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Six-Month Report

DEVELOPMENT OF IMPROVED AND NEW IUDS

April 1 — September 30, 1978

International Fertility Research Program
Research Triangle Park, North Carolina 27709
USA

INTERNATIONAL FERTILITY RESEARCH PROGRAM

Six-Month Report

DEVELOPMENT OF IMPROVED AND NEW IUDS

April 1, 1978 - September 30, 1978

Contract AID/pha-C-1111

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INTERNATIONAL FERTILITY RESEARCH PROGRAM

Six-Month Report

DEVELOPMENT OF IMPROVED AND NEW IUDs

April 1, 1978 - September 30, 1978

Contract AID-pha-C-1111

SUMMARY

The three main objectives of this contract are the development of postpartum IUDs that have low expulsion rates when inserted immediately following removal of the placenta, medicated IUDs that will reduce IUD-related bleeding side effects and a prescriptive approach to IUD insertion that will improve contraceptive performance by matching the IUD type to the woman's individual attributes.

Satisfactory progress has been made towards each objective. Additional data have been generated on the hand insertion of the IFRP postpartum suture Loops showing an expulsion rate at six months that compares well with interval Loop insertion rates. The postpartum suture Cu-T is performing equally well. New postpartum inserters have been developed and are available for clinical trials. All five models of antibleeding medicated IUDs (two AMCA, three Trasylol) are scheduled for testing in animals for excessive osmotic swelling and associated tissue inflammation. AMCA-medicated clinical trials will start as soon as ongoing prehysterectomy insertions are complete and evaluated. A decision tree for prescribing an IUD according to a woman's particular physical characteristics is being developed.

The extension of the IUD contract from September 30, 1978 to April 30, 1979 provides necessary additional time to complete project objectives. By the end of the contract period, solid data on postpartum devices will be available and studies of both IUD-delivered drugs, AMCA and Trasylol, will be underway. The prescriptive approach to IUD insertion will be undergoing clinical evaluation.

I. POSTPARTUM IUDS AND INSERTERS

The three types of postpartum IUDs developed under this contract are: 1) the Lippes Loop and Cu-T IUDs with biodegradable No. 2 Chromic gut suture projections that extend 0.5 cm in the anterior and posterior directions from the fundal limb; 2) the Lippes Loop and Cu-T IUDs with biodegradable gelatin/polylactic acid or collagen lateral extensions on the fundal limb; and 3) the Lippes Loop IUD with molded (nondegradable) anterior-posterior projections on the fundal limb.

Preliminary evaluations of these devices have led to a concentration of research effort on the IUD models with biodegradable No. 2 Chromic suture projections. Clinical trials with the postpartum suture Loop have indicated favorable results (six-month life table expulsion rate for 286 cases pooled from four centers is 11.2 $\pm$ 2.4 per 100 women). See Table I for net life table rates for pregnancy, expulsion and removal for pain and bleeding.

Also, preliminary data from clinical trials of the postpartum suture Cu-T show favorable results (six-month life table expulsion rate for 100 women is 8.8 $\pm$ 2.3 per 100 women). See Table II for net life table rates for pregnancy, expulsion and removal for pain and bleeding for the suture Cu-T.

Table I. Net life table rates for pregnancy, expulsion and removal for pain and bleeding of the postpartum suture Loop. Data pooled from insertions in Santiago, Cairo, Manila and Bandung. (N = 286, 1083.5 woman-months of exposure)

NET LIFETABLE RATES PER 100 WOMEN

Event	1 mo.	3 mo.	6 mo.
Pregnancy	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.6
Expulsion	4.4 ± 1.3	6.5 ± 1.5	11.2 ± 2.4
Removal, B/P	0.4 ± 0.4	1.2 ± 0.7	2.3 ± 1.1
Removal, Med.	0.4 ± 0.4	0.4 ± 0.4	0.9 ± 0.7

The Bandung study was discontinued after 44 insertions because of operator difficulties. The expulsion rate for the pooled data minus Bandung at three months is 4.7 ± 1.5 per 100 women and at six months is 8.8 ± 2.3 per 100 women.

Table II. Net life table rates for pregnancy, expulsion and removal for pain and bleeding of the postpartum suture Cu-T. (N = 100, 374.0 woman-months of exposure)

NET LIFE TABLE RATES PER 100 WOMEN

Event	1 mo.	3 mo.	6 mo.
Pregnancy	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Expulsion	1.0 ± 1.0	4.5 ± 2.2	6.2 ± 2.7
Removal, B/P	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Removal, Med.	0.0 ± 0.0	0.0 ± 0.0	3.4 ± 3.3

There has been high physician acceptance of the retentive suture concept. The lateral extension and the nondegradable projection concepts, however, have not proven attractive to either physicians or acceptors and, consequently, studies of these designs have been discontinued. The Chromic suture devices are simple to manufacture and could be an appropriate LDC technology requiring no significant capital investment.

The 1200 suture Loop cases approved by the Protection of Human Subjects Committee (PHSC) have been assigned to investigators for evaluation. On the basis of the suture Loop clinical data noted above, the PHSC has removed the quota on the number of suture Loops that may be evaluated. Among the 286 cases reported to the IFRP (including 44 patients in the study that was terminated because of insertion difficulties experienced by the physician), there have been no uterine perforations. Reports of pelvic infections include five cases of mild salpingitis, three cases of mild endometritis and one case of moderate endometritis. For the 11 women who have had their IUDs removed, investigators have not reported any removal difficulties.

Having collected preliminary data on the safety and efficacy of immediate postpartum insertion of the suture Loop, the IFRP is proceeding with further evaluation of this device. On the recommendation of the Core Advisory Committee, the IFRP will evaluate immediate postabortal (late first trimester and second trimester) insertions of the suture Loop. (See Appendix A for Minutes of CAC meeting on June 2, 1978.) Initially, the IFRP will conduct comparative post-midtrimester abortion trials of the suture Loop versus a plain Loop D in the United States and Singapore. Comparative immediate postpartum hand insertion of the suture Loop versus the plain

Loop D is scheduled to begin in November 1978 in Mexico and Chile. This study will evaluate the importance of the hand insertion technique as compared to the suture projections in reducing the expulsion rate of the Loop. Later studies will compare the hand technique against the inserter technique.

Expanded straight and comparative trials of postpartum suture Loops will be initiated, principally among the regional fertility research programs associated with the IFRP. Some of these programs have specifically requested that they be allowed to evaluate this device for inclusion in their national postpartum family planning programs.

A Tapered-Photoreduced Loop with No. 2 Chromic sutures is being tested in a 200-case study in Bangladesh. Preliminary results will be detailed in the next report.

A number of contributors are being considered for straight and comparative clinical trials of the suture Cu-T.

New inserter designs for the postpartum suture Loop and suture Cu-T, incorporating the suggestions made by the Core Advisory Committee, have been developed and are ready for clinical evaluation.

Summary: Postpartum IUDs and Inserters

The research and development work for IUD designs that improve IUD retention when inserted immediately postpartum is complete. Clinical evaluation is ongoing under Contract AID-pha-C-1172.

II. MEDICATED IUDS

Five contributions to antibleeding medicated-IUD technology are being developed by the IFRP for clinical evaluation:

1. AMCA/HEMA/Estane 5716 Loop;
2. AMCA/EVA Loop;
3. Trasylol/EVA Loop;
4. Trasylol/HEMA Loop; and
5. Trasylol/Collagen Loop.

Plans to develop Trasylol-medicated Cu-T IUDs have been temporarily halted as a result of the in vitro data of Zaneveld and Goodpasture which show that the addition of copper to sterilized EVA/Trasylol solutions can produce up to 70% less Trasylol activity than either the sterile or nonsterile samples of EVA/Trasylol. (See Appendix B.) The reduced activity of Trasylol shown in vitro presumably would require much higher drug loads to achieve desired effects. Therefore, for the present, controlled-release Trasylol delivery systems will be tested on the Loop vector. Should they prove efficacious in reducing excessive IUD-related bleeding from this vector they may later be considered candidates for the Cu-T, if further tests so warrant.

An in vitro test is underway to validate the EVA/AMCA and EVA/AMCA with Copper release rate data that have been generated by En-Cas Analytical Laboratories.

AMCA/HEMA/Estane 5716 Loop

This device has been available for testing since the last reporting period. The AMCA/HEMA/Estane 5716 Loop was developed under subcontract by Southern Research Institute in Birmingham, Alabama. Independent release rate analyses suggest that the SRI-developed system offers a consistent release pattern in the vicinity of the desired limits, i.e., 75 μg/day/100 days. (See Appendix C.)

AMCA/EVA Loop

The AMCA/EVA Loop was developed at IFRP by means of a process that homogeneously incorporates the drug into an ethylene vinyl acetate matrix. Release rate analyses on modified Loops with AMCA-medicated sections suggest that a therapeutic dose can be delivered for at least three months. (See Appendix D.)

By September 1978, only two of the AMCA devices initially selected to provide prehysterectomy insertions had been inserted; therefore, devices have been supplied to another clinic to expedite the process of evaluating the devices for unacceptable swelling characteristics.

Trasylol/EVA Loop

This device is fabricated by the same process developed by the IFRP to make AMCA/EVA Loops. Release rate analyses suggest that this device is capable of delivering a therapeutic dose for a period of at least three months. Although the release rate is below the target level of 100 μg/day, it is believed to be high enough to have an effect on menstrual blood loss. (See Appendix B.)

Trasylol/HEMA Loop

Bend Research undertook development work on a Hydron matrix for Trasylol release in October 1977. Difficulties, including insufficient physical integrity and excessive osmotic swelling, have delayed completion of this project. There is some concern that the relative instability of the Hydron matrix reduces its suitability for long-term intrauterine applications.

Trasylol/Collagen Loop

Dr. Milos Chvapil is under contract to develop and fabricate 100 Trasylol-medicated devices using a biodegradable collagen matrix. Initial tests show a release rate of 1.4 mg over 14 days representing a 25% release of the active drug. (See the progress report in Appendix E.) Later verbal reports suggest that a diffusion mechanism is more important in drug release than erosion, and that greatly increased drug capacity is possible in part due to the slow dissolution rate of the collagen polymer. The expected date of completion for this project is October 30, 1978.

Tests

In vitro tests on the above medicated devices were designed primarily to assay for drug release and secondarily to measure their physical characteristics. Just as copper has been shown to inhibit the activity of Trasylol, ethylene oxide gas sterilization has been shown to have little or no effect in depotentiating the drug in tests conducted by Goodpasture. (See Appendix B.)

A rabbit study has been planned to permit evaluation of the osmotic swelling characteristics of all the above experimental devices and to record any

inflammatory tissue reaction associated with the medicated as well as with control devices. The study will commence in early November 1978. The PHSC will be notified of the results, with special reference to the swelling properties of the devices, in order to avoid the time-consuming requirement for prehysterectomy insertions prior to Trasylol-medicated device studies.

Progestacoil

During the Medical Advisory Committee IUD Subcommittee in June 1978, concerns about the possible association between ectopic pregnancy and the use of progesterone-releasing devices resulted in the Progestacoil being given a low priority for clinical evaluation.

Summary. Medicated IUDs

The research and development work on IUD designs that reduce IUD-related bleeding is nearly complete. To provide clinical verification, clinical trials under AID Contract 1172 will be initiated as soon as practicable.

III. PRESCRIPTIVE IUD INSERTION

Work on the development of a protocol to be used in the clinical evaluation of an IUD prescription method is nearly complete. A decision-tree guide with numbered tabs has been developed that will permit the selection of an IUD type for each acceptor. A comparative study is being designed to investigate what effect use of this method will have on event and continuation rates of IUD users. A description of the decision-tree and how to use it will be published next year by Wheeler in Hafez, ed., IUD and Biodegradable Delivery Systems. (See Appendix F for abstract.)

One hundred Uterine Cavity Depth Sounds II, which measure the distance from the inner os to the fundus, have been assigned to IFRP-associated clinicians for further evaluation. More exact knowledge of uterine cavity and cervical length and their relationship to IUD-related events is needed in order to better develop the prescriptive approach to IUD insertion.

IV. OTHER

The implementation of a computer storage and retrieval system for library materials has been initiated. All IUD project literature references have been loaded to facilitate categorical access.

The development of a technique of nonsurgical female sterilization is a major priority for many family planning programs. A T-shaped IUD has been investigated as a vector to deliver a tubal-occluding dose of quinacrine hydrochloride. Dr. Zipper (Chile) is evaluating the safety and efficacy of this substance for female sterilization.

V. PUBLICATIONS

An article entitled, "Development of a Postpartum IUD" by Laufe, Wheeler and Friel has been submitted for publication. A chapter entitled, "Use of Biodegradable Materials to Improve IUD Performance: I. Postpartum Retention; II. Bleeding Side Effects" by Laufe and Friel and a chapter entitled, "Decision Guide for Selecting IUDs for Individual Acceptors" by Wheeler are being prepared for publication next year in a book edited by E.S.E. Hafez.

VI. PLANS FOR NEXT PERIOD

The goal of developing improved intrauterine devices that are adaptable to indigenous production has been furthered by the establishment of an IUD fabrication facility in Mexico City. A subcontract under AID-pha-C-1172 has been executed with PIACT de Mexico to continue the IUD fabrication work initiated under the IUD Contract. By the end of November, the Mexican workshop will be fabricating postpartum suture Loops. By December 1978 the Mexican workshop will be equipped to fabricate medicated EVA Loops. Before the end of the contract period, it will be possible to use this facility to make production quantities of any of the above approved medicated devices.

Clinical evaluation of the postpartum devices and inserters will continue in straight and comparative trials. A special study to investigate the efficacy of hand insertion versus inserter insertion will be initiated.

At least two AMCA-medicated Loop studies will be started in IFRP centers that have the capabilities of doing menstrual blood loss studies. Application to the PHSC will be made to begin clinical evaluation of a Trasylol-medicated device, whose selection will be determined by the outcome of the ongoing animal study.

Protocols for clinical evaluation of IUD prescription methods will be tested in a pilot study.

VII. RECOMMENDATION ON CURRENT NEEDS

Work to date does not indicate a need to modify project objectives as stated in the contract.

VIII. ADMINISTRATIVE REPORT

Data on staff salaries, consultants, and expenditures are provided in Appendix G.

INTERNATIONAL FERTILITY RESEARCH PROGRAM

IUD Core Advisory Committee Meeting
June 2, 1978

The IUD Core Advisory Committee Meeting was held in the Key Bridge Marriott Hotel, Arlington, Virginia on 2 June 1978. The names of the participants are as follows:

Dr. Michael Burnhill	Ms. Elizabeth Maguire
Dr. Antonio Scommegna	Dr. Leonard Laufe
Dr. Gebhard Schumacher	Mr. Robert Wheeler
Dr. Lourens Zaneveld	Dr. Patrick Friel
Dr. Jaime Zipper	Dr. Alfredo Goldsmith
Dr. J. Joseph Speidel	Mr. Robert Taylor
Dr. James Shelton	Ms. Beth Young

Dr. Laufe welcomed the Committee in his introductory remarks. The minutes of the 16 December 1977 meeting were approved.

1. Postpartum IUDs

a. Young reported the postpartum data for the Cu-T and Loop D with Chromic sutures. Laufe explained that the Bandung cancellation was due to poor insertion technique. He proposed a postabortion mid-trimester series of Cu-Ts with Chromic sutures. Scommegna suggested that a gentle curettage be used following prostaglandin abortions, especially when followed by immediate IUD insertion.

b. Wheeler queried the Committee whether copper on the crossarm of the Cu-T 220 C or 380 A, when embedded in uterine tissue, would likely cause tissue reactions, inflammation, granuloma, etc Scommegna confirmed his experience with these reactions in the peritoneal cavity of the rabbit. During an examination of radiographs of Cu-T 200s (provided by Dr. Hefnawi) that showed a high incidence of embedment (15-20%), Wheeler observed that cases of penetration were asymptomatic. To this Scommegna added that he knew of a series of hysterectomies performed on copper-IUD-wearing women that showed no problems in utero. He also questioned whether the series of 80 radiographs were a random selection from the 200 patients in the study. Wheeler said that he would check on this.

c. Burnhill, referring to Laufe's proposed postabortal suture Cu-T study, suggested comparison with the suture Lippes Loop. His misgivings about copper devices focus on embedment, perforation, expense, and lack of adaptability to the postpartum or postabortal uterus. When queried, Zipper reported that he experienced very few problems in removing the Cu-T. Burnhill also suggested that IFRP initiate a good blind comparative, hand vs. inserter, of immediate postplacental insertion of Chromic suture Loop versus plain Loop. The Committee agreed and Wheeler noted that it could be implemented concurrently with proposed field trials of the suture Loop. Shelton and Speidel expressed interest in completing a tightly controlled

comparative study before the initiation of massive clinical trials. Laufe explained that, in his experience, there was tremendous enthusiasm in LDCs for the suture Loop and that he preferred IFRP-controlled studies of the device to uncontrolled experimentation. The concensus of the Committee was that concurrent trials could be initiated but that the highest priority should be attached to the comparative Loop study.

d. With respect to the postpartum inserters for the Loop and Cu-T, prototypes of which were passed around the table, Schumacher suggested, 1) that an orientation mark be hot-stamped at the 12 o'clock position on both inserters and b) that a straight section be added to the ends of the Y-shaped arms of the postpartum Loop inserter. The Committee concurred.

2. Medicated IUDs

a. Wheeler explained the fabrication technique developed at IFRP for incorporating drugs into an EVA matrix. Zaneveld discussed the preliminary Trasylol data prepared at his lab that was generated using such devices (see attachment I). Schumacher reported on his telephone conversations with Bayer Research Center personnel (see attachment II), the most recent of which revealed that Trasylol ought not be de-activated by the fabrication process. These findings were confirmed by Zaneveld's early data, although his experiment does show that the sterile Trasylol/EVA strip is somewhat less active than the non-sterile sample. Although the copper seemed to be suppressing the inhibitor activity of Trasylol, Zaneveld noted that a recent telephone report from his lab indicated that this effect was diminishing. An additional series of experiments is being planned with Dr. Zaneveld on EVA formulations of Trasylol as well as AMCA to more clearly pinpoint the effect of copper, the effect of ETO sterilization, and the effect of EVA on the activity of the drugs. Zaneveld reported that he was also doing analyses for Bend Research on Trasylol samples being developed under contract for the IFRP.

b. Wheeler explained the concept behind the Medicated IUD Study Design which will enable analysts to estimate IUD-related blood loss using a smaller study population than hitherto possible. Shelton suggested that the pre-insertion patients be stratified to equalize the MBL values. The Committee endorsed the Medicated IUD Study Design.

c. Zaneveld presented a protocol to test the release of Trasylol in rabbits for its effect on fertilization and implantation. The Committee was referred to Dr. Hans-Werner Denker's work on the blockage of implantation by Trasylol. Denker reports that Trasylol was especially effective at inhibiting blastolemmase when applied intrauterally to rabbits. Scommegna noted that if Trasylol has contraceptive action it might permit the use of a T-vector without copper. Burnhill, who also supported the protocol, raised the question of whether teratogenic effects had been examined in animal studies. Shumacher noted that Bayer had performed a complete evaluation of the administration of the drug in gram quantity dosages many years ago and had found it to be safe. Still, the Committee recommended

that a literature search be implemented in order to establish the results clearly for the record. In the booklet published by Bayer entitled Trasylol, Code number E 293-795/38008, p. 15, it is reported that, "Pregnant rats of the FB 30 strain received intravenous injections of Trasylol in doses of up to 80,000 KIU/kg on five successive days during the first three months of pregnancy. The treatment did not result in any impairment of either the behaviour of the dams or the development of the foetuses." In addition, Dr. Schneils of Bayer reported in a letter to the IFRP (Friel) dated 24 August 1976 that in Bayer-administered tests in rabbits at doses up to ten times the therapeutic dose "there was no evidence of any adverse effect of Trasylol on fetal development." Returning to Zaneveld's Protocol, Speidel objected to a study of the contraceptive efficacy of Trasylol if its long range release was not feasible. Burnhill expressed interest in the possibility of a non-steroidal medicated IUD and Scommegna inquired whether Trasylol was among the many compounds screened by Dr. Moyer. It is believed that it was not among them. Although a 1-2 year release term could be easily predicted, there is no evidence at this time that pinpoints the daily dose needed to inhibit implantation. This protocol may be reconsidered by IFRP if clinical evaluation of the drug's antibleeding properties indicates any improvement in the device's pregnancy rate. Zaneveld, who will spend several weeks in Essen doing research with Dr. Tauber, offered to look into the literature on Trasylol's teratogenicity.

3. Photoreduced and Tapered Loops, et al.

a. Taylor reported on the subject devices, noting that the data were insufficient to provide a clear indication of the success of the modified Loop devices. Further analysis of the data, as it becomes available, will include multivariate techniques that may explain the high variation between centers that was noted in these preliminary data.

b. The early data on the TR-11 are satisfactory. Further modifications will be pursued if lower side effects are established in current studies.

c. The Committee was informed of the problems reportedly experienced by Dr. Newton with the Popcouncil Postpartum T, the use of which recently has been discontinued in Mexico. Dr. Aznar in Mexico City reports that there is a flaw at the juncture of the crossarm and stem that weakens the structural integrity of the device. IFRP is examining the problem with a view to discontinuing its use in ongoing clinical studies if this report is confirmed.

d. Wheeler presented to the Committee drawings of the new Hasson IUD. The Committee requested prototypes but none are available. It was agreed that the concept appears sound, but there is no money available in the IUD Contract at this time to undertake such a major project.

e. The Progestacoil IUD developed at IFRP was discussed briefly and it was agreed that for some women this device is potentially preferable because of its extremely low pregnancy rate.

4. Mexican IUD Workshop

a. Friel reported on his trip with Wheeler to Mexico City (PIACT) and Juarez (Guadalupe de la Vega) to evaluate potential sites for an IUD Workshop. The Committee endorsed the concept of such a workshop to fabricate modified IUDs using in-country resources.

b. Wheeler discussed the visit with Syntex, S.A. in Mexico City where pellets had been made up for Dr. Zipper's Quinacrine studies.

Summary

1. Highest priority assigned to the implementation of a blind comparative study of the suture Loop vs. the standard Loop, hand vs. inserter.

2. Initiate as soon as possible a mid-trimester postabortion comparative study of suture Cu-T vs. suture Loop D. Singapore was suggested as possible site.

3. The Committee urged that AMCA-medicated IUD studies be initiated soon. The Committee asked Dr. Zaneveld to obtain Trasylol data/biopsies reportedly available through Dr. Tauber and to visit Bayer scientists with regard to ongoing work on Trasylol, including its heat stability and teratogenicity.

4. Continue Estimation of Blood Loss work and Prescriptive IUD Insertion modelling.

5. The Committee endorsed the concept of an IUD workshop in Mexico.

6. Pursue TR series thickness hypothesis if current TR-11 studies show improved performance over the Loop D.

7. Continue Progestacoil development.

FINAL REPORT - IFRP # 4602

COMPARATIVE EVALUATION OF THE ACTIVITY OF TRASYLOL RELEASED IN VITRO FROM EVA/TRASYLOL DEVICES -A PRELIMINARY STUDY

Prepared by Jessie C. Goodpasture

Terminology and Abbreviations

EVA - trasylol releasing device sent to us by the IFRP. These devices release approximately 100 μg of trasylol per day.

IUD - a plastic "T" intrauterine device.

Plain IUD - the non-copper containing IUD.

Copper IUD - IUD with 4 copper bands.

PUFS - "Physiological Uterine Fluid Solution", the buffer used for these studies This buffer imitates as closely as possible the ionic composition of uterine fluid reported by Kar et al., in the American Journal of Obstetrics and Gynecology, 101 966, 1968 It consists of the following Na(OH) - 81 mEq/l, KOH - 3.5 mEq/l, $NaHCO_3$ - 36 mEq/l, $CaCl_2$ - 3 mEq/l, glucose - 500 mg/l, lactic acid - 115 mg/l, Penicillin - 60,000 units/l, streptomycin - 60 mg/l. The final pH is adjusted with HCl to 7.84, the pH of human uterine fluid. Care must be taken in the sequence of dissolving the chemicals so that the $CaCl_2$ does not precipitate.

Trasylol - bovine pancreatic trypsin inhibitor supplied by the Bayer Facotories

Trypsin - bovine pancreatic trypsin from Sigma Chemical Company.

ETA sterilization - sterilization by ethylene tetraoxide gas.

Objectives

The purpose of this preliminary study was to investigate whether the activity of trasylol released from EVA devices is affected by 1) ETO sterilization, and/or 2) the presence of copper released from an IUD. In

order to determine this, three EVA devices were tested by incubation in a physiologically suitable medium (PUFS), and the activity of the released trasylol from each of these three devices compared over a three month period. One sterilized device was kept in the presence of a copper IUD, and a second sterilized device kept in the presence of a plain IUD. A third, non-sterilized device was kept only in the presence of the physiological medium (i.e., without an IUD).

Methodology

The copper and plain IUD's were cut into 3 pieces (in order to fit into the container) and each IUD placed in a separate 10 ml beaker. A sterilized EVA device was added to each of the two beakers. The non-sterilized EVA device was placed in a third beaker by itself. Ten ml of PUFS was added to each beaker, the beakers sealed with parafilm, and placed in a 37°C, temperature controlled, moist chamber. The buffer was replaced every day for the first week (when the release of trasylol was presumably the greatest), every third day for the next 3 weeks, and once a week for the next two months. Each time, the removed solution was tested for the total amount of trasylol activity.

The basis for the assay of trasylol activity is the quantitative determination of the amount by which a known trypsin activity is inhibited by a test solution containing trasylol. 0.1 ml of the standard trypsin solution at pH 8.0 is incubated at room temperature with 0.1 ml of the trasylol test solution for 5 minutes. The pH of 8.0 of the trypsin solution is achieved by having dissolved the trypsin in 50 mM tris buffer containing 50 mM $CaCl_2$, pH 8.0. As control, the same amount of trypsin is incubated with PUFS only. Immediately following the 5 minute incubation period, 0.1 ml of the solution is added to a cuvette with 2.9 ml of 50 mM tris buffer, pH 8.0, containing

50 mM $CaCl_2$, and 0.5 mM α-N-benzoyl-L-arginine ethyl ester (BAEE). The hydrolysis of BAEE by trypsin is followed spectrophotometrically at 253 nm. A molar absorption coefficient of 1150 M^{-1} cm^{-1} is used to convert change in absorbance to μmoles of BAEE hydrolyzed. One unit of trypsin activity is defined as that amount causing the hydrolysis of 1 μmole of BAEE per minute at 25°C. One unit of trypsin inhibitory activity is defined as that amount of inhibitor necessary to inhibit one unit of trypsin activity. The amount of trypsin activity that is inhibited by a given solution is calculated by subtracting the activity in the presence of the test solution from the control activity:

Control Activity Minus Activity In Presence of Test Solution =

Amount of Trypsin Activity Inhibited.

Duplicate tests were performed each time. In case these differed, a third test was performed. This was only rarely necessary.

Standard solutions of trasylol were prepared containing known concentrations of the inhibitor. A standard curve was prepared with these solutions which exhibited the amount of trypsin activity inhibited vs. the known concentrations of trasylol. From this curve, the trypsin inhibitory activity found in a solution could be converted to μg of active trasylol (Table I).

In order to insure that the presence of copper in the test solution did not interfere with the assay system used, one copper IUD (cut into 3 pieces) was placed into 10 ml of PUFS and allowed to incubate (sealed) at 37°C for 24 hours. The PUFS was then replaced with a fresh 10 ml of the buffer. Following a second 24 hour period of incubation, the effect of this solution (which contained high amounts of copper) on the hydrolysis of trypsin was determined. The "second day" solution was used because this was a more

reasonable representation of the actual amount of copper in the solution, with the exception of the first day.

Results

The EVA devices incubated with the copper IUD's appeared to be more rigid and "stiffer" than the devices in the plain IUD + EVA or the non-sterilized EVA solutions. No explanation can be offered for this at the present time.

The copper of the "second day solution" did not have an effect on the activity of the trypsin, i.e., it neither inhibited the enzyme nor activated it.

The results of the trasylol tests can be found in Tables I, II and III. The activity of the released trasylol was essentially the same for the non-sterilized EVA (Table I) as for the plain IUD + sterilized EVA (Table II). After the initial burst in trasylol release, the average amount of active trasylol liberated from both EVA devices is 134 µg per day for two days. An average of 35 µg per day of active trasylol is released for the next 20 days. Thereafter, an average of 16 µg per day of active trasylol is released.

The solutions obtained from the copper IUD + sterilized EVA (Table III) consistently produced less trasylol activity than either the plain IUD + sterilized EVA or the non-sterilized EVA solutions, averaging approximately 40% less trasylol activity during the first week and 70% less thereafter until the end of the three month period of testing.

Conclusions

(1) Active trasylol is released from EVA devices (sterilized and non-sterilized) *in vitro* for at least three months.

(2) ETO sterilization of EVA devices does not affect the activity of trasylol.

(3) In the presence of a copper IUD, the activity of trasylol is significantly less than in the presence of a plain IUD.

(4) After 4 days, the amount of active trasylol released from all the devices tested is much less than the desired 100 μg per day release rate.

The observations of the effects of copper on the levels of active trasylol found are of particular concern because it is the intention of the IFRP to attach trasylol releasing devices to copper IUD's. The study should be repeated with more devices, however, before any definitive conclusions can be reached.

TABLE I

RELEASE RATE OF ACTIVE TRASYLOL FROM A NON-STERILE EVA/TRASYLOL DEVICE

Date	Time Elapsed Between Tests (hours)	Total Units of Trypsin Inhibitory Activity[a]	Equivalent µg of Trasylol[b]
05/20	30.5	354.78	3,608
05/21	17.5	14.09	153
05/22	23.5	12.91	142
05/23	23.5	6.78	79
05/24	32.0	6.52	77
05/25	16.0	1.57	27
05/26	24.0	3.39	45
05/29	69.0	4.07	52
06/01	74.5	6.99	81
06/04	68.0	4.28	54
06/07	70.0	6.47	76
06/11	98.5	7.51	87
06/14	64.5	4.90	60
06/17	72.5	4.54	57
06/23	140.0	8.50	97
07/07	331 5	7.25	84
07/14	275.5	7.30	85
07/21	160.0	5.06	62
07/31	239.5	6.37	75
08/06	148.0	3.76	49
08/14	188.0	2.35	34
08/21	174.5	6.10	72

[a]Total units (in 10 ml solution) of active trasylol released in X number of hours, expressed as units of trypsin inhibitory activity (see text for details).

[b]As determined with known, standard concentrations of trasylol (see text for details).

TABLE II

RELEASE RATE OF ACTIVE TRASYLOL FROM A STERILE EVA/TRASYLOL DEVICE IN COMBINATION WITH A PLAIN IUD

DATE	Time Elapsed Between Tests (hours)	Total Units of Trypsin Inhibitory Activity[a]	Equivalent µg of Trasylol[b]
05/20	30.5	313.04	3,184
05/21	17.5	10.96	122
05/22	23.5	10.70	119
05/23	23.5	6.00	71
05/24	32.0	3.91	50
05/25	16.0	1.57	27
05/26	24.0	3.39	45
05/29	69.0	10.12	113
06/01	74.5	14.50	158
06/04	68.0	8.03	92
06/07	70.0	12.68	139
06/11	98.5	10.90	121
06/14	64.5	3.86	50
06/17	72.5	4.59	57
06/23	140.0	9.76	110
07/07	331.5	7.46	86
07/14	275.5	5.74	69
07/21	160.0	2.71	38
07/31	239.5	3.91	50
08/06	148.0	4.49	56
08/14	188.0	3.34	44
08/21	174.5	5.63	67

[a]Total units (in 10 ml solution) of active trasylol released in X number of hours, expressed as units of trypsin inhibitory activity (see text for details).

[b]As determined with known, standard concentrations of trasylol (see text for details).

TABLE III

RELEASE RATE OF ACTIVE TRASYLOL FROM A STERILE EVA/TRASYLOL DEVICE IN COMBINATION WITH A COPPER IUD

Date	Time Elapsed Between Tests (hours)	Total Units of Trypsin Inhibitory Activity[a]	Equivalent µg of Trasylol[b]
05/20	30.5	281.74	2,867
05/21	17.5	6.26	74
05/22	23.5	8.74	99
05/23	23.5	3.65	48
05/24	32.0	1.83	29
05/25	16.0	0.91	20
05/26	24.0	1.57	27
05/29	69.0	1.04	21
06/01	74.5	1.72	28
06/04	68.0	0.10	12
06/07	70.0	1.20	23
06/11	98.5	0.89	20
06/14	64.5	0.52	16
06/17	72.5	0.16	12
06/23	140.0	4.17	53
07/07	331.5	1.57	26
07/14	275.5	0	0
07/21	160.0	0	0
07/31	239.5	1.30	24
08/06	148.0	0.89	20
08/14	188.0	1.57	26
08/21	174.5	2.82	39

[a]Total units (in 10 ml solution) of active trasylol released in X number of hours, expressed as units of trypsin inhibitory activity (see text for details).

[b]As determined with known, standard concentrations of trasylol (see text for details).

FINAL REPORT - IFRP # 4714

THE ACTIVITY OF TRASYLOL RELEASED FROM EVA DEVICES IN VITRO IN THE PRESENCE OF COPPER AND PLAIN IUD'S

Prepared by: Jessie C. Goodpasture

Terminology and Abbreviations

See Final Report - IFRP # 4602, with the exception that in the present study, a Copper IUD is an IUD with 7 copper bands instead of 4 copper bands.

Objectives

In the previous study (IFRP # 4602), data indicated that the activity of trasylol released from EVA devices was significantly less in the presence of a copper IUD than in the presence of a plain IUD. However, this was only based on a comparison between two single devices. The purpose of the present study was: 1) to perform a more definitive study in order to determine the activity of released trasylol in the presence of copper IUD's compared to plain IUD's and 2) to test the effect of copper IUD's as compared to plain IUD's on the activity of trasylol directly.

Methodology

The methods employed were identical to those used in IFRP project # 4602, except that the length of the study was one month rather than three months. Six non-sterile EVA devices were evaluated, three in the presence of copper IUD's and three in the presence of plain IUD's. The devices were randomly selected from about 10 devices sent to us by the IFRP. The devices and IUD's were incubated in 10 ml of PUFS. The PUFS solutions were changed every day

for the first week and every third day for the next three weeks. All solutions were tested for trasylol activity (see IFRP # 4602).

In order to determine the direct effect of copper IUD's on the activity of trasylol, copper and plain IUD's were submerged in PUFS containing a known amount of trasylol, and the trasylol activity measured at two predetermined time intervals. To do so, 10 ml of PUFS containing 150 μg trasylol were added to each of four 10 ml beakers. Two copper IUD's and two plain IUD's were cut into three pieces each in order to fit into the beakers, and each IUD added to a separate beaker. The beakers were sealed with parafilm and kept at 37°C in a temperature controlled, moist chamber. After 3 days and 6 days, 0.2 ml aliquots were removed and assayed for trasylol activity.

Results

The release rates of active trasylol for each of the 6 devices are presented in Tables I and II. Starting on the third day of evaluation, all 3 devices that were in combination with plain IUD's (Table I) showed greater release rates of active trasylol than the devices in the presence of copper IUD's (Table I). This relationship became more pronounced as time passed until 1½ weeks at which time the plain IUD + EVA device solutions contained an average of 91% more active trasylol than the copper IUD + EVA device solutions. After the first 1½ weeks, the amount of active trasylol present remained higher in the plain IUD + EVA solutions, but the magnitude of this difference decreased until, after the third week of evaluation, trasylol activity became low and approximately equal for both types of IUD's.

These results correspond with the results obtained during the preliminary study (IFRP # 4602) except that somewhat higher levels of active trasylol were

generally found in the first study than were found in the present study in non-copper containing experiments. The one month data obtained in both projects are combined and averaged for the copper IUD's and plain IUD's, and presented in Table III. These are graphically presented in Figure 1. The differences between the plain and copper IUD's are obvious from this table and graph.

In the second part of the present study, the effect of incubating plain IUD's as compared to copper IUD's with trasylol in solution was observed. All 4 IUD's used in this study were submerged in equal amounts (150 µg) of trasylol. The amount of active inhibitor measured was significantly less in solutions containing copper IUD's vs. those containing plain IUD's (Table IV). On the third day of incubation, plain IUD + trasylol solutions exhibited twice as much trasylol activity as the copper IUD + trasylol solutions. At present we have no explanation for the observation of somewhat low activity on day 3 in the plain IUD + trasylol solutions. Of the original 150 µg of active trasylol added to the copper IUD's, an average of only 29 µg (19%) remained active by the sixth day, whereas the total amount was active up to that day in the presence of the plain IUD's.

Discussion and Conclusions

It is clear from the above presented data that the amount of active, released trasylol is substantially greater in solutions in which EVA devices are incubated with plain IUD's as compared to copper IUD's, supporting the results obtained from the preliminary investigation (IFRP # 4602). The results of the experiments in which IUD's were incubated with known amounts of trasylol in solution strongly indicate that this effect is due to a direct

effect of copper on trasylol in solution, rather than an effect on the rate of inhibitor released from the matrix of the EVA device. Therefore, since the highest possible levels of active trasylol released from a device are desired, the combination of a copper IUD with a trasylol releasing device is contraindicated.

It is of concern that the amount of active trasylol found was much less than the total amount released from the device (supposedly 100 μg/day). One wonders if this may be due to the "packaging" process employed in making of the EVA/Trasylol device, since heat and high pressure are used simultaneously during the process. Although lyophilized trasylol is stable to heating at 110°C for 9 to 10 times according to Bayer Factories, the combination of high pressure with heating may increase the denaturation effect on trasylol. Thus, the innermost packaged trasylol, which is subjected to many heat-presssure treatments, could therefore be substantially denatured, whereas the trasylol in the outer elements of the matrix may not be denatured, or only slightly denatured.

It is recommended that this be further investigated, perhaps by observing the activity of trasylol released from a device constructed in a different fashion.

TABLE I

RELEASE RATE OF ACTIVE TRASYLOL FROM EVA/TRASYLOL DEVICES IN COMBINATION WITH PLAIN IUD's

Date	Time Elapsed Between Tests (hrs.)	PLAIN IUD + EVA #1		PLAIN IUD + EVA #2		PLAIN IUD + EVA #3	
		Total Units of Trypsin Inhibitory Activity[a]	Equivalent µg Active Trasylol[b]	Total Units of Trypsin Inhibitory Activity	Equivalent µg Active Trasyol	Total Units of Trypsin Inhibitory Activity	Equivalent µg Active Trasyol
07/08	23.5	469.57	4771	373.57	3798	281.74	2867
07/09	24.0	26.30	277	26.61	280	26.30	277
07/10	27.5	11.06	123	16.28	176	12.21	134
07/11	22.0	6.68	78	10.12	113	7.10	83
07/12	23.0	7.62	88	10.33	115	10.17	144
07/13	22.0	8.50	97	10.12	113	8.66	98
07/14	28.0	11.63	128	7.93	91	12.68	139
07/17	69.0	10.90	121	8.66	98	10.85	121
07/20	67.0	4.49	56	2.30	34	5.84	70
07/23	79.0	1.30	24	0.99	21	4.75	59
07/27	88.0	2.97	41	2.66	38	5.69	68
07/31	96.5	1.15	22	0.89	20	1.98	31
08/03	71.5	0.63	17	1.83	29	2.50	36
08/06	76.0	0.94	20	1.41	25	1.72	28

[a]Total units (in 10 ml solution) of active trasylol released in X number of hours, expressed as units of trypsin inhibitory activity (see text for details).

[b]As determined with known, standard concentrations of trasylol (see text for details).

TABLE II

RELEASE RATE OF ACTIVE TRASYLOL FROM EVA/TRASYLOL DEVICES IN COMBINATION WITH COPPER IUD's

Date	Time Elapsed Between Tests (hrs.)	COPPER IUD + EVA #1		COPPER IUD + EVA #2		COPPER IUD + EVA #3	
		Total Units of Trypsin Inhibitory Activity[a]	Equivalent µg Active Trasylol[b]	Total Units of Trypsin Inhibitory Activity	Equivalent µg Active Trasyol	Total Units of Trypsin Inhibitory Activity	Equivalent µg Active Trasyol
07/08	23.5	419.48	4264	440.35	4475	384.00	3904
07/09	24.0	24.63	260	21.08	224	17.32	186
07/10	27.5	4.59	57	5.11	62	6.99	81
07/11	22.5	3.08	42	2.50	36	3.29	44
07/12	23.0	2.09	32	1.72	28	1.15	22
07/13	22.0	1.77	29	2.35	34	1.77	29
07/14	28.0	0.94	20	1.62	27	0.37	14
07/17	69.0	0	0	0.31	14	0.57	16
07/20	67.0	0	0	0.52	16	0	0
07/23	79.0	0	0	0	0	0	0
07/27	88.0	2.24	33	2.14	32	2.09	32
07/31	96.5	1.10	22	0.83	19	0.89	20
08/03	71.5	0	0	0.26	13	0.78	18
08/06	76.0	2.50	36	0.63	17	3.18	43

[a]Total units (in 10 ml solution) of active trasylol released in X number of hours, expressed as units of trypsin inhibitory activity (see text for details).

[b]As determined with known, standard concentrations of trasylol (see text for details).

TABLE III

AVERAGE RELEASE RATE OF ACTIVE TRASYLOL FROM 8 EVA/TRASYLOL DEVICES IN COMBINATION WITH COPPER AND PLAIN IUD'S

			COPPER IUD + EVA		PLAIN IUD + EVA	
Days of Incubation	Average hours elapsed between tests	Cummulative hours elapsed	µg of active trasylol released[a]	Cummulative µg of active trasylol released	µg of active trasylol released	Cummulative µg of active trasylol release
1	27	-	*[b]	-	*	-
2	21	48	*	-	*	-
3	26	74	74.8	-	138.0	-
4	23	97	45.0	119.8	86.3	224.3
5	28	125	27.8	147.6	91.8	316.1
6	19	144	28.0	175.6	83.8	399.9
7	26	170	22.0	197.6	100.8	500.7
10	69	239	12.8	210.4	113.3	614.0
13	71	310	11.0	221.4	79.5	693.5
16	73	383	3.0	224.4	49.0	742.5
19.5	79	462	30.0	254.4	71.5	814.0
23.5	98	560	20.3	274.7	48.5	862.5
26.5	68	628	11.8	286.5	33.0	895.5
29.5	74	702	27.0	313.5	32.5	928.0

[a]See Methodology section for details.

[b]For purposes of clarification and simplicity, figures for the first two days have been ommited since they were all high and similar for each device in both groups (copper vs. plain).

Fig.1. Average release rate of active trasylol from 8 EVA/Trasylol devices in combination with Copper (•-----•) and Plain (✱---✱) IUD's.

- Jessie C. Goodpasture

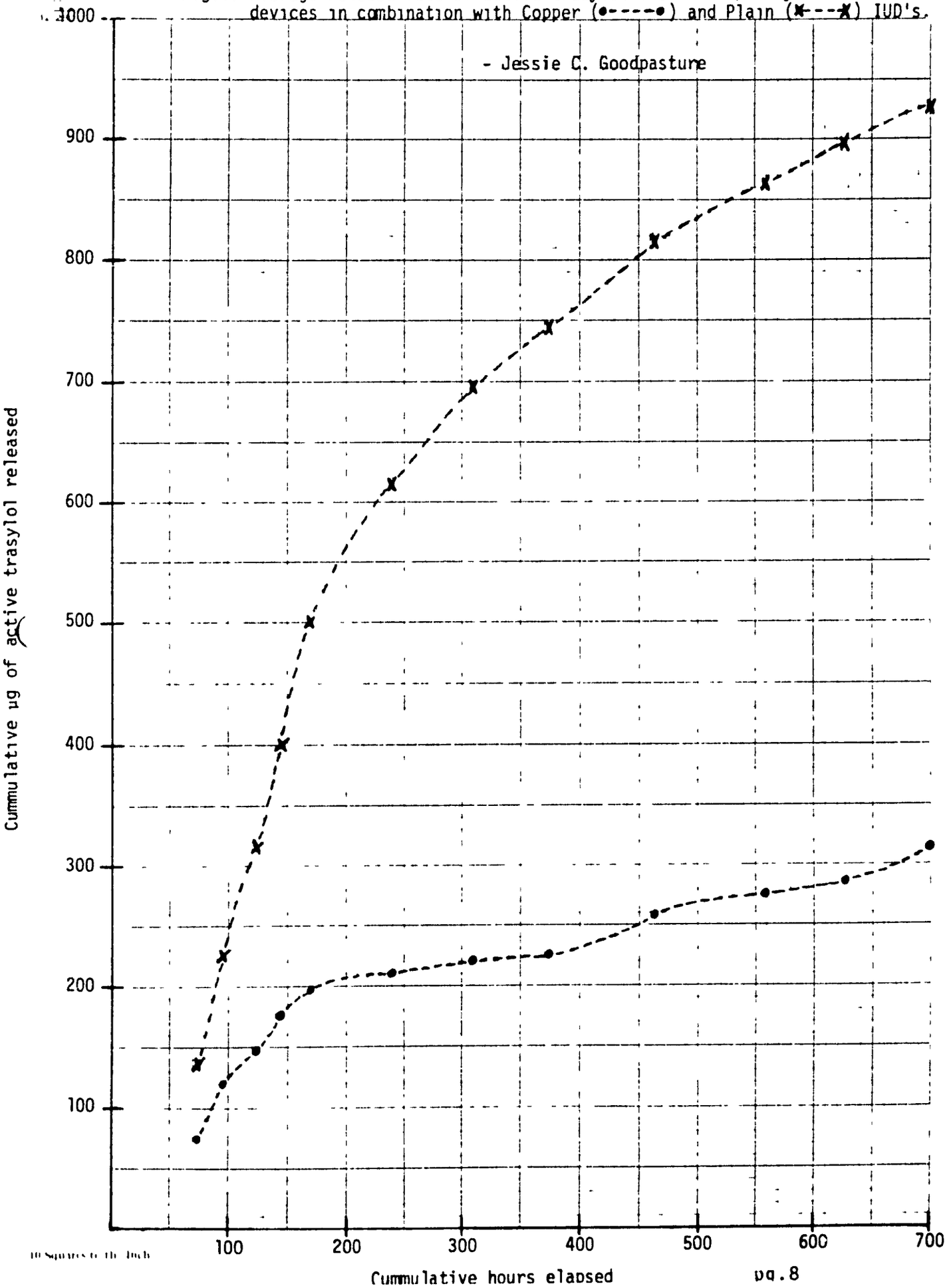

TABLE IV

EFFECT OF PLAIN AND COPPER IUD'S ON TRASYLOL ACTIVITY

Experiment[a]	Equivalent µg Active Trasylol Measured Following 3 Days of Incubation[b]	Equivalent µg Active Trasylol Measured Following 6 Days of Incubation
Copper IUD #1 + Trasylol	62	31
Copper IUD #2 + Trasylol	65	26
Plain IUD #1 + Trasylol	117	144
Plain IUD #2 + Trasylol	118	154

[a]See text for experimental details. All solutions contained 150 µg of active trasylol on day 1.

[b]See text for details regarding incubation and measurement of trasylol activity.

EN-CAS ANALYTICAL LABORATORIES

1409 J South Stratford Rd. • Winston-Salem, N. C. 27103
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LABORATORY REPORT

EN-CAS Job #: 78-051 (PO 4522-1)

Customer: International Fertility Research Program

Subject: AMCA Release from Medicated Lippes Loops No. 9043-45-II and 9043-45-S.

Experimental:

The devices were immersed in 100 ml portions of pH 7.1 physiological saline buffer at 37°C. A fine stream of air was bubbled into the solutions to provide agitation. After 7, 14, 21 and 28 days of immersion, the solutions were decanted for analysis and replaced with 100 ml of fresh buffer. A buffer blank was run along with the samples in order to monitor any interferences. AMCA was analyzed by the Fluoram fluorescence method as previously described. Visual observations of the devices were periodically made to determine whether the membrane coating remained intact during the immersions.

Results:

As shown in the accompanying table, both devices yielded quite uniform release rates. However, the release rate from the 45-II device was 2-3 times that from the 45-S device. The membrane coating remained intact throughout the study. There was no evidence of the unraveling or disintegration seen in some similarly constructed devices previously tested in our laboratory.

EXPOSURE PERIOD (DAYS)	AMCA Released (ug/day) 9043-45 II (E6278)	9043-45-S (E6279)
0-7	104	47
7-14	93	33
14-21	93	29
21-28	91	34

Approved: Charles Ganz, Ph.D., President — Date: 3/21/78

EN-CAS ANALYTICAL LABORATORIES
MONITORING • TESTING • METHODS DEVELOPMENT • CONSULTING

1409 J South Stratford Rd. • Winston-Salem, N. C. 27103
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Laboratory Report

En-Cas Job #: 78-192 (PO#4642)

Customer: International Fertility Research Program

Subject: Study of AMCA Release from five Medicated Intrauterine Devices

Experimental: The five devices were immersed in 100 ml of pH 7.1 physiological saline buffer held at 37° C. A fine stream of air was bubbled through the solutions to ensure continuous mixing. After 7, 14, 21 and 28 days of immersion, the buffer was removed for analysis and replaced with 100 ml of fresh buffer. A buffer blank was run along with the samples to monitor possible interferences.

AMCA analysis we performed by the Fluoram fluorescence method as previously described.

Results:

Exposure Time (days)	AMCA Released ug/day				
	9043-45C (E 7207)	9043-45H (E 7208)	9043-43~~H~~ W (E 7211)	9043-43X (E 7209)	9043-45BB (E 7210)
0-7	49	59	49	197	47
7-14	54	63	59	109	59
14-21	46	79	59	190	54
21-28	43	73	63	169	51

Ref: 3/147 CG

Date: 7/1/78

Approved by:

Charles Ganz, Ph.D.,
President

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LABORATORY REPORT

En-Cas Job #: Part of 78-219 (P.O.#4680)

Customer: International Fertility Research Program

Subject: Release of AMCA from Medicated EVA-Coated IUD's

Experimental: The 3 devices were immersed in 100 ml of pH 7.1 physiological saline buffer held at 37°C. A fine stream of air was bubbled through out the solutions to ensure continuous mixing. After 7, 14, 21 and 28 days of immersion, the buffer was removed for analysis and replaced with 100 ml of fresh buffer. A buffer blank was run along with the samples to monitor possible interferences.

AMCA analysis was performed by the Fluoram fluorescence method as previously described.

Each device was blotted and weighed after 28 days of exposure to determine if any weight gain had occurred.

Results:

Exposure time (days)	AMCA Released ug/day		
	78-016B E-7366	78-016C E-7367	78-016A E-7368
0-7	1616	1808	1760
7-14	401	607	401
14-21	336	427	370
21-28	261	341	273
Wt. at 28 days	757 mg	781mg	780mg

Approved by: Charles Ganz, Ph.D., President

8/17/78
Date

PROGRESS REPORT ON THE CONTRACT

IUD-TRASYLOL DELIVERY SYSTEM

Prepared for

INTERNATIONAL FERTILITY OF RESEARCH PROGRAMS

By

Milos Chvapil, M.D., Ph.D.

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1. Test if Components of the Polymerizing System Interfere with the Biological Activity of Trasylol in Trypsin Inhibition Test

2. In Urokinase Test

3. Development of Polymer for IUD-Trasylol

4. Kinetics of the Release of Trasylol from Collagen Based Polymer

IUD - TRASYLOL DELIVERY SYSTEM

REPORT ON THE COMPLETED PHASE

The Following Experiments were Performed

1. TEST IF COMPONENTS OF THE POLYMERIZING SYSTEM INTERFERE WITH THE BIOLOGICAL ACTIVITY OF TRASYLOL IN TRYPSIN INHIBITION TEST

We obtained two shipments of Trasylol

Sample 1 - approximately 150 mg Trasylol BE - KI/73

Sample 2 - 3 g Trasylol BE - Ki/73

Sample 1 was kept at least 3 months at room temperature in cloud vial. On Sephadex G-10 column it showed two peaks, the major (∿70%) at 7,000 daltons molecular weight and the smaller peak at 500 daltons, fuming ∿30% of the total area. This finding urged us to call Dr. Fried, as we felt that our sample was not homogenous This finding was reproduced.

Sample 2 showed only one peak at 7,000 daltons.

General

1) Reaction of Trasylol with HCOH was carried out with 4 mg Trasylol in .5 mls ddH_2O reacted with 0.05% HCOH (final concentration). This was vortexed for 15 min. at room temperature

2) Column purification of Trasylol + HCOH (and control Trasylol) Sephadex G-15-20 was carried on UV absorption analysis and protein (Micro-Lowry) was completed on sample before it was placed on the column (protein = 4.300 mg/ 5 mls). Only one peak was found, tested at 280 nm, 340 nm and by Micro Lowry (see graph 1)

3) HCOH was analyzed in Trasylol - HCOH sample by a colorimetric reaction. NH - acetate buffer (pH 6) and

pentanedione form color reaction with HCOH. No HCOH was found in the sample of Trasylol + HCOH after going through the column.

4) Trypsin Inhibitory Capacity (method enclosed as Appendix A)
   a Calibration curve to determine concentration of Trypsin to be used (Graph B).
   b. Trasylol inhibiting curve (on freshly weighed Trasylol - Graph C)
   c Trasylol + HCOH inhibiting curve (on freshly weighed Trasylol and freshly reacted Trasylol, as in #1 - Graph D).

Results

	Absolute 410	Average 410 nm
Control	.449 - .451	450
Trasylol	309 - 310	.3095
Trasylol + HCOH	281 - .278	2795

Trasylol + HCOH appears to inhibit better than Trasylol

Conclusion

Formaldehyde, used as a crosslinking agent in developing collagen derived polymer vehicle for Trasylol, does not affect the biological activity of Trasylol.

TRYPSIN INHIBITORY CAPACITY in the U.S. Department of Health, Education and Welfare Alpha - Antitrypsin Laboratory Manuel. Procedure by·

1) Eriksson, B.F. Studies in a antitrypsin deficiency. Acta Scand. 177 1-85 (Suppl 432), 1975.

2) Eilanger, B.F., Kokowsky, N , and Cohen, W. The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem Biophys 96 271-278, 1961

Reagents

1) 0 0025 M HCl solution
2) Trypsin - standard liquid solution from Reheis Chemical Co. (stock #024-00)
3) Benzoyl-DL-arginine-p-nitroanilicle hydrochloride (BAPNA) 435 mg to 1000 mls H_2O (dd)
4) 0.1 M Tris - 0.02 M $CaCl_2$ pH 8 2
5) 30% (w/v) HOAC

Procedure (General) - will refer to this

1) Warm reagents and samples to 25°C.
2) 2.8 mls Tris buffer into sample, standard serum and control tubes.
3) 3.0 mls Tris buffer into sample, standard serum and control blank tubes.
4) Add 15 µl samples (trasylol) to sample or standard.
5) Add 200 µl of Trypsin solution at 15 second intervals and incubate for 15 min. at 25°C (mix) (No Trypsin to blanks).
6) Add 3 0 ml of BAPNA in 15 sec intervals to all tubes (mix).
7) 10 minutes after addition of BAPNA to the first tube, add 750 µl of 30% acetic acid in 15 sec. intervals.
8) Absorbance is read at 410 nm on spectrophotomer.

To determine the concentration of Trypsin to use, follow the general procedure and set tubes up as below (step 4 not included) (standards in duplicate).

(GRAPH A)

Tube	Tube #	Tris	Sample	Trypsin*	BAPNA	Acetic
Standard	1,2	2 8 mls	--	.25 % (in 200 µl)	3 0 mls	750 µl
Standard Blank	3	3.0 mls	--	--	↓	↓
Standard	4,5	2 8 mls	--	.10 % (in 200 µl)	↓	↓
Standard Blank	6	3 0 mls	--	--	↓	↓
Standard	7,8	2 8 mls	--	.05 % in 200 µl)	↓	↓
Standard Blank	9	3.0 mls	--	--	↓	↓
Standard	10,11	2 8 mls	--	025% (in 200 µl)	↓	↓
Standard Blank	12	3 0 mls	--	--		

(GRAPH B)

Tube	Tube #	Tris	Sample	Trypsin*	BAPNA	Acetic
Standard	1,2	2 8 mls	--	05 %	3 0 mls	750 µl
Standard Blank	3	3 0 mls	--	--	↓	↓
Standard	4,5	2 8 mls	--	.025%	↓	↓
Standard Blank	6	3 0 mls	--	--	↓	↓
Standard	7,8	2 8 mls	--	.02 %	↓	↓
Standard Blank	9	3 0 mls	--	--	↓	↓
Standard	10,11	2.8 mls	--	01 %	↓	↓
Standard Blank	12	3 0 mls	--	--		

* Percentage in final concentration is 200 µl.

Trasylol inhibitor curve

1) Mix fresh Trasylol to form a calibration curve
2) Follow instructions from general procedure.
3) Tubes set-up as follows (standards in duplicate).

(GRAPH C)

Tube	Tube #	Tris	(Trasylol) Sample Final Concentration	Trypsin	BAPNA	Acetic
Control	1,2	2.8	--	200 µl	3.0 ml	750 µl
Control Blank	3	3.0	--	--		
Standard	4,5	2.8	1 µg in 15 µl	200 µl		
Standard Blank	6	3.0		--		
Standard	7,8	2.8	10 µg	200 µl		
Standard Blank	9	3.0		--		
Standard	10,11	2.8	15 µg	200 µl		
Standard Blank	12	3.0		--		
Standard	13,14	2.8	25 µg	200 µl		
Standard Blank	15	3.0		--		

Trasylol and HCOH inhibitor curve

1) Mix fresh Trasylol and add 0.05% HCOH Mixing is completed by vortexing for 15 min at room temperature.
2) Follow instruction in general procedure.
3) Standards in duplicate
4) Tubes set up as follows

(GRAPH D)

Tube	Tube #	Tris	(Trasylol + HCOH) Sample Final Concentration	Trypsin	BAPNA	Acetic
Control	1,2	2 8	--	200 µl	3 0 ml	750 µl
Control Blank	3	3.0	--	--		
Standard	4,5	2 8	1 µg in 15 µl	200 µl		
Standard Blank	6	3.0		-		
Standard	7,8	2.8	10 µg	200 µl		
Standard Blank	9	3 0		--		
Standard	10,11	2.8	15 µg	200 µl		
Standard Blank	12	3.0		--		
Standard	13,14	2 8	25 µg	200 µl		
Standard Blank	15	3.0		--		

Run Column Samples

1) Trasylol (dilute 1-10) (∿12.18 µg protein run in test).
2) Trasylol + HCOH (dilute 1-10) (∿11.9 µg protein run in test).
3) Follow instruction in general procedure
4) Set-up test tubes as follows (duplicates)

Tube	Tube #	Tris	Sample	Trypsin	BAPNA	Acetic
Control	1,2	2 8 ml	--	200 µl	3.0 ml	750 µl
Control Blank	3	3 0 ml	--	--	↓	↓
Trasylol Standard	4,5	2.8 ml	15 µl	200 µl	↓	↓
Blank	6	3.0 ml	↓	--	↓	↓
Trasylol + HCOH	7,8	2 8 ml	↓	200 µl	↓	↓
Blank	9	3 0 ml	↓	--	↓	↓

	Tube #	Results	Average Absolute 410	Protein
Control	1	.449	450	
	2	451		
Trasylol	4	309	.3095	∿12.18
	5	310		
Trasylol	7	.281	.2795	∿11.9
+ HCOH	8	.278		

Trasylol + HCOH appears to inhibit better than Trysylol

K&E 10 X 10 TO THE INCH 46 0700
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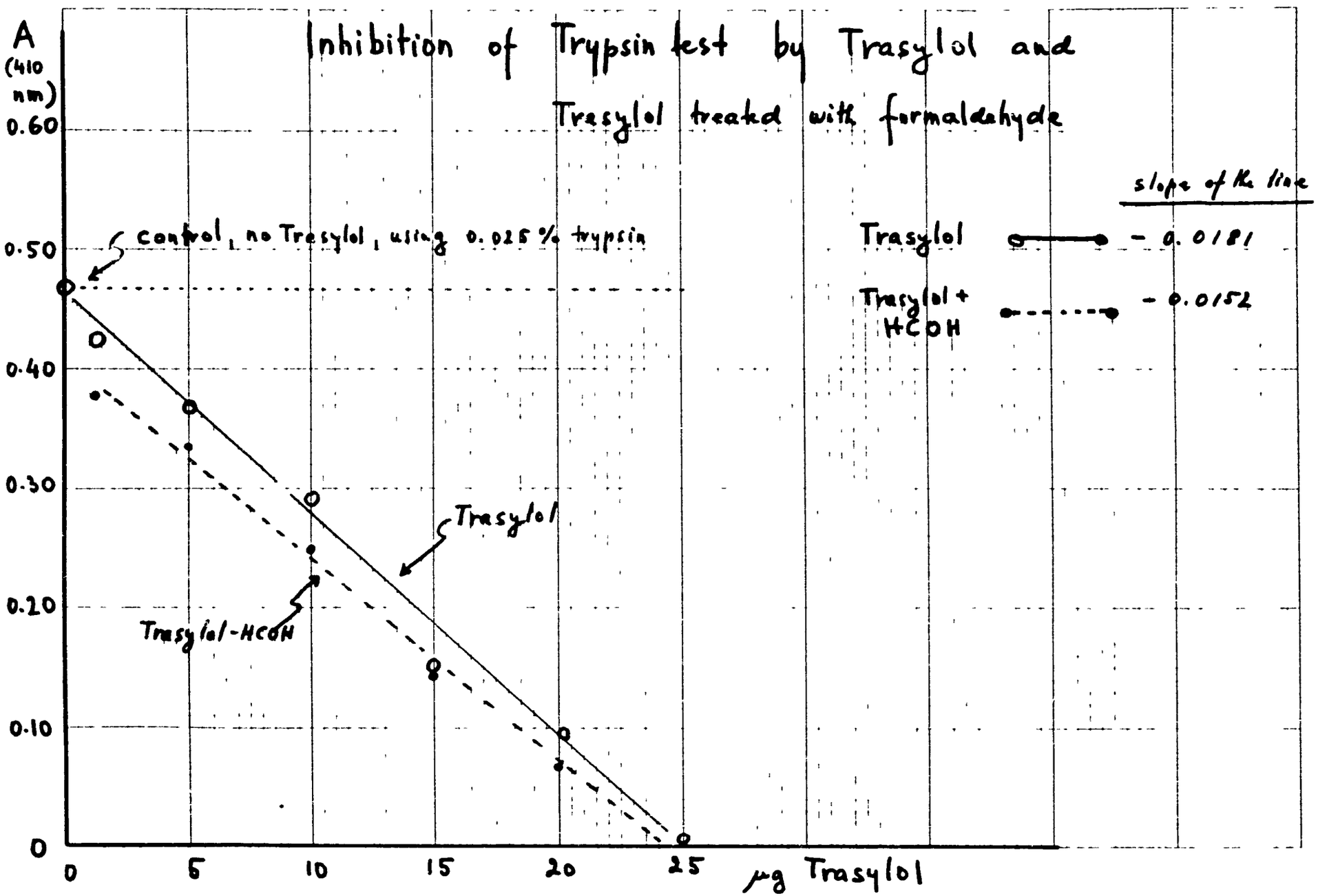
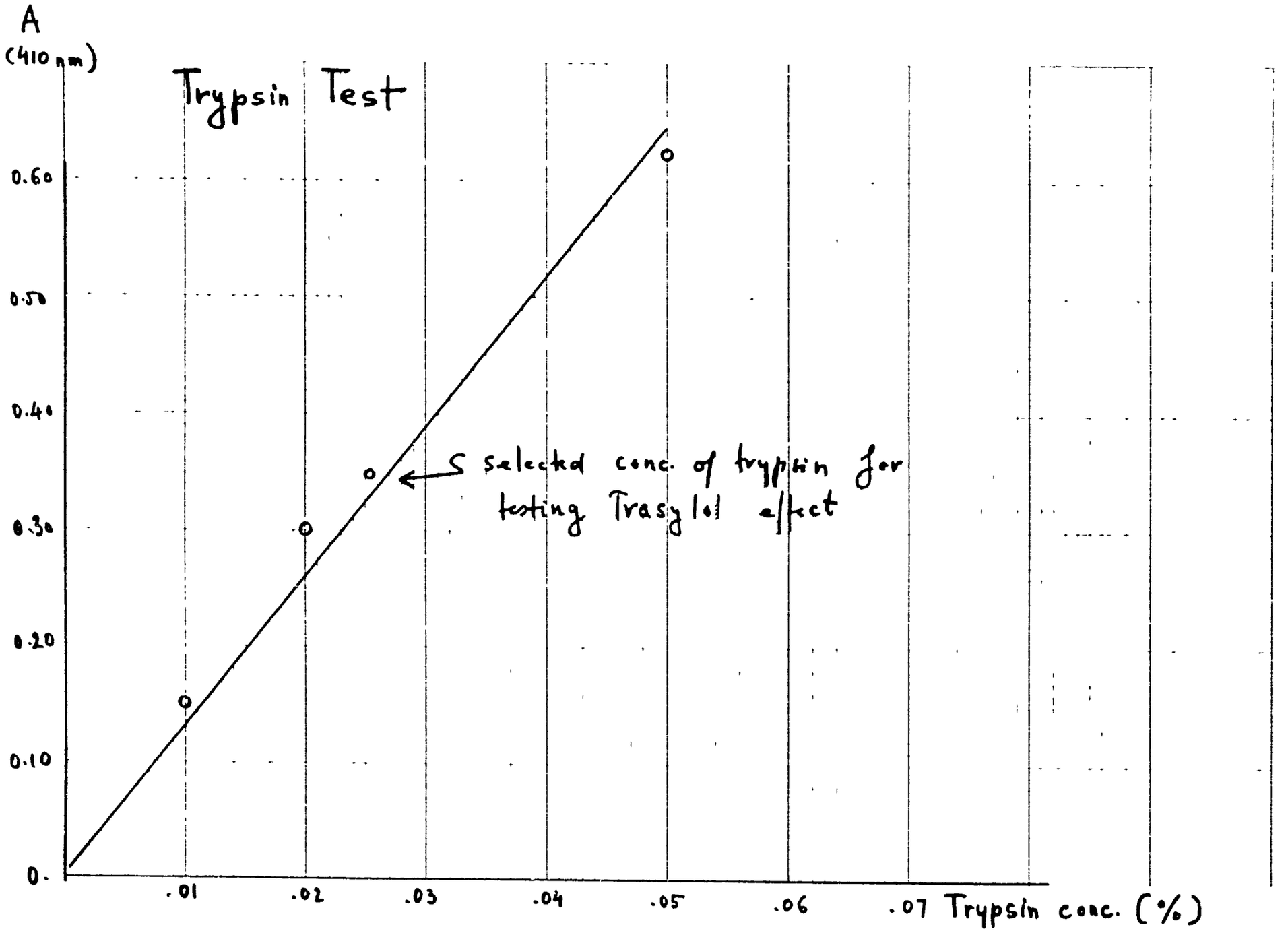


46 0700


A
(410 nm)
Trypsin Test
0.60
0.50
0.40
0.30
0.20
0.10
0.
.01
.02
.03
.04
.05
.06
.07
Trypsin conc. (%)
selected conc. of trypsin for testing Trasylol effect

2. In Urokinase Test

Q What is the concentration of Trasylol that inhibits the lytic activity of urokinase on fibrin plates by approximately 50%?

M/M Urokinase stock solution [233 0u/ml] in saline (UK*)
Trasylol [20mg/100ml] in saline (T*)
Urokinase-trasylol solutions

0.25μg = 0 05ml T* + 1.0ml UK* + 2 95ml saline
0.50μg = 0 10ml T* + 1.0ml UK* + 2.90ml saline
1.00μg = 0.20ml T* + 1.0ml UK* + 2.80ml saline

Urokinase solution [58 2u/ml] in saline
Standard fibrin paltes

Place 0 1ml UK solution and Trasylol-UK solutions on plates (n=9).
Incubate in humid incubator (37°) for 24 hours.
Determine lytic areas by plarimetry.

R

AGENT	LYTIC AREA (mm^2)	PER CENT INHIBITION
5.82u UK	1753	N/A
5 82u UK + 0 25μg T	1140	65 1
5.82u UK + 0 50μg T	808	46.1
5.82u UK + 1 00μg T	0	100 0

1.0μg Trasylol completely inhibits the activity of 5.82u urokinase. Lower concentrations appear to inhibit the activity in a dose related manner. The concentration 0 50μg causes approximately 50% inhibition and can be used in further studies.

On all plates that had lytic activity, there was a small island of unlysed fibrin either floating in the lysed clot or attatched lightly to the plate This probably represents an interaction of trasylol and fibrin resulting from the application of the trasylol-urokinase solution to the fibrin plates.

Trasylol-urokinase solutions

Dilute Trasylol 20mg/100ml (0.2mg/ml) with normal saline.

0.05ml (0.01mg Trasylol) to 1.0 ml= Test 1

0.10ml (0.02mg Trasylol) to 1.0 ml= Test 2.

0.20ml (0.04mg Trasylol) to 1.0 ml= Test 3

To 1, 2, and 3 add 1ml urokinase solution (233 u/ml) and 2ml normal saline. Final concentrations are

1 2.5 g Trasylol + 58.2 u UK/ml

2. 5.0 g Trasylol + 58.2 u UK/ml

3 10.0 g Trasylol + 58.2 u UK/ml

Dispense 0.1ml of each solution on fibrin plates. Also dispense 0.1 ml urokinase (58.2 u/ml) on fibrin plates.

Quantities per plate are

UK. 5.82 u

1. .25 g T + 5.82 u UK

2. .50 g T + 5.82 u UK

3 1.0 g T + 5.82 u UK

Q· What are the effects of various concentrations of glutaraldehyde on the lytic activity of urokinase?

M/M Urokinase stock solution [233.0u/ml] in saline (UK*)
Urokinase solution [58.2u/ml] in saline
Glutaraldehyde [1.25%] in saline (G*)
Glutaraldehyde-urokinase solutions

0.01% = 0.025ml G* + 0.5ml UK* + 1.475ml saline
0.02% = 0 050ml G* + 0.5ml UK* + 1 450ml "
0.04% = 0 100ml G* + 0.5ml UK* + 1.400ml "
0.08% = 0 200ml G* + 0 5ml UK* + 1.300ml "
0.10% = 0 250ml G* + 0.5ml UK* + 1.250ml "

Standard fibrin plates

Place 0.100ml urokinase solution and Glutaraldehyde-urokinase solution on plates (n=2)
Incubate in humid incubator (37°) for 24 hours.
Determine lytic area by planimetry.

R

AGENT	LYTIC AREA (mm^2)	% INHIBITION
5.82u UK	1753	N/A
0.01% G in 5 82u UK	0	100
0.02% G in 5.82u UK	0	100
0.04% G in 5.82u UK	0	100
0 08% G in 5 82u UK	0	100
0.10% G in 5.82u UK	0	100

Glutaraldehyde completely inhibits the activity of 5.82 u urokinase at all concentrations tested. It cannot be utilized in the present model.

Q· What are the effects of dialysed trasylol and formaldehyde-treated dialysed trasylol on the activity of urokinase?

M/M Dialysed Trasylol and dialysed HCOH-Trasylol [5mg/ml] as received were diluted to 0.2mg/ml with saline
Solutions were prepared of both dialysed trasylol and dialysed HCOH-trasylol such that the final concentration of trasylol would be 5μg/ml in urokinase [58.2u/ml]

0.1ml of both solutions and 0.1ml of straight UK [58.2u/ml] were placed on standard fibrin plates

Areas of lysis were noted after incubation for 24 hr at 37°

R 1)

AGENT	LYSIC AREA (mm^2)	% INHIBITION
5.82u UK (n=3)	1556	N/A
0.5μg dialysed Trasylol in 5.82u UK (n=10)	1615	no inhibition
0.5μg dialysed HCOH-Trasylol in 5.82u UK (n=9)	1398	10

Evidently, dialysis removed all trasylol from the sample not treated with formaldehyde. A small and probably insignificant amount of inhibition is noted for the formaldehyde treated trasylol. It is not possible to attribute this to the activity of trasylol in view of the absence of such activity in the control sample.

2) Extracted trasylol and HCOH trasylol

5.82u UK (n=3)	1410	N/A
0.5μg Extracted trasylol in 5.82u UK (n=6)	756	53.6
0.5μg Extracted HCOH-trasylol in 5.82u UK (n=6)	717	50.9

The treatment of trasylol with formaldehyde does not significantly alter the inhibitory effects of the substance on the lytic activity of urokinase.

3. Development of polymer for IUD - Trasylol

The optimal polymer should meet the following requirements

1) Biodegradable for a period of 4^{-5} months (which would give reasonable release of the drug during requested 100 days post implantation).
2) No swelling of the final product above the requested 4 mm.
3) Sufficient mechanical strength.
4) Controlled delivery of known quantity of the drug within 100 days post implantation.

Procedure - a total of seven (7) various polymer mixtures were studied. The variables were

1) Concentration of gelatin.
2) Presence of glycerin - to keep pliability
3) Addition of fibrous collagen - to obtain enough tensile strength.
4) Degree of tanning with formaldehyde or glutaraldehyde.

It was found that optimal mechanical and swelling characteristics has a mixture containing:

65% collagen fibers

35% polymerizing solution with glycerin

The final polymer (7251) does not swell in physiological media as shown in the table.

Time (hr)	Weight (g)	Diameter of the Tubing (mm)
0	0.287	4.10
1.0	0 296	4.13
1.5	0 297	4.13
18.0	0.297	4.13

Incubated in 0.9% saline at 37°C.

4 KINETICS OF THE RELEASE OF TRASYLOL FROM COLLAGEN BASED POLYMER

Aim of the study

1 Prepare polymer with Trasylol

2 Study the rate of the release of Trasylol from the polymer incubated in saline at 37° without starting

3. Study the release of Trasylol, starting with 6 hours of incubation, then at daily intervals for a total of 14 days.

Measurements

1. Trypsin inhibition test

2. Micro Lowry protein

3 Hydroxyproline release

While test 1 & 2 should indicate the release of Trasylol, parameter 3 refers to degradation (solubilization) of the collagen based polymer in the incubation medium

Methods

1 Preparation of Polymer-Trasylol in the form of 4 mm thick tubing with a 2 mm central opening

3 g collagen paste is mixed with 2 ml solution B to form a homogenous viscous gel Add 20 mg Trasylol and mix thoroughly The polymerization is initiated with 0.2 ml 25% glutaraldehyde This paste was injected through a syringe in a mold of a tubing. Polymerization was completed in 60 - 90 minutes

We obtain 37 cm long tubing having a wet weight of 5.0 g and a water content of 78%.

2. Incubation procedure

A .4 g segment of the collagen polymer-Trasylol tubing was incubated at 37° in 25 ml of 0.9% NaCl under sterile conditions. 0.5 ml volume samples were collected after 6 hours of incubation and then at daily intervals upto 14 days. Theoretically, there should be 5 6 mg Trasylol present on the incubated sample

Results

1. Release of protein into the incubation medium (Figure 1)

The determination of protein by Micro-Lowry procedure showed that, from the theoretical 5 6 mg Trasylol present in the incubated polymer, 1.4 mg was eluted during the 14 days. This represents 25% release of the active substance in 14 days. The release was continuous and linear with time of incubation

The analysis of protein on the eluate by Micro-Lowry met with several difficulties which I would like to report in case somebody reproduces our results Both glutaraldehyde and resorcinol interfere with Lowry procedure, both giving positive reaction and contributing to the colour formation For these reasons, we obtained excessive values of protein released

In order to eliminate the positive interference of both glutaraldehyde and resorcinol, we absorbed these compounds to Sephadex G-15 according to the procedure of Neal and Florini (Anal. Biochem. 55 328, 1973) In principle, swollen Sephadex G-15 in 0.1 M ammonium sulfate is mixed with the eluent. The eluent is disabled and protein (Trasylol) recovered after spinning the system. The interfering substances are trapped within Sephadex matrix.

2 Release of collagen into the medium (Figure 2)

The incubation medium poorly mimics the situation of the IUD-Trasylol in the uterus environment The presence of inflammatory cells, granulocytes and macrophages, in the uterus with the IUD should enhance the rate of collagen polymer degradation

In our model system, 800 μg of hydroxyproline was released from the polymer This amounts to 5 9 mg collagen (Hyp x 7 34 = collagen) Assuming that the polymer tubing (2 4 g weight and having 22% solids) contains approximately 528 mg collagen protein, the amount of dissolved collagen would be between 1 to 2%

3 Trypsin inhibition test

Knowing, from Lowry protein determination, that quantities amounting to tens of milligrams of protein (globular) were released from the collagen polymer, we used only 15 μl aliquots of the incubation medium for the very sensitive trypsin inhibition test The sample was analyzed as such, without any additional treatment

The results are summarized in Table 1

TABLE 1

TRYPSIN INHIBITION TEST TUBING
THE RELEASE OF TRASYLOL FROM COLLAGEN POLYMER

Time of Incubation Days	% Inhibition
1	0 0
3	1.48
5	1 82
7	2.36
10	3.82
12	3 28
14	4 01

The results showed a certain increasing trend indicating that more Trasylol is present in the eluate with a duration of the incubation.

Conclusion

Trasylol is released from the collagen polymer tubing, 25% of the drug being eluted in 14 days At the same time there is a minimal change in collagen polymer structure. The released drug was biologically active, as indicated by Trypsin inhibition test.

Fig. 1

Rate of the release of the globular protein from Trasylol-polymer

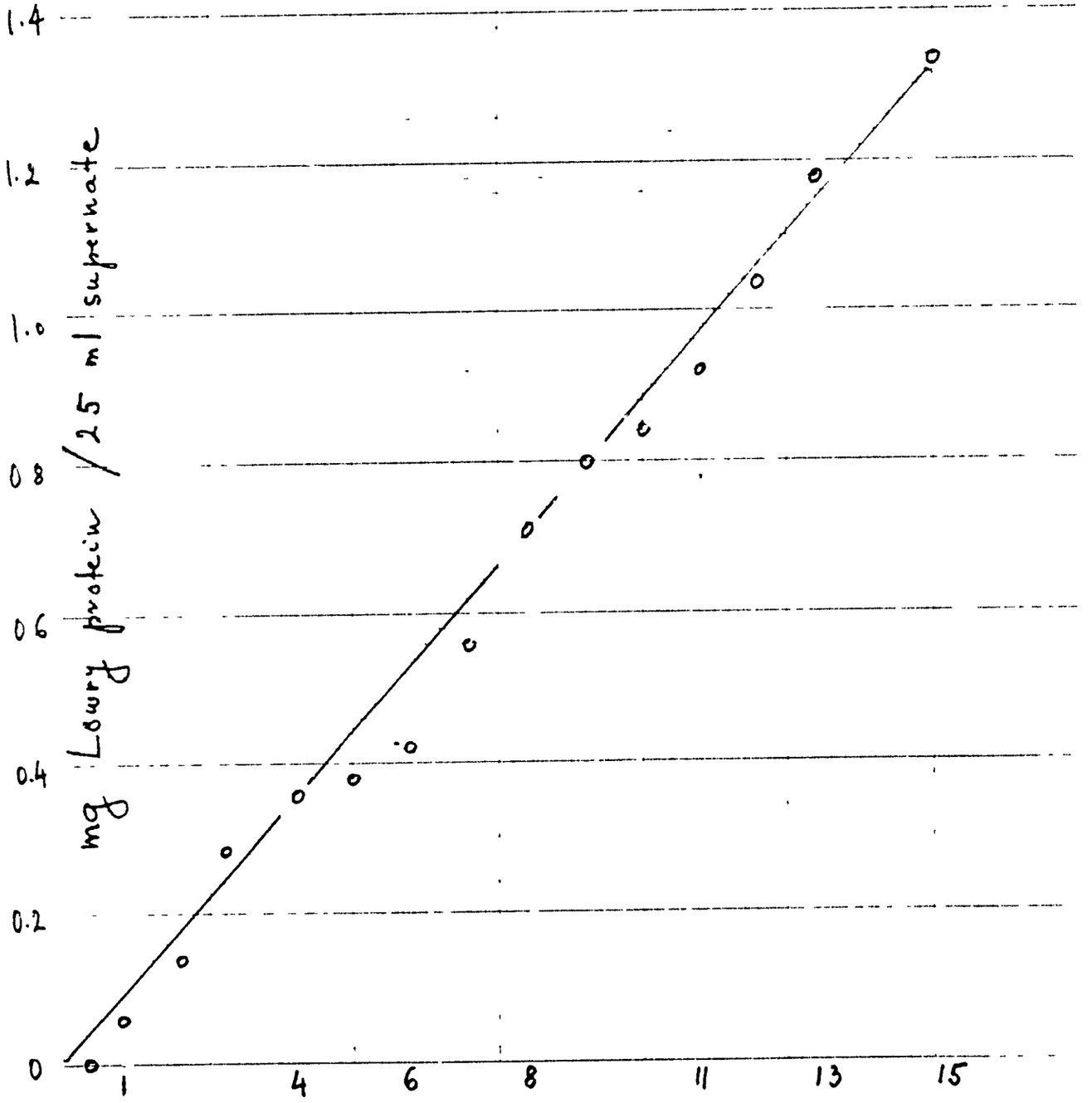


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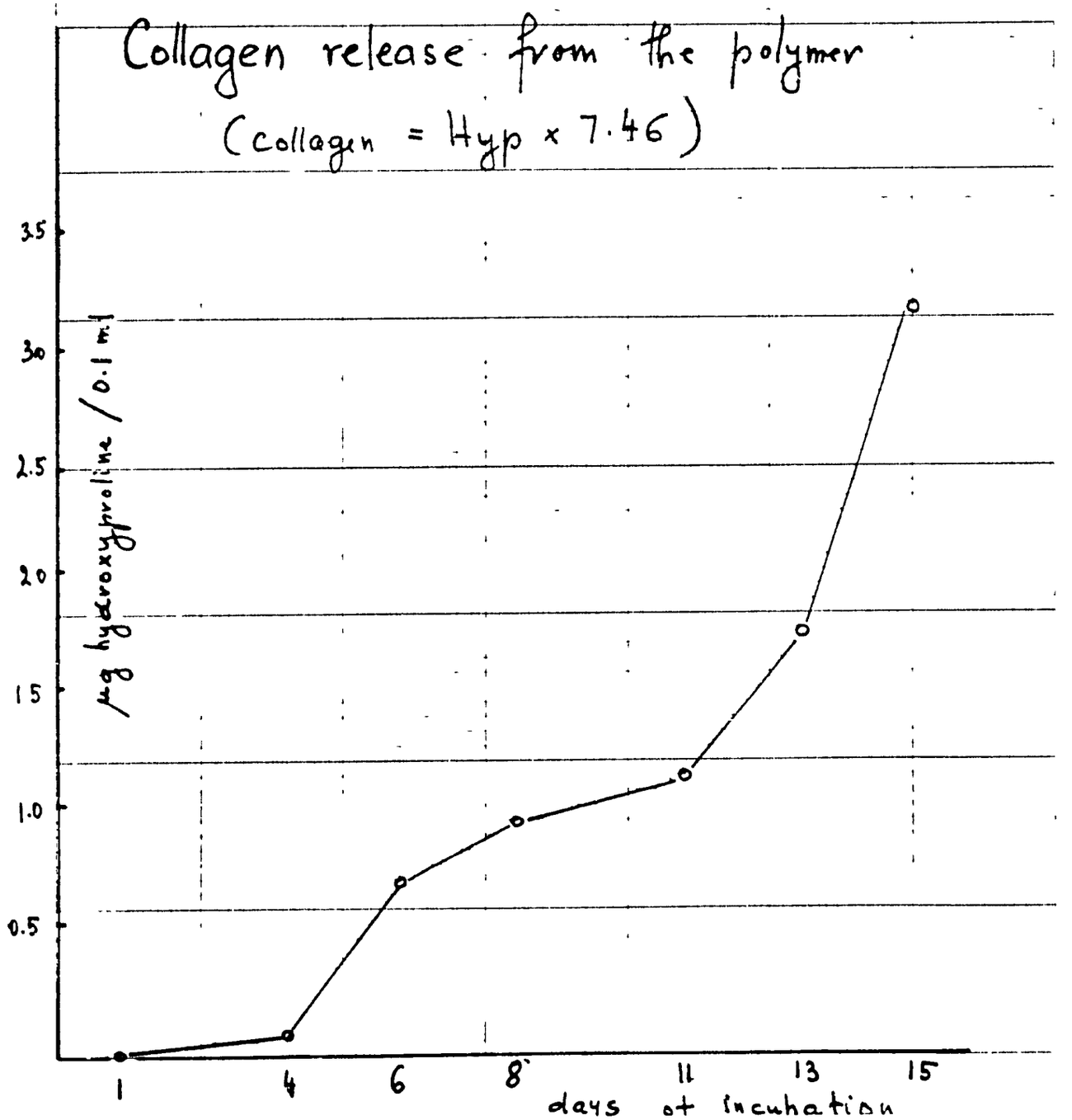
Fig. 2.


Collagen release from the polymer
(collagen = Hyp x 7.46)
μg hydroxyproline / 0.1 ml
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1
4
6
8
11
13
15
days of incubation

46 0700

IUD & BIODEGRADABLES
Abstract Form
Deadline: October 1, 1978

Please complete and return to:

Dr. E.S.E. Hafez
Department of YN/OB
Medical Re[illegible] Building
Wayne State [illegible]ersity
550 E. Canfield
Detroit, Michigan 48201, U.S.A.

DECISION GUIDE FOR SELECTING IUDS FOR INDIVIDUAL ACCEPTORS

R G. Wheeler
International Fertility Research Program, Research Triangle Park, North Carolina 27709, U.S.A

Providers of contraceptive services can improve the use effectiveness of IUDs by selecting subjects and IUDs according to a decision system based on cluster analysis. The general principle is illustrated by using continuation rate as the dependent variable and IUD acceptor attributes recorded on the IUD study admission form as the independent variables

Computers were used to classify women into groups which had unique combinations of attributes and groups which had unique average continuation rates and variances The Searching For Structure computer program, developed by the Department of Social Sciences, University of Michigan, was used to make a cluster analysis on several thousand IUD admissions and follow-up records at the International Fertility Research Program The result is a decision tree for selecting IUDs for individual acceptors

The Provider is guided along the first branch of the decision tree by selecting one of the two possible answers to a series of questions The question and branching process continues until an end category is reached This process requires from three to ten questions, depending on the path taken At the end of the last branch the provider will find the predicted event, rates, and standard deviations for several types of IUDs

The convenience of only one prediction tree for all events offsets any improvement in the standard error of estimate that would be obtained with a separate tree for each event Knowledge of the expected outcome of IUD contraception will enable the provider to select an IUD with the highest probability of continued effective use.

SALARIES

AID/pha-C-1111

Name and Position	4-1-78 to 9-30-78	Adjustments	4-1-78 to 9-30-78
Laufe, L, Medical Director	6238.98		6238.98
Wheeler, R., Bioengineer	19228.08		19228.08
Greenhill, B., Laboratory Assistant	5119.56		5119.56
Friel, Pat, IUD Project Manager	9008.52	(768.13)	8240.39
Barnes, E., Secretary	5200.08	(385.00)	4815.08
Smith, A., Parttime Assistant	1319.56		1319.56
Other	---	961.38	961.38
Total	46114.78	(191.75)	45923.03

CONSULTANCY

AID/pha-C-1111

4-1-78 to 9-30-78

Name	Days Worked		Fees Paid
Brenda Sawyer	32 days @ $43.85/day	-	$ 1,403.20
Jaime Zipper	17.5 days @ $100/day	=	1,750.00
Gebhard Schumacher	1 day @ $150/day	=	150.00
Antonio Scommegna	1.5 days @ $155/day	=	232.50
Lourens Zaneveld	1.5 days @ $109/day	=	163.50
	Total		$ 3,699.20

EXPENDITURES

AID/pha-C-1111

Twelve-month Period Ending September 30, 1978

Category	Year-to-date 10/77 - 9/78
Salaries and wages	$ 90.779.00
Fringe benefits	20,943.00
Overhead	58,814.00
Consultants	9,951.00
Travel	8,090.00
Equipment	6,126.00
Expendable supplies and other direct expenses	50,608.00
Subcontracts	13,646.00
Publication costs	920.00
Occupancy	4,410.00
	$ 264,287.00