

PROGRESS REPORT

Contract for: Research Services directed toward the development
of a combined agent for Disease Prophylaxis and Contraception.

Contract No. AID/csd - 2822

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June 30, 1970 - December 31, 1970

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OBJECTIVES

As under the terms of the contract, the project is striving to formulate a dual purpose vaginal preparation which would have properties of both prophylaxis against venereal disease and certain other genital infections and contraception for the female and prophylaxis for the male.

PROGRESS

In meeting these objectives, two lines of activities have been followed.

Realizing that once the vaginal product is developed, it must be field tested both in the United States and abroad, a working relationship has been developed with the Allegheny County Health Department and the Pittsburgh Shadyside Free Clinic. The cooperation and assistance of Dr. Hugh B. Robins, Director of the County Health Department, and Mr. H. Balisky, Director of the Venereal Disease Clinic, has been obtained. The County Health Department is currently supplying N. gonorrhoeae cultures from their clinic patients for our laboratory testing. Several meetings have been held with the Health Department staff concerning the feasibility of conducting a questionnaire study of the female clinic patients. This questionnaire would be designed to yield useful information to both the clinic and the project in terms of developing both a population for the clinical testing and one in which techniques of motivation for use can be developed. Determining the behavior and attitude of these women towards VD and contraception would be especially helpful. We feel this is important because they would probably represent a large percentage of the users of our product. Once this questionnaire has been completed and the confidence of the patients has been won, it is expected to be possible to utilize the clinic as a milieu for testing our product.

In the same respect, liaisons have been initiated with Mr. John Pfahler, Medical Coordinator and Head of the Shadyside Free Clinic. This clinic is

run strictly by volunteer help. Dr. B. Singh has been assisting in this clinic carrying out comparative studies on various techniques for diagnosis of gonorrhoea in preparation for future field work in the epidemiologic studies of the products to be field tested. At the same time he is establishing the professional contacts which will provide the basis for utilizing the clinic as a site for field testing. He has found a significant percentage of VD in this clinic population. Since this clinic attracts a different clientele than the County Health Department Clinic, it can provide the opportunity for testing our product on a broader population.

Our main emphasis thus far, however, has been on perfecting laboratory testing techniques, and methodologies.

An extensive review of the literature on prophylaxis, testing procedures, and other aspects of venereal disease has been compiled (Appendix A).

We attempted to establish contact with the various drug companies which produce vaginal contraceptives and antiseptics which the project is expecting to review. We sent letters to each pharmaceutical company stating the purpose and objectives of our study requesting their cooperation. Most of the companies responded promptly, sending free samples of their products for our laboratory tests. Any products we could not obtain in this manner, we have purchased through our contract on the open market.

Thirty-nine different vaginal products have been obtained to date and tested for their efficacy against N. gonorrhoeae (Appendix C), T. pallidum (Appendix B), and C. albicans (Appendix D).

The standard microbiological methodologies for testing these products were followed. The slide immobilization test for T. pallidum (Table I), time exposure technique for N. gonorrhoeae (Table II), and C. albicans (Table III), and the standard plate dilution techniques also for C. albicans (Table IV) were used.

In testing the efficacy of these various products against T. pallidum, each product was diluted to 50, 20, 10, and 1% of its original concentration. A drop of the test solution was placed on a slide next to a drop of T. pallidum suspension. The time required by the test solution to immobilize the spirochetes was measured through microscopic examination (Table 1).

Solutions of N. gonorrhoeae and C. albicans were exposed to varying concentrations of diluted test samples for 1, 5, and 10 minutes. After exposure the organisms were plated out on standard media and read 48 hours later for inhibition of growth. Results of these tests are in Tables 2 and 3.

The effectiveness of varying concentrations of these products was also tested when incorporated in the media against 10^5 to 10^6 microorganisms of C. albicans. Each dilution was plated out on media containing 50, 20, 10, and 1% concentrations of test samples. These plates were then read for inhibition of growth 48 hours later (Table 4).

We have also begun to test certain preparations for their spermicidal abilities (Appendix E). In this test varying concentrations of the test material are mixed with semen, until the highest concentration which will immobilize the spermatozoa in 20 seconds is determined.

Certain aspects of the laboratory work have proceeded less rapidly because of delays in procurement of supplies and equipment occasioned by the necessity for following AID contractual requirements for procurement. Although the contract was effective as of July 1, it was not executed until July 28. This delay in execution prevented the purchase of supplies for nearly one month which greatly impeded laboratory progress. A two month wait for animal cages due to delay in obtaining clearance from the AID contracting officer followed by a quarantine of the animal quarters due to suspected TB exposure has also slowed the "tooling up" of the laboratory. Progress was also slowed because of the lack of certain organisms in stock cultures in the collection of the

usual suppliers. This lack necessitated an unexpected search for other suppliers. The laboratory is now equipped with the basic essential materials for testing, and work is proceeding according to the original plan.

With delivery of the rabbit cages in December 1970, the move will be made to test certain preparations in vivo which have been shown to have promise in vitro.

Work is now proceeding to develop experimental protocols and procedures appropriate to the materials to be tested following, in general, the procedure utilized in the VDRL for prophylactic studies.

FUTURE PLANS

The preliminary in vitro findings have shown in various contraceptives and "vaginal antiseptic" agents a degree of activity higher than anticipated. This suggests the feasibility of starting clinical studies somewhat sooner than originally planned.

Discussions with the World Health Organization staff and with health officials of several countries are expected to be carried out to clearly establish the fact that because of political considerations, it will be necessary to carry out precedent as well as concurrent parallel studies within the U.S.A. For reasons already well discussed, the plans for field testing against genital and venereal infections in man call for establishment of baseline data on incidence and prevalence in certain well-defined population groups. These techniques, laboratory, epidemiologic, sociologic, and administrative are being developed in order to establish this baseline data.

CURRENT NEEDS IN THE FIELD

At the recent board meeting of the American Social Health Association, the national voluntary association in the United States dealing with venereal disease, there was considerable discussion of the ways and means by which it might be possible to deal with the resurgence of gonorrhoea in the United States. While it has been possible through applied public health methods to reverse the trend of increase in syphilis, it has been impossible to do anything in gonorrhoea. Thus the rates have been showing increases of up to 10% per year over the past few years. It is anticipated that this trend will continue throughout the U.S.A. and the world. It was the consensus of the Board that it has become essential to begin to approach the gonorrhoea problem as one requiring cooperation of the patient. This cooperation will require the use of prophylaxis.

In view of these developments, it appears that a National VD Advisory Board will be established by the Public Health Service, the Department of HEW and the American Social Health Association according to present plans. It is felt very probably that one of the earliest recommendations will be to reintroduce the concept of prophylaxis into venereal disease control activities. At this stage of thinking it is anticipated that this will call for a major health education effort utilizing not only the condom, but other preparations which have in the past and hopefully in the future will be effective prophylactic agents.

In reporting on our pro-con studies it was the reaction of the Board that if such a preparation can be made available, it would be a most important element in an augmented national program undertaken in the U.S.A. The Board also in view of its relationships with the International Union against Venereal Disease and the ~~treponematoses~~ has recognized the international implications of such a preparation and has indicated its hope that it may be possible at an early

date to count on effective preparations that can be put into venereal disease control programs.

J. C. Cutler is a member of the Board of the American Social Health Association and a member of the VD advisory committee so that the Association has been well briefed on the progress of the project to date.

At the appropriate time and with appropriate health educational techniques, the pro-con preparation to be tested will be introduced and followed.

The regulations of FDA will be followed, and steps have been taken to ascertain what requirements must be met so as to be able to design clinical work in accord with these official requirements.

It now appears that clinical work can be started within the very near future and discussions have been inaugurated with AID so as to be able to work out the method of financing the necessary local staff.

APPENDIX A
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APPENDIX B

METHOD OF TESTING TEST SAMPLES vs SYPHILIS SPIROCHETES

Treponema pallidum (Nichol) were harvested from infected rabbits using standard techniques. Suspensions of T. pallidum were made in saline solution mixed with 20% rabbit serum. The number of spirochetes were examined and counted per dark-field (40x). Each test sample was diluted to varying concentrations with sterile water. In preliminary screening, one drop of the test dilution was placed on a microscope slide next to one drop of the T. pallidum suspension and the drops mixed with an applicator. The time required to immobilize the spirochetes was noted microscopically and recorded. Preliminary findings were confirmed by a more quantitative method in which 0.5 ml of spirochete suspension was mixed with 0.5 ml of test dilution in a test tube. At various intervals microscopic examination was made of this mixture. Proper controls were used in all these tests.

APPENDIX C

METHOD FOR TESTING TEST SAMPLES vs NEISSERIA GONORRHOEAE

In testing the efficacy of test samples against Neisseria gonorrhoeae a standard suspension of N. gonorrhoeae was made in sterile saline or Trypticase Soy Broth by measuring the percent transmission on a spectrophotometer. Each test sample was diluted to varying concentrations using sterile water. To 1 ml of diluted test sample 0.1 ml of bacterial culture containing 10^5 to 10^6 organisms was added and mixed. After intervals of 1, 5, and 10 minutes, a chocolate agar plate was inoculated, using a 3 mm loop. The plates were incubated in a CO_2 incubator and read 24 to 48 hours later. Proper controls were used, and the bacterial growth was confirmed by the oxidase reaction and gram stain. The effect of pH was also considered in these experiments.

APPENDIX D

TEST METHOD FOR CANDIDA ALBICANS

In testing samples for efficacy against C. albicans, two methods were used, the first method was the same as that used for N. gonorrhoeae with the exception that Sabourand Maltose agar plates were used and incubated at 37° instead of chocolate agar plates. In the second method 1.0 ml quantities of diluted test samples were mixed with 10 ml of Sabourand Maltose agar before preparing the plates. After the plates solidified, they were inoculated with C. albicans standard culture (10^5 to 10^6 organisms / 0.1 ml) using a 3mm loop. The plates were incubated at 37° and read after 48 hours.

APPENDIX E
TEST FOR SPERMICIDAL QUALITY

The spermicidal quality of the contraceptives and vaginal antiseptics was determined by using the Modified Sander-Cramer Dilution Test. Under this method the test material is diluted to varying concentrations with a saline solution. 0.5 ml of this dilution is then mixed with 0.1 ml semen and examined microscopically under high dry power (40x). The greatest concentration of material capable of immobilizing the spermatozoa within 20 seconds is determined. Results are then confirmed by examining five fields for sperm motility. All the contraceptives and vaginal antiseptics which are found to be effective will be tested further by using the International Planned Parenthood Agreed Test for total spermicidal power.

The source of spermatozoa is human semen obtained through volunteers payed a nominal fee. This approach has been approved by the University Committee on Human Experimentation.

TABLE I

Screening of Test Material for Efficacy against *T. pallidum*

| Test Material * | Slide Immobilization Test | |
|----------------------------|---|-----------------------------------|
| | Lowest Effective Concentration (Percent) | Time To Immobilize (Minutes)** |
| Betadine Vaginal Gel | 10 | 1.5 |
| Candepatin Vaginal Tablets | 10 | 2.0 |
| Certane Vaginal Jelly | 10 | 1.5 |
| CN 23577 | 10 | 4.5 |
| CN 35458 | 10 | >5.0 |
| CN 59895 | 10 | >5.0 |
| CN 60563 | 10 | >5.0 |
| CN 60684 | 10 | >5.0 |
| CN 67013 27 | 10 | 1.5 |
| CN 7828 | 10 | >5.0 |
| CN 83402 B | 0.1 | 1.5 |
| Contra Creme | 20 | 1.5 |
| Cooper Creme | 10 | 1.5 |
| Cortone Acetate | 20 | 1.5 |
| Delfen Foam | 10 | 1.5 |
| Emko Concentrate | 10 | 1.5 |
| Emko Concentrate + agent A | 10 | 1.5 |
| Emko Concentrate + agent B | 1 | 1.5 |
| Immolin Vaginal Cream-Jel | 10 | 1.0 |
| Iso-sol-Argyrol | 100 | 1.5 |
| Koromex A Vaginal Jelly | 50 | 1.5 |
| Lanesta Gel | 20 | 1.5 |
| Lorophyn Suppositories | 10 | 1.5 |
| Milex Crescent Jelly | 50 | 1.5 |
| Neo-Silvol | 1 | 5.0 |

TABLE I (continued)

Screening of Test Material for Efficacy against *T. pallidum*

| Test Material* | Slide Immobilization Test | |
|-------------------------|---|-----------------------------------|
| | Lowest Effective Concentration (Percent) | Time to Immobilize (Minutes)** |
| Ortho Creme | 1 | 1.5 |
| Ortho Gynol Jelly | 10 | 1.5 |
| Penigin | 10 | 1.5 |
| Penigin C | 10 | 1.5 |
| Preceptin Gel | 1 | 5.0 |
| Progonasyl | 50 | 1.5 |
| Propion-Gel | 50 | 1.5 |
| Ramses Vaginal Jelly | 20 | 2.0 |
| Silver Protein | 0.1 | 1.5 |
| Sporostacin Cream | 10 | 1.5 |
| Trib Vaginal Cream | 10 | 1.5 |
| Trimo-San Vaginal Jelly | 20 | 1.5 |
| Vabal-D Cream | 10 | 1.5 |
| Vagisec Liquid Douche | 1 | 1.5 |

* Additional material tested: Delfen Cream 1% concentration and time to immobilize 1.0 minutes.

** Observation for immobilization was discontinued after 5 minutes. Further dilutions were not tested on materials which were found to be unsatisfactory on initial screening. Minimum time for slide preparation and observation, 1.0 to 1.5 minutes, considered as the baseline.

Source: Laboratory reports on testing done under the direction of Dr. Singh.

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TABLE II

Screening of Test Material for Efficacy against N. gonorrhoeae

| Test Material | Test for Inhibition of Growth | | | |
|----------------------------|---|-------------------------|------|-----|
| | Lowest Effective* Concentration (Percent) | Exposure Time (minutes) | | |
| | | One | Five | Ten |
| Betadine Vaginal Gel | 10 | - | - | - |
| Candectin Vaginal Tablets | 50 | + | + | + |
| Certane Vaginal Jelly | 50 | + | - | - |
| CN 23577 | 10 | + | + | ** |
| CN 35458 | 10 | + | + | ** |
| CN 59895 | 10 | + | - | ** |
| CN 60563 | 10 | + | + | ** |
| CN 60684 | 5 | + | - | ** |
| CN 67 013 27 | 0.1 | - | - | ** |
| CN 7828 | 10 | + | + | ** |
| CN 83402 B | 0.1 | - | - | ** |
| Contra Creme | 50 | + | + | ** |
| Cooper Creme | .1 | - | - | ** |
| Delfen Cream | 50 | + | + | + |
| Delfen Foam | 50 | ± | ± | ± |
| Emko Concentrate | 50 | - | - | - |
| Emko Concentrate + agent A | 50 | - | - | - |
| Emko Concentrate + agent B | 50 | - | - | - |
| Iso-sol-Argyrol | 50 | - | - | ** |
| Koromex A Vaginal Jelly | 10 | - | - | ** |
| Lanesta Gel | 50 | - | - | ** |
| Milex Crescent Jelly | 10 | - | - | ** |
| Neo Silvol | 10 | + | - | ** |
| Ortho Creme | 10 | - | - | ** |
| Ortho Gynol Jelly | 50 | + | - | ** |

TABLE II (continued)

Screening of Test Material for Efficacy against N. gonorrhoeae

| Test Material | Test for Inhibition of Growth | | | |
|---------------------------|--|-------------------------|------|-----|
| | Lowest Effective* Concentration (Percent) | Exposure Time (minutes) | | |
| | | One | Five | Ten |
| Penigin | 1 | - | - | - |
| Penigin C | 1 | - | - | - |
| Preceptin Gel | 1 | - | - | ** |
| Progonasyl | 50 | - | - | - |
| Ramses Vaginal Jelly | 50 | + | + | + |
| Silver protein | 5 | - | - | ** |
| Sporostacin Vaginal Cream | 50 | + | + | ** |
| Trimo-San Vaginal Jelly | 50 | - | - | - |
| Vabal D Cream | 50 | - | - | - |
| Vagisec Liquid Douche | 50 | + | + | + |

* If the material was not effective at 50%, no further testing was done.
Minus (-) indicates no growth, plus (+) indicates growth.

** Not tested at 10 minutes

Source: Laboratory reports on testing done under the direction of Dr. Singh

V. Ashmun
J.H. Gerende
1/4/71 rr

TABLE III

Screening of Test Material for Efficacy against C. albicans

| Test Material** | Test for Inhibition of Growth | | | |
|----------------------------|---|-------------------------|------|-----|
| | Lowest Effective Concentration (Percent) | Exposure Time (minutes) | | |
| | | One | Five | Ten |
| Betadine Vaginal Gel | 1 | - | - | - |
| Candectin Vaginal Tablets | 10 | + | - | - |
| CN 23577 | 50 | + | ± | - |
| CN 35458 | 50 | - | - | - |
| CN 59895 | 50 | + | + | + |
| CN 60563 | 50 | + | + | + |
| CN 67 013 27 | 10 | - | - | - |
| CN 7828 | 50 | - | - | - |
| CN 83402B | 10 | - | - | - |
| Contra Creme | 50 | + | + | + |
| Cooper Creme | 50 | + | ± | ± |
| Cortone Acetate | 50 | + | + | + |
| Delfen Cream | 50 | + | + | + |
| Delfen Foam | 50 | + | + | + |
| Emko Concentrate | 50 | + | + | + |
| Emko Concentrate + agent A | 50 | + | + | + |
| Emko Concentrate + agent B | 50 | + | + | + |
| Immolin Vaginal Cream Jel | 50 | + | + | + |
| Iso-sol Argyrol | 50 | + | + | - |
| Koromex A Vaginal Jelly | 50 | + | - | - |
| Lanesta Gel | 50 | + | + | + |
| Lorophyn Suppositories | 50 | + | ± | - |
| Milex Crescent Jelly | 50 | + | + | ± |
| Neo-Silvol | 10 | + | + | + |
| Ortho Creme | 50 | + | - | - |

TABLE III (continued)

Screening of Test Material for Efficacy against C. albicans

| Test Material | Test for Inhibition of Growth | | | |
|---------------------------|--|-------------------------|------|-----|
| | Lowest Effective Concentration (Percent) | Exposure Time (minutes) | | |
| | | One | Five | Ten |
| Ortho Gynol Jelly | 50 | + | + | + |
| Penigin | 50 | + | + | + |
| Penigin C | 50 | + | + | + |
| Preceptin Gel | 50 | + | + | + |
| Progonasyl | 50 | + | + | + |
| Propion-Gel | 50 | + | + | + |
| Ramses Vaginal Jelly | 50 | + | + | + |
| Silver Protein | 10 | - | - | - |
| Sporostacin Vaginal Cream | 50 | + | + | + |
| Trib Vaginal Cream | 50 | + | + | + |
| Trimo-San Vaginal Jelly | 50 | + | + | + |
| Vabal-D Cream | 50 | + | + | + |
| Vagisec Liquid Douche | 50 | + | + | + |

* If the material was not effective at 50%, no further testing was done. Minus (-) indicates no growth, plus (+) indicates growth.

** Additional Material tested: Vabal D Base and Emko Concentrate (no active ingredient) Both were at 50% concentration, growth observed at 1, 5 and 10 minutes.

Source: Laboratory reports on testing done under the direction of Dr. Singh.

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TABLE IV

| Growth of <u>C. albicans</u> in Presence of Test Material in the Medium | | |
|---|--|-------------------------|
| Test Material | Lowest Effective* Concentration (Percent) | Growth (48 hours) ** |
| Betadine Vaginal Gel | 50 | - |
| Candephine Vaginal Tablets | 1 | - |
| Contra Creme | 50 | + |
| Cooper Creme | 50 | + |
| Cortone-Acetate | 50 | + |
| Delfen Cream | 50 | + |
| Delfen Foam | 50 | + |
| Emko Concentrate | 50 | + |
| Emko Concentrate + agent A | 50 | + |
| Emko Concentrate + agent B | 50 | + |
| Emko Concentrate + spermicide | 50 | + |
| Emko Concentrate (no active ingredient) | 20 | + |
| Immolin Vaginal Cream-Jel | 50 | + |
| Koromex A - base only | 50 | + |
| Koromex A Vaginal Jelly | 10 | - |
| Lanesta Gel | 50 | + |
| Lorophyn Suppositories | 1 | - |
| Milex Crescent Jelly | 50 | + |
| Ortho Creme | 50 | + |
| Ortho Gynol Jelly | 50 | + |
| Penigin | 50 | + |
| Penigin C | 50 | - |
| Preceptin Gel | 50 | + |
| Progonasyl | 50 | + |
| Propion Gel | 50 | - |

TABLE IV (continued)

Growth of C. albicans in Presence of Test Material in the Medium

| Test Material | Lowest Effective* Concentration (Percent) | Growth ** (48 hours) |
|---------------------------|--|-------------------------|
| Ramses Vaginal Jelly | 50 | + |
| Silver Protein | 10 | - |
| Sporostacin Vaginal Cream | 10 | - |
| Trib Vaginal Cream | 50 | + |
| Trimo San Vaginal Cream | 10 | - |
| Vabal D Base | 50 | + |
| Vabal D Cream | 1 | - |
| Vagisec Liquid Douche | 20 | + |

* If the material was not effective at 50%, no further testing was done. Minus (-) indicates no growth, plus (+) indicates growth.

** Results from duplicate plates. Other products and dilutions have not been tested as yet.

Source: Laboratory reports on testing done under the direction of Dr. Singh.

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1/5/71 rr

FINANCIAL REPORT

AID/csd - 2822

June 30, 1970 - December 31, 1970

January 15, 1971

| Category | Allocation | Expenditure | Balance |
|------------------------------------|--------------------|---------------------|----------------------|
| Salaries and Wages | \$ 152,997.00 | \$ 17,665.10 | \$ 135,331.90 |
| Fringe Benefits | 15,299.00 | 1,766.51 | 13,532.49 |
| Cooperating Country Nationals | 128,400.00 | - | 128,400.00 |
| Consultants Fees | 17,000.00 | - | 17,000.00 |
| Travel and Transportation | 30,506.00 | 502.19 | 29,983.81 |
| Other Direct Costs | 24,600.00 | 42.46 | 24,557.54 |
| Subcontracts | 61,000.00 | - | 61,000.00 |
| Equipment, Materials, and Supplies | 64,400.00 | 5,105.61 | 59,334.39 |
| Nonexpendable | \$30,340.00 | 2,106.60 | 28,233.40 |
| Expendable | 34,100.00 | 2,999.01 | 31,100.99 |
| | | | |
| | <u>Total</u> | <u>\$ 25,101.87</u> | <u>\$ 469,140.13</u> |
| Overhead | 86,956.00 | 9,747.22 | 77,208.78 |
| On Campus | 82,236.00 | 9,747.22 | 72,208.78 |
| Off Campus | 4,720.00 | - | 4,720.00 |
| | | | |
| | <u>Grand Total</u> | <u>\$ 34,849.09</u> | <u>\$ 546,348.91</u> |