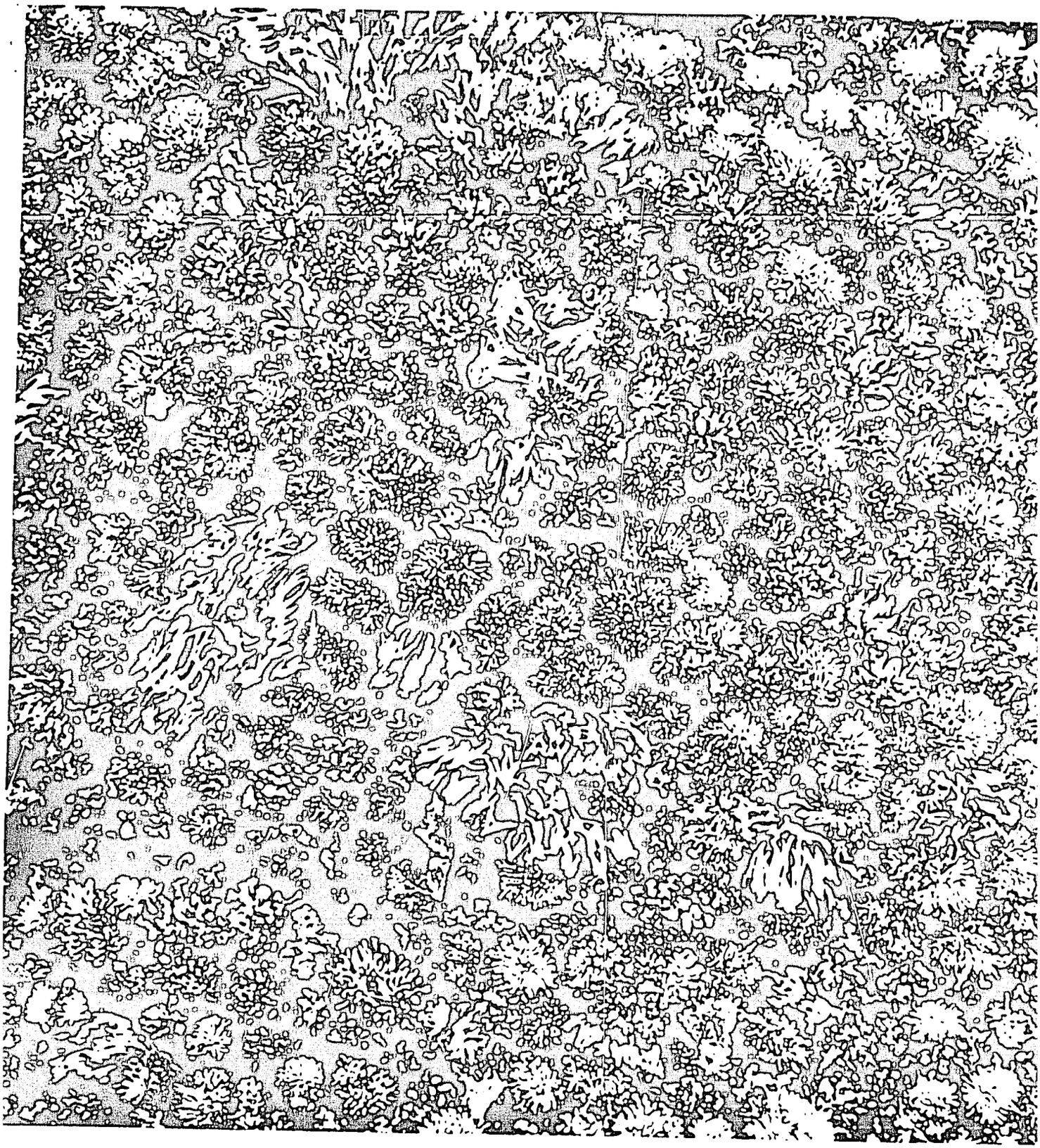


Figure 12

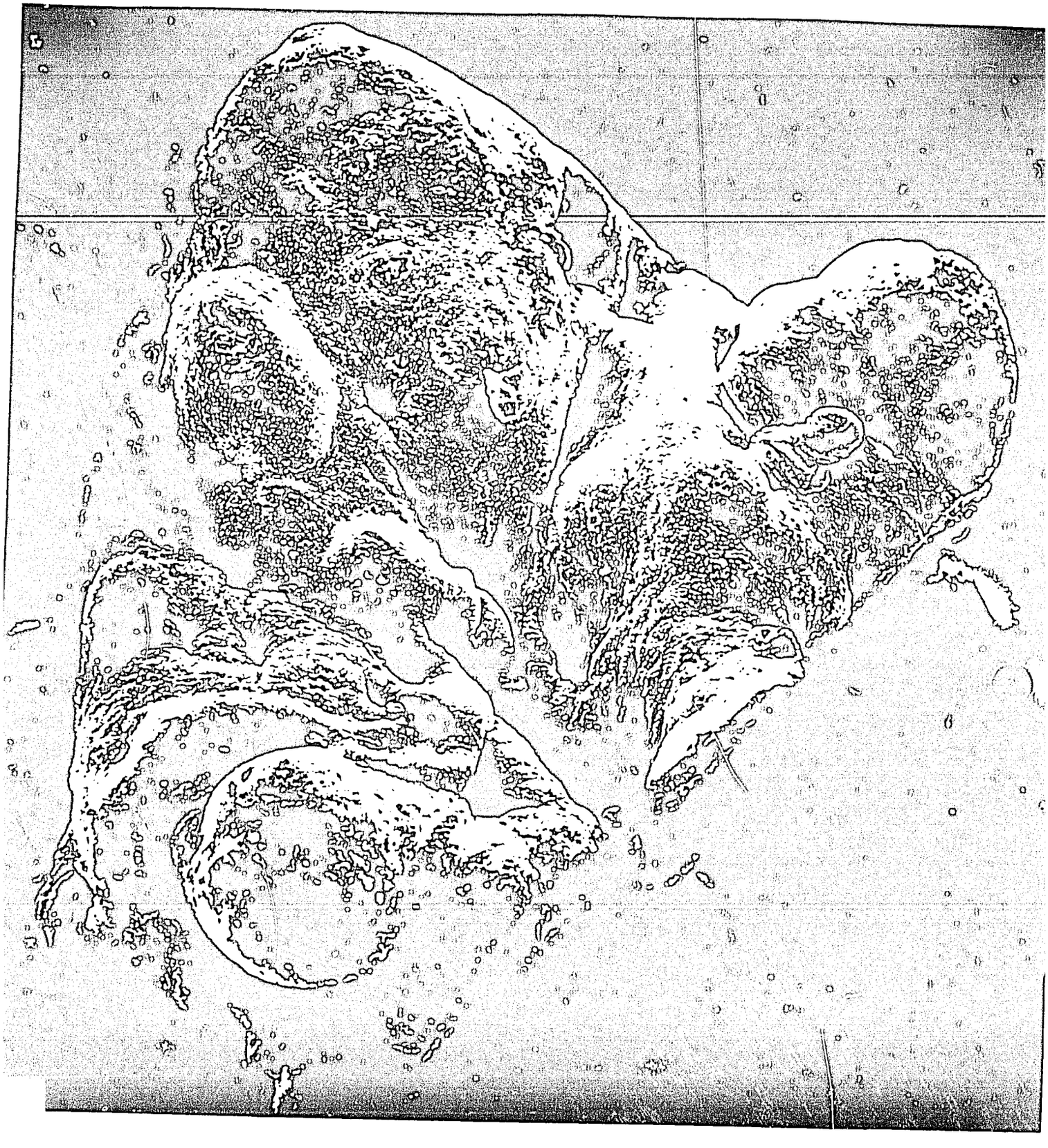


Figure 13

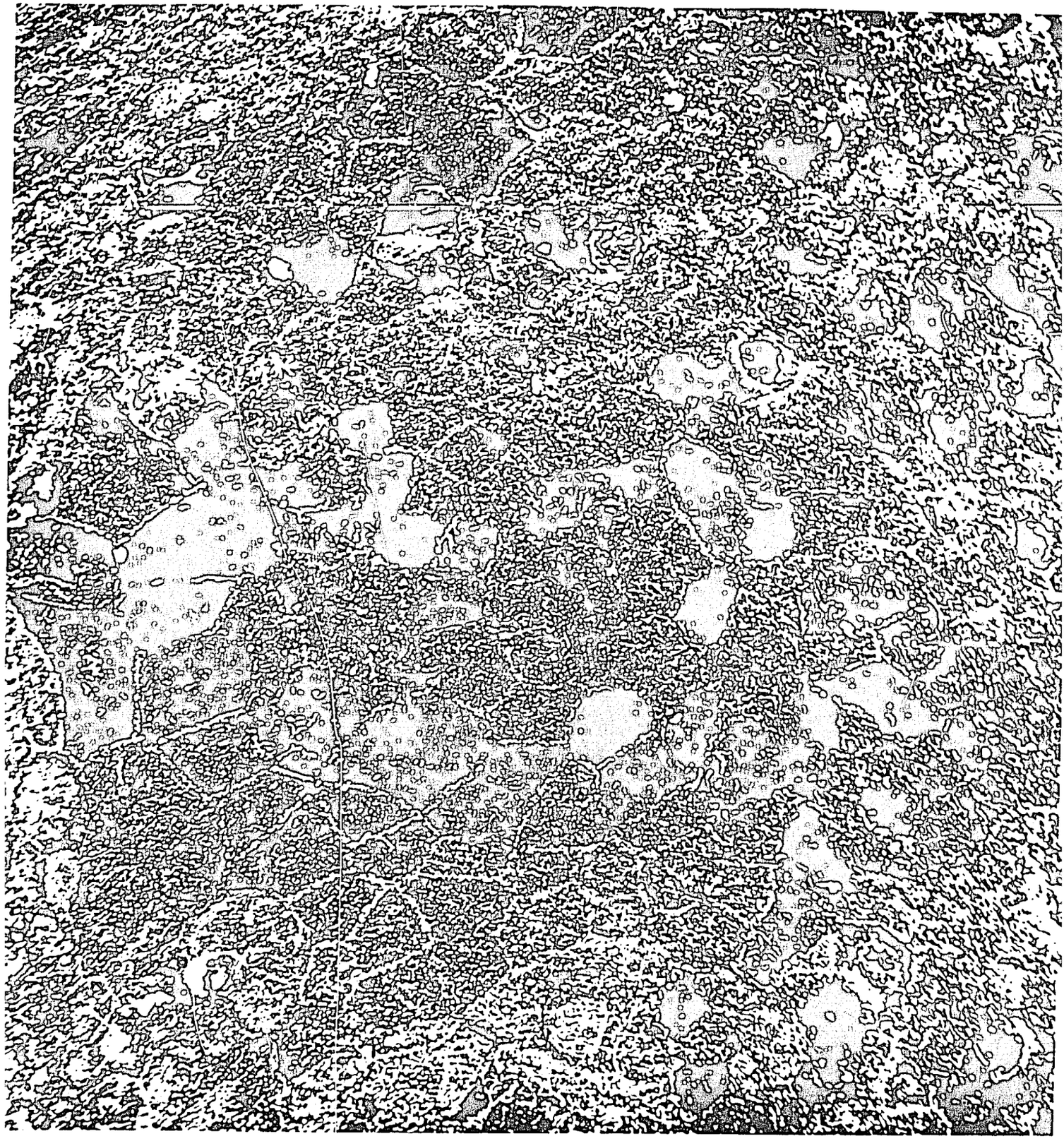


Figure 14

Figure 15

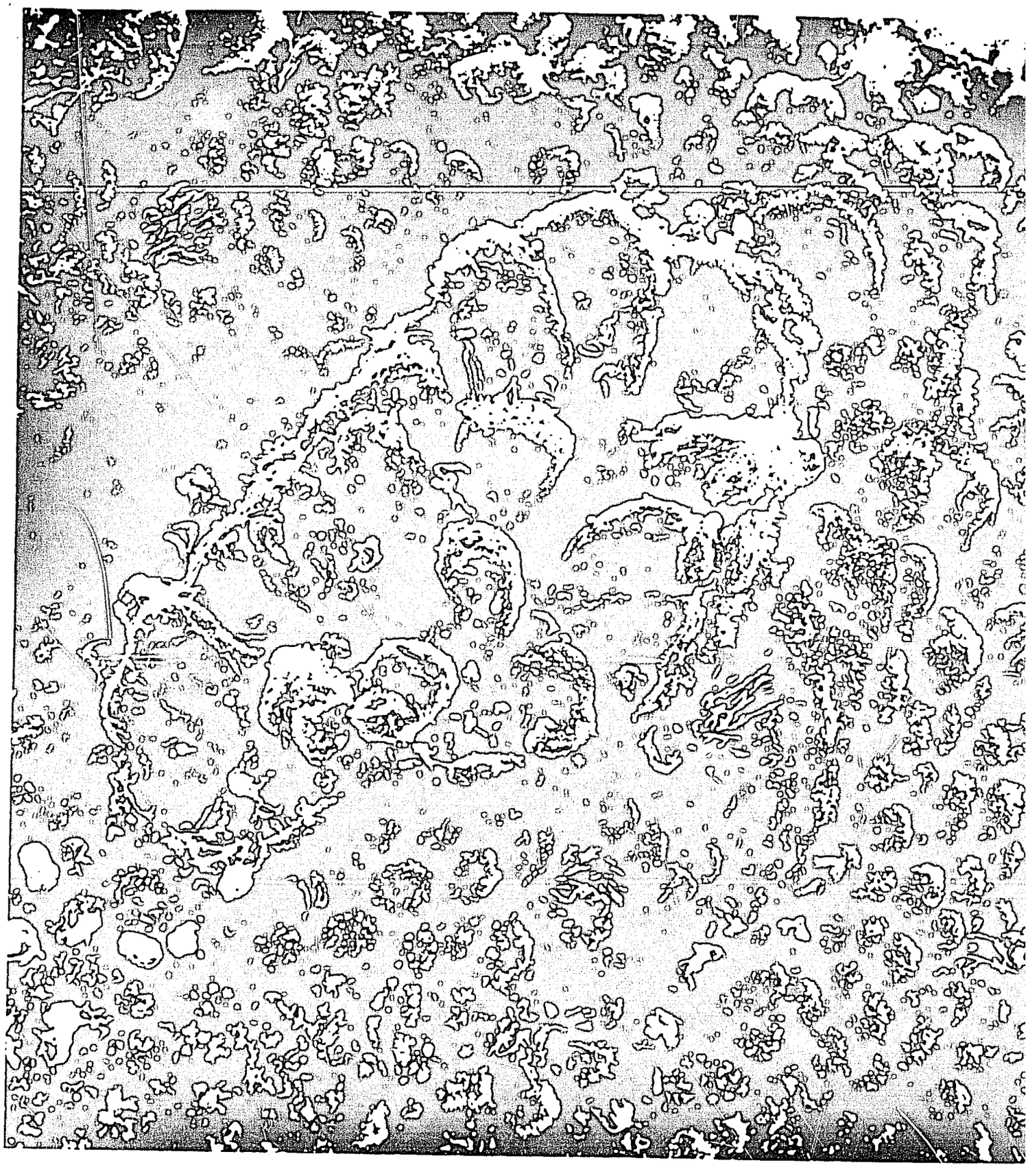


Figure 16

Figure 17

Figure 18

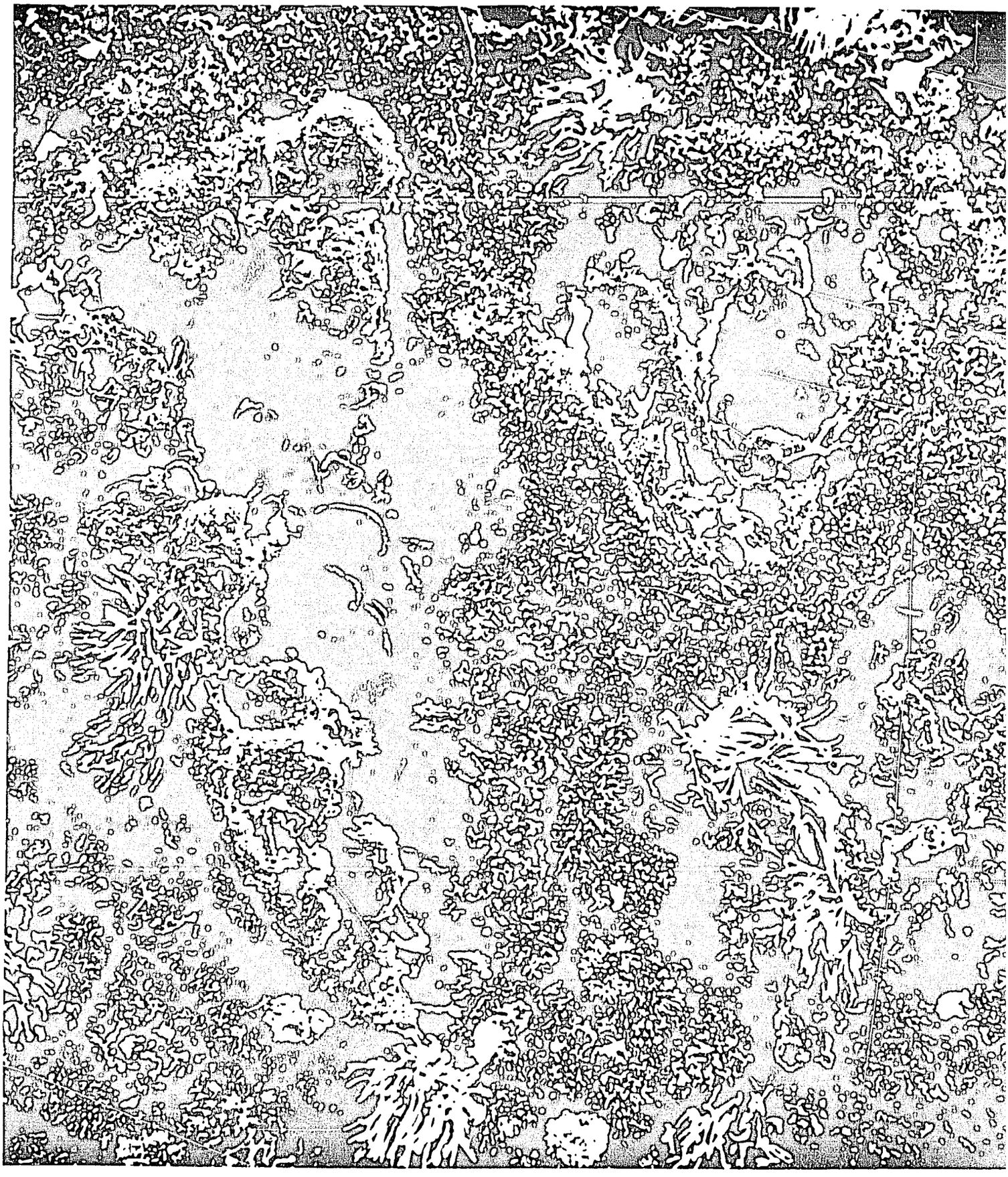


Figure 19

Figure 20

Figure 21

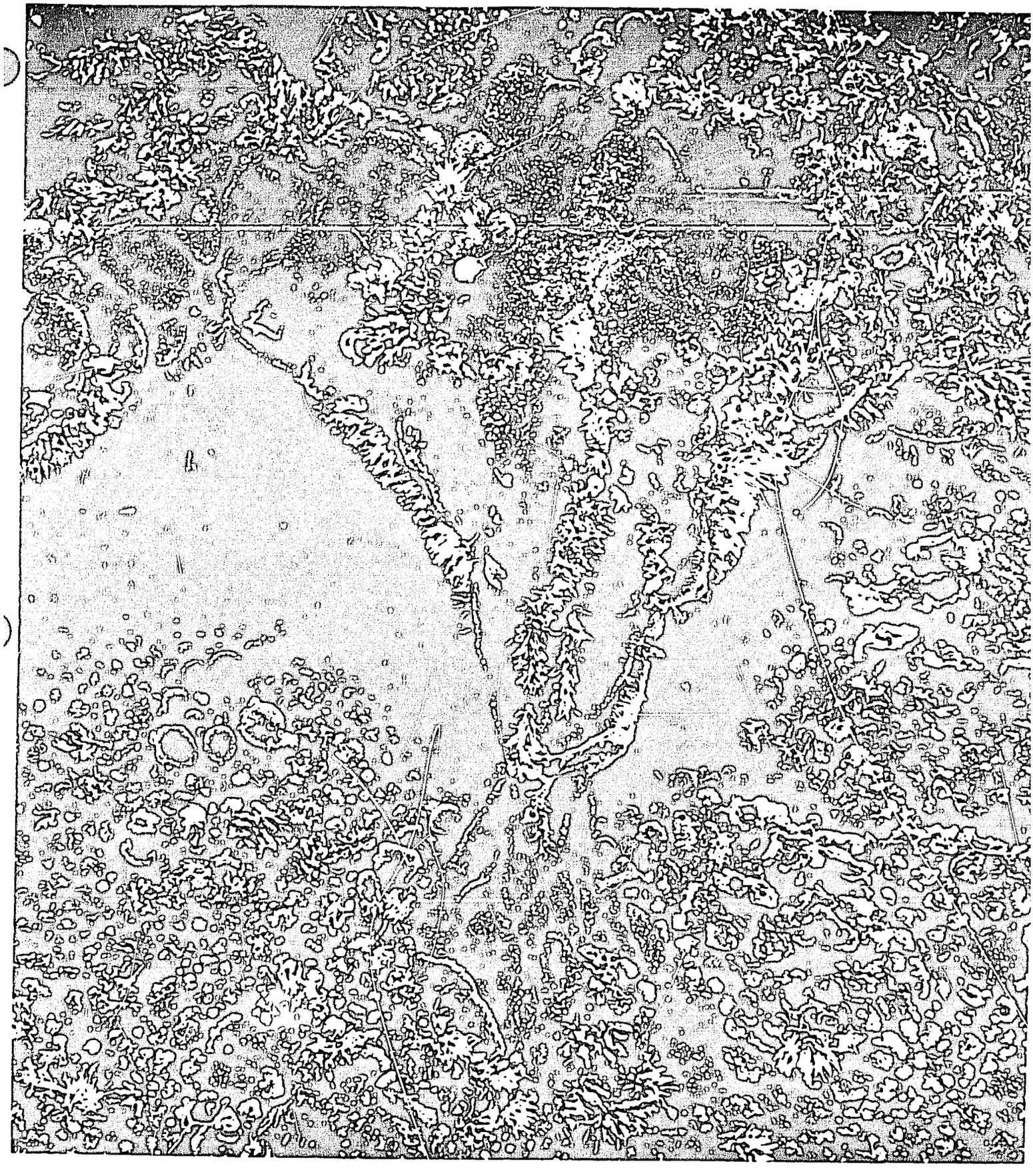


Figure 22

NAME: PARFR NET90

Subject's Initials: NH

DOB W 292-86

AGE SP# (N-V) C,CD30

SEX F DATE 5-24-86

RACE INS BALMACEDA

ADDRESS:

PHYSICIAN: Biopsy date: May 12, 1986. Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 1.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 25.

Initial should b NH. Correct by WHW.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 292-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures collectively 0.8 cm, with individual segments ranging up to 0.3 cm. The endometrial glands are tortuous, with incipient secretory exhaustion, and are located in a stroma which exhibits a moderate degree of diffuse predecidualization.

EADjh
5-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: July 18, 1986.
Patient No. 01.

PHYSICIAN: Day-63 PI

CLIN. DX: PARFR's NET-90 Study

DOB

AGE

SP# W 502-86

SEX F

DATE 7-29-86

RACE

INS (NH) 63d PI

BALMACEDA

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY:
a) INSUFFICIENT FOR DIAGNOSIS.
b) CERVICAL EPITHELIUM SHOWING MICROGLANDULAR HYPERPLASIA AND SQUAMOUS METAPLASIA.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 502-86.

MICROSCOPIC DESCRIPTION: The biopsy measures 1.1 cm collectively with individual segments ranging up to 0.7 cm. The specimen consists mostly of mucus. The endocervix is also represented and features squamous metaplasia with focal microglandular hyperplasia. No endometrium is available for evaluation.

EADjh
7-30-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS:

PHYSICIAN:

CLIN. DX: Biopsy date: May 09, 1986. Control (baseline) biopsy for PARFR's NET-90 Study. Patient No. 02.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SP# W 291-86

SEX F

DATE 5-24-86

RACE

INS (LTS) C,CD23

BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 18.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 291-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures collectively 1.1 cm, with individual segments measuring up to 0.5 cm. Some of the endometrial segments appear to be from lower uterine segment. Otherwise, the endometrial glands show subnuclear vacuolization with some supranuclear vacuolization. The stroma is moderately cellular.

EADjh
5-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: July 14, 1986.
Day-63 PI

PHYSICIAN: PARFR's NET-90 Study.

CLIN. DX: Patient No. 02.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 503-86

SEX F DATE (LTS) 63d PI 7-29-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: EARLY PROLIFERATIVE ENDOMETRIUM.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 503-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.8 cm with individual segments ranging up to 0.2 cm. Several well preserved segments of endometrium are available for study. Tubular endometrial glands are uniformly separated by basophilic stroma. There is little palisading of nuclei but scattered mitoses can be seen in glands and stroma.

EADjh
7-30-86

NAME: PARFR NET90

ADDRESS:

PHYSICIAN:

CLIN. DX: Biopsy date: May 05, 1986. Control (baseline) biopsy for PARFR's NET-90 Study. Patient No. 03.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 277-86

SEX F DATE (M-O) C,CD22 5-24-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 24.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 277-86.

MICROSCOPIC DESCRIPTION:

An aggregate of endometrial segments measures 1.1 cm collectively and up to 0.6 cm individually. Much of the specimen consists of endocervical mucus, but there is an adequate representation of endometrium consisting of tortuous, secretory glands with basally located nuclei. Cuffing around spiral arterioles is moderately well marked.

EADjh
5-28-86

E. A. Bowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: July 11, 1986. Day-63 PI.

PHYSICIAN: PARFR'S NET-90 Study.

CLIN. DX: Patient No. 03.

DOB

AGE

SP# W 504-86 (MO) 63d PI

SEX F

DATE 7-29-86

RACE

INS BALMACEDA

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: LIMITED REPRESENTATION, COMPATIBLE WITH WEAK PROGESTATIONAL EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 504-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.7 cm and individually up to 0.1 cm. Several superficial strips of intact endometrium are available for study. The endometrial glands and simple in structure and appear secretorily exhausted. A mild degree of predecidual change is noted in the stroma.

EADjh
7-30-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: April 29, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 04.

DOB

AGE SP# W 255-86

SEX F DATE (OPC) C,CD23 5-5-86

RACE INS BALMACEDA

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 18.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 255-86.

MICROSCOPIC DESCRIPTION: The biopsy consists of an aggregate of segments measuring collectively 1.5 cm and, individually, up to 0.3 cm. The endometrial glands are slightly tortuous, show prominent subnuclea vacuolization and slight supranuclear vacuolization. The endometrial stroma is moderately cellular, with focal areas of edema.

EADjh
5-5-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: July 11, 1986
Day-63 PI.

PHYSICIAN: PARFR's NET-90 Study.

CLIN. DX: Patient No. 04.

DOB

AGE

SEX F

RACE

SP# W 505-86

DATE (OC) 63d PI 7-29-86

INS BALMACEDA

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: BASAL ENDOMETRIUM, SUGGESTIVE OF PROGESTATIONAL EFFECT, MINIMAL REPRESENTATION.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 505-86.

MICROSCOPIC DESCRIPTION: The biopsy consists of a single segment measuring 0.1 cm. This small segment of endometrium is from the basal zone and includes a small amount of adjacent myometrium. The glands are quite simple and may be secretorily exhausted. The stroma appears fibrous.

EADjh
7-30-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB W 236-86

ADDRESS:

AGE SP# (SG) C,CD22

PHYSICIAN: Biopsy date: April 22, 1986. Control (baseline) biopsy for PARFR's NET-90 Study. Patient No. 5.

SEX F DATE 4-25-86

CLIN. DX:

RACE INS BALMACEDA

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 236-86.

MICROSCOPIC DESCRIPTION: The biopsy consists of multiple segments measuring in aggregate 1.0 cm and, individually up to 0.3 cm in length. Several of the biopsy segments are endocervical. The endometrium is secretory, characterized by moderately tortuous glands with basal nuclei and luminal secretion. The stroma is edematous and the spiral arterioles are readily identified, with early stromal cuffing.

EADjh
4-28-86

E. A. Dowling, M.D.

UNIVERSITY of SOUTH ALABAMA

COLLEGE OF MEDICINE
ELECTRON MICROSCOPY CENTER

MOBILE, ALABAMA 36688
TEL. 205/460-6258

June 27, 1986

Dr. Jose Balmaceda
Department of OB/GYN
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, Texas 78284

Dear Dr. Balmaceda:

I received an endometrial biopsy from you yesterday that was taken on 06-24-86 from SG (Patient No. 05) for PARFR's Poly NET-90 Microsphere Study. The biopsy was numbered W-421-86 and was obtained on Day-63 post-injection.

Unfortunately, there was no endometrial tissue in the biopsy material. Recuts of biopsy material revealed only additional blood and endocervical mucus. I could not find even a fragment of endometrium to evaluate.

This letter is for your records, and will serve as the only report on the biopsy from SG at Day-63 post-injection.

Please let me know if you have any questions.

Sincerely yours,

Walter Wilborn

Walter H. Wilborn, Ph.D.
Professor of Anatomy;
Director, EM Center

NAME: PARFR NET90

ADDRESS: Biopsy date: February 7, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 06.

DOB

AGE

SP#: W 71-86

SEX: F

DATE: (MC) C,CD22 2-18-86

RACE

INS: BALMACEDA

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY:

a) SECRETORY ENDOMETRIUM, DAY 23.

b) SMALL FRAGMENTS OF CERVICAL EPITHELIUM SHOWING SQUAMOUS METAPLASIA.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 71-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial fragments measures 1.1 cm collectively and, individually, up to 0.4 cm. Multiple small segments of endometrial glands and stroma are available for study. The glands are tortuous with luminal secretion. The stroma is moderately edematous with beginning decidualization of the stroma around the spiral arterioles. A few tiny strips of endocervical epithelium are also visualized, featuring squamous metaplasia.

EADjh
2-18-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: May 20, 1986.

PHYSICIAN: Patient No. 06.

CLIN. DX: Day-63 post-injection.

DOB W 312-86

AGE SP# (M-C) 63d PI

SEX F DATE 5-28-86

RACE INS BALMACEDA

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY EXHAUSTION COMPATIBLE WITH PROGESTATIONAL EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 312-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 1.2 cm, with individual segments ranging up to 0.2 cm. The endometrial glands are small and simple, featuring secretory exhaustion. The stroma is mildly cellular, even somewhat spindly in some areas. The vasculature of the endometrial stroma is not prominent. A few strips of transformation zone are present as well as endocervical mucosa. There is a mild degree of squamous metaplasia.

EADjh
5-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: March 06, 1986.
Control (baseline) biopsy
PHYSICIAN: for PARFR's NET-90 Study.

CLIN. DX: Patient No. 07.

ISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 136-86

SEX F DATE (SM) C,CD22 3-13-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY
ENDOMETRIUM, DAY 22.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 136-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures up to 1.5 cm, with individual segments measuring 0.3 cm. The biopsy features considerable technical artifact, suggesting a lack of prompt fixation, or immersion in saline. The glands are tortuous, with nuclei which are basally located. Secretion cannot be observed in the lumen in many of the glands possibly due to artifact. Wispy secretion is noted focally. Some supranuclear vacuolization is noted, but is difficult to discriminate from artifact. The stroma is markedly edematous; once again artifact makes precise evaluation somewhat difficult.

EADjh
3-14-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB W 290-86

AGE SP# (S-M) 61d PI

SEX F DATE 5-24-86

RACE INS BALMACEDA

ADDRESS: Biopsy date: May 13, 1986.
Patient No. 07.

PHYSICIAN: Day-61 post-injection.

CLIN. DX:

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY EXHAUSTION COMPATIBLE WITH PROGESTATIONAL DRUG EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 290-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures 1.1 cm, with individual segments measuring up to 0.3 cm. The endometrial glands are small, simple, featuring secretory exhaustion. The stroma appears somewhat edematous, spiral arterioles are not conspicuous, but there are many small, dilated capillaries.

EADjh
5-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB W 111-86

AGE SP# (MP) C,CD21

SEX F DATE 3-11-86

ADDRESS: Biopsy date: March 04, 1986.
Baseline (Control) biopsy for PARFR's NET-90 Study.

PHYSICIAN:

RACE INS BALMACEDA

CLIN. DX: Patient No. 08.

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23.

TISSUE REMOVED:

2 SLIDES

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 111-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures up to 1 cm, with individual segments measuring up to 0.3 cm. There is an adequate representation of secretory endometrium. The glands have basal nuclei and a small amount of luminal secretion. The stroma is moderately edematous and the spiral arterioles are prominent, without fully developed cuffing. This is consistent with early day 23.

EADjh
3-11-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: May 13, 1986.
Day-63 post-injection.

PHYSICIAN: Patient No. 08.

CLIN. DX:

TISSUE REMOVED:

2 SLIDES

DOB W 313-86

AGE SP# (M-P) 63d PI

SEX F DATE 5-28-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: MILD SECRETORY EXHAUSTION, COMPATIBLE WITH PROGESTATIONAL EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 313-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure 2.5 cm in aggregate, with individual segments ranging up to 0.4 cm. Much of the specimen consists of mucus and blood clot. The endometrial glands are small and simple, featuring secretory exhaustion. The stroma is mildly cellular with no prominence of the vasculature.

EADjh
5-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: February 14, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 09.

TISSUE REMOVED:

1 SLIDE

DOB W 86-86

AGE SP# (RC) C,CD23

SEX F DATE 2-20-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY:
a) SECRETORY ENDOMETRIUM, DAY 23.
b) A FEW FRAGMENTS OF BENIGN ENDOCERVICAL MUCOSA.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of one slide bearing PARFR NET90 accession number W 86-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures 0.7 cm, with individual segments ranging up to 0.2 cm. The glands are moderately tortuous and contain a watery secretion in the lumen. The nuclei are basal. The stroma is moderately edematous and decidual cuffing is beginning around the spiral arterioles. A few small fragments of benign endocervical mucosa and mucus are also present.

EADjh
2-20-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: April 18, 1986.
Patient No. 9.

PHYSICIAN: Day-63 post-injection.

CLIN. DX:

TISSUE REMOVED:

2 SLIDES

DOB W 237-86

AGE SP# (RLC) 63 d PI

SEX F DATE 4-25-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: PROLIFERATIVE ENDOMETRIUM.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 237-86.

MICROSCOPIC DESCRIPTION: The endometrial biopsy consists of segments which measure 1.1 cm collectively and up to 0.1 cm individually. The specimen consists of blood clot and mucus with multiple tiny segments of endometrial glands and stroma. A number of the endometrial glands are tubular with a moderate degree of palisading of nuclei as well as mitotic activity. The surrounding stroma available for study is limited but appears moderately cellular with multiple small blood vessels featuring prominent endothelium.

EADjh
4-28-86

E. A. Dowling, M.D.

127

NAME: PARFR NET90

ADDRESS: Biopsy date: February 7, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 10.

TISSUE REMOVED:

1 SLIDE

DOB W 70-86

AGE SP# (LV) C,CD23

SEX F DATE 2-18-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 24.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of one slide bearing PARFR NET90 accession number W 70-86.

MICROSCOPIC DESCRIPTION: Multiple segments of endometrium measure collectively 1.6 cm and individually, up to 0.5 cm. Multiple segments of well preserved endometrium are available for study. The glands are tortuous, with luminal secretion. The stroma features a significant degre of decidualization, both in a periarteriolar distribution, but also more diffusely throughout the stroma without, however, a distinct compact layer.

EADjh
2-18-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: April 18, 1986.
Patient No. 10.
Day-63 post-injection.

PHYSICIAN:

CLIN. DX:

ISSUE REMOVED:

2 SLIDES

DOB

AGE

SP# W 238-86

SEX F

DATE (LMV) 63 d PI 4-25-86

RACE

INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SHEDDING OR MENSTRUAL ENDOMETRIUM FEATURING PROGESTATIONAL EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 238-86.

MICROSCOPIC DESCRIPTION: The endometrial segments measure in aggregate 1.1 cm with individual segments ranging up to 0.2 cm. Most of the specimen consists of blood clot. The endometrium is represented by a few small fragments in which stromal crumbling is in evidence, indicative of early shedding. The glands are simple and widely spaced in a cellular stroma which features early decidual changes as well as infiltration by many leukocytes.

EADjh
4-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: February 4, 1986

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 11.

TISSUE REMOVED:

2 SLIDES

DOB W 57-86

AGE SP# (RG) BALMACEDA

SEX DATE 2-13-86

RACE INS C,D24

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 19.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 57-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial fragments measures 0.7 cm collectively and, individually up to 0.3 cm. Many of the pieces are from the lower uterine segment, but there are two fragmen which are representative of the functional endometrium. The glands are moderately tortuous with variable subnuclear vacuolization. In some of the glands there is a slight amount of secretion. The stroma is markedly edematous.

EADjh
2-13-86

E. A. Dowling, M.D.

ME: PARFR NET90

DRESS: Biopsy date: April 18, 1986.
Patient No. 11.
'SICIAN: Day-63 post-injection.

:LIN. DX:

'ISSUE REMOVED:

2 SLIDES

DOB
AGE
SEX F
RACE

SP# W 239-86
(RG) 63 d PI
DATE 4-25-86
INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SUPPRESSED ENDOMETRIUM COMPATIBLE WITH PROGESTATIONAL EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 239-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 1.1 cm, with individual segments ranging up to 0.4 cm. Multiple small segments of endometrium are intermingled with some mucus. The endometrial stroma features numerous small empty spaces, possibly a freezing artifact. A number of the tissue segments prove to be endocervical. The endometrial glands are small and simple, widely spaced in a moderately cellular stroma in which spiral arterioles are difficult to identify. There are no venous lakes.

EADjh
4-28-86

E. A. Dowling, M.D.

NAME: PROTOCOL PARFR NET90 (BALMACEDA)

ADDRESS:

PHYSICIAN:

CLIN. DX: Biopsy date: January 28, 1986
Control (baseline) biopsy for PARFR's NET-90 Study.
Patient No. 12.

TISSUE REMOVED:

1 SLIDE

DOB W 43-86

AGE SP# (MS) C.D25

SEX F DATE 2-3-86

RACE INS

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: PROBABLE SECRETORY ENDOMETRIUM FROM LOWER UTERINE SEGMENT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

The specimen consists of one slide bearing number W 43-86.

FROZEN SECTION DX:

Protocol PARFR NET 90 accession

MICROSCOPIC DESCRIPTION: An aggregate of endometrial fragments measures 1.2 cm and, individually, up to 0.4 cm. The specimen consists of several fragments of endometrium, interspersed with blood clot, cervical mucus and strips of endocervical epithelium, together with exfoliated squamou cells. For this reason, the biopsy is difficult to evaluate and it cannot be established that we are dealing with a normal day 25 endometrium. This is probably because the sample comes predominantly from the lower uterine segment. Individual endometrial glands appear to be secretory, especially those which are dissociated from the stroma.

EADjh
2-3-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: April 18, 1986.
Patient No. 12.
Day-63 post-injection.

PHYSICIAN:

CLIN. DX:

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 240-86

SEX F DATE (MS) 63 d PI 4-25-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)
ENDOMETRIAL BIOPSY: SUPPRESSED ENDOMETRIUM COMPATIBLE WITH PROGESTATIONAL EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 240-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 1.2 cm, with individual segments ranging up to 0.3 cm. The biopsy consists partly of mucus and blood clot, and partly of tiny strips of endometrial epithelium, individual glands dissociated from stroma, and a few segments in which glands and stroma are seen in appropriate relationship. The endometrial glands consist of a ring of epithelial cells which present very little cytoplasm and no mitotic activity. The intervening stroma is moderately cellular, without decidualization.

EADjh
4-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: February 7, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 13.

TISSUE REMOVED:

1 SLIDE

DOB W 69-86

AGE SP# (DA) C,CD20

SEX F DATE 2-18-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of one slide bearing PARFR NET90 accession number W 69-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures 0.9 cm with individual segments measuring up to 0.1 cm. Approximately 40% of the specimen consists of mucus and blood clot. The endometrial glands are tortuous with a moderate amount of luminal secretion. The stroma shows a moderate degree of prominence of the spiral arterioles with incipient periarteriolar cuffing. Stromal edema is only slight.

EADjh
2-18-86

E. A. Dowling, M.D.

ME: PARFR NET90

DOB

AGE

SP# W 241-86

ADDRESS: Biopsy date: April 18, 1986.
Patient No. 13.
Day-63 post-injection.

SEX F

DATE (DA) 63 d PI 4-25-86

PHYSICIAN:

RACE

INS BALMACEDA

CLIN. DX:

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SUPPRESSED ENDOMETRIUM, COMPATIBLE WITH PROGESTATIONAL EFFECT.

TISSUE REMOVED:

2 SLIDES

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 241-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 1.3 cm, with individual segments ranging up to 0.2 cm. Much of the biopsy consists of mucus and blood clot. However, there are several segments of endometrial glands and stroma available for study. These are superficial strips featuring widely spaced, simple endometrial glands without evident secretion or mitotic activity. The stroma is moderately cellular but cannot convincingly be recognized as presenting decidual features. Spiral arterioles are inconspicuous. There are no venous lakes.

EADjh
4-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: February 5, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 14.

TISSUE REMOVED:

2 SLIDES

DOB W 67-86

AGE SP# (CA) C,CD24

SEX F DATE 2-18-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: LATE SECRETORY ENDOMETRIUM.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 67-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures up to 1.1 cm, and individual segments range up to 0.2 cm. There is considerable shattering and contraction artifact, as well as hemorrhage in the numerous small endometrial segments. The glands are of secretory quality, quite tortuous, but the relationship to stroma is not well shown because of fixation artifact. Nevertheless, we are dealing with a late secretory endometrium.

EADjh
2-18-86

E. A. Dowling, M.D.

Interstate 2

ME: PARFR NET90

DRESS:

YSICIAN:

LIN. DX:

Biopsy date: April 18, 1986.
Patient No. 14.
Day-63 post-injection.

DOB

AGE

SP# W 242-86

SEX F

DATE (CA) 63 d PI 4-25-86

RACE

INS BALMACEDA

ISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: INSUFFICIENT FOR DIAGNOSIS.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 242-86.

MICROSCOPIC DESCRIPTION: Biopsy segments measure 0.8 cm in aggregate and, individually, up to 0.4 cm. The specimen consists mostly of endocervical mucus together with a few strips of endocervical mucosa and a few tiny segments of endometrial epithelium and stroma. The endometrium is insufficient for diagnosis.

EADjh
4-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90 Subject's initials: LO

ADDRESS: Biopsy date: January 31, 1986

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 15.

TISSUE REMOVED:

1 SLIDE

DOB W 54-86

AGE SP# (LC) BALMACEDA

SEX F DATE 2-13-86

RACE INS C,D24

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 18.

Initia
be LO.
Correc
WHW.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of one slide bearing PARFR NET90 accession number W 54-86.

MICROSCOPIC DESCRIPTION: Segments of endometrium measure up to 0.7 cm on the slide and individually up to 0.2 cm. The endometrial glands show strong, subnuclear vacuolization and a mild degree of supranuclear cytoplasmic vacuolization, without luminal secretion. The stroma is cellula with no significant edema.

EADjh
2-13-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: April 15, 1986.
Patient No. 15.

PHYSICIAN: Day-63 PI.

CLIN. DX:

TISSUE REMOVED:

2 SLIDES

DOB W 224-86
AGE SP# (LO) 63 d PI
SEX F DATE 4-22-86
RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)
ENDOMETRIAL BIOPSY:
a) SUPPRESSED ENDOMETRIUM COMPATIBLE WITH PROGESTATIONAL EFFECT (MINIMAL REPRESENTATION.
b) FOCAL SURFACE METAPLASIA AND STROMAL CRUMBLING SUGGESTIVE OF EARLY MENSTRUATION.
c) FRAGMENTS OF STRATIFIED SQUAMOUS EPITHELIUM AND ENDOCERVICAL MUCOSA.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 224-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures 0.8 cm, with individual segments ranging up to 0.2 cm. The biopsy consists of an admixture of mucus, endocervical mucosa, strips of squamous epithelium and a minimal representation of the endometrium itself. A few of the endometrial segments show stromal collapse suggestive of early menstruation. Overlying one of the segments of collapsed endometrial stroma, the surface epithelium is metaplastic and features several enlarged hyperchromatic nuclei. The endometrium proper is minimal for definitive evaluation, but appears to represent suppression compatible with progestational effect.

EADjh
4-22-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: February 4, 1986

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 16.

TISSUE REMOVED:

2 SLIDES

COB W 56-86

AGE SP# (DJ) BALMACEDA

SEX F DATE 2-13-86

RACE INS C,D24

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: PROLIFERATIVE ENDOMETRIUM, INAPPROPRIATE FOR THE STATED DAY OF THE CYCLE (DAY 24).

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 56-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial fragments measure 1 cm on the slide and, individually up to 0.4 cm. There is an admixture of endocervical mucus, blood clot and well defined endometrial fragments. The endometrial glands are tubular with considerable palisading of nuclei, focal dilatation of the lumen and mitotic activity. The stroma is cellular and also features readily identified mitoses.

EADjh
2-13-86

E. A. Dowling, M.D.

NAME: PARFR NET 90

DOB W 225-86

AGE SP# (DJ) 63 d PI

SEX F DATE 4-22-86

RACE INS BALMACEDA

ADDRESS: Biopsy date: April 15, 1986.
Patient No. 16.
Day-63 PI.

PHYSICIAN:

CLIN. DX:

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: MINIMAL REPRESENTATION OF ENDOMETRIUM SHOWING SUPPRESSION COMPATIBLE WITH PROGESTATIONAL EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 225-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures 0.7 cm collectively and individually, up to 0.3 cm. Microscopically we are dealing with an admixture of mucus, blood clot, endocervical mucosa and a few tiny strips of endometrium. Endometrium is insufficient for definitive evaluation but appears to consist of suppressed endometrial glands in a moderately cellular stroma.

EADjh
4-22-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: February 5, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 17.

TISSUE REMOVED:

1 SLIDE

DOB W 68-86
AGE SP# (TS) C,CD24
SEX F DATE 2-18-86
RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 24.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of one slide bearing PARFR NET90 accession number W 68-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures collectively 0.7 cm with individual segments ranging up to 0.3 cm. Approximately 50% of the biopsy consists of endocervical mucu and blood clot. There are also segments of normal appearing endocervical mucosa. The endometrial tissue is in the secretory phase with glands which are tortuous, featuring luminal secretion. The spiral arterioles are prominent and show perivascular cuffing with decidualized stroma.

EADjh
2-18-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB W 226-86

AGE SP# (TS) 63 d PI

SEX F DATE 4-22-86

RACE INS BALMACEDA

ADDRESS: Biopsy date: April 15, 1986.
Patient No. 17.

PHYSICIAN: Day-63 PI.

CLIN. DX:

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: MILD SUPPRESSION, SUGGESTIVE OF PROGESTATIONAL EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 226-86.

MICROSCOPIC DESCRIPTION: The aggregate of endometrial segments measures 0.7 cm, with individual segments measuring up to 0.2 cm. Small tubular endometrial glands are uniformly distributed in an endometrial stroma which is only moderately cellular, the constituent stromal cells exhibiting relatively little cytoplasm. The glands are lined by a single layer of epithelium with no evidence of palisading and only a rare mitotic figure.

EADjh
4-22-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: January 31, 1986

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 18.

TISSUE REMOVED:

2 SLIDES

DOB W 55-86

AGE SP# (CM) BALMACEDA

SEX F DATE 2-13-86

RACE INS C,D24

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 24.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 55-86.

MICROSCOPIC DESCRIPTION: An endometrial biopsy presents an aggregate of fragments of tissue measuring 1.2 cm. The individual fragments range up to 0.4 cm. There is an admixture of endometrium, endocervical mucus, and a few fragments of normal endocervical mucosa. The endometrial glands are tortuous and filled with pale staining secretion. The stroma i focally edematous. In other areas, the spiral arterioles are prominent and are surrounded by a cuff of developing decidua. There is a tendency for the decidualized stroma to become confluent, focally.

EADjh
2-13-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS:

PHYSICIAN:

CLIN. DX: Patient Number 18.
Biopsy date: April 08, 1986.
Day-63 post-injection.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 204-86

SEX F DATE CMK.63 d PI 4-15-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY EXHAUSTION COMPATIBLE WITH PROGESTATIONAL DRUG EFFECT (MINIMAL REPRESENTATION).

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 204-86.

MICROSCOPIC DESCRIPTION: Segments of endometrium measure collectively 0.8 cm, with individual segments measuring up to 0.2 cm. Most of the specimen consists of blood clot and mucus. A few small segments of intact endometrium are found. In such areas the glands are small and simple, spaced by abundant moderately cellular stroma without evident decidualization. Elsewhere the endometrium is represented only by individual glands and surface strips of epithelium.

EADjh
4-16-86

E. A. Dowling, M.D.

NAME: PROTOCOL PARFR NET90 (BALMACEDA)

ADDRESS:

PHYSICIAN: Biopsy date: January 24, 1986

CLIN. DX: Control (baseline) biopsy for PARFR's NET-90 Study. Patient No. 19.

TISSUE REMOVED:

1 SLIDE

DOB

AGE SP# W 41-86

SEX F DATE CM.C, D-21 2-3-86

RACE INS

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, APPROXIMATELY DAY 22.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

The specimen consists of one slide bearing number W 41-86.

FROZEN SECTION DX:

Protocol PARFR NET90 accession

MICROSCOPIC DESCRIPTION: The specimen consists of an aggregate of endometrial fragments measuring 0.7 cm and, individually, up to 0.3 cm. Some of the specimen consists only of endocervical mucus and benign appearing endocervical mucosal cells. The endometrium is well represented in two of the fragments. The glands are secretory and tortuous, lying in an edematous endometrial stroma. Spiral arterioles are not prominent, but the sample is too limited to make such an observation reliable. There is no evidence of subnuclear vacuolization at this time.

EADjh
2-3-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: April 08, 1986.

PHYSICIAN: Patient No. 19.
Biopsy date: April 08, 1986.

CLIN. DX: Day-63 post-injection.

DOB

AGE SP# W 202-86

SEX F DATE CM.63 d PI 4-15-86

RACE INS BALMACEDA

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SMALL FRAGMENTS OF ENDOMETRIUM, INSUFFICIENT FOR DEFINITIVE EVALUATION.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 202-86.

MICROSCOPIC DESCRIPTION: Multiple segments from the endometrium measure in aggregate 1.3 cm, with individual segments ranging up to 0.3 cm. The biopsy consists of blood clot, mucus, a fragment of stratified squamous epithelium, a fragment of myometrium, a few tiny segments of endometrial glands and stroma, and isolated endometrial glands, together with strips of surface epithelium. The small segments of intact endometrium show predominantly stroma which is moderately cellular, without convincing evidence of decidualization. The few glands seen in these segments are simple, tubular, possibly representing secretory exhaustion. The sample is too small for definitive evaluation.

EADjh
4-15-86

E. A. Dowling, M.D.

NAME: PROTOCOL PARFR NET 90 (BALMACEDA)

ADDRESS: Biopsy date: January 28, 1986

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 20.

TISSUE REMOVED:

1 SLIDE

DOB

AGE SP# W 42-86

SEX F DATE (LV) C.D21 2-3-86

RACE INS

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 19.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

The specimen consists of one slide bearing number W 42-86.

FROZEN SECTION DX:

Protocol PARFR NET 90 accession

MICROSCOPIC DESCRIPTION: An aggregate of endometrial fragments measures 0.9 cm and, individually, up to 0.4 cm. Multiple small endometrial fragments represent an adequate biopsy of the endometrium. The glands are secretory, with a residual feature of subnuclear vacuolization. The stroma is moderately edematous and spiral arterioles are readily identified, but show no perivascular cuffing.

EADjh
2-3-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS:

PHYSICIAN: Patient No. 20.

CLIN. DX: Biopsy date: April 08, 1986. Day-63 post-injection.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 203-86

SEX F DATE LV.63 d PI 4-15-86

RACE INS BALMACEDA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY EXHAUSTION COMPATIBLE WITH PROGESTATIONAL DRUG EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 203-86.

MICROSCOPIC DESCRIPTION: Segments of endometrium measure in aggregate 0.7 cm, with individual segments measuring up to 0.3 cm. The sample of endometrium is quite adequate for evaluation. Endometrial glands are widely separated by a moderately cellular endometrial stroma. Decidualization of the stroma is not prominent. The individual glands are very small and simple, presenting a pattern of secretory exhaustion. The spiral arterioles are inconspicuous. No venous lakes are noted.

EADjh
4-16-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: February 14, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 1.

TISSUE REMOVED:

1 SLIDE

DOB

AGE

SP#

SEX F

DATE

RACE

INS

W 77-86
(JAP) ARCHER
2-19-86

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 19.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of one slide bearing PARFR NET90 accession number W 77-86.

MICROSCOPIC DESCRIPTION: Multiple segments of endometrium measure up to 0.9 cm and, individually up to 0.3 cm. The endometrial glands show distinct subnuclear vacuolization, moderate tortuosity. some luminal secretion and focal supranuclear vacuolization. The stroma is focally edematous.

EADjh
2-19-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: August 19, 1986.
PARFR's NET-90 Study.

PHYSICIAN: Patient No. 01.

CLIN. DX: Day-93 Post-injection.
Injection No. 2.
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SEX F

RACE

SP# W 556-86 (JAP) 93d PI

DATE 8-26-86

INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: APPARENT SHEDDING AND EARLY PROLIFERATIVE CHANGE (MINIMAL REPRESENTATION).

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 556-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.4 cm with no segment measuring as much as 0.1 cm. Fifty percent of the specimen consists of endocervical cells and mucus. The remaining endometrial segments show some stromal crumbling and stromal collapse. The endometrial glands are distorted, but feature regenerative change in the form of mitotic activity.

EADjh
8-26-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB

AGE

SP#

SEX F

DATE

RACE

INS

W 102-86
(MAC) Archer
3-4-86

ADDRESS: Biopsy date: February 24, 1986.
Baseline biopsy--Control Biopsy--
for PARFR's NET-90 Study.

PHYSICIAN:

CLIN. DX: Patient No. 02.

TISSUE REMOVED:
1 SLIDE

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of one slide bearing PARFR NET90 accession number W 102-86.

MICROSCOPIC DESCRIPTION: The endometrial segments measure 0.7 cm in aggregate and, individually, up to 0.3 cm. The endometrial glands are tortuous, with basal nuclei and a slight amount of luminal secretion in many of the glands. The stroma is edematous and there is beginning predecidual cuffing around spiral arterioles.

EADjh
3-4-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB

AGE

SP#: W 586-86

ADDRESS: Biopsy date: August 28, 1986.
Day-90 PI.

SEX: F

DATE: (MAC) 90d PI 9-4-86

PHYSICIAN: PARFR's NET-90 Study
Patient No. 02.

RACE

INS: ARCHER

CLIN. DX: Injection No. 2.
About 6 months T M with NET.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: WEAK, PROLIFERATIVE EFFECT, POSSIBLY ESTROGEN EFFECT MODIFIED BY A PROGESTATIONAL AGENT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 586-86.

MICROSCOPIC DESCRIPTION: The endometrial biopsy consists of segments which measure collectively 0.7 cm and individually up to 0.1 cm. Several small segments of intact endometrium are available for study. The endometrial glands are uniformly distributed in a moderately cellular stroma. The constituent glands vary slightly in size, but for the most part are simple and tubular. The surface epithelium has many cilia, while the constituent glands show no evident mitotic activity.

EADjh
9-8-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: February 10, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 St udy.

CLIN. DX: Patient No. 3.

DOB

AGE

SEX F

RACE

SP#

DATE

INS

W 74-86

(RDB) ARCHER

2-19-86

TISSUE REMOVED:

1 SLIDE

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 25.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of one slide bearing PARFR NET90 accession number W 74-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures up to 0.8 cm, with individual segments measuring up to 0.2 cm. The endometrial glands are tortuous and secretory. The stroma shows prominence of spiral arterioles, with moderate decidual cuffing, and incipient formation of a compact zone.

EADjh
2-19-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB

AGE

SP#: W 601-86

ADDRESS: Biopsy date: September 05, 1986.
Day-89 PI.

SEX: F

DATE: (RDB) 89d PI 9-12-86

PHYSICIAN: PARFR's NET-90 Study.

RACE

INS: ARCHER

CLIN. DX: Patient No. 03.

Injection No. 2.
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: INSUFFICIENT FOR DIAGNOSIS.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 601-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.5 cm, and individually up to 0.1 cm. The specimen is inadequate for diagnosis, since it consists almost entirely of mucus and endocervical epithelium, with only a few individual endometrial glands noted.

EADjh
9-15-86

E. A. Dowling, M.D.

IE: PARFR NET90

DRESS: Biopsy date: March 12, 1986.
Control (baseline) biopsy
SICIAN: for PARFR's NET-90 Study.

N. DX: Patient No. 04.

SUE REMOVED:

2 SLIDES

DOB W 142-86
AGE SF# (SMK) C,CD23
SEX F DATE 3-20-86
RACE INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 142-86.

MICROSCOPIC DESCRIPTION: The endometrial segments measure collectively 0.3 cm, with individual segments measuring 0.1 cm. The specimen is difficult to evaluate precisely, since surface epithelium is not visualized. However, the glands are moderately tortuous with basal nuclei and luminal secretion. Where spiral arterioles can be identified, there is a suggestion of early perivascular cuffing.

EADjh
3-21-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: September 15, 1986
PARFR NET-90 Study

PHYSICIAN: 94-days PI

CLIN. DX: Patient No. 04.

Injection No. 2.
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SEX F

RACE

W 636-86

SPO (SMK) 94d PI

DATE 9-22-86

INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: WEAKLY PROLIFERATIVE ENDOMETRIUM.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 636-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.5 cm and, individually up to 0.3 cm. Several small segments of intact endometrium are available for study. Most of the endometrial glands are small and tubular, uniformly distributed in a spindle stroma. An occasional endometrial gland is cystically dilated. The glands are lined by a single layer of cells. There are a few, small, subnuclear vacuoles and occasional mitoses.

EADjh
9-22-86

E. A. Dowling, M.D.

AME: PARFR NET90

DDRESS: Biopsy date: March 24, 1986.
Control (baseline) biopsy for PARFR's
HYSICIAN: ARCHER NET-90 Study. Repeat biopsy.

LIN. DX: Patient No. 05.

ISSUE REMOVED:
2 SLIDES

DOB
AGE SP#
SEX F DATE
RACE INS

W 170-86
(EJ) C,CD23
4-1-86

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)
ENDOMETRIAL BIOPSY: SECRETORY
ENDOMETRIUM, DAY 19.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PAR R NET90 accession number W 170-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures 0.9 cm collectively and, individually, up to 0. cm. Several of the segments consist only of endocervical mucosa. The endometrial glands are secretory with well developed subnuclear vacuolization and a slight amount of luminal secretion. The stroma is edematous in a patchy manner. In some of the glands the nuclei have resumed a basal location.

EADjh
4-2-86

E. A. Dowling, M.D.

NAME: PARFR NET90 Subject's Initials: EJ DOB W 680-86
AGE SP# (EV) 92d PI
ADDRESS: Biopsy date: September 26, 1986. Day-92 PI. SEX F DATE 10-6-86
PHYSICIAN: PARFR's NET-90 study. RACE INS ARCHER
CLIN. DX: Patient No. Not Given, but found to be No. 05. Injection No. 2 . About 6 months TM with NET.
TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: PROLIFERATIVE ENDOMETRIUM.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 680-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.5 cm and individually up to 0.3 cm. Multiple small segments of intact endometrium are available for study. Small tubular glands are uniformly spaced in a spindle, edematous stroma. The constituent glands show active mitosis. Mitotic activity is also identified in the stroma.

EADjh
10-7-86

E. A. Dowling, M.D.

ME: PARFR NET90

DRESS:

YSICIAN:

N. DX: Biopsy date: April 09, 1986. Control (baseline) biopsy for PARFR's NET-90 Study. Patient No. 6.

SUE REMOVED:

2 SLIDES

DOB

AGE SP# W 209-86

SEX F DATE (CLG) C,CD22 4-15-86

RACE INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: CHANGES COMPATIBLE WITH OVULATION SUPER-IMPOSED UPON AN ANOVULATORY CYCLE.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 209-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures 0.8 cm collectively, with individual segments measuring up to 0.2 cm. The endometrial glands are variably distributed throughout stroma which exhibits only a slight degree of edema. A number of the glands are cystically dilated, while ciliation is a prominent feature on the luminal aspect. However, the glands do exhibit some degree of tortuosity and the nuclei, while predominantly basal, also in some of the glands, feature subnuclear vacuolization. This does not appear to be a normal secretory endometrium and suggests the possibility of ovulation superimposed upon an anovulatory cycle.

EADjh
4-16-86

E.A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: October 13, 1986.
Day-90 PI.

PHYSICIAN: PARFR's NET-90 Study.
Patient No. 06.

CLIN. DX: Injection No. 2
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

DOC W 718-86

AGE SP# (CLG) 92d PI

SEX F DATE 10-17-86

RACE INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY EXHAUSTION COMPATIBLE WITH PROGESTATIONAL DRUG EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 718-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.6 cm and, individually, up to 0.1 cm. Small, simple glands lie in a moderately cellular stroma and exhibit secretory exhaustion.

EADjh
10-21-86

E. A. Dowling, M.D.

AME: PARFR NET90

DDRESS: Biopsy date: April 25, 1986. Control (baseline) biopsy for PARFR's NET-90 Study.

HYSICIAN:

LIN. DX:

Patient No. 07.

ISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 250-86

SEX F DATE (BJS) C,CD23 4-30-86

RACE INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 250-86.

MICROSCOPIC DESCRIPTION: The endometrial biopsy consists of an aggregate of segments which measure collectively up to 0.8 cm and, individually, up to 0.2 cm. The endometrial glands are tortuous and convoluted, with only a small amount of luminal secretion in some of the glands. The nuclei are basally located and there is supranuclear vacuolization. The endometrial stroma is moderately cellular, spiral arterioles are prominent, with a surrounding cuff of cellular stroma.

EADjh
5-1-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: October 24, 1986.
Day-90 PI.

PHYSICIAN: PARFR's NET-90 Study.

CLIN. DX: Patient No. 007.
Injection No. 2.
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SP#: W 761-86

SEX: F

DATE: (BJS) 90dPI 10-31-86

RACE

INS: ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: INSUFFICIENT FOR DEFINITIVE DIAGNOSIS, INACTIVE EPITHELIUM, OR POSSIBLY ATROPHIC EPITHELIUM (SEE COMMENT BELOW).

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 761-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.7 cm and individually up to 0.3 cm. Most of the specimen consists only of mucus. The endometrium is represented only by strips of surface epithelium, individual glands, and one minute segment of surface epithelium with associated stroma. While the specimen is insufficient for diagnosis, the constituent glands and surface epithelium appear atrophic, rather than suppressed, as by a progestational agent.

EADjh
11-3-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS:

PHYSICIAN:

CLIN. DX:

Biopsy date: May 07, 1986.
Control (baseline) biopsy for PARFR's NET 90 Study.

Patient No. 08.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SEX F

RACE

SP#

DATE

INS

W 281-86
(BEV) C,CD23
5-24-86
ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: INSUFFICIENT FOR DEFINITIVE EVALUATION, MOSTLY ENDOCERVICAL MUCOSA.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 281-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.6 cm, with individual segments measuring up to 0.3 cm. Only one tiny segment of endometrium is available for study, the remainder consisting of endocervical mucosa and mucus.

EADjh
5-28-86

E. A. Dowling, M.D.

NAME: PARFR NET 90

DOB

AGE

SP# W 799-86

ADDRESS: Biopsy date: November 04, 1986.
Day-92 PI.

SEX f

DATE (BEV) 92d PI 11-17-86

PHYSICIAN: PARFR's NET-90 Study.

RACE

INS ARCHER

CLIN. DX: Patient No. 008.
Injection No. 02.
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: FRAGMENTS OF SUPERFICIAL ENDOMETRIUM AND INDIVIDUAL GLANDS INSUFFICIENT FOR DEFINITIVE DIAGNOSIS.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 799-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 1.5 cm and, individually up to 0.4 cm. Most of the specimen consists of mucus and blood clot. The endometrium is represented only by individual glands, strips of surface epithelium and a few minute fragments in which glands and stroma are seen in normal relationship. In the latter areas the glands are tubular and do not show convincing secretory changes. The stroma is moderately cellular without evidence of predecidua.

EADjh
11-19-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB

AGE

SPO

SEX F

DATE 5-24-86

RACE

INS

W 280-86
(VL) C,CD26

ARCHER

ADDRESS: Biopsy date: May 07, 1986.
Control (baseline) biopsy for
PARFR's NET-90 Study.

PHYSICIAN:

CLIN. DX:

Patient No. 09.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY
ENDOMETRIUM, DAY 19.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 280-86.

MICROSCOPIC DESCRIPTION: Endometrial biopsy consists of two segments of tissue measuring collectively 0.5 cm. One of the segments appears to be from the lower uterine segment. The second segment features mildly tortuous glands with a moderate degree of subnuclear vacuolization and some luminal secretion. The glands are situated in a cellular endometrial stroma. However, the relationship to surface epithelium is not well shown, making precise phasing difficult.

EADjh
5-28-86

E. A. Dowling, M.D.

1/2 -108-1

NAME: PARFR NET90

DOB

AGE

SP# W 819-86

ADDRESS: Biopsy date: November 14, 1986.
Day 90 PI 2nd dose NET IM.

SEX F

DATE (VL) 90d PI 11-21-86

PHYSICIAN: Patient No. 009.
PARFR's NET-90 Study.

RACE

INS ARCHER

CLIN. DX: Injection No. 2.
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: APPARENT NON-SECRETORY ENDOMETRIUM, INSUFFICIENT FOR DEFINITIVE EVALUATION.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 819-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.7 cm and, individually up to 0.2 cm. Much of the specimen consists only of mucus. There are several small strips of endocervical mucosa, one of which shows squamous metaplasia. The representation of the endometrium is limited to two minute fragments. Only in one of these is there reasonable representation of endometrial glands which appear tubular, relatively simple, with no discernable mitotic activity. The stroma is composed of spindle cells without evidence of predecidua.

EADjh
11-21-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB W 325-86

AGE SPO (JC) C,CD23

SEX F DATE 6-2-86

RACE INS ARCHER

ADDRESS: Biopsy date: May 23, 1986. Control (baseline) biopsy for PARFR's NET-90 Study.

PHYSICIAN:

CLIN. DX:

Patient No. 10.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 24

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

MICROSCOPIC DESCRIPTION:

The endometrial segments measure collectively 1.0 cm, with individual segments ranging up to 0.3 cm. The endometrial glands are secretory and markedly tortuous. The stroma is moderately edematous and shows well defined cuffing around spiral arterioles.

EADtms
6-2-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB

AGE

SP#: W 865-86

ADDRESS: Biopsy date: December 01, 1986.
Day-93 PI.

SEX: F

DATE: (JC) 93d PI 12-5-86

PHYSICIAN: PARFR's NET-90 Study
Patient No. 010.

RACE

INS: ARCHER

CLIN. DX: Injection No. 2.
About 6 months TX with NET.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SUPPRESSED ENDOMETRIUM WITH MINIMAL EVIDENCE OF ESTROGEN ACTIVITY.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 865-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.5 cm and, individually up to 0.1 cm. Several minute segments of endometrium are available for study. A few segments of endocervical mucosa are intermingled with the endometrium. The endometrial glands vary from simple tubular type to somewhat larger mildly dilated structures. The constituent cells are arranged for the most part in a single layer and the luminal aspect is conspicuously ciliated in some of the glands. The endometrium has an atrophic and focally even fibrotic quality and features a number of dilated, thin walled venous channels.

EADjh
12-5-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS:

PHYSICIAN:

CLIN. DX:

Biopsy date: June 02, 1986.
Control (baseline) biopsy for PARFR's NET-90 Study.

Patient No. 12.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W347-86

SEX DATE (RN) C, CD24
6-9-86

RACE INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 25

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.8 cm, with individual segments ranging up 0.4 cm. The endometrial glands are tortuous and secretory, with basally located nuclei. They are surrounded by a stroma which shows advanced decidualization, with beginning evidence of a superficial, compact zone.

6-10-86
EADtms

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB

W 874-86

ADDRESS: Biopsy date: December 03, 1986.
Day-95 PI.

AGE

SP# (RKW) 95d PI

PHYSICIAN: PARFR's NET-90 Study.
Patient No. 12.

SEX F

DATE 12-9-86

CLIN. DX: Injection No. 02.
About 6 months TM with NET.

RACE

INS ARCHER

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: PROBABLE SECRETORY EXHAUSTION, MINIMAL REPRESENTATION.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 874-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 1.3 cm and individually up to 0.6 cm. Most of the specimen consists of mucus. There is also a segment of normal endocervical mucosa. The endometrium is represented only by a few minute segments of intact glands and stroma. In only one minute segment are glands and stroma seen in their normal relationship. These glands appear small and simple and present numerous epithelial snouts on the luminal aspect. The surrounding stroma is moderately cellular. No mitotic activity can be identified in the limited sample.

EADjh
12-10-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: June 04, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX:

Patient No. 13.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W353-86

SEX DATE (PSR) C, CD23

6-10-86

RACE INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

MICROSCOPIC DESCRIPTION:

The biopsy segments measure in aggregate 0.6 cm, with individual segments measuring up to 0.3 cm. Multiple intact segments of endometrium are available for study. Some appear to be from the lower uterine segment. Elsewhere, we are dealing with moderately tortuous, secretory endometrial glands with basally located nuclei. The stroma shows prominence of the spiral arterioles with stromal cuffing.

6-10-86
EADtms

E. A. Dowling, M.D.

1/2 -1C6-1

NAME: PARFR NET90

ADDRESS: Biopsy date: December 04, 1986.
Day-94 PI.

PHYSICIAN: PARFR's NET-90 Study.

CLIN. DX: Patient No. 13.
Injection No. 2.
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SEX F

RACE

SP# W 897-86

DATE (PSR) 94d PI 12-16-86

INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: MINIMAL PROGESTATIONAL EFFECT.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 897-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.8 cm and individually up to 0.2 cm. Multiple small segments of intact endometrium are available for study. The endometrial glands are simple, tubular, varying only slightly in size, and are without mitotic activity. In some of the glands there is a minimal degree of subnuclear vacuolization. The surface epithelium is pseudostratified columnar, with prominence of both cytoplasmic protrusions and cilia. The stroma is mildly edematous, the cells stellate, and a rare mitosis can be identified.

EADjh
12-16-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS:

PHYSICIAN:

CLIN. DX:

Biopsy date: June 10, 1986.
Control (baseline) biopsy
for PARFR's NET-90 Study.

Patient No. 14.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SP#

SEX F

DATE

RACE

INS

W 370-86
(JMR) C,CD24
6-16-86
ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY
ENDOMETRIUM, DAY 23.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 370-86.

MICROSCOPIC DESCRIPTION: The endometrial biopsy consists of segments of tissue measuring collectively 0.6 cm, and individually up to 0.2 cm. The biopsy features tortuous, secretory endometrial glands with basally located nuclei. The stroma is moderately edematous, but there early predecidual cuffing around the spiral arterioles.

EADjh
6-16-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: December 16, 1986.
Day 93 PI.

PHYSICIAN: PARFR's NET-90 Study.
Patient No. 14.

CLIN. DX:
Injection No. 2.
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SP# W 937-86 (JMR) 93d PI

SEX F

DATE 12-24-86

RACE

INS ARCHER

PATHOLOGIC DX.: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY EXHAUSTION COMPATIBLE WITH PROGESTATIONAL DRUG EFFECT, MINIMAL REPRESENTATION.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 937-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 1.1 cm and, individually up to 0.3 cm. Virtually all of the specimen consists of endocervical mucus. There are three small intact segments of endometrium in which the constituent glands are small, simple and secretorily exhausted. The stroma has an almost fibrous quality.

EADjh
12-29-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS:

PHYSICIAN:

CLIN. DX:

Biopsy date: June 11, 1986.
Control (baseline) biopsy for PARFR's NET-90 Study.

Patient No. 15.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP#

SEX F DATE

RACE INS

W 371-86
(JLT) C,CD23
6-16-86

ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, APPROXIMATELY DAY 18, WITH GLANDULO-STROMAL ASYNCHRONY.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 371-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.9 cm with individual segments ranging up to 0.2 cm. The endometrial glands are mildly tortuous and secretory, showing subnuclear vacuolization and a small amount of luminal secretion. Some of the segments of endometrium show an even more immature glandular secretory development, with tubular glands and only minimal subnuclear vacuolization. The stroma on the other hand, is more advanced, features edema, and some beginning predecidual cuffing around spiral arterioles.

EADjh
6-16-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB

AGE

SP# W 898-86

ADDRESS: Biopsy date: December 10, 1986.
Day-93 PI.

SEX F

DATE (JT) 93d PI 12-16-86

PHYSICIAN: PARFR's NET-90 Study.

RACE

INS ARCHER

CLIN. DX: Patient No. 15.
Injection No. 2.
About 6 months TM with NET.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: WEAKLY PROLIFERATIVE ENDOMETRIUM.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 898-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 1.2 cm and individually up to 0.3 cm. Most of the specimen consists of mucus and blood clot. Multiple very small segments of intact endometrium are available for study. The endometrial glands are simple, tubular with moderate numbers of mitoses. The stroma is quite edematous and the cells variably stellate or spindle. Mitoses can also be identified in the stroma rarely. There is no convincing progestational effect.

EADjh
12-16-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: June 30, 1986. Control (baseline) biopsy for

PHYSICIAN: PARFR's NET-90 Study.

CLIN. DX: Patient No. 16.

TISSUE REMOVED:

2 SLIDES

DOB W 443-86

AGE SPE (RMT) C,CD23

SEX F DATE 7-7-86

RACE INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 443-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.7 cm with one segment measuring as much as 0.6 cm. The endometrial glands are tortuous with abundant secretion in the lumen. The nuclei are basally located. The stroma features prominence of spiral arterioles with some residual stromal edema and early periarteriolar cuffing.

EADjh
7-9-86

E. A. Dowling, M.D.

NAME: PARFR NET90

DOB

AGE

SP#

W 422-86

(PER) C,CD22

SEX F

DATE 7-1-86

ADDRESS: Biopsy date: June 25, 1986. Control (baseline) biopsy for PARFR's NET-80 Study.

PHYSICIAN:

RACE

INS ARCHER

CLIN. DX: Patient No. 17.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23, WITH MODERATE GLANDULO-STROMAL ASYNCHRONY.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing Ortho Protocol accession number W 422-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 1.5 cm and, individually, up to 0.3 cm. The endometrial glands are mildly tortuous with only a trace of subnuclear vacuolization remaining. The stroma appears histologically more advanced, featuring edema, prominence of spiral arterioles and initial periarteriolar cuffing.

EADjh
7-2-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: July 03, 1986.
Control (baseline) biopsy for
PHYSICIAN: PARFR's NET-90 Study.

CLIN. DX: Patient No. 18.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 446-86

SEX F DATE (MAM) C,CD19 7-14-86

RACE INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: EARLY SECRETORY ENDOMETRIUM, DAY 16.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 446-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.8 cm with individual segments ranging up to 0.2 cm. Multiple small segments of endometrium are available for study, although surface epithelium is not well represented. The endometrial glands are mildly tortuous and show slight dilatation of the lumen in some instances. There is palisading of the nuclei and early subnuclear vacuolization. The endometrial glands and stroma still show a rare mitosis.

EADjh
7-14-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: August 01, 1986.
Control (baseline) biopsy for
PHYSICIAN: PARFR's NET-90 Study.

CLIN. DX: Patient No. 19.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 521-86

SEX F DATE (CAL) C,CD25 8-5-86

RACE INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 25, WITH SLIGHTLY ATYPICAL FEATURES (SEE COMMENT BELOW).

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 521-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.8 cm and individually up to 0.2 cm. Multiple small segments of endometrium are available for study. Some of these appear to be from the lower uterine segment and exhibit dilated, but secretory, glands in a more fibrous than average stroma. The remaining segments show marked tortuosity of the glands and prominent cytoplasmic snouts on the luminal aspect, associated with a watery secretion in the lumen. The spiral arterioles are prominent in some of the segments and there is beginning development of a compact, subepithelial zone.

EADjh
8-5-86

E. A. Dowling, M.D.

1/2-106-1

NAME: PARFR NET90

ADDRESS: Biopsy date: August 05, 1986.
Control (baseline) biopsy for
PHYSICIAN: PARFR's NET-90 Study.

CLIN. DX: Patient No. 20.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SP# W 530-86 (MJM) C,CD22

SEX F

DATE 8-11-86

RACE

INS ARCHER

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)
ENDOMETRIAL BIOPSY: SECRETORY
ENDOMETRIUM, DAY 22.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 530-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.8 cm and individually up to 0.4 cm. Multiple intact segments of endometrium are available for study. The glands are moderately tortuous, secretory, with basally located nuclei. The stroma is moderately cellular without cuffing of the spiral arterioles.

EADjh
8-12-86

E. A. Dowling, M.D.

^ME: PARFR NET90

DRESS: Biopsy date: March 06, 1986.
Control (baseline) biopsy
YSICIAN: for PARFR NET-90 Study.

LIN. DX: Patient No. 1.

DOB

AGE SP# W 132-86

SEX F DATE (HOC) C,CD24 3-11-86

RACE INS SAXENA

ISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, EARLY DAY 23.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 132-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures up to 0.8 cm, with individual segments ranging up to 0.3 cm. The endometrial glands are moderately tortuous, with a small amount of luminal secretion and basally situated nuclei. The stroma is markedly edematous, without distinct evidence of perivascular cuffing, although the process appears to be incipient.

EADjh
3-12-86

E. A. Dowling, M.D.

1/2-100-1

NAME: PARFR NET90

ADDRESS: Biopsy date: August 21, 1986.
Patient No. 01.

PHYSICIAN: PARFR's NET-90 Study.
Day-68 Post-injection.

CLIN. DX: Injection No. 2.
About 5 months TM with NET.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SP# W 563-86

SEX F

DATE (HOC) 68d PI 8-26-86

RACE

INS SAXENA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: PROLIFERATIVE ENDOMETRIUM, MINIMAL REPRESENTATION.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 563-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.4 cm with no segment measuring as much as 0.1 cm. Most of the specimen consists of blood clot. The endometrium is represented by tiny segments of tissue with prominent freezing artifact. The endometrial glands are tubular and feature mitotic activity.

EADjh
8-26-86

E. A. Dowling, M.D.

ME: PARFR NET90

DRESS: Biopsy date: March 19, 1986. Control (baseline) biopsy for PARFR NET-90 Study.

HYSICIAN:

LIN. DX: Patient No. 3.

SSUE REMOVED:

3 SLIDES

DOB W 164-86
AGE SP# (LIS) C,CD25
SEX F DATE 3-25-86
RACE INS SAXENA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)
ENDOMETRIAL BIOPSY: SMALL FRAGMENT OF SECRETORY ENDOMETRIUM, INSUFFICIENT FOR DEFINITIVE EVALUATION.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of three slides bearing PARFR NET90 accession number W 164-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures up to 0.7 cm with individual segments measuring up to 0.3 cm. The specimen consists predominantly of endocervical mucus with entrapped leukocytes and strips of normal endocervical epithelium. One tiny segment of endometrium is represented. This appears to be secretory but is insufficient for definitive evaluation.

EADjh
3-26-86

E. A. Dowling, M.D.

AME: PARFR NET90
DDRESS: Biopsy date: March 26, 1986. Control (baseline) biopsy for PARFR's NET-90 Study.
HYSICIAN: SAXENA
LIN. DX: Patient No. 4.

DOB
AGE SP#
SEX F DATE
RACE INS

W 172-86
(DOT) C,CD29
4-1-86

ISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: LATE SECRETORY ENDOMETRIUM WITH EARLY MENSTRUAL CHANGES.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 172-86.

MICROSCOPIC DESCRIPTION: An aggregate of tiny endometrial segments measures collectively 0.7 cm with no segment measuring as much as 0.1 cm. Much of the sample consists of mucus and blood clot. The endometrial segments are quite tiny and show considerable artifact in the form of dissociation of the stroma. Because of this artifact it is difficult to assign a precise date to the phase of what is evidently a secretory phase of the cycle. In all probability we are looking at early menstruation.

EADjh
4-2-86

E. A. Dowling, M.D.

1/2-106-1

NAME: PARFR NET90

DOB

AGE

SP#

W 600-86

ADDRESS: Biopsy date: September 08, 1986.
Day-64 PI.

SEX F

DATE

(RSB) 64dPI
9-12-86

PHYSICIAN: PARFR's NET-90 Study.

RACE

INS

SAXENA

CLIN. DX: Patient No. 04.
Injection No. 2.
About 5 months TM with NET.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: INSUFFICIENT FOR DIAGNOSIS.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 600-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure collectively 0.6 cm and individually, up to 0.1 cm. The specimen is inadequate for diagnosis since it consists almost entirely of mucus. The endometrium is represented only by a few glands, dissociated from stroma.

EADjh
9-15-86

E. A. Dowling, M.D.

AME: PARFR NET90
DDRESS: Biopsy date: March 28, 1986. Control (baseline) biopsy for Ortho's NET-90 Study. Patient No. 5.
HYSICIAN: SAXENA
LIN. DX:

DOB
AGE
SP#
SEX F
DATE
RACE
INS

W 184-86
(MGB) C,CD17
4-1-86

ISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 20.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 184-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures 1 cm collectively, with individual segments measuring up to 0.7 cm. Much of the specimen consists of endocervical mucus. The endometrial glands are moderately tortuous, with basally located nuclei. Secretory changes are difficult to discern in most of the glands, possibly because the tissue is from the lower uterine segment. The endometrial stroma is slightly edematous with inconspicuous spiral arterioles.

EADjh
4-2-86

E. A. Dowling, M.D.

AME: PARFR NET90

DRESS: Biopsy date: April 01, 1986. Control (baseline) biopsy for Parfr's NET-90 Study.

HYSICIAN:

LIN. DX: Patient No. 6.

ISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 191-86 (RSB) C,CD24

SEX F DATE 4-4-86

RACE INS SAXENA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 22.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 191-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures up to 1 cm in greatest dimension, with individual segments ranging up to 0.6 cm in greatest dimension. Much of the specimen consists of blood clot and mucus. Several small fragments of endometrium are available for study and feature considerable stromal artifact, possibly freezing artifact. The individual glands are mildly tortuous, with luminal secretion and basally located nuclei. The stroma is apparently edematous, without evident perivascular cuffing.

EADjh
4-7-86

E.A. Dowling, M.D.

NAME: PARFR NET90

DOB W 198-86

AGE SP# (KYK) C,CD24

SEX F DATE 4-8-86

RACE INS SAXENA

ADDRESS: Biopsy date: April 04, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 7.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: FRAGMENTS OF ENDOCERVICAL MUCOSA AND LOWER UTERINE SEGMENT, INSUFFICIENT FOR DIAGNOSIS.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 198-86.

MICROSCOPIC DESCRIPTION: An aggregate of endometrial segments measures up to 0.8 cm, with individual segments measuring up to 0.3 cm. The specimen consists of endocervical mucus, strips of benign endocervical mucosa and a few tiny fragments of lower uterine segment.

EADjh
4-9-86

E. A. Dowling, M.D.

ME: PARFR NET90

DRESS: Biopsy date: April 18, 1986. Control (baseline) biopsy for Parfr's Net-90 Study.

IYSICIAN:

.IN. DX: Patient No. 8.

DOB

AGE SP# W 231-86

SEX F DATE (CHB) C,CD22 4-22-86

RACE INS SAXENA

SSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 18.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 231-86.

MICROSCOPIC DESCRIPTION: An aggregate of tissue segments measures 0.7 cm collectively and, individually up to 0.1 cm. The endometrial glands are slightly tortuous and feature striking subnuclear vacuolization, without more than incipient, supranuclear vacuolization. The stroma is moderately cellular, without significant edema.

EADjh
4-23-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: April 18, 1986. Control (baseline) biopsy for PARFR's NET-90 Study.

PHYSICIAN:

CLIN. DX: Patient No. 9.

DOB

AGE SP# W 232-86

SEX F DATE (MHL) C,CD21 4-22-86

RACE INS SAXENA

ISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 20.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 232-86.

MICROSCOPIC DESCRIPTION: An aggregate of biopsy segments measures collectively 0.6 cm with individual segments measuring up to 0.2 cm. The glands are moderately tortuous with predominantly basally located nuclei and luminal secretion in evidence. The stroma is mildly edematous.

EADjh
4-23-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: May 09, 1986.
Patient No. 10.
Control (baseline) biopsy for
PARFR's NET-90 Study.

PHYSICIAN:

CLIN. DX:

Patient No. 10.

TISSUE REMOVED:

2 SLIDES

DOB

AGE

SP#

SEX F

DATE

RACE

INS

W 287-86
(SRB) C,CD24
5-24-86

SAXENA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY:
a) INSUFFICIENT FOR PHASING.
b) FOCAL ENDOMETRITIS AND MYOMETRITIS WITH HYALINIZATION, SUGGESTIVE OF RECENT PREGNANCY.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 287-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.7 cm, with individual segments measuring up to 0.1 cm. Much of the specimen consists of blood clot. Both endometrium and myometrium are represented in the biopsy. The myometrium is characterized by separation of the fibers by fibrous tissue and inflammatory cells, associated with areas of hyalinization. No recognizable decidua, or trophoblast can be identified. Nevertheless, these focal areas suggest a recent pregnancy, although reaction to a foreign body, such as an intrauterine device, should also be considered. Otherwise, the endometrial glands and stroma are insufficient for definitive evaluation.

EADjh
5-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: May 14, 1986.

PHYSICIAN: Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 11.

DOB

AGE SP# W 295-86

SEX F DATE (DEW) C,CD23 5-24-86

RACE INS SAXENA

ISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 19.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 295-86.

MICROSCOPIC DESCRIPTION: The endometrial biopsy consists of an aggregate of segments measuring 0.7 cm, with individual segments measuring up to 0.1 cm. The representation of endometrium is small but adequate. The glands are mildly tortuous and some still show a minimal degree of subnuclear vacuolization. Secretion is present in the lumen and the stroma is mild edematous.

EADjh
5-28-86

E. A. Dowling, M.D.

W-811-86. MDM. Saxena. PARF
Poly NET Study. Day-64 PI #
About 5 months TM with NET.
Patient No. 14. BX 11-17-8

UNIVERSITY of SOUTH ALABAMA

November 21, 1986

COLLEGE OF MEDICINE
ELECTRON MICROSCOPY CENTER

MOBILE, ALABAMA 36688
TEL. 205/460-6258

Dr. Brij Saxena
Room A-267
Cornell University Medical College
525 East 68th Street
New York, NY 10021

Dear Dr. Saxena:

I received an endometrial biopsy from you on November 18th that was taken on 11-17-86 from MOM (Patient No. 011) for PARFR's Poly NET-90 Microsphere Study. The biopsy was obtained on Day-64 Post-Injection (2nd injection).

Unfortunately, there was no endometrium for evaluation in the biopsy material. Recuts showed only more of the same--endocervical mucus and a fragment of cervical tissue.

This letter is for your records, and will serve as the only report on MOM's biopsy, which is labeled W-811-86 in my records.

Please call me if you have any questions.

Sincerely yours,

Walter Wilborn

Walter H. Wilborn, Ph.D.
Director, EM Center;
Professor of Anatomy

cc: Dr. Alfredo Goldsmith, PARFR

NOTE: INVESTIGATOR GAVE WRONG INITIALS & SUBJECT NUMBER. CORRECT INFORMATION WAS OBTAINED AFTER LETTER WAS SENT DR. SAXENA & DR. GOLDSMITH OF PARFR. THE CORRECT INITIALS ARE MDM, AND THE CORRECT NUMBER IS 14.

Walter Wilborn

NAME: PARFR NET90

DOB

AGE

SP# W 308-86

SEX F

DATE (KRO) C,CD27 5-26-86

RACE

INS SAXENA

ADDRESS:

PHYSICIAN: Biopsy date: May 21, 1986. Control (baseline) biopsy for PARFR's NET-90 Study.

CLIN. DX: Patient No. 12.

TISSUE REMOVED:

2 SLIDES

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 23.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 308-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.6 cm, with individual segments ranging up to 0.7 cm. Endometrial glands are tortuous and secretory, with basally located nuclei. While the surface is not noted, the stroma features prominence of spiral arterioles edema and early periarteriolar cuffing.

EADjh
5-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS: Biopsy date: May 23, 1986. Control (baseline) biopsy for PARFR's NET-90 Study.

PHYSICIAN:

CLIN. DX: Patient No. 13.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP# W 318-86 (MGR) C,CD

SEX F DATE 5-28-86

RACE INS SAXENA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)
ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 24.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION: **FROZEN SECTION DX:**

The specimen consists of two slides bearing PARFR NET90 accession number W 318-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 0.9 cm, with individual segments ranging up to 0.4 cm. Some fixation or freezing artifact is present. However, the glands are mildly tortuous with basally situated nuclei and some luminal secretion. The stroma features early decidualization, rather diffusely distributed, with a mild degree of generalized edema.

EADjh
5-28-86

E. A. Dowling, M.D.

NAME: PARFR NET90

ADDRESS:

PHYSICIAN:

CLIN. DX: Biopsy date: May 27, 1986. Control (baseline) biopsy for PARFR's NET-90 Study. Patient No. 14.

TISSUE REMOVED:

2 SLIDES

DOB

AGE SP#

SEX F DATE

RACE INS

W 326-86
(MDM) C,CD21
6-4-86
SAXENA

PATHOLOGIC DX: (GROSS AND MICROSCOPIC)

ENDOMETRIAL BIOPSY: SECRETORY ENDOMETRIUM, DAY 17.

UNIVERSITY OF SOUTH ALABAMA
HEALTH SERVICES LABORATORY

LABORATORY OF SURGICAL PATHOLOGY

GROSS DESCRIPTION:

FROZEN SECTION DX:

The specimen consists of two slides bearing PARFR NET90 accession number W 326-86.

MICROSCOPIC DESCRIPTION: The biopsy segments measure in aggregate 1.4 cm with individual segments ranging up to 0.3 cm. The glands remain generally tubular but show prominent subnuclear vacuolization. A few mitoses can be identified. The stroma is edematous.

EADjh
6-4-86

E. A. Dowling, M.D.