

AVIATION MEDICINE SPECIAL (CAM) REPORTS
VENEREAL DISEASES - NUMBERED ABSTRACTS AND REPORTS

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VENEREAL DISEASES REPORT #1 (abstracted)

COMMITTEE ON MEDICAL RESEARCH
of the
OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT
Abstract of MS. for publication
October, 1943

GLUTAMINE AS AN ESSENTIAL GROWTH FACTOR FOR CERTAIN STRAINS OF
NEISSERIA GONORRHOEA. A. Gordon Gould, (Harvard Medical School,
Dept. of Bacteriology).

There have been in the literature contradictory statements
regarding the influence of cystine on the growth of the gonococcus.
To a certain extent the experiments described in this paper explain
the inconsistencies and indicate that cystine may reasonably be
expected to promote the growth of certain strains while inhibiting
that of others.

It appears that repeated transfer of gonococcal cultures on
certain types of media lead to the acquisition by the strain of a
particular growth factor. This has been found to occur in meat
infusion, in red blood cells, and in yeast. Attempts at isolation of
this factor and a study of its properties led to the belief that it
was identical with glutamine. This has proved to be the case, and
synthetic glutamine can be substituted for natural sources of the
growth factor with complete satisfaction.

It appears that when the requirement for glutamine develops in
a strain it then becomes sensitive to the presence of cystine evidently
in much the same way that bacterial growth is inhibited in many cases
by the sulfonamide drugs through the blocking of an enzyme system by a
closely related but not identical compound.

The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical
Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101
Constitution Avenue, Washington 25, D. C.

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VENERICAL DISEASES REPORT #2 (Abstracted)

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Ven. Dis.COMMITTEE ON MEDICAL RESEARCH
of the
OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT
Abstract of Interim Report
27 August, 1943BIOLOGICAL FALSE POSITIVE REACTIONS
IN THE SEROLOGY OF SYPHILIS. Herbert
Lund, M. D. (Western Reserve University).
Aug. 27, 1943.Abstract: (Of four bimonthly reports for
1942-43).

As has been previously reported, the use of larger serum-antigen ratios in flocculation tests for syphilis increases the sensitivity of the reaction (Lund; J. of Syphilis, Gon. & Ven. Dis. 26:1-15, 1942). It has been shown further that the use of large volumes of serum brings about reactions of flocculation in the sera of nonsyphilitic disease and of normal persons. This work enlarges the above observations and is particularly directed at the study of the distribution and nature of false reactions.

Technic. The method used is that of a centrifugal-resuspension technic using comparatively large volumes of serum (up to 2 cc. of whole serum). It is necessary to dilute this serum 1:4 in order to produce effective centrifugation. Kline exclusion antigen is used in most of the tests. The Kehn, Eagle, Hinton, and Boerner, Jones and Leukens antigens are also used in certain experiments. A colloidion particle antigen has been developed and used.

Serum-Antigen Ratio, Quantitative Relationships. Experiments are run to see if the proportion of combined antigen to free antigen is constant no matter what the volume of the reacting system. Small volumes of syphilitic sera were mixed with various volumes of saline, and the efficiency of the reaction was determined by titrations at various time intervals. Experiments were run to study the effect of initial combination of serum and antigen in small volumes followed by dilution with saline to larger volumes. In like manner the effect of removal and replacement of the suspending fluid was studied. The results indicate the following: the use of large volumes of serum in a centrifugal-resuspension technic leads to greater sensitivity. A constant ratio of the masses of combined and free reagin is almost but not quite maintained no matter what the volume of the reacting system. There is a little larger proportion of free reagin in the larger volumes even after equilibrium is reached. A little more reagin elutes upon replacement of the suspending fluid in a large volume system as compared with the small volume system. Equilibrium is reached more slowly when the volume of the reacting system is large.

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False-Positive Reactions Obtained by Using Large Serum Volumes.

The use of large volumes of serum frequently produces false-positive flocculation tests. The reactions are more common in young people and decidedly more common in females as shown by the following chart of percent of positive reactions with 2 cc. of serum:

	<u>MALES</u>					<u>FEMALES</u>				
Age	<u>10-19</u>	<u>20-29</u>	<u>30-39</u>	<u>40-49</u>	<u>50-59</u>	<u>10-19</u>	<u>20-29</u>	<u>30-39</u>	<u>40-49</u>	<u>50-59</u>
Pos.	64	48	43	32	34	64	69	58	50	56
Neg.	36	52	57	68	66.	36	31	42	50	44

Tuberculosis, scarlet fever, pregnancy, and menstruation did not seem to affect the incidence of the reactions.

The Nature of the False Reactions Obtained by Using Large Serum Volumes.

1. The reactions tend to be characteristic of certain individuals.
2. They are more readily obtained by certain antigens, specifically those with large antigen particles, the Kline and Boerner, Jones and Leukens antigens exceeding Eagle and Kahn antigens.
 - 2a. The reactions are not related to the type of stock antigen; for example, a Kahn stock antigen if concentrated and cholesterolized in the manner used for Kline antigens behaves in a manner similar to the Kline antigen.
 - 2b. The false reactions are not related to the chemical nature of the sterols. Colloidal particle antigens behave the same way.
3. The false reactions occur after inactivation but not with raw serum.
4. The substance responsible for the reactions is more thermostable than the reagin of syphilis.
5. On one fractionation made by Dr. Neurath on a normal serum containing this reacting substance reactions were found in the GII globulin fraction and to a lesser extent in the GI globulin fraction.
6. False reactions are increased in number and occur readily with all types of antigens if the ionic concentration is temporarily lowered sufficiently to precipitate euglobulin.
7. False reactions appear to occur in a peculiar high-volume zone.
8. The reactions are more readily produced in a centrifugal-resuspension technic than in other techniques of flocculation, even when the same serum-antigen proportions are used.
9. The false reactions are subject to mer variation in titer and are more inconstant than reactions due to reagin. This is possibly related to the temperature at which the test is performed.



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VENEREAL DISEASES REPORT #3 (Abstracted)

COMMITTEE ON MEDICAL RESEARCH
of the
OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT
Abstract of MS. for publication
5 October, 1943

GROWTH REQUIREMENTS OF NEISSERIA GONORRHEA R. Gordon Goula, Lewis
W. Kane, and J. Howard Mueller. (Harvard Medical School, Dept. of
bacteriology)

It is shown that most strains of the gonococcus when freshly obtained
from patients are capable of growing although sparsely in a relatively
simple medium containing only substances of known composition. These in-
clude only two amino acids, glutamic acid and histidine, a small amount
of glutathione is included, and the only other organic components are glu-
cose, starch and agar. These materials together with inorganic salts
constitute the medium.

Growth on such a medium is sparse but definite and is greatly im-
proved by the addition of other more complex materials. It is shown that
an acid hydrolysate of commercial casein contains a factor which it has
not been possible to identify with any of the known amino acids which
exert a marked stimulatory effect. Similarly meat infusion contains
another substance probably chemically different from the first which also
produces greatly increased growth. It has not been possible to isolate
or identify either of these stimulating substances, although a certain
amount of information has been obtained about their behavior.

Evidence has been obtained that the action of starch in the medium
is to protect the organism against an inhibitory effect which is exerted
on its growth by certain batches of agar. The nature of this inhibition
and the antagonism of the starch has not been explained beyond the state-
ment that it is probably some kind of a colloidal effect.

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Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101
Constitution Avenue, Washington 25, D. C.



VENEREAL DISEASE REPORT #1 (Abstracted)

Justina H. Hill
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Ven. Dis.

COMMITTEE ON MEDICAL RESEARCH
of the
OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT
Abstract of Annual Report
1 September, 1943

STUDIES AND INVESTIGATIONS IN CONNECTIONS WITH THE
ESTABLISHMENT OF A GONOCOCCAL INFECTION IN EXPERI-
MENTAL ANIMALS BY METHODS APPLICABLE TO THE STUDY
OF VENEREAL DISEASE: (Justina H. Hill) (The Johns
Hopkins University).

Part I. The purpose of the investigation has been primarily to develop a method by which the action of chemical agents upon gonococci on mucous surfaces can be evaluated from the point of view of prophylaxis or of therapy.

1: It was found possible to infect mice by testicular inoculations, and the possible use of this method to measure differences in virulence of gonococci is suggested, but it was not possible to increase virulence by this method or to establish consistent local infections by the other methods investigated in this series.

2: Variations of the suspensions of gonococci used for vaginal or urethral inoculations of mice: no significant results were obtained in this series.

3: Treatment of the vaginal or urethral mucosa with agents which might increase penetration or susceptibility: no significant results were obtained, although prolonged viability of the organisms was observed with or after the use of certain detergents.

4: Systemic treatment of the experimental animals: the major steps in this investigation have been (1) the intraperitoneal injection of human serum; (2) the substitution of horse serum for human serum, and (3) the substitution of blood albumin for horse serum. With each of these steps a number of variations have been studied to determine optimum conditions for a given agent or to determine the limits of its action. Treatment with a wide variety of agents has been included, including refrigeration, the use of benzene, ammonium chloride, sodium carbonate, an atmosphere of 8 to 10% carbon dioxide, histamine, phenobarbital, rabbit serum, normal, anti-mouse serum rabbit serum, charcoal, carmine, and egg albumin.

By far the best results have been obtained by the use of serum albumin. A method has been evolved by which the prophylactic action of drugs may be studied and by which a heavy growth of gonococci in the untreated controls is obtained 24 hours after inoculation. The application of this method to the study of selected drugs is now progressing as rapidly as possible, and variations in the method are also being studied. The number of organisms which should be killed in such tests is discussed and it is suggested that experiments be made with two orders of culture dilutions; one of 1,000,000 and one of 100,000,000 or-

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ganisms. The explanation of the effect of successful systemic treatment is not yet known and is being investigated by parallel experiments. Other factors than the drop of temperature appear to be involved. The greater part of the investigation can now be devoted to the actual testing of agents.

Part II. Comparisons have been made of five in vivo methods of evaluating the action of drugs against gonococci by the chick embryo method and of two in vitro methods. Fifty percent end points have been determined whenever possible. The 2 hour in vitro test has been found adequate, the 2 minute in vivo test more rigid but difficult to use in the evaluation of long series of drugs. In view of the questionable significance of either in vitro or chick embryo tests in regard to the killing of gonococci on mucous surfaces, it is recommended that these be subordinated at present to the experimental method in the mouse. (See Part I.)

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COMMITTEE ON MEDICAL RESEARCH
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OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT

Venereal Diseases Report #5 (Abstracted)
Abstract of Final Report
31 December, 1943

LYMFOGRANULOMA VENEREUM AND CHANCOID. Frank
C. Combes, M.D., Orlando Canizares, M.D., and
Simeon Eli Landy, M.D. (New York University Col-
lege of Medicine)

The purpose of this investigation was to evaluate the prophylactic action of the sulfonamides in various ointment bases as recommended by the Subcommittee on Venereal Diseases and prepared by Dr. Marvin Thompson and arsenicals and surface active agents as supplied by Drs. Harry Eagle and Geoffrey Rake. In this work, extending over a period of 6 months, cultures of H. ducreyi were utilized.

Results showed that a 10% solution of zephiran in water and 2% solution in propylene glycol will afford 100% protection if applied within one hour of inoculation. Zephiran 2% , aqueous, was effective in 70% of cases if applied within three hours of inoculation and 81% effective if applied within 1 hour. In less than 2% dilutions the degree of protection was too low to be of practical value.

Calomel 33% and sulfathiazole 15% in an oil-in-water emulsion ointment base, prepared by Dr. Marvin Thompson and known as A440-Mod #2 Batch #3 SC-1005, gave 85% effective prophylaxis 6 hours after inoculation.

All the arsenicals and other surface active agents with the exception of zephiran were unsatisfactory.

The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

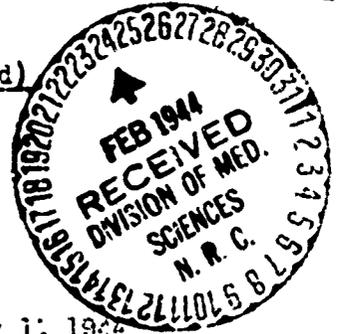
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Classification (Open)
Ven. Dis.

Venereal Diseases Report No. 6 (Abstracted)



COMMITTEE ON MEDICAL RESEARCH
of the
OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT

ABSTRACT OF FINAL REPORT

DATE: January 1, 1944

NAME OF RESPONSIBLE INVESTIGATOR Elvin A. Kasat and Ian Moore, in association with Bernard D. Lavis and Ad Harfis, U. S. P. H. S.

SUBJECT Biologic False Positive Serologic Tests for Syphilis.

CONTRACT NO. OLActr-25

Serologic tests on serum fractions separated by electrophoresis in the Tiselius apparatus showed the antibody of syphilitic and of numerous false positive sera to have an electrophoretic mobility intermediate between that of beta and gamma globulin, although no separate component was visible at that position. This is consistent with the fact that Wassermann antibody in even high titer sera forms only a very small fraction of the total protein.

Ultracentrifugation of syphilitic and false positive sera and of syphilitic gamma globulin showed a concentration of the Wassermann antibody in the sediment, indicating a higher mean molecular weight for the antibody than for the bulk of the globulin. A component of sedimentation constant higher than the bulk of the globulin was found to constitute approximately 2-5 per cent of the protein in a normal human serum. Concentration of this heavy material by repeated ultracentrifugation of syphilitic and normal serum resulted in concentration both of the syphilitic and of the "normal" Wassermann antibody (Cf. Lund), which formed only a small part of the rapidly sedimenting material. This material was not electrophoretically homogeneous, the Wassermann antibody being associated with the slower of the two components present.

Methods developed for dissociating Wassermann antibody from specific floccules by 15% NaCl and ether or by alcohol and ether yield preparations of 30 to 87% purity as measured by the amount of protein removable by antigen floccules. The purified antibody was essentially homogeneous electrophoretically with a mobility of 1.0×10^{-5} cm²/volt-sec., intermediate between that of beta and gamma globulin. In the ultracentrifuge it was found to have two components, of sedimentation constants 7 and 19 Svedbergs, respectively, corresponding to the globulins of molecular weight 160,000 and 990,000 which have been observed in numerous animal sera. Since the proportion of precipitable antibody exceeded the proportion of heavy component, part of the active purified antibody must have been associated with the light globulin component.

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The serological titer of purified antibody was not increased by preliminary inactivation, as is usual with whole sera. The complement fixation and flocculation titer of the purified antibody was increased two to four times by the addition of serum albumin or inactivated whole normal serum.

Purified antibody was obtained from three false positive sera; the failure of the procedure when applied to several others is ascribed to their low titer. Studies on mixtures of purified antibody with false positive sera showing anomalous patterns of response to various tests (Kahn, Kline, Mazzini, Eagle, Kelmer) provided no evidence for the presence in these sera of an inhibiting factor affecting any of these tests.

The procedures of electrophoresis, ultracentrifugation, and purification of antibody have thus failed in our hands to offer any promise of distinguishing biologic false positive from syphilitic sera.

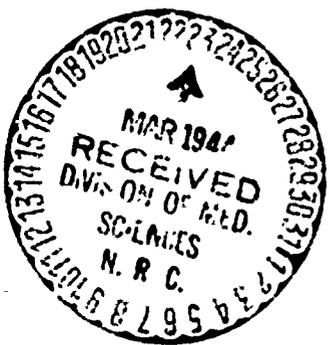
The ammonium-sulfate fractionation method of Mourath was applied to 9 syphilitic and 9 false positive sera. Although syphilitic antibody precipitated less completely with GI in our experiments than in those of Mourath, and several syphilitic sera behaved like false positives, we were able in several instances to confirm his observation that the reacting substance of certain false positive sera precipitates at higher salt concentrations than the antibody of many of the syphilitic sera studied. The irregularities encountered with the syphilitic sera, however, suggest that the most important step in further evaluation of this procedure would be the testing of a large series of low titer syphilitic sera.

The observation of Mourath that globulin fractions of false positive sera are sometimes serologically more active than the original serum was confirmed for both salt-precipitated and electrophoretically separated globulins. Since the effect was slight, barely exceeding serological error in most cases, and since it was also observed in a syphilitic serum, it seems unlikely that this will prove to be a reliable differential criterion.

Dr. F. A. J. ...
#5286

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Moore - Kabat J. ...
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Ven. Diseases



COMMITTEE ON MEDICAL RESEARCH
of the
Office of Scientific Research and Development

VENEREAL DISEASES REPORT # 7 (ABSTRACTED)
Abstract of MS. for Publication
25 February 1944

ELECTROPHORETIC, ULTRACENTRIFUGAL, AND IMMUNOCHEMICAL
STUDIES ON WASSERMANN ANTIBODY. Bernard D. Davis,
Dan H. Moore, Elvin A. Kabat, and Ad Harris. (Columbia
University, College of Physicians and Surgeons).

Methods developed for dissociating Wassermann antibody from specific floccules by 15% NaCl and ether or by alcohol and ether yielded preparations of as high as 87% purity, as measured by the amount of protein removable by antigen. Serologic tests on serum fractions separated by electrophoresis, and electrophoretic analysis of a preparation of purified antibody, showed the Wassermann antibody to have a mobility intermediate between beta and gamma globulin. Ultracentrifugal concentration of whole syphilitic serum or electrophoretically separated gamma globulin showed a relative concentration of antibody in the sediment, indicating that a portion of the antibody has a higher molecular weight than the bulk of the globulin. Examination of purified antibody solutions showed that the antibody was associated with both a light and a heavy globulin component, of sedimentation constant 7 and 19 Svedbergs, respectively. The serologic titer of purified Wassermann antibody was nonspecifically increased two to four times by the addition of serum albumin or inactivated whole serum. Electro-phoresis, ultracentrifugation, and purification of antibody failed to show significant differences between a number of syphilitic and false positive sera.

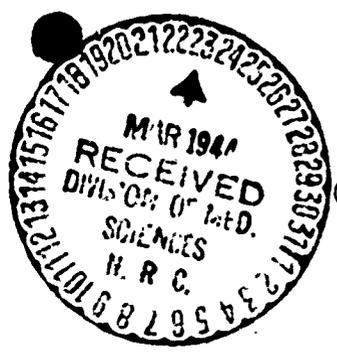
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Dr. Forbes
1#5287

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Moore - Kabat
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Ven. Diseases



COMMITTEE ON MEDICAL RESEARCH
of the
Office of Scientific Research and Development

VENEREAL DISEASES REPORT # 8 (ABSTRACTED)
Abstract of MS. for Publication
25 February 1944

THE ANTICOMPLEMENTARY ACTIVITY OF SERUM GAMMA GLOBULIN.
Bernard D. Davis, Elvin A. Kabat, Ad Harris, and Dan. H.
Moore. (Columbia University, College of Physicians and
Surgeons).

Electrophoretically separated gamma globulin from a number of normal human sera was highly anticomplementary, as little as 0.04 mg. of protein giving complete inhibition of hemolysis under the conditions used. Purified Wassermann antibody, prepared by dissociation of the specific precipitate, was anticomplementary in similar amounts. The anticomplementary action was decreased by heating at 56°C. for one-half hour, and was abolished by addition of approximately equal amounts of albumin or beta globulin. It is suggested that the data offer the first evidence of an easily dissociable compound between complement and antibody, postulated by Heidelberger, Weil, and Treffers in their theory of the mechanism of complement fixation.

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COMMITTEE ON MEDICAL RESEARCH
of the
OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT

Venereal Diseases Report #9 (Abstracted)
Abstract of MS. for Publication
29 March 1944

THE EXPERIMENTAL PROPHYLAXIS OF CHANCEROID DISEASE. Frank C. Combes, M.D. and Orlando Canizares, M. D.

The purpose of this investigation was to evaluate the prophylactic action of the sulfonamides in various ointment bases as recommended by the Subcommittee on Venereal Diseases and prepared by Dr. Marvin Thompson and of arsenicals and surface active agents as supplied by Drs. Harry Eagle and Geoffrey Rake. In this work extending over a period of six months cultures of H. aucreyi were utilized.

Results showed that a 10% solution of zephiran in water and 2% solution in propylene glycol will afford 100% protection if applied within one hour of inoculation. Zephiran 2%, aqueous, was effective in 70% of cases if applied within three hours of inoculation, and 81% effective if applied within one hour. In less than 2% dilutions the degree of protection was too low to be of practical value.

Calomel 33% and sulfathiazole 15% in an oil-in-water emulsion ointment base prepared by Dr. Marvin Thompson and known as A440-Mod #2 Batch #3 SC-1005 gave 85% effective prophylaxis six hours after inoculation.

All the arsenicals and surface active agents with the exception of zephiran were unsatisfactory.

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Venereal Diseases No. 10

COMMITTEE ON MEDICAL RESEARCH

Of The

OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT

ANNUAL REPORT



DATE February 1, 1944

NAME OF RESPONSIBLE INVESTIGATOR Dr. Arthur Schoch & Dr. L. J. Alexander

SUBJECT Intensive Arsenotherapy of Early Syphilis CONTR. OR NO. OEICAR-84

During the 12 months' period, starting February 1, 1943, and ending February 1, 1944, we have treated a total of 503 patients with syphilis by a tri-weekly mapharsen schedule. (Eagle and Hogan)

For evaluation of final treatment results, more time is obviously needed. (All data on patients mentioned above have been sent to Dr. Harry Eagle, (USPHS, Johns Hopkins Hospital, Baltimore, Maryland).

The duration of treatment for all patients was 8 weeks. Patients with early syphilis received bismuth once a week (a total of 9 injections) in addition to the mapharsen administered.

Two hundred and seventy-four patients with early syphilis were treated with mapharsen and bismuth; 229 patients with untreated latent syphilis were treated for 8 weeks with mapharsen alone.

In addition, follow-up studies were made on over 400 patients with early syphilis treated last year with the tri-weekly mapharsen schedule for 8 weeks without bismuth.

During the last year there were no fatalities on the tri-weekly schedule. Only one major treatment reaction was encountered.

Fifteen patients were discarded from the latent group because of positive spinal fluids. Only one patient in the group of early syphilis had a positive spinal fluid. This was a neuro-recurrence.

Results of treatment in the majority of instances of early syphilis were satisfactory with reference to disappearance of lesions and reversal of STS to negative. As would be expected, reversal of STS in the group of latent patients is exceedingly slow.

2-Annual Report

PENICILLIN THERAPY OF EARLY SYPHILIS

Under the direction of the Penicillin Panel, patients with early syphilis were started on penicillin therapy by Schedule II, starting November 15, 1944.

Sixty-six patients have been started on penicillin therapy and 51 have completed treatment. Only one has failed to complete treatment.

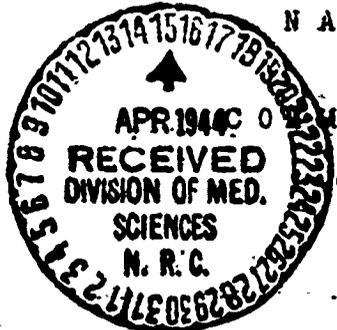
For results of treatment, consult the Penicillin Panel (Drs. Moore, Mahoney, and Wood). This problem is being continued.

*Dr. Forbes
Invent*

**PLEASE RETURN PROMPTLY TO
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**CLASSIFICATION OF THIS REPORT
HAS BEEN REDUCED TO
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NATIONAL RESEARCH COUNCIL
Division of Medical Sciences
Acting for the
COMMITTEE ON MEDICAL RESEARCH
of the
Office of Scientific Research and Development
Subcommittee on Venereal Diseases
of the
Committee on Chemotherapeutics and Other Agents

Venereal Diseases Report #11

DETAILED PRESENTATIONS OF RESULTS OF PENICILLIN THERAPY.
IN EXPERIMENTAL AND CLINICAL SYPHILIS, MADE AT A MEETING
OF THE PENICILLIN PANEL OF THE SUBCOMMITTEE ON VENEREAL
DISEASES. MARCH 7, 1944.

At the meeting of the Penicillin Panel of the Subcommittee on Venereal Diseases 7 March, 1944, the several investigators who have been studying the effect of penicillin in syphilis of experimental animals and human beings reported their experiences. These reports are reproduced herewith. In each instance the name of the responsible investigator and institution or facility precedes the report, the names of junior associates being omitted for brevity. Likewise in some instances the reports have been edited for brevity by the Chairman of the Panel.

The reports follow, in the order of their presentation.

RESULTS OF PENICILLIN THERAPY IN EXPERIMENTAL SYPHILIS IN RABBITS

J. F. Mahoney, Senior Surgeon, U. S. P. H. S.
Venereal Disease Research Laboratories
U. S. Marine Hospital, Stapleton, S. I.

One-hundred and fifty units of penicillin per kilogram of body weight administered at 3 hour intervals for a total of 16 injections over a 48 hour period (total 2400 units/kg) is approximating the curative level in experimental rabbit syphilis. In a large number of treated animals not any evidence of infection has been noted, either through node transfer or tissue transfer from scrotal or testicular lesions, from animals which have received in excess of that amount of treatment.

Further to establish this threshold a large group of animals, all of which displayed the characteristic lesions of experimental rabbit syphilis, have been treated with the above indicated dosage for 72 hours. Tissue and node transfers were effected at the completion of therapy and gland transfers will be carried out at the expiration of 4 months post-treatment observation. If this routine fails to disclose survival of spirochetes it will be considered as lending support to the thesis that a curative therapy requires something less than 72 hours with a total dosage equal to 3600 units per kilogram of body weight.

In a small group of animals, (30), a subcurative penicillin therapy was carried out and the animals allowed to pass into the latent stage of experimental disease. After a post-treatment lapse of days, the animals have been re-treated utilizing a supposedly curative therapy. This group of animals is at present under observation. Subsequent developments will be observed with interest because of the inferences as to the treatment of latent disease which may be drawn.

In an additional group of 25 animals the efficacy of penicillin therapy in latent disease in rabbits is being studied. The animals in this group have been allowed to pass into the latent phase of experimental disease and have been treated with injections of 150 units of penicillin per kilogram of body weight at 3 hour intervals for 72 hours (total 3600 units/kg). At the completion of 4 months post-treatment observation, lymph node transfers will be carried out in an effort to determine the sterilizing influence of this therapy.

Harry Eagle, U. S. P. H. S. and Johns Hopkins University
V. D. Research and Post-graduate Training Center OEmmr-215

Present status of studies on the therapeutic efficacy of penicillin in experimental syphilis in relation to the frequency and number of injections (cf. Table I):

1. Single injections, whether intramuscular, intravenous or subcutaneous, have not effected cure at the largest dosages used (16,000 units per kg.).

~~RESUMED~~ 2. Four intramuscular injections, whether given at intervals of 1 hour, 4 hours, 1 day, or 4 days, have not effected cure at the largest dosage used (a total of 32,000 units per kg., corresponding to approximately two million units in man). It is of interest to point out in this connection that the dose required to render a testicular chancre temporarily darkfield negative bears no relation to the curative dose. On some of the schedules involving four injections, a total dose of as little as 200 units per dose of penicillin has caused spirochetes to disappear from a testicular chancre; but 160 times that dose has failed to effect cure, as shown by positive lymph node transfers six weeks after treatment.

3. Eight injections, given intravenously twice daily over a four day period, have failed to effect cure at 16,000 units per kg. Thirty-two thousand units per kg. may, however, be effective. An experiment involving the same schedule of injections, but given intramuscularly rather than intravenously, is in progress.

4. Sixteen intramuscular injections, repeated at 15 minute intervals over a period of four hours, have failed to cure at a dosage of 16,000 units per kg. The same number of injections given at 1 hour, 4 hours, and daily intervals is, however, proving effective. The preliminary results so far available do not permit a definite evaluation of the curative dose; but it will apparently be a small fraction of that required when only four injections are given. It should be possible to appraise the relative efficacy of these three schedules within a few months.

5. Schedules involving a) eight injections at 4 hourly intervals, b) 16 injections given twice daily and at two hourly intervals, c) 32 injections given hourly, and d) an intravenous drip, will be undertaken in the near future. These new schedules, plus those enumerated above and listed in Table I, will give information as to the curative dose of penicillin in experimental syphilis when given over a time period varying between 10 seconds to 16 days, and at a frequency of injection varying from 15 minutes to 4 days.

Table I

The Effect of the Number, Frequency, and Route of Injections on the Therapeutic Efficacy of Penicillin in Syphilitic Rabbits.

Treatment begun 4 to 6 weeks after intratesticular inoculation.

Total number injections	Interval between injections	Total duration of treatment	Route of administration	Dose required to render chancre temporarily dark-field negative, units/kg.	Curative dose ¹ units/kg.
1			intramuscular	> 1,000	> 16,000 [↓]
1			subcutaneous	> 16,000	> 16,000
1			intravenous	> 16,000	> 16,000
4	1 hour	3 hours	intramuscular	400—1,000	> 32,000
	4 hours	12 hours	intramuscular	400—1,000	> 32,000
	1 day	3 days	intramuscular	< 2,000	> 32,000
	4 days	12 days	intramuscular	< 200	> 32,000
3	twice daily	3 days	intravenous	160—640	> 16,000*
	twice daily	5 days	intramuscular		
16	15 minutes	4 hours	intramuscular	800—1,600	> 16,000*
	1 hour	15 hours	intramuscular	200—400	*
	4 hours	60 hours	intramuscular	< 200	*
	1 day	15 days	intramuscular	< 400	*

* Experiments incomplete.

[↓] The entry "curative dose > 16,000 units" means that all animals treated at that dosage, or less than that dosage, were treatment failures (spirochetes demonstrable in the testicular lesion more than three days after the completion of treatment, or more commonly, lymph nodes infectious for normal animals 6 weeks after the completion of treatment). Doses larger than those indicated were not tried. Although definitive results are not yet available, it seems clear that schedules involving 16 injections will prove far more effective than those involving only 4 injections.

PENICILLIN THERAPY IN EARLY SYPHILIS

J. F. Mahoney, Senior Surgeon, U.S.P.H.S.
Venereal Disease Research Laboratories
U. S. Marine Hospital, Stapleton, S. I.

I. It is desired to record the following information in regard to the four (4) patients who constituted the original group treated for early syphilis with penicillin. At the time of treatment all had seropositive primary syphilis. The period of post-treatment observation now exceeds 9 months. All have remained free of clinical evidence of syphilis. The serologic reactions reversed to and have remained negative. Spinal fluid examinations have not disclosed central nervous system involvement. Not any evidence of delayed toxicity has been noted.

II. A total of 30 patients displaying evidence of early syphilis have been treated up to the present. Routine therapy has consisted of intramuscular injections of 20,000 units of the drug administered at 3 hour intervals for a total of 60 injections and a total of 1,200,000 units of penicillin. It has not been necessary to alter this routine or to discontinue the therapy because of untoward effects or toxic manifestations. In spite of the care which has been exercised in the selection of patients for treatment, a shrinkage in the material has occurred due to lapse from observation.

III. Fifty-two of the patients have been followed for 40 days or longer. Of this group, 22 have undergone complete reversal of serologic findings and have remained seronegative throughout the observation period. In 29 instances there has been a definite trend toward seronegativity as indicated by the quantitative serologic procedures. Eight additional patients had only low titer serologic reactions at the completion of treatment and these reversed to negative early in the post-treatment period.

IV. Of the patients who were well into the seropositive phase of the disease at the time of treatment, a definite serology pattern appears to be discernible when multiple tests are used in the serology follow-up. The quantitative procedures indicate an increase in titer during and immediately following treatment. This upstroke is followed by a uniform and consistent decline in titer until complete negativity is reached.

In patients in whom pre-treatment serology is of high titer, the rate of post-treatment decline is more protracted. The inference is inescapable that possibly an increase in the amount of drug and an increased period of treatment is indicated in this type of infection, or that repeated courses may be found efficacious.

V. In one instance the classification of treatment failure is considered to be justified. This patient displayed a satisfactory trend toward negativity for two months following treatment. The decline then halted, remained stationary for a short period and subsequently returned to a titer higher than the original point. This patient did not display evidence of clinical relapse. Re-treatment with penicillin was carried out and subsequent serologic findings indicate a resumption of the decline in titer.

In 8 patients, cellulitis at the site of injection was produced by a particular brand and lot of penicillin. By changing to another brand of drug the treatment was continued and the reaction subsided without further complications.

Exfoliative dermatitis has been observed in two patients. In one the reaction was moderately severe and persisted with severe itching and scaling for 3 weeks. Patch tests were positive in one of the dermatitis patients with a brand other than that which caused the reaction. Three other cases of patch test sensitivity have been observed in workers with the drug who had not been treated.

In a total of 850 patients treated with penicillin for gonococcal infection there have been three instances of the therapy masking or altering a concomitantly acquired syphilis. In each, there was an absence of primary lesions and the clinical and serologic manifestations of syphilis were observed shortly after the completion of the gonorrhoea therapy. These instances suggest the advisability of serologic scrutiny of patients for a period of at least 3 months following penicillin therapy for gonorrhoea.

Oscar Cox, Massachusetts Memorial Hospitals, Boston, Mass.

Forty patients have been admitted for penicillin therapy. All cases were given 1,200,000 units in 60 injections over a 7 $\frac{1}{2}$ day period. Six are still under treatment: thirty-four have completed treatment.

Three of these were seronegative when treatment was started:

No. 293496 - Hinton, Wassermann, and Kahn positive 2 days after treatment was started: 2 days later serologic tests were negative and have remained so for 81 days.

No. 298596 - Hinton positive 14 days after treatment started, negative at 21 days, and positive at 28 days. Forty-nine days after beginning of treatment Wassermann was doubtful. All other serologic tests done at weekly intervals have been negative, last on the 56th day.

No. 299241 - Four days after treatment was started Hinton was doubtful, Wassermann positive, Kahn positive. Following discharge from the hospital he was by error sent to sea. The next serologic tests done 111 days after beginning of treatment were Hinton negative, Wassermann negative, Kahn not done. At 133 days Hinton, Wassermann, and Kahn were all negative.

Of the 34 who have completed treatment, 6 have been observed for less than one month. In none of these has the serologic tests reversed to negative but the titre is falling.

Twelve have been observed between one and two months. Of these 3 have become sero-negative: the titre is falling in the remaining 9.

Six have been observed from two to three months. In three the serologic tests have reversed to negative and in three the titre is falling.

Ten have been observed for more than 3 months. In 5 the serologic tests have reversed to negative, in 4 the titre has fallen, but in one the titre has risen. This patient, No. 299098, is a 21 year old colored female. On admission she had secondary syphilis. Hinton, Wassermann, and Kahn were positive; spinal fluid negative. The lesions on the vulva were darkfield positive. She was given 20,000 units of penicillin intramuscularly every three hours for a total of 1,280,000 units. The lesions on the vulva became darkfield negative within 12 hours and were healed on the 5th day. She was not seen again until 106 days after beginning of treatment at which time the skin lesions had healed, the Hinton was positive (5 units), Wassermann negative, Kahn positive (2.5 units). Five subsequent serologic tests have shown a steady rise in the titre. The last one done 140 days after the beginning of treatment was Hinton 120 units, Wassermann 120 units, Kahn 40 units.

Twenty-five of the 34 patients who have completed treatment had an elevation of temperature a few hours after beginning treatment: all returned to normal within 36 hours.

Lesions became darkfield negative in from 6 to 13 hours; average 11 hours.

No case has had a clinical relapse.

C. W. Barnett, Stanford University, San Francisco, Calif.

The patients so far treated for early syphilis number 26, of whom 9 with primary syphilis, 15 with secondary manifestations and 2 with relapses. Thirteen were white and thirteen were colored. Sixteen were males. Two of the females were pregnant during penicillin therapy, one delivered a macerated fetus, known to have been dead for a month, and in the other, the pregnancy is progressing normally.

In spite of steadily increasing social service activity, the follow up is expected for various reasons to be disappointing.

One patient consulted a private physician a few weeks after therapy, who upon hearing her story and finding a positive Wassermann insisted on giving her immediate antisyphilitic treatment. Since syphilis is so widely treated on serologic evidence alone, this may prove to be a serious follow up problem.

Two methods of treatment have been used. The first eight patients received 200,000 units of penicillin in 2 liters of glucose or saline by continuous day and night intravenous drip, for five days, a total of 1,000,000 units. In some of these, multiple thrombosis necessitated that the last day's treatment be given by continuous dialysis.

The other 18 patients received 5000 units intramuscularly in 1 cc. of saline every 3 hours for 60 doses, a total of 300,000 units.

The rates of darkfield reversal, the healing of lesions, reactions and serologic results are shown in Tables 1 and 2.

Table I (Barnett)

Penicillin in Early Syphilis

Results	<u>Type of Therapy</u>	
	<u>Intramuscular</u>	<u>Intravenous</u>
Darkfield negative	15.6 hrs. (7-29)	10 hrs. (8-14)
Involution	17.5 days (4-45)	14 days (2+24)
Reactions		
Herxheimer	2	1
Fever	5	2
Both	6 (3 grade III)	1

Table II (Barnett)

Serologic Results in Early Syphilis

Type of Treatment

Initial Titer (Kolmer)	<u>Intramuscular</u>		<u>Intravenous</u>		<u>Total</u>	
	No.	Days	No.	Days	No.	Days
>32 units						
Result						
* Improved	4	(27-73)	2	(37-103)	6	(27-103)
Unchanged	2	(15-16)	0		2	(15-16)

Initial Titer

<32 units

Result

Remained negative	4	(0-62)	1	55	5	(0-62)
Reversed	3	(50-85)	0		3	(50-85)
* Improved	1	42	1	121	2	(42-121)
Unchanged	1	15	3	(5-126)	4	(5-126)
Relapsed (nonreactive)	0		1	55	1	55

* Improved = reduced to a titer of 1/4 or less.

In only one or two instances have pre-treatment C.S.F. examinations been made. We had been following our usual plan in early syphilis, of examining the C.S.F. after serologic reversal or at about 6 months. Six C.S.F. examinations up to 4 months after treatment have been made. One of these showed a group II fluid with a pleocytosis of 26 cells one month after the completion of therapy. No clinical evidence of neurosyphilis could be found.

The results of penicillin therapy are encouraging but from the slowness of serologic reversal, it seems that 300,000 units may be an insufficient dose. The inadequacy of this dose is also to be expected from blood level measurements. With constant intravenous drip at 200,000 units per day, there is maintained a steady penicillin level of 0.1 to 0.2 units per cc. with an average value of 0.16. After the administration of an intramuscular injection of 5000 units, the blood level was found to be 0.04 units at 1/2 and one hour, but none could be detected after two and three hours.

W. Barry Wood, Jr., Barnes Hospital, St. Louis, Mo.

Thirteen patients with early syphilis have been treated with penicillin, using three different therapeutic schedules.* For convenience of consideration, these patients are grouped as follows:

Group I: Treatment employing 1,200,000 units of penicillin, in 60 i.v. doses, administered at 2-hour intervals (5 day total). Four patients were so treated. Three had darkfield positive genital chancres (all seropositive). One patient had genital darkfield positive chancre, perineal papulo-hypertrophic lesion, and marked central nervous system involvement on spinal fluid examination. Under treatment, in all four cases, lesions became darkfield negative in 4-12 hours. Subsequently, quantitative Kahn tests became negative in 2 cases in 1 and 2 months' time; after four months observation, quantitative Kahns have reached low titre range in the remaining 2 cases.

Group II: Treatment employing 1,100,000-1,200,000 units of penicillin, administered in doses of 20,000 units, 8 such doses daily, for 7-7½ days. Two cases of darkfield positive secondary syphilis and two cases with genital primary lesion treated by intravenous administration of the drug in this dosage schedule; two cases showing darkfield positive secondary lesions were treated by the intramuscular administration of the drug in the same dosage schedule. All cases showed negative darkfield tests 9-18 hours after the beginning of therapy. There has been a steady decline of quantitative Kahn titre in these cases: 2 cases showed negative Kahns in 2 months; 2 cases showed very low titre Kahns in 1 and 2 months; in one case Kahn value is halved; in one case follow up has been unsatisfactory. Four cases had slight to moderate fever after beginning of therapy.

Group III: Treatment schedule + 300,000 units of penicillin, 320 mg. napharsen over 8-day period. Three cases so treated. Two cases showed primary genital darkfield positive lesions; one showed secondary cutaneous syphilis. Lesions became darkfield negative in 9-12 hours. Case of cutaneous syphilis showed marked cutaneous Herxheimer, with fever and acute pharyngitis. One patient has shown marked decline of Kahn to low titre range in two weeks; in two cases Kahn has steadily declined over one and one half months' period of observation.

* All patients in groups I and II were treated before a specific treatment schedule had been assigned to this clinic.

One case of early taboparesis treated, using 1,200,000 units of penicillin in 5 days (as in Group I). Spinal fluid changes were typical. Over a period of two months the patient's mental status was greatly improved; spinal fluid showed a moderate decline in the cell count. At the end of two months, aortic regurgitation was found to be present and was considered to be due to a therapeutic paradox.

Francis G. Blake, Yale University, New Haven, Conn.

Twenty-five cases of early syphilis have been treated with penicillin at the New Haven Hospital since August 1943. These may be divided into two groups:

I:- 13 cases treated between August 1943 and January 1944 with 10,000 units i.m. every 2 hours for 3 days (a total dose of 960,000 units). Of these 4 had darkfield positive seropositive primary syphilis, 3 had early secondary syphilis, and one was congenital syphilis in a 2 months old infant (2,500 units i.m. q. 2 h. for 3 days). Of the first 5, 4 are now seronegative, one is still seropositive. Of the remainder, 3 have been lost to follow-up, the balance show diminishingly positive serology. All in whom there are follow-ups are clinically asymptomatic. Four cases had Herxheimer reactions of which 3 were febrile and cutaneous, grade II or III, one was febrile only, grade II. Darkfields became negative between 28 to 26 hours except in one case which required 66 hours.

The second group consists of 12 cases treated since Dec. 28, 1943, with 5,000 units of penicillin q. 3 h. i.m. to a total of 300,000 units + 320 mapharsen. Of these 4 had seropositive primary syphilis, 8 had early secondary syphilis. The initial response of lesions was satisfactory in all cases. Four had febrile Herxheimer reactions, grades I to III. They are being followed with quantitative Kahn reactions. Sufficient time has not elapsed for any of them to become seronegative.

Captain William Leifer (M.C., A.U.S.) Fort Bragg, N.C.

CLINICAL MATERIAL:- 46 patients have received intensive penicillin treatment of syphilis, between November 17, 1943, and February 29, 1944. Treatment consisted of 60 consecutive intramuscular injections of 20,000 units of penicillin, at 3 hour intervals, for a total dosage of 1,200,000 units.

There were 13 white and 33 colored patients, 44 male and 2 females. Nine had seronegative primary syphilis, 11 seropositive primary syphilis, and 26 secondary syphilis.

Ten patients had some abnormality of the spinal fluid; 2 of these had seropositive primary syphilis, and 8 had secondary syphilis. Five patients had an elevated cell count of the spinal fluid, varying from 6 to 13 cells. Three had a doubtful Wassermann reaction in 1.0 c.c. of fluid, the remaining 2 had positive Wassermann reactions of the fluid.

REACTIONS:- Every patient had some soreness of the gluteal muscles, but in only one instance was it really severe.

Herxheimer reactions occurred in 41 of the 46 patients. Twenty-six had a febrile reaction only; 1 had a cutaneous exacerbation; and 14 had combined febrile and cutaneous reactions.

Reactions appeared, after the first day of treatment, in 8 patients. These consisted of: (1) Herpes simplex labialis (1 case) - this followed a severe febrile Herxheimer reaction. (2) Herpes proiesitalis (1 case). (3) Generalized pruritus without eruption (1 case). (4) Urticaria (3 cases). (5) Abdominal cramps (1 case). (6) Erythema multiforme - like eruption (1 case).

No serious reaction was encountered, and in every case treatment was completed as planned.

DISAPPEARANCE OF TREPONEMES:- Darkfield observations were made in 23 patients after onset of treatment, and showed treponemes absent at 6 hours (4 patients); 7 hours (2 patients); 8 hours (3 patients); 9 hours (8 patients); 12 hours (5 patients); and 15 hours (1 patient).

This does not give a true picture of the rate of disappearance of the treponemes, since the darkfield examinations were usually done only at 6, 9, 12 and 15 hours after the onset of treatment.

HEALING OF LESIONS:- Healing of syphilitic lesions was as rapid, if not more so, as with any other form of therapy, including intensive arsenotherapy. A general statement may be made that the rate of healing varied with the degree of infiltration of the lesion. The ordinary mucous patch, macular or papular syphilodema, moist papule and chancre were healed by the time treatment was completed, or very shortly thereafter. The exceptionally large, deeply indurated and ulcerated chancre, and the larger, more infiltrated papular lesions required a longer time. One patient had severe alopecia of the scalp, eyebrows and body; when re-examined 46 days after starting treatment, there was an almost complete regrowth of hair.

No instance of treatment resistance was observed, and there has been no report, as yet, of infectious relapse.

SEROLOGIC TESTS FOR SYPHILIS: Results of the quantitative Kahn tests of blood can be reported now in only 12 patients:

Diagnosis	KAHN TEST			No. of Days
	Before Treatment	At 8 Days	Most Recent	
Sero-negative primary	0	Doubtful	0	82
	0	0	0	70
	0	10 U	0	47
	0	0	0	38
Sero-positive primary	Doubtful	20 U	0	63
	4 U	4 U	3 U	43
	Doubtful	Doubtful	0	32
Secondary	40 U	40 U	4 U	94
	40 U	80 U	40 U	46
	10 U	40 U	Doubtful	45
	20 U	20 U	10 U	38
	4 U	20 U	Doubtful	37

Thus, 6 of the 12 patients have maintained or achieved sero-negativity for periods of 32 to 82 days after the inception of treatment. Two additional patients have shown a reduction from 40 units and 20 units to doubtful in 45 and 37 days respectively. The remaining 4 patients have all had a reasonable decline in the titer in the period that they have been observed.

There has been no evidence of serologic relapse or serum-fastness.

Lieutenant E. E. Barksdale (M.C.) U.S.N.R.
U. S. Naval Hospital, Bethesda, Md.

Number of cases started treatment in Sept. 1943	5
Number negative	3
Number partly negative	1 (o)
Number positive	1 (x)
(o) Case of syphilitic meningitis treated with penicillin I.T., I.M. and I.V. clinically cured.	
(x) Case of condyloma D.P. positive, clinically cured but still seropositive.	
Number of cases started treatment in Oct. 1943	7
Number negative	4
Number partly negative	3
Number positive	0
1 Case treated with 15,000 u. q. 3 h for 5 days. Relapsed, retreated with 20,000 q 3 h for 60 doses. Now only partially positive. 1 Case now becoming negative. 1 Case still Kolmer positive only.	
Number of cases started treatment in Nov. 1943	4
Number negative	3
Number partly positive	0
Number positive	1
Case still positive had secondary syphilis	
Number of cases started treatment in Dec. 1943	3
Number negative	2
Number partly positive	0
Number positive	1
Case still positive had secondary syphilis	
Number of cases started treatment in Jan. 1944	24
Number negative	1
Number partly positive	16
Number positive	7
Number of cases started treatment in Feb. 1944	26
Number negative	0
Number partly positive	8
Number positive	18
Total cases treated 69. 53 received 20,000 u. q 3 h for 60 doses.	

The relapse in one case of primary syphilis and the slow serologic response in secondary syphilis has given us the impression that certain cases may require larger doses. It is impossible to maintain a constant blood penicillin level with I.M. injections. On the three-hour routine there is practically no penicillin in the blood by the time the three-hour period is up.

We have treated a few cases of secondary syphilis by giving 160,000 I.V. q 3 h for the first 48 hours and then 160,000 q 24 h for 7 days and find that a more or less constant blood penicillin level can be obtained, approximately 10 times higher than that with the I.M. route. We feel that the clinical response in these cases is better, and one of the cases of secondary syphilis was negative 3 weeks after the treatment was discontinued.

Harold N. Cole, Western Reserve University, Cleveland, Ohio

I:- Our first experiments on the penicillin treatment of acute syphilis were carried out on the Medical Service at the University Hospital with a small group of five patients given 400,000 Oxford units by continuous intravenous drip over a period of 48 hours and a further total dosage of 800,000 units by the intramuscular route given every three hours for five days. This was then followed up with a further group of five patients given a total dosage, intramuscularly, of 1,200,000 units in five days, injections being administered every two hours.

With the first group, spirochaetes disappeared in nine to ten hours in three cases, but in the other two apparent degenerated forms were seen for nine and twelve days respectively. Primary lesions healed in from four to twenty days, but in two patients with secondary lesions response was slow, 21 and 32 days. However, the seronegative primary has never had any serologic change in four months and the other four became completely seronegative in 4, 3, 8 and 14 weeks respectively. (Chart I)

In the second group, one patient was lost after the second week. The response in the remainder, however, was gratifying. *S. pallida* disappeared in 5, 6, 7 and 14 hours respectively. No darkfield was possible in one. Secondary lesions healed within ten days in all cases. Serologic blood negativity was achieved in ten to twelve weeks in three of the patients and practically so (4 units) in the last.

II:- The remainder of the study has been devoted to 1000 units of penicillin intramuscularly every 3 hours for a total of 60,000 units as studied in 31 cases. In a further uncompleted group of 29 cases the same dosage of penicillin has been supplemented with a daily intravenous injection of mapharsen .040 Gm. given half way between injections of penicillin.

The first half of the study comprises 31 patients; 12 males and 19 females. There were 1 white and 11 colored males and 2 white and 17 colored females. Spirochetes usually disappeared in from 9 to 20 hours, with a few cases showing persisting organisms at 21, 24, 27, 28, 30, 33 and 48 hours respectively. All were still negative 24 hours after the last negative examination and many were repeatedly examined thereafter with negative results.

The primary lesions healed in 7 to 10 days, despite repeated curettage in the hunt for organisms.

Secondary lesions healed mostly in the period of 6 to 8 days (21 cases) with additional cases on the 12th, 14th, 15th and 24th days.

Serologic study of these 31 cases shows 4 patients to have become seronegative over a period of 6 to 13 weeks. One has remained seronegative throughout. One patient, pregnant, was delivered at home and has thus far not (Chart II)

been rechecked. Two cases have been lost, one at one week and another at 2 weeks. The titer is unchanged in 7 cases. There is a decreasing titer in 15 and increasing titer in 1 (32 - 128)

III:- A further group of 29 patients has been treated with 60,000 units penicillin plus 320 mgm. mapharsen. Most have been followed for 5 weeks or less; one for 8 weeks and another 7. There were 15 males and 14 females - 1 white and 14 colored males and 3 white and 11 colored females.

Darkfield studies of rate of disappearance of organisms post-therapy showed that most of them fell in the period 8 to 9 hours: - one at 7 hours, two at 8 hours, six at 9 hours, three at 11 hours, three at 14 to 15 hours, then two at 23 hours and one at 28 hours. The last were extensive secondary moist papules.

Three chancres healed on the 6th day, four on the 8th and three on the 10th. This showed little difference from the results with penicillin alone.

With secondary eruptions, complete healing was seen in six on the 7th day, in nine on the 8th, in six on the 9th, in one on the 10th, one on the 13th and one on the 26th day.

Serologic study of these 29 cases is of too short duration to reveal much. In every case followed at least 4 weeks there is a decrease in titer. In four instances the patients have temporarily, at least, become seronegative. In 15 cases titer is decreasing, but all these cases must be followed further before any conclusions may be drawn.

RELAPSE:- There is one example of serologic progression in the series of patients treated with 60,000 units of penicillin alone. The first titer was 28, in one week 256, 6th, 7th and 8th weeks thereafter 128. We have hesitated to do anything yet. In another from this same group, first titer was 32, in two weeks 128, four weeks 32, six weeks 64, nine weeks 32 and eleven weeks 64. This patient, again, is being followed without treatment.

In one instance there is a question of recurrent chancriform relapse or of reinfection, which, if either, not yet settled.

Two pregnant women with early syphilis have been treated, in neither of whom is the status of the child yet certain.

REACTIONS:- In the first five cases treated with continuous intravenous drip there was a rise in temperature in several and a definite Herxheimer reaction with accentuation of the eruption. Since then, other patients have been observed

CHART I (Cole)

CONTINUOUS INTRAVENOUS DRIP PENICILLIN 400,000 UNITS AND 800,000 INTRAMUSCULARLY.

Weeks	0	1	2	3	4	5	6	7	8	9	10	11	12	13
	256	80	80	80	80			32		16		8		0
	32													
TITER	32	32	32	32		16		0	0	0	0	0	0	0
BEFORE	16			0	0	0	0	0	0	0	0	0	0	0
AND	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FOLLOWING	128		64		32	16		0	0	0	0	0	0	0
THERAPY.														

TOTAL 1,200,000 BY INTRAMUSCULAR ROUTE IN FIVE DAYS: EVERY TWO HOURS.

64	16											(4 months)	4)
128		128										(3 months 16 - 4 months)	0)
128			16									(4 months)	0)
16		16		(Lost)									
128	64	16				4				0		(4 months)	0)

CHART II (Cole)

PENICILLIN 100G UNITS EVERY THREE HOURS. TOTAL 60,000 UNITS.

Weeks	0	1	2	3	4	5	6	7	8	9	10	11	12	13
	64	128	128				32					32		
	128	64			64	32	32			16		32		
	256	128		128		64		64				32		
TITER	128	64		64	64	32			16		32			
BEFORE	32	128	(lost)											
AND	128	256	128	(lost)										
FOLLOWING	128	32						64		8				
THERAPY	32	128				64	64							
	128	128	128				128	64	32					
	128	128	128		64					8	2	neg.		
	64	(No follow-up yet.				Baby delivered at home).								
	64	64	64	64		32			32			32		
	64			32		16								
	128	64		64	64	64			16		16			
	32	16	4				neg.							neg.
	32			16	8	8		8						
	128		16	32										
	32	128		64	64	128	128	64		32				
	64			32			4							
	16		64	8					neg.			neg.		
	64				32	(3 mo. 32-questionable sparse papules trunk-								
	4	8	16	2	4				neg.				(D.F. neg.)	
	128	128	(No further follow-up)											
	32	64		64	64	64						32	32	
	4	16			4									
	32	256					128	128	128					
	256	128				16								
	32		128		32		64			32			64	
	16	64	32		32	16	16	32	16				4	2
	64	64			32	32		32		32	16	16		
	8	8	4		16	8	8							

closely for this reaction, but with the low dosage of penicillin employed little has been observed. No untoward reaction from the use of penicillin has been seen at any time.

S. W. Becker, The Chicago Intensive Treatment Center, Chicago, Ill.

From January 12 to February 29, 1944, 79 patients completed treatment, 51 with penicillin and mapharsen (5000 units penicillin every three hours for a total of 300,000 units - mapharsen 0.040 G, daily for a total of 0.320 G.) and 28 with penicillin alone (10,000 units in 1.0 c.c. NaCl. every three hours for a total of 600,000 units. The penicillin was administered in rotation into muscles of arms and thighs, using each arm and thigh twice daily. Of the 79 patients 8 had darkfield positive seronegative primary syphilis, 16 had darkfield positive, seropositive primary syphilis; 45 had early secondary syphilis; 10 had recurrent secondary syphilis.

All lesions responded rapidly. At the end of eight days, about one-third had healed and two-thirds were healing. Darkfield examination was made at short intervals. After 6 hours, 25% penicillin treated were negative; 33 1/3 penicillin and mapharsen negative. After 12 hours, 90% penicillin cases negative; 97% penicillin and mapharsen cases negative. After 24 hours, 100% penicillin cases negative; 97% penicillin and mapharsen negative. All darkfield examinations were negative after 30 hours.

REACTIONS: - There were no significant local reactions. For statistical purposes, generalized reactions in first 24 hours are called Herxheimer reactions.

Of 79 patients, 10 had no reactions at all, 55 had Herxheimer reactions. Seventeen of these had no later reactions, 38 had later reactions.

Febrile reactions were more frequent following penicillin alone (75%) as compared with 66% for penicillin and mapharsen. However, the reactions were less severe with penicillin alone. Eighty-one percent of febrile reactions following penicillin did not go above 100°F as contrasted with 50% of reactions from penicillin and mapharsen. There were 5 mild medicamentous eruptions. No reactions necessitated complete cessation of treatment.

With incomplete data, it is our impression that accentuation of the generalized secondary eruption is less pronounced after penicillin alone than after penicillin and mapharsen.

Follow-up (7 to 28 days duration):- There were no mucocutaneous relapses.

Quantitative Kahn titers: 2 remained negative, Of the positives: 25 remained same as pre-treatment. 22 were lower than pre-treatment, 26 were higher than pre-treatment. This number includes 10 patients with insignificant rises. 4 patients did not return.

Two patients with precocious tertiary lesions responded promptly as to healing of lesions.

Arthur G. Schoch, M. D.
Dallas Syphilis and Venereal Disease Clinic

OELicmr-84

Since November 15, 1943, we have completed treatment, as per Schedule II (300,000 units in 7½ days), on 107 patients with primary and secondary syphilis, all dark-field positive.

In all but seven, spirochaetes disappeared in less than twenty-four hours. In two, follow-up dark-fields were positive at twenty-five and one-half and at forty-eight hours. In five the dark-field data were inadequate.

In this group there are seven cases of early syphilis associated with pregnancy. Three of these patients received, as per Schedule II, a total of 300,000 units of penicillin; one patient received 600,000 units; the other three patients received 1,200,000 units.

Six patients, all with secondary syphilis, had positive spinal fluids (post-treatment and pre-treatment). All of these spinal fluids gave a Wassermann reaction positive in 1/4 cc. and larger amounts only. There have been no spinal fluids of the parietic type (group 3). All Colloidal gold curves were negative, except one. This one was a high first zone curve.

We have observed two cases of reinfection!

We have observed two cases of serologic relapse, both occurring in the third month following completion of treatment. There has been no clinical relapse.

Quantitative serologic titers on all patients followed the usual curve toward serologic negativity when tested by quantitative Kolmer, Kline, Mazzini, and, recently, the quantitative Kuhn test.

To summarize, it is our impression that we are treating patients on a low dosage schedule and must expect a certain number of failures. It is furthermore our impression that another three to six months' observation period will bring to light the majority of failures in this particular group.

Evan W. Thomas, M.D., Bellevue Hospital, New York

OELicmr-104

The use of penicillin in the treatment of early infectious syphilis began at Bellevue Hospital on Nov. 17, 1943. This report deals with the first 150 cases treated. By March 6, 181 patients had been treated or started on treatment. Of the 150 patients in this report, 14 were seronegative primary, 30 were seropositive primary and 101 were secondary cases. In addition 1 early latent case with pleocytosis and 4 dark-field positive reinfections or relapses were treated.

Toxic Effects: No toxic effects of any consequence have been noted. Herxheimer reactions with relatively high temperatures have occurred but no more frequently than when arsenotherapy has been used. Four cases of urticaria were noted during treatment. All recovered before treatment was finished in spite of the fact that treatment was continued according to schedule. With one batch of penicillin, low grade temperatures were noted in most of the patients treated but there were no subjective complaints.

Results of Treatment: All 14 seronegative primary cases have remained seronegative with one exception who had a Wassermann titer of 2.4 when last seen. This is not a significant rise. Of the seropositive primary cases 8 have become seronegative in less than 10 weeks. None of the secondary cases are completely seronegative as yet but the titers are falling satisfactorily in all cases with the exception of patients who relapsed. The fall of Wassermann titers is similar to that noted following the rapid treatment of early syphilis with arsenotherapy or combined arsenotherapy and fever. It is noted that patients with seropositive primary or early secondary syphilis frequently have fairly marked rises in Wassermann titers during the 8 day period of treatment with penicillin, but in all cases marked fall in titers has been observed one week after the completion of treatment. This also is similar to our experience with other forms of rapid therapy for early syphilis.

Results of Treatment with 60,000 units: Ten patients were treated with 60,000 units of penicillin in a period of 7½ days. Seven of these had secondary syphilis, 2 had seropositive primary and 1 seronegative primary syphilis. Two of the secondary cases had clinical and serologic relapses under our observation and one secondary case was reported to have a serologic relapse while under observation in Chicago.

The first patient who relapsed was treated from 11/17/43 to 11/24/43. His titer before treatment was 190. On 12/21/43 the Wassermann titer was 36. On 1/18/44 the quantitative Kahn test showed a definite rise in titer and on 2/1/44 the Wassermann titer had risen to 170. A generalised papular rash and darkfield positive moist papules on the genitalia appeared the last week in January.

The second patient had a Wassermann titer of 200 on 1/18/43 when treatment was started. On 12/30 the Wassermann titer was 31. On 1/3/44 the patient noted lesions on the vulva which were darkfield positive. The Wassermann titer on 1/28/44 was 77.

The third patient had a Wassermann titer of 290 on 11/17/43 when treatment was started. On 12/14/43 the Wassermann titer had fallen to 49. In January, 1944 the patient moved to Chicago where a quantitative Kahn test on 1/22/44 was reported as 2 units. On 2-5-44 the Kahn titer had risen to 20 units. Nothing was reported about evidences of clinical relapse.

Treatment with 200,000 units: Ten patients were treated with 200,000 units of penicillin in a period of 7½ days. Eight were cases with secondary syphilis; 2 had seropositive primary syphilis. Two of these patients relapsed.

One started treatment on 11/22/43 when his Wassermann titer was 150. On 12/30/43 the Wassermann titer had fallen to 19. On 2/5/44 patient noted moist papules on penis and on 2/3/44 the Wassermann titer had risen to 94.

The second relapse in this series started treatment on 11/30/44 when the Wassermann titer was 23. The titer rose to 51 by the time treatment was completed. On 1/22/44 the titer had fallen to 8.9. On 2/1/44 the titer rose to 26; on 2/7/44 it was 63 and on 2/17/44 it was 84. No mucocutaneous lesions were noted but the spinal fluid on 2/17/44 showed pleocytosis and increased protein although the Wassermann and colloidal gold tests were negative.

Treatment with 600,000 units:- All remaining cases (130) have been given 600,000 units in 7½ days. No relapses have been observed.

Early G.M.S. Syphilis Case Treated with 600,000 units:

This patient was a white female who had had two negative blood Wassermann tests, the last test having been on 8/12/43. On 11/4/43 she had an incomplete abortion and her STS was positive. On 12/7/43 she was transferred to us for antisyphilitic treatment. No lesions of syphilis were noted. A spinal fluid examined 12/7/43 showed 10 cells, faint trace Pandy, total protein of 20 and the Wassermann test was positive in amounts over 0.25 cc. she was treated with 600,000 units penicillin in 7½ days. The blood Wassermann titer on 12/7/43 was 250. By 2/17/44 the Wassermann titer had fallen to 21 but on 2/29/44 it was 80. A spinal fluid examined 2/29/44 contained 220 cells per c.mm., Pandy 2 plus, total protein 38.

Note: The first 20 patients treated included the 60,000 unit and 200,000 unit series. The same lot of penicillin was used in both series.

PENICILLIN THERAPY

OEicmr-403

In Late Syphilis, Congenital Syphilis, Syphilis in Pregnancy, Serologic Fastness
John H. Stokes, University of Pennsylvania

Method of treatment: A single system of treatment was employed - 1,200,000 Oxford units, at 25,000 units every 4 hours, intramuscularly, around the clock, for 8 days. The dosage for 2 infants was approximately the adult dosage scale adjusted to weight and multiplied by 2. One infant received 4 times the weight-adjusted scale. It was felt that the curative dose for early syphilis introduced by Mahoney should be used as a base-line for estimating the effect of penicillin in the types of syphilis treated. Once its values and limitations were established, rational variations could be made. Since January 27th, 1944, 4 patients have been re-treated--2 with identical, and 2 with double doses, but no conclusions are offered as yet. No penicillin has been used intraspinally or intravenously.

Classification by Diagnosis of Cases Studied (Stokes)

<u>Neurosyphilis</u>	
Asymptomatic neurosyphilis.....	14
Primary optic atrophy in tabes.....	8
Paresis, symptomatic (simple dementia).....	2
Meningovascular.....	2
Gumma of cord (suspected).....	2
	<u>28</u>
<u>Early syphilis in pregnancy.....</u>	7
<u>Congenital syphilis (excluding neurosyphilis)</u>	
Interstitial keratitis.....	4
Infantile.....	2
8th nerve deafness, early.....	1
	<u>7</u>

<u>Serologic fastness</u>	
Latent syphilis.....	2
Congenital syphilis.....	2
	<u>4</u>
<u>Summa of Soft palate</u>	1
<u>Charcot hip (early)</u>	1
<u>Gangrenous balanitis in late syphilis</u>	1
<u>Early syphilis (age 14 months)</u>	1
	<u>50</u>

General Impressions of Penicillin Therapy: Our experience with penicillin confirms Mahoney's as to its spirillicidal activity in man. Broadly speaking, it behaves as an arsphenamine minus the reaction-producing effects and complications, and plus a therapeutic drive or impetus which we were inclined to estimate in the technic employed, as from 50 to 100 percent greater. There were indications that the scale employed was inadequate to "cure" the late infection or reduce the clinical and laboratory manifestations to normal in the cases thus far treated, within the period of observation. Because neurosyphilis offers a particularly good array of guide-posts, it is easiest to demonstrate both effectiveness and short-comings in this group.

Spirillicidal Effects: These were observed in pregnant women and were if anything more striking than in the currently reported male material. In only one case did *Spirochaeta pallida* in the lesions survive to the 12th hour (75,000 units); and in one case, only to the 3th hour (50,000 units).

Involution of Lesions: This was so rapid in pregnant women with condylomas that only pigmented traces remained after 72 to 120 hours. The one visible extensive gummatous process perforating the soft palate of an elderly woman healed in approximately 30 days, after the manner and at the rate to be expected of an efficient arsphenamine. A gangrenous balanitic, rapidly progressing, with loss of most of the glans and part of one of the corpora cavernosa, healed in 35 days under 392,500 units--5.5 days, and the patient has had intercourse satisfactorily since.

Treatment Reactions and Therapeutic Shock or Herxheimer Effects: A negligible local burning sensation occurs at the injection site, lasting 30 to 60 seconds (25,000 and 50,000 units per cc. distilled water). One case of urticaria was observed, lasting 2 days, improving but not clearing completely (spontaneously?) on change of drug to that of another manufacturer; and on re-treatment 39 days later, replaced by burning and slight erythema of the skin which did not go on to dermatitis or urticaria. Profuse sweating was observed in several patients not ordinarily hyperidrotic. One complained of a burning sensation in the nose. After 48 needle punctures there was slight traumatic reaction, controlled by "sprinkling" the injections all over the area provided by the two buttocks.

The therapeutic shock effects were minimal, possibly because after one or two observed in the nervous system, we reduced the first 48 hour dosage to half when such reaction was expected or began to appear; or paused a half-day or a day between the 48th and subsequent hours of treatment. Even in the pregnant women in whom no such modification was made, no lesion flares were observed. In no case did 4-hour temperatures go above 100.4°F.

Nonetheless, what we interpreted as definite symptomatic flare or shock effects were noted in neurosyphilis, especially, and were occasionally suggested in serologic responses of both blood and spinal fluid. Two pregnant women experienced pains suggesting threatened abortion, disappearing after pause, treatment resumed uneventfully. A case of paresis with simple dementia developed convulsions at the first day after the 4th penicillin injection, which stopped after a pause of 24 hours, treatment being resumed with no serologic but a good symptomatic result, and no residue. Emotional instability and excitement of mild grade was observed in two patients with parietic formulas, but 12 others failed to show such effects. One primary optic atrophy was worse the second week subjectively, better the third week.

Aggregate Therapeutic Effects on Titered Blood Serologic Reactions:

The result in late syphilis is diagrammatically presented in Chart I. The varying period of observation affects the whole group, but the trend seems supported by those observed over 30 days. The tendency is to produce a drop in titer and a leveling off without achievement of a negative in about 1/3 the cases; a drop and a leveling off followed by a rise in about an equal proportion, and in about 1/5 a preliminary rise (Herxheimer or provocative effect?) was followed by a decline and leveling off, but not to normal. Thus approximately 80 percent showed at least temporary reduction in reagin titer. Twenty-eight percent were unchanged or ultimately showed higher titers (possible delayed provocatives?). Curiously, the only complete reversal was in a low titer serologic-fast case.

Therapeutic Effects on the Spinal Fluid: None achieved normality within the range of observation from 8 to 57 days, and 18 cases over 30 days, thus representing 2 spinal fluid examinations following the institution of penicillin. The most frequent change is a drop in cell count and protein, with a decline in reagin titer next, and changes of significance in the colloidal mastix least frequent. The trend at 1,200,000 units in 8 days, is fifty-fifty between improvement and no-change. No early provocative effect and only one seemingly unfavorable change (possibly late provocative) was observed. Charts II, III, and IV.

Our 2 best results on the spinal fluid are presented in Charts III and IV.

Blood Serologic Response, Penicillin Investigation
(Excluding Early Syphilis and Syphilis in Pregnancy--)
1,200,000 OU in 8 days

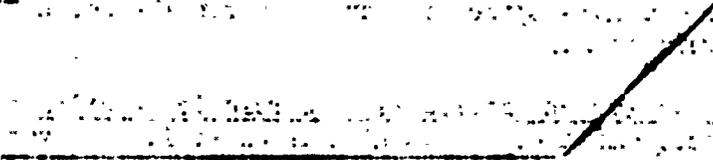
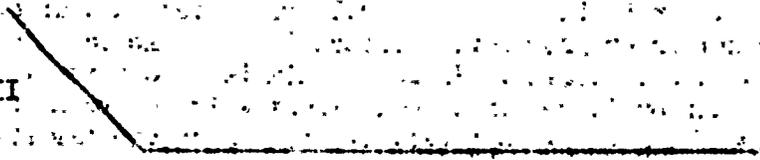
Type of Curve	All Cases	30 days ob.
<p>I</p> 	8	4
<p>II</p> 	4	1
<p>III</p> 	14	9
<p>IV</p> 	12	12
<p>V</p>  <p>Normal</p>	1	1
<p>VI</p> 	4	

CHART II

Response of Spinal Fluid--Penicillin Investigation, Neurosyphilis (Stokes)
 1,200,000 OU in 8 days. 22 Cases

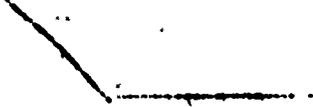
Type of Response	Comment	Cells	Protein	W.R. Kolmer	Coll.
	No case				
	No case				
	Drop, but not to normal	11	12	7	4
	Drop, rise (relapse?)			1	1
 Normal	No case				
	No significant change	11	10	14	17

CHART IIIPenicillin Investigation (Stokes)

Case No. 8 Untreated Preperesis (Total Penicillin: 1,200,000 O.U.)

Days Post Penicillin	Quantitative Kline (Blood)	Cells	CSF Wass. (Kolmer)	Protein (Pandy)	Mastic
0	64	103	4444	4 f	2444411000
17	8	29	1244	2 f	2221100000
46	32	11	0012	1 f	2211000000
74	32	6	0112	7	1110000000

Chart IVPenicillin Investigation

Case No. 11 Late Congenital Syphilis (Neurosyphilis)

Total Penicillin: 1,200,000 O.U.

Days Post Penicillin	Quantitative Kline (Blood)	Cells	CSF Wass. (Kolmer)	Protein (Pandy)	Mastic
0	32	32	1244	4 f	5332210000
13	4	16	0122	1 f	2111000000
40	4	8	0011	7	1111000000

Comment on Special Aspects of Syphilis--Neurosyphilis: Our material supports the following brief summary. Asymptomatic neurosyphilis improves under the standard unit therapy employed, and this beneficial effect includes even some Type III fluids, in congenital as well as acquired syphilis which had resisted large amounts of arsphenamine and heavy metal therapy. Other cases including one that had had fever (hyperthermia) and tryparasamide, were unimproved. There seems to be no available basis for prediction as to which case will respond and which not. In symptomatic neurosyphilis, paresis responded in the earlier simple demented phase but late deterioration was uninfluenced. There is no necessary immediate correlation between symptomatic and serologic improvement. In subsequent reports we hope to present encephalographic studies by Doctors Gammann and Scott, still in their inconclusive beginnings.

In 4 cases of primary optic atrophy, the results are as yet inconclusive. No one of 4 cases has been thus far lastingly the worse for penicillin. In one case with a clinical picture suggesting an arachnoiditic process, improvement in the spinal fluid followed 2 Swift-Ellis treatments, without change in visual acuity. After penicillin, a steady decline in the blood reagin titer from 16 units to 2 units took place, with a see-saw cell count and drop in protein, but unchanged Kolmer Wassermann and attenuated colloidal mastix reaction in the fluid. The first fluid following re-treatment with penicillin showed no further change. The fields are questionably improved. The visual acuity, originally OD 6/60 and OS 2/100, is now OD 6/100 and OS 4/100. The other three cases showed neither favorable nor unfavorable progress and were relatively far-advanced. An early 8th nerve deafness in an adult congenital syphilitic showed a hearing drop following penicillin, followed by improvement. Diffuse meningovascular syphilis, one case, showed temporary improvement, decreased ataxia, disappearance of hyperreflexia and Babinski; but subsequently resumed unfavorable symptomatic progression in other signs. A patient with Type III fluid lost her severe headaches, without fluid change (only 8 days observation).

Interstitial Keratitis: Of four cases, two, both adults who had had prolonged previous treatment, including fever, without complete arrest, improved symptomatically, the photophobia disappearing first, and subsequently other inflammatory manifestations improved but vascularity remained unchanged. One of these cases was dramatic. He returned to work after months of incapacity, 5 days after completion of the penicillin routine, and has remained stationary since. His serologic titer dropped from 64 to 2--and rose to 16, 75 days later. Two cases were failures, one far advanced with much corneal damage and ulceration, the other an early case now regarded as tuberculous.

Infantile Congenital Syphilis: Of two cases, one--a premature (7 1/2 months) infant of 4 1/2 pounds--died apparently a cardiac death (patent ductus arteriosus?--autopsy refused) 16 days post-penicillin, reckoned from first injection. Roentgenograms of the long bones just after penicillin treatment was completed had already demonstrated disappearance of an osteitis syphilitica present before treatment was begun. The second infant, age 41 days, weight 6.5 pounds, with eruptive lesions, became darkfield negative between the 20th and 24th hour, dose reduced from 2,000 to 1,000 units because of cyanosis and dyspnea. Blood titer rose, fluctuating between 64 to 123 units; roentgenograms showed a change 15 days post-penicillin with definite improvement in the extensive epiphysitis, diaphysitis and periostitis of all of the long bones. The general condition rapidly improved; snuffles disappeared; liver was reduced in size; and the baby gained weight.

Serologic Fastness: One congenital case treated routinely for 10 years, dropped from 32 to 8 units in 52 days. Only one case dropped to complete negativity from 4 units (quantitative Kline) in 23 days, and thereafter has fluctuated on the borderline of weak positive (less than one unit). Two cases are substantially unchanged in 36 and 46 days.

Early Syphilis in the Pregnant Woman: Seven cases have been treated (charts of progress omitted from this report). The treatment of 5 was begun before the fifth month. One is now in the eighth month. Our pregnant women have shown rapid spirillicidal effect and rather prolonged titer plateaus which have caused us concern lest at the dosage level used the child had been unaffected or uncured by the penicillin and, still syphilitic, be maintaining an infection on a positive reagin status in the mother which would otherwise have responded. The last-minute results however, have again encouraged us to see the pregnancies through on the original penicillin session.

CONCLUSIONS

This paper is a summary of a brief experience and should be read for details. Penicillin in a close approximation to the initial Mahoney time-dosage scheme is extremely effective in the benign forms of late syphilis, effective to a less but still a remarkable degree in resistant manifestations, including neurosyphilis. In congenital syphilis, early and late, it shows evidence of marked effectiveness. It cannot be expected to restore irreparable damage. In infants it is effective. The results in pregnant women with early infectious syphilis are promising but as yet inconclusive. The absence of significant reaction to penicillin has been conspicuously apparent and this opens the way for more intensive and varied use. Among the possibilities, delayed absorption methods (penicillin in oil, etc.) to do away with hospitalization and to prolong effects; larger intramuscular dosage per injection; longer sessions; the combination of penicillin with other antisyphilitic therapy, including fever, suggest themselves. Therapeutic shock (Herxheimer) effects, we believe, occur, may be serious, can be guarded against by reduction of initial dosage.

Russel Nelson, Johns Hopkins University, Balto., Md.

LATE BENIGN SYPHILIS: We have treated a total of 14 patients with late cutaneous, osseous, or visceral syphilis. These patients have received total doses of 60,000; 300,000; 600,000 and 1-2 million units of penicillin intramuscularly given every three hours for seven to ten days. All lesions have healed and none has shown relapse in periods up to six months after treatment. We believe the lesions to have healed as fast or faster than they would have under standard treatment schedules with arsenicals. There have been no blood serologic reversals and there does not appear to be a trend towards falling blood serologic titer. Two patients with late congenital syphilis and interstitial keratitis have been treated. One of these received a total of 3,900,000 units in 21 days. There was an initial improvement followed by little or slow change for seven to ten days but final clearing in 23 days. The patient has remained asymptomatic for three months after treatment. The other patient had recurring interstitial keratitis and also what was thought to be a gumma of

of the cornea, a condition of extreme rarity to our consulting ophthalmologists. The acute lesions developed while under treatment with arsenicals. She was treated with 1,280,000 units of penicillin in eight days. There was almost complete clearing in 13 days and there has been no relapse of the ocular lesion in the three months since treatment was given!

NEUROSYPHILIS: Nine patients with clinical general paresis have been treated with 2-4 million units of penicillin given every three hours intramuscularly over a period of ten to fourteen days. The first patient was given two courses of intramuscular treatments and is the only patient who has been treated intra-spinally. He received a total of five intrathecal injections of 10,000 units each. Most of these patients are early dementing and dilapidating paretics. One was severely ill and died three weeks after treatment was started; one improved but had to be committed to a state hospital. The others all show a slight symptomatic improvement in periods up to six months. The spinal fluid cell counts and total protein content all have decreased, usually in the second week after treatment; spinal fluid Wassermann titers have not significantly changed.

Seven patients with late asymptomatic neurosyphilis and Group III spinal fluids have been treated. The periods of follow-up observation usually do not exceed one month. Spinal fluid cell counts and protein content again diminish but Wassermann titers have not changed.

Seven of the patients with late benign syphilis have shown minor Group II changes in the spinal fluid. In the two-three weeks following treatment, three have had normal spinal fluids and there was no change in four. Five of the patients with early syphilis have shown similar minor changes. All have had normal spinal fluids 8 to 19 days after treatment was started.

Four patients with acute syphilitic meningitis have been treated with intramuscular penicillin alone for periods of seven to ten days in total doses of 600,000 to 4,000,000 units. The results have been dramatic. Symptomatic improvement begins at once and is complete within the first week. Two of the patients now have negative blood and spinal fluid Wassermann reactions, the other a falling titer, and the last has only recently completed treatment.

TREATMENT-RESISTANT EARLY SYPHILIS: We have treated six patients with arsenic resistant early cutaneous syphilis. These patients have received total doses of 60,000; 600,000 or 2,000,000 units in seven to ten days. Spirochetes disappear, and the lesions heal just as they have in other cases of early syphilis. One patient has shown a serologic reversal on the 41st day after treatment. There have been no relapses.

UNCOMPLICATED EARLY SYPHILIS: Fourteen patients with darkfield positive early syphilis have been treated on a schedule of 1,000 units intramuscularly every three hours for 60 doses - a total of 60,000 units in 7½ days. This series was undertaken at the request of the chairman of the Panel in order to add information in this low dose group and help settle differences which had earlier appeared in two other clinics. Our patients have done well. The lesions have all healed though the rate may be slower. Spirochetes have disappeared from the lesions in from 10 to 60 hours (average 19 hours); in most instances less than 24 hours. Three patients have had serologic reversals at the 5th, 6th and 9th weeks respectively. There have been no relapses in intervals up to 3½ months.

HERXHEIMER REACTIONS: Cutaneous and febrile Herxheimer reactions have been observed in 22 of 47 patients with early syphilis. This appears to be independent of the dosage schedule. Nine of the reactions fall into Group I, nine into Group II, and only four into Group III.

We were surprised to observe febrile reactions in the first 24 hours of treatment in one third of the cases of late syphilis and neurosyphilis. These, we interpret as being Herxheimer febrile reactions and in one half of the instances were of the Group III variety. No evidences of local Herxheimer has been observed in late syphilis.

REACTIONS TO PENICILLIN: One patient with neurosyphilis receiving 20,000 units intramuscularly every three hours developed, on the 15th day, swelling of one eye and the dorsa of both hands. This had the appearance of angioneurotic edema. No other abnormalities were noted and the reaction did not appear with subsequent test doses of the same lot of penicillin. No other reactions have been observed.

Paul O'Leary, Mayo Clinic, Rochester, Minn.

I have been using the penicillin assigned to me on January 23, 1944 by the Subcommittee on Venereal Diseases of the National Research Council for the treatment of patients with neurosyphilis.

To date, I have treated 17 patients of whom 4 had asymptomatic neurosyphilis, 7 had the early clinical manifestations of general paresis, and 2 were advanced (institutionalized) paralytics. One patient had a fulminating type of tabes dorsalis. One case had optic neuritis and a recurrent secondary papular syphiloderma, one child had interstitial keratitis and one had recurrent secondary papular lesions with severe headaches.

Obviously it is too early to make any deductions from these few cases. The clinical improvement in the cutaneous lesions, all late, has been striking, while the serologic changes in the spinal fluids have been insignificant thus far. However, there has been a reduction in the titre in each case. I have given penicillin intravenously, intramuscularly, and intraspinally and have noted no difference in the results. The last four paralytics (early) I have treated have received four million Oxford units intramuscularly while undergoing malaria therapy. Those who were given penicillin intravenously received either a 400,000 or a 1,000,000 unit course twice a day by the continuous drip method for ten days.

Experience with various infectious diseases has shown that when given intravenously about one-third as much penicillin need be used as when given intramuscularly. Herrell and Heilman found the effective concentration of penicillin in the blood for various infectious diseases to be .06 Oxford units per c.c. of blood which can be readily maintained with a dose of 40,000 Oxford units given intravenously twice a day. It would be of considerable help in the treatment of patients with syphilis if we could establish the effective penicillin blood level for *S. pallida*. Whether larger doses, more injections or amplification of the program by chemotherapy or fever therapy is necessary for the neurosyphilitic is one of the issues now confronting us.

Efforts to recover penicillin from the spinal fluid following intramuscular or intravenous (3 cases each) injection have been unsuccessful. Likewise, following the injection of penicillin intraspinally none was recovered from the spinal fluid after thirty-six hours. It might be that the use of the calcium salt rather than the sodium salt would increase this factor somewhat, as we have found the calcium salt decidedly more stable.

The only reactions of significance were: (1) febrile reactions (temperature 104°) following intravenous injection in one patient. It was due to improper technique in the preparation of the solution and continued use of the same batch of penicillin was uneventful. (2) Mild phlebitis in three cases on the intravenous procedure. (3) One patient with mild urticaria. (4) Treatment was discontinued in one patient who was uncontrollable. (5) "ID" reaction on palms.

I first started giving 20,000 Oxford units twice a day for ten days by the continuous drip method and it required about ten hours to give each dose. I have increased it so that now I am giving 50,000 Oxford units twice a day intravenously for ten days (one million units) to patients with asymptomatic neurosyphilis, and the early paretics are receiving 50,000 units intramuscularly every three hours during a malarial fever course of twelve days (4,800,000 Oxford units).

The outstanding serologic reversal occurred in one patient with asymptomatic neurosyphilis who was given 320,000 Oxford units intravenously in ten days in September, 1943. The serologic improvement has consisted in decreasing the cell count from 31 to 2 lymphocytes, reduction of the blood titre from 160 to 20 units and a slight change in the colloidal gold curve.

Walsh McDermott, New York Hospital, New York

In the past six weeks, we have treated eleven patients with late syphilis. Two patients had interstitial keratitis and nine neurosyphilis. Penicillin therapy was a complete failure in the two patients with interstitial keratitis, as judged both by response at the time and during a follow-up period of two and four weeks respectively.

The first patient was an eight-year-old, 32-kilo boy who received 1,000,000 units in fourteen days. The second patient, a twenty-eight year old woman, received almost 5,000,000 units in two courses separated by a week. All material was given by the intramuscular route. Although the penicillin therapy was completely ineffective, the ocular lesion of the boy cleared promptly under artificially induced fever, and the second patient is now also receiving fever therapy.

The nine patients with neurosyphilis have various clinical manifestations of the disease, including five with general paresis; but, with one exception, examination of the spinal fluids of the entire group showed the Group III or parietic formula pattern. One of these neurosyphilitics has an active gumma of the nasal septum, and another has a minimal aortic insufficiency.

Our regimen consists of the daily administration of 300,000 units of penicillin intramuscularly for a two week period, making a total dose of 4,000,000 units.

Examination of the spinal fluids at the end of treatment of the first five patients to complete this regimen has shown definite, although slight, changes in three. In these three instances, the cell counts and the total proteins have become normal. The abnormal gold curves have become less marked, and the titre of the Wassermann reaction has dropped by one dilution. In one patient, an advanced paralytic, a completely first zone gold curve has become normal in the two week period of therapy.

In regard to the clinical results, all that can be said this early is that one patient, a paralytic who was deteriorating rapidly when treatment was instituted, reversed this trend while receiving therapy, and, in the opinion of the attending psychiatrists, is showing definite signs of improvement ten days after the cessation of therapy.

Harry Solomon, Boston Psychopathic Hospital

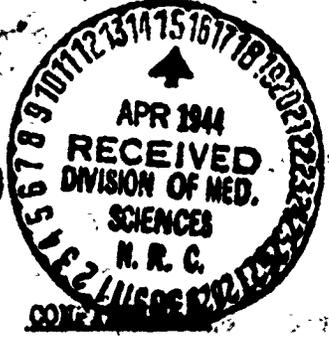
Four patients with paresis have so far been treated, but insufficient time has elapsed to permit comments.

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Dr. Forbes

5718

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of the
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VENEREAL DISEASES #12

REPORTS TO A CONFERENCE HELD UNDER THE AUSPICES OF THE SUB-COMMITTEE ON VENEREAL DISEASES 9 FEBRUARY 1944, ON THE CHEMICAL PROPHYLAXIS ON VENEREAL DISEASE.

The Minutes of a Conference on the chemical prophylaxis of gonorrhoea, held in Washington on 9 February 1944, have been separately circulated. It is desirable that two reports of experimental work presented at that meeting be circulated to persons particularly interested in the field from clinical or experimental standpoints. These are the reports of Drs. John F. Mahoney and C.J. Van Slyke on the prevention of gonococcal infection in experimentally infected human male volunteers; and the report of Dr. Justina Hill on the prophylaxis of gonococcal infection in the vaginae of immature female mice.

THE PREVENTION OF GONOCOCCAL INFECTION IN EXPERIMENTALLY INFECTED HUMAN MALE VOLUNTEERS (M-3169). Drs. John F. Mahoney and C.J. Van Slyke.

The report of Drs. Mahoney and Van Slyke is as follows (Those comments within this report included in italics were inserted by the Chairman on the basis of discussion brought out at the meeting):

In the application submitted to the National Research Council for financial support of a program of human experimentation as a means of investigating some of the problems connected with prophylaxis in gonorrhoea, the following statement was included: "It is first proposed to establish a uniform infecting dose of intra-urethral injected gonococci. Should uncertainties render difficult or hazardous the drawing of sound conclusions in the subsequent phases, the abandonment of the project at that point will be recommended." In keeping with this attitude, and in view of the character of the results which have been produced in the initial phase of the work, it is felt that a critical review of all aspects of the study should be undertaken at this time.

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The evidence which has been produced supports the belief that the information needed for the main purpose of the study will not be attainable through use of the present experimental methods. This is based upon the lack of success in the development of an infecting routine which will produce a constant pattern of infection in the volunteer groups. Without this background the evaluation of prophylactic agents becomes impossible.

Several additional modifications of the infecting technique remain to be studied. However, these do not offer hope of markedly altering the present picture. It is hoped that this group will contribute advice in the following premises:

1. The advisability of concluding the study at this point.
2. The advisability of altering the basic objective of the work and proceeding with the study of problems in immunology and bacteriology, utilizing human volunteers.
3. The continuation of the study along the present lines in the search for a uniformly reliable infecting technique.
4. The advisability of continuing the study along the present lines altered to include the investigation of sulfonamide resistance and other pertinent problems.

The administrative aspects of the study are probably familiar to all. The work has been housed in the Federal Penitentiary at Terre Haute, Indiana. Volunteers are secured from the inmates of this institution with the privilege of securing additional volunteers from other institutions should the need arise. The volunteers have been given a gratuity of \$100.00. Each has received an engraved certificate of merit, and a letter of commendation to the Parole Board has been submitted for each participant.

Two medical officers and two bacteriologists were detailed to the work. Laboratory and clinic facilities entirely adequate for the purpose of the study have been installed. The details of clinic management and of technical methods employed in the culture diagnosis and in the development of strains will not be presented other than to state that all technical procedures were in the hands of skilled workers and that not any concessions which would detract from the high standards of the work were permitted.

The degree of cooperation which has been exhibited by the prison officials has been of the highest order and has left nothing to be desired. The attitude of the volunteers toward the work as regards willingness to participate and to follow instructions has been far more wholehearted than was remotely anticipated.

The detailed results which have been produced will be presented in chronological order with some brief comments as to the reason underlying each of the variations of technique which were employed. These details are summarized in Table I.

Table I
COMMITTEE ON MEDICAL RESEARCH
of the
OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT

Interim Report
February 9, 1944

NAME OF RESPONSIBLE INVESTIGATOR: Senior Surgeon J. F. Mahoney, USPHS
SUBJECT: Prevention of gonococcal infection in experimentally infected human male volunteers
CONTRACT NO.: M-3169

SUMMARY

Date	Strain	Inoc. Vol.	Inoc. Pos.	Inoc. Conc.	Inoc. Depth	Urin. Post	Inoc. Pros.	Inoc. Mass.	Inoc. Min.	Notes
10/12	850	5y	5	0	#5	0.1	3/4	1 h.		
10/19	850	5y	3	0	#10	0.2	1-1/2	1-3/4		X
10/19	1111	4y	3	0	#10	0.2	1-1/2	1-3/4		X
10/20	TH#1	20	3	3	#10	0.2	1-1/2	1-3/4		XX
10/20	TH#2	20	3	3	#10	0.2	1-1/2	1-3/4		XX
10/27	TH#2	27	5	0	#10/250	0.05	1/2	1 h.		
11/9	TH#1	18	1	1	#10	0.2	1-1/2	1 h.		XX
11/9	TH#1	18	3	2	#5/2	0.05	1/2	1 h.		
11/9	TH#1	18	3	1	#5/10	0.05	1/2	1 h.		
11/26	TH#1	15	3	1	#5	0.1	3/4	1 h.		
12/4	TH#1	23	3	1	#10	0.1	3/4	1 h.		
12/15	TH#1	7	4	0	#5	0.1	3/4	1-1/2		
12/18	TH#1	10	3	0	#10	0.2	1-1/2	1-1/2		

* Refers to age dating from first isolation from human; transferred on media 0. 2 d.

Date	Strain Age	No. of Vol.	No. Mcf. Pos.	Inoc. Conc.	Dose	Depth	Urin. Post	Pros. 5 min. held	Inoc. held	No. Mass.	Notes
12/23	TH#5	3	1	#10	0.2	1-1/2	1-1/2	-	-	-	Positive at 31 days
12/23	TH#6	3	1	#10	0.2	1-1/2	1-1/2	-	-	-	
12/24	TH#7	3	2	#10	0.2	1-1/2	1-1/2	-	-	-	
1/1/11	TH#7	3	1	Dir*	-	1/2	1-1/2	-	-	-	Direct
2/1/11	TH#7	3	2	Dir*	-	1/2	1-1/2	Yes	-	-	Direct
3/1/11	TH#7	3	2	Swab*	-	1/2	1-1/2	Yes	-	-	Swab from dish
4/1/11	TH#7	3	3	#5*	0.1	1/2	1-1/2	-	-	-	In 5% Mucin-all positive
5/1/11	TH#7	3	2	#5*	0.1	1/2	1-1/2	Yes	-	-	In 5% Mucin- 2 of 3 Positive
6/1/11	TH#7	3	1	#5*	0.1	1/2	1-1/2	Yes	-	-	In broth- 1 of 3 Positive
7/1/11	TH#7	3	1	#5*	0.1	1/2	1-1/2	Yes	-	-	Plus engorgement
1/15	TH#7	3	0	#10*	0.1	1/2	1-1/2	Yes	-	-	In 10% Mucin
1/15	TH#7	3	0	#10*	0.1	1/2	1-1/2	-	-	-	In 10% Mucin
1/19	TH#7	3	1	#5*	0.1	1/2	1-1/2	Yes	-	-	In 5% Mucin- 1 of 3 Positive
1/19	TH#7	3	1	#5*	0.1	1/2	1-1/2	-	-	-	In 5% Mucin- 1 of 3 Positive
1/22	TH#7	3	3	#10	0.2	1-1/2	1-1/2	-	XX	-	All Positive
1/25	TH#7	3	0	#10	0.2	1-1/2	1-1/2	-	XX	-	
1/27	TH#7	1	0	#10	0.2	1-1/2	1-1/2	-	XX	-	
1/27	TH#7	3	0	#10/2	0.2	1-1/2	1-1/2	-	XX	-	
1/27	TH#7	3	0	#10/10	0.2	1-1/2	1-1/2	-	XX	-	
1/27	TH#7	3	0	#10	0.2	1/2	1-1/2	-	XX	-	

* Excess inoculum allowed to escape - glans penis thoroughly massaged for 5 minutes

It will be noted that the use of 4 and 5 year old strains, strains number 850 and 1111, as employed on October 12th and October 19th, 1943, was without results in any of eleven volunteers.

On October 20th two groups of 3 volunteers each were exposed to strains TH#1 and TH#2, respectively. The organisms were 20 days old and 0.2 cc. of a fresh broth suspension identical in density with a McFarland Standard Suspension #10 containing about 450 million living organisms per cc. was introduced 1-1/2 inches into the urethra. The inoculum was retained for 5 minutes. The volunteers developed typical infections; 5 within 46 hours, the 6th within 85 hours. The volunteers infected with strain TH#1 were promptly cured by sulfathiazole. The three infections produced by TH#2 strain, which was secured from a female patient with a sulfonamide-resistant infection, were also nonresponsive to sulfathiazole but responded promptly to penicillin.

Since the investigations of October 20th showed that experimental gonococcal infections were produced in all 6 volunteers, and since the infecting dose and method used on October 20th seemed unduly severe, the next step, taken on October 27th, was aimed at producing experimental infections by a method which would markedly decrease the immediate local irritation and which coincided more closely with the concept of the normal mechanism of infection of the male anterior urethra. For this purpose strain TH#2 was employed in 5 volunteers. The organisms used were 27 days old instead of 20 days; the suspension of organisms was 1/250 as concentrated as the original McFarland #10 suspension; the inoculum was deposited only 1/2 inch instead of 1-1/2 inches into the urethra; and only 0.05 cc. instead of 0.2 cc. of the suspension of gonococci was introduced. In addition, the suspension was not retained in the urethra, and the penis was not massaged. Irritative phenomena were very slight, and not any of the five volunteers developed an infection.

By November 9th it had been well established that Strain TH#2 was sulfonamide-resistant, so strain TH#1 was used for the experiments on that day, having been recovered 18 days earlier from the volunteers exposed on October 20th. On this day, November 9th, one volunteer was treated exactly as were the six successfully infected volunteers on October 20th, that is, he was exposed to 0.2 cc. of McFarland #10 strength suspension of gonococci in fresh broth which was deposited 1-1/2 inches into the urethra and retained for 5 minutes during which time the penis was stretched and massaged. A typical infection was present within 46 hours.

Also on November 9th, two groups of 3 volunteers each were exposed to further broth dilutions of the same suspension of gonococci in the following manner: 0.05 of the inoculum was deposited 1/2 inch into the urethra, the excess being allowed to escape and the penis was not massaged. Infections developed in four and five days, respectively, in two of the three volunteers injected with one-half strength McFarland #5 suspension, while only after 18 days did one of three volunteers develop an infection following the injection of one-tenth strength McFarland #5 suspension.

The above results appeared to indicate that a moderate increase in the in-

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tensity of the inoculating attack should yield a uniformly successful inoculation regime. Therefore, on November 26th, three more volunteers were exposed to a 15 day old suspension of strain TH#1. The suspension was of a concentration McFarland #5 which was twice as strong as employed November 9th, the inoculating dose was 0.1 cc. instead of 0.05 cc. and was deposited 3/4" instead of 1/2" into the urethra. Only one of three volunteers developed an infection. This was present within 48 hours.

On December 4th the suspension of organisms of strain TH#1 was 23 days old but was used exactly as on November 26th except that the concentration of the organisms was increased from a McFarland #3 to a McFarland #10 density. Of the 3 volunteers exposed only one developed an infection: this was at 7 days.

These puzzling results indicated that possibly the age of the strain was of major importance in the production of the disease, so strain TH#1 at 7 days of age was employed on December 15th exactly as used on November 26th when one of 3 volunteers was infected with 15 day old strain TH#1 organisms. Even with identical technique and younger organisms, all 4 of the volunteers exposed on December 15th failed to reproduce the disease.

On December 18th strain TH#1 at 10 days of age was used in three volunteers exactly as employed in the infected volunteers of October 20th and the one volunteer of November 9th, except that the inoculum was not retained and the penis was not massaged. As compared, however, to the partially successful experiments with the other two groups on November 9th and to the single groups on November 26th and December 4th where retention and massage also were omitted, the organisms of this suspension were 5 to 13 days younger, the concentration of the suspension was 2 to 20 times stronger, the inoculating dose was 2 to 4 times greater, and the introduction of the organisms was 2 to 3 times deeper into the urethra, not any of the 3 volunteers was infected. Following this experience it was decided to forego further studies with strain TH#1 and to examine other freshly-isolated strains.

On December 23rd strains TH#5 and TH#6, each 8 days old, and on December 24th, strain TH#7, 7 days old, served as the inoculating organism in one of 3 groups of volunteers each. The concentration of the organisms in fresh broth was McFarland #10. The amount of the inoculum was 0.2 cc. and was deposited 1-1/2 inches into the urethra without retention or penile massage, except that the penis was stretched and massaged 3 times after a lapse of 15 minutes. STRAIN TH#5 produced an infection in one of the 3 volunteers but only after 31 days (sic): STRAIN TH#6 also produced one infection: this at 2 days. STRAIN TH#7 produced 2 infections in the 3 volunteers exposed. These infections, were noted at 3 and 4 days, respectively, were characterized by a moderately profuse yellow purulent discharge, were not accompanied by severe discomfort, and were readily susceptible to sulfathiazole therapy. For these reasons strain TH#7 was selected for future studies.

On January 11th strain TH#7 was used in the exposure of 7 groups of 3 volunteers each.

- Group 1. By means of cotton-tipped toothpicks a liberal amount of yellowish purulent material was conveyed from an infected untreated volunteer into the urethras of the 3 volunteers. The gonococci-laden pus was rubbed well over the distal 1/2 inch of the urethral mucosa. One infection developed at the end of 4 days.
- Group 2. This group of 3 volunteers was treated exactly as Group 1, except that each volunteer had, immediately preceding inoculation, received a prostatic massage which produced ample prostatic secretion at the urinary meatus. Two of the 3 volunteers in this group reproduced the disease by the end of the 4th and 6th day, respectively.
- Group 3. For each of the 3 volunteers a cotton-tipped toothpick was rubbed well over the surface of 7 day old strain TH#7 growing luxuriantly on the surface of solid "V" media in Petri dishes (separate dishes for each volunteer), and conveyed directly to the distal 1/2 inch of the urethra following thorough prostatic massage as in Group 2. Two of the 3 volunteers developed typical gonorrhoea at 3 and 5 days, respectively.
- Group 4. In this group of 3 volunteers, equal parts of 10% mucin and McFarland #10 suspension of 7 day old strain TH#7 gonococci in fresh broth were thoroughly mixed. The inoculum was therefore considered to be a 5% mucin solution with a concentration of McFarland #5, and 0.1 cc. was introduced 1/2 inch into the urethra. All three volunteers developed typical gonococcal infection by 3, 4, and 13 days, respectively.
- Group 5. The 3 volunteers in this group were given prostatic massages immediately preceding identical exposure as carried out in Group 4. Two of the 3 volunteers showed typical infections at 5 and 8 days, respectively.
- Group 6. Strain TH#7 at 7 days of age was prepared in fresh broth to a concentration of McFarland #5. The amount of the inoculum was 0.1 cc. and was deposited 1/2 inch into the urethra immediately following prostatic massage. Only one of the 3 volunteers was infected - this by 4 days.
- Group 7. The 3 volunteers in this group were treated exactly as those in Group 6 with the additional step whereby pronounced venous congestion was secured distally to a constricting band placed around the penis close to the suspensory ligament. This congestion was maintained for 5 minutes following the introduction of the inoculum. Only one of the 3 volunteers was infected - this at 4 days.

In the seven groups exposed on this day (January 11th) there was not any attempt made to retain any excess amount of inoculum. In every instance thorough manual massage of the glans penis was carried out by the volunteer under close scrutiny. In 19 of the volunteers this massage was continued for exactly 5 minutes. In 2 men massage was ordered discontinued at 3-1/2 minutes because of pronounced turgescence. These latter men did not develop gonorrhoea.

Encouraged by the results of the mucin experiments discussed in Groups 4 and

5 of January 11th wherein 5 of 6 volunteers were infected, 6 men were exposed on January 15th to double the concentration of organisms and of mucin used on January 11th. The strain employed was TH#7, which was again 7 days old, was from the same patient, and inoculation was followed by 5 minutes of massage of the glans penis. Three of the 6 volunteers had previously had prostatic massage. All 6 men failed to develop the infection.

It was then considered that the concentration of mucin may have been too great (10%) on January 15th, so again 6 volunteers were exposed on January 19th exactly as Groups 4 and 5 had been exposed on January 11th, except that the strain TH#7 organisms (again from the same patient) were 8 instead of 7 days old. Three of the volunteers had previous prostatic massage and three had not. The glans penis was massaged vigorously by the volunteer for 5 minutes following inoculation. One infection developed in each group at 6 and 10 days respectively. Obtaining 5 infections in 6 volunteers on January 11th, followed by complete failure to infect anyone of 6 men on January 15th, and by infecting only 2 of 6 men on January 19th, the indications were that further studies with a mucin-containing inoculum would be of questionable value.

In reviewing the studies up to this point it will be noted that the method employed on October 20th with 6 volunteers, and again on November 9th with one man, was the only one which uniformly had produced infections. This method required 0.2 cc. of McFarland #10 suspension to be introduced to a depth of 1-1/2 inches and retained for 5 minutes while the pendulous portion of the urethra was stretched and massaged. Although the objections to this method in any study involving the testing of the usefulness of any topically applied prophylactic agent are obvious, it did appear to offer a reliable means of producing experimental gonococcal infections in the male urethra. This method was therefore employed on January 22nd, using an 18 day old growth of strain TH#7. All 3 volunteers had typical infections by the end of three days.

Although this drastic method had worked with 3 different strains on 3 different occasions to infect all 10 volunteers exposed, it was decided to test this procedure again in 3 volunteers. Accordingly, on January 25th strain TH#7, 17 days of age, was employed. All 3 volunteers remain free of the infection to date.

Without delaying for the results expected in the group inoculated on January 25th, strain TH#7 of 19 days of age, was used for experiments carried out on January 27th. One man was exposed exactly as called for by this method. Six men were similarly exposed except that the number of organisms in the inoculum was reduced one-half for 3 of the volunteers, and to 1/10 for the other 3. Three volunteers were also exposed exactly as required by this method except that the 0.2 cc. of McFarland #10 inoculum was introduced only 1/2 inch into the urethra for retention and massage for 5 minutes. Not any of the 10 men exposed on January 27th have developed the infection to date.

The above discussion has been limited by following a chronological order. The following tables are concerned with grouping the various inoculating methods which have been employed.

Table II

McFarland #10 in Broth. No Massage - no retention.
In 3/4"

<u>Date</u>	<u>Strain</u>	<u>Age</u>	<u>No. Vol.</u>	<u>No. Infected</u>	<u>Dose</u>
Dec. 4	TH #1	23 d.	3	1	0.1
<u>In 1-1/2"</u>					
Dec. 18	TH #1	10 d.	3	0	0.2
Dec. 23	TH #5	8 d.	3	1	0.2
Dec. 23	TH #6	8 d.	3	1	0.2
Dec. 24	TH #7	7 d.	3	2	0.2
<u>Total</u>			<u>15</u>	<u>5</u>	

Table III

McFarland #5 in Broth. No Massage - no retention.
In 1/2"

Jan. 11	TH #7	7 d.	3	1	0.1
Jan. 11	TH #7	7 d.	3	1 (venous congestion)	0.1
<u>In 3/4"</u>					
Nov. 26	TH #1	15 d.	3	1	0.1
Dec. 15	TH #1	7 d.	4	0	0.1
<u>In 1-1/2"</u>					
Jan. 11	TH #7	7 d.	3	1	0.1
Jan. 11	TH #7	7 d.	3	1 (venous congestion)	0.1
<u>Total</u>			<u>19</u>	<u>5</u>	

Table IV

McFarland #10 in Broth. Retained and Massaged 5 minutes
In 1-1/2"

<u>Date</u>	<u>Strain</u>	<u>Age</u>	<u>No. Vol.</u>	<u>No. Infected</u>	<u>Dose</u>
Oct. 20	TH #1	20 d.	3	3	0.2
Oct. 20	TH #2	20 d.	3	3	0.2
Nov. 9	TH #1	18 d.	1	1	0.2
Jan. 22	TH #7	18 d.	3	3	0.2
Jan. 25	TH #7	17 d.	3	0	0.2
Jan. 27	TH #7	19 d.	<u>1</u>	<u>0</u>	0.2
	<u>Total</u>		<u>14</u>	<u>10</u>	

And on January 27th with TH #7 - 19 days old - 1-1/2" - retained and massaged 5 minutes: McFarland #10/2 - none of 3 infected.

McFarland #10/10 - none of 3 infected.

And again on Jan. 27th with TH #7 - 19 days old - 1-1/2" - retained and massaged 5 minutes: McFarland #10 - none of 3 infected.

Total 23 volunteers 10 infected

Table V

MUCIN EXPERIMENTS

No retention - Glans Penis massaged 5 minutes - 1/2" depth.

January 11th TH #7 - 7 d. - McFarland #5 - Mucin 5%

3 of 3 without prostatic massage: - infected

2 of 3 with " " : - infected

January 19th TH #7 - 8 d. - McFarland #5 - Mucin 5%

1 of 3 without prostatic massage: - infected

1 of 3 with " " : - infected

January 15th TH #7 - 7 d. - McFarland #10 - Mucin 10%

3 of 3 without prostatic massage: - no infections

3 of 3 with " " : - no infections

Summary: With prostatic massage 9 volunteers; 3 infected

Without " " 9 " ; 4 "

T A B L E VIII

Incubation Period

1 day - 6 infections	7 days - 1 infection
2 days- 3 "	8 days - 1 "
3 days- 6 "	10 days - 1 "
4 days- 8 "	12 days - 1 "
5 days- 3 "	13 days - 1 "
6 days- 2 "	18 days - 1 "
	31 days - 1 "

The study has thus far been characterized by the singular absence of any obvious factor or factors which might be expected to presage the success or failure of any method explored. The most plausible explanation for the erratic results appears to rely upon the well-known fact of individual variations in susceptibility to many diseases. For gonorrhoea this may readily be assumed but cannot, in any way, be considered as proved.

Volunteers have been used only once, so that no information is available as to continued susceptibility or resistance.

If the study is to be continued, it may be possible to evaluate the merits of the following plans aimed at producing a uniformly reliable infecting technique:

1. The cultivation of the inoculated organisms on media containing pooled human sera.
2. The use of multiple strains of gonococci in the infecting inoculum.
3. The use of certain concentrations of various vaginal bacteria, together with the gonococci as the inoculum.
4. Preliminary selection of the volunteer by accepting only those with a low bacteriolytic titer against the organism employed (Spink and Keefer).
5. Selection of new strains.
6. Selection of a new strain from a female patient for each prophylactic survey.
7. Irrigation of the urethra by mechanical, chemical or biologic agents, including alcohol, previous to the introduction of the inoculum.
8. Combinations of two or more of the above suggested means.

A review of the data does not suggest that within a limit of 4 weeks from the time of securing the strain of gonococci is the age of the inoculum of importance. Nor can it be shown that the depth of introduction of the inoculum, the concentration of the inoculum above McFarland #5/2, the amount of the inoculum above 0.05 cc., the retention of the inoculum, the massage of the penis or of the prostate as employed, or an absence or presence of a history of previous gonorrhoea, have been significant factors in the production of the disease.

Again, if it is desired to continue the investigation, it may be advantageous to consider the inclusion of certain studies having to do with the production of sulfonamide-resistance by subminimal therapeutic doses, the course, validity, and response to various antigens of the gonococcus complement fixation

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Table I. Method Summary. Comparison of Efficacy of Agents Injected Intraperitoneally.

Agent	Dosage		Time	Results				
	Ml. Inj.	No. of Injs.		No. of Expts	Tot. No. of Mice	No. Vaginal Cultures	%	Mean Colony Count/10 ⁶ cult.
	-	-	-	12	77	11	14.4	25.6
Saline	2	2	pre & 8 hrs	6	23	7	30.4	25.8
		1	pre	5	33	5	15.2	19.0
Norse Serum	2	2	pre & 8 hrs	8	48	19	39.5	62.9
	2	1	pre	2	15	5	33.3	39.0
Egg Albumin, Merck	2 1:10	1	pre	3	39	37	94.8	160.0
Milk Powder	2 1:10 or 1	1	pre	5	46	44	95.6	127.8
Blood Albumin - Difco	2 1:20	2	pre & 8 hrs	8	81	78	96.3	257.8
	"	1	pre	8	44	42	95.4	163.8
Acacia	2 1:10	1	pre	1	4	4	100	171.2
Agar	2 1:100	2	pre & 8 hrs	2	7	7	100	180.8
	"	1	pre	2	8	7	87.5	181.4
Charcoal	2 1:10	1	pre	3	12	11	91.6	117.4
Mucin	1	1	pre	1	9	8	88.2	115.8

tests of Price and/or Kolmer, the persistence of sulfonamide-resistance of gonococci passed through successive (human) hosts; the relationship of the bacteriolytic titer against the infecting gonococcus before, during, and after treatment, as well as other related problems.

* * * * *

Certain technical details not included in Dr. Mahoney's report were also submitted. These are not reproduced here except the statement that the culture medium used was the McLeod medium.

Dr. Mahoney summarized the Report by saying that in his opinion the results obtained indicate that the study probably cannot be pushed to a successful conclusion insofar as it concerns prophylactic agents; or that at least this would require time and the expenditure of money which goes beyond the present contract.

There ensued a general discussion of Dr. Mahoney's Report in which certain suggestions for further studies were made, as follows:-

Dr. Herrold suggested that for the inoculum there be employed a suspension of gonococci in equal parts of non-inactivated rabbit serum, plus triple distilled water.

Dr. Miller emphasized the fact that Table VI of the Mahoney-Van Slyke Report suggested not a variation in susceptibility of the host, but instead variation in strain virulence, since strain TH#7, taken from the patient on January 4, produced 12 of 18 infections, on January 8 one of 17, and on January 11th 5 of 12. Dr. Miller made certain suggestions with regard to transfer of organisms on culture media and to volunteers.

Dr. Hill discussed the possible association and colony type of virulence, and also suggested that it would be desirable to use for transfer to patients cultures which had been grown on artificial media for approximately 6 hours.

Drs. Herrold, Hill, and Miller agreed to write out their suggestions and to transmit copies to Doctors Mahoney and Van Slyke.

THE PROPHYLAXIS OF GONOCOCCAL INFECTION IN THE VAGINAE OF IMMATURE FEMALE MICE (OEMcmr-197). Dr. Justina Hill

The Report of Dr. Justina Hill is summarized in two tables - 1 and 2 - which are appended herewith.

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 Table II. Prophylactic Experiments, ¹⁵ Treatment 2 Hours after Inoculation

Prophylactic Treatment per vaginam unless otherwise indicated.		No. of Expts.	Act.No. of Lines	# Vaginal Cultures 24 hrs after Inoc.	
Agent	Dilution			No.	Per cent
Water	-	18	128	101	78.8
Argyrol	1:10	3	30	0	0
	1:30	1	7	0	0
	1:200	1	5	1	20
	1:1000	1	5	3	60
	1:2000	1	5	4	80
Protargol	1:50	2	17	2	11.6
	1:100	1	9	2	22.2
	1:200	2	14	7	50.0
Silver Picrate in Jelly	1:400	7	50	14	21.2
Jelly base	-	3	25	16	65.0
Arsenical #3 in Water	1:200	1	6	0	0
	1:400	2	23	19	82.6
	1:500	3	12	7	58.3
	1:1000	4	19	12	63.1
	1:2500	3	14	10	71.4
	1:5000	4	20	13	65.0
Arsenical #85 in Water (see below)	1:200	1	7	1	14.3
	1:400	2	22	16	72.7
	1:500	3	12	5	41.6
	1:1000	8	29	14	48.2
	1:2500	3	13	10	55.5
	1:5000	4	19	15	79.0
Arsenical #115 in water	1:200	4	13	9	50
	1:1000	4	13	5	46.1
	1:2000	2	10	8	80
	1:4000	2	10	10	100
Arsenical #114 in water	1:200	2	9	2	22.2
	1:1000	2	9	2	22.2

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Arsenical # D-1001a	1:200	4	23	11	47.8
	1:1000	3	11	7	62.6
Arsenical No. 1002	1:200	2	13	1	76.9
	1:1000	1	7	2	50.0
	1:5000	1	5	2	40.0
Sulfadiazine	8gm/60kg per os	1	11	5	45.4
	1:100 Na salt per vag.	1	6	5	33.3
	Crystals per vag.	1	9	4	44.4
Sulfamerizine	4gm/60kg per os	1	4	2	50
	8gm/60kg per os	1	11	6	55.5
	Crystals per vag.	1	12	5	41.6
Sulfathiazole (see below)	4gm/60kg per os	3	24	21	87.5
	Crystals per vag.	2	23	18	78.2
Calomel Oint. in Lanolin & Petrolatum	30%	3	23	3	13.0
Lanolin- Petrolatum Base	-	2	11	8	72.7
Army Base No. 1	-	1	9	8	88.8
S-2060	15% Thiazole	2	15	8	53.3
S-2037	Calomel 30%	2	15	1	6.6
Army Prop. No. 1	S-2060 C-2037	4	29	0	0

Army Base No. 2	-	1	8	5	62.5
E - 2059	15% Thiazole	2	15	8	53.5
C - 2036	30% Calomel	2	14	0	0
Army Proph. No. 2	S-2059# C-2036	4	32	1	3.1
No. 85	1:1000 in Base A	1	9	0	0
	Base A	-	-	-	-
No. 85	1:1000 in Base A	1	10	6	60
	Base B	1	10	6	60

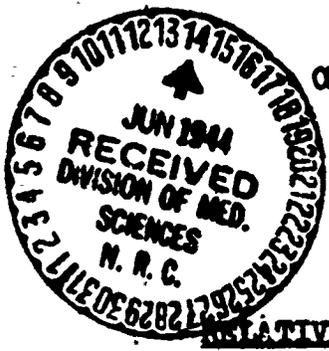
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VENEREAL DISEASES REPORT #13 (ABSTRACTED)
Abstract of Final Report
30 April 1944

RELATIVE PROPHYLACTIC EFFECTIVENESS AGAINST SYPHILIS OF
ONTIMENTS CONTAINING CALOMEL IN DIFFERENT PARTICLE SIZES.
William L. Fleming, M. D. (University of North Carolina).

The prophylactic effectiveness against syphilis of ointments containing calomel in different particle sizes was tested. The base for the ointments was composed of equal parts of hydrous lanolin and white petrolatum. The different calomel particle sizes tested were 100 micra, 5 micra, and 1 micron. In testing, one of various amounts of the different particle size ointments was applied to a superficial incision in the skin of a clipped area of a rabbit's back one hour after inoculation of the scratch with syphilitic testicular emulsion. Protection was judged by lack of chancre formation and by negative lymph node transfers six months after inoculation. The effectiveness of the calomel ointments was inversely proportional to the particle size of calomel. The 100 micra particle size ointment was almost completely ineffectual.

Four phenyl arsenoxide derivatives (7-a, D-66, 90-c, D-1001A) obtained from Dr. Harry Eagle were tested for prophylactic effectiveness against syphilis. The compounds were tested in solution in propylene glycol. Concentrations of 0.8, 0.4, 0.2, 0.1, and 0.05% were tested with all of the compounds except 90-c, with which the 0.05% concentration was omitted and the 1.6% concentration added. These compounds were tested by rubbing them into superficial incisions in the skin of clipped areas on rabbits' backs four hours after inoculation. Partial protection was obtained with the two higher concentrations. D-1001A proved to be the most effective compound tested.

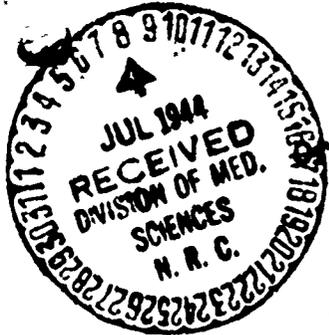
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The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

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COMMITTEE ON MEDICAL RESEARCH
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VENEREAL DISEASES REPORT #14 (ABSTRACTED)
Abstract of MS. for Publication
20 May 1944

THE ENHANCEMENT OF VIRULENCE OF GONOCOCCUS FOR THE
MOUSE. C. Phillip Miller, M.D. (with the technical
assistance of Edward Tamani, University of Chicago)

The virulence of a strain of gonococcus for white mice was enhanced by persistent animal passage until the virulence reached a level which enabled inocula of less than 10 gonococci to initiate lethal infections with regularity. One hundred and thirty animal passages during the course of 15 months were required to raise and stabilize virulence at this level.

This strain surpassed all others in its response to mouse passage. Results with ten other strains out of a total of 70, were sufficiently successful to bring the minimum lethal doses within the range in which death could be the result only of a genuine infection rather than of the toxic action of the gonococci themselves.

The strain's ability to retain virulence during subcultivation on artificial media increased gradually throughout the course of the series of animal passages. In the beginning each subculture resulted in appreciable loss of virulence, but toward the end of the series four subcultures could be interposed without loss of virulence. The experimental gonococcal infection produced by this strain proved highly satisfactory for testing in vivo such chemotherapeutic agents as the sulfonamides and penicillin.

Methods and media are described. Inoculations were made intraperitoneally with gonococci suspended in mucin. Death usually occurred between 18 and 36 hours. The pathological changes were those of generalized sepsis.

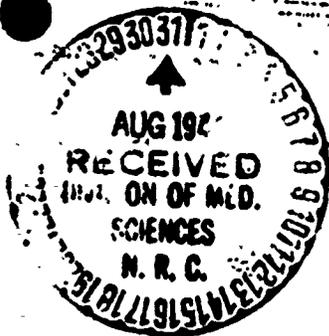
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COMMITTEE ON MEDICAL RESEARCH
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Office of Scientific Research and Development

VENEREAL DISEASES REPORT #15 (ABSTRACTED)
Abstract of MS. for publication

26 May, 1944

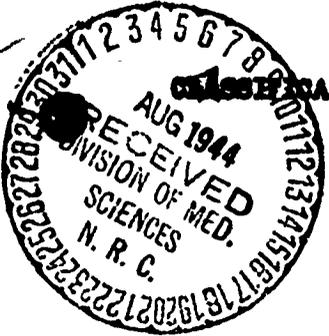
THE LOCAL CHEMICAL PROPHYLAXIS OF EXPERIMENTAL SYPHILIS WITH PHENYL ARSENOXIDES. Harry Eagle, Ralph B. Hogan, and Ralph Fleischman. (U. S. Public Health Service, Johns Hopkins Hospital).

1. Nine trivalent arsenicals (the p-OCH₂CONH₂, p-CONH₂, p-SO₂NH₂, p-NHCONH₂, 3-NH₂-4-CONH₂, 3-NH₂-4-COOH, p-SO₂NHC₂H₄OH, p-CH₂CONHCH₂CONH₂, and p-OCH₂COOH phenyl arsen-oxides) have been studied with respect to prophylactic activity in rabbit syphilis. One-half cc. of solutions in propylene glycol was rubbed for four minutes over a superficial skin incision, at varying intervals before and after its inoculation with a suspension of T. pallidum (Nichols strain) containing 10⁷ organisms per cc.
2. All the compounds were found to be effective, approximately in proportion to their treponemicidal activity. The concentration necessary to protect half the animals when applied four hours after inoculation varied from 0.06 to 0.15% in the case of the more active compounds to 1.5% in the case of the least active compound.
3. The effective concentration of the p-CONH₂ phenyl arsenoxide varied with the time interval between inoculation and application. The prophylactic efficacy of this compound, although definite, was somewhat less pronounced when it was applied before inoculation.
4. The effect of the p-CONH₂ compound and, presumably, of the other arsen-oxides here tested, was due to a direct treponemicidal action on the organisms in the skin itself, and not to a systemic effect on an established infection.
7. The stability of the selected compounds here discussed, the time interval over which they remain effective, the low concentration necessary within reasonable time periods, and the absence of local irritative effects at those concentrations all offer promise that some of these compounds may be of value in the prophylaxis of the human disease.

The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

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COMMITTEE ON MEDICAL RESEARCH
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Final Report

July 1, 1944.

NAME OF RESPONSIBLE INVESTIGATOR: Medical Director J. F. Mahoney, USPHS
SUBJECT: Prevention of Gonococcal Infection in Experimentally Infected Human Volunteers.
CONTRACT NUMBER: M-3169

Effective at the close of business on June 30, 1944 the above-designated investigation was terminated.

The progress of this study has been reported in bimonthly progress reports dated September 7, 1943; November 1, 1943; January 3, 1944; and May 1, 1944. A detailed interim report was made on February 9, 1944 and a memorandum concerning the current status of the study was submitted on March 7, 1944.

In the course of the study a total of 247 patients were experimentally inoculated, of which 82 developed gonorrhoea. In spite of the use of different strains of N. gonorrhoea, modifications in methods of cultivating the organism and of inoculation, it was found impossible to infect with a degree of regularity which would be required in the testing of prophylactic agents. As a consequence termination of the project was recommended.

A final, complete report of the study is in preparation and will be submitted for approval for publication.

This document contains information affecting the national defense of the United States within the meaning of the Espionage Act, U. S. C. 50, 31 and 32. Its transmission or the revelation of its contents in any manner to an unauthorized person is prohibited by law.

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VENEREAL DISEASES REPORT No. 17 (ABSTRACTED)

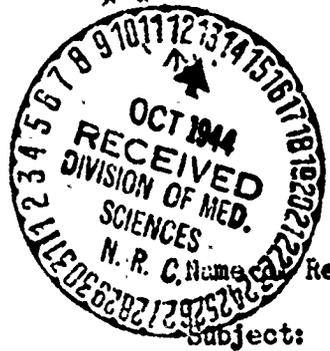
COMMITTEE ON MEDICAL RESEARCH

of the

Office of Scientific Research and Development

Summary, Annual Report

July 15, 1944
Contract No:
OEM-cmr 197



Responsible Investigator: Justina H. Hill

Subject: Studies and investigations in connection with the establishment of a gonococcal infection in experimental animals by methods applicable to the study of venereal disease.

Investigations have been undertaken along the following 3 lines:

1. studies of prophylactics by the method previously described
2. studies of the agents which prolong the survival time of gonococci and the inflammatory response in the vaginal mucosa of the prepuberal mouse.
3. studies of the gonococcus for the purpose of selection and stabilization of the inoculum.

1. Studies of Prophylactics by the method previously described, i.e. intraperitoneal injection of 2 ml. of 5 per cent blood albumin, followed by vaginal inoculation, treatment 2 hours after inoculation, culture 24 hours after inoculation. The results are summarized as follows:

Prophylactic	% of recoveries of gonococci 24 hrs after inoculation
0	89.1
Army Base No. 1	73.1
Army Prophylactic No. 1	10.7
S-2060, 15% sulfathiazole in Army Base No. 1	51.4
C-2037, 30% Calomel " " " " "	12.3
Army Base No. 2	66.6
Army Prophylactic No. 2	1.3
S-2059, 15% sulfathiazole in Army Base No. 2	58.2
C-2036, 30% Calomel " " " " "	66.6
50% Petrolatum, 50% Lanolin base	72.7
Calomel Ointment, U.S.P.	12.5
Sulfadiazine <u>per os</u> , 8 gms/60kgm	45.4
" <u>crystals per vaginam</u>	44.4
Water <u>per vaginam</u> , control for the above	81.8

The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

Agent Given Intraperitoneally	% of recoveries of Gonococci 24 hrs. after inoculation
0	19.4
Sodium Chloride, 2m., once, before inoculation	20.0
Horse Serum, 2 m., once, before inoculation	33.3
Horse Serum, 2 m., twice, before & 8 hrs after inoculation.	39.5
Egg Albumin, powdered, 2 ml. 5%, once, before inoculation	62.5
" " " " " 10% " " "	94.4
Milk Powder, 2 ml. 10%, once, before inoculation	93.8
Mucin, Granular, 1 ml. 5% " " "	74.4
Blood Albumin, 2 ml. 5% " " "	89.4
" " " " " , twice, before & 8 hrs after inoculation	96.3

In smaller series marked effect was also noted with 2 ml. 10% acetic, i.p., - or 2 i.p. injections of 1 per cent agar, 1 i.p. injection of 2 ml. of 1 per cent charcoal, and with 1 oral administration of 0.3 ml. of 24 % alcohol. Results with histamine acid-phosphate, insulin and phenobarbital were not good.

Application of this method to tests in mice inoculated intravenously or subcutaneously with Streptococcus 203 or with a Type 1 pneumococcus showed that the intraperitoneal injection of blood albumin (1) increased the mortality and (2) shortened the duration of life.

3. Studies of the Gonococcus for the Purpose of Selection and Stabilization of the Inoculum.

During the course of this investigation it has become evident that the gonococcus is an exceptionally labile organism, which degrades rapidly upon the usual media. Observations are being made upon the types of colonies obtained on a variety of media from clinical cases, in order to determine the types associated with acute, untreated human gonorrhoea. A study is being made of a number of different media, of the influence of various physical and chemical factors upon the type of growth and from the point of view of stabilization of the inoculum. These studies are being paralleled by observations in the fertile egg, from the point of view of finding a medium by which the organism can be "smoothed".

It is believed that only by a combination of reduction of host resistance and use of undegraded gonococci that a true animal infection can be established on the mucosa and that control of the inoculum is essential for all types of in vitro or in vivo experimentation with N. gonorrhoeae.

The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

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VENEREAL DISEASES REPORT No. 18

COMMITTEE ON MEDICAL RESEARCH
of the
OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT

ANNUAL REPORT NO. II

Date: August 1, 1944



NAME OF RESPONSIBLE INVESTIGATOR: Marvin R. Thompson

SUBJECT: (1) Chemoprophylaxis in Gonorrhoea, Venereal Lymphogranuloma and Chancroid.
(2) Study of Sulfonamides in Ointment Bases.

CONTRACT NO.: OEMcmr-204.

The investigations of the Warner Institute group led to the adoption of one of their formulas for the chemoprophylaxis of venereal disease, for use by the Armed Forces.

Stabilization studies on the calomel-sulfathiazole ointments were continued throughout the year, with results as set forth in the preceding bimonthly progress reports under this contract.

It should be pointed out that the research efforts of the Warner Institute group, for the second year of their contract, were proscribed by the membership of the Subgroup on Vehicles.

At the request of Dr. Harry Eagle, various arsenical compounds, supplied by him, were incorporated into two types of ointments. The comments and suggestions of Dr. Eagle were studied and samples submitted to him.

This contract expired July 31, 1944 and no application was made for renewal.

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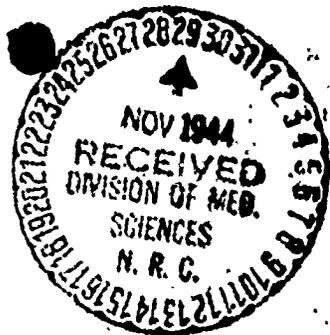
Office of Scientific Research and Development

VENEREAL DISEASES REPORT #19

Received for distribution 14 November 1944

RECENT ADVANCES IN THE BACTERIOLOGY OF GONORRHOEA

Justina H. Hill, D.C.*



(Paper presented before the Clinical Society of Genito-Urinary Surgeons, April 1, 1944, and to be published in the Transactions of that Society.)

During the past two years bacteriologic work in our laboratories has included intensive research on the gonococcus. The purpose of this report is to summarize briefly the three main aspects of this work as follows: first, a constant effort to improve laboratory methods for the recovery and identification of gonococci from clinical material; second, studies of drug resistance; and third, research pertaining to the development of a mucosal infection in animals and of a method for the evaluation of chemoprophylactics.**

1. Routine Procedures. It is now recognized that the use of culture methods is essential except in acute, untreated urethritides or in frank chemotherapeutic failures. No exact diagnosis in asymptomatic gonorrhoea in the male or in almost any type of this disease in the female can be made by microscopic examination. No rigid criterion of cure can be established which does not include the use of a series of cultures made after the patient's system is free from all traces of the chemotherapeutic agent which has been employed.

Although a number of satisfactory media for primary isolations of the gonococcus have been described, most of these require the use of meat and are somewhat difficult of preparation. On the basis of a preliminary investigation of the influence of a number of substances upon the growth of the gonococcus, Miss Huffer and I found that a simple meat-free medium, a cystine, glucose, chocolate blood agar, gave results comparable to those obtained with the usual media prepared from a meat base (Huffer and Hill, 1943). While it is our opinion that further investigation will result in even better media, the trend toward simpler formulae and ease of preparation facilitates the inclusion of gonococcus culture methods in routine laboratory procedure. The question of the development of satisfactory media for the transportation of specimens from outlying clinics to central laboratories is of the most urgent importance, if the culture method is to be as widely applicable as it should be.

Improvement of methods for the identification of the gonococcus would be of value. The fermentation tests which form the classic means of identification are time-consuming and must be carefully watched for the detection of errors. Following the recent work of Phair and his associates (Phair, Smith and Root, 1943), Miss Huffer has immunized a number of chickens with gonococci and has also worked with polyvalent anti-gonococcal chicken serum prepared by Sharp and Dohme. While in general agglutination titers with these sera have been higher with gonococci than with meningococci, some cross agglutination with meningococci has been found and some difficulty with auto-agglutination has occurred. At present

*With the collaboration of M.E. Turner, S.V. Huffer and E.E. Well.

**The work described in this part of this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

the method permits rapid identification of a given organism as a species of Neisseria and it is hoped that by further refinement differentiation can be obtained within the species N. Gonorrhoeae of carrier, avirulent or virulent strains, a matter of public health importance. This work is temporarily being postponed until immunizations can be made which will yield more highly titered antisera and until better definition can be obtained of the phases of the organisms used as antigens.

2. Drug Resistance Standardization and improvement of methods of determining drug resistance are pertinent at this time, when the increased incidence of sulfonamide resistant gonorrhoea is being established in many widely separated areas and when the evaluation of penicillin is in the same state as that of the sulfonamides at the time of their introduction. In this country no base line was ever established of the normal resistance of gonococci to the sulfonamides, so that we have today no accurate basis of comparison for the determination of increase of gonococcal resistance. The time to determine such a base line of normal penicillin resistance of the gonococcus is now, before the drug has been widely used. If this can be done by valid methods, we shall be able ten years from now to determine by statistical comparison whether or not increased penicillin resistance has developed. If such studies are to be made with any chemotherapeutic agent, some agreement on methods must be reached and the media employed for the determination of resistance should be free from any substances which interfere with the action of the drug being studied. In the case of the sulfonamides, the elaboration of such inhibitor-free media is difficult because of the general use of peptone in media which support the growth of the gonococcus. Following the charcoal adsorption methods of MacLeod (MacLeod 1940), we have prepared a sulfonamide inhibitor-free medium which permits exact determination of sulfonamide resistance, with no masking of any of the effect of drug action by the presence of inhibitors (Hill, Petroskas and Huffer 1944). The problem of obtaining penicillin-inhibitor free media promises to be much simpler. In view of the generally favorable response of gonorrhoea to penicillin therapy, it seems improbable that it will be shown, as in the case of colon group bacilli, that gonococci themselves, elaborate penicillin inhibitors.

3. Animal Inoculations with Gonococci. Review of previous attempts to infect animals with gonococci (Hill 1944) has shown that only two methods are now available, inoculation of the chick embryo and use of the intraperitoneal mucin technique. The object of this investigation is to establish if possible a mucosal infection in animals by a method suitable for statistically significant studies of chemoprophylaxis. The two main approaches to the problem are modification of the resistance of the animal host and stabilization of the inoculum. We are not prepared at this time to make conclusions in regard to the latter aspect of the problem except to state that the gonococcus is an organism of extreme lability and that the development of means by which it can be stabilized in a state corresponding to its most virulent or infective form presents many difficulties. We have, however, been able to so modify host resistance as to prolong the survival of gonococci on the vaginal mucosa of the preputeral mouse. Gonococci normally disappear from this surface with great rapidity. We are at present able to recover them 24 and in some cases 48 hours after inoculation and this prolongation

of survival is accompanied by an inflammatory response and active phagocytosis. The means by which this prolongation is obtained consists of the intraperitoneal injection before vaginal inoculation of one of a number of substances, including milk, egg albumin, blood albumin or mucin. By the use of such methods it is now possible to evaluate the effect of prophylactics on the vaginal mucosa, since satisfactory recoveries of gonococci can be made from the controls 24 hours after inoculation in from 70 to 95 per cent of the animals. While this whole problem is one of great complexity, its investigation has both afforded an in vivo method of evaluating chemoprophylactics which can serve as a screening test and has forced us to realize our ignorance of many of the fundamental biologic reactions of the gonococcus. Much remains to be learned concerning the virulence patterns of the gonococcus and the solution of this problem is of importance both in regard to this particular research and in relation to the clinical and public health aspects of gonorrhoea.

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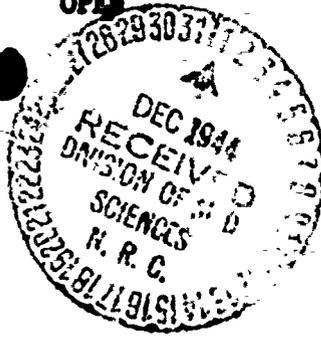
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COMMITTEE ON MEDICAL RESEARCH
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VENEREAL DISEASES REPORT #30 (ABSTRACTED)
Abstract of Paper for Presentation
Received 11 October 1944

**SYPHILITIC IRITIS WITH PARTICULAR REFERENCE TO THE
HERXHEIMER REACTION AS A DIAGNOSTIC AID AND THE EFFECT
OF DIFFERENT TREATMENTS, INCLUDING PENICILLIN. J. V.
Klauder and G. J. Dublin.**

How valid is the diagnosis of syphilitic iritis based solely on a positive Wassermann reaction, or such diagnosis in the late stage of syphilis or when the duration of infection is unknown? What are the criteria of the efficacy of anti-syphilitic treatment to justify the conclusion that iritis was caused by syphilis? Antisyphilitic treatment exerts a non-specific effect on iritis. Judgment at times is difficult, since local treatment and non-specific therapy exert favorable action on iritis. The purpose of this paper is to discuss these considerations and also the Herxheimer reaction of the ocular lesion as evidence of syphilitic causation. The intensification of the inflammatory process (constituting the Herxheimer reaction) was evaluated by slit lamp examination conducted before and soon after antisyphilitic treatment. The Herxheimer reaction as observed through the corneal microscope has heretofore not been employed on diagnosis.

Of 33 patients with syphilitic iritis, three were treated with penicillin. The case record of one is detailed; the flare up of the ocular lesion (Herxheimer reaction) after penicillin is described; the period required for the iritis to become quiescent and the effect on skin and mucous membrane lesions are discussed.

In order to avoid too pronounced Herxheimer reaction and too rapid retrogression of the inflammatory lesion (therapeutic paradox), reduced initial doses of penicillin were employed—10,000 units for the first four injections in contrast to 50,000 units employed in treatment of early syphilis. Total dosage of 2,400,000 units of penicillin was administered in treatment of two patients, and 1,200,000 units of penicillin was administered in treatment of one patient with syphilitic iritis who had associated secondary syphilis.

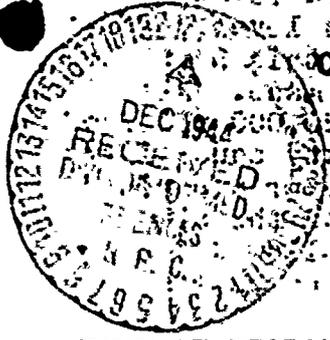
The iritis of the three patients treated with penicillin became quiescent within eight days. This compared with two to five weeks in patients treated with chemotherapy and fever combined with chemotherapy.

The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

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COMMITTEE ON MEDICAL RESEARCH
OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT

VENEREAL DISEASES REPORT No. 21

Date: August 29, 1944

NAME OF RESPONSIBLE INVESTIGATOR: Dr. R. V. Platou

SUBJECT: The effect of penicillin in congenital syphilis

CONTRACT NO. QEMcmr-461

An investigation into the effects of penicillin in the treatment of congenital syphilis was initiated in this clinic on 4/12/44.

This report deals with preliminary observations secured after treating 33 infants and children in various stages of the disease, ranging in age from 11 days to 11 years, and in weight from 2.6 to 33.8 kilograms. Thirty-two were colored, 1 white.

Of this entire group of 33 cases, 29 had received no previous antiluetic therapy; 2 had no more than two or three injections of bismuth, administered months or years previously; and 2 had received conventional therapy with arsenical and bismuth preparations but were included for penicillin therapy because of interstitial keratitis and juvenile paresis.

Diagnoses were established in all cases by careful history and physical examination, investigation of the maternal status, with particular reference to the duration of her disease and the quantitative aspects of any therapy received during pregnancy, serologic tests done according to at least three techniques in at least two different cooperating laboratories, x-rays of long bones in all patients under two years of age, dark field examinations of all known or suspected infectious lesions, careful objective evaluation of all lesions thought to be syphilitic in origin, routine complete spinal surveys just preceding treatment.

Cases were selected for treatment on the basis of medical need, socioeconomic factors independently evaluated, the likelihood of securing adequate follow-up observations, the presence of diagnostic criteria which could be objectively evaluated or measured, and lack of previous therapy (exceptions noted in paragraph one).

Incidental studies of penicillin levels in sera related to dosage changes, in cephalin flocculation and prothrombin levels, local and systemic reactions to penicillin, and the effect of this particular therapeutic regime on associated diseases have been made. All these cases received the prescribed dosage schedule of 20,000 units of penicillin per kilogram of body weight, divided into sixty equal intramuscular injections and spaced at three hour intervals over a period of seven and one-half days. A charted system of rotating the sites of injection was used, similar to that conventionally followed for insulin injections in diabetic children. Each group of cases was treated by penicillin of a different manufacture.

In the first group of 9 cases treated, 1 could be classed as latent and 8 presented manifest active lesions. In the single latent case there was no apparent clinical change, but a prompt fall in Kahn titre to zero was followed three months later by a return to the previous level. Of the 8 with manifest lesions, all showed marked improvement, and all superficial lesions healed during therapy. Follow-up observations have been possible in 7 cases; quantitative Kahn titres declined to zero during or following therapy in 3, progressively declined in 1, and fluctuated in 2. In 2 of 3 cases having positive spinal fluid findings prior to treatment, both became negative; and 1 has not been re-examined.

Among 16 cases treated in the second group, there were 4 previously untreated cases which could be classed as latent; in 1 of these the quantitative titre became negative during therapy, progressively declined in 1, is fluctuating in 1, and could not be evaluated in the fourth. Of 12 cases with manifest lesions, titres fell to zero during treatment in 3, during the ensuing three months in 2, are progressively declining in 5, and are fluctuating in 2.

Again in this group, marked clinical improvement was noted. In the observations made thus far, the involution of skeletal lesions seen in the x-rays does not appear to be accelerated by penicillin, though data for this observation have not been completely analyzed. Spinal fluid examination, positive in 4 of these cases before treatment, became negative in 3, and it has not been re-examined in the fourth.

In the third group of 8 cases, there were 2 with no manifest lesions; adequate diagnostic criteria were present, however, and no previous therapy had been given. In 1 of these previous Kline and Kolmer reactions strongly positive have been followed by a progressive decline in Kahn titre; in the other previously strongly positive Kline and Kolmer reactions were followed by persistently negative Kahn tests, and one positive Kolmer sixty-one days after penicillin was begun.

Of 6 patients presenting manifest lesions, marked improvement was noted in all during and after treatment. While all cases had characteristic histories and lesions together with strongly positive or positive Kline and Kolmer tests, only 2 of these 6 had measurable reagin titres by the Kahn technic immediately preceding initiation of penicillin therapy; the first of these shows a decline to zero at 33

days but a return to 3 reagin units at 61 days, while the second shows a decline from a pre-treatment level of 200 reagin units to 80 reagin units 43 days following.

For the 4 patients with negative Kahn titres prior to therapy, adequate follow-up data are not available, though marked serologic fluctuations have been noted in 2, and 2 are apparently remaining negative.

* * * * *

While sufficient time has not elapsed for proper evaluation, the following tentative statements seem justified:

(1) Of 33 cases treated with the 20,000 unit schedule, only 7 had no manifest lesions and could best be classed as latent.

(2) Of 7 cases classed as latent, for which the serologic titre is the only criterion of improvement, there has been reversal during therapy in 1, progressive decline in 2, fluctuations in 3, and 1 could not be evaluated.

(3) For 26 cases showing manifest lesions of congenital syphilis, marked clinical improvement has been apparent for all, comparative roentgenologic observations are not yet possible, superficial lesions cleared dramatically, and the following serologic results have been observed: 8 became negative during or following treatment, 7 progressively declined, 7 are fluctuating, and 4 could not be evaluated.

(4) In these 33 cases, 8 had positive spinal fluid surveys, 2 with clinical juvenile paresis, the other 6 with no unusual symptoms and signs referable to the nervous system. Re-examination of spinal fluid in 5 of these cases showed reversal to normal in all, while the spinal fluid has not yet been re-examined in the other 3.

(5) Only 1 case of interstitial keratitis was treated on this schedule. While the patient volunteers a sense of improvement, there are no objective evidences thereof.

(6) There appears to be no discernible difference in the therapeutic efficiency of the various lots of penicillin employed so far in this study.

(7) It seems reasonable to expect improved results with the next group of patients being treated with a larger dosage schedule (40,000 units/kg.), or by increasing the dosage of penicillin in each case sufficient to produce a serologic reversal during therapy.

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COMMITTEE ON MEDICAL RESEARCH
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VENEREAL DISEASES REPORT No. 22

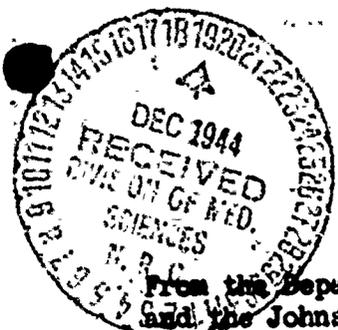
(Received 16 November 1944)

ACUTE SYPHILITIC MENINGITIS TREATED WITH PENICILLIN*

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The recent demonstration that penicillin is of value in the treatment of syphilis (1,2,3) makes it important to record promptly and in as much detail as possible the results obtained in various types of syphilitic infection. Within the first year of use of the drug, interest has naturally centered in early syphilis (1,2), which provides the readiest yardstick of therapeutic efficacy, and is moreover of most importance to the Armed Forces and to the public health.

Aside from uncomplicated primary and secondary syphilis, investigative effort has been devoted largely to neurosyphilis. All types of neurosyphilis have been treated (3). The ultimate test of the efficacy of penicillin in neurosyphilis may well rest on its effect in the serious late forms of the disease, especially paresis. Several years may be necessary before an opinion can be formed. Early neurosyphilis, however, either asymptomatic or with the typical manifestations of acute syphilitic meningitis, provides an opportunity to obtain some information rapidly.

This paper is a preliminary report of the effect of penicillin on acute syphilitic meningitis in ten patients treated at The Johns Hopkins Hospital. In all patients treated, the drug employed was sodium penicillin. The route of administration was intramuscular for reasons to be discussed later. Dosage and duration of treatment were variable, but no other form of treatment was given with or after penicillin in any of the patients here reported.

Case Reports

Case 1. (JHH 305141) A 19 year old Negress was admitted to The Johns Hopkins Hospital on October 19, 1943, complaining of severe headache and vertigo. These symptoms began three weeks before admission, and seven days later were followed by partial but increasing deafness, first on the right and then on both sides. The right side of her face became paralyzed four days prior to admission. There was no history of earlier manifestations of syphilis.

Vision in the left eye, the macular region of which showed a small scar, was limited to light perception and was said to have been bad for years. The fundi were otherwise normal. Bilateral nerve deafness, a right facial paralysis of the peripheral type, a macular rash, and a few darkfield positive genital

*The work in this paper was done under a contract recommended by the Committee on Medical Research of the Office of Scientific Research and Development.

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papules, which the patient had not noticed, were present. There was 50 per cent hearing loss on the right side and 45 per cent on the left by audiometer test. Physical examination was otherwise normal.

The Eagle flocculation titer of the blood was 4 units. The spinal fluid, which was clear and colorless, contained 53 mononuclear cells per cubic millimeter and 25 mgm. of protein per 100cc. The Wassermann reaction was positive with 0.4 cc. but not with smaller amounts, and the mastic test was negative. The erythrocytes sickled, and a biologic test for pregnancy, suggested by the fact that her last menstrual period had been early in September, was positive.

Treatment with 20,000 Oxford units of sodium penicillin intramuscularly every three hours was started on October 22 and continued for 62 injections, a total of 1,240,000 units in seven and one half days. There was no Herxheimer reaction. The mucocutaneous lesions healed promptly. Improvement in hearing and in facial strength, which was first apparent on the fourth day of therapy, continued, so that by the eleventh day after the start of treatment there was no evidence of facial weakness. By the thirteenth day hearing had improved on the left to only 15 per cent loss, although there was still 55 per cent hearing loss on the right.

On November 5, fifteen days after the start of treatment, the spinal fluid contained 29 mononuclear cells per cubic millimeter and 37 mgm. of protein per 100 cc. The Wassermann reaction was now negative with 1.0 cc. and the mastic test gave a flat reading. After discharge the patient was followed in the clinic where her clinical course was uneventful. On January 5, 1944, the Eagle blood flocculation test was negative. The spinal fluid contained no cells and 17 mgm. of protein per 100 cc. The mastic test was negative in all dilutions and the Wassermann reaction was negative with 1.0 cc. Hearing continued to improve; on January 14, there was 12 per cent hearing loss on the right and 3 per cent on the left, and on February 8, six per cent loss on the right and 3 per cent on the left. Frequent blood Eagle flocculation tests gave negative or doubtful results except on one occasion, April 13, when a titer of one unit was reported.

(Insert Table I)

On April 22, because of severe pre-eclampsia, she was admitted to the obstetrical service where she was found to have a marginal placenta praevia. The following day she was delivered by version and extraction. The child showed no evidence of syphilis, darkfield examination of scrapings from the umbilical vein showed no spirochetes, and serologic tests on cord blood were negative. The child has been carefully followed for 124 days and so far has shown no clinical, serologic, or roentgenologic evidence of congenital syphilis. On April 27, the patient's spinal fluid was normal.

On May 10 she returned to the hospital in a severe hemolytic crisis, a manifestation of sickle cell anemia. She recovered rapidly from this and has since been asymptomatic although Eagle flocculation tests of the blood have continued to be frequently doubtful. The course is summarized in Table I.

Case 2. (JHH 308125) A 21 year old Negress was admitted to The Johns Hopkins Hospital on November 26, 1943. A year previously she had developed intermittent, diffuse headaches which 10 months later had increased in frequency

TABLE I
The Course under Penicillin Therapy of Case 1.

Date	Day after penicillin treatment	Treatment	Blood STS, Titer in units	Spinal Fluid	Remarks
10-20-43			4	53 cells 25 mgm.% protein WaR pos. with 0.4cc Mastic 0000000000	Headache 3 wks Hearing: Rt. 50% loss Lt. 45% loss Rt. VII nerve pale DF+ genital lesion Early pregnancy Sickle cell trait
10-22-43	0-8	Penicillin 1,240,000 units.			VII nerve normal 4th day; headache etc. gone.
11-3-43	13				Hearing:- Rt. 55% loss. Lt. 15% loss
11-5-44	15		D*	29 cells 37 mgm.% protein WaR neg. with 1.0cc Elastic 0000000000	
11-22-44	31				Improved
1-5-44	75		0	Normal	
1-14-44	84				Hearing:- Rt. 12% loss Lt. 3% loss
1-20-44	90		0		
2-3-44	104		D		
2-7-44	109				Hearing:- Rt. 6% loss Lt. 3% loss
2-17-44	116		0		
3-16-44	144		0		
4-13-44	172		1		Hearing:- Rt. 3% loss Lt. no loss
4-27-44	186		D	Normal	Delivery, Apr. 23, normal child
5-10-44	197		D		Sickle cell anemia crisis
6-8-44	226		D		Sl. headache
6-15-44	233		0		
6-29-44	247		D		Asymptomatic
7-13-44	261		D		
8-31-44	310		0		Asymptomatic

*In this and subsequent tables D = reaction

and duration and three days prior to admission had become constant and refractory to aspirin. She vomited once on the day of admission. Four months prior to that, because of a rash on the volar surfaces of her forearms, she had consulted her private physician who, finding her blood serologic test to be positive, had given her six intravenous injections, the last one three weeks before admission. This treatment caused the disappearance of the rash (presumably secondary syphilis), but the headache did not improve. There was no history of earlier manifestations of syphilis.

Essential findings of the admission physical examination were a very stiff neck, three diopters of papilledema on the right with a few small retinal hemorrhages and slight papilledema on the left. The other cranial nerves were normal. The skin and mucous membranes were normal, and there were a few small superficial lymph nodes. Visual acuity was 20/30 right and 20/30 left. Visual fields were normal except for enlargement of the right blind spot.

The Eagle blood flocculation titer was 16 units. Roentgenograms of the skull, sinuses, and chest were normal. Because a brain tumor might have been present, it was thought advisable to precede lumbar puncture by ventriculography. The ventricles were normal in size and shape, so that a lumbar puncture subsequently was done. The spinal fluid, which had a groundglass appearance and was under an initial pressure of 190 mm. of water, contained 1280 cells per cubic millimeter, of which 87 per cent were mononuclear and 12 per cent polymorphonuclear, and 100 mgm. of protein per 100cc. The Wassermann reaction was positive with 0.05 cc. but negative with smaller amounts, and the mastic curve was 555553100.

Treatment was started on December 14 with 10,000 Oxford units of sodium penicillin intramuscularly every three hours for sixty-four injection, 640,000 units in seven and one half days. There was no Herxheimer reaction. Her temperature had varied between normal and 102° F. previously, but from the second day of therapy on she was afebrile.

(Insert Table II)

By December 16, two days after treatment was started, the headache ceased, and the following day diminution in the papilledema was apparent. On December 26 the spinal fluid, which was clear and colorless, contained 80 mononuclear cells per cubic millimeter and 37 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.1 cc. but not with 0.5 cc., and the mastic curve was 555432100.

After discharge she remained well. By January 17, 1944, there was no papilledema. On February 3, the spinal fluid contained 37 mgm. of protein per 100 cc., but no cells were seen. The Wassermann reaction was positive with 0.2 cc. but not with 0.1 cc., and the mastic curve was 5543100000. On March 9 there were 37 mgm. of protein per 100 cc. of spinal fluid, but no cells were found. The Wassermann reaction was positive with 0.4 cc. but not with 0.2 cc., and the mastic curve was 5555421000. The Eagle blood flocculation titer diminished and on April 20 was 2 units. The patient was last seen on September 7, 1944, and was asymptomatic, the blood Eagle flocculation test being doubtful. The spinal fluid abnormalities continue to regress. The findings were listed in Table II.

Case 3. (JHH 315662) A 27 year old Negro male was admitted to The Johns Hopkins Hospital on March 10, 1944, complaining of having had several convulsions. In August, 1943, because of a penile sore, (presumably primary syphilis), he had been given elsewhere some antisyphilitic treatment (details not exactly known but consisting of about 20-25 hip and arm injections), which continued irregularly until December. The lesion healed promptly, but late in February, 1944, he became somewhat drowsy and developed headaches. He had one generalized convulsion on March 4 and three subsequent ones which resulted in his admission on March 10.

The patient appeared acutely and seriously ill. The neck was moderately stiff and the sensorium quite clouded. No cranial nerve abnormalities could be made out, and no other physical findings of note were present.

The Eagle blood-flocculation test was negative. The spinal fluid, which was clear and colorless, contained 102 cells per cubic millimeter and 105 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.05 cc. but not with smaller amounts, and the mastic curve was 555543100.

Treatment was started on March 11 with 10,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 doses, 600,000 units in seven and one half days. A rise in temperature to 100.6° F. nineteen hours after the start of treatment was thought to be a Herxheimer reaction. During treatment the headache and drowsiness cleared strikingly, and no further convulsions occurred. On March 19 the spinal fluid contained 42 cells per cubic millimeter and 19 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.4 cc. but not with smaller amounts, and the mastic curve was 3210000000.

(Insert Table III)

After discharge the patient remained well except for several spells of weakness and nervousness. On April 12 the spinal fluid, which contained 4 cells per cubic millimeter, gave a negative Wassermann reaction with 1.0 cc. and a negative mastic test. He was well when examined on August 24, 1944. Table III illustrates the course.

Case 4. (JHH 312211) An 18 year old Negro male was admitted on January 22, 1944, in a stupor following repeated convulsions. On November 2, 1943, he had been seen in the Syphilis Clinic of The Johns Hopkins Hospital with a papular rash, enlargement of the superficial lymphnodes, and a darkfield positive penile lesion. Antisyphilitic treatment was begun and five injections of 0.06 gm. of napharsen and two injections of 0.2 gm. of bismuth were given within nine days before the patient stopped treatment. An effort made to have him treated in a city clinic was, due to his uncooperativeness, a failure. The lesions healed promptly, and he was asymptomatic until January 22, 1944, when after a mild headache, he had a series of generalized convulsions which resulted in his hospital admission.

At that time he was stuporous, but neither then nor the next day when he had recovered consciousness could any neurologic or other abnormalities be found.

Table II
The Course under Penicillin Therapy of Case 2

Date	Day after penicillin treatment	Treatment	Blood STS, titer in units	Spinal Fluid	Remarks
Sept. '43		6x Arsenic	4		Secondary syphilis(?)
11-26-43			4		Severe headaches Papilledema Negative ventriculogram
12-14-43	0-8	Penicillin 640,000 units	16	1200 cells 100 mgm.% protein WuR. pos. with .05cc Mastic 555553100	Papilledema Headache
12-26-43	12		32	80 cells 37 mgm.% protein WuR. pos. with 0.1cc Mastic 5555432100	Asymptomatic Much less papilledema
1-4-44	20		16		Improvement continues
1-17-44	34		12		
2-3-44	48		4	0 cells 37 mgr.% protein WuR. pos. with 0.2cc Mastic 5543100000	Optic discs normal
2-24-44	67		4		
3-9-44	81		4	0 cells 37 mgm.% protein WuR. pos. with 0.4cc Mastic 5555421000	
3-23-44	95		2		
4-20-44	123		2		
7-6-44	200		1	12 cells 22 mgm.% protein WuR. pos. with 0.6cc Mastic 2211000000	Asymptomatic
9-7-44	263		D...		Asymptomatic

TABLE III
The Course under Penicillin Therapy of Case 3.

Date	Day after penicillin therapy	Treatment	Blood STS, titer in units	Spinal Fluid	Remarks
Aug. '43		20-25 Bi-smuth and Arsenic	(No titer)		Chancre
3-10-44	0-8	Penicillin 600,000 units	0	102 cells 105 mgm.% protein WaR.pos. with 0.05 cc Mastic 5555543100	Convulsions Headache Drowsy
3-19-44	9		D	42 cells 19 mgm.% protein WaR.pos. with 0.4cc Mastic 3210000000	Greatly improved
3-30-44	20		0		Asymptomatic
4-12-44	33		D	Normal	Asymptomatic
4-20-44	41		0		Asymptomatic
5-18-44	69		0		Asymptomatic
6-15-44	97		0		Asymptomatic
7-13-44	125		0		Asymptomatic
8-24-44	167		0		Asymptomatic

The Eagle blood flocculation titer was 2 units. The spinal fluid contained 70 lymphocytes per cubic millimeter and 25 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.4 cc. but negative with smaller amounts. The mastic curve was 1100000000.

Treatment was started on the day of admission with 10,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 injections, 600,000 units in seven and one half days. There was no Herxheimer reaction, nor did subsequent convulsions occur. On January 30 the spinal fluid contained 15 cells per cubic millimeter and 25 mgm. of protein per 100 cc. The Wassermann reaction was negative with 1.0 cc., and the mastic test was negative.

(Insert Table IV)

The patient has remained well. The Eagle test was negative on March 16 and subsequently. The spinal fluid on July 6 was normal. Table IV summarizes the findings.

Case 5. (JHH 32207) A 38 year old Negress was admitted to the neurosurgical service on May 21, 1944, complaining of double vision of three weeks' duration. This and a mild headache, which had since been intermittently present, had developed on May 1. On May 13 she was seen by a physician who found that her right sixth nerve was paralyzed. On May 17 she had severe attack of rotary vertigo followed by nausea and repeated vomiting. Tinnitus and partial deafness developed on the right, associated with dizziness and staggering. The next day the right side of her face felt tight. She was admitted to another hospital and found to have right sixth and seventh nerve paralysis and lateral nystagmus on looking to the left. She was discharged after arrangements had been made for her to come to The Johns Hopkins Hospital where she was admitted to the neurosurgical service on May 21.

Essential findings on physical examination were complete paralysis of the right sixth and seventh nerves, complete loss of hearing on the right by audiometer test, normal hearing on the left, nystagmus to the left on lateral gaze, staggering to the right, large pupils reacting neither to light nor on accommodation, normal visual fields, and protrusion of the tongue slightly to the left, although it could be moved to the right.

Following examination of the spinal fluid a diagnosis of syphilitic meningitis was made and the patient transferred to the medical service on May 25.

The Eagle blood flocculation titer was 2 units. The spinal fluid, which was clear and colorless, contained 70 lymphocytes per cubic millimeter and 118 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. but negative with smaller amounts, and the mastic curve was 5554310000.

(Insert Table V)

Treatment was started on May 26 with 20,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 injections, 1,200,000 units in seven and one half days. Her temperature rose to 100.2° F. twenty-

four hours after the start of therapy. This was the highest temperature recorded while she was in the hospital and was thought to represent a Herxheimer reaction. During treatment there was slight improvement in the sixth nerve paralysis, she was able to walk without staggering, the headache ceased, and the tinnitus diminished. On June 3 the spinal fluid contained 80 cells and 86 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. but not with smaller amounts, and the mastic curve was 5555431000. On June 14 she could hear but could not understand a phonographic test record with the right ear, but there was only slight improvement in function of the sixth and seventh nerves. On June 29, 1944, the spinal fluid contained 8 lymphocytes, 58 mgm. of protein per 100 cc., a positive Wassermann reaction with 0.4 cc., and a mastic curve of 2322100000. Ninety-eight days after treatment (August 31, 1944), the Eagle flocculation titer was negative and great improvement had taken place in the function of the sixth and seventh cranial nerves on the right. An audiometer test on September 6, 1944, showed no improvement in the hearing on the right.

Case 6. (JHH 161626) A 30-year-old Negro male was admitted to The Johns Hopkins Hospital on May 15, 1944, complaining of moderate headache of a month's duration. In November 1942, as a family contact, a routine blood serologic test was negative. He had had an untreated penile sore in March, 1944, but not other symptoms. Examination showed a very slight left facial weakness of the peripheral type, a few darkfield-positive papules on the face and abdomen, a darkfield positive scar on the prepuce, and moderately enlarged inguinal lymphnodes. An audiometer test was normal.

The spinal fluid, ground-glass in appearance, contained 1450 lymphocytes per cubic millimeter and 158 mgm. of protein per 100 cc. The Wassermann test was anticomplementary with 0.03 to 1.0 cc. and gave a mastic curve of 555554400.

On May 16 treatment was started with 20,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 doses, or 1,200,000 units in seven and one half days. There was no Herxheimer reaction and the papules, which became darkfield negative in 13 hours, healed promptly. During treatment the headaches ceased, and the slight facial paralysis cleared. On May 24 the spinal fluid was clear and colorless, and contained 177 cells per cubic millimeter and 62 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.1 cc. but negative with 0.05 cc., and the mastic curve was 5555420000.

(Insert Table VI)

After discharge the patient remained asymptomatic and when seen on June 1 was well. The Eagle titer, which before treatment on May 15 had been 16 units and had risen two days later to 256 units and three days after that to 512 units, had fallen to 32 units. One hundred and fourteen days after treatment (September 7, 1944), the Eagle flocculation test had become negative. On September 15 the spinal fluid contained 7 cells and 26 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.8 cc; the mastic curve was 4431000000. Table VI summarizes the findings.

TABLE IV

The Course under Penicillin Therapy of Case 4.

Date	Day after penicillin treatment	Treatment	Blood STS titer in units	Spinal Fluid	Remarks
11-2-43		Mapharsen .06 x 5 Bismuth x2	32		Secondary syphilis
1-21-44				34 cells 45 mgm.% protein WaR.pos.with 0.4cc Mastic 3210000000	Convulsions Delirium Headache
1-22-44	0	Penicillin 600,000 units	2	70 cells 25 mgm.% protein WaR.pos.with 0.4cc Mastic 1100000000	
1-23-44	1				Mentally clear
1-30-44	8		4	15 cells 25 mgm.% protein WaR.neg.with 1.0cc Mastic 0000000000	Asymptomatic
2-10-44	19		0		Asymptomatic
3-16-44	54		0	Normal	Asymptomatic
5-9-44	78		0		Asymptomatic
7-6-44	106		0	Normal	Asymptomatic
9-14-44	176		0		Asymptomatic

TABLE V
The Course under Penicillin Therapy of Case 5

Date	Day after penicillin treatment	Treatment	Blood STS titer in units	Spinal Fluid	Remarks
5-25-44			2	70 cells 118 mgm.% protein WaR.pos.with 0.2cc Mastic 555431000	Paralysis, rt.VI, VII, VIII nerves.
5-26-44	0-8	1,200,000 units penicillin			
6-3-44	9		1	80 cells 86 mgm.% protein WaR.pos.with 0.2cc Mastic 5555431000	Symptomatic improvement
6-8-44	14		D		Improved
6-15-44	21		1		VII nerve unim- proved
6-29-44	35		D	8 cells 58 mgm.% protein WaR.pos.with 0.4cc 2322100000	VI nerve greatly improved VII unimproved
7-27-44	63		D		VI nerve, only slight weakness VII nerve improved
8-31-44	98		0	8 cells 51 mgm.% protein WaR.pos.with 0.6cc Mastic 5554300000	Improvement continues
9-6-44	104				Audiometer: no improvement

TABLE VI
The Course under Penicillin Therapy of Case 6.

Date	Day after penicillin treatment	Treatment	Blood STS titer in units	Spinal Fluid	Remarks
11-18-42			0		Family Contact
March '44					Untreated penile lesion
5-15-44	0	Penicillin 1,200,000	16	1450 cells 158 mgm.% protein W.R. pos. with .02 cc Mastic 555554400	Facial weakness, lt. Headaches and dizziness - 3 wks. DF+ penile lesion Hearing normal
5-24-44	8		516	177 cells 62 mgm.% protein W.R. pos. with .10cc Mastic 555420000	Asymptomatic Facial weakness gone
6-1-44	16		32		Asymptomatic
7-6-44	51		4	55 cells 40 mgm.% protein W.R. pos. with 0.6cc Mastic 432100000	Asymptomatic
9-7-44	114		0		Asymptomatic
9-15-44	121			7 cells 26 mgm.% protein W.R. pos. with 0.8cc Mastic 443100000	Asymptomatic

Case 7. (JHH 137279) A 21 year old Negress was admitted to The Johns Hopkins Hospital on March 7, 1944, complaining of deafness of 3 weeks' duration. In September, 1941, she was seen in The Johns Hopkins Hospital Out-Patient Department with pelvic inflammatory disease, and a routine serologic test for syphilis was negative. Her serologic test had also been negative in May, 1943, but the following January she had received elsewhere three intravenous injections of mapharsen in two weeks for an illness characterized by a sore throat, rash, and genital sore. The lesions healed promptly, and she remained well until the middle of February when a transitory stiff neck, persistent headache, bilateral partial deafness and tinnitus, and a right facial paralysis developed. She came to the hospital and was admitted for treatment.

The only abnormalities on examination were a right facial paralysis of the peripheral type, bilateral nerve deafness with 25 per cent hearing loss in each ear by audiometer test, and slight enlargement of the superficial lymphnodes.

The result of the Eagle blood flocculation test was doubtful. The spinal fluid was clear and colorless and contained 57 cells per cubic millimeter and 37 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. but negative with 0.1 cc., and the mastic curve was 5543110000.

(Insert Table VII)

Treatment began on March 9 with 10,000 Oxford units of sodium penicillin every three hours for 60 injections, 600,000 units in seven and one half days. There was no Herxheimer reaction. Hearing was subjectively better by the third day of therapy and by March 20 there was only 12 per cent hearing loss on each side. The facial paralysis did not improve during treatment but by March 23 was less and by April 6 was completely gone. On March 17 the spinal fluid contained 20 cells and 33 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.6 cc. but not with smaller amounts, and the mastic curve was 1110000000. All Eagle tests of the blood subsequent to the first one gave negative results. When seen on July 20, 1944, the patient was quite well. The spinal fluid on June 15, 1944, contained no cells and 22 mgm. of protein per 100 cc. The Wassermann reaction was negative with 1.0 cc., and the mastic curve was 0000000000. The course is given in Table VII.

Case 8. (JHH 312861) A 21 year old white man was admitted to The Johns Hopkins Hospital on January 31, 1944, complaining of headache of three weeks' duration. Early in 1942 he had had a penile sore followed in June, 1942, by a generalized rash and sore throat. In September, 1942, his blood serologic test was found to be positive. Treatment was started and given regularly so that from this time until December, 1943, he received seventeen injections of 0.6 gm. neoursphnamine, nineteen injections of 0.06 gm. mapharsen, and twenty injections of bismuth. Nevertheless, six months after the start of therapy and in spite of its active continuation, ulcerative lesions appeared on his lower legs and persisted for five weeks. After these healed he remained well until three weeks before admission when he developed an intermittent, severe left-sided headache, became increasingly irritable, and began to have occasional spells of dizziness. These symptoms resulted in his admission on January 31, 1944.

At that time there were several pigmented almost circular scars up to three centimeters in greatest diameter on each lower leg. The only other abnormality found on physical examination was a small penile scar on the coronal sulcus. Visual fields, audiometric tests, and vestibular tests were normal.

The Eagle blood flocculation titer was 12 units. The spinal fluid, which was clear and colorless, contained 225 leukocytes per cubic millimeter, of which 220 were lymphocytes, and 50 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.05 cc. but not with smaller amounts, and the mastic curve was 5554321000.

Treatment was started on February 2, 1944, with 50,000 Oxford units of sodium penicillin intramuscularly every three hours for 80 doses, 4,000,000 units in ten days. A transitory rise in temperature from normal to 102.4°F. twenty hours after the start of therapy was the only manifestation of a Herxheimer reaction. In several days the patient became completely asymptomatic. On February 12 the spinal fluid contained 130 lymphocytes per cubic millimeter and 50 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.1 cc. but not with 0.05 cc., and the mastic curve was 5544431000.

(Insert Table VIII)

After discharge he continued to be asymptomatic. On May 4 the Eagle titer was 3 units, and the spinal fluid contained 22 cells per cubic millimeter and 37 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. but not with smaller amounts, and the mastic curve was 4321000000. One hundred and thirty-nine days after treatment (July 20, 1944) the serologic test for syphilis was 1 unit, the spinal fluid contained 8 cells and 40 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.4 cc., and the mastic curve was 4321000000.

Case 9. (JHN 313431) A 30 year old Negress was admitted to The Johns Hopkins hospital on February 7, 1944, complaining of a moderately severe sore throat which had begun one month previously and had been followed in a week by hoarseness and a rash. There was no history of earlier manifestations of syphilis.

Although she was hoarse, she did not appear very ill or uncomfortable. Examination showed a generalized, darkfield positive, papular rash, injection and edema of the pharynx and tonsils which were covered with exudate, and slightly enlarged cervical lymph nodes.

The Eagle blood flocculation was 256 units and the spinal fluid was normal.

Treatment with 1,000 Oxford units of sodium penicillin intramuscularly every three hours was begun on February 9 and continued for 60 injections, 60,000 units in seven and one half days. There was no Herxheimer reaction, and the lesions which became darkfield negative in eleven hours healed completely in two weeks.

TABLE VII
The Course under Penicillin Therapy of Case 7.

Date	Day after penicillin treatment	Treatment	Blood STS titer in units	Spinal Fluid	Remarks
Sept. '41			0		P.I.D.
May '43			0		
Jan. '44		Waphursen .04 x 3.	+ (no titer)		Early syphilis
3-7-44			D	57 cells 37 mgm.% protein W.R. pos. with 0.2 cc Mastic. 5543110000	Headache; stiff neck. Palsy rt. VII nerve.
3-8-44					Hearing loss: Rt. 25% Lt. 25%
3-9-44	0-8	Penicillin 600,000 units			
3-17-44	8		0	20 cells 33 mgm.% protein W.R. pos. with 0.6cc Mastic 1110000000	Marked improvement
3-20-44	11				Hearing loss: Rt. 12% Lt. 12%
3-23-44	14		0		Right VII improved
4-6-44	28		0		Right VII paralysis gone.
5-4-44	56		0		Asymptomatic
6-8-44	91		0		Asymptomatic
6-15-44	98			Normal	Asymptomatic
7-20-44	133		0		Asymptomatic

TABLE VIII
The Course under Penicillin Therapy of Case 8.

Date	Day after penicillin treatment	Treatment	Blood STS titer in units	Spinal Fluid	Remarks
June '42 to Jan. '44		36xarsenic 20xbismuth	.. + (no titer)		early syphilis eruption legs 6 mos. after treatment started
1-25-44			6	350 cells 75 mgm.% protein Wak pos. with .05cc Mastic 5554321000	Headache, dizziness, nervousness
1-31-44			12	225 cells 50 mgm.% protein Wak pos. with 0.5cc Mastic 5554321000	Headache, dizziness, nervousness
2-2-44	0-8	Penicillin 4,000,000 units			
2-10-44	10		12	130 cells 50 mgm.% protein Wak pos. with 0.1cc Mastic 5524431000	Rapid loss of all symptoms
5-4-44	92		3	22 cells 37 mgm.% protein Wak pos. with 0.2cc Mastic 4321000000	Asymptomatic
7-20-44	139		1	8 cells 40 mgm.% protein Wak pos. with 0.4cc Mastic 4321000000	Asymptomatic

It was difficult to get the patient to return to the clinic after discharge, but when seen on March 16 she was asymptomatic, there were no lesions, and the Eagle titer was 64 units. Early in April, after transitory diplopia and unsteadiness, the left side of her face became paralyzed, she became partly deaf, and a week later genital lesions appeared.

On May 4, when she was readmitted, a left facial paralysis of the peripheral type and numerous darkfield positive vulvar condylomata lata were present. An audiometer test showed 30 per cent loss of hearing on the right and 15 per cent loss on the left.

The Eagle blood flocculation titer was 32 units. The spinal fluid, which was clear and colorless, contained 48 cells per cubic millimeter and 40 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. of spinal fluid but not with smaller amounts, and the mastic curve was 2431000000.

Treatment was begun on May 6 with 20,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 injections, 1,200,000 units in seven and one half days. There was no Herxheimer reaction. The condylomata, which became darkfield negative in 12 hours, healed promptly, but at the end of treatment there was no change in the facial paralysis. On May 14 the spinal fluid contained 14 cells per cubic millimeter and 33 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.4 cc. but negative with 0.2 cc., and the mastic curve was 1221000000.

After discharge she was not seen until June 1 at which time the facial palsy had entirely cleared, there were no symptoms, no neurologic or mucocutaneous lesions, and the Eagle titer was 32 units. Seven weeks later, July 6, 1944, the Eagle titer was 8 units. The spinal fluid then was normal except for a positive Wassermann reaction with 0.6 cc. The patient was last seen on August 24, 1944, at which time she was asymptomatic and the spinal fluid was normal. Table XI illustrates the course.

(Insert Table IX)

Case 10. (JMH 312453) A 19 year old white male referred to us by Dr. Angus MacLean complained of severe diminished vision of one month's duration. There had been poor visual acuity in the left eye since early childhood related, according to the patient, to an operation performed on the eye at the age of 6. The nature of this operation is unknown though it seems likely that it was an attempt to correct a strabismus. His general health had been excellent except for this finding. In October, 1943, he was classified 4F by Selective Service because of defective vision.

There was no history of any venereal disease and specifically no knowledge of early syphilis though sexual activity was promiscuous for the year prior to admission. The onset of the present illness was abrupt with severe loss of vision about one month before entry. This improved temporarily at the end of two weeks only to become worse again.

Upon admission to the hospital on February 26, 1944, examination revealed a healed annular penile lesion and a moderate general enlargement of the lymphnodes.

One *T. pallidum* was seen upon darkfield examination of material removed from one of the lymphnodes. The visual acuity was considerably reduced being: R.E. 4/200, L.E. 20/200. Ophthalmoscopic examination revealed bilateral swelling of the optic discs and surrounding retinal edema. The blood Eagle flocculation test was 64 units. The spinal fluid contained 2 cells and 22 mg. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. and the mastix curve was 1100000000.

Treatment with sodium penicillin was started on February 28, 1944. The drug was given intramuscularly every three hours for eleven days. A Herxheimer reaction was feared so initial doses consisted of only 1000 Oxford units given every three hours for eight doses. No reaction was observed, and larger doses were employed so that the total amount given in 11 days amounted to 2,008,000 Oxford units.

(Insert Table X)

Improvement in vision began on the fifth day and at the end of one week he was able to read small print. At the end of the 9th day of treatment the visual acuity was: R.E. 20/50, L.E. 25/200. The swelling of the optic discs and retinal edema had likewise improved and was completely gone by the 18th day from onset of treatment. Visual acuity in the right eye returned to normal by the 46th day, and there has been no further improvement in the vision of either eye since that time. The residual poor vision on the left is explainable on the basis of amblyopia ex anopsia. The blood Eagle flocculation titre has shown a steady fall so that when last examined on September 8, 1944, it was 8 units. Spinal fluid abnormalities have persisted as is shown in Table X.

Discussion

The results obtained in these ten patients are briefly summarized in Table XI.

In all patients except three (Cases 5, 8 and 10), infection with syphilis was recent, - of less than 8 months' duration. In Case 8 the duration of infection was 20 months. In Case 5 there was no history permitting the duration to be defined; the collateral evidence of the patient's race, sex, and age suggests that it may have been of some years standing. In Case 10 the onset of infection cannot be defined, but was probably recent.

Four patients (Cases 1, 5, 6 and 10) had had no previous antisyphilitic treatment of any sort, and two of these (Cases 1 and 6) had associated mucocutaneous lesions of early syphilis. The other six patients all had received varying amounts of antisyphilitic treatment given for early syphilis. This previous treatment was grossly inadequate in all except Case 8. These six cases fall into the category of neurorecurrence. It is of particular interest that in one of them (Case 9), the initial treatment for early syphilis had been a course of 60,000 units of penicillin, a dosage now demonstrated (2) to be inadequate.

The clinical manifestations were typical of acute syphilitic meningitis in all, including headache (all cases), stiff neck (3 cases), cranial nerve palsies

TABLE IX.

The Course under Penicillin Therapy of Case 9

Date	Day after penicillin treatment	Treatment	blood STS titer in units	spinal Fluid	Remarks
2-7-44	0	Penicillin 60,000 units	256	Normal	Early syphilis DF+ genital lesions
2-24-44	16		128		Lesions heal- ed.
3-16-44	37		64		Asymptomatic
5-4-44	86 Day after second course		32	48 cells 40 mgm.% protein WdR pos. with 0.2cc Mastic 2431000000	DF+ genital lesions; Headache, dizziness. Left VII nerve paralysis. Hearing loss: Rt. 30% Lt. 15%
5-6-44	0-8	Penicillin 1,200,000 units			
5-14-44	9		64	14 cells 33 mgm.% protein WdR pos. with 0.4cc Mastic 1221000000	Symptomatic improvement; left VII improved
6-1-44	27		32	0 cells 10 mgm.% protein WdR pos. with 0.8cc Mastic 1100000000	Left VII Normal. No symptoms
7-6-44	62		8	5 cells 19 mgm.% protein WdR pos. with 0.6cc Mastic 0000000000	Hearing normal
8-24-44	111		8	Normal	Asymptomatic

TABLE X
The Course under Penicillin Therapy in Case 10.

Date	Day after treatment	Treatment	Blood STS titer in units	Spinal Fluid	Remarks
Oct. '43					Rejected Sel.Ser. because of poor vision L.E.
1-13-44					Sudden almost complete loss of vision
1-25-44			*	4 cells 40 mgm.% protein WaR.pos.with 0.4cc Mastic 1100000000	R.E. 20/100 L.E. 20/100 Bilateral neuroretinitis
2-26-44			64	2 cells 22 mgm.% protein WaR.pos.with 0.2cc Mastic 1100000000	R.E. 4/200 L.E. 20/200 DF+ lymph node puncture
2-28-44		Penicillin course begun tot. dosage 2,008,000 units in 11 days			
3-1-44	3				R.E. 4/200 L.E. 20/200
3-3-44	5				R.E. 20/200 L.E. 25/200
3-5-44	7		128		R.E. 20/70 L.E. 25/200
3-7-44	9				Vastly improved R.E. 20/50 L.E. 20/200
3-10-44	12			4 cells 37 mgm.% protein WaR.pos.with 0.6cc Mastic 1222200000	Discharged, greatly improved
3-16-44	18		64		Optic discs and retina normal. R.E. 20/50 L.E. 20/200
3-27-44	29		32		Further improved
4-13-44	46		16		R.E. 20/20 L.E. 20/200
4-27-44	60		12		
9-8-44	193		8	27 cells 40 mgm.% protein WaR.pos.with 0.6cc Mastic 5431000000	R.E. 20/30 L.E. 20/200

(5 cases), papilledema (2 cases), convulsions, drowsiness and stupor (2 cases).

(Insert Table XI)

The dose of penicillin employed ranged from 600,000 to 4,000,000 units; the duration of treatment was seven and one half to eleven days. These dosages and time intervals are not suggested as the optimum and were chosen because of similar treatment schedules employed at the time in the treatment of uncomplicated early syphilis. In general, the earlier patients treated received the smaller dose of 600,000 units. Later, as information accumulated as to the direct relationship between total dosage and relapse in early syphilis (1, 2) and the comparative ineffectiveness of doses less than 1,200,000 units, larger doses have been employed in the meningitis cases.

As previously stated, the route of administration was intramuscular in all these cases. Before this work was begun, we were of course aware of the observations of Kaefer and Rammelkamp (4) that penicillin injected intravenously or intramuscularly did not appear in the cerebrospinal fluid in any significant quantity. Furthermore, McDermott, Eagle, and Nelson (5) were unable to demonstrate penicillin in the cerebrospinal fluid in patients with early syphilis, late neurosyphilis, and tuberculous meningitis. Nevertheless, in neurosyphilis there is reason to believe that the effectiveness of a chemotherapeutic agent does not necessarily depend on the ability of that agent to penetrate the cerebrospinal fluid, but instead, on the basis of penetrability into the diseased tissues (meninges, blood vessels, parenchyma) (6). The effectiveness of intramuscular treatment in early neurosyphilis and the lack of necessity for use of the intrathecal route, is borne out by the results presented here.

To the date of writing (September 15, 1944), the patients treated have been followed for 104 to 310 days after the start of penicillin therapy (8 of the 10 for 4 months or longer). No clinical relapses have so far been observed, and re-treatment, whether with penicillin or by some other method, has not yet been required. (One patient, Case 10, has shown a relapse in the spinal fluid findings after 193 days and is to be retreated). Judging from the observed incidence of relapse in patients with uncomplicated early syphilis treated with 600,000 units (relapse rate about 20-25 per cent) (2), relapse in some of these patients with acute syphilitic meningitis is certainly to be expected.

The immediate symptomatic result has been dramatically favorable in all 10 cases. Headache and stiff neck have disappeared in 24-48 hours. Cranial nerve paralyses have disappeared completely in all cases except one (Case 5), in which slight residuals remained after 98 days. Four patients had nerve deafness (Cases 1, 5, 7 and 9) and in three, improvement was gradual, requiring respectively 110, 98, and 139 days for return to complete or nearly complete normal as objectively measured by audiometer tests. One patient has shown no improvement in deafness during 104 days of observation. Two patients (Cases 3 and 4) had no further convulsions after penicillin was started.

Three patients (Cases 3, 5 and 8) had mild febrile Wernheimer reactions (highest temperature 102.4°F), but this did not occur in the other seven cases.

Continued on Next Fiche!

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AVIATION MEDICINE SPECIAL (CAM) REPORTS
VENEREAL DISEASES - NUMBERED ABSTRACTS AND REPORTS

FICHE 2 OF 2

NAS_0003164

In none was there any clinical exacerbation suggesting therapeutic shock, nor were there any other reactions from treatment.

One patient (Case 3) had a doubtful blood serologic test for syphilis before treatment, which became and remained negative thereafter. Three cases (Cases 4, 5 and 7) with low titer tests (each 2 units) at the start of treatment have become seronegative. In the other 7 cases the serologic titer is falling toward negative in all.

The effect upon the spinal fluid is even more remarkable. The original cell counts before treatment varied from 42 to 1450. In all patients the cerebrospinal fluid was retested on the day of or immediately following the cessation of penicillin therapy, and in all, an abrupt fall in cell count was observable even at this early period. At the last observation, cell counts were normal (assuming a count of 10 cells or less per cubic millimeter to be normal) in 8. In one patient (Case 2 whose original cell count was 1280) the cell count was 12, and in only one patient (Case 10) has there been an increase in cell count during observation.

The protein content was abnormally high in nine of the ten cases (range 37-158 mgm. per 100 cc.). In all of these, there was a fall toward normal; in four cases, the protein has reached a normal level (25 mgm. per 100 cc. or less).

The Wassermann test of the spinal fluid was positive before penicillin treatment in all cases, varying in titer from 0.05 - 0.8 cc. In all cases, there was improvement, the extent of which varied with the strength of the original reaction and the duration of observation. Improvement has been slower than with cell count or protein content but so far has been continuous and progressive in all cases. Five patients (Cases 1, 3, 4, 7 and 9) have reverted to negative (with 1.0 cc. of fluid), and these are the cases which have also shown the greatest improvement in the blood serologic tests for syphilis.

The mastic curve, abnormal in all patients except Case 1 and ranging in type from relatively moderate to extreme zone I (paretic type), has likewise improved in all except one (Case 10), and has become negative in five.

So far as can be determined from this small number of cases and from the relatively short time in which they have been followed, there is no relationship between total dosage of penicillin and the clinical or eventual serologic result, or in the rapidity with which serologic improvement occurs.

Schedule advised for the Treatment of Acute

Syphilitic Meningitis

In spite of the observations in the preceding paragraphs, it is believed, on the basis of the results accumulating in uncomplicated early syphilis (2), that patients with acute syphilitic meningitis should receive a total of 2,000,000 to 3,000,000 Oxford units of penicillin administered by the intramuscular route every 3-4 hours day and night over a minimum period of 3-16 days. There appears to be no reason to suggest intravenous or intrathecal administration;

TABLE XI
Summary of Clinical, Serologic

Case	Duration syphilis	Previous treatment	Neurological manifestations	Penicillin Therapy		Days of observation after penicillin	Symptomatic response
				Tot. Units	Days of Rx.		
1	few mos.	none	Rt. N. VII, bilateral N. VIII, headache, vertigo	1,240,000	8	310	Complete relief
2	4 mos.	5 x As.	Headache, stiff neck, papilledema	640,000	8	263	Complete relief
3	7 mos.	2-25 x As & Bi	Headache, stiff neck, convulsions, stupor	600,000	7 1/2	167	Complete relief
4	4 mos.	6 x Maph 2 x Bi	Headache, convulsions, stupor	600,000	7 1/2	176	Complete relief
5	?	none	Headache, Rt. VI, VII and VIII paralysis	1,200,000	7 1/2	104	Improved; slight residual cranial nerve weakness.
6	3 mos.	none	Headache; left VII palsy	1,200,000	7 1/2	121	Complete relief
7	5 mos.	3 x Maph	Headache, stiff neck, rt. VII and bilateral VIII paralysis	600,000	7 1/2	133	Complete relief
8	20 mos.	36 x As. 20 x Bi.	Headache, vertigo, "nervousness"	4,000,000	10	199	Complete relief
9	4 mos.	60,000 u. penicillin	Left VII, bilateral N. VIII palsy	1,200,000	7 1/2	111	Complete relief
10	?	none	Bilateral neuro-retinitis	2,000,000	11	193	Complete relief

and Spinal Fluid Result in All Patients

Blood STS Titer Units at start of Ex.	Cerebrospinal Fluid									
	At Start of Treatment					Last Observation				
	Just Obs.	Cells	Prot. mg.%	Walt	Mastic	Cells	Prot. mg.%	Walt	Mastic	
4	0	53	25	4 with 0.4cc	000000000	0	25	0 with 1.0cc	000000000	
10	D	1280	100	4 with 0.05cc	5555430000	12	22	4 with 0.6cc	211000000	
0	0	102	105	4 with 0.05cc	4321000000	4	33	0 with 1.0cc	000000000	
2	0	70	25	4 with 0.4cc	1100000000	0	22	0 with 1.0cc	000000000	
2	0	70	118	4 with 0.2cc	5554300000	8	51	4 with 0.6cc	555430000	
6	4	1450	158	4 with 0.2cc	5555554000	7	26	4 with 0.8cc	443100000	
D	0	57	37	4 with 0.2cc	5543110000	0	25	0 with 1.0cc	000000000	
6	1	225	50	4 with 0.5cc	5554321000	8	40	4 with 0.4cc	432100000	
32	8	48	40	4 with 0.2cc	2432100000	4	20	0 with 1.0cc	000000000	
64	8	2	40	4 with 0.2cc	1100000000	47	40	4 with 0.6cc	543100000	

the former is probably relatively ineffective (2), the latter unnecessary. All patients with acute syphilitic meningitis treated with penicillin should be closely followed with clinical re-examinations (especially for the detection of infectious mucocutaneous or neurorelapse) and with laboratory studies of blood and spinal fluid. These follow-up examinations should be made at least as often as every three months during the first year after treatment, every six months for another 2-3 years, and yearly thereafter. It should not be forgotten that although symptomatic (and often serologic) improvement can be secured in acute syphilitic meningitis with almost any form of modern chemotherapy or fever therapy, the eventual prognosis of this condition in terms of development of late neurosyphilis, especially paresis and tabes, is grave (6) (7).

SUMMARY

1. In ten cases of acute syphilitic meningitis treated by us with penicillin, the immediate results (98 to 310 days after treatment) are excellent, both from clinical and laboratory standpoints.

2. Although penicillin does not appear in the cerebrospinal fluid even after frequent intramuscular administration, the drug is effective in acute syphilitic meningitis when given by the intramuscular route.

3. None of the ten patients treated has so far developed any evidence of clinical relapse, though one has shown relapse in the spinal fluid findings.

4. The total amount of penicillin administered to any one patient varied from 600,000 to 4,000,000 units; the duration of treatment varied from 7½ to 11 days.

5. On the basis of present information, the treatment schedule advised for acute syphilitic meningitis is a total dosage of 2,000,000 to 3,000,000 Oxford units of penicillin, administered every 3-4 hours day and night for from 8-16 days.

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VENEREAL DISEASES REPORT #25 (ABSTRACTED)

Abstract of Annual Report

WORK DONE BETWEEN JULY 1, 1943 AND JUNE 30, 1944

NAME OF RESPONSIBLE INVESTIGATOR Dr. Geoffrey W. Rake

Prophylaxis (1) against lympho-
granuloma venereum; (2) against

SUBJECT all venereal diseases

CONTRACT NO. W709 md 238

Vehicles for Venereal Prophylactics

Dr. W. G. Christiansen and Dr. W. S. Jones

The investigation of more stable ointments was continued. Additional ointments were prepared and sent to Dr. Calvery for study. Preparations received from Dr. Calvery were tested for stability and for dissolved lead and mercury.

Syphilis

Dr. G. W. Rake, Dr. W. B. Dunham and Dr. D. H. Hamro

As a result of the work of Dr. John Mahoney and his collaborators, studies were conducted on the use of penicillin as a prophylactic against syphilis. Using Eagle's technique for producing skin chancres, suspensions of the Nichols strain of T. pallidum were rubbed into incisions in the backs of rabbits. In most cases, 4 or 5 hours after infection a single injection of penicillin was administered intravenously or intramuscularly. The rabbits were observed for 3 months or more for the development of chancres. Lymph node transfers were then made from the rabbits without obvious syphilitic skin lesions. The details of the experiments together with the results are given in Table III.

Granuloma Inguinale

Dr. W. B. Dunham and Dr. G. W. Rake

A suspension of the 70th egg passage of the presumed agent isolated by Dr. Katherine Anderson was very kindly made available to us 1-5-44. We have carried the strain for 50 additional passages by inoculation into the yolk of 5-6 day eggs.

Table III

Summary of Experiments on Use of Penicillin for Prophylaxis Against Syphilis.

Total Units/kg.	Hours After Infection	Route	No. of Rabbits	No. Developing Local Lesions	Lymph Node Transfer		No. positive
					No. donor rabbits Without Skin Lesion	No. With Skin Lesion	
Skin Infection							
<u>Experiment I</u>							
55,000	4	I.V.	2	0			
Controls	---	---	2	2			
<u>Experiment IV</u>							
49,500	4	I.V.	3	1	2		0
49,500	4, 48, 96	I.V.	2	0	2		0
4,950	4	I.V.	3	3		1	1
4,950	4, 48, 96	I.V.	2	2			
82,500 in 0.5 ml.	4	Rubbed into Wound	2	2		1	1
Controls	---	---	3	3			
<u>Experiment V</u>							
66,000	5	I.M.	5	0	5		1
49,500	5	I.M.	5	4	1		0
Controls	---	---	3	3		1	1
<u>Experiment VI</u>							
66,000	4	I.M.	5	5			
Crystalline							
49,500	4	I.M.	4	4			
Controls	---	---	5	5			
Testicular Infection							
<u>Experiment II</u>							
55,000	4	I.V.	4	4			(Orchitis)
Controls	---	---	3	3			
<u>Experiment III</u>							
16,500	4, 48, 96	I.V.	3	3			
Controls	---	---	3	3			

Complement Fixation Reaction. The sediments of pooled yolks from infected eggs were used as antigens for testing 16 sera from patients diagnosed as suffering from granuloma inguinale. Twelve gave positive reactions while 4 were negative. There was no fixation with any of 10 normal sera tested.

Skin Test Antigens. Suspensions of washed bacteria treated in various ways gave reactions in normal subjects and so were not satisfactory for diagnostic skin tests.

Agglutination. Three of the sera that gave complement fixation reactions caused agglutination of washed bacteria.

Chemotherapy. Mixtures of drugs and infected yolk were allowed to stand at room temperature for 2 hours and were then injected into eggs. The concentration of the agents mentioned below are the final concentrations before inoculation into the eggs. Fudin (sodium antimony bisacetochole disulfonate of sodium) was effective at a concentration of 12 mg./ml., but not at 6 mg./ml. Tartar emetic (antimony and potassium tartrate) was effective at 20 gammas per ml. but not at 10 gammas per ml. Bismuth and potassium tartrate was effective at 1 mg./ml. but not at 0.25 mg./ml. Penicillin was effective at 128 units per ml., but not at 32 units per ml. Streptothricin was effective at 14 units per ml., but not at 7 units per ml. Flavaclidin was partially effective at 49 units per ml. but not at 25 units per ml. Sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine were all effective at 8 mg./ml. but not at 4 mg./ml. Homosulfanilamide had no demonstrable activity at 16 mg./ml. Copper sulfate gave variable results, the end-point of effectiveness being about 0.06% though in one test 0.015% was partially effective. This variability is probably due to an irregular degree of binding of copper ions by the protein present in the yolk. In most tests, Zephiran (alkyl dimethyl benzyl ammonium chloride) in a dilution of 1:64,000 was effective while a dilution of 1:128,000 was ineffective or only partially effective. However, mixtures of copper sulfate and Zephiran were active in which each component was at a lower concentration than had been found effective when employed alone. Thus a mixture of copper sulfate 0.0075% and Zephiran 1:256,000 was effective. Mercurous chloride, 1/2 saturated at 24°C., was effective, while half this concentration was ineffective. Saturation with other killed the organisms in 2 hours. They were apparently unaffected by M/15 phosphate solutions within the range from pH 5.5 to pH 9.0. Heating to 56°C. for 30 minutes killed the organism.

The above tests, it should be emphasized, are screening tests for the drugs and may include two types of action, bactericidal at the high concentration in vitro, and bacteriostatic or bactericidal later in the eggs.

Preservation. Mixtures of equal volumes of infected yolk and normal rabbit serum were sealed in glass ampoules and stored at 24°, 0°, -25° and -72°C. All suspensions were infectious for eggs a month later. The suspension preserved at -72°C. maintained a greater virulence than those kept at the 3 other temperatures.

Animal Inoculations. Inoculations in mice and rabbits by various routes have failed to produce demonstrable infections.



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VENEREAL DISEASES REPORT No. 24 (Abstracted)Abstract of Final Report
14 December 1944THE EFFECT OF HEAVY METAL INUNCTIONS IN THE PROPHYLAXIS OF
EXPERIMENTAL SYPHILIS IN RABBITS. G. H. Carpenter*

Studies have been made of the efficacy of heavy metal inunctions in the prophylaxis of experimental syphilis in rabbits. Two compounds of phenyl arsenoxide, identified as D-66 and D-124 and supplied by Dr. Harry Eagle, were employed. The hair was closely clipped from the mid-dorsal region of a 5 - 6 lb. rabbit after which three scratches 1 cm. long and deep enough to cause fine bleeding points, were made in the skin of this area. A suspension of Treponema pallidum (Nichols strain) containing 10^7 motile spirochetes per ml. was dropped onto the scarifications. Four hours later the arsenical compound in concentrations of .05, 0.1, 0.2, and 0.4 per cent dissolved in propylene glycol were applied and massaged over the inoculated area. Darkfield examinations were made on animals developing suspicious lesions, to determine the presence of Treponema pallidum. Animals which showed no lesions during a 6 months' period of observation were sacrificed and lymph node transfers were made to normal rabbits as tests for cure.

Compound D-66 in a concentration of 0.4 per cent protected 55 per cent of the animals treated. No protection was observed in the lower concentrations tested.

Compound D-124 in a concentration of 0.4 per cent protected up to 80 per cent of the rabbits treated. Lower concentrations protected to a lesser degree.

* The study was carried out with the collaboration of Dr. Ruth A. Book and Dr. F. Leslie Dorn

Table I
The Preliminary Results of Penicillin Therapy in Infantile
Congenital Syphilis: 39 Infants followed for 4 - 12 months

Clinical Results

Course uneventful . . . 37
Clinical relapse . . . 2

<u>Serologic Results</u>	<u>Cases</u>	<u>Per cent</u>
Reversed to negative	21	54
Reversed to doubtful	4	10
Still positive but titer declining	9	23
Serologic relapse	5	13

The dosage schedules so far employed have corresponded to a total dose of 1 - 2 million units in 7-1/2 days for an adult with early acquired syphilis.

The results so far obtained in infants indicate that the present total dose and time-dose relationship schedule is not entirely satisfactory. Other modifications of this schedule should be studied with due attention to analogous results secured by various schedules in early acquired syphilis in adults. For the present, we believe that an increase in total dose with the same time-dose relationship should be tried first. Accordingly, we recommend temporarily a total dose of 40,000 Oxford units per kilogram of body weight, given in 60 intramuscular injections over a 7-1/2 day period.

Attention is called to the vital necessity of adequate supportive care in acutely ill infants.

Definite relapse, serologic or clinical, should be retreated at double the original dosage. A relapse is defined as either a persistently rising serologic titer, or clinical evidence of progression of the disease. Persistent positivity of the blood serologic test alone is not yet to be considered an indication in itself for retreatment.

A detailed report of these and additional cases will be made by this group at a later date.

Summary

Penicillin is an effective agent in the treatment of infantile congenital syphilis, but the optimum method of its use has not yet been defined.

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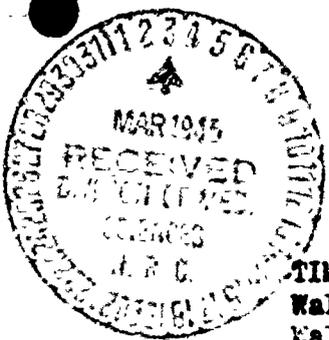
McDermott
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COMMITTEE ON MEDICAL RESEARCH
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VENEREAL DISEASES REPORT No. 26

Interim Report
10 January 1945

TIME-DOSE RELATIONSHIPS OF PENICILLIN THERAPY. Parts I - IV:
Walsh McDermott, Maria Benoit, and Rebeckah DuBois. Part V:
Walsh McDermott, Helen Steinberg, and Willetta Haynes.



- I. The concentration and duration of penicillin levels in the serum following the use of various dosages, routes, and vehicles of administration.
- II. The relation of the concentration and duration of penicillin effect to the inhibition of bacterial populations in vitro.
- III. Regimens used in early syphilis.
- IV. The relation of the concentration and duration of penicillin in the serum to the concentration in spinal fluid and dialysates.
- V. The effect of penicillin upon the clotting of blood. in vitro.

When subcurative penicillin therapy is administered to patients infected with organisms susceptible to penicillin, the clinical and laboratory evidence of infection disappears completely, to be followed after an interval by relapse. In most instances, this state of remission is indistinguishable by laboratory and clinical means from that of cure. Such remissions may be induced by the use of a small or a large amount of penicillin, and apparently with equal facility. It is obvious that the comparative value of different penicillin regimens for the treatment of a given disease cannot be determined from the number or type of remissions induced, as they are all more or less alike and occur following such a wide range of dosage. It is only by a study of the incidence of the relapses which follow the various regimens that such an evaluation is possible.

In infections caused by the pneumococcus or Group A streptococcus, the total duration of this remission which follows subcurative penicillin therapy is usually only 24 hours. When relapse can be detected in such a short period of time, the evaluation of different treatment systems can be made in a similarly short period and a large number of programs may be studied by trial and error.

In the less acute infections such as bacterial endocarditis and syphilis, the interval of remission before relapse may be a period of weeks or months, and the time required for the comparative evaluation of various systems of treatment is

correspondingly prolonged.

Therefore, it is of obvious importance to attempt to find some means whereby the penicillin regimens of least promise for the successful treatment of these subacute infections can be eliminated in advance, and only the more hopeful ones subjected to clinical trial.

The following investigations have been conducted in an attempt to obtain information which would be of help in this problem.

Methods

The amount of penicillin in the sera was measured by the Rummelkamp dilution method with these qualifications:

1. The complete inhibition of the test organism in the greatest dilution of unknown serum, which is the end-point of this method, was determined by the inoculation of blood broth with the unknown serum-test organism mixture at 48 hours. In the original Rummelkamp technique, this end-point is determined by streaking a blood agar plate at 24 hours with the unknown serum-test organism mixture. The more rigid demonstration of inhibition of the organism by the 48-hour inoculation method will result, not infrequently, in lower determinations of penicillin in a given serum, but the results are more consistently reproducible.

2. The Rummelkamp method requires more than a threefold dilution of the unknown serum for the performance of the test. Therefore, if concentrations of penicillin in the serum when thus thrice diluted are too low to exhibit antibacterial action, they cannot be measured. As a result, the concentration, 0.078 unit per cc., is the lowest penicillin level which can be measured with accuracy in an unknown specimen. It should be emphasized that this 0.078 u./cc. concentration is almost four times the concentration required to inhibit completely the hemolytic streptococcus used as a test organism.

The penicillin standard used was checked against a reference standard of penicillin calcium which was supplied for this purpose by Dr. Albert C. Hunter of the Federal Food and Drug Administration. The test organism was a Group A streptococcus, C-203, M.V. A penicillin concentration of 0.02 unit will completely inhibit for 48 hours 1 to 43,000 of these organisms per cubic centimeter.

In short, the method used for the determination of the penicillin levels in the sera was somewhat less sensitive than many tests now in use, but gave consistent results.

The penicillinase was obtained through the courtesy of Dr. Robert D. Coghill, of the Bureau of Agricultural and Industrial Chemistry of the U. S. Department of Agriculture.

I. The Concentration and Duration of Penicillin Levels in the Serum Following the Use of Various Dosages, Routes, and Vehicles of Administration.

Studies were made of the serum penicillin levels obtained after the single intramuscular injection of various amounts of penicillin in aqueous solution.

As had been noted by previous investigators, following a single dose of 25,000 units, a serum level of .078 u/cc or above was maintained for approximately two hours. However, it was found that progressive increases in the amount of penicillin in aqueous solution given in a single intramuscular injection were associated with a considerable prolongation of serum levels of 0.078 unit or more. Thus, the administration of single doses of 200,000 units was associated with maintenance of such serum levels for 3 or 4 hours, whereas the single injection of 300,000 units was followed by a level of 0.078 or higher for 4 to 5 hours. Increasing the single dose above 300,000 units to 500,000 and 800,000 units resulted in only slight prolongation of the 0.078 unit level to 6 hours in a few instances.

(See Fig. 1)

When a single injection of 500,000 units was followed within two hours by another injection of 100,000 - 500,000 units, the 0.078 unit level was maintained for 6 or 7 hours following the first injection. Thus it is possible, by the use of aqueous penicillin in single, or quickly repeated double injections, to maintain a serum level of 0.078 unit of penicillin for 5 to 6 hours.

(See Fig. 2)

The use of 3 per cent beeswax in peanut oil as a vehicle for intramuscular penicillin administration has been shown by Romansky to retard the absorption and hence prolong the serum level of the penicillin. In his published curves (U. S. Army Bul., 81:47, 1944), levels of 0.078 were maintained for 5 to 7 hours following the single intramuscular injection of 100,000 units of material incorporated in peanut oil and beeswax.

(See Fig. 3)

It is evident that maintenance of serum levels of this order (0.078 unit or higher) for a period of approximately 7 hours can be effected by the use of either large doses of aqueous penicillin or much smaller doses of penicillin in beeswax.

A study therefore was made of the duration of penicillin action which could be achieved by the use of large doses of penicillin in 4 and in 5 per cent beeswax. The material available consisted of a mixture of penicillin calcium in peanut oil and 3, 4, or 5 per cent beeswax. The 5 per cent beeswax preparation contained 75,000 units of penicillin per cc. The 3 per cent and the 4 per cent preparations contained 100,000 units of penicillin per cc.

As may be seen in Figure 4, the administration of a single dose of 150,000

units of penicillin was followed by a serum level of 0.078 u/cc for 7 to 9 hours. The rapid fall of the concentration to below a detectable level during the second hour, which occurred in one of the patients, is thought to be due to an irregular absorption from the beeswax depot, and not to a fault of the technique. In support of this finding are the facts that the penicillin concentration in this patient at the end of the first hour was at the 0.078 u/cc or lowest detectable level, and that none of the previous studies of serum levels produced by aqueous penicillin showed values which did not fall on a straight line.

Serum levels of 0.078 u/cc or higher were prolonged for more than 12 hours after a single dose of 300,000 units of penicillin. During the first eight hours after injection, the serum level was maintained at the relatively high level of 0.312 units. There was no evidence of irregularity of absorption from the depot in the studies of these two patients.

In Figure 4, may be seen the serum penicillin concentrations attained following single intramuscular injections of 300,000 units of penicillin in oil and 4 per cent beeswax. Although in one subject an initial high peak occurred, while in another detectable concentrations (i.e., .078 u/cc or higher) were not found at all during the period of observation, the serum penicillin concentrations ranged for the most part between .078 and .312 u/cc and were maintained for 9 to 12 hours. (That Subject 1 absorbed appreciable, albeit undetectable, amounts of material is established by the fact that *Treponema pallidum* disappeared from surface lesions of syphilis present as rapidly as is generally observed following the administration of penicillin.

From these preliminary studies with the beeswax vehicle, it would seem that by this method it is possible to maintain significant levels of penicillin action in the serum for periods longer than 12 hours after a single dose, provided that sufficient penicillin is used. The questions of the irregularity of absorption and the tolerance of the patient to repeated doses of penicillin in this vehicle are yet to be studied.

Aside from the fact that the aqueous method requires the use of much more penicillin to prolong levels for 6 or 7 hours, there is a possibly important difference between the two methods of administration. With the aqueous vehicle, extremely high levels of penicillin are attained for the first few hours of the 7-hour period. On the contrary, when penicillin is administered in beeswax, no such peak concentrations above the 0.078 - 0.312 u/cc level are usually attained because of the slower absorption. The possible implications of this difference will be discussed subsequently.

The duration of serum penicillin levels of two concentrations, 0.078 and 0.156 unit, are presented in Table I.

TABLE I

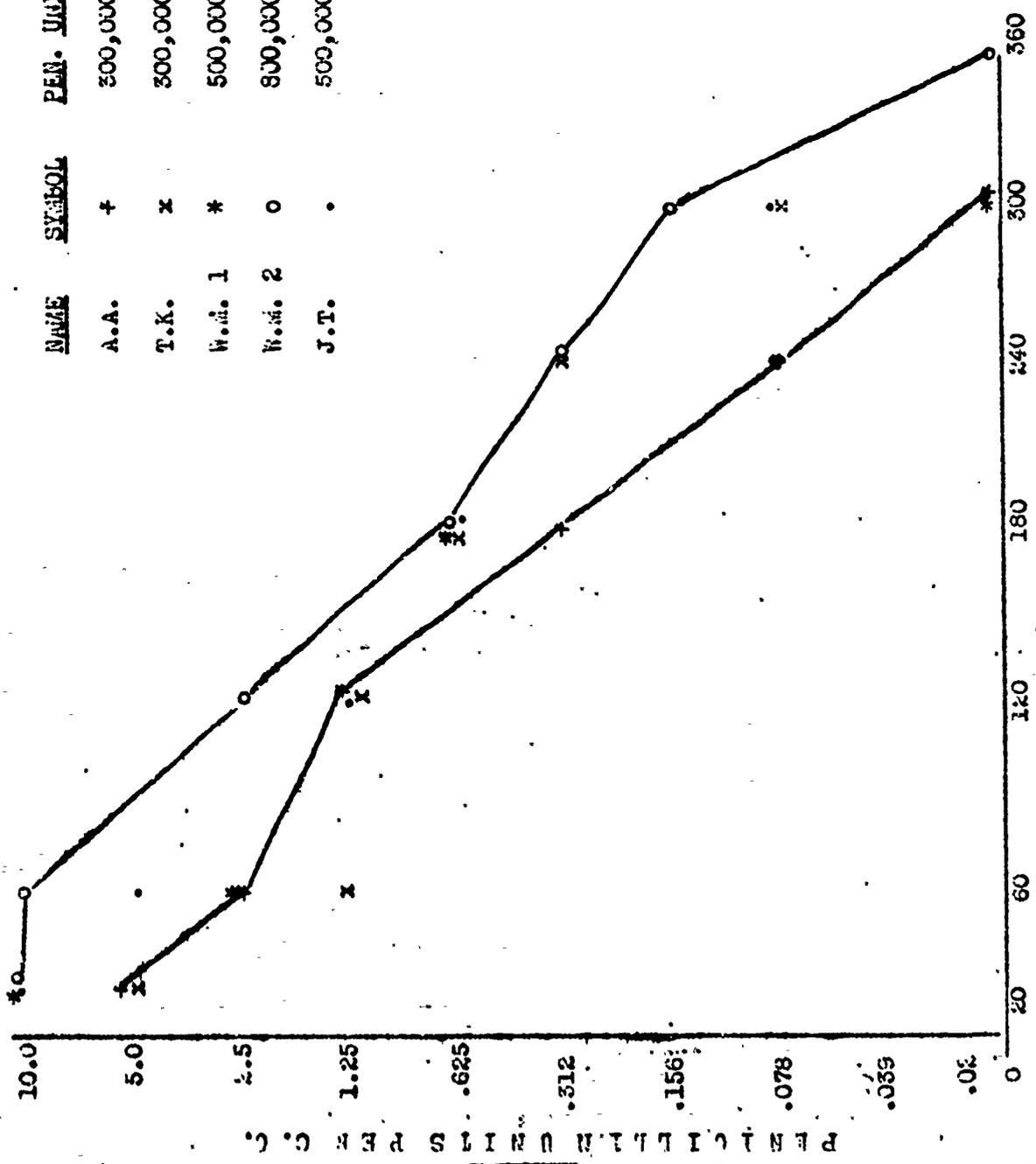
<u>Duration of Serum Penicillin Level</u>	<u>0.078 Unit</u>	<u>0.156 Unit</u>	<u>Peak</u>
	<u>hours</u>	<u>hours</u>	<u>units</u>
200,000 units aqueous	3 - 4	2 - 3	5.0
300,000 " "	4 - 5	3 - 4	5.0
500,000 " "	4 - 5	3 - 4	10.0
800,000 " "	5 - 6	5	10.0
500,000 " " plus 100,000 at 2 hours	6	5	10.0
500,000 plus 500,000 at 2 hours	7	6	20.0
100,000 units 3% beeswax	5 - 7	4 - 6	0.156
300,000 " 4% "	9 - 10	9 - 10	0.625
150,000 " 5% "	7 - 9	5 - 8	0.312
300,000 " 5% "	12 or more	12	0.625

Serum levels of penicillin can be maintained at various concentrations by continuous intravenous administration. It should be noted that the serum concentrations obtained by the use of this method in general clinical practice are by no means as constant as is generally believed. This is particularly the case when the total 24 hour dose is relatively low. In one patient receiving 160,000 units per day by intravenous drip, serum levels ranging from 0.156 unit to zero, (i.e., somewhere less than .078 u/cc to zero), were obtained at various times during the 24 hour period. With the use of a larger daily dosage, such unusually low levels can be avoided. However, unless a constant watch of the rate of flow through the drip and the oral intake of fluid is maintained, irregular serum levels will occur. Because the unusual vigilance necessary is so wasteful of manpower, and because phlebitis and other reactions to the drip make its prolonged use impracticable, only a few studies of the administration of penicillin by this route were made, and it is believed that the method is the least desirable in the treatment of the subacute infections.

HIGH LOSSES - 300,000 - 800,000 UNITS OF PENICILLIN

TIME OF TAP
AFTER PEN. ADMIN.

NAME	SYMBOL	PEN. UNITS	TIME OF TAP AFTER PEN. ADMIN.
A.A.	+	300,000	4 hours
T.K.	x	300,000	20 minutes
W.M. 1	*	500,000	
W.M. 2	o	800,000	
J.T.	.	500,000	6 hours

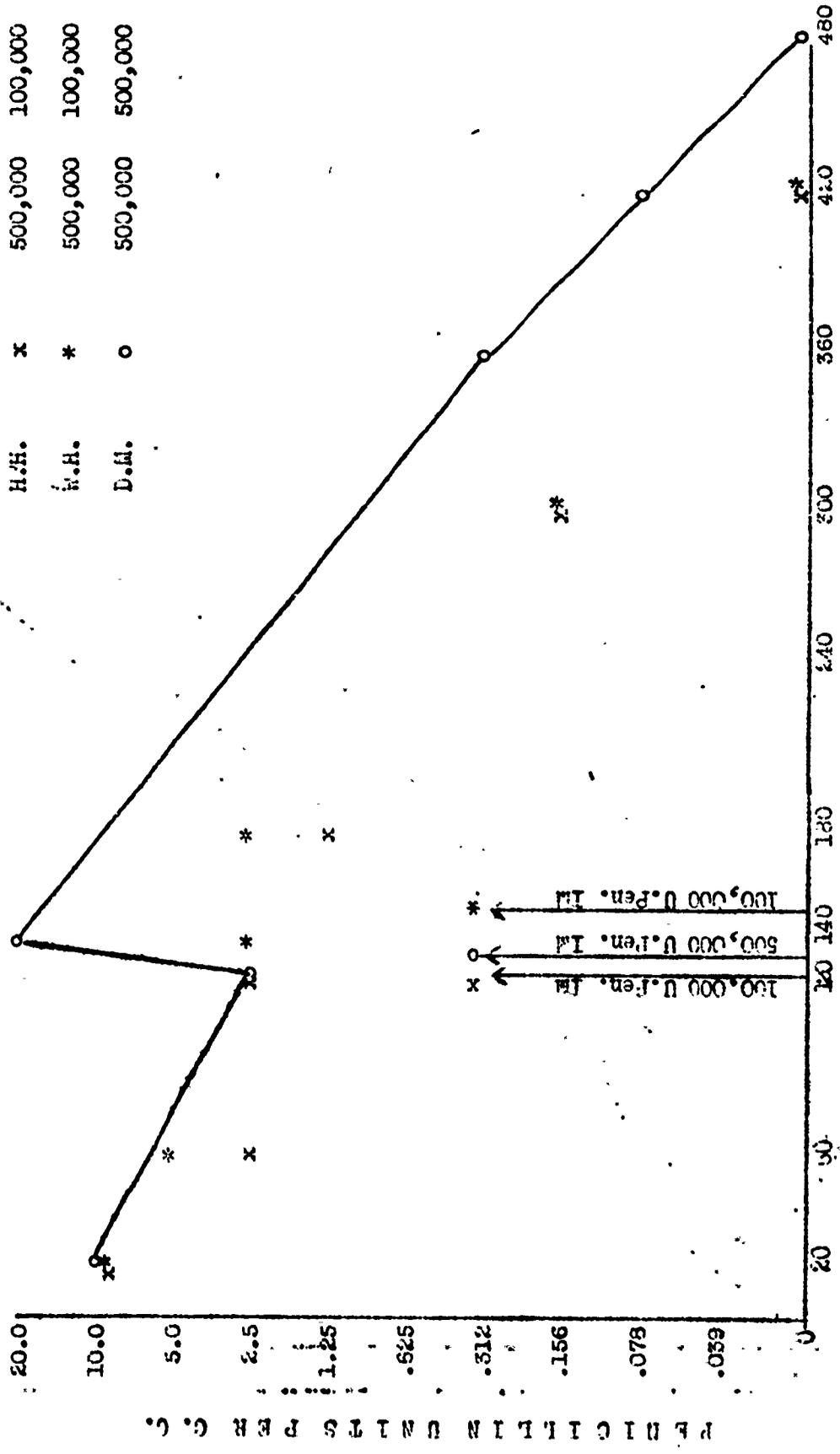


HIGH DIVIDED DOSES -- 500,000 - 1,000,000 UNITS OF PENICILLIN

UNITS OF PENICILLIN	
1st Dose	2nd Dose
500,000	100,000
500,000	100,000
500,000	500,000

NAME SYMBOL

H.H. x
 H.H. *
 D.M. o



TIME-MINUTES AFTER PENICILLIN ADMINISTRATION

Figure 41
 PENICILLIN UNITS PER G.C.

SINGLE INJECTION TREATMENT OF GONORRHEA
 WITH PENICILLIN BEESWAX-PEANUT OIL MIXTURE

BLOOD LEVELS

— Patient # 10 Calcium Penicillin 100,000 U. 3% Beeswax
 - - - Patient # 11 " " 100,000 U. 3% "
 - - - - Patient # 12 " " 100,000 U. 3% "

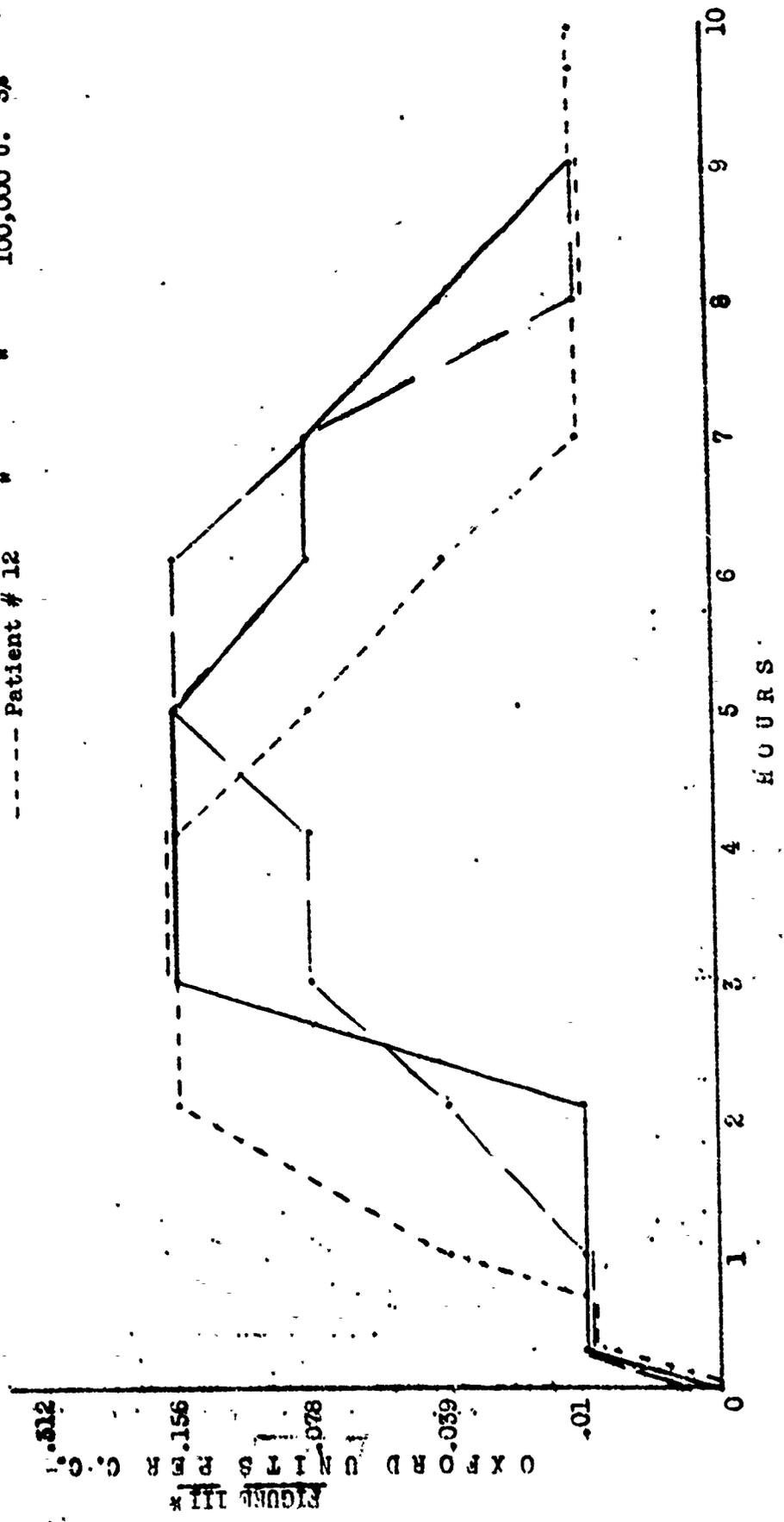
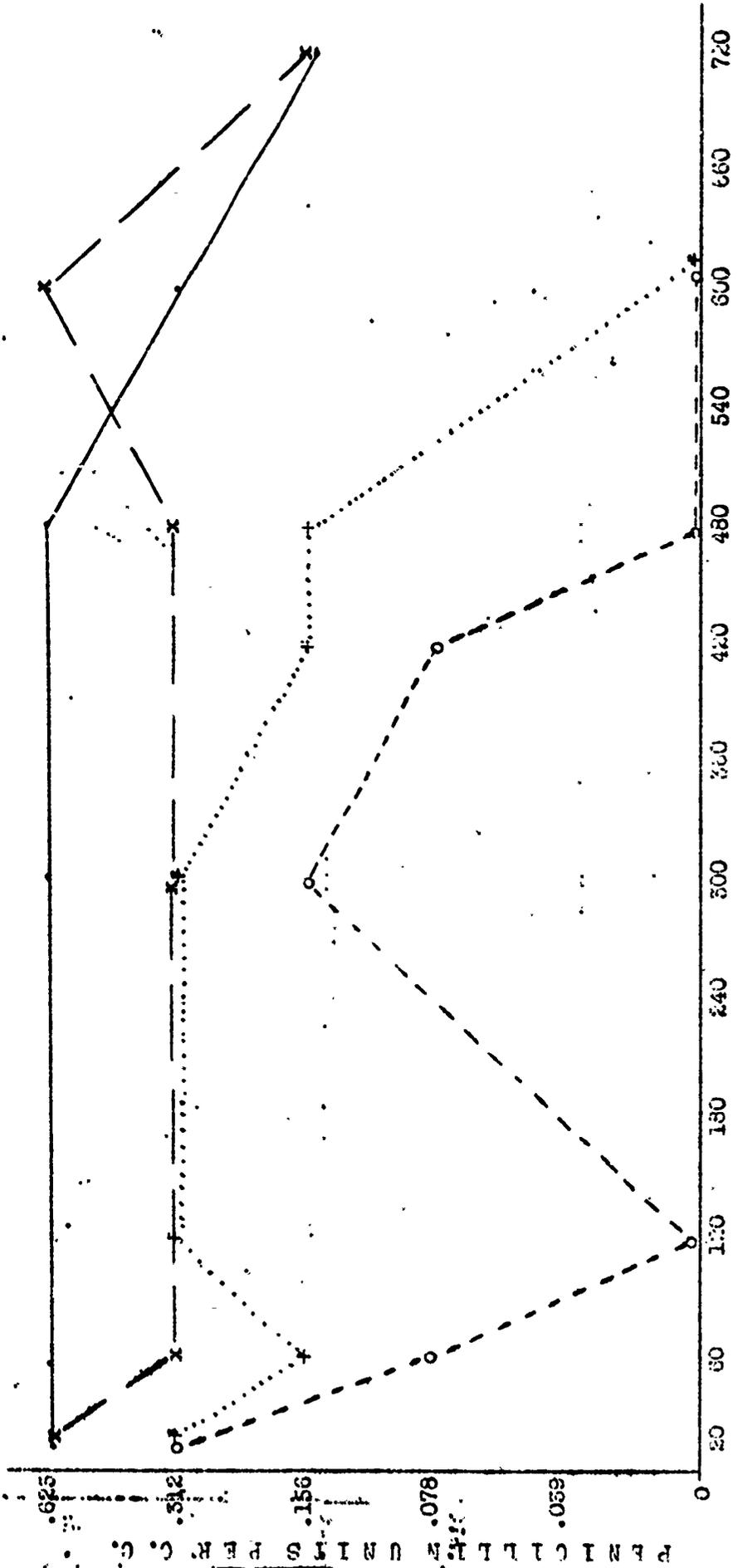


FIGURE III*
 OXFORD UNITS PER C.C.

*A. J. Romanek, U. S. Army Publ., 31:97, October 1944.

PENICILLIN 5% BEESWAX

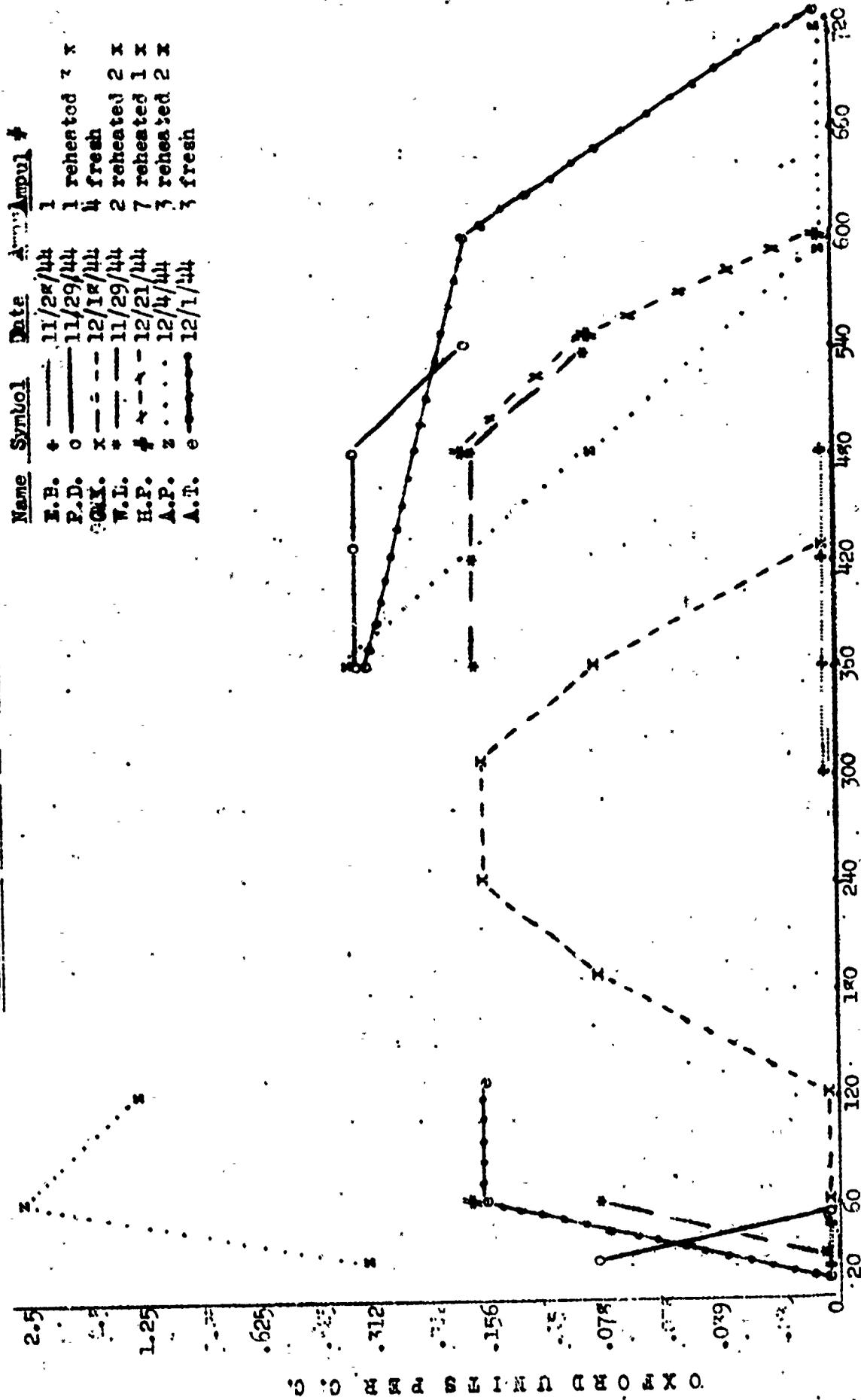
NAME	SYMBOL	PENICILLIN UNITS	NAME	SYMBOL	PENICILLIN UNITS
E.A.	+	140,000	E.L.	•	280,000
J.W.	o	140,000	Z.S.	x	280,000



TIME - MINUTES AFTER PREPARATION

FIGURE 2A
PENICILLIN UNITS PER G.

FIGURE IV-A
 PENICILLIN HEMAZIN 1/4 - DOSE OF 100,000 UNITS



MINUTES AFTER PENICILLIN ADMINISTRATION

PERCENT HEMAZIN

II. The Relation of the Concentration and Duration of Penicillin Effect to the Inhibition of Bacterial Populations in vitro.

Sufficient evidence is available from clinical studies to permit the conclusion that the constant maintenance of a detectable penicillin level in the serum is not an essential for successful penicillin therapy. In some of the clinical regimens used successfully, the penicillin has been administered in such a manner that poorly sustained peaks of concentration with total periods of demonstrable penicillin action of relatively short duration have been alternated with longer periods during which no penicillin can be detected in the serum. The treatment of pneumococcal pneumonia with 400,000 units of penicillin in 96 hours is an example of such a regimen.

Of the two methods of intramuscular administration under consideration, such peaks are prevented in one, yet with both the maintenance of a detectable serum concentration is possible with relatively infrequent injection. This raises the question of whether the poorly sustained but high peaks of penicillin concentration are of therapeutic importance or merely represent penicillin which is in excess of that utilizable for the destruction of organisms. This question of a possible "flash action" of penicillin is one facet of the great problem in the penicillin treatment of subacute and chronic infections today, i.e., whether high level therapy is superior to prolonged treatment at the minimally effective level.

The following experiments were designed to study the time-dose relation of penicillin action on a population of organisms of a susceptible strain.

The same strain of organisms (C-203 M.V., a beta hemolytic Group A streptococcus), which was the test organism in the penicillin determinations, was used. It has been demonstrated repeatedly that 1 - 43,000 of these organisms per cc. are completely inhibited for 48 hours by 0.02 unit of penicillin.

To four tubes containing 10 cc. of 8 per cent blood broth, penicillin was added so that the final concentrations were 0.02, 0.08, 0.63, and 10.0 units per cc. These concentrations were chosen to be comparable to the various concentrations attained in patients following the intramuscular injection of penicillin in the aqueous or beeswax vehicle. All four tubes were inoculated with the organism obtained from an actively growing (18-hour) culture and were then placed in the water bath at 37° c.

At hourly intervals an aliquot of 0.3 cc. was removed from each of the four cultures. The penicillin activity in these aliquots was destroyed by the addition of penicillinase. Determinations of the number of viable organisms present in each aliquot were then made by the pour plate method.

In this manner it was possible to compare the action of the four penicillin concentrations hour by hour. The results of a typical experiment are shown in Figure 5:

In this graph the number of organisms present in the four penicillin concentrations are plotted against the hours of penicillin activity. As may be seen, after an initial rise, the fall in bacterial population was progressive during the first five hours. The destruction of organisms occurred as quickly and was

of the same order of magnitude whether they were subjected to 0.03, 0.625, or 10.0 units. The rate of destruction of the culture containing only 0.02 unit was not identical with that which occurred with the three higher concentrations, but even with this lowest concentration, the curve approaches the others after five hours of penicillin action. This lowest concentration, which is only one-quarter of the minimal concentration of penicillin which can be detected in the serum, is the smallest amount of material which will inhibit the test organism, so that minute unavoidable errors in dilution to this concentration might be such as to vary the antibacterial effect to this slight degree.

As may be seen from the curves, the same number of organisms are destroyed per hour of penicillin action (except for 0.02), regardless of the concentration of penicillin, until at 5 hours less than 5 per cent of the organisms remain viable. The complete destruction of this small fraction of the bacterial population, however, requires many hours for completion. This phenomenon, that the addition of penicillin to a culture of susceptible bacteria will result in the killing of most but not all of the organisms in a relatively short period of time, is well known in chemotherapy and has been observed by Hobby, by Dubos, and by others with penicillin. It is generally assumed that in any bacterial population there is some variation in the susceptibility of the individual bacterial cells to the therapeutic agent used and that the minority remaining after the initial effect of the agent represent the more resistant organisms.

It is obvious that the subsequent behavior to penicillin, of this apparently untouched minority, is of importance, and studies on this point are continuing.

The next experiments were devised to observe the subsequent course of the remaining 5 per cent of the original penicillin-treated culture when the penicillin action was stopped at 5 hours by the addition of penicillinase. Cultures containing comparable small inocula of untreated organisms were introduced at this point as controls. In the media used for these controls, large inocula of organisms had been subjected to 0.08 and 10 units of penicillin for the 5-hour period, penicillinase had been added, and the media rendered sterile by passage through a Seitz filter. The pH of all of the cultures was identical at this 5-hour point, which really represented the start of this experiment.

As shown in Figure 6, the control inocula showed the usual 4-hour lag before entering into the logarithmic phase of growth. This lag phase was slightly prolonged in the medium which had previously contained the 10 units of penicillin. However, the lag phase, before logarithmic growth, of the cultures which had been previously exposed to the penicillin was appreciably prolonged, by 2 or 3 hours, over the control cultures. This prolongation of the lag phase was of approximately the same order of magnitude in the bacteria which had received 5 hours of treatment at the 0.08 unit level, as it was in the organisms treated at the 10 unit concentration. It is probable that the slight prolongation of lag phase seen in the organisms previously exposed to the 10 units of penicillin compared with the organisms in 0.08 unit is merely within the limits of the method, and may be associated with the similar slight prolongation in the control medium, which had previously contained 10 units of penicillin.

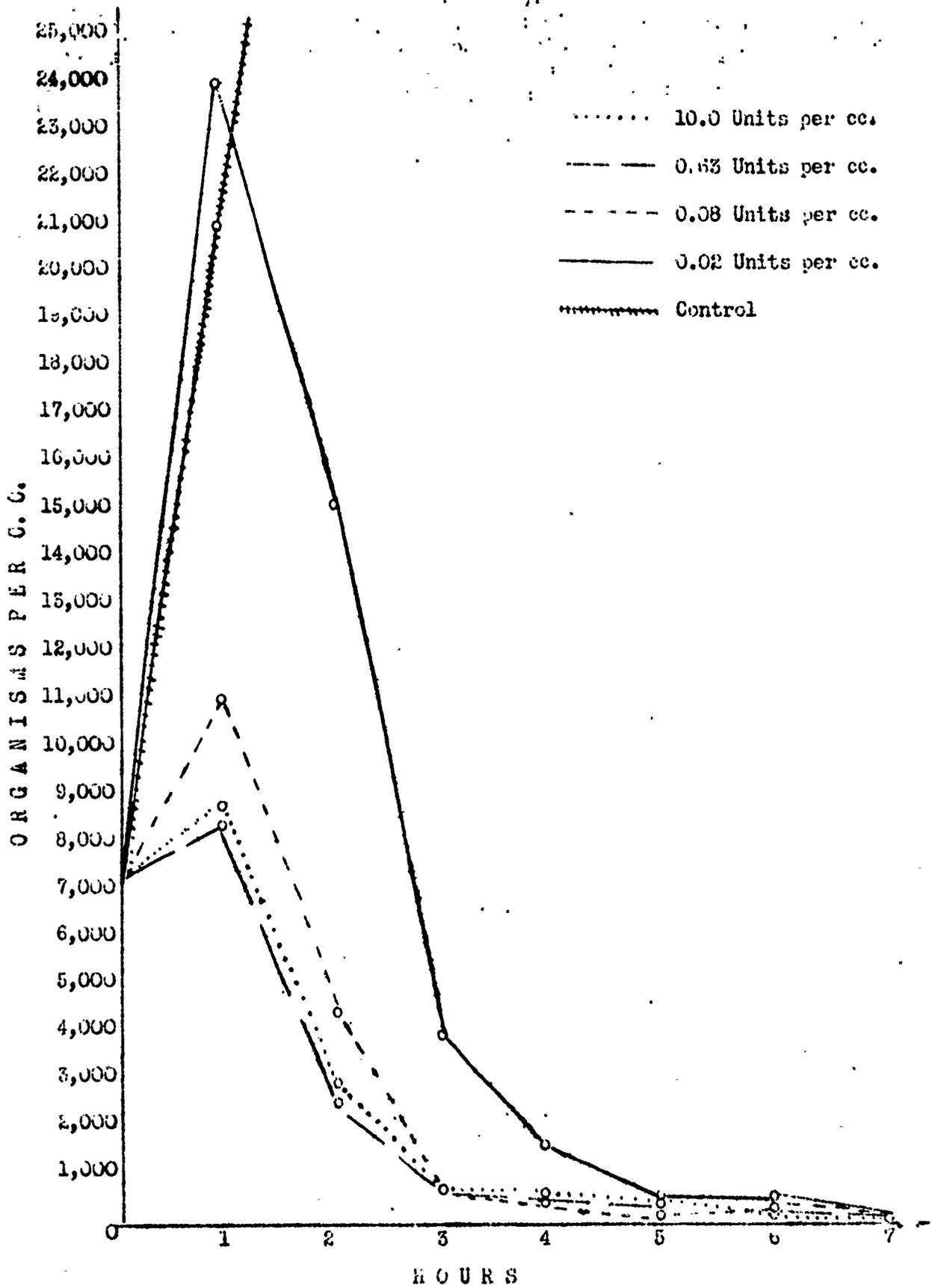
From these experiments with streptococci in vitro, it would seem that:

1. When the minimal concentration of penicillin which will completely inhibit an inoculum of this strain of bacteria in a 48-hour period is added to a culture of such bacteria, more than 95 per cent of the organisms will be destroyed in a relatively short period of time (5 to 10 hours).
2. Increasing the penicillin concentration above this minimal concentration by 125 times does not alter the rate or extent of this penicillin effect per unit of time.
3. The approximately 4 per cent of the original bacterial population which is not destroyed in the early hours of exposure to penicillin is not entirely eliminated until it has been in contact with penicillin for 30 to 40 hours.
4. When the penicillin action is stopped after 5 hours, the lag phase before the onset of active multiplication of these remaining organisms is prolonged by 2 to 3 hours to a total lag phase of 5 to 7 hours.
5. This prolongation of the lag phase before active multiplication of the organisms reappeared was approximately the same whether the bacteria had been treated previously with 0.08 or with 10.0 units of penicillin.

The applicability of these studies of the time-dose relationship of penicillin action on streptococci in vitro to the problem of infections in man is as yet impossible to evaluate. However, there are striking parallels between the action of penicillin in the two situations. In both there is an immediate marked diminution in the number of organisms present, and in both the cessation of penicillin action short of cure is followed first by a latent period and then by relapse. This period of latency in human infections is of variable length. Even in the most acute infections caused by the pneumococcus and the beta hemolytic streptococcus, it is usually prolonged for at least 24 hours. With the bacterial cultures, both the phenomena of immediate destruction of 95 per cent of the organisms and the lag phase before multiplication of the survivors appears seem to be unaffected by marked increases in the concentration of penicillin above the minimal effective level. Certain clinical observations would indicate that the same phenomena are likewise independent of penicillin concentration in man. Tillett has noted that, when single doses of penicillin, as varied as 10,000 and 300,000 units are given to patients with pneumococcal pneumonia, the resulting remissions of the signs of infection and latent periods before relapse are identical. Similar observations have been made with the use of small doses of penicillin in the treatment of streptococcal, gonococcal, and spirochetal infections.

In short, the results of these experiments in vitro would indicate, and certain clinical experience support, the thesis that once the minimal effective concentration of penicillin is attained, remission will occur; that the presence of much greater concentrations per unit of time serves no useful purpose; and that only by maintenance of the minimal effective level for a period much longer than that necessary to produce the original remission can relapse be prevented. It would seem possible, because of the prolongation of the quiescent phase in previously treated organisms observed in these studies in vitro, that this prolonged maintenance of an effective level need not be absolutely continuous.

FIGURE V
THE EFFECT OF VARYING CONCENTRATIONS OF PENICILLIN ON STREPTOCOCCI



Obviously, the absolute values of the height of the minimal effective level (or "effective level", as it will be hereinafter designated) and the time at that level necessary for cure will vary with the different species of organisms and with the histopathology of the particular infection. The factor of the histopathology of the infection is probably of much more importance in the treatment of the chronic infections such as bacterial endocarditis and neurosyphilis than it is in the therapy of infectious syphilis.

III. Regimens Used In Early Syphilis

The minimal concentration of penicillin which will produce complete inhibition of the *Treponema pallidum* in vitro has not been determined as yet. Penicillin concentrations ranging from 0.01 to 0.02 unit are sufficient for the inhibition of the Group A streptococcus and many other organisms. Inocula of the Reiter's strain of spirochetes can be inhibited completely in vitro for a 7-day period by penicillin concentrations of 0.02 to 0.04 unit per cc. And, as noted above, in the treatment of humans with infectious syphilis, amounts of penicillin which would produce serum concentrations of only approximately this order (0.01 - 0.04 unit) are associated with a rapid disappearance of the organisms from the surface lesions followed by a healing of the lesions themselves quite comparable to the effects observed after larger doses. So that in the absence of exact information about the penicillin sensitivity of the *Treponema pallidum* but utilizing both this information obtained from its response to penicillin in vivo and data on the sensitivity of other organisms in vitro, it would seem that a serum penicillin concentration of 0.078 unit per cc. is probably well above the "effective level" for the spirochete.

Assuming this to be the case, and also assuming the validity of the hypothesis that the duration of treatment at this level is the most important determining factor of cure, it is important to contrast various regimens for the treatment of infectious syphilis with one another from the standpoint of the number of minutes or hours during which this level is maintained, and to compare these data with the so-far recorded relapse rates in these several regimens.

In Table II, ten regimens which have received trial are listed in the order of the total period of time, measured for convenience in hours, during which a serum level of 0.078 unit of penicillin per cc. would be maintained. In the right-hand columns are indicated such data as are at present available on the relapse rates for the various systems. As it has been found that the critical period for relapse is between the 113th and the 163th day, the relapse rate in per cent is indicated only for those groups which have been followed for that period.* Obviously, because of insufficient information, it is impossible to make a direct comparison of the number of hours of the 0.078 unit level with the relapse rate. However, certain features are of interest.

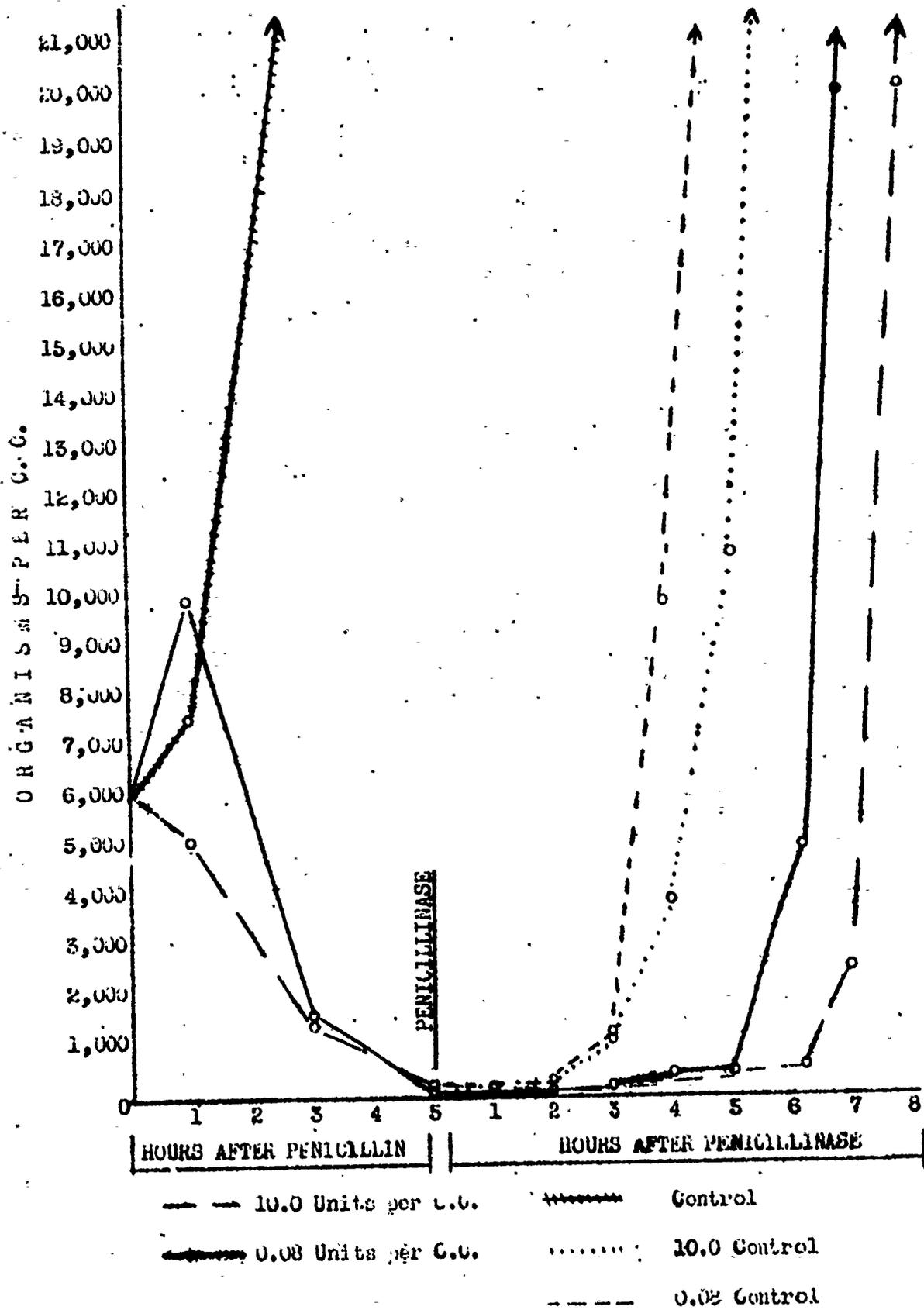
Only two of the systems show 90 or more hours at the 0.078 unit level. The greatest number of hours at such levels attained by any 4-day system was only 75, which is 20 hours less than the maximum possible in a 4-day period.

With the exception of the three systems listed near the bottom of the chart which have been discontinued as unsatisfactory, the cumulative relapse rates for the systems providing less than 90 hours "effective level" are not yet available. However, the fact that relapses have already occurred on those regimens in such small groups of patients in so short a period of follow-up makes it seem probable that all of these systems will eventually prove to be unsatisfactory.

The relapse rate in the 90-hour regimen is 7.1 per cent, a figure which compares favorably with that attained by any previously used system of therapy of infectious syphilis, but one which is still high. In the 120-hour system, on relapse has already been noted in the small group followed for a short period of time.

* Figures on relapse rates obtained from J. E. Moore, Chairman, Subcommittee on Venereal Diseases.

FIGURE VI
LAG PHASE OF GROWTH OF PENICILLIN-TREATED AND UNTREATED STREPTOCOCCI



It seems probable; therefore, that even the most satisfactory of the present regimens falls short of the attainable ideal in terms of the prevention of relapse. If the total period of an 0.078-unit level proves to be valid as a yardstick, it is suggested that 140 to 160 hours at that level might be responsible for a still lower relapse rate.

Two additional qualifications accompany the use of the total period at a given level as a yardstick. To have maximum effectiveness, it is probable that the total hours at a given serum concentration should not be spread over too long or too short a period of time.

If spread over too long a period, it is possible that the number of hours each day at the given concentration would be too few to be effective. If the total number of hours at the desired concentration is produced in too short (i.e., shortest possible) a period, it might detract from the over all efficiency of the system. This latter qualification would hold true only if the prolongation of the lag phase of growth observed in organisms treated with penicillin in vitro also occurred with spirochetes in the body.

If this did occur, the continuous production of a certain serum concentration of penicillin for an arbitrarily chosen number of hours might not be so effective as if that same number of hours at that concentration were produced intermittently. Hobby's observations that resting organisms are much less affected by penicillin than are organisms in the stage of multiplication and growth might have a bearing in regard to the necessity for the maintenance of penicillin concentrations continuously during this lag phase of growth.

However, with the assumption that the total period of time during which a 0.078 unit level of penicillin is maintained is an index of the efficacy of a given system, the following regimens which will produce such a level for total periods ranging from 90 to 180 hours have been calculated.

In Table III may be seen five such regimens which require eight or twelve intramuscular injections daily of aqueous penicillin. Although the total amounts of penicillin required are of the same order of magnitude as used in some of the regimens which have already received clinical trial, a considerable prolongation of penicillin action would be attained.

Because of the relatively long (10-15 days) period of hospitalization necessary for these regimens which require multiple daily injections, a series of regimens have been outlined which would produce the desired serum concentrations for the desired period of time with the use of only one (or two closely spaced) intramuscular injections each day. Such treatment systems conceivably could be administered on an ambulatory basis. Calculation of the prolonged maintenance of serum penicillin concentrations following the intramuscular injection of large doses in the aqueous or beeswax vehicle are made on the basis of the observations reported above.

In Table IV may be seen the number of days of treatment necessary to produce total periods of 0.078 unit levels of 160, 120, and 90 hours, respectively, when only one treatment is administered each day. Excluding the 90-hour regimen as probably unsatisfactory, it would require 10 to 21 daily treatments of 150,000 to 300,000 units of penicillin in oil and 4 or 5 per cent beeswax to produce such levels of this total duration. In order to achieve the desired time-dose relationships by the use of aqueous penicillin in single daily injections, it would be

necessary to administer almost a million units a day for three or four weeks.

In Table V may be seen the calculated number of days of treatment which would be necessary to produce serum penicillin concentrations of at least 0.078 unit per cc. for 120 to 160 hours when the penicillin is administered twice daily. The systems employing 300,000 units of penicillin with 5 per cent beeswax are the shortest possible regimens which will produce the desired levels for 120 and 160 hours, respectively. In regard to these latter systems, it must be remembered that, as mentioned above, there might be certain theoretical disadvantages in compressing all of the penicillin therapy of a given system into the minimal period of time.

As may be seen in Table VI, 120 to 160 hours at the desired level may be attained in 9 to 11 days by the administration of aqueous penicillin three or four times daily. However, as with the other regimens in which aqueous penicillin is administered at infrequent intervals, prohibitive amounts of penicillin are required.

In Table VII are indicated the duration of levels of 0.078 unit in the various regimens used in the treatment of neurosyphilis and bacterial endocarditis. The two columns at the bottom of the table merely illustrate the obvious fact that when more than 300,000 units of penicillin is given per day at two-hour intervals there is no increase in the total number of hours during which a level of 0.078 or 0.156 unit is maintained. However, if it can be demonstrated in a given infection that because of the histopathology of the lesion, or an unusual penicillin resistance of the organism, the maintenance of even higher levels is required, such increase in daily dose above 300,000 units might be advantageous.

The final evaluation of the ideal penicillin regimen for the treatment of early syphilis must await a prolonged period of study. Obviously, it is impossible to prove at this time that these suggested penicillin regimens are superior to those which have already been under investigation. However, as it would seem at present that the length of time of penicillin action is the most important factor in the success of a given regimen, it is possible that consideration of this time factor might help to limit the choice of regimens for study to those with the most promise.

TABLE III
PENICILLIN REGIMENS FOR EARLY SYPHILIS WITH 120 TO 180 HOURS OF SERUM PENICILLIN
CONCENTRATION OF AT LEAST .078 UNIT/CC.

Total Penicillin Units	Days	Daily Dose Units	Single Dose Units	Injections Per Day	Hours/day of .078 u.	Total period of levels of .078 u.
1,600,000	10	160,000	20,000	8	12	120
2,080,000	13	160,000	20,000	8	12	155
3,200,000	10	320,000	40,000	8	16	160
2,400,000	15	160,000	20,000	8	12	180
3,000,000	10	300,000	25,000	12	22 - 23	220

TABLE IV

ONE INJECTION OF PENICILLIN PER DAY

Individual dose	To Attain Total 0.078 Unit Level		
	For 160 hours	For 120 hours	For 90 hours
800,000 u. aqueous	29 days (25.2)*	22 days (17.6)*	16 days (12.8)*
300,000 u. with 4% beeswax	18 " (5.9)*	14 " (4.2)*	10 " (3.0)*
150,000 u. with 5% beeswax	21 " (3.1)*	16 " (2.4)*	12 " (1.8)*
300,000 u. with 5% beeswax	13 " (3.9)*	10 " (3.0)*	8 " (2.4)*

Two Injections Within Two Hours

500,000 u. plus 500,000 u.	23 days (23.0)*	17 days (17.0)*	13 days (13.0)*
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* = millions of units of penicillin

TABLE V
TWO INJECTIONS OF PENICILLIN PER DAY
12 HOURS APART

Individual dose	To Attain Total 0.078 Unit Level		
	For 160 hours	For 120 hours	For 90 hours
800,000 u. aqueous	14 days (23.3)*	11 days (17.6)*	8 days (12.8)*
300,000 u. with 4% beeswax	9 " (5.4)*	7 " (4.2)*	5 " (3.0)*
150,000 u. with 5% beeswax	10 " (2.8)*	8 " (2.2)*	6 " (1.6)*
300,000 u. with 5% beeswax	6½ " (3.6)*	5 " (2.8)*	4 " (2.4)*

* = millions of units of penicillin

TABLE VI

THREE INJECTIONS OF PENICILLIN PER DAY

AT 8 A.M., 3 P.M. AND 10 O.M.

Individual dose	To Attain Total 0.078 Unit Level		
	For 160 hours	For 120 hours	For 90 hours
300,000 u. aqueous	11 days (9.9)*	9 days (8.1)*	6 days (5.4)*

FOUR INJECTIONS OF PENICILLIN PER DAY

AT 8 A.M., 12 A.M., 4 P.M. AND 8 P.M.

200,000 u. aqueous	11 days (8.8)*	9 days (7.2)*	6 days (4.8)*
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* = millions of units of penicillin

TABLE VII
NEUROSYPHILIS

Total Penicillin	Days	Total Daily Dose	Individual Dose	Injections		Duration 0.078	Duration 0.156
				Per day	Total		
2,000,000	6-7	300,000	25,000	12	80	9,600	
4,000,000	13-14	300,000	25,000	12	160	19,200	
13,700,000*	24	500-600,000	40-50,000	12	268	34,560	

BACTERIAL ENDOCARDITIS

2,800,000	14	200,000	25,000	8	112	13,440	
4,200,000	14	300,000	25,000	12	168	20,160	
4,200,000	21	200,000	25,000	8	168	20,160	
5,600,000	28	200,000	25,000	8	224	26,880	
8,400,000	28	300,000	25,000	12	336	40,320	30,240
16,800,000	28	600,000	50,000	12	336	40,320	30,240

* This regimen has not actually been used for neurosyphilis, but has been used in bacterial endocarditis.

SUMMARY

1. Studies of penicillin action on streptococci in vitro show that 24 or more hours of penicillin at a certain concentration are necessary for the complete inhibition of a culture, even though 95 per cent of the organisms are destroyed within the first five hours. The time required for the complete inhibition of the culture cannot be shortened by increasing the concentration of penicillin above the minimal effective level.

2. Certain clinical observations of penicillin action in a variety of infections, including syphilis, suggest that here also only short periods of penicillin action are necessary for the immediate destruction of a large number of the organisms, but prolonged penicillin action is necessary for cure. In addition, there is some evidence that in vivo, as in vitro, large increases in the concentration of penicillin for short periods do not shorten this period of time necessary for cure.

3. It has been shown that by the use of sufficiently large quantities of aqueous penicillin in a single injection, it is possible to prolong the period of detectable serum concentrations for 6 or 7 hours with the production of an initial high concentration. If sufficient penicillin in beeswax is administered in a single injection, these initial high concentrations are not obtained, but the period of a detectable serum concentration can be prolonged for 9 to 12 or more hours.

4. As both the studies in vitro and clinical experience lend support to the thesis that it is not the production of multiple peaks of high concentrations of penicillin but the length of the action at low concentrations which is the important factor, it is suggested that this penicillin action time be given consideration in the planning of new regimens for the treatment of syphilis and the other sub-acute infections. In calculations involving the use of penicillin in beeswax and other inhibitors of absorption, this time of action of the penicillin is the only available index for comparison with the other systems.

5. There is a possibility, as indicated by the in vitro experiments, that once the initial effect of the penicillin action has occurred, subsequent action need not be absolutely continuous to obtain the maximum effect.

6. Analysis of the regimens under investigation for the treatment of early syphilis reveals that in any given system, irrespective of the total amount of penicillin or the number of days of treatment, there is a wide variation in the total period of time during which detectable concentrations of penicillin are present in the serum, depending upon the timing and size of the individual doses.

7. Using a serum concentration of 0.078 unit of penicillin as an arbitrary standard, a number of regimens for the treatment of early syphilis have been calculated which would produce this concentration for total periods of 90, 120, and 160 hours by a minimum of intramuscular injections.

IV. The Relation of the Concentration and Duration of Penicillin in the Serum to the Concentration in Cerebrospinal Fluid and Dialysates.

Whether penicillin passes into the central nervous system in effective concentrations following parenteral administration is an important problem in the treatment of meningitis and neurosyphilis. Several studies of the passage of penicillin from blood to spinal fluid in subjects with and without meningitis have been made. It was established by Rammelkamp and Keefer (1) that, both following single parenteral injections and the continuous administration of penicillin for 24 hours by intravenous drip, it was impossible to detect penicillin in the cerebrospinal fluid of subjects who did not have meningitis. Fleming (2), and more recently Rosenberg and Sylvester (3) have reported that, in the presence of purulent meningitis, penicillin can be demonstrated in the cerebrospinal fluid after parenteral administration.

Rammelkamp and Keefer concluded on the basis of their study that penicillin be given intrathecally in the presence of meningitis. This method has been given extensive trial and is unquestionably effective. Whether meningitis can be treated with equal effectiveness solely by the parenteral route is not established as yet.

It must be remembered that the problems of the penicillin therapy of purulent meningitis and of neurosyphilis, although similar, are not necessarily identical. In both types of infection involvement of the meninges and the parenchyma of the brain may occur to a varying degree, but in many syphilitic infections the parenchymatous involvement is the more extensive. For this reason it is possible that the most effective method for the administration of penicillin for the one type of infection might not be the ideal method for the other.

Because of the importance of establishing the most effective route for the administration of penicillin in the therapy of neurosyphilis, an investigation has been made of the penetration of penicillin into the cerebrospinal fluid of neurosyphilitics following intravenous and intramuscular administration.

Methods: The subjects for the clinical study were patients with neurosyphilis and infectious syphilis who were receiving penicillin therapy on the medical service of The New York Hospital. Determinations were made on a few patients with late latent syphilis and on a few with other infections which involved the central nervous system. The tests for penicillin activity of the cerebrospinal fluids were made by the Rammelkamp technique described above and in some instances by direct inoculation test (4). The direct inoculation method is a simple qualitative test for the presence of concentrations of penicillin which are too low to be detected by the other methods. Although cerebrospinal fluid is a poor medium for the culturing of most bacteria, if it is enriched by the addition of 0.5 per cent casein hydrolysate it will support the growth of the Group A streptococcus. Five cc. of the spinal fluid to be tested is enriched in this manner and is inoculated directly from an actively growing culture of the test organism. The tubes are then incubated along with suitable controls and the test is considered to be positive when the streptococci are completely inhibited in the unknown sample for 24 to 48 hours. If the growth of the organisms is retarded but not completely inhibited in the test specimen, the result is considered to be doubtful, and when growth in both the unknown sample and the control are identical, the test is regarded as negative. It has been established that 0.02 unit of penicillin is necessary for the complete inhibition of this test organism for a 48-hour period. Most of the "positive"

results obtained by this technique reflect a penicillin concentration in the specimen of cerebrospinal fluid of approximately this amount. In the instances in which the organism is completely inhibited for the entire 48-hour period in a specimen of fluid in which no penicillin can be demonstrated by the Rammekamp technique, the penicillin concentration may range anywhere between 0.02 and 0.078 unit per cc.

Results: Thirteen patients received a single intramuscular injection of penicillin, and at various periods thereafter cerebrospinal fluid was obtained for penicillin determination. The period of time between the injection of the penicillin and the withdrawal of the spinal fluid is indicated in Table I. The concentration of penicillin in the serum is the amount present at the time of the lumbar puncture. As in each instance only one injection of penicillin was given, the serum concentration of penicillin was at or above the concentration listed in the table for the entire period previous to the lumbar puncture.

No penicillin could be detected by the Rammekamp method in any of the spinal fluids. The range of dosage in these patients was from 25,000 to 500,000 units, the serum concentrations at the time of withdrawal of the fluid varied from 0.078 to 5.0 units, and the period of time during which the penicillin had opportunity to pass into the spinal fluid was from 30 to 360 minutes.

The failure of the penicillin to appear in the cerebrospinal fluid of these subjects is not surprising when it is realized that to effect the interchange between serum and spinal fluid of a number of diffusible substances requires periods ranging from 4 to 24 hours. (5). In only two of these subjects were detectable concentrations of penicillin present in the serum for as long as 4 hours before withdrawal of the fluid, and in both of these two the inoculation test showed the presence of penicillin. The significance of this latter finding will be discussed subsequently.

In order to provide an adequate period of time for the serum concentrations of penicillin to have the opportunity of passing over into the cerebrospinal fluid, determinations have been made on a series of subjects who had been receiving penicillin intramuscularly at frequent intervals for periods ranging from four days to two weeks.

Thirty-two subjects received penicillin on systems of administration which would ensure either a continuous or virtually continuous penicillin concentration in the serum of 0.078 to 0.156 unit. As may be seen in Table II, in no instance was any penicillin action demonstrable in the cerebrospinal fluids of these 32 subjects. The presence or absence of syphilitic inflammation of the central nervous did not affect this finding.

Determinations were made on three subjects who had received 160-300,000 units of penicillin by continuous intravenous drip for a 24-hour period before the cerebrospinal fluid was withdrawn. One of these subjects had neurosyphilis, and the other two had latent syphilis and subacute bacterial endocarditis, respectively. In these three subjects the serum concentrations of penicillin at the time of the lumbar puncture ranged from 0.156 to 0.625 unit per cc. However, no penicillin was detectable in any of the cerebrospinal fluids of these three subjects, one of whom had inflammatory disease of the central nervous system.

Because of the possibility that a change in the dynamics of the fluid system secondary to repeated withdrawals might influence the transfer of penicillin across the blood-brain barrier, the following observations were made.

Three patients (two with neurosyphilis, one with latent syphilis) were given 25,000 units of penicillin at two-hour intervals for 24 to 72 hours before the first lumbar puncture, and the administration at this dosage was continued throughout the period of investigation. At 24, 48, and 72 hours, cerebrospinal fluid was withdrawn and tested by the Rammelkamp method for the presence of penicillin. There was no evidence of penicillin action in any of these nine fluids. In short, tests for the presence of penicillin in the cerebrospinal fluid of 38 subjects who had been given penicillin by various parenteral routes were uniformly negative by a technique which permits the detection of penicillin concentrations as low as 0.078 unit per cc. The presence of syphilitic inflammation of the nervous system, repeated withdrawals of the cerebrospinal fluid, and the maintenance of a serum concentration of 0.078 to 0.125 unit of penicillin for at least 24 hours before the spinal fluid was tested, did not influence the results.

The more sensitive "inoculation" method described above was used to test for penicillin in 28 spinal fluids obtained from the subjects who had received 25,000 units of penicillin intramuscularly for a 7- or 14-day period before the lumbar puncture. The serum concentrations of penicillin on this regimen range for the most part between 0.078 and 0.156 unit. In 9 of the 28, a "doubtful" result was obtained, i.e., the spinal fluid slowed but did not completely inhibit the growth of the test organism. In no instance was a definitely positive test (i.e., 0.02 unit or more) obtained in this group of subjects nor in the one patient tested who had received 160,000 units by continuous drip for 24 hours. However, when sufficiently high concentrations of penicillin were maintained in the serum of five subjects for periods of 4 hours or longer (Figure 1), the presence of penicillin in the cerebrospinal fluid of all five was demonstrable by this inoculation test. As may be seen in Table III, in only two specimens was the test organism inhibited for more than 24 hours. For the reasons given above, in the remaining three specimens of cerebrospinal fluid the concentration of penicillin was probably just under the 0.02 unit level. It may also be seen in Table IV that when the cerebrospinal fluid was withdrawn at too short an interval after the administration of the penicillin, it exhibited little or no inhibitory effect upon the test organism.

An explanation for this failure to demonstrate more than a minute amount of penicillin in the cerebrospinal fluid after systemic administration might be at hand if it could be shown that penicillin is bound to a high degree to certain nondiffusible constituents of plasma such as albumen. In order to investigate this possibility, observations have been made on the distribution of penicillin between human serum and its dialysate in vitro, and serum and ascitic fluid in vivo.

The details of the methods used were essentially the same as those of Davis (6) and have been described elsewhere (7). Only the sodium salt of penicillin was used. The concentrations of penicillin in the serum and in the dialysates were determined by the same Rammelkamp dilution method (4) used for the cerebrospinal fluid determinations. It should be recognized that the percentage of error in determinations by any dilution method is high and that the data presented here have only rough quantitative value. However, the agreement in the values obtained in duplicate specimens was fairly close.

As may be seen in Table IV, when penicillin sodium was added to normal human serum in vitro, an appreciable amount passed through the membrane into the buffer. When the penicillin was added to the buffer, comparable amounts of material permeated the membrane into the serum (Table II A & B). When the serum of a patient who had received 100,000 units of penicillin by intramuscular injection was dialysed against the buffer, an appreciable amount of the penicillin passed through the membrane into the buffer (Table VI A & B).

For the study of the diffusibility of penicillin through a living membrane in vivo, observations were made on 5 subjects with ascites. Penicillin administration was continued for at least 24 hours before the ascitic fluid was withdrawn to ensure an adequate period for the diffusion of penicillin into the ascitic fluid. The dosage of penicillin was such that a continuous or virtually continuous serum concentration of 0.078 unit or higher should have been maintained. Hypoproteinemia was present in two of the patients, one of whom had a peritoneal infection (pneumococcus peritonitis) as well. In the other patients the serum proteins were within the normal range and no infection was present.

As may be seen in Table V, it was possible in every instance to demonstrate that appreciable amounts of penicillin diffused through the peritoneal membrane. The discrepancy between the values obtained in the serum at the time of the withdrawal of the ascitic fluid, and the values in the ascitic fluid itself, is undoubtedly due to the fact that the height of the serum concentration varies considerably over a 24-hour period, when the penicillin is administered by repeated intramuscular injections.

In another patient with a moderate degree of hypoproteinemia but with considerable ascites (Table V), the cerebrospinal fluid was withdrawn after 24 hours of penicillin therapy. No penicillin was detectable by the Rammelkamp method in this cerebrospinal fluid.

DISCUSSION

From the clinical observations which confirm the previous observations of others (1, 8, 9), it is evident that when a detectable concentration of penicillin is maintained in the serum long enough for equilibrium between blood and cerebrospinal fluid to occur, no penicillin is detectable in the cerebrospinal fluid by the methods used. When the penicillin concentration in the blood is maintained at a level significantly higher than is usually attained in the treatment of patients with meningitis and neurosyphilis, only a small amount, approximately 0.02 Oxford unit, is demonstrable in the cerebrospinal fluid. The presence of syphilitic infection of the central nervous system did not alter this distribution of penicillin between blood and cerebrospinal fluid.

From the dialysis studies, it would appear that a significant fraction of the penicillin present in serum is readily diffusible through membranes in vitro and in vivo.

There are at least three conceivable mechanisms which might explain the failure to demonstrate more than a minute amount of penicillin in the cerebrospinal fluid of these subjects. As mentioned previously, it is possible that the penicillin could be bound to a high degree to certain non-diffusible constituents of serum such as albumen. Another possibility is that the molecular structure of penicillin is such that it would fail to pass through the blood-brain barrier even though it were freely diffusible from the serum. And thirdly, assuming the transport of penicillin across the barrier, it is conceivable that it might be rapidly inactivated by some constituent of nerve tissue or cerebrospinal fluid.

It would seem from these studies, however, that the hypothesis that the failure of penicillin to appear in the cerebrospinal fluid is due to a binding with the nondiffusible constituents of serum is not tenable. The hypothesis that penicillin does pass through the blood-brain barrier but is rapidly inactivated within the nervous system has not been investigated in any detail. However, it can be stated that cerebrospinal fluid does not inactivate penicillin in vitro, and following intrathecal administration it is possible to demonstrate its presence in the cerebrospinal fluid for at least 24 hours (10).

In the absence of more information than is at present available concerning the molecular structure of penicillin, the relation of this structure to the problem of transport across the blood-brain barrier cannot be studied. Certain observations of Shannon (11), however, are of interest in this connection. He has demonstrated that certain sulfanilic acid derivatives, which are diffusible from the plasma to the extent of at least 50 per cent, show a wide variation in the ability to pass the blood-brain barrier. This phenomenon is presumably dependent upon variations in chemical structure. He also points out that the inability to pass the blood-brain barrier "appears to be as true for the capillary plexes in brain substance as for those that are responsible for the formation of cerebrospinal fluid, i.e., if these be different anatomical structures."

As stated above, there is no evidence from this study that the distribution of penicillin between blood and cerebrospinal fluid is affected by the presence of inflammatory changes within the central nervous system. However, the involvement of the central nervous system in the group investigated was of a subacute or chronic nature. In meningitis the distribution of certain substances such as calcium and phosphate between blood and cerebrospinal fluid is so altered that increased amounts of these substances are present in the fluid (5). In some instances this increase in calcium concentration may be explained satisfactorily on the basis of the increased protein content of the cerebrospinal fluid (12). The values for the total proteins in 48 cerebrospinal fluid specimens examined in this study ranged from 21 to 357 mg. per 100 cc. In only six specimens was the value 100 or more mg. per 100 cc. Furthermore, if the protein concentration were the determining factor in the distribution of penicillin, it would be expected that penicillin administered intrathecally into a cerebrospinal fluid of normal protein content would appear promptly in the serum with its much greater content of protein. It was shown by Rammelkann and Keefer (10) that this transfer of penicillin from cerebrospinal fluid to blood occurred to a significant extent only in the presence of meningitis. Fleming (2) and more recently Rosenberg and Sylvester (3) have demonstrated the presence of penicillin in the cerebrospinal fluid following the parenteral administration of the material to subjects with purulent meningitis. The latter authors, using a modification of a turbidometric method of bioassay described by Foster (13), found concentrations ranging from 0.03 to 0.35 unit per cc. within 60 to 140 minutes after the parenteral administration of 20-40,000 units of penicillin. It is impossible to make a direct comparison of these values with the values obtained in this study because of the differences in the methods used for bioassay. Turbidometric methods of assay, however, contain certain sources of error not present in the plate methods and as the authors point out, there was considerable variation among the different subjects. In three of the eight subjects only minute amounts of penicillin were present. The concentrations of penicillin in the blood and the protein content of the cerebrospinal fluids were not reported. These interesting results await further confirmation.

In short, the available evidence would indicate that save for the possible exception of some instances of purulent meningitis, the parenteral administration of penicillin is not followed by the appearance of appreciable amounts of the material in the cerebrospinal fluid.

The question immediately arises, is it an essential for the proper chemotherapy of an infection of the central nervous system that an effective concentration of the chemotherapeutic agent be present in the cerebrospinal fluid? It is generally assumed that in the use of antibiotics in the treatment of infection the distribution of the agent at the site of the infection is the important factor and not the concentration of drug attained in urine, tears, and other secretions and excretions. The primary site of infections of the central nervous system is not the cerebrospinal fluid itself, but the parenchyma and supporting structures of the brain and spinal cord. Is it conceivable that an agent could be present in effective concentration in the tissues of the central nervous system and yet show only traces in the cerebrospinal fluid? It would appear that this could be possible if either the material were fixed or inactivated by the tissue cells, for which there is no supporting evidence, or if the rate of diffusion into the fluid was so much slower than the rate of the production and absorption of the fluid itself, that equilibrium could not be attained. It would seem possible that the passage of the small amounts of penicillin into the cerebrospinal fluid which can be demonstrated is effected not only through the choroid plexus, but also through diffusion into the interstitial fluid of the brain and thence into the fluid of the subarachnoid space. Wallace and Brodie (14) and Shannon (11) believe that the evidence from studies on the distribution of thiocyanates, bromides and iodides with reference to the central nervous system supports the view that the brain is in equilibrium with this interstitial fluid. The possibility that the major part of the penicillin which passes through the blood-brain barrier does so through this interstitial fluid which forms only a fraction of the total cerebrospinal fluid present at any one time is a very real one. It would seem, however, that if anything more than relatively small amounts of penicillin were being transported through this route, equilibrium with the fluid in the subarachnoid space would occur during the continued parenteral administration of penicillin. Furthermore, in Shannon's investigations of the distribution of sulfanilamide and other sulfanilic acid derivatives in the cat, there was a close correlation in general between the concentration of a drug in the cerebrospinal fluid and that present in the brain tissue.

Therefore, although it cannot be assumed that an effective concentration of penicillin is not present in the tissues of the central nervous system following parenteral administration merely because little or no penicillin is detectable in the cerebrospinal fluid, yet it would appear that the height of the concentration so attained must be definitely limited.

The concentration of penicillin which would be most effective at the site of any individual infection is not established as yet. Presumably the height of the concentration necessary varies, depending in part on the species of infecting organism and the histopathology of the lesion. The penicillin-susceptible organisms which commonly infect the central nervous system are the meningococcus, the Group A streptococcus, the pneumococcus, the *Treponema pallidum*, and the *Staphylococcus aureus*. With the exception of the *Staphylococcus aureus*, these organisms despite the individual variations among them, are sensitive to the effects of relatively low concentrations of penicillin. This can be demonstrated by *in vitro* tests for some and the rapid disappearance of others from lesions *in vivo* when small amounts of penicillin are administered. Therefore, it might be anticipated that only small concentrations of penicillin in the central nervous system would be required for the effective therapy of infections produced by these organisms, provided that the pathological changes of the infection were not of such a nature as to interfere with the distribution or action of the penicillin.

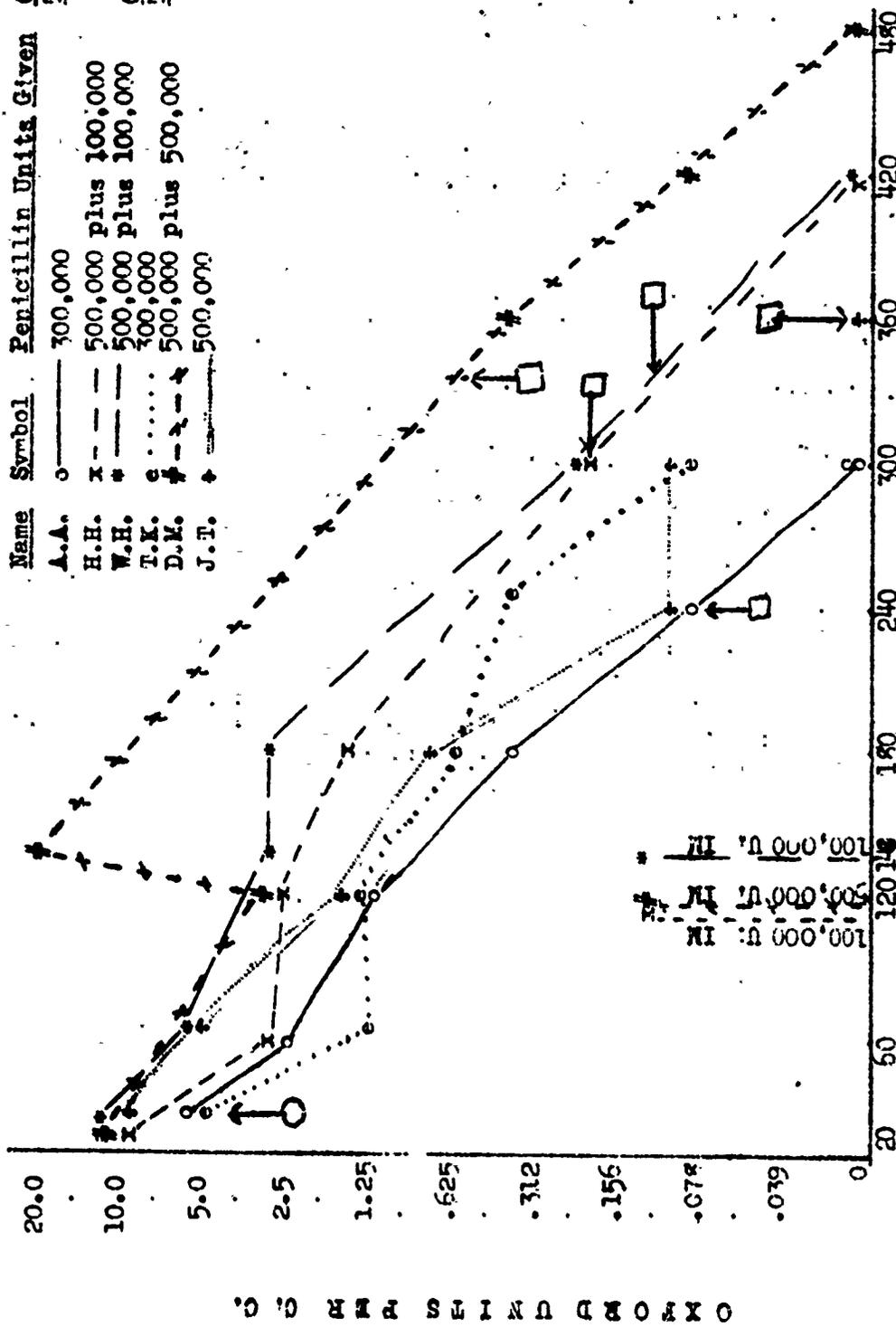
Certain clinical investigations lend support to this concept. It has been shown by Nelson and Duncan (15) in a series of ten patients with acute syphilitic meningitis that excellent therapeutic results were attained by the use of penicillin, which was administered solely by parenteral routes. In the treatment of less acute forms of neurosyphilis, definite clinical improvement and rapid disappearance of abnormalities in the cell count, protein content and colloidal gold test have been observed repeatedly following the administration of penicillin by intramuscular injection (16). Price and Hodges (17) have recently reported in detail the successful treatment of two patients with purulent meningitis by the sole use of penicillin administered systemically. In one patient the infecting organism was a Type III pneumococcus and in the other, a meningococcus. In a footnote the authors mention the successful treatment by this method of two additional patients with meningococcus meningitis. They also report on four other patients with purulent meningitis who received penicillin by the parenteral route only. Two of these patients had received considerable sulfonamide therapy previous to the inauguration of the penicillin and both patients recovered. The other two patients had fulminating infections which were fatal within eight hours of admission to the hospital. Unfortunately, in neither the series of Nelson and Duncan nor that of Price and Hodges was the cerebrospinal fluid tested for the presence of penicillin. However, the improvement in the less acute forms of neurosyphilis mentioned above has occurred without the demonstrable presence of penicillin in the cerebrospinal fluid (18).

Thus it is evident from these clinical studies that the concentration of penicillin which can be attained at the site of the infection in the central nervous system is sufficient to be effective in many patients with neurosyphilis and at least in some patients with purulent meningitis. However, the development of purulent meningitis caused by the pneumococcus has occurred in patients who were already receiving parenteral penicillin therapy for systemic infections due to the organism (19). Also, several patients with pneumococcus meningitis have failed to respond to penicillin therapy until it was administered intrathecally (19). In addition in a few instances, the parenteral administration of penicillin to patients with meningococcus meningitis has not been followed by improvement (20)(21). In one of these cases, the administration of sulfonamide and antimeningococcus serum was followed by a satisfactory outcome (21).

It is conceivable that the divergent results obtained from the parenteral use of penicillin in neurosyphilis and its similar use in purulent meningitis might be due to the different pathologic changes characteristic of the two types of infection. Despite the fact that penicillin is not inactivated by the presence of purulent exudates, it has been a general experience that such collections of pus must be drained surgically or by aspiration before a satisfactory therapeutic result can be attained. There is reason to believe, as discussed above, that the penicillin concentration attainable in the central nervous system following systemic administration can reach only a certain relatively low level. The maximum level of the concentration of penicillin achieved in this manner might be so close to the minimal amount which would be effective against the various infecting organisms that the factor of safety would be small. Thus with a syphilitic infection, even in the presence of active meningitis, as the rate of progress of the infection is relatively slow, the continued presence of small amounts of penicillin is presumably all that is necessary for effective therapy. With a pyogenic infection, on the contrary, although in many instances the concentration attainable by parenteral administration might be sufficient, yet if considerable purulent exudate were present, or if the infection were progressing rapidly, the penicillin concentrations attainable by the sole use of the parenteral routes might be dangerously inadequate.

"INOCULATION TEST" FOR THE PRESENCE OF PENICILLIN IN THE CEREBROSPINAL FLUID AFTER INTRAMUSCULAR INJECTIONS

OF LARGE DOSES OF PENICILLIN



MINUTES AFTER PENICILLIN ADMINISTRATION

Obviously the only way in which the problem can be solved satisfactorily is by the careful study of comparable series of patients with infections of the central nervous system who have received penicillin solely by the parenteral or solely by the intrathecal route. It would seem that no useful purpose would be served by conducting such a study on patients with neurosyphilis as the clinical and cerebrospinal fluid results attained following the administration of penicillin intramuscularly are so promising. In the treatment of purulent meningitis, however, it would seem that on the basis of the available information the administration of penicillin by the intrathecal route cannot be abandoned, particularly in the more severe types of infection.

SUMMARY

1. Using a dilution technique of bioassay, no penicillin was demonstrable in the cerebrospinal fluids obtained from 55 patients who had received penicillin in various dosages by parenteral routes. The presence of neurosyphilis did not alter these results.

2. Approximately 0.02 unit of penicillin was demonstrable in the cerebrospinal fluids of patients who had received one or two intramuscular injections of 300 - 500,000 units of penicillin three to four hours previously.

3. At concentrations ranging from 0.078 to 1.25 unit of penicillin per cc. of serum, penicillin is diffusible through artificial membranes in vitro and into ascitic fluid in vivo. Thus the failure of the penicillin to appear in the cerebrospinal fluid is not because it is bound, to a high degree, to nondiffusible elements in the serum.

4. The interpretation of these findings with respect to the penicillin treatment of neurosyphilis and purulent meningitis is discussed. It is suggested that as the immediate results from the intramuscular penicillin treatment of syphilitic meningitis and other forms of neurosyphilis are so promising, it is unnecessary to use the intrathecal route for the treatment of these conditions. In contrast, in the treatment of purulent meningitis, particularly with the patients who are critically ill, it would seem that the administration of penicillin by the intrathecal route cannot be abandoned.

TABLE I.

PENICILLIN DETERMINATION OF C. S. F. AFTER SINGLE INTRAMUSCULAR DOSE

Number of Subjects	Single Dose units	Serum Concentration	Minutes before Withdrawal of C. S. F.	Penicillin Determination	
				Rammelkamp	Inoculation
1	25,000	.625	30	0	0
1	25,000	.078	90	0	0
1	50,000	.625	30	0	0
2	100,000	1.25	30	0	0
		1.25	30	0	0
1	100,000	.625	120	0	0
1	160,000	.625	100	0	0
2	200,000	.625	120	0	0
		.312	120	0	doubtful
1	200,000	.078	220	0	
1	500,000	5.0	20	0	0
1	300,000	.078	240	0	positive
1	500,000	.078	360	0	positive

TABLE II
PENICILLIN DETERMINATIONS OF SPINAL FLUIDS OBTAINED FOLLOWING PROLONGED,
FREQUENTLY REPEATED, INTRAMUSCULAR ADMINISTRATION OF PENICILLIN

Number of Subjects	Therapy	Route	Serum concentration (estimated)		C. B. F. Rammelkamp dilution
			Height	Duration	
32	25,000 u. q.2 hrs.	I.M.	.078-.156	7-14 days	No Detectable Penicillin
	"	"	"	"	

TABLE III

"INOCULATION TEST" FOR THE PRESENCE OF PENICILLIN IN THE CEREBROSPINAL FLUID
AFTER INTRAMUSCULAR INJECTIONS OF LARGE DOSES OF PENICILLIN

Units I.M.	Peak of serum concentration	Minutes above .156 u. before L.P.	Minutes at or above .078 μ . before L.P.	Results: (positive = approximately .02 units or more)
25,000	.625	60	30	negative
	.625	60	90	negative
50,000	.625	60	30	negative
100,000	1.25	90	100	negative
160,000			60	doubtful
			160	negative
200,000	5.0	180	115	doubtful
300,000	10.0	240	20	negative
	10.0	240	240	positive (18 hrs.)
500,000	10.0	240	360	positive (48 hrs.)
500,000 plus 100,000 in 2 hrs.	10.0	300	300	positive (24 hrs.)
	10.0	300	335	positive (26 hrs.)
500,000 plus 500,000 in 2 hrs.	20.0	330	330	positive (48 hrs.)

TABLE IV.

PENICILLIN DIALYSIS IN VITRO

		Serum penicillin level before dialysis	Serum penicillin level after dialysis	Buffer penicillin level after dialysis	Period of dialysis	pH of Buffer
(A and B = duplicates) u/cc=Oxford units per cc)	I	A 8.6 u/cc	0.312 u/cc	0.156 u/cc	68 hrs.	7.4
		B 8.6 u/cc	0.312 u/cc	0.156 u/cc	68 hrs.	7.4
	II	A 0	0.312 u/cc	0.078 u/cc	68 hrs.	7.4
		B 0	0.312 u/cc	0.078 u/cc	68 hrs.	7.4
	III	A 8.6 u/cc	0.312 u/cc	0.156 u/cc	68 hrs.	7.2
		B 8.6 u/cc	0.312 u/cc	0.156 u/cc	68 hrs.	7.2
Penicillin added to buffer to give concentration 8.6 u/cc	IV	A 8.6 u/cc	0.312 u/cc	0.156 u/cc	68 hrs.	7.4
		B 8.6 u/cc	0.312 u/cc	0.312 u/cc	68 hrs.	7.2
	V	A 1.25 u/cc	0.625 u/cc	0.156 u/cc	24 hrs.	7.3
		B 1.25 u/cc	0.625 u/cc	0.312 u/cc	24 hrs.	7.3
Patient given 100,000 U. penicillin I.M. Blood withdrawn 10 minutes later	VI	A 1.25 u/cc	0.625 u/cc	0.312 u/cc	24 hrs.	7.3
		B 1.25 u/cc	0.312 u/cc	0.312 u/cc	24 hrs.	7.3

TABLE V

PENICILLIN DIALYSIS IN VIVO

Subj. Kg.	Diagnosis	Pen.therapy before Abdominal Paracentesis	Dosage Inj. of Pen.	Interval from last Concentration in serum	Penicillin Concentration in serum	Pen. Conc. in Ascitic fluid	Serum Protein Total Alb. Glob.	Fluor Tbt. Prot
1	15.3 nephrotic syndrome Pneumo- coccus Type A perito- nitis	51 hours	2,500 U. c. 2 hrs. I.M.	30 mins.	.625 u/cc	1.24 u/cc	4.9	
1	15.1 "	48 hours	1,500 U. c. 3 hrs. I.M.	2 1/2 hours	.625 u/cc	.312 u/cc	5.5	1.5 4.0 .13
1	20.5 nephrotic syndrome fib peri- tonial in- fection	96 hours	1,000 U. c. 3 hrs. I.M.	3 hours	.075 u/cc	.312 u/cc	5.5	0.14 5.36 0.1
2	60 Laennec's Hepatic Cirrhosis	22 hours	25,000 U. c. 2 hrs. I.M.	20 mins.	.625 u/cc	.312 u/cc	4.5	2.3 2.2 1.0
3	50.2 Valvular Heart Dis. Rheumatic Congestive Failure	15 hours	25,000 U. c. 2 hrs. I.M.	3 hours	.156 u/cc	.312 u/cc	5.4	3.4 2.0
4	70.6 Laennec's 58.1 Hepatic Cirrhosis	18 hours	15,000 U. c. 2 hrs. I.M.	4 1/2 hours	0	.156 u/cc	2.5	
5	87 "	22 hours	25,000 U. c. 2 hrs. I.M.	2 hours	.156 u/cc	.156 u/cc	7.7	2.2 5.5 .6

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V. The Effect of Penicillin upon the Clotting of Blood In Vitro.

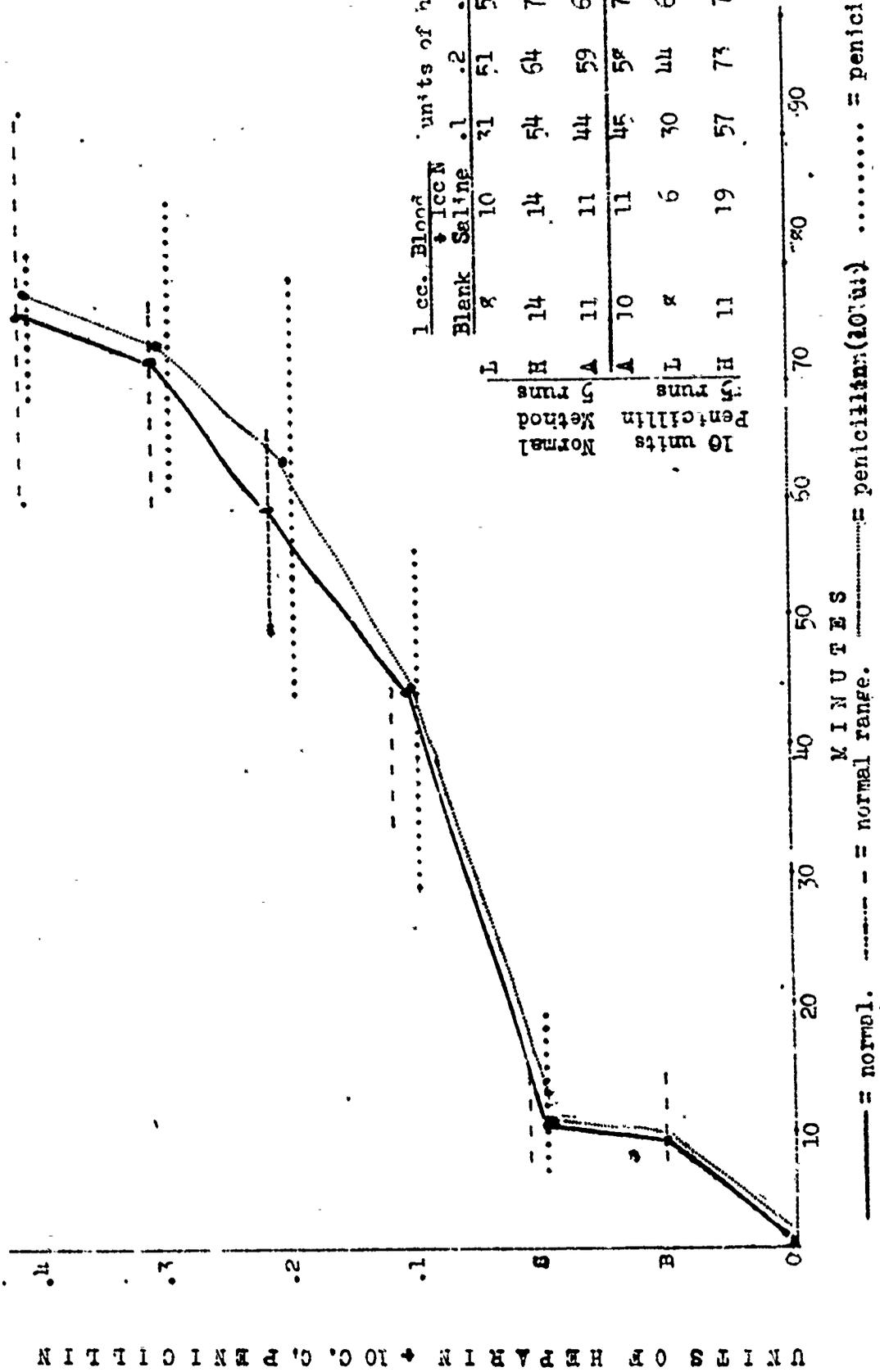
Greaves (1) has observed that the addition of sodium penicillin to fibrinogen in distilled water is followed by the formation of a jellylike clot. He suggested that this observation might provide an explanation for the frequent occurrence of venous thromboses which follow the intravenous administration of penicillin. The development of phlebitis both at the site of, and remote from, the injection of penicillin has occurred not infrequently in clinical practice. However, it is not established that such phlebitis is actually any more common following penicillin than it is after therapy with other agents which are administered by continuous intravenous drip to patients who are in bed with acute infections. However, because in certain of the studies of the pharmacology of penicillin presented above, such high (10 to 20 units/cc.) concentrations of penicillin were present in the blood, and because of the importance of the question in relation to the chemotherapy of bacterial endocarditis, it was felt advisable to study the effects of such penicillin concentrations upon the clotting of blood in vitro.

The technique used for the determination of the clotting time was that of Waugh and Ruddick (2), with certain minor modifications. With this method, the normal clotting of the blood is prolonged by the use of measured amounts of heparin. It is thus possible to measure a decrease in the clotting time as well as the increased clotting time measurable by the other techniques.

A composite curve of five experiments is shown in Figure 1. In this graph may be seen the time of clotting of a series of duplicate specimens of blood. To one specimen of each pair of duplicates, sufficient sodium penicillin was added to produce a final concentration of 10.0 units/cc.

As may be seen in the figure, there was no demonstrable difference in the speed of clotting of the blood specimens whether or not the penicillin was present.

FIGURE I
AVERAGE (+ RANGE) CURVE OF CLOTTING TIME OF BLOOD + 10 UNITS OF PENICILLIN (AS COMPARED WITH NORMAL)



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COMMITTEE ON MEDICAL RESEARCH
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OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT
FEDERAL DISEASES REPORT No. 26 (Abstracted)

Abstract of Interim Report
10 January 1945

TIME-DOSE RELATIONSHIPS OF PENICILLIN THERAPY. Parts I - IV:
Walsh McDermott, Maria Benoit, and Rebeckah DuBois. Part V:
Walsh McDermott, Helen Steinberg, and Willetta Haynes.

- I. The concentration and duration of penicillin levels in the serum following the use of various dosages, routes, and vehicles of administration.
- II. The relation of the concentration and duration of penicillin effect to the inhibition of bacterial populations in vitro.
- III. Regimens used in early syphilis.
- IV. The relation of the concentration and duration of penicillin in the serum to the concentration in spinal fluid and dialysates.
- V. The effect of penicillin upon the clotting of blood in vitro.

These studies have been conducted in an effort to obtain information about the proper time-dose relationships of the penicillin therapy of the subacute infections such as bacterial endocarditis or syphilis. First, the height and duration of the serum concentrations of penicillin which could be attained after the administration of large doses of material in the aqueous or beeswax vehicle were determined. With the aqueous vehicle, extremely high levels of penicillin are attained for the first few hours of a 6 or 7 hour period. On the contrary, when penicillin is administered in beeswax, no such peak concentrations are usually attained because of the slower absorption. This raises the question of whether the poorly sustained but high peaks of penicillin concentration are of therapeutic importance or merely represent penicillin which is in excess of that utilizable for the destruction of organisms. This question of a possible "flash action" of penicillin is one facet of the problem of whether high level therapy is superior to prolonged treatment at the minimally effective level.

With the use of penicillinase, the effects per unit of time of penicillin concentrations comparable to those obtained in vivo, upon cultures of Group A streptococci in vitro, were studied.

From these studies and clinical experience, support is obtained for the thesis that once the minimal effective concentration of penicillin is attained, remission will occur; that the presence of much greater concentrations per unit of time serves no useful purpose; and that only by maintenance of the minimal

The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

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effective level for a period much longer than that necessary to produce the original remission can relapse be prevented.

In Part III, an analysis was made of the penicillin regimens which have been used in the treatment of early syphilis on the basis of the available information concerning the penicillin level necessary to inhibit the *Treponema pallidum*, and with the assumption that the duration of treatment at this level is the most important determining factor of cure. Also, with this assumption (that the total period of time during which an 0.078 unit/cc. serum level of penicillin is maintained is an index of the efficacy of a given system) a number of other regimens for the penicillin treatment of early syphilis have been calculated.

Results in Summary:

I. From these preliminary studies with the beeswax vehicle, it would seem that by this method it is possible to maintain significant levels of penicillin action in the serum for periods longer than 12 hours after a single dose, provided that sufficient penicillin is used. The questions of the irregularity of absorption and the tolerance of the patient to repeated doses of penicillin in this vehicle are yet to be studied.

Aside from the fact that the aqueous method requires the use of much more penicillin to prolong levels for 6 or 7 hours, there is a possibly important difference between the two methods of administration. With the aqueous vehicle, extremely high levels of penicillin are attained for the first few hours of the 7-hour period. On the contrary, when penicillin is administered in beeswax, no such peak concentrations above the 0.078 - 0.512 u/cc level are usually attained because of the slower absorption. The possible implications of this difference will be discussed subsequently.

II. In short, the results of these experiments in vitro would indicate, and certain clinical experience support, the thesis that once the minimal effective concentration of penicillin is attained, remission will occur; that the presence of much greater concentrations per unit of time serves no useful purpose; and that only by maintenance of the minimal effective level for a period much longer than that necessary to produce the original remission can relapse be prevented. It would seem possible, because of the prolongation of the quiescent phase in previously treated organisms observed in these studies in vitro, that this prolonged maintenance of an effective level need not be absolutely continuous.

Obviously, the absolute values of the height of the minimal effective level (or "effective level", as it will be hereinafter designated) and the time at that level necessary for cure will vary with the different species of organisms and with the histopathology of the particular infection. The factor of the histopathology of the infection is probably of much more importance in the treatment of the chronic infections such as bacterial endocarditis and neurosyphilis than it is in the therapy of infections syphilis.

III. 1. Studies of penicillin action on streptococci in vitro show that 24 or more hours of penicillin at a certain concentration are necessary for the complete inhibition of a culture, even though 95 per cent of the organisms are destroyed within the first five hours. The time required for the complete inhibition of the culture cannot be shortened by increasing the concentration of penicillin above the minimal effective level.

2. Certain clinical observations of penicillin action in a variety of infections, including syphilis, suggest that here also only short periods of penicillin action are necessary for the immediate destruction of a large number of the organisms, but prolonged penicillin action is necessary for cure. In addition, there is some evidence that in vivo, as in vitro, large increases in the concentration of penicillin for short periods do not shorten this period of time necessary for cure.

3. It has been shown that by the use of sufficiently large quantities of aqueous penicillin in a single injection, it is possible to prolong the period of detectable serum concentrations for 6 or 7 hours with the production of an initial high concentration. If sufficient penicillin in beeswax is administered in a single injection, these initial high concentrations are not obtained, but the period of a detectable serum concentration can be prolonged for 9 to 12 or more hours.

4. As both the studies in vitro and clinical experience lend support to the thesis that it is not the production of multiple peaks of high concentrations of penicillin but the length of the action at low concentrations which is the important factor, it is suggested that this penicillin action time be given consideration in the planning of new regimens for the treatment of syphilis and the other sub-acute infections. In calculations involving the use of penicillin in beeswax and other inhibitors of absorption, this time of action of the penicillin is the only available index for comparison with the other systems.

5. There is a possibility, as indicated by the in vitro experiments, that once the initial effect of the penicillin action has occurred, subsequent action need not be absolutely continuous to obtain the maximum effect.

6. Analysis of the regimens under investigation for the treatment of early syphilis reveals that in any given system, irrespective of the total amount of penicillin or the number of days of treatment, there is a wide variation in the total period of time during which detectable concentrations of penicillin are present in the serum, depending upon the timing and size of the individual doses.

7. Using a serum concentration of 0.078 unit of penicillin as an arbitrary standard, a number of regimens for the treatment of early syphilis have been calculated which would produce this concentration for total periods of 30, 120, and 180 hours by a minimal of intramuscular injections.

In Part IV are presented the results of a study of the relation of the concentration and duration of penicillin in the serum to the concentration in cerebrospinal fluid and dialysates.

Results in Summary:

IV. 1. Using a dilution technique of bioassay, no penicillin was demonstrable in the cerebrospinal fluids obtained from 55 patients who had received penicillin in various dosages by parenteral routes. The presence of neurosyphilis did not alter these results.

2. Approximately 0.02 unit of penicillin was demonstrable in the cerebrospinal fluids of patients who had received one or two intramuscular injections of 500-500,000 units of penicillin three to four hours previously.

3. At concentrations ranging from 0.078 to 1.25 unit of penicillin per cc. of serum, approximately one-third of the penicillin is freely diffusible through artificial membranes in vitro and into ascitic fluid in vivo. Thus the failure

of the penicillin to appear in the cerebrospinal fluid is not because it is bound, to a high degree, to nondiffusible elements in the serum.

4. The interpretation of these findings with respect to the penicillin treatment of neurosyphilis and purulent meningitis is discussed. It is suggested that as the immediate results from the intramuscular penicillin treatment of syphilitic meningitis and other forms of neurosyphilis are so promising, it is unnecessary to use the intrathecal route for the treatment of these conditions. In contrast, in the treatment of purulent meningitis, particularly with the patients who are critically ill, it would seem that the administration of penicillin by the intrathecal route cannot be abandoned.

In Part V, the failure of a high concentration (10 units per cc.) of penicillin to affect the clotting of blood in vitro is reported.

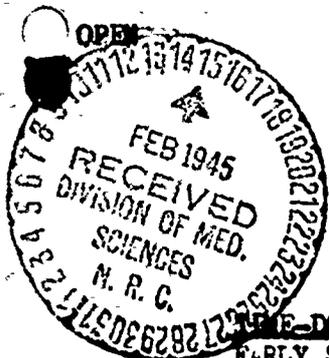
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COMMITTEE ON MEDICAL RESEARCH
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VENEREAL DISEASES REPORT No. 27 (Abstracted)
Abstract of MS for Publication
25 January 1945



TIME-DOSE RELATIONSHIPS OF PENICILLIN THERAPY. III. REGIMENS USED IN
EARLY SYPHILIS. Walsh McDermott, Maria Benoit, and Rebeckah DuBois.

1. Observations of the action of penicillin on infectious syphilis suggest that only short periods of penicillin action are necessary for the immediate destruction of a large number of the organisms, but prolonged penicillin action is necessary for cure. In addition, there is some evidence that in vivo, as with bacterial cultures in vitro, large increases in the concentration of penicillin for short periods do not shorten this period of time necessary for cure.
2. Analysis of the regimens under investigation for the treatment of early syphilis reveals that in any given system, irrespective of the total amount of penicillin or the number of days of treatment, there is a wide variation in the total period of time during which detectable concentrations of penicillin are present in the serum, depending upon the timing and size of the individual doses.
3. As both studies in vitro and clinical experience lend support to the thesis that it is not the production of multiple peaks of high concentrations of penicillin but the length of the action at low concentrations which is the important factor, it is suggested that this penicillin action-time be given consideration in the planning of new regimens for the treatment of syphilis. In calculations involving the use of penicillin in beeswax and other inhibitors of absorption, this time of action of the penicillin is the only available index for comparison with the other systems.
4. There is a possibility, as indicated by in vitro experiments, that once the initial effect of the penicillin action has occurred, subsequent action need not be absolutely continuous to obtain the maximum effect.
5. Using a serum concentration of 0.078 unit of penicillin as an arbitrary standard, a number of regimens have been calculated which would produce this concentration for total periods of 90, 120, and 160 hours by a minimum of intramuscular injections.

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The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

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COMMITTEE ON MEDICAL RESEARCH
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VENEREAL DISEASES REPORT No. 28 (Abstracted)
Abstract of MS for Publication
19 January 1945

THE TRANSFER OF PENICILLIN INTO THE CEREBROSPINAL FLUID FOLLOWING PARENTERAL ADMINISTRATION. Walsh McDermott, Harry Eagle, and Russell A. Nelson.

This paper presents the results of a study of the relation of the concentration and duration of penicillin in the serum to the concentration in cerebrospinal fluid and dialysates.

Results in Summary:

1. Using a dilution technique of bioassay, no penicillin was demonstrable in the cerebrospinal fluids obtained from 55 patients who had received penicillin in various dosages by parenteral routes. The presence of neurosyphilis did not alter these results.
2. Approximately 0.02 unit of penicillin was demonstrable in the cerebrospinal fluids of patients who had received one or two intramuscular injections of 300-500,000 units of penicillin three to four hours previously.
3. At concentrations ranging from 0.078 to 1.25 unit of penicillin per cc. of serum, approximately one-third of the penicillin is freely diffusible through artificial membranes in vitro and into ascitic fluid in vivo. Thus the failure of the penicillin to appear in the cerebrospinal fluid is not because it is bound, to a high degree, to nondiffusible elements in the serum.
4. The interpretation of these findings with respect to the penicillin treatment of neurosyphilis and purulent meningitis is discussed. It is suggested that as the immediate results from the intramuscular penicillin treatment of syphilitic meningitis and other forms of neurosyphilis are so promising, it is unnecessary to use the intrathecal route for the treatment of these conditions. In contrast, in the treatment of purulent meningitis, particularly with the patients who are critically ill, it would seem that the administration of penicillin by the intrathecal route cannot be abandoned.

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COMMITTEE ON MEDICAL RESEARCH
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VENERICAL DISEASES REPORT #29 (ABSTRACTED)
Abstract of Interim Report
22 March 1945

TREATMENT OF EARLY SYPHILIS WITH PENICILLIN.
L. W. Shaffer, Detroit Department of Health.

(1)—A total of 267 patients with primary or secondary syphilis have been treated with penicillin from 3 April 1944 to 15 March 1945 through the Social Hygiene Clinic of the Detroit Department of Health. The cases were approximately evenly divided between males and females--70% colored and 30% white.

(2)—52 patients treated from 2 April 1944 to 27 June 1944; received 30 intramuscular injections of penicillin, 10,000 units each at 3 hr. intervals (300,000 units in 3-3/4 days). 17 of these cases have not been seen for 3 or more months and are classified as lost. In the 35 cases remaining under observation results to date may be classified as follows: Primary syphilis - good results in 82%, questionable results in 9% and failures in 9%. Secondary syphilis - good results in 58.5%, questionable results in 12.5% and failures in 29%.

(3)—83 patients treated from 9 July 1944 to 13 November 1944, receiving 15 injections 80,000 units penicillin at 6 hr. intervals (1,200,000 in 3-3/4 days). 21 of these cases have been lost. Of the 62 cases still under observation, 25 were primary and 37 secondary syphilis. Good results to date were secured in 96% of the primary cases, questionable results in 4% and failures in none. In the secondary cases good results were secured in 67.6%, questionable results in 21.6% and failures in 10.8%.

(4)—Early syphilis complicated pregnancy in 7 cases. Such patients received a total of 1,200,000 units penicillin. 1 case is lost. The other 6 cases have been delivered. Two babies have been negative to follow-up to date; 4 babies were seropositive at birth and for a short time thereafter but have progressed to seronegativity.

(5)—107 patients have been treated since 13 November 1944 with 1,200,000 or 2,400,000 units penicillin (20,000 units q. 3 hrs. x 60 or 120). Since there has not been sufficient length of observation to warrant an appraisal of results in this group they will be appraised in a future report.

(6)—Numerous cases arise that call for a differentiation between infection and superinfection and clinical relapse. This is impossible to do with accuracy in many cases. The mediate susceptibility of adequately treated cases of early syphilis receiving penicillin to reinfection or superinfection makes the investigation and control of all contacts of great importance.

The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

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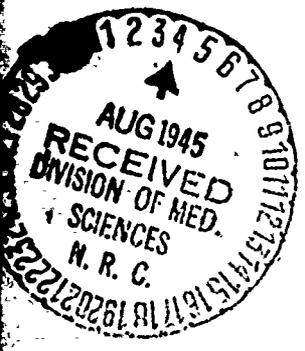
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COMMITTEE ON MEDICAL RESEARCH
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RS/D49-14

VENEREAL DISEASES REPORT No. 30 (Abstracted)
Abstract of Final Report
June 15, 1945

EVALUATION OF CHEMICAL PROPHYLACTICS AGAINST
GNOCOCCUS. C. Phillip Miller



The results of the investigation are presented under three headings:

1. In Vitro Tests of Gonococidal Action: For a preliminary estimation of their relative prophylactic value, a number of chemical substances were tested for their gonococidal activity by the following method: Large numbers of live gonococci were suspended in broth containing varying concentrations of the germicide for 2 hours at 37°, removed by centrifugation and cultured on fresh rabbit blood agar containing 0.065 per cent cystine to determine the number of viable survivors. This method was designed to test the ability of the germicide to kill all of the members of a large bacterial population.

Most of the substances tested were derivatives of phenylarsineoxide submitted by Dr. Harry Eagle of the U.S. Public Health Service. The most potent was found to be compound "S", a proprietary of unknown composition. It was followed by the unsubstituted phenylarsineoxide which was more active than any of its derivatives. Listed in the order of diminishing potency, as determined by this method, the compounds arranged themselves in the following order:

"S"	unknown (proprietary)	7a	4	CONHC ₂ H ₄ OH	indistinguishable
1	unsubstituted phenylarsineoxide	140		unknown	as to potency
	protargol 2%				
2Aa	4 NHCONH ₂				
"PD"	unknown (proprietary)	56	3	NHCOCH ₃	"
D5A		136	3	NHCONH-4	"
12	4 SO ₂ NH ₂				
85	4 OCH ₂ CONH ₂				
32	4 NHCOCH ₃	115B	4	SO ₂ NHC ₂ H ₄ OH	
38B	3 NH ₂ -4-CONH ₂				
		A5	4	NHCOCH ₂ NH ₂	"
myrol 5%	indistinguishable	D66	4	NH(CH ₂) ₂ CONH ₂	"
3Aa	4 CONH ₂				as to potency
144	4 CONHCH ₂ CN				
		92	4	CONHCH ₂ CH ₂ CONH ₂	"
		37a	3	NH ₂ -4-CONH	"
4	3 CONH ₂	90B	4	CONHCH ₂ CONH ₂	

The complete report, filed in the offices of the Committee on Medical Research and the Division of Medical Sciences, National Research Council, is available to authorized persons either on short loan or for examination at 2101 Constitution Avenue, Washington 25, D. C.

Rabbits' serum in concentrations of 10 per cent or mucin in concentrations of 0.4-1 per cent reduced the gonococidal activity of most of the active germicides sufficiently to require a 4-fold increase in the concentration in the chemical to compensate for it.

2. In Vivo Experiments: In an effort to provide a method for testing prophylactic agents in vivo, attempts were made to develop a localized gonococcal infection in an easily accessible site in an available laboratory animal. After numerous preliminary trials, attention was concentrated on the eye of the rabbit and led to a number of procedures among which the following deserve mention: Two Methods for producing gonococcal conjunctivitis in the rabbit which were fairly successful: (a) implantation of gonococcus as a secondary invader on the conjunctiva of rabbits suffering from infectious myxomatosis and (b) inoculation of the conjunctiva and closing and sealing the lids. Both of these were abandoned in favor of (c) inoculation of the anterior chamber.

Experimental gonococcal infection of the anterior chamber was produced by the injection of gonococci directly into the anterior chamber of a rabbit prepared by suitable anesthesia. The gonococci were found to invade the tissues of the eye and to produce a severe inflammatory reaction. In approximately 1/3 of the animals, the infection became chronic and persisted for as long as 14 weeks, the maximum period of observation.

The proportion of successful inoculations, as determined by culture after 24 hours, depended on the number of gonococci injected.

Ninety-three per cent of "takes" followed injection of approximately 20,000,000 gonococci, the standard inocula for the tests on the prophylactic agents. The percentage fell with diminishing numbers of gonococci to 45 per cent with inocula of approximately 200 gonococci.

Serological studies on a series of these rabbits showed that most of them developed positive complement-fixation reactions sooner or later, indicating an immune response to this localized infection. The agglutination reaction was slower and less certain in its development. Preliminary examination of histological material indicate a pathological picture of panophthalmitis.

The prophylactic action of a variety of chemical agents, solutions and ointments, was tested on this experimental infection by injecting the prophylactic agent into the eye 1 to 2 hours after inoculation. 24 hours later the eye was enucleated and dissected. Cultures were made of the aqueous humor, the lens and macerated ciliary body on rabbit blood agar containing 0.065 per cent cystine.

The results are summarized in Table 2 which presents the findings on most of the ointments tested. The results with aqueous solution of argyrol and protargol are added for purposes of comparison. All of the ointments containing 15 per cent sulfathiazole and 30 per cent calomel made up in aqueous or "vanishing cream" bases were highly effective in preventing infection; whereas, the same ingredients made up in an oily base failed in 70 per cent of the tests. In the aqueous base either 15 per cent sulfathiazole or 30 per cent calomel alone was effective in the amounts used in these experiments (0.05 to 0.1 ml.). Other experiments with smaller quantities of

TABLE 2

PROPHYLACTIC ACTION OF VARIOUS AGENTS AGAINST EXPERIMENTAL GONOCOCCAL INFECTION IN THE RABBIT'S EYE

Code No.	Ingredients		Base	No. In-oculated	Eyes Yielding Positive Cultures	
					No.	% positive (i.e. failure of prophylaxis)
SC-2004	<u>sulfathiazole</u>	<u>calomel</u>				
	15%	30%	oily s	24	17	70%
	15	---	oily b a	6	5	63
Base No. 1	---	30	oily a m	6	5	83
	---	---	oily s e			
SC-2020	15	30	watery s	53	0	0
S-2059	15	---	watery b a	26	0	0
C-2036	---	30	watery a m	28	1	4
SC-2030	15 (merazine)	30	watery s e	32	1	3
SC-2023	15 (iazine)	30	watery e	23	3	10
Base No. 2	---	---	watery			
SC-1005	15	30	watery	34	1	3
SC-3003	15	30	watery	22	1	5
SC-AL-C	15	30	watery	32	2	8
SC-2039	5	10	watery	6	0	0
SC-2040	5	10	watery	6	1	12
SC-2041	5	10	watery	6	1	12
	<u>phenylarsineoxide</u>					
AS-2048	0.1% (p-CO ₂ NH ₂)		oily	16	13	81
AS-2049	" "		watery	16	5	31
AS-2052	0.1% (p-SO ₂ NH ₂)		oily	16	15	93
AS-2053	" "		watery	15	8	53
Base No. 1 alone (control)			oily	2	2	100
Base No. 2 alone (control)			watery	29	20	69
Base N-40 alone (control)			oily	22	22	100
	Argyrol 5%		aq. sol.	15	9	60
	Argyrol 10%		aq. sol.	14	7	50
	Protargol 2%		aq. sol.	15	8	53
	Protargol 5%		aq. sol.	13	10	76
Untreated Controls				249	237	95

the prophylactic agent showed that sulfathiazole or calomel alone were less effective than the two together. The substitution of sulfadiazine or sulfamerazine for sulfathiazole seemed to reduce its effectiveness slightly. The phenylarsinoxido compounds were decidedly less effective than sulfathiazole or calomel. Argyrol and protargol which were included for comparison were found to be poor gonococidal agents in vivo.

The superiority of ointments made up in an aqueous base as compared with ointments containing the same ingredients in an oily base is believed to be due to the ability of the former to spread and "wet" the surfaces of the structures lining the anterior chamber. A striking difference was observed in the behavior of the two ointments after introduction into the anterior chamber. Those of the vanishing cream type could be easily dispersed by a little gentle massage and made to spread over the structures bounding the anterior chamber. The greasy ointments, on the other hand, could not be spread successfully.

Conclusion: Ointments containing 15 per cent sulfathiazole and/or 30 per cent calomel made up in aqueous base are highly effective in the prevention of the experimental gonococcal infection of the rabbit's eye. Similar ointments made up in oily bases are much less effective.

Penicillin introduced directly into the anterior chamber was found to be highly effective in sterilizing that region of gonococci. Approximately 2.5 units sufficed in all of 7 eyes on which it was tried, and 1.0 unit was successful in 19 of 22. When injected beneath the conjunctiva, the infection was brought under control if large amounts were used, 11 or more injections of 10,000 units each at hourly intervals.

When administered by intramuscular or intravenous injection, very large quantities of penicillin were required to sterilize the anterior chamber of gonococci. Eleven to thirteen doses of 10,000 units each were successful in only 2/3 of the rabbits. When intramuscular administration was supplemented by treatment designed to assist the penetration of penicillin into the anterior chamber (repeated aspiration of aqueous humor, administration of miotics and of dionin) smaller doses of intramuscular penicillin sufficed to sterilize the eye.

3. The Development by Gonococci of Resistance to Penicillin

A. In Vitro Experiments: Since gonococci grow more readily on solid than in liquid media, blood agar plates containing known concentrations of penicillin were used. Plates containing several concentrations were inoculated with gonococci and incubated overnight. Every day or two, transfers were made to media containing higher concentrations of penicillin. One strain, after two months, was able to grow on agar containing 6.2 units of penicillin per ml.

Recent experiments indicate that penicillin fastness can be acquired more rapidly if cultivation on ordinary agar is interspersed between culture on penicillin containing agar instead of keeping the strain continuously on the latter.

B. In Vivo Experiments: Preliminary observations which were interrupted by the termination of the contract suggest that resistance to penicillin was beginning to develop in a strain of gonococci which was being passed, (1) through mice by intraperitoneal inoculation in sucin and (2) through rabbits by inoculation of the anterior chamber.

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COMMITTEE ON MEDICAL RESEARCH
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OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT
VENEREAL DISEASES REPORT No. 31
Abstract of MS for Publication
19 September 1945

THE TREATMENT OF EARLY SYPHILIS WITH PENICILLIN, Arthur G. Schoch, M.D.,
and Lee J. Alexander, M.D.

In a series of over 900 patients with early syphilis, who were treated with sodium penicillin at the Dallas Syphilis and Venereal Disease Clinic, several systems of treatment have been investigated.

Observations indicate that total sodium penicillin doses of less than 1.2 million units are inadequate, but 2.4 million units are probably adequate, particularly if five injections of bismuth salicylate, administered on alternate days, are added to the penicillin schedule. The intramuscular injection interval of 3 hours, and a treatment period of 7½ days proved the most effective. The number of reinfections observed were relatively high, which logically leads to the recommendation that intimate contacts be treated simultaneously with the patient.

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