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The Use of penicillin in treating nonpyogenic infections, particularly syphilis

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THE USE OF PENICILLIN IN TREATING NON-PYOGENIC INFECTIONS, PARTICULARLY SYPHILIS

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FOREWORD

Knowledge about penicillin increases on a logarithmic scale as time advances on an arithmetical scale. This paper is an attempt to present a few aspects of penicillin therapy as far as known to the first week in April, 1945. Since that time so many articles on the same subjects as this thesis have appeared that another thesis of equal size could probably be written.

Consequently, although I regret the imperfections and incompleteness of this thesis, I feel that I was fortunate in writing it at a time when all the knowledge about one or two branches of penicillin therapy could still be presented with reasonably completeness.

To my advisor, Dr. A. Ross McIntyre, for his helpful advice and for reading the manuscript; to the entire library staff, who repeatedly uncovered material which I might otherwise have missed; and to Mrs. Paul Bell, who typed the final draft, I wish to acknowledge my indebtedness and express my thanks.

April 27, 1945.

INTRODUCTION

Less than two decades ago Charles Thom (136), a world authority on molds, wrote a book on the Penicillia, in which he indicted them as follows:

"The molds of the genus Penicillium share with the Aspergilli and the Mucors a noisome pre-eminence as weeds. They rot our fruit, attack our vegetables and meats, injure our stored grain, spoil our soft drinks and our bottled water, contaminate our pantries and kitchens and even attack our bodies. They infect and at times destroy the usefulness of solutions and moist precipitates, discolor fibers, wood, paper stock, stored paper and sometimes our books. In the laboratory they infest and often invalidate every kind of culture operation, bacteriological, mycological, or phanerogamic. To offset these activities, chemists have gathered a little return by using them in biochemical investigations and the cheese industry has capitalized on their enzymic activity to ripen such cheeses as Camembert and Roquefort. Otherwise their possibilities of usefulness remain mostly unknown, but their presence is thrust upon us so frequently that some means of identifying them is very desirable.."

It was from one of the members of this obnoxious family that Dr. Alexander Fleming (47) a few years later, in 1928, was to extract the wonder drug Penicillin, which

possibly will be the greatest single medical discovery of the century.

HISTORY OF PENICILLIN

Penicillin was discovered partly by accident. Dr. Alexander Fleming (47) had been interested for many years in the destruction of bacteria by leucocytes. In 1922 (54) he described lysozyme, a powerful antibacterial ferment occurring naturally in human tissues and secretions, especially tears, and in egg white and elsewhere. In 1928 in the laboratories of St. Mary's Hospital in London, while working with staphlococcus variants, he was using a number of culture plates which were set aside on the laboratory bench and examined from time to time. These plates inevitably became air contaminated, and after a time, there developed a mold colony around which, in his own words, "The staphlococcus colonies had become transparent and were obviously undergoing lysis". This was sufficiently striking and unusual that he preserved the original plate and subcultured it. (Until recently all of the penicillin used clinically had been produced from subcultures of this original tube). It was found that broth in which the mold had grown for a week or two acquired marked inhibitory properties to many common bacteria, even when diluted 500 to 800 times. The mold was identified later as Penicillin notatum (49). It had

never before been regarded as of any importance, and in his book on the Penicillia, Thom (136) devoted only a few lines to it. It had originally been found on rotting branches of Hyssopus in Norway, and had been described at other times by other authors, but Thom attributes it to Westling. Fleming christened the active substance "Penicillin"*. He found that some species of bacteria were highly susceptible, others not at all, to the action of this new antibiotic. He also found that the penicillin had no poisonous effects on white blood cells, nor was it toxic when injected into animals.

Fleming (49) made the first applications of the new substance in bacteriological studies. Since it suppressed the growth of the streptococci and staphlococci normally found in the mouth, but not the growth of influenza bacilli, its addition to a throat swab culture permitted the latter organism to grow uncontaminated by ordinary mouth flora.

Unfortunately, although the potential value of penicillin was recognized and the mold exhaustively studied, it was very unstable and attempts to extract the active principle from the broth and concentrate it met with little success. These, together with the small number of

*Although Dorland's Dictionary and the British Broadcasting Company pronounce penicillin with the accent on the second syllable, Dr. Fleming accents the third, i.e., pennysil'-lin (13).

septic cases in peacetime, and the advent of the sulfonamides, prevented much further interest and its clinical use was not seriously pursued. In his original (1929) paper, Fleming (47) stated: "It may be an efficient antiseptic for application to or injection into areas infected with penicillin-sensitive microbes", and in 1931: "It is quite likely that it... will be used in the treatment of septic wounds." (48)

Beyond publishing several papers on the use of penicillin in the laboratory to separate susceptible from non-susceptible organisms, and experimentally on dressings for septic wounds, Fleming apparently did not pursue the matter further. (48,49)

It was to the problem of extracting penicillin in purified form to which the Oxford workers, headed by H. W. Florey (26) turned their attention when war in Europe became imminent, and there appeared to be great need for some method of control of infection more effective than those then in use. Falk (45), whose interest in the matter was that of a close observer, states that the full story of the rediscovery of penicillin nine years after Fleming's work has not been told, probably because Professor Florey has not wanted to detract from the honor due Professor Fleming. Florey was interested in antibacterials of cellular origin and he and others read

Fleming's original 1929 paper on penicillin, and found it difficult to understand why the study of penicillin had practically lapsed for nine years. This was probably due mainly to difficulties in purification.

Florey and co-workers borrowed a strain of the Fleming Penicillium notatum and recultured it. Florey, being busy, asked Dr. Norman Heatley to work with Dr. Abraham Chain, and they, in 1938, succeeded in purifying and standardizing penicillin, and by late Spring of 1940 had produced enough for animal experiments. These experiments were well planned and were immediately and brilliantly successful, and were published in an epoch-making paper, "Penicillin as a Chemotherapeutic Agent" (26). The importance of these dramatic results were understood at once, and practically the whole Sir William Dunn Institute of Pathology was turned over to penicillin research, the work being financed mainly by the British Medical Research Council. This was at Britain's darkest hour--at the time of the fall of France.

Clutterbuck, Lovell and Raistrick (32) had found in 1932 that the mold could be grown on a purely synthetic liquid medium but they did not succeed in extracting penicillin from the medium in stable form. They had found, however, that if the medium was acidified and shaken with ether, the penicillin passed into the ether. Unfortunately,

when they tried to get rid of the ether and concentrate the penicillin by evaporation, most of the penicillin activity was lost. One observation which this group made confirmed a similar observation of Fleming's (49), and this suggested that penicillin was not too unstable to handle; that was, that its activity might, under certain conditions, be maintained in the original medium for some weeks.

One of the first steps in the progress was the elaboration by Heatley (33) of a quick test for penicillin which made the cup assay of penicillin-containing fluids possible. It was also Heatley who had developed and supervised the production procedures at Oxford.

The crucial observation was made that when ether containing penicillin was shaken with water containing the right amount of alkali, the penicillin passed from the ether back into water. In this way penicillin could be extracted from the crude brew and partially purified. The processes now used for the preparation of penicillin all depend at one stage or another on transference of penicillin from a water solution to an organic solvent and back. The isolation from the original medium of a protein and salt-free product made possible a study of the bacterial and pharmacological properties in detail.

The benefits to be derived from such a chemothera-

peutic agent for the treatment of the wounded in the armed forces were obvious to the medical profession of the embattled peoples of Britain, and arrangements were made by the Rockefeller Foundation for Florey and Heatley to visit the United States. England, at that time, was being extensively bombed and did not appear to be the place to undertake large scale production of so delicate and capricious a product. Accordingly, the Rockefeller Foundation brought Drs. Florey and Heatley to the United States to enlist help on the problem. They were immediately referred to the National Academy of Science and to Dr. Charles Thom, head Mycologist of the United States Department of Agriculture. Work on the problem was begun almost at once. (33).

By December 1941 at the Northern Regional Research Laboratory at Peoria, A. J. Moyer, a microbiologist, had found that the addition of corn steeping liquor to the medium upon which the mold grew increased the yield by at least ten times, and strain selection increased the yield of penicillin from two up to forty Oxford units per cc. This was possibly the greatest single factor in making commercial production of penicillin feasible. A still further extension of this work increased the yield from two to two hundred units per cc. in small flasks. A yield of one hundred units per cc. represents only .06 mg. of

pure drug, and half is lost in recovery.

The work in Peoria has been largely supported by the United States Department of Agriculture, helped by the Committee on Medical Research of the Office of Scientific Research and Development. Several commercial companies in this country were also encouraged to undertake the mass production of this much needed drug. The increased requirements for antibacterial agents, brought about by the war, served as a great stimulus to the commercial manufacture of penicillin (33).

After the entry of the United States into the war late in 1941, work was speeded up. In order to expedite clinical research, the Committee on Medical Research of the Office of Scientific Research and Development appointed the Chairman of the Committee on Chemotherapeutic and Other Agents of the National Research Council, Dr. Chester Keefer, to supervise the distribution of all stocks available for therapeutic trial. Under this arrangement clinical investigation of penicillin has been conducted on as wide a scale as supplies of material have permitted. As reports from individual research workers and investigative groups have been received, they have been collated, and summarized by the Committee on Chemotherapeutic and Other Agents. The first report of this body, embracing the results obtained in 500 cases of

various infections treated by 22 groups of investigators, appeared in August 1943 (81). From a study of this, and of independent reports published both in America and England, it is obvious that the discovery of penicillin constitutes the greatest forward step in chemotherapy since the advent of the sulfonamides, and in many respects the new agent appears far superior to the sulfonamides or to any other group of therapeutic compounds.

Penicillin has now reached the stage at which its efficacy in a wide variety of diseases has been established, although its full clinical potentialities are yet to be realized. At the present time, the demands of the armed forces have been fully met, and penicillin is now available for use in civilian practice without restriction. Effective March 15, 1945, producers and distributors may sell penicillin through normal commercial channels; 1,280,000 ampules, each containing 100,000 units, for the period March 15 to March 31, and 1,500,000 ampules monthly, each containing 100,000 units, may be expected. This per month figure is four times the total U. S. production just one year ago (33).

The fact that the supply of penicillin has been limited and strictly controlled until its true value was accurately assessed and fully established has been, in a way, a very good thing, for it has prevented use of the mold by

unscientific investigators and the consequent overburdening of the literature with false or equivocal claims, which must later be disproved, and our thinking revised. In contrast to most investigative problems, one is impressed by the total agreement on basic principles, and to a large extent, on finer details. One may be fairly sure that when an author presents a statement as a fact, there will soon be corroboration from independent and equally reliable sources. Even in tentative conclusions there is usually agreement, and nowhere have I encountered direct challenge of findings or retraction of a claim. Investigation and experiment have been carried out on a plane of total objectivity, by cautious men with facilities for checking all results, but more important, by men who possess true zeal for scientific truth.

PRODUCTION OF PENICILLIN

There are four methods of production: surface, submerged, bran and continuous flow.

1. Surface culture: This is the old standard method. Spores are seeded on the surface of flasks of various size containing culture media. The yield is better in small flasks because diffusion of the nutrient is better if the medium is shallow, and because the concentration is greater, recovery costs are less. Commercially, production is accomplished in one or two quart bottles or in large

flat pans.

The advantages of this method are high yield, simpler equipment, and especially safety, as contamination of a few flasks does not destroy the whole run. The chief objection is the high labor cost of handling so many small units.

2. Submerged culture: This is done in large vats. The mycelium of the mold grows submerged. This is the best method when it will work. To prevent surface growth, the vat must be agitated and rotated and the media supplied with abundant sterile air. Not all strains, including the notatum, grow well with this method. There is, of course, the greater danger of contamination, as the conditions are ideal for growth of bacteria, and the sterilization of air is a not inconsiderable problem.

The biggest advantage of this procedure is the saving of labor.

3. On moist bran, the yield is high, but bran is a poor conductor of the heat the mold produces, and bran is hard to sterilize.

4. Clifton (31) has devised a method of large-scale penicillin production, modifications of which are now largely used commercially. A medium of yeast extract is allowed to trickle over a column of wood shavings on which the mold is growing. This closely resembles the

commercial method of production of vinegar. Substitution of corn steep liquor more than doubled the production of penicillin. The author claims that this method has the advantage of continuous production and requires a minimum of equipment and handling. The chief disadvantage is the possibility of contamination.

RECOVERY OF PENICILLIN

After filtration or centrifugation of the broth culture to remove the mycelium (and the bran if it was used), a clear yellow to brown solution remains, which still contains enough nutrients so that contaminants can very quickly destroy all the penicillin if given a chance. The broth is therefore protected by chilling, asepsis or use of a disinfectant.

The concentration of penicillin is 30 to 100 units per cc., or .002-.006% of penicillin. Since it is so unstable to pH, metals and other reagents, there are still difficult problems.

Penicillin as used clinically is the sodium or calcium salt of a reasonably strong organic acid. The pH is set at 2.-3.0 and all free organic acids are extracted with a solvent such as ether, chloroform or amyl acetate, at a low temperature, and rapidly, due to the instability of the penicillin. It is then combined with sodium bicarbonate to form sodium penicillin. The final solution

must be frozen and dried in that state, like plasma, and is done either in bulk or in small ampules.

Finally, it is a powder, pale yellow to dark brown, of a potency of 100-500 units per mg., and is a mixture of the sodium salts of most of the organic acids in the original broth, with 8-30% of sodium penicillin.

ORGANISMS USED

Penicillin production is not confined to Penicillium notatum, although this is the organism most commonly associated with it. Penicillium notatum is a member of a large aggregate of forms commonly designated as the Penicillium chrysogenum group. There are a number of the group which are good penicillin producers. Over 200 strains of molds of the notatum-chrysogenum group were tested in an effort to find better penicillin-producing strains, and at least two superior ones have been obtained. An extensive program is under way at various research centers seeking richer sources of penicillin.

CHARACTERISTICS OF PENICILLIA

Reid (138) investigated a large number of various hypomycetes, and found that only Fleming's mold had any antibacterial effect. Later investigation has shown that many of the Penicillia produce penicillin; some of these products are toxic. Strain selection has resulted in high commercial yields.

The media used for penicillin production has been extensively modified. Fleming (47,49) stated that the mold grew in a variety of differential media, but best in trypsin digest broth. Later, Abraham (4) recommended Clutterbuck's (33) modification of the synthetic Czapek-Dox media containing sodium nitrate, potassium acid phosphate, potassium chloride, magnesium sulfate ferrous sulfate and glucose. This formula was modified by McKee and Rake (109), who substituted brown sugar for glucose. Hobby et al (86) reduced the amount of brown sugar and made other slight changes. Kochalaty (97) substituted manganese for iron and others stated that the addition of zinc was helpful. Taylor (135) claims that the growth of Penicillium notatum was far better on Amigen than on any other substrate.

The addition of corn steep liquor to the media at the Northern Regional Research Laboratory in the early days of American production doubled the yields of penicillin and was a long step forward in making commercial production possible (33).

Growth of Penicillia: Only known identified strains of the highest potency were selected, and these were rigidly controlled to prevent degeneration. The number of vegetative transfers were reduced to a minimum. A convenient method of keeping cultures at a high level was

by allowing sporulation of the mold mixing the spores with dry sterile sand, and drying the mixture at low temperatures. As needed, spores from this master race were sown upon suitable media.

The mold flourishes at 28° C. with a range of 20-36° C., but fails to grow at 37° C. It grows at a wide range of oxygen tensions but not anaerobically. The production of penicillin is in part dependent on the pH of the media, being maximum slightly below the neutral point.

PRODUCTION PROGRAM

In April 1944 there were 21 plants being erected in the United States and Canada at a total cost of twenty million dollars, mostly financed by private capital. Production in the early months of manufacture was as follows:

January to May 1943	400	million units
June	425	do
July	763	do
August	906	do
September	1,787	do
October	3,873	do
November	4,846	do
December	9,184	do
January 1944	13,550	do
February	18,700	do
March (estimated)	40,000	do

No later published figures have been found, but the amount now released monthly for civilian use alone is several times as great as the total U. S. production a year ago. 3

PURIFICATION OF PENICILLIN

Drs. McPhillamy and Wintersteiner of the Squibb Institute for Medical Research were the first to isolate pure crystalline penicillin (11). When pure, it has a potency of 1,650 units per mg.

Isolation was difficult because of the instability of penicillin. It is destroyed by dilute acid and alkali, by many heavy metals such as zinc, cadmium, copper and mercury, and by primary alcohols, ketonic and oxidizing agents. The stable alkali and alkaline earth salts are very soluble in water, and no organic cation forming a relatively insoluble salt was found.

Purification, therefore, is limited to distribution between different solvents and water, and to adsorption methods. Chromatographic methods have been used extensively. These combined with a reduction process with aluminum amalgam have yielded penicillin products from which salts could be made, the purest material at Oxford being 1000 units per mg., and is capable of inhibiting growth of certain bacteria in a dilution of about 1:50,000,000.

PHYSICAL AND CHEMICAL PROPERTIES OF PENICILLIN

Penicillin is a highly unstable acid which reacts chemically to form salts and esters. Although the sodium salt so far has been most generally used, the

calcium salt is more stable, less hygroscopic, and equally effective. The sodium salt is a light orange-brown powder having a slight odor. It is very soluble in water, and although soluble in alcohol, is inactivated by this solvent. Clinically penicillin is frequently administered in sodium chloride or glucose.

Penicillin in the dry state is stable to light but is adversely affected by heat in direct relation to the temperature. When stored at temperatures below 10 C., however, it retains practically its full activity for several months. Aqueous solutions can be kept at refrigerator temperatures for several days without significant loss of potency.

The chemical formula of penicillin is still in dispute. According to Abraham (1), the nitrogen content appeared to increase with the purity of the product. On the assumption that the product is practically pure, the molecular weight is about 640. Although the Oxford workers (35) state that their product is nitrogen free, Meyer et al (113) state that all of their highly active and pure preparations analyzed for one nitrogen atom, with a probable formula of $C_{14}H_{19}NO_6$ or $C_{14}H_{17}NO_5 + H_2O$. Abraham et al (1) announced earlier that the formula was $C_{34}H_{32}O_{110}N_2BA$ (as the barium salt), and stated that there were no oxygen-methyl or nitrogen-methyl groups.

Meyer and colleagues (112) believe that penicillin does not contain a free primary amino group. Abraham et al (1) contend that the penicillin is the salt of a strong dibasic acid, and that there is evidence of one carbonyl, one latent carboxylic and two acetylatable groups, and at least five carbon-methyl groups. This group found no easily reducible double bond.

Garrod (69), in seeking more potent molds, suggests the other possible approach to production--synthesis of penicillin. When the structure of penicillin is known it may be possible by varying it to obtain a wider range of activity. The isolation of pure sodium penicillin by Squibb and Company (11) crystals was a step forward in this direction. The pure salt has an activity of about 1650 Oxford units per milligram. Due to difficulties in purification and subsequent loss of potency, impure penicillin is used clinically and material with a potency of 100 units or less per milligram yields satisfactory therapeutic results. Increasing the purity decreases the toxicity, and untoward effects are caused mainly by products of low potency, that is, these effects are due to impurities.

EFFECT ON BACTERIA

Penicillin, a potent antibiotic, differs from chemical antibiotics-the so-called antiseptics--in the manner

in which its antibacterial influence is exerted. Its safety also appears largely due to this difference in action.

The chemical antiseptics in general are protoplasmic poisons, in adequate concentration quickly destructive not only to the invading bacteria, but to leucocytes and tissue cells of the host as well. Penicillin, by contrast, is not a protoplasmic poison, but accomplishes its antibacterial influence by affecting the functional activity of certain microorganisms. It induces certain morphologic changes in these organisms, accompanied by interference with their normal biologic processes and reproduction.

This antibacterial activity of penicillin does not affect all organisms. Greatest efficacy is manifested against the aerobic and anaerobic gram-positive bacteria. Against gram-negative organisms penicillin exerts, as a rule, little or no effect, except that the gram-negative Neisseria are particularly sensitive to its actions.

Dubos (43a), in an attempt to explain this difference in penicillin-sensitivity, suggests that the chemical group responsible for the gram-staining reaction may be involved in the reaction to penicillin. He explained this to be in accordance with Ehrlich's theory that antiseptics and antibodies owe their activity to their respective

ability to react with chemically reactive groups (known as receptors) in the bacterial cell.

Below are listed the organisms so far found to be sensitive and those considered to be resistant to penicillin. This list must not be considered conclusive. Some organisms have not been tested clinically: others have been found to be either more or less sensitive in vivo than in vitro.

CLINICAL INDICATIONS

A. Penicillin is the best therapeutic agent available for the treatment of:

1. All staphylococcic infections with and without bacteremia:
   Acute and chronic osteomyelitis
   Carbuncles--soft tissue abscesses
   Meningitis
   Cavernous or lateral sinus thrombosis
   Pneumonia--empyema
   Carbuncle of kidney
   Wound infections--Burns
   Endocarditis

2. All cases of clostridia infections:
   Gas gangrene
   Malignant edema

3. All hemolytic streptococcic infections with bacteremia and all serious local infections:
   Cellulitis
   Mastoiditis with intracranial complications, i.e., meningitis, sinus thrombosis, etc.
   Pneumonia and empyema
   Puerperal sepsis
   Peritonitis
   Endocarditis

4. All anaerobic streptococcic infections:
   Puerperal sepsis
   Localized infections elsewhere

5. All pneumococcic infections of:
   Meninges
   Pleura
   Endocardium
   All cases of sulfonamide-resistant pneumococcic pneumonia

6. All gonococcic infections, especially those complicated by:
   Arthritis
   Ophthalmia
   Endocarditis
   Peritonitis
   Epididymitis

7. All cases of Anthrax

8. All cases of chronic pulmonary suppuration in which surgical treatment is contemplated.

9. All meningococcic infections failing to respond to sulfonamides

10. All cases of bacterial endocarditis due to susceptible organisms.

B. Penicillin is effective in the following diseases, but its position has not been definitely defined:
   1. Syphilis
   2. Actinomycosis
   3. Diphtheria, especially in horse-serum sensitive patients

C. Penicillin is of questionable value in mixed infections in which the predominating organism is of the gram-negative flora--i.e.,
   1. Ruptured appendix with peritonitis
   2. Liver abscesses
   3. Urinary tract infections
   4. Also in rat bite fever due to streptobacillus moniliformis

D. Penicillin is contraindicated in the following cases because it is ineffective:
   1. All gram-negative bacillary infections:
      Typhoid--Paratyphoid
      Dysentery
      E. Coli

H. Influenzae
B. Proteus
B. Pyocyaneus
Br. Melitensis (undulant fever)
B. Tularense (tularemia)
B. Friedländer

2. Tuberculosis
3. Toxoplasmosis
4. Histoplasmosis
5. Acute rheumatic fever
6. Lupus erythematosis diffuse
7. Infectious mononucleosis
8. Pemphigus
9. Hodgkin's disease
10. Acute and chronic leukemia
11. Ulcerative colitis
12. Coccidiomycosis
13. Malaria
14. Poliomyelitis
15. Blastomycosis
16. Nonspecific iritis and uveitis
17. Moniliasis
18. Virus infections
19. Cancer (111)

Whether the effect of penicillin in causing cessation of bacteria to multiply and slowly to die is due to a bactericidal or bacteriostatic effect, according to Garrod (70), is not clear, and the mechanism of the effect is unknown, although the peculiar changes in bacterial morphology first observed by Gardner (68) suggest that at least the process of division is inhibited. Hobby et al (86, 87, 88, 89, 90) believe that bacteriocidal action takes place only if multiplication occurs. Penicillin is apparently not destroyed nor absorbed from solution by bacteria, and lysis of organisms does not occur. Gardner (68) found microscopic changes in all susceptible rod-

shaped organisms that showed any inhibition. These changes consisted in lengthening of cells, mainly due to a failure of fission; growth proceeds but division and separation of the cells do not follow in due course. Fleming (50) is in complete accord with these findings of Gardner's.

These findings are in contrast to the action of the sulfonamides which cause a slowing of the rate of growth, and is similar to that of gramacidin, which prevents multiplication, but penicillin acts more slowly than either.

ABSORPTION, EXCRETION, AND DISTRIBUTION IN THE TISSUES

Absorption and excretion of penicillin are dependent chiefly on route of administration, dosage, and status of the kidneys. The drug may be given locally, parenterally, and of late, orally. For local application, it may be applied with sulfanilamide as a powder, or as a cream mixed in soft paraffin and lanette wax. Bodenham states that this method is particularly effective in the treatment of burns (19,30). Fleming (52) was the first to show that penicillin diffuses very slowly into the cerebrospinal fluid and that for meningitis, to be effective, it must be injected intrathecally. This observation has since been confirmed by many other observers (126).

In the past, the chief routes used have been the

intravenous and intramuscular. Herrell (85) believes that the continuous intravenous route insures more uniform and continuous contact with the bacteria. He claims that periodic intravenous injections of relatively large amounts of penicillin, followed by intervals during which the amount in the blood is almost negligible, may do much to encourage the development of penicillin-fast or penicillin-resistant pathogens. Rammelkamp and Keefer (127) recommend the intravenous route for severe infections when high blood levels must be reached rapidly. Lyons (101) speaking from experience in an Army hospital, believes that in unskilled hands the incidence of thromboses after intravenous injection is great enough to make the intramuscular route preferable. This has been the method most widely used, chiefly because the administration of the drug is often done by other than medical personnel. Subcutaneous injection, according to Rammelkamp and Keefer (127) causes local soreness and penicillin should not be given by that route. However, Dr. John L. Gedgoud (70a), of the University of Nebraska College of Medicine, routinely administers penicillin subcutaneously to pediatric patients, with uniformly good results. It was claimed after early trials that penicillin given subcutaneously did not establish sufficiently high blood levels to be therapeutically effective, a claim that has not been proved in practice.

Penicillin which is given intravenously or into sites available to the blood stream is rapidly excreted by the kidneys, so that by three hours after injection little is found in the blood. Attempting to maintain therapeutically adequate blood levels, is, as Florey (70) states "like trying to fill a bathtub with the plug out". Injected locally into wounds, joint or other cavities, excretion is not nearly so rapid.

Methods of slowing penicillin absorption from the site of injection or excretion via the kidneys have recently occupied the attention of many investigators.

Romansky and Rittman (131) employed a beeswax-peanut oil mixture as a vehicle for penicillin calcium. The calcium salt was used because it is less hygroscopic than the sodium salt and forms a better mixture with the oil. The mixture (5% beeswax in peanut oil plus penicillin) is sufficiently liquid to be injected with an ordinary needle. The mixture delayed absorption and excretion and maintained a detectable level in the blood for six to seven hours, and in the urine for 30-32 hours. From the standpoint of ease of handling, preservation, etc., the mixture was satisfactory, and clinically it cured acute gonorrheal urethritis in single doses of 100,000 units, a total amount identical with that used by

multiple injections. Commenting on this experiment, Armstrong et al (14) suggest that perhaps a long-continued low concentration of penicillin is more effective therapeutically than separated spikes of higher concentration.

Trumper and Hutter (137) employed a slightly different method of delayed absorption. They preceeded and followed the injection of penicillin in the deltoid muscle by the application of an ice bag to the area. Bacteriostatic levels of penicillin were found in the blood for six hours or longer. Because of relapse in some of their patients, they suggest that two or three injections in 24 hours, instead of the usual six or eight, may suffice for the treatment of gonorrhea. It is hard to discover any advantage this method of administration has over that of Romansky and Rittman(131).

Rammelkamp and Keefer (127) noted that excretion of penicillin was inhibited in patients with renal failure. This suggested the possibility of excretory blockade as a means of maintaining adequate blood levels over longer periods. Rammelkamp and coworkers (124,127) employed diodrast for this purpose, Beyer and colleagues (18) paraaminohippuric acid. Both of these substances decrease the rate of renal excretion of penicillin about two-thirds, and maintain blood penicillin titers at a therapeutic level for six hours compared to about two

hours for non-blockade controls. The mechanism of this delay is that both penicillin and the other preparations are excreted by the renal tubules in addition to glomerular filtration, and by competing for tubular excretion, less penicillin is excreted in a given length of time.

Rammelkamp and Helm (135) found that gastric juice destroys penicillin rapidly, especially at body temperature, but that penicillin is quickly absorbed when introduced directly into the duodenum. Hydrochloric acid, not pepsin, is apparently responsible for the action of gastric juice on penicillin. In two subjects with pernicious anemia, the absorption of penicillin given orally was greater than that observed in normal subjects.

Rammelkamp and Keefer (133,137) attempted oral administration of penicillin in normal subjects, but found by blood and urinary level studies that there was poor absorption, and that this was only slightly increased by simultaneous administration of sodium bicarbonate, was shown by Rammelkamp and Helm (135). Since patients with achlorhydria attained adequate blood levels after oral ingestion of penicillin, and since introduction of the drug into the duodenum resulted in similar findings, it was reasoned that penicillin was destroyed in the gastric juice. Powell and Jamieson (133) found that although huge (30 mg.)

doses of penicillin orally were required, experimentally infected mice could be protected, thus proving that under some circumstances the oral route was possible therapeutically. Further studies of the matter had to wait until supplies of penicillin became more plentiful, since when attention has been turned to the less efficient but more convenient oral method of administration.

In the light of these findings, Charney et al (30) and Free et al (67) studied penicillin excretion through the kidneys after combined administration of penicillin and buffer salts, with a buffer range not reaching alkaline pH values. (It is known that penicillin is sensitive to alkaline as well as acid reactions.) These investigators found trisodium citrate to be a suitable buffer. Figures of urinary excretion of penicillin are not of course an accurate yardstick of the therapeutic effect penicillin might exert in the body, but are proof that it is absorbed from the gastrointestinal tract. This theory was proved clinically in patients with gonorrhea who received penicillin combined with buffer. With sodium citrate, the effective dose of penicillin was comparable to the doses routinely used in parenteral administration, and the blood penicillin level was higher and more prolonged than if no buffer was given. McDermott and colleagues (107) report the treatment of 13 pneumonia patients with oral

penicillin with results similar to those following intramuscular administration of penicillin, although the size of the oral doses is not stated. The same group administered penicillin orally in various mixtures and concluded that oil and oil with beeswax mixtures may be superior to either buffered or unbuffered penicillin for oral administrations. Libby (98) reported similar conclusions, finding suspensions of penicillin salts in cottonseed oil, enclosed in gelatin capsules, maintained ordinary therapeutic levels if given repeatedly.

The oral route will probably become the method of choice in the future, when methods are devised to protect the drug from the action of gastric secretions, and when the drug becomes plentiful and cheap enough that it need no longer be used with scrupulous economy.

Abraham and Chain (2) and Rammelkamp and Keefer (127) found that absorption of penicillin from the rectum is poor, and only a small amount appears in the urine. This is probably due to inactivation of the antibiotic in the lower bowel by enzymes from Escherichia coli.

Penicillin is absorbed very slowly from joint cavities, bursae or pleural cavities, hence the local action is prolonged in these areas (127).

INDICATIONS FOR PENICILLIN

The commoner uses of penicillin are well known and

merit little more than mention here. At the present time the use of the drug is modified by limitation of supply, cost and tedious methods of administration. Because of its potency and greater range of effect, when compared with the sulfonamides, certain types of bacterial infections demand immediate use of penicillin. Most common of these are those caused by staphlococcus only partially responsive to the sulfonamides. Staphylococcemia mortality has been reduced to less than 20% (40,85). Acute osteomyelitis, cavernous sinus thrombosis, carbuncles, furuncles, lung, brain and mediastinal abscesses, palmar space, tendon sheath and wound infections have responded well in many cases, and better than to any previous treatment.

Early controversy concerning the value of penicillin in the treatment of subacute bacterial endocarditis has been dissipated by more recent studies, in which large doses have established the drug as definitely curative in a large proportion of adequately treated cases (41,99).

All cases of anaerobic streptococcal infections such as puerperal sepsis and empyema deserve penicillin treatment, as it is more effective than the sulfonamides.

Penicillin is quickly curative of pneumococcal pneumonia, meningococcal meningitis and gonorrhea, but as these diseases respond adequately to the sulfonamides, it

is not likely that they will be treated with penicillin until it becomes freely available, cheap and can be administered by mouth. Only the complications of these diseases and those resistant to the sulfonamides are now treated with penicillin.

STORAGE OF DRY PENICILLIN

Penicillin is stable for at least one year when stored in its original container under refrigeration at a temperature of 10° C. (50° F.) or below.

While Penicillin in its unopened package is reasonably stable at ordinary temperatures, it is rapidly inactivated by high temperatures and may undergo slow deterioration even at room temperature.

In the dry form, Penicillin is very hygroscopic. After absorbing moisture from the atmosphere, it tends to lose potency even if stored at low temperatures.

PREPARATION OF SOLUTIONS

Penicillin is extremely soluble. It may be dissolved in small amounts of sterile, pyrogen-free, distilled water, sterile physiologic saline solution, or sterile 5 per cent dextrose solution.

All Penicillin solutions not used immediately should be kept under refrigeration at 10° C. (50° F.) or below. Since Penicillin gradually loses potency after it is put into solution even though properly stored, it is preferable

not to use solutions more than 24 hours old.. No solution should be used 48 hours after preparation.

Penicillin is packaged in sterile containers, and solutions need not be sterilized before administration, so long as solvents used are sterile and solutions are not contaminated.

A. For Intravenous Injection:

1. The dry Penicillin may be dissolved in sterile physiologic salt solution in concentrations of 1,000-5,000 units per cc. for direct injection through a syringe.

2. The dry Penicillin may be dissolved in sterile normal saline or 5 per cent dextrose solution in lower dilution (25-50 units per cc.) for constant intravenous therapy.

B. For Intramuscular Injection:

1. The total volume of individual injections should be small, i.e., 5,000 units per cc. of sterile normal saline. It may also be given by constant intramuscular drip--120,000 units in 250 cc.

C. For Topical Applications:

1. The dry form of the sodium salt is irritating and should not be used alone in this concentrated form.

2. Solutions of 250 units per cc. of physiologic salt solution are satisfactory. For resistant or more intense infections this concentration may be increased to 500 units per cc.

DOSAGE AND ADMINISTRATION

The dosage will vary from one patient to another depending on the type and severity of infection. Recovery has followed in many serious infections following 40,000-

50,000 units a day; in others, 100,000-120,000 units or even more are necessary. The objective in every case is to bring the infection under control as quickly as possible. The following recommendations are made at the present time with a full realization that revisions may be necessary as experience accumulates.

Penicillin is excreted rapidly in the urine so that following a single injection it is often impossible to detect it in the blood for a period longer than 3 to 4 hours. It is well to use repeated intramuscular or intravenous injections every 3 or 4 hours, or to administer it as a continuous infusion.

A. In serious infections, with or without bacteremia, an initial dose of 15,000 or 30,000 units with continuing dosage as:

   1. Constant intravenous injection of normal saline solution containing Penicillin so that 3,000 to 5,000 units are delivered every hour, making a total of 48,000 to 120,000 units in a 24-hour period. One-half the total daily dose may be dissolved in a liter of normal saline solution and allowed to drip at the rate of 30 to 40 drops per minute.

   2. If continuous intravenous drip is undesirable, then 10,000 to 20,000 units may be injected intramuscularly every 3 to 4 hours.

   3. After the temperature has returned to normal, Penicillin may be stopped and the course of the disease followed carefully.

B. In chronically infected compound injuries, osteomyelitis, etc.

1. The dosage schedule should be 5,000 units every 3 hours or 10,000 units every 4 hours parenterally with local treatment as indicated. This dosage schedule may have to be increased, depending upon the seriousness of the infection, and response to treatment. Best results are obtained in these cases when Penicillin is combined with adequate surgical treatment.

C. Gonorrhea

1. Twenty thousand units every 3 hours intramuscularly for 5 doses. The minimum dosage has not been worked out completely. Results of treatment should be controlled by culture of exudate.

D. Empyema

1. Penicillin in normal saline solution is injected directly into the empyema cavity after aspiration of pus or blood. This is done once or twice daily, using 30,000 or 40,000 units depending on the size of the cavity, type of infection, and number of organisms. Penicillin solutions should not be used for irrigation. It requires at least 6 to 8 hours for a maximum effect.

E. Meningitis

1. Penicillin does not penetrate the subarachnoid space in appreciable amounts, so that it is necessary to inject it into the subarachnoid space or intracisternally. Ten thousand units diluted in normal saline solution in a concentration of 1,000 units per cc. may be injected once or twice daily, depending upon the clinical course and the presence of organisms. Intravenous or intramuscular injections are usually given concomitantly.

F. Bacterial Endocarditis

1. Penicillin is the best agent available for the treatment of bacterial endocarditis. It is as effective alone as when combined with heparin. Treatment should be continued for 3 weeks or longer, depending upon the individual case. The intramuscular route is the one of choice, although in a few instances it may be desirable to use the continuous intravenous route in order to obtain

maximum effect. For best results the dosage should range from 200,000 to 300,000 units a day.

G. Local Infections

1. The sodium salt of Penicillin in dry form is irritating to wound surfaces and should not be used alone in this concentrated form. Solutions in physiologic saline, suspensions in mineral oil or petrolatum, or admixtures with a suitable powder base are satisfactory.

2. For topical application, Penicillin may be diluted to concentrations of 250-500 units per Gm. Isotonic saline containing 250 units per cc. may be applied on compresses. Small Dakin tubes are inserted in the compresses and additional amounts of Penicillin are administered through the tubes every 8 hours. It is imperative that the Penicillin be trapped at the site of the lesion for a minimum period of six to eight hours. Penicillin cannot be used effectively as an irrigation. (111)

TOXICITY OF AND ALLERGY TO PENICILLIN

In animals, penicillin has produced toxic effects only when huge doses are given, and these reactions have become less as purer preparations of the drug have been given. Abraham and co-workers (4) were able to produce severe reactions in mice by the intravenous injection of a preparation containing 40-50 units per mg. But Florey and Jennings (55) reported that 30 mg. of a preparation containing 250-325 units of penicillin per mg. could be given to mice without ill effects, thus indicating that purification reduces the acute toxicity of penicillin.

Hobby, Meyer and Chaffee (89) pointed out that a cardinal symptom of toxicity in mice was respiratory

embarrassment--choking, gasping, rapid respiration. The presence of these pulmonary phenomena following toxic doses into mice (30,000 units per kg.) was confirmed by Robinson (130). Within the limits of therapeutic doses (100 units per kg.) none of the animals was harmed.

No complete histologic study of animals dying of large doses of penicillin has been encountered. According to Hobby and co-workers (89) no pathology was noted other than congestion of the lungs. Hamre et al (76), giving large doses over a few days time, found that the most common finding for all species of animals (guinea pigs, mice and rabbits), was foci of necrosis in the liver.

According to Hamre and colleagues (76) all animals given penicillin subcutaneously showed a severe reaction at the site of injection. This may account for the widespread prejudice against the use of this route in humans, a prejudice which is only of late being broken down. (70a)

Clinically, no seriously significant toxic effects have been observed following systemic therapy with either the sodium or the calcium salt of penicillin. According to Garrod (69) one thousand times the concentration of penicillin necessary for therapeutic action can be produced in the blood without ill effect. Treatment with the drug is governed not by fear of overdosage but only by anxiety to employ so precious a remedy with utmost possible

economy. Impurities remaining after extraction were believed to be the prime causes of reactions (55). Further refinement of the drug seems to have reduced these reactions listed by Lyons (101):

1. Chills with or without fever after intravenous injections.
2. Eosinophilia--20 to 30%.
3. Burning pain at the site of intramuscular injections.
4. Headache.
5. Faintness and flushing of the face.
6. Unpleasant taste following parenteral injection.
7. Tingling of the testes.
8. Muscle cramps.
9. Femoral phlebothrombosis.

All of the symptoms listed have been mild and have caused no difficulty aside from temporary discomfort to the patient. Transient azotemia has been recorded in a few cases by the Floreys (56) and by Lyons (100). Albuminuria or other significant evidence of renal damage has not been noted in these cases, and such rise in non-protein nitrogen content of the plasma as has occurred appears to have been unimportant. For all practical, clinical purposes penicillin has few or no toxic properties, and within any reasonable limit may be determined on the basis of clinical requirements without consideration of possible toxic effects.

Urticaria following penicillin administration has been observed in a few cases but has never been serious, since it usually disappears when penicillin is withheld for a day or several days. Lyons (101) reports urticarial

reactions to penicillin occurring in 13 of 309 cases, or 5.7%, in an early series. In one patient with osteomyelitis, who received a second course of penicillin after an interval of a week, massive, generalized urticaria appeared and persisted until the drug was stopped. The patient's past history of sensitiveness was negative. Lyons (101) presents evidence to indicate by skin tests etc., an obvious allergy to penicillin, and states that, "the course of the urticaria was independent of continuation or cessation of treatment", but the patient lost his urticaria immediately on cessation of treatment. Criep (36) challenges this statement of Lyons' that the course of the urticaria was independent of continuance or cessation of treatment. His case followed a course almost identical to that of Lyons' patient, with similar skin test findings.

It is significant that the reports in the literature for 1944 contain scant mention of urticarial complications, which provides ancillary evidence for the contention that reactions to penicillin are caused more by impurities than by the drug itself.

The reactions to penicillin therapy in syphilis reported by Lentz (97), Moore (114), Stokes (133), Leifer (96) and others and described in more detail elsewhere, are probably due to impurities in the drug, or are the reactions

caused by stirring up a quiet syphilitic focus which any antisyphilitic drug would do.

ASSAY OF PENICILLIN

Foster (60) was the first to formulate the requirements of a suitable assay for penicillin. The material to be tested must require no pre-treatment, the test must have a high accuracy, the results must be available with reasonable promptness, the readings must be unequivocal, and it must be demonstrated that only one member of the Penicillia is present. The methods of assay may be classified under one of the following basic principles:

1. The Oxford cup methods. The progenitor of this method was that of the hole or agar cup technic described by Fleming (47,51). Foster et al (61,62,63, 64,65) modified the method by implanting tiny glass or porcelain cylinders containing penicillin in nutrient plates heavily seeded with Staphlococcus aureus. The Plates were incubated twelve to sixteen hours at 37° C., and the surrounding zone of bacterial inhibition read. A standard solution produced an average zone of inhibition of 24 mm., which was defined as a Florey unit. According to Coghill (33), it was at the insistence of Florey that the term "Oxford" unit was

substituted for the original designation "Florey unit".

2. Serial dilution method: This is done in tubes containing nutrient broth or upon agar plates. The essential points of the plate method are as follows: various amounts of the sample are mixed with ten cc. of melted agar, which after solidification, is streaked with a test organism. Concentration of penicillin is determined by comparing degrees of inhibition.

Since the Florey (Oxford) unit as defined above did not apply to the serial dilution method, Florey and Jennings (57) defined this unit in terms of the latter as follows: A unit is that amount of penicillin which, when dissolved in 50 cc. of meat extract broth, completely inhibits the growth of a test strain of Staphlococcus aureus. Expressed differently, a meat extract broth containing one unit of penicillin per mg. just inhibits the growth of Staphlococcus aureus in dilutions of 1 to 50,000.

3. Turbinimetric: Foster (60,61,63) et al stated that the inhibition of growth of a culture of Staphlococcus aureus in liquid media was a function of the penicillin concentration, and that the turbidity of a mixture as measured by a colorimeter was in inverse ratio to the strength of the penicillin. Recently

the technic has been modified by the substitution of Bacillus adhaerans for Staphlococcus aureus. The time required for an end-point was reduced to three to five hours. This method is particularly suitable for use in assaying substrates.

Foster and Woodruff (63,64,65) have summarized the disadvantages of these methods:

1. Oxford cup: Affected by the pH of the media. Organisms and penicillin must be inoculated within a very short time of one another, and the presence of a halo may make the reading of the end point difficult.

2. Serial dilution: (a) in liquid broth--sterile samples are necessary or else sterility must be effected by passage through a Seitz filter. The end point may not be definite. (b) plate method--this is affected by changes in pH; the presence of bacteria containing penicillinase may affect the end point, and the method is very tedious.

3. Turbinimetric: A large quantity of the sample is necessary, and it is too difficult for routine use.

The Oxford unit as later defined (7) is the one most used in the United States, Great Britain and Canada. The potency of such a unit is such that .01-.02 of a unit per cubic cm. inhibits the growth of 26 sensitive strains of Staphlococcus.

The Food and Drug Administration has set up a new standard, consisting of pure crystalline penicillin, which is our National Standard in the United States. Its value is 1650 units of penicillin per mg., and it is believed to be reasonably close to the Oxford unit (33).

The purposes of penicillin assay are first, to determine the penicillin content of manufactured samples, and second, to determine blood or serum levels, to check adequacy of dosage. Although no hard and fast rules can as yet be laid down, concentrations of .156 Oxford units per cc. are considered adequate for most infections. As the supplies become greater and costs less, clinicians will be less interested in maintaining minimum adequate levels, as determined by laboratory procedures, but will supply dosages known to be adequate.

PENICILLIN IN NON-PYOGENIC INFECTIONS

Besides its use in the pyogenic infections, penicillin is finding wide application in the treatment of diseases caused by a variety of organisms, many of which are unaffected by the sulfonamides, and has proved to be effective in vitro or in experimental infections in animals in some conditions so far not treated with penicillin in the human. In addition, it

has proved to be disappointing in many diseases, clinical and experimental. Its clinical applicability in all conditions except those caused by the more common pus-formers is still not definitely outlined. An attempt will be made to give a resume of the work so far done in the use of penicillin in these non-pyogenic diseases.

DISEASES CAUSED BY BACILLI

1. PENICILLIN IN ANTHRAX

Fleming (47) in his original paper observed that the growth of Bacillus anthracis was inhibited in vitro by a penicillin concentration of 1:10, compared to 1:400 for Staphlococcus aureus. Abraham et al (4) found that the growth of one strain of Bacillus anthracis was inhibited in vitro at the same concentration as that which inhibited four different strains of Staphlococcus aureus. Heilman and Herrell (84) found that 100% of mice inoculated with ten times the lethal number of anthrax bacilli could be protected if penicillin therapy was started at once, while all the controls died.

Three female wool workers with smears and/or cultures of cutaneous lesions positive for Bacillus anthracis were treated with total dosages of penicillin ranging from 150,000 to 475,000 units. All showed rapid

healing, subsidence of clinical signs and symptoms, and disappearance of the organisms from the lesions, with complete recovery. So far no cases of anthrax with bacteremia have been treated with penicillin. The Committee on Chemotherapy of the National Research Council has received reports on four human cases of anthrax treated with penicillin (9). All survived.

2. CHANCROID OF THE CERVIX

While this condition is not commonly seen in white women, Day (38) reports on two cases. The diagnosis was made by ruling out the usual causes of a red, edematous, boggy cervix. Only one case was treated with penicillin, 30,000 units intramuscularly every three hours, for five days. In a week the pelvic examination revealed no abnormality.

3. DIPHTHERIA

Corynebacterium diphtheriae has been shown to be susceptible in vitro to penicillin. Although specific antitoxin is the remedy of choice in the treatment of diphtheria, when it is unavailable, or when there is a hypersensitivity to it, it is desirable to have an alternative medication.

A recent report to the Committee on Medical Research of the Office of Scientific Research and Development (10) states: "Diphtheria has occurred in German

prisoners of war and where antitoxin was unavailable or sensitivity present, penicillin has proved very effective." No details are given. Sweeney et al (134) without citing experience, state that penicillin is of no value in diphtheria.

In cases where, in spite of administration of antitoxin, the local infection still presents a threat to life, for example, laryngeal diphtheria, administration of penicillin would seem desirable. It must be remembered, however, that penicillin has no specific action in the body against toxins already formed (10).

4. RAT BITE FEVER DUE TO STREPTOBACILLUS MONILIFORMIS

Although rat bite fever caused by the Spirillum minus responds quite well to arsenotherapy, the less frequently seen Streptobacillus form has never been satisfactorily treated with drugs. Altmeier et al (5) report on three children who were definitely diagnosed, who were treated with penicillin. A ten month old infant received 302,500 units, a 2 month old boy 212,500 units and a 4½ year old child exhausted the available supply after receiving 132,000 units. All had had positive blood cultures and high fevers. The fever disappeared rapidly with treatment, and blood cultures became negative. The insufficiently treated case took

longer but recovered. Recovery for all three was a matter of days, compared to weeks or months for cases reported in the older literature, some cases developing a crippling arthritis and there was some mortality.

5. GAS GANGRENE AND TETANUS

McIntosh and Selbie (108) found that in mice, infected with Clostridium welchii, injection of penicillin in solution at the site of infection, combined with subcutaneous use of specific antitoxin, gave higher survival times and doubled the number of survivals than either agent alone, and that penicillin is better than proflavine or sulfonamides in synergistic action with antitoxin. Craig et al (34) reports on one case in which crepitus was present and cultures positive for Clostridium welchii and hemolytic streptococci, which was successfully treated with penicillin topically and with large doses intramuscularly, in addition to antigas serum and blood transfusions. There was rapid control of the toxemia in 48 hours. McKnight et al (110) report a case of gas gangrene treated with tetanus antitoxin, sulfathiazole and amputation which, when moribund, was saved by penicillin administered topically, and by intravenous and intramuscular injection (this, an early

case, was widely publicized in the newspapers). Harvey (77) reports on a case in which the organisms were eliminated, although the patient died, apparently of toxemia. Buxton and Kurman (23) report on two cases with severe symptoms of tetanus and history of possible infection, which responded rapidly and completely to a combination of tetanus antitoxin and penicillin. It was their conclusion that the addition of penicillin to tetanus antitoxin is apparently a life-saving measure. Jeffrey and Thomson (93) report on 33 cases of gas gangrene in military casualties in Italy, in which radical surgery with full supportive therapy, gas gangrene antiserum, plus penicillin resulted in a total mortality of 36.4%. McAuley and Gearhart (106) report a patient who, when near death from gas gangrene, recovered sufficiently on huge doses of penicillin, so that amputation could be performed. The patient lived. Pickett(130a) reports two injured soldiers who developed gas gangrene, who were cured by large doses of penicillin.

DISEASES CAUSED BY SPIROCHETES

1. LEPTOSPIROSIS ICHTEROHEMORRHAGICA (WEIL'S DISEASE)

This is a disease found chiefly in oriental countries among persons who work in water. The animal reservoir is the rat population, which discharges the spirochetes

in the urine. Serum is of some value if given early in the disease, but arsenic and bismuth are not useful.

Abraham et al concluded from in vitro tests that Leptospira icterohemorrhagica was relatively insensitive to the action of penicillin. Heilman and Herrell (80), however, were able to protect guinea pigs injected with infected blood containing several times the lethal dose of leptospirochetes. Of 14 untreated animals, three died after a few days, and most of the others developed generalized lesions of leptospirosis. Of 14 treated animals, none had any abnormal temperature during observation, and none died. Seven showed no lesions of leptospirosis, and the other seven died of what seemed to be the toxic effects of penicillin. (Penicillin is relatively toxic for guinea pigs.) Smaller doses of the antibiotic gave better protection. In a final experiment, using smaller doses of penicillin over longer periods, none of 32 treated pigs had any fever during treatment, and all but one of the controls suffered from elevated temperature, some very markedly. None of the treated and most of the untreated animals died of leptospirosis.

Cross (37) reports a case of Weil's disease which failed to improve on Leptospira icterohemorrhagicae

antisérum. When treated with a slow intramuscular drip of penicillin, a total of 800,000 units over seven days, the patient recovered slowly. Guinea pig inoculations with organisms recovered from this case also showed the efficacy of penicillin in this condition.

2. YAWS

No record of the animal experimental treatment of yaws with penicillin could be found in the literature.

Whitehill and Austrian (139) report on 17 cases treated in a British Colonial Service Hospital. The lesions on all these patients had been progressing, and spirochetes morphologically identical with Treponema were isolated from the lesions. In all but one, the serum Kahn tests were positive before treatment with penicillin was started. The patients received 15,000 units of penicillin every four hours for five days, a total of 400,000 to 500,000 units. The treponemata rapidly disappeared, and all the lesions were healed in three weeks.

3. VINCENTS STOMATITIS

In a preliminary study of the treatment of this condition in the United States Navy it was found that topical application of sodium penicillin in normal saline solution, 500 to 1000 units per cc., applied twice daily arrested inflammation of the gingivae in

oral fusospirochetosis in humans, clinically and microscopically, after six to eight applications. Craig et al (34) report good results in the same condition by the use of 20,000 units intramuscularly each two hours for two to three days, a regime which seems unnecessarily intensive, as does the schedule of Sweeney et al (134), who cured all of 43 patients by the use of 25,000 units intramuscularly each three hours, to total dosage of 600,000 to 1,000,000 units. A novel and ingenious approach to the same problem is that of the British workers MacGregor and Long (102), who incorporated penicillin into oral pastilles. Penicillin in the amounts of 100-1000 units was mixed with melted gelatin base to which .1% nipagin was added. When hardened, the mixture was cut into squares. Intelligent patient cooperation was required, for the investigators prescribed that the pastille was to be held in the buccal sulcus, not chewed or sucked, and left until dissolved (about 45 minutes), then immediately replaced by a new one. Twenty-five cases of acute ulcerative gingivostomatitis (Vincent's type) were treated by this method. All the patients became asymptomatic in 24 hours. Epithelialization of the ulcerated areas had so improved that in all cases treatment was stopped in five days. After the first

24 hours, spirochetes were absent, and fusiform bacilli reduced, and in 72 hours none of either was found. No other therapy was used (the pastilles were also found useful in acute streptococcus tonsillitis).

4. RELAPSING FEVER

This tick and louse borne disease caused by a variety of spirochetes of the Borrelia group, has been treated in the past with arsenicals, but the results have been far from satisfactory. Heilman and Herrell (82) found that in vitro there was no difference in the motility of their test strain between controls and those treated with a concentration of penicillin which inhibit bacteria. The organism used was Borrelia novyi. However, in vivo, when treatment with penicillin was started 32 hours after mice were injected intraperitoneally with blood from infected rats, improvement was noted in most of the treated mice 24 to 36 hours after treatment was started, while the illness of the untreated controls had become more severe. There was complete absence of spirochetes from the blood smears of the treated group in two to three days, and total mortality was under five percent, compared to 75 percent mortality in the controls. While there were only four relapses in the treated mice (about 15 percent), all the

surviving controls had relapses. It is pointed out that as all the strains causing relapsing fever are closely related, it is likely that penicillin will be effective in all cases of the disease.

Eagle et al (43a) report similar results in experimental relapsing fever in mice and rats, but end on a less hopeful note by calculating that to cure 95% of both mice and rats 400,000 units of penicillin per kg. would be needed. If this can be translated to man, 35,000,000 units would be needed for cure. Moreover, the dose needed for rats is about half the quantity of penicillin which has been found to be fatal for that species. Lourie and Collier (100), after similar experiments, conclude that penicillin appears to be more effective than neoarsphenamine in experimental relapsing fever.

RICKETTSIAL INFECTIONS

1. TYPHUS

In the past, and especially in wartime, typhus has frequently assumed epidemic proportions, with high mortality. There has never been any satisfactory treatment, and control has centered around suppression of the rat and louse population.

In testing the effectiveness of penicillin on the

causative agent of typhus, Moragues (115) infected mice in groups with doses of Rickettsia of variable size, from overwhelming to minimum lethal dosage. A crucial point in these experiments was the determination of the presence or absence of secondary bacterial infection. It was concluded that this secondary infection was not present in any of the mice used, so that only the Rickettsia caused death. Penicillin definitely protected the treated mice from typhus when given in divided doses, if the disease was not overwhelming. Assuming the penicillin like the sulfonamides exerts its bacteriostatic action by interfering with metabolic activity, the apparent vulnerability of Rickettsiae to penicillin suggests that they possess enzyme systems which enable them to carry on a certain amount of independent metabolic activity.

The results would seem to justify a thorough clinical trial of penicillin in human typhus. So far no such trial has appeared in the literature.

2. ROCKY MOUNTAIN SPOTTED FEVER

Edmunds (44) reports of a case ill with typical symptoms of Rocky Mountain spotted fever, and a history of tick bites, and increased agglutination dilution for Rocky Mountain spotted fever. 100,000 units of penicillin given by constant intravenous drip caused the

temperature of the patient to fall in two hours from 104° F. to 100° F., with improvement in the symptoms. The patient recovered. Notwithstanding the good results in this case, the author hesitates to draw conclusions from this single instance of its use.

VIRUS DISEASES

1. TRACHOMA

Trachoma is one of the few diseases which responds to the sulfonamides. Gilford (70b) reports on four patients, all Russian nationals, who were treated in a British hospital for trachoma. Every case showed a well marked vascularization of the cornea and pannus formation. Two patients were treated with penicillin drops of a concentration of 16,000 units per cc., each three hours for three days. The others, serving as controls, were given the standard treatment of sulfathiazole by mouth and mercuric oxychloride drops and copper sulfate locally. The patients treated with penicillin were objectively well at the end of three days, but in the controls the conjunctivae was still inflamed and the photophobia undiminished. On penicillin drops the disease cleared quickly. Unfortunately the patients could not be observed over a long period.

2. ORNITHOSIS AND PSITTACOSIS

Because of the multiple hosts, Meyer (112) has suggested that it is desirable to replace the term "psittacosis" by the term "ornithosis", since the primary source of infection is not always psittacine in origin.

During the past decade, an atypical pneumonia variously designated as "virus" or "focal" pneumonia or "acute pneumonitis" has become an important clinical entity, and the clinical course of this and of ornithosis are very similar. To date no treatment has proved of value in clinical or experimental ornithosis. A sulfa-resistant fever in an unusual case of pneumonia should lead one to suspect a virus infection.

In seeking to determine the effectiveness of penicillin on the virus of ornithosis, Heilman and Herrell (79,83) inoculated eighty mice with large doses of the virus. Of the forty which received 1000 units of penicillin daily for seven days beginning 17 hours after inoculation, only two, or 5%, died, while 35, or 88%, of the controls died, and these mostly during the first week. The two treated mice which died did so on the 14th and 15th days. Many of the mice which recovered harbored the virus in the liver or spleen.

Experimental confirmation of clinical experience that penicillin is ineffective against virus diseases is reported by Parker and Diefendorf (120) of the Western Reserve University School of Medicine. Equine encephalomyelitis, psittacosis, St. Louis encephalitis and two strains of vaccinia virus were tested by these investigators. Two methods were used to determine virucidal effects. By the first method, the virus was cultivated in Rivers-Li culture medium consisting of a suspension of chick embryonic cells in Tyrode solution, with or without penicillin. After five days' incubation the penicillin containing cultures and the non-penicillin controls were titrated for virus. By the second method, both virus and penicillin were injected into the yolk sac of intact chick embryos or placed on the chorioallantoic membranes. Development of lesions on the membrane or death of the embryo was taken as a criterion of virus growth.

The results of experiments in which viruses were cultivated in Rivers-Li medium showed that the rates of growth in the presence of 10 units of penicillin per cc. within the limits of the experimental error are identical with the rates in the non-penicillin controls. Similar negative results were obtained in many of the

intact chick embryos. In one series of experiments 14 day embryos were inoculated on the chorioallantois with a quantity of vaccinia virus sufficient to give 15 to 50 lesions on each membrane. In the therapeutic tests 500 units of penicillin were injected into each egg either 24 hours before the inoculation, or at the time of the inoculation. Difference could not be distinguished between the control and the treated embryos either in the size or number of lesions when the membranes were examined two days later. Negative results were obtained also with St. Louis encephalitis and with equine encephalomyelitis virus.

Slight therapeutic results were obtained with psittacosis. Ninety percent mortality of embryos injected with psittacosis virus was reduced to eighty percent by one or more yolk sac injections of 500-600 units of penicillin.

These positive results are of little clinical interest, since maintenance of a comparable blood concentration of penicillin in man would require the daily injection of several million units. On the whole, Parker's experiments confirm the current clinical belief that penicillin is without practical therapeutic value in virus infection. In a similar study with the virus of psittacosis, the same investigators (130)

found that the mortality of treated mice was 8%, of untreated mice, 100%. Thirty days after the inoculation all of the survivors were sacrificed and at autopsy showed enlarged spleens, and most of them harbored the virus in the liver and spleen. These two experiments would indicate that penicillin protected the animals from the virus of ornithosis, but did not eliminate the virus, and may have inhibited the multiplication of the virus or prevented further invasion of the cells of the host; that is, an acute and fatal infection was converted into a chronic and benign one.

OTHER VIRUSES

In an effort to determine the effect of penicillin on certain viruses, Parker and Diefendorf (130) inoculated chick embryos with various viruses. In the doses used, penicillin was without effect on the multiplication of the viruses of vaccinia, St. Louis encephalitis or equine encephalomyelitis, or on the course of the disease induced in chick embryos by vaccinia or equine encephalomyelitis. Penicillin did have a very definite effect on the course of the disease induced in chick embryos by the viruses of psittacosis and of meningo-pneumonitis. It is to be noted, however, that the doses used were large. To maintain a comparable blood level concentration of the drug

would require the injection into man of several million units daily. The practical importance of this observation may, therefore, be questioned. Craig et al (34) report the failure of penicillin to affect the course of one case of acute epidemic parotitis and three cases of infectious mononucleosis. Many other cases of both experimental and clinical failure of penicillin in virus diseases could be cited, with equal lack of profit.

MISCELLANEOUS CONDITIONS

Wollgast (140) reports three cases of actinomycosis of the cervical region. One of these was treated for eight weeks, the others for one week, with large doses of penicillin, with indifferent or no results. All three responded promptly to sulfonamide therapy.

Snow (132) reports a case of American leishmaniasis treated both intramuscularly and topically with penicillin, with no significant change until therapy was instituted. Neghme (117) showed that mice infected experimentally with Trypanosoma cruzi showed no significant differences between treated animals and controls, both before, during and after penicillin treatment.

Foley et al (59) tested the susceptibility of seven strains of Listerella to penicillin. This organism, al-

though apparently not a pathogen, is widely distributed and has been thought to cause infectious mononucleosis. The seven strains studied all grew freely in forty times the concentration of penicillin necessary to inhibit completely the growth of other representative gram positive organisms. Craig (34) reports three cases of infectious mononucleosis, mentioned above, which did not respond to penicillin therapy.

Boland et al (31) treated ten patients with acute rheumatoid arthritis with intensive penicillin therapy, and failed to bring about any definite improvement. Craig et al (34) treated one case, with similar results. Sweeney et al reports that penicillin has no curative value in disseminated lupus nor in trichophytosis. Sweeney et al (134) and Craig (34) also agree that penicillin is of no value in malaria nor filariasis.

In the treatment of rheumatic fever, Foster et al (66) treated 38, and Watson et al eight, young adults, most of them as soon as diagnosed, and all the patients received intensive, prolonged penicillin therapy. Sweeney et al (134)and Craig et al (34), reporting on smaller numbers of patients, agree with the other groups of investigators, that penicillin failed to alter the course of the disease.

CONTRAINDICATIONS

Penicillin is not indicated in the gram negative bacillary infections such as typhoid, para-typhoid, undulant fever, tularemia, dysentery and infections produced by Hemophilus influenzae, Bacillus proteus, Bacillus pyocyaneus, Bacillus Friedlander and organisms of the coli-aerogenes group. It is of no value in ulcerative colitis, tuberculosis, rheumatic fever, Hodgkin's disease, virus diseases, blood dyscrasias, parasitic infestations, including those caused by simple cell forms, and all fungus, yeast, blastomycotic and neoplastic diseases (94,106,111).

PENICILLIN IN THE TREATMENT OF SYPHILIS

Encouraging results have been obtained in the treatment of all forms of syphilis with penicillin, but all the work so far published is still presented with caution. Although no cases have been followed for the long periods of time necessary to be certain of cure, clinical and laboratory findings indicate that a method may be developed to eradicate syphilitic infection in a few weeks rather than in the lengthy intervals required with arsenotherapy.

PENICILLIN IN THE TREATMENT OF EARLY SYPHILIS

The earliest report on the use of penicillin in syphilis was made by Mahoney (104) on four cases of

primary syphilis in males, each with a single penile chancre of an average duration of eight days, all with positive serological tests at the start. The treatment was 25,000 units of penicillin intramuscularly each four hours for 48 doses, over a period of eight days, a total of 1,200,000 units. Complete disappearance of the spirochetes from the lesions occurred within 16 hours; carefully controlled serodiagnostic tests showed that the reacting substance usually associated with spirochete activity in early syphilis disappeared from the blood rapidly and completely. The report concluded: "Should later experiments confirm the impression to be gained from the present pilot study, a rebuilding of the entire structure of syphilis therapy may become imperative."

Interest in penicillin treatment of syphilis was greatly stimulated, and in September 1944 several reports were published simultaneously. Moore et al (114) stated that "Early syphilis is at present under investigation in 23 clinics or research centers". Moore's own group used four treatment schedules, identical except for single dosage and total dosage. The patients were treated for seven and one-half days, at three hour intervals for a total of sixty intra-

muscular injections, the individual doses being one, five, ten and twenty thousand Oxford units, and the total quantity 60, 300, 600 thousand and 1,200,000 units of the sodium salt. (See Table I). Some, in addition to penicillin, received 320 mg. of mapharsen, a known subcurative dose, during the 7½ days of therapy.

Of the 1418 patients treated by Moore's group, 177 had seronegative primary, 379 seropositive primary, 698 uncomplicated and 67 complicated early secondary syphilis, and 97 various types of recurrent (usually previously treated) secondary syphilis. At the time of the report, most of these had been observed for nine weeks or longer.

Mahoney et al (105), reporting at the same time as Moore, treated a similarly varied group of patients, but a total of only 100, and used only a single treatment schedule, 20,000 units each three hours for 60 doses, a total of 1,200,000 units in 7½ days.

Both Mahoney (105) and Moore (114) reported that regardless of the single or total dose of penicillin, organisms disappeared from open lesions in every case in from six to sixty hours. There were more failures among the patients with secondary lesions than in those with primary lesions in Mahoney's cases, but this

was not marked except that seronegative results were obtained earlier, 84 days being the minimum for reversal of positive serological test. Nine of Mahoney's cases were classed as probable failures, which corresponds closely with Leifer's[96] finding of three cases of outright failure out of 25 cases of secondary syphilis, or 12% failures, three months after a course of 1,200,000 units of penicillin administered in a manner comparable to that of Moore and Mahoney. From the probable failures, Mahoney was led to conclude that there are probably some persons with syphilis who are resistant to penicillin as some are to arsenic therapy, and as some gonorrhea patients are resistant to sulfonamides. It is felt that certain host factors are largely responsible for determining whether or not an agent, as penicillin, will be effective in infections which are amenable, as a rule to treatment, although strain characteristics (as in gonorrhea) may play a role. It is felt that one of the most important problems in chemotherapy is the delineation of this essential factor and the development of ways in which it may be favorably influenced.

In Moore's cases, healing was less prompt with the 60,000 units in eight days schedule than with the three

other, larger dosage schedules, being, in the latter, as rapid as with arsenicals, and apparently having as its minimum period tissue response after elimination of the irritating organisms.

Serological response, Moore reports, shows a trend regardless of total dosage, toward reversal within 20 days from the start of treatment. With the 300,000 to 1,200,000 unit schedules the trend is uniform, but with the 60,000 unit schedule this reversal is slower and less pronounced. The reversal is identical with that of arsenic therapy, customary or intensive.

Further, of those with seronegative primary syphilis, there were only two out of 48 who had relapses to positive serology in the brief period (about nine weeks) followed, and the quantity of penicillin (60,000 to 1,200,000 units) made no difference in this trend; that is, results were satisfactory in 95.8% of cases of primary seronegative syphilis for the brief time studied.

Moore reports that of 10 cases of patients with early syphilitic meningitis treated in a manner identical to that described above, all the patients received symptomatic relief and that spinal fluid ab-

normalities improved.

The optimal time-dose relationship for penicillin in early syphilis so far determined is: The 20-fold (60,000 to 1,200,000 units) doses in 7½ days all have profound effect on clearing of lesions, healing and serological reversal. In seronegative primary syphilis, no statements as to minimum effective dose can be made, as all were effective, and a still smaller amount may prove adequate. In seropositive primary and early secondary, any dose of less than 600,000 units total allowe relapses in 5%, and 1,200,000 units total, relapses in 2%, in the short observation period of about nine weeks. (Table II)

In the 94 patients who received 320 mg. of mapharsen, a known subcurative dose, plus either 60,000 or 300,000 units of penicillin, only one relapse occurred (114). It is perhaps to be expected that certain patients with early syphilis will prove to be resistant to penicillin exactly as some patients are to arsenotherapy. But it is possible that those resistant to one will not be the same ones resistant to the other and that a combination of the two forms of treatment will eventually prove to be more effective than any method of use of either alone.

Barksdale (16) reports a case of early syphilis which ended fatally ten days after receiving 1,200,000 units of penicillin intramuscularly, the death occurring from a cause totally unrelated to the treatment. A pathological examination of the body tissues with special stains failed to reveal any spirochetes anywhere. In view of the spirochetemia and general tendency of the organisms to invade tissues, especially the regional lymph nodes, in the early stages of the disease, this is especially significant, as it suggests that the spirochetes are destroyed rather than driven into the deeper tissues.

Moore (114), after treatment of his comparatively small group, concluded that penicillin as so far employed is not suitable for mass application to large numbers of cases, since it requires hospitalization with skilled treatment day and night. Methods must be developed for use of penicillin on an ambulatory basis.

A warning note in the use of penicillin in the treatment of gonorrhea is sounded by Greydon (73), Shafer and Zakon (131a), Van Slyke (138), Ricchiuti (129), Canizares (23), and by many others. Penicillin is the first therapeutic agent to be effective in the treatment of both syphilis and gonorrhea. However, 100,000 units of penicillin is ample for cure of

the average case of gonorrhea and this will render noninfectious but will not cure early syphilis, and a patient with syphilis so treated will promptly show a relapse. Shafer and Zakon cite a case of intraurethral chancre in a male in which this occurred. Van Slyke (138) urges that this possibility be avoided by making microscopic tests of lesions before beginning treatment with penicillin for gonorrhea, and serological tests after penicillin treatment is concluded, and some time later when the serology would more likely be positive. He emphasizes the masking effect of even small amounts of penicillin on spirochetal lesions. Canizares (23) states that the period of observation for syphilis of patients treated for gonorrhea with penicillin should be one year and preferably longer.

One group of patients to whom penicillin will prove especially valuable is the small number resistant to arsenic and bismuth. Nelson and Duncan (118) believe there is an increase in this phenomenon in the United States in recent years, although in 1936 Beerman (17), who noted such an increase in Europe, stated that no such increase was occurring in the United States at that time. Until penicillin became available, there was no effective method of treating such cases except

with fever therapy induced by malaria or artificially, and such treatment is difficult and expensive.

Nelson and Duncan (118) report on six patients, each of whom had early syphilis and in all of whom the cutaneous and general lesions had shown either progression or failure to heal under adequate treatment with trivalent arsenic, and usually with bismuth. Treatment and course on penicillin therapy was in general the same as in patients with early syphilis not previously treated with metals and arsenicals. On the basis of what was learned, the authors would use 2,400,000 units intramuscularly in sixty 40,000 unit doses, at three or four hour intervals. On this regime infectiousness was quickly controlled and the serological response reversed. The writers feel that it may be tentatively concluded that previous conventional treatment does not prepare the patient for a superior response to penicillin treatment.

In some 3000 patients with early syphilis so far treated with penicillin, no examples of penicillin resistance have so far been encountered, and in one case treated with 60,000 units, later use of 1,200,000 units brought about prompt healing (114). This rather impressive result would seem to refute the warning sounded

by Dawson and Hobby (39) after in vitro studies. Admitting that the only criterion for an in vitro test of the action of a drug on spirochetes is rendering them immotile, they warn that in the treatment of human syphilis, care must be exercised to administer a sufficient quantity of penicillin to cure the patient rapidly; otherwise, there will be the possibility of producing a penicillin-resistant strain of spirochetes. The basis for this warning is as follows: In studies of the use of penicillin in the prophylaxis of syphilitic skin infections in the rabbit, it was found that a strain from an insufficiently treated rabbit was more resistant to the action of penicillin than was the control strain, based solely on comparisons of motility. As indicated above, these findings would suggest the development of penicillin fastness in inadequate penicillin therapy in humans, but clinically no such phenomenon has been observed.

TABLE I

Disappearance time of spirochetes from primary lesions (Moore).

UNITS OF PENICILLIN PER INDIVIDUAL DOSE	DISAPPEARANCE TIME IN HOURS
1,000	21
5,000	20
10,000	19
20,000	14
40,000	13

TABLE II

Seropositive early syphilis, nine or more weeks after completion of penicillin treatment (Moore).

TOTAL PENICILLIN	NUMBER CASES	SATISFACTORY RESPONSE (REVERSED OR FALLING TITER)	UNSATISFACTORY RESPONSE (NO CHANGE OR RELAPSE)
60,000 units	38	57.8%	42.1%
60,000 units plus Mapharsen 320 mg.	26	76.9%	23.0%
300,000 units	79	82.1%	17.7%
300,000 units plus Mapharsen 320 mg.	24	91.6%	8.3%
600,000 units	109	88.0%	12.0%
1,200,000 units	62	90.3%	9.6%

CONGENITAL SYPHILIS

There is a long-held tenet among syphilologists that any woman who has had syphilis should be treated throughout each pregnancy. This is well borne out by the studies of Dippel (42) on fetal autopsy findings. Two of nine fetuses of Wassermann-negative mothers yielded spirochetes. It is not known how many slightly infected babies are born alive to Wassermann negative mothers. Miller (113) cites one case in which nothing was found until the child was nine years old. Dippel (42) found no spirochetes in fetal tissues prior to the eighteenth week of gestation. It is obvious from this that the syphilitic mother must receive therapy prior to the eighteenth week if her child is to be protected against syphilitic infection.

Arsenotherapy in pregnancy for the treatment of syphilis leaves much to be desired, as it is subcurative to the mother and, in five to eight percent of cases, fails to be wholly preventive to advancement of the disease in the mother, and involvement of the fetus. In addition, it is somewhat dangerous to both. A drug curative to both mother and fetus during pregnancy is greatly to be wished for.

In the treatment of the syphilitic pregnant woman,

if the fetus is already infected, curative doses of an antisyphilitic agent must traverse the placental membrane. This is often uncertain for arsenicals.

PENICILLIN IN THE PREVENTION AND TREATMENT OF CONGENITAL SYPHILIS

In an attempt to evaluate penicillin in the prevention of congenital syphilis, Lentz et al (97) treated 13 pregnant women with symptomatic early syphilis and two with early latent syphilis. Seven of these were reported upon after delivery, the maximum observation period being 316 days post-partum, the average being 84 days. One of these received a total of 2,400,000 units of penicillin in 50,000 unit doses, the others 1,200,000 units of penicillin in 25,000 unit doses, all administered intramuscularly every four hours for eight days. (One patient failed to receive the last two injections.) In all cases treatment was started between the fifth and eighth lunar months of pregnancy.

Lentz reports that all the infants born were full term at birth except one, and it appeared healthy. All were symptom free and seronegative at the time of the report, although three had had positive cord blood tests at birth. The titer of these three fell sharply and became normal in less than a month, with no tendency to revert to positive. In the period of observation only

three mothers became seronegative, and this at variable times, but all were clinically normal.

Lentz pointed out that the permeability of the placental membrane to penicillin was at that time unknown. More recently, Greene and Hobby (71) reported an experiment to determine placental permeability to penicillin. Four women in active labor were given penicillin within two hours of delivery. A comparison of maternal and placental penicillin serum levels showed that significant amounts of penicillin pass the placental barrier, although fetal blood levels were below maternal levels.

This passage of penicillin into fetal circulation is demonstrated clinically by Lentz' finding of no stillbirths or neonatal deaths in his series of 14 (half of whom had not delivered at the time of the report). This would be an unusual result if there had been no anti-syphilitic treatment.

Lentz (97) also reports on five cases of congenital syphilis in young infants treated with penicillin. There is no report on whether or not there was antepartum arsenotherapy. The plan of treatment was a total dose of penicillin of 16,000 to 19,000 units per pound of body weight, or 80,000 to 350,000 units total dosage.

This is in excess of that given adults per pound of body weight. With this regime two of the five died, neither of causes directly attributable to the therapy, but Lentz concluded that as with arsenotherapy, it may be wise to wait until a congenital syphilitic infant is at least two months of age before starting penicillin therapy, and to start with small doses and continue treatment for three or four weeks.

Moore et al (114) report briefly on 30 infants treated with 30,000 units of penicillin per kilogram, or roughly one-half to two-thirds of Lentz' dosage, that is, the equivalent to a total dosage of 1,200,000 units total in an adult, based on body weight. This group states that the results are analagous to early acquired adult syphilis, with rapid healing of lesions and less rapid reversal of serology. Platoux (121) reports that of 38 cases, 35 had four or more months of follow-up, and of these all but one were apparently cured of all manifestations. This one had only X-Ray relapse and a sharp rise in serological titer. His dosage corresponded to that used by Moore (114).

Stokes et al (133) report that interstitial keratitis presents rather equivocal though at times dramatically favorable results after penicillin treatment, but this is not as yet interpretable in relation to

time-dosage. Two of the treated cases were made worse. Other congenital lesions responded comparably well with late neurosyphilis; that is, the progress of the disease was arrested but damage already done was, of course, unimproved. Three of Stokes' cases of eighth nerve deafness showed no improvement.

Lentz et al (97) recommend that, in view of the demonstrably incompletely curative effect of a total of 1,200,000 units of penicillin in not less than ten percent of cases, twice this amount is a proper and presumably safe amount for the pregnant woman in good condition, with reduced individual doses for the first 36-48 hours. This amount will clear the mother of infective surface lesions and protect a good proportion of offspring from early or immediate manifestations.

It is to be remembered that the cases reported are early syphilis complicating pregnancy. There are as yet no known studies of latent syphilis of unknown duration complicating pregnancy treated with penicillin. Moreover, in the woman the ultimate test of cure of her syphilis will always remain her ability to give birth to normal children in subsequent pregnancies, even though no further antisyphilitic treatment is administered (97). Thus it will be several years before the

use of penicillin for treatment of such cases can be evaluated with reasonable certainty.

PENICILLIN IN THE TREATMENT OF LATE SYPHILIS

The published reports on the use of penicillin in late syphilis are few in number as yet, but include a fair number and variety of cases. Stokes et al (133) report the results obtained by eight groups of investigators, working entirely independently. Lacking precedent, each of the eight investigators groped his way into the problem. Most of the patients were treated in accordance with the dosage schedules which had been found by Mahoney et al (104) to be effective in early syphilis, and with regard for the physiological and pharmacological properties of penicillin in the body. Total dosages of 1,200,000 units or less, in 25,000 unit doses each three or four hours, were contrasted to 2,000,000 to 4,000,000 units, in individual doses of 25,000-50,000. Many types of late syphilis were treated, including 56 cases of paresis, and neurosyphilis of all forms totaled 123 cases. Some cases had previously been treated with fever or with arsenic and bismuth.

In neurosyphilis, judgment of improvement was made on the basis of reduction in cell count, total protein, intensity of colloidal test, complement-fixation test,

and clinical improvement. The results were, in many cases, spectacular, from both a clinical and serological standpoint. Stokes (133) commented that "Penicillin has distinctly beneficial serological effects on neurosyphilis, including early and late manifestations, not excepting tabes and paresis, and including asymptomatic neurosyphilis. Its action on gummatous manifestations of skin, mucosae and bones is striking and complete".

SYMPTOMATIC RESULTS

Craig et al (34) treated 74 cases of latent and central nervous system syphilis with large doses of penicillin. For each, 40,000 units were given each three hours, for 100 injections or a total of 4,000,000 units in 12½ days for latent, and twice that number for neurosyphilis, concluding with ten units of artificial fever accompanied by ten 60,000 unit injections of penicillin intravenously. The results were excellent and Craig feels that these schedules are optimal at the present time.

Of Stokes' thirty cases of paresis and taboparesis classed as simple demented, only six failed to improve, and one grew worse. Half improved fifty percent or more. The transformation in orientation, speech,

handwriting, encephalographic findings, disappearance of ptosis and violent headache, were often striking. As with every form of treatment for syphilis, deteriorated cases made no response, or at best, less than the others. Of ten patients with various forms of meningovascular neurosyphilis, six showed varying grades of improvement. Lesions of benign gummatous syphilis of skin and bones healed under a dosage of approximately 300,000 units in twelve to forty-six days with few failures. Cases with optic neuritis and iritis due to syphilis showed improvement.

A patient with a nodular syphiloderm of the nose was given a course of penicillin at the Mayo clinic by O'Leary and Herrell (119), in doses of 20,000 units twice daily for eight days, a total of 320,000 units. Three weeks after the course was completed, the skin over the area was normal except for slight residual pigmentation. There was no reversal of the positive serologic findings. There had been no other clinical findings. This is the only instance encountered anywhere of giving penicillin only twice a day.

LABORATORY AND PATHOLOGICAL RESULTS

Stokes (133) found that irrespective of the system of treatment used, and in all types of syphilis, penicillin causes a reduction of syphilitic reagin titer

in the blood in from fifty to sixty percent of late cases. Only five seroresistant cases were treated, and of these, one was made negative and four were improved. In neurosyphilis, Stokes observed that the commonest change is a drop in cell count and total protein. Although the work so far done is only suggestive, it appears that neurosyphilis with a low cell count has a less favorable prognosis under penicillin treatment than patients with a high cell count.

REACTIONS TO PENICILLIN IN SYPHILOTHERAPY

Apart from the reactions to penicillin described elsewhere and caused by allergy in the patient, or pyrogenic impurities in the drug, there are certain reactions to penicillin worthy of mention. These are usually a Herxheimer-like reaction or therapeutic shock. Mahoney et al (105) reported that 86% of 100 patients with early syphilis had a Herxheimer-like reaction on the first day of penicillin treatment. Leifer reported 87% reactions on a group of the same size, but in Moore's (114) larger series (1418 patients), there were only 59% with reactions. The symptoms consisted usually of fever alone, or an exacerbation of secondary syphilis, or both. The fever was usually mild, reaching about 103 F., but was occasionally higher. All ulcerative

and cutaneous lesions promptly receded, and there were no severe toxic reactions. A few patients showed exfoliative dermatitis, and this may be moderately severe, requiring several weeks to return to normal. Reactions other than these are infrequent and inconsequential, and consist of urticaria, erythema multiforme, generalized pruritis, herpes simplex, gastro-intestinal disturbances, or secondary fever. No therapy was required other than cessation of penicillin, or reduction in dose, until the subsidence of the untoward symptoms. There was apparently no loss of therapeutic effect.

Stokes et al (133) reports that therapeutic shock may occur in late syphilis under the usual rule that an active syphilitic process in a vital structure may be gravely and even fatally damaged by a large dose at the start of treatment. Twenty-three per cent of Stokes' late cases reported reactions interpretable as Herxheimer or therapeutic shock effects, most of them with fever, and this sometimes rose to 105.5 F. In a few cases there were more serious symptoms, as convulsions, mania, and hallucinations, and a few showed allergic manifestations or gastro-intestinal symptoms. Reactions in penicillin-treated late syphilis are, however, fewer than in early, and on the whole not serious.

In two of Lentz' (97) 14 pregnant women, threatened abortion, as evidenced by spotting and lower abdominal cramps, occurred, in one 18 hours, in the other 48 hours after the start of penicillin. The drug was immediately discontinued, but resumed in full dosage in 24 hours without a recurrence of symptoms. This, according to Lentz, could be considered a type of Herxheimer reaction occurring in a grossly diseased area and would possibly fall in the category of placental shock, occasionally seen after arsenotherapy without preparatory treatment in pregnant women with active syphilis.

RECOMMENDED DOSAGES IN SYPHILIS

The low toxicity of penicillin causes the problem of its use in syphilis, as in other conditions caused by susceptible organisms, to be one of utmost economy in the use of the limited supplies of a precious drug. Lentz (97) suggests that in the pregnant woman with early syphilis, a total dose of twice 1,200,000 units in eight days should be used, since the lesser quantity results in failure in about ten percent of cases. There should be reduced individual doses in the first 36-48 hours, to prevent placental or therapeutic shock. In early syphilis, there was a good response to the eight-day, 1,200,000 units schedule in many cases, and

since there is as yet no evidence that the spirochetes become penicillin fast, retreatment can be used in cases which do not respond to the first course. Moore's (114) results in combining mapharsen with penicillin deserve further study, especially while supplies of penicillin are limited. Leifer (96) holds the tentative opinion that it is best to use higher doses than the 1.2 and 2.4 million unit schedules, and that the future may reveal the need for prolongation of the treatment beyond seven and one-half days. Stokes (133) asserts that, offhand, there was no striking difference recognizable between the shorter time (three hours contrasted to four hour intervals) in the treatment of late syphilis, except the more frequent induction of Herxheimer reactions in the shorter interval schedule, and this could be avoided by reduction in the dosage the first 24-48 hours. He offers that the findings in late neurosyphilis suggest that good effects may be secured by less than the maximum dosage so far employed, as some patients with asymptomatic neurosyphilis can achieve almost normal spinal fluid on 1,200,000 units and completely achieve them on retreatment with the same amount. Pushing the patient "over the hump" to a partial self cure is a recognized principle with some aspects of late neurosyphilis.

This is in direct contrast to the statement of Craig et al (34) that 4,000,000 unit totals in latent, and 8,000,000 unit courses combined with fever therapy and still more penicillin in neurosyphilis appear optimal at present. Since penicillin is of such low toxicity, it would appear that no objection, except cost and unavailability of the drug could be raised to the more liberal dosages of Craig.

For congenital syphilis, schedules adjusted to body weight, but otherwise comparable to adult regimes, appear to meet the problem adequately.

A warning note is sounded by Hough (91) concerning the necessity of adequate treatment of syphilis. He recalls that almost immediately following the introduction of arsphenamine, there was an epidemic of early neurosyphilis in Europe, due to inadequate treatment. The cause of so many cases of neurorelapse was inadequacy of the treatment. Hough believes that if syphilis, early or late, is to be treated with penicillin, it should be done with a view to protection of the future of the patient as well as to arrest infectiousness. He reminds us that penicillin penetrates the cerebrospinal fluid inadequately. Some of the new drugs, as penicillin, may not completely sterilize the patient's tissues,

and by rapidly destroying many but not all of the spirochetes, thus may materially lower a very essential factor in cure--the patient's immunity.

The case of late syphiloderm (119) successfully treated at the Mayo Clinic, with 300,000 units total given in twice daily injections, causes one to wonder if perhaps the constant blood penicillin level so assiduously sought by most workers is not, after all, absolutely necessary.

The question of the route of administration of penicillin is in dispute among those treating syphilis as among other therapists. Barksdale (16), by determining quantitative blood penicillin levels on those patients treated with intramuscular injections every three hours, found that it was impossible to maintain a constant level of penicillin, and indeed for one-third of the time, there was no penicillin detected in the blood by any test. This made him think that the continuous intravenous drip might be the procedure of choice, and he states that by giving 2,080,000 units intravenously in 9 days, his group was able to maintain a blood level ten times as high as by intramuscular injection, with no relapses in 11 patients with primary or secondary syphilis, and no positive serological find-

ings beyond the 14th week. Barksdale, therefore, holds the opinion that the intravenous method may be superior. Stokes (133), however, in the treatment of late syphilis, reports that relapse appears to be more frequent after intravenous than after intramuscular administration of comparable doses. No conclusive statement can be made at the present time, but the proponents of the intramuscular route have numbers on their side at least.

RESULTS

There is one point on which complete unanimity of opinion seems to have been achieved by all investigators in the use of penicillin. This is the fact that the results, although encouraging, must be considered preliminary. It is of utmost importance that this fact be universally realized, and it will be extremely unfortunate if the lay press overstimulates the hopes of the public. Although immediate results are comparable with the best of other intensive treatment schedules, this by no means indicates that the Treponema pallidum has been completely destroyed throughout the body. Early recurrence or late sequelae may yet occur. The most careful observations and control over a period of perhaps twenty years before any conclusion can be

drawn concerning the ultimate effect. Dosage so far has been purely tentative. The optimum time-dosage relation has not been worked out. It is obvious then that the penicillin treatment of syphilis must be regarded as an experimental procedure for many years to come. New methods of administration may have to be devised. Penicillin excretion is known to be rapid, which necessitates repeated injections at intervals of three or four hours. In the future, this may be amended by slowing renal excretion or absorption from the injection site.

CONCLUSIONS

1. Penicillin, a new antibiotic derived from a mold, is remarkable for its potency, lack of toxicity and wide range of applicability. It is useful in some some conditions caused by bacteria which have not heretofore been amenable to any treatment, notably subacute bacterial endocarditis.

2. While practically all the experimental and clinical work done so far indicates that the parenteral is the route of choice in administering penicillin, recent studies have shown that, with increasing supplies, it may be possible to give penicillin orally.

3. Penicillin, since it is excreted very rapidly, must be given at frequent intervals, or absorption or excretion must be slowed, in order to maintain adequate blood levels. This is a problem on which much work is being done at the present time.

4. Dosage of penicillin may be computed with regard for rapidly bringing a disease under control, and without regard for possible toxic effects. Dosage varies with the causative agent, the degree of involvement,- and the route of administration. Since the cost of penicillin is still high, minimum effective doses are being sought for the conditions in which it is useful.

5. Penicillin is useful in many diseases caused by gram-positive organisms, in several spirochetal diseases, and in some Rickettsial infections. It has not

been definitely proved to be useful in most virus diseases nor in parasitic diseases, nor against gram-negative organisms except the Neisseria group. It is of no value against the toxins of bacteria, nor against conditions not of biotic origin.

6. Penicillin has been shown by preliminary studies to be strikingly effective against syphilis in all stages in short, intensive courses of treatment. It protects the fetus of an infected mother and causes remissions in neurosyphilis. The work so far done suggests that penicillin is probably the best antisyphilitic agent so far discovered. The final judgment of results must not be made, however, until many years have elapsed.

7. There is as yet no indication that penicillin therapy alone is capable of replacing diagnostic acumen, laboratory studies and general medical supervision.

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