University of Nebraska Medical Center
BREAKTHROUGHS FOR LIFE

University of Nebraska Medical Center
DigitalCommons@UNMC

MD Theses

Special Collections

1946

Oral administration of penicillin

Jack Samuel Debusk
University of Nebraska Medical Center

This manuscript is historical in nature and may not reflect current medical research and practice. Search PubMed for current research.

Follow this and additional works at: https://digitalcommons.unmc.edu/mdtheses

Recommended Citation

Debusk, Jack Samuel, "Oral administration of penicillin" (1946). *MD Theses*. 1378.
https://digitalcommons.unmc.edu/mdtheses/1378

This Thesis is brought to you for free and open access by the Special Collections at DigitalCommons@UNMC. It has been accepted for inclusion in MD Theses by an authorized administrator of DigitalCommons@UNMC. For more information, please contact digitalcommons@unmc.edu.

THE ORAL ADMINISTRATION OF PENICILLIN

JACK S. DEBUSK

SENIOR THESIS

PRESENTED TO THE COLLEGE OF MEDICINE,

UNIVERSITY OF NEBRASKA, OMAHA, 1946.

Introduction

The development of penicillin as a chemotherapeutic agent has long since passed the stage of preliminary investigation, although it has been only five years since the first clinical trials were made. At present, new methods of administration and new uses for the drug are being sought, while its therapeutic properties are being more critically analyzed and evaluated. The constant aim is to make this drug more adaptable in the hands of the average medical practitioner, and to determine its specific applicability in order that it may be administered less empirically.

A great step toward making penicillin a more usable therapeutic agent has been made in the recent investigations of the oral route of administration. A review of the experimental work performed, a discussion of the various problems encountered in this work, and a presentation of the conclusions which may be derived from the results of these investigations are the primary objectives of this paper.

Contents

DISCOVERY AND DEVELOPMENT OF PENICILLIN

The discovery of penicillin was preceded by many observations of the phenomenon of antibiosis. Pasteur and Joubert (82), in 1877, were aware that certain air-borne organisms inhibited the growth of the anthrax bacillus, and they suggested that this phenomenon might be of use in the treatment of infections. We now know that this inhibition of growth produced by certain micro-organisms is due to the elaboration by the antagonistic microbe of certain products which have definite chemical and biologic properties.

The first serious attempts to apply an antibiotic for the purpose of treatment were made by Emmerich and Loew (27) in 1899. These observers suggested that the products of Bacillus pyocyaneus could be used for treating anthrax and diphtheria; however, pyocyanase never has proved of important clinical value.

By this time we have all heard or read several accounts of the accidental manner in which Alexander Fleming (30) discovered penicillin. Any discussion of penicillin, however, would be incomplete without mention of this great scientist and his acute observation, which provided the corner stone upon which penicillin has grown to become the master drug of 1945.

A pupil of Sir Almroth Wright, Fleming had been studying the destruction of bacteria by leukocytes just preceding the first World War. (33) Later, during the war, while studying septic wounds, he noticed that, in general, chemical antiseptics were more destructive to leukocytes than to bacteria. Having previously demonstrated that leukocytes in pus possess a marked antibacterial activity, he realized the futility of using chemical antiseptics in treating septic wounds and, therefore, sought other agents which would be antibacterial without affecting leukocytes or other cells.

In 1922 Fleming (33) described lysozyme, a powerful antibacterial ferment which he found occurring in egg white and in human tissues and secretions. Then in September 1928, (30) while working in the laboratories of the Inoculation Department, St. Mary's Hospital, London, he observed during a routine examination of culture plates a clear area around a large colony of mold which was contaminating a culture of staphylococcus. His long years of experience and study of bacterial inhibitors prompted his interpretation of this phenomenon as an indication that the mold was producing some kind of bacteriolytic substance which caused the lysis of staphylococcus colonies nearby. Subcultures of this mold were grown in broth for one or two weeks and it was

found that the broth "had acquired marked inhibitory, bactericidal and bacteriolytic properties to many of the more common pathogenic bacteria".

The colony of mold, according to Fleming's description, is a "white, fluffy mass" which enlarges rapidly, and sporulates in a few days, the center becoming a dark green which darkens to almost black in old cultures. A bright yellow color appears and diffuses into the medium in four or five days. At times a reddish color can be observed in the growth.

The pH of the broth on which the mold grows varies from 8.5 to 9.0. Acid is produced in three to four days in glucose and saccharose broth, while no acid is produced in lactose, mannite or dulcite broth after seven days. Growth is best at 20 degrees C. and slow at 37 degrees C., with no growth occurring anerobically.

Fleming and la Touche, a mycologist, identified the organism as a member of the penicillium, most closely resembling Penicillium rubrum. However, a later examination of this organism by Thom*, at the request of Clutterbuck and his associates (18), revealed that it was not Penicillium rubrum, as Fleming had supposed, but a

* Mycologist in the U. S. Department of Agriculture at Washington, D. C.

strain closely related to Penicillium notatum Westling in the Penicillium chrysogenum Thom series.

The active agent of this mold was found to be readily filterable, and the name "Penicillin" was used by Fleming to denote "the filtrate of a broth culture of the particular penicillium with which we are concerned". He reported that this bacterio-inhibitory agent was very active toward Streptococcus pyogenes, pneumococci, staphylococci, gonococci and meningococci, and that it was less active toward Corynebacterium diphtheriae and Bacillus anthracis. He stated that it did not affect members of the coli-typhoid group and other intestinal bacilli, enterococci, Friedlander's pneumobacillus, Hemophilus influenzae and gram-negative cocci of the mouth. These observations were confirmed later by Clutterbuck et. al. (18).

The first use made of penicillin was the isolation of Hemophilus influenzae (Pfeiffer) and other organisms insensitive to penicillin, using the filtrate to inhibit contaminants. (30) Fleming later observed that any organism sensitive to penicillin is, in most cases, insensitive to tellurite, and vice versa. By combining penicillin and potassium tellurite, he isolated the enterococcus which is insensitive to both. (31)

Penicillin was found to be non-toxic to animals in enormous doses and non-irritant locally. It did not interfere with leukocyte formation or function to a greater degree than did ordinary broth, and it was suggested that it might be an efficient antiseptic for application to, or injection into, areas infected with penicillin-sensitive microbes. (30)

The next work done on penicillin was reported in 1935 by Roger D. Reid of Pennsylvania State College. (92) He found that a 1:100 dilution of a broth filtrate obtained from a strain of Penicillium inhibited sensitive microbes. He noted also the effect of light, gases and temperature upon penicillin, and attempted to isolate the active substance by distillation at low temperatures.

Five years later, Bornstein, (10) working at the Beth Israel Hospital, New York, made a report of the antibacterial action of penicillin in broth cultures, using a strain of Penicillium notatum obtained from Fleming. His observations and those of Reid (92) agreed with those of the Oxford workers published later. (15)

Ten years after the discovery of penicillin, the practical application of the substance had not been investigated. In 1939 and 1940, Dubos et al. (24)(25)(58)(59) published a series of reports on the antibacterial

effect of an extract of a soil bacillus against gram-positive cocci. This extract was divided into three active substances, the most active of the three being gramacidin. All three substances showed marked bacteriocidal activity against gram-positive organisms in vitro, but gramacidin proved to be toxic in vivo. The discovery of these substances is important in relation to penicillin because the reports of Dubos stimulated a renewal of interest in the potential properties of penicillin. The first workers who became active in developing penicillin were Chain, Florey et al. (15) who had been working on lysozyme in the laboratories of the Sir William Dunn School of Pathology at Oxford. They decided that a systematic investigation of the chemical and biological properties of substances produced by bacteria and molds would likely be very profitable, and began work at once.

In August 1940 the results of these investigators were reported in Lancet. (15) This report included details of the methods of purification of penicillin, and studies of the bacteriostatic properties of penicillin in vitro. It also contained information about the chemotherapeutic action of penicillin in animals. These workers used a fairly purified sodium salt of penicillin in

their investigations. They experienced great difficulty in producing enough of the material, nearly 100 liters of the mold brew being required to obtain enough penicillin to treat one patient. They first grew the mold in flasks, and later in large vessels when more material was required. To prevent inactivation of penicillin by contact with heavy metals, bedpans were used as containers in which to grow cultures of Penicillium notatum.

In 1941 these same investigators (3) presented a more detailed report of the early experiments and of the preparation of a fairly purified product of penicillin. This report contained the first actual data of the possible clinical value of the material. After much labor, enough of the material had been acquired for injection into a human being. Penicillin was given intravenously to five patients with staphylococcic and streptococcic infections, and by mouth to one baby with a persistent staphylococcic urinary infection. It was applied locally as drops to four patients with eye infections, in one of whom a penicillin ointment was also applied to a rash on the face. A favorable therapeutic response was obtained in all cases. Temperature fell and the general and local condition improved. Also spirits and appetite

of the patients were improved. Seven of these patients had serious infections in the treatment of which sulfonamides or other forms of chemotherapy or surgery had been tried without benefit. Two of them died. One of the two was not given penicillin until late in the course of the infection, and the supply lasted for only five days. The other patient died because of a ruptured mycotic aneurysm. The penicillin used contained only 40-50 Oxford units per milligram and, also, dosages were small and continued only 4-6 days.

This work provided very strong evidence that penicillin was deserving of much more study, and that it would likely be very valuable to the allied armed forces if it could be produced in large quantities. It was obvious, however, that the intensive bombing of England, which was going on at that time, together with the very limited personnel, would make impossible any large scale production of penicillin in England. Therefore, in the summer of 1941, through the sponsorship of the Rockefeller Foundation, Florey and one of his associates, N. G. Heatley, who devised methods of assay of penicillin in connection with the experiments at Oxford, visited the United States. (19) They were very anxious to stimulate interest in penicillin and also to secure help in large-scale production.

These men were very soon referred to Charles Thom, principal mycologist in the U. S. Department of Agriculture at Washington, D.C. Florey also consulted the Committee on Medical Research of the Office of Scientific Research and Development. Soon he and his colleague were taken to the Northern Regional Research Laboratory, Department of Agriculture, in Peoria, Illinois, to consult with a group of micro-biologists in the fermentation division under the direction of Robert D. Coghill. Members of the staff of this laboratory had had extensive experience in mold fermentations and it was thought that they were the logical investigators to begin work on penicillin. (19)

A short time later, Florey visited the laboratories of the Mayo Clinic where he consulted with W. E. Herrell and Dorothy Heilman, who had been working with penicillin since the early part of 1941. Heatley supplied Herrell with approximately 100 milligrams of penicillin, half of which had been prepared in the laboratories at Oxford and contained 42 units of penicillin per milligram; the other half had been recovered from the urine of one of the patients treated at Oxford, and it contained 20 units per milligram. (50)

Florey then returned to Washington for further

consultation with those connected with government agencies. Arrangements had been made with Dr. A. N. Richards, Chairman of the Committee on Medical Research of the Office of Scientific Research and Development for consultation with commercial drug houses interested in the development of penicillin. In the fall of 1941 Florey returned to England to continue his studies with his colleagues at Oxford. He later carried the experiences of the group to the battlefronts of North Africa and Sicily, and later still to our allies in Russia. Because of his great contributions to the war effort, he was knighted, as was Professor Fleming, by his Majesty, King George VI of England. (50)

There were several drug companies, Merck, Squibb, Pfizer, Abbott and Winthrop, who pioneered the development of penicillin in this country. They had begun experimentation on production methods when a second meeting was held in December, 1941. (19) Two difficulties were quite apparent at that time. One was that the drug houses were afraid to pool their information for fear of prosecution under the Sherman Anti-Trust Laws, and, secondly, the yields of penicillin were so low that it looked impossible to produce a kilo of crude penicillin, which was thought to be the minimum amount needed for

its proper clinical evaluation.

It was indeed fortunate that by that time A. J. Moyer, an associate of Coghill, (19) had found a way of increasing the yields of penicillin by at least tenfold. This consisted of adding corn steeping liquor to the medium upon which the mold grew. Coupled with his efforts in strain selection, the addition of this liquor increased yields of penicillin from two up to forty Oxford units per cc. Dr. Heatley of Oxford remained several months at the laboratory in Peoria and trained the workers there in the proper techniques involved in the cylinder plate method of assay which he had devised in England.

Besides the workers at Peoria, several other groups of recognized, competent clinical investigators were assigned the task of obtaining data regarding the clinical applicability and use of penicillin. (19) Under the chairmanship of Dr. Chester S. Keefer of the Evans Memorial Hospital in Boston, Massachusetts, the selected groups of investigators began their work on the clinical testing program. Dr. Keefer had the difficult job of deciding who should get penicillin and who should be denied. He had to keep in mind that it was fundamental that a comprehensive picture of the effectiveness of

the material be obtained, especially because of our military concern at that time. He examined all applications for penicillin and performed an excellent job of allocating the small available supplies of penicillin to types of cases where it was sure to be of value, and where accurate information of its use and effectiveness could be obtained. Such a program enabled the Committee on Medical Research to get the maximum amount of information with the very least expenditure of the precious material.

In August 1943, Keefer (63) tabulated the reports of 500 cases of various infections treated by 22 groups of investigators accredited to the above-mentioned committee. Since this was a great milestone in the development of penicillin as a chemotherapeutic agent, the conclusions of this report, which summarized all the essential clinical information on penicillin up to that time, are presented below:

> "Penicillin has been found to be most effective in the treatment of staphylococcic, gonococcic, pneumococcic and hemolytic streptococcus infections. It has been disappointing in the treatment of bacterial endocarditis. Its effect is particularly striking in sulfonamide resistant gonococcic infections.
>
> "While the dosage schedule requires additional investigation, it seems clear that the average patient requiring intravenous or intramuscular injections for serious staphylococcic infections requires a

> total of between 500,000 and 1,000,000 Oxford units, and the best results have been observed when treatment is continued for at least ten days to two weeks. At least 10,000 units should be given every two or three hours at the beginning of treatment, either by continuous intravenous injection or by interrupted intravenous or intramuscular injections.
>
> "Satisfactory results are obtained in sulfonamide resistant cases of gonorrhea following the injection of 100,000 to 160,000 units over a period of forty-eight hours.
>
> "Patients with pneumococcic pneumonia frequently recover following the use of 100,000 units given over a period of three days. This is especially important in sulfonamide resistant pneumococcic infections. It may be necessary to give between 60,000 and 90,000 Oxford units daily for four to seven days to get a maximum effect.
>
> "In the treatment of empyema or meningitis it is advisable to use penicillin topically by injecting it directly into the pleural cavity or the subarachnoid space."

The armed forces recognized the possibilities and potentialities of producing large amounts of penicillin, and Mr. Fred J. Stock, (26) chief of the Drugs and Cosmetics Section, a branch of the Chemicals Division of the War Production Board, urged industry to tackle this problem. During 1942 only experimental quantities of penicillin had been produced. Most of the construction program was carried out during the months of October, 1943 to March, 1944. Only 400 million units of penicillin were produced during January to June of 1943, but this was increased to a production of 9.1 billion units dur-

ing the month of December, 1943, with a total production for the year of 21,192,000,000. The production in March 1944 was estimated to be at least twice the total production in 1943, and for the year 1945 the production was expected to be three to four times that of 1944. (19)

In September, 1943, A. L. Elder was appointed as Penicillin Coordinator. At this time, also, the War Production Board's subsidiary, the Office of Production Research and Development, came into the picture, and with the assistance of L. A. Monroe established supplementary projects at the University of Wisconsin to study fermentation, and at Pennsylvania State College to improve recovery methods. (19)

In May, 1944, production of penicillin had reached sufficient quantity to enable the release of limited quantities for civilian use. (4) This was handled by the Penicillin Distribution Unit which was set up in Chicago, Illinois by the War Production Board. Hospitals placed orders for penicillin through this unit, and where possible the drug was dispensed immediately to those who ordered it. This plan was continued until March 15, 1945 at which time it was announced that producers and distributors could sell this drug through normal commercial channels. (4) Rapid sales were soon reported by

retailers. (6) The drug was moved from the wholesalers' warehouses first to the hospitals who had priority, and then to druggists for prescription sales. It was released in vials containing 100,000 units of sodium penicillin.

The supply of penicillin was fairly adequate in most communities and no shortages were reported until November, 1945. At that time it was reported (5) that there was apparently a definite shortage of penicillin for injection, and many physicians were asking the reason for this. It has been suggested (5) that manufacturers stop making any doubtfully acting mixtures and that a better quality of corn steep liquor be used until the shortage is alleviated. Export controls and directives giving hospitals first call on penicillin production have also been proposed. (5)

The development of penicillin, from both the standpoint of chemotherapy and production, represents a true modern miracle. Probably no drug has ever been so thoroughly investigated and received so much publicity in such a short space of time as has penicillin. All possible resources have been utilized, and many men and women have worked relentlessly to put this valuable, life-saving drug in the hands of both the doctor on the battlefield and the family physician. It has been used

empirically in many cases, but each day the medical profession is coming closer to a true evaluation of its many properties. The next step is the synthesis of the pure material. When this is accomplished, the production program will have been completed.

PRODUCTION OF PENICILLIN*

There are three main methods of producing penicillin, surface culture, submerged culture and bran culture. Several species of mold are used, although Penicillium notatum is the one commonly associated with penicillin. (19)

1. Surface Culture: This is the original standard method which was used for all the early production of penicillin. Spores of the mold are seeded on the surface of a culture medium and a heavy pellicle or mat of mold mycelium forms.

Larger yields are obtained when smaller containers are used, e.g. 200 cc. Erlenmeyer flasks, although in commercial production larger containers are used. This is due, in part, to the fact that in small flasks the depth of the medium is only 1 to 2 cm. and nutrients diffuse to the mycelium better. Commercially the yield per container is more important than the yield per cc. and the depths are increased to 3 to 4 cm.

On proper culture medium, yields of 200 Oxford units per cc. can be obtained in small Erlenmeyer flasks in 6 to 7 days, while commercial yields from bottles or pans vary from 50 to 100 units per cc. in 7 to 11 days.

*This section, "Production of Penicillin" has been taken almost entirely from a report by R. D. Coghill of the Fermentation Division, Northern Regional Research Laboratory, Peoria, Ill. See No. 19 in bibliography.

The main advantage of this method of production over the others which will be described is that contamination of the fermentation, if it occurs, does not destroy a whole run. Contamination, of course, by species of Pseudomonas, Bacillus subtilis, and even certain strains of Escherichia coli, results in destruction of the penicillin by the enzymes produced by these organisms. The very high labor cost incurred in this type of production is the most serious objection to its use. 100,000 to 125,000 bottles are required to process 10,000 gallons of medium, which requires several days in even the largest bottle plants.

2. Submerged Culture: Here the mold fermentation is conducted in large vats, where the mycelium grows in a submerged condition. It is necessary to aerate and agitate the culture in some manner, in order to prevent the surface growth which the organism prefers. To accomplish this, the shaken flask can be used, or the cultures can be placed in vats equipped with aerators and agitators.

The mold grows as small pellets instead of the large ones seen in surface culture, the size of the pellets varying inversely with the amount of inoculum used. It is interesting to note that the strain of P. notatum, NRRL 832, is only half as good as NRRL 1249.B21,

in surface cultures, the reverse being true under submerged conditions. This indicates the great strain variability of Penicillium notatum. In small shaken flasks, yields of 50 to 100 Oxford units per cc. can be realized in 4 to 5 days. In vat cultures, yields of 40 units per cc. in two days can be obtained consistently, and as high as 80 units per cc. on occasion.

As previously mentioned, the big advantage of the submerged method of operation is the saving in labor. However, the conditions under which the fermentation is run are ideal for the development of contamination, and any stray organisms which might enter at one place or another grow abundantly in the nutrient medium, with its essentially neutral pH, and necessary aeration. Also, a little contamination at the end of the run will completely eliminate the penicillin in a very short time. From this it is easily seen that the successful production of penicillin by this method is dependent upon the experience of the operators, and that one very slight slip may easily ruin a whole run of penicillin.

3. Bran Culture: This method consists in growing the mold on moist bran. The bran may be spread thinly in trays or processed in a rotary drum. It is first sterilized and then inoculated with a culture of Penicillium notatum. After 2 to 4 days' growth, the substrate

may contain as much as 200 to 400 units of penicillin per gram of dry bran. It is then placed in percolators and the penicillin is thereafter recovered, following its extraction by a suitable solvent.

Two outstanding difficulties are encountered with this method of production, namely, that bran is difficult to sterilize, and that it is likely to hold the heat produced during fermentation so that the temperature is elevated above 24 degrees C., at which point penicillin production occurs best. A big advantage of this method however, is that large amounts of penicillin can be produced with much less labor than is required in the surface culture method previously described.

4. Recovery Methods: The mold mycelium, or bran, is first removed by the centrifuge or by filtration, leaving a clear, yellow to brown solution. To prevent contamination, this broth must be protected by chilling, by the addition of a disinfectant, or by aseptic handling. Any slight contamination at this point will destroy the run as there is very little penicillin present. The usual content of 30 to 100 units per cc. is equivalent, roughly, to between 0.002 and 0.006 of 1 per cent of penicillin.

The pH of the broth is then set between 2.0 and 3.0 and all of the free organic acids are extracted into a

solvent such as ether, chloroform, or amyl acetate. Penicillin is very unstable at this pH so the extraction must be carried out at as low a temperature as possible, and as quickly as possible. The solution of the penicillin is then shaken out with aqueous sodium bicarbonate to obtain a solution of the sodium salt, or with a calcium base to yield the calcium salt.

Penicillin must be packed absolutely dry because of its fundamental instability in aqueous solution. However, extensive decomposition takes place if it is dried at ordinary temperatures, so it has to be frozen and dried from that state. This freeze-drying operation may be carried out in bulk or in the final ampule. The final product is a powder ranging from pale yellow to dark brown, and it generally contains 100 to 500 units per mg. It is rigorously tested for potency, sterility, toxicity and pyrogens, all of which is under the control of the Food and Drug Administration. (19)(64)

STANDARDIZATION OF PENICILLIN

The basis for the methods of assay commonly used for determining the potency of penicillin solutions is the inhibition of bacterial growth. As this paper is not intended to be primarily a discussion of the methods of assay of penicillin, a detailed description of the various technics will not be presented. However, a brief review of the methods and their variations as used by different investigators will perhaps give some idea as to how the work is done.

1. The Oxford Cup method was designed by Heatley and described in the original reports of the Oxford workers. (3) Heatley later described it himself in more detail. (47) In this method penicillin is allowed to diffuse out into agar plates from small glass cups, and the diameters of the zones of inhibition of bacterial growth are measured. The Oxford investigators (3) originally set up a standard--"a partially-purified solution of purely arbitrary strength. It is made up in dilute phosphate buffer, is saturated with ether, and is kept in the ice-chest. It gives an average assay value of 24 mm.". Their arbitrary unit, then, was "that amount of penicillin which when dissolved in 1 cc. of water gives the same inhibition as this standard". This was called the

"Oxford unit" and is at present used interchangeably with the term "Florey unit".

According to Kirby and Rantz, (67) the Oxford method is not satisfactory for clinical use because the concentrations of penicillin, especially in the blood, are not large enough to produce adequate zones of inhibition. This method, however, has been the one most generally used for routine assay purposes. (50)

Various modifications of the cup method have been made. Filter paper discs saturated with penicillin have been substituted for the cylinders by Vincent and Vincent, (99) while Bacillus subtilis has been substituted for Staphylococcus aureus as the test organism by Foster and Woodruff. (39)(41) The first alteration is claimed to save time, while the second is made because larger zones of inhibition are seen on the plate and the edges of the zone are knife-sharp, thus reducing the error in measuring the diameter of the zones.

2. Fleming (30) was the first to utilize the serial dilution method. In such a method, the smallest amount of penicillin which will completely inhibit bacterial growth in solid or liquid media is the standard with which unknowns are compared. Foster and Woodruff described a variation of this method in which a broth

dilution was used. (40) This method was alleged to have an accuracy of 85 per cent. A quick assay method, which was also a modified serial dilution technic, using hemolysis as an indicator, was described by Rake and Jones. (87)

The unit of penicillin was defined by Florey and Jennings, (35) who used another serial dilution method, as "that amount of penicillin which when dissolved in 50 ml. of meat extract broth just inhibits completely the growth of the test strain of Staphylococcus aureus". In other words, if the material contained one unit per milligram it would just inhibit the growth of the test strain of Staphylococcus aureus in a dilution of 1:50,000. If inhibition were complete at a dilution of 1:20,000,000, the activity of the penicillin would be 400 units per milligram.

3. In turbidimetric methods a range is selected in which bacterial growth is proportional to penicillin concentration, and the standard curve is constructed from turbidimetric measurements of the bacterial suspensions. In other words, growth in various dilutions of unknown penicillin samples is compared with a standard curve of inhibition, run daily side by side with the unknowns. The potency of the unknown is then computed with respect to the standard reference sample of established potency. (37)

Various modifications of this method have been suggested by Foster and Wilker, (38) by Joslyn, (61) and by Lee and associates. (69) Likewise, other turbidimetric methods have been described by McMahan (74) and by Holmes and Lockwood. (57)

Foster, (37) and Foster and Woodruff (40) described this method as the most accurate (less than 15 per cent error) means of determining penicillin concentrations. However the technic is rather difficult, and a great deal of experience is required to carry it out satisfactorily.

4. The tissue culture method of assay has been found by Heilman (48) to be a satisfactory method for determination of the amount of penicillin present in given samples. This method has the advantage of requiring small amounts of penicillin for performance of a large number of tests, and, too, very consistent results are obtained.

Methods of Assay Suitable for Determining the Presence of Penicillin in Body Fluids

While the methods of assay of penicillin previously mentioned are satisfactory for the titration of penicillin lots during production, and for some clinical studies, their accuracy is not sufficiently high to be used in studies of a chemical nature. Therefore, various methods

have been devised for more accurate determination of levels of penicillin in body fluids. The two most extensively used methods were described by Rammelkamp (88) and by Fleming. (32)

1. The procedure described by Rammelkamp has been used widely and is excellent for routine determinations. The test is said to be sensitive to concentrations as low as 0.0039 unit of penicillin per 0.2 cc. of the solution. The amount of unknown fluid required is small, (0.2 to 0.9 cc.). This procedure is another form of the serial dilution technic. Kirby and Rantz (67) have modified the technic to give a narrower range of dilutions, and consequently, greater accuracy.

2. The second method, described by Fleming, (32) is a modification of the Wright slide cell technic. This has been found by Herrell and Heilman (49) to be more reliable than the Rammelkamp method. However, Kirby and Rantz (67) state that the serial dilution technic is unquestionably the most suitable for routine clinical use.

3. The turbidimetric method of determining penicillin concentrations has gained in popularity (61)(69) since Foster's description. (37) However, attempts to

adapt turbidimetry to clinical use met with two serious difficulties, according to Kirby and Rantz. (67) (a) Turbidities and colors of body fluids made comparison with standards difficult, and (b) even with highly enriched nutrient broth there was a marked nonspecific stimulation of growth of the organisms, especially with blood plasma and chest fluid. Except for highly specialized research procedures the turbidimetric technic will probably be employed very little.

Other methods of assay of penicillin in body fluids have been described by Cooke, (20) Kakavas (62) and others, but since they are modifications of procedures already mentioned they will not be described here.

International Penicillin Standards

An international conference was held for the purpose of establishing standards for penicillin. (98) The following are paragraphs taken from the recommendations of that conference.

> "The International Unit equals the specific penicillin activity contained in 0.6 microgram of the International Penicillin Standard.
>
> "The International Penicillin Standard is a specimen of the pure crystalline sodium salt of Penicillin II or G." There is also an "International Penicillin Working Standard, the specific activity of which has been determined in relation to that of the International Standard. The International Penicillin Working Standard for general distribution shall, for the

present, consist of a calcium salt of penicillin.

"2.7 micrograms of the present International Penicillin Working Standard" shall "be accepted as containing one International Unit of Penicillin."

The final check on the commercial penicillin products is made as follows, according to a report by Keefer. (64) He states in his article that "Before distribution in interstate commerce, each manufacturer must submit samples of each batch of any penicillin product to the Food and Drug Administration for examination and must obtain a certificate showing that the batch complies with the applicable regulations".

PHYSICAL AND CHEMICAL PROPERTIES OF PENICILLIN

It is commonly known that in the free state penicillin exists as an organic acid which reacts chemically to form salts and esters. Abraham and Chain (2) showed penicllin to be a dibasic acid which is most unstable. Labile, free carboxyl groups are thought to be the main reason for its instability. Penicillin and its salts, when exposed to air, or when heated, lose activity and it has been shown (2) that bacteria contaminating preparations of penicillin out of the air will destroy activity.

Penicillin is very soluble in either, alcohol, acetone, ethyl acetate, amyl acetate, cyclohexanon and dioxane, while it is less soluble in benzene, chloroform and carbon tetrachloride. Chain (14) states that free penicillin is soluble in water up to 5 milligrams per cubic centimeter. It is unstable when treated with dilute acids, alkalies, primary alcohols, oxidizing agents and heavy metals, but reducing agents do not affect it so readily. At a pH range of 5 to 7 it is fairly stable, but is quite unstable below pH of 5 and above 7.

Sodium and Calcium Salts

The sodium and calcium salts of penicillin have been widely investigated, (3)(15)(51)(54) although sev-

eral other salts have been prepared. The sodium salt is very hygroscopic and is easily destroyed by changes in the hydrogen ion concentration of the surrounding medium, and by oxidizing. Also, heat, primary alcohols and heavy metals will alter it, necessitating storage of this salt at temperatures not higher than 5 degrees C. However, when the salt is dissolved in physiologic saline or glucose solution, it will remain potent at least 48 hours at room temperature. (66)

The calcium salt is not hygroscopic, (35)(36)(54) and can be stored, in the dry state, in sealed ampules and at room temperature for as long as six months without loss of potency. (54) It was thought by Clark et al. (17) that the calcium salt was unsafe for intramuscular and intravenous use because of certain toxic reactions following injections into experimental animals. However, studies carried out by Herrell and associates at the Mayo Clinic, (51)(52)(54)(55) and confirmed by Gyorgy and Elmes, (44) proved the calcium salt to be satisfactory not only for local use but also for the various forms of systemic therapy. It was shown to be even less toxic for cellular elements than the sodium salt.

Other Salts and Esters

Other salts of penicillin which have been prepared

include those of barium, (2) strontium (13) and ammonium. (56)(75) Meyer, Hobby and Chaffee (76) prepared methyl, ethyl, n-butyl and benzohydryl esters of penicillin, while Meyer, Hobby and Dawson (77) provided for reaction of the free acid of penicillin with the corresponding diazo compounds. These esters were found to be insoluble in neutral or slightly alkaline buffers, but very soluble in benzene, and could not be precipitated from chloroform-benzene solutions by dry ammonia. Activity in vivo was higher than in vitro, presumably due to hydrolysis of the esters with subsequent liberation of active penicillin. The importance of these esters is that they are not destroyed by gastric juices and therefore can be experimentally administered by mouth. However, the dose of methyl and ethyl esters required to protect experimental animals against certain infections is so high that it will kill fifty per cent of a group of animals to which it is administered. The problem now is to prepare higher esters which will prove less toxic, and which will retain antibacterial activity, in the hope that a form of penicillin suitable for oral administration can be produced.

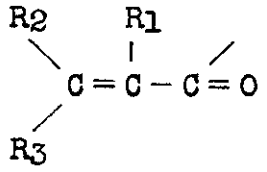
Effect of Heavy Metals Oxidation and Reduction

Heavy metals, such as copper, lead, zinc and cadmium greatly inactivate penicillin, while it is affected to a lesser degree by nickel, mercury and uranium. (2) The color of Fehling's solution is changed from blue to green, although not because penicillin reduces the solution. Potassium permanganate and hydrogen peroxide completely oxidize, and thereby stop all antibacterial action of penicillin. Reducing agents are not so effective in killing the power of penicillin as is shown by hydrogenation experiments carried out by Abraham and Chain. (2)

Chemical Analysis

The empirical formula $C_{24}H_{32}O_{10}N_2Ba$ (M.W.645), or $C_{23}H_{30}O_9N_2Ba$ was suggested by Abraham and Chain. (2) These men thought it very evident that there was one ketonic, two acetylatable and one latent carboxylic group. At least they found proof that penicillin possessed an extremely unstable chemical configuration. Holiday (2) made spectrographic examinations of the barium salt and concluded that it had a polysubstituted hydroaromatic ring structure, that the acidic groups were not conjugated with the chromophore responsible for the absorption, and that a trisubstituted alpha-beta

unsaturated ketone grouping was possibly present. He suggested the following formula and thought that R_1 and R_2, and R_2 and R_3 might each be cyclically combined.



Subsequently other investigations were carried out concerning purification and chemistry of penicillin. A strontium salt was used (13) and the formula $C_{24}H_{34}O_{11}NSr$ proposed. It was also pointed out (13) that when the molecule of penicillin was broken down, the following were formed: (a) a colorless, water-soluble acid which appeared on further hydrolysis to be a simple peptide, (b) a yellow insoluble pigment ($C_{16}H_{20}O_6$ or $C_{16}H_{18}O_5H_2O$), and (c) acetaldehyde, accompanied by very small amounts of and alpha-beta unsaturated aldehyde. These investigators found no carbon dioxide liberated during analysis of penicillin, and they suggested that the pigment was not a quinone. They thought also that the material titrated as a monobasic acid, but that it was more likely enolic in character.

The ammonium salt of penicillin was studied by Meyer and his associates. (75) Although this salt was fairly stable, the stability was increased by acetylation,

and they found that the free acids of the acyl derivatives formed fine needles which were about as active in vitro as the original substance. These investigators proposed the formula $C_{14}H_{19}NO_6$ or $C_{14}H_{17}NO_5$ plus H_2O. Their product was strongly dextrorotatory and possessed an absorption maximum on spectrographic examination of 2750 Angstrom units. In potency this penicillin was inferior to the preparation of Catch et al.,(13) the Oxford unit value being 240 units per milligram, while that of the strontium salt contained 500 units per milligram.

TOXICITY OF PENICILLIN

It seems that, in general, penicillin has been considered to be non-toxic. Fleming reported in his original work (30) that penicillin was no more toxic to animals to which he gave 20 cc. than was an equal amount of nutrient broth. The injection of 0.5 cc. into the peritoneal cavity of a mouse produced no toxic reactions, and no signs of irritation were noted in human conjunctiva which was irrigated with penicillin every hour for a day.

Later, the Oxford workers studied the toxic effect of penicillin on normal mice, rats and cats. Their preparation of penicillin was "purified only to the stage of recovering the sodium salt from a first ether extract", and contained probably less than four or five units per milligram. (3) Only negligible toxic reactions and temporary blood changes were produced by the injection of 10 mg. of this preparation in rats. No histological changes were found in any of the organs except "some evidence that the tubule cells of the kidney were damaged" in one rat. In the mice no toxic reactions were observable. No effect was noted on the blood pressure, heart beat and respiration of cats after the intravenous injection of 40 mg. which produced

a blood concentration of 1:5000 immediately after injection.

These same workers (3) however, found that a preparation of penicillin containing 40 to 50 units per milligram produced marked toxic reactions in a mouse and caused shivering attacks in afebrile human subjects, with a rise in temperature lasting about an hour. This preparation was found to contain pyrogens and when a more highly purified substance, containing 250-325 units per mg., was injected into four mice, no reactions occurred. (35) This indicates that more highly purified preparations of penicillin are much less toxic, or, that penicillin preparations may contain impurities which cause toxic reactions.

Gyorgy (44) used a calcium salt containing 230 units per milligram, in doses fifty times the therapeutic dose for human subjects, and found no toxic reactions in mice when it was injected intramuscularly or intraperitoneally. Doses of 160,000 and 400,000 units of the calcium salt in aqueous solution did not produce toxic effects in human beings, these doses being given by intravenous infusion over a twenty-four hour period. This was contrary to the findings of Florey and Jennings (35) who noted the calcium salt

to be toxic for mice. It agreed, however, with recent work of Herrell and Nichols, (54) who reported that the calcium salt is safe for injection in human beings.

Other workers, Robinson, (93) Hamre et al. (46) and Lyons, (72) also have considered that toxic reactions occur as the result of impurities contained in the preparations of penicillin, rather than through any toxic property of the drug itself. They all found that more highly purified material containing from 250 to 500 units per milligram was less toxic than the more crude preparations. The common reactions included chills, fever, headache, faintness, flushing of the face, eosinophilia and other minor miscellaneous conditions. A report of 500 cases by Keefer (63) indicated that reactions were observed in 69 cases, 13.8 per cent, with essentially the same complications as shown above.

Lyons (72) reported that urticaria was the commonest single complication in a series of 209 cases. This reaction was found in 5.7 per cent of the cases, and was thought to be due to toxic impurities.

Penicillin itself, however, has not escaped blame for producing some toxic reactions. Recent literature has contained many reports of such manifestations as

urticaria,(63) fever, thrombophlebitis, (7) gastro-intestinal reactions, (79)(97) contact dermatitis, (8)(9)(86) vesicular dermatitis (43) and bullous dermatitis (dermatitis medicamentosa). (80) In an excellent article, Cormia, Jacobsen and Smith (21) reported a study dealing with reactions in some 2,000 soldiers receiving prolonged courses of penicillin. The reactions were classified as follows: 1. urticaria, complicated by (a) angioneurotic edema, (b) shock, (c) convulsions, and (d) psychotic depression; 2. serum sickness-like syndrome; 3. acute syncope; 4. transient miliaria-like eruptions; 5. erythemato-vesicular eruptions, at times simulating dermatophytosis; 6. erythema nodosum; and 7. epididymitis.

These men (21) stated that the reactions to penicillin were of two main types, "those appearing shortly after first exposure to the drug and those occurring at a later date as a result of developing sensitization". The reactions were seen in about 0.5 per cent of those patients who received penicillin, and were of both early and late types, being due to an existing hypersensitiveness or to a developing sensitization, respectively.

It was concluded that both types of reaction may be severe enough to require discontinuation of the peni-

cillin therapy. Also, these workers found that a previous acute fungus disease may be the basis of increased reactivity to penicillin.

Regarding the mechanism of reactions to penicillin, Cormia et. al.(21) stated that "it appears that the principal shock tissue in penicillin reactions is in the blood vessels rather than the epidermis, although the latter may be sensitized in some instances". The fact that the epidermis can become sensitized is indicated by reports of other workers also. (65)(86)(96) Erythema is very prominent even in the vesicular reactions,(21) and the involvement of deeper blood vessels may be the precipitating factor in the reactions complicated by shock.

Fineberg, (28) prepared an extract of Penicillium spores and tested to see if patients who had clinical reactions to penicillin were sensitive to this extract. These patients were not sensitive to the spores. To further test commercial penicillin for toxic products, Silvers (96) used a patch test, obtaining a positive reaction with commercial penicillin and a negative test with crystalline penicillin. This tends to confirm the impression that impurities in commercial penicillin are responsible for the toxic reactions obtained. However, Pyle and Rattner (86) obtained a positive patch test

when crystalline penicillin was used. The toxicity of the corn steep liquor in which Penicillium is grown has been tested by the use of skin tests, and this has shown (86)(65)(10) that such extracts of corn are not the cause of the reactions.

Whatever may be the cause of the toxic reactions to penicillin, the essential thing for the ordinary clinician to remember is that such reactions do occur, although they are relatively uncommon, and that they may be severe. In due time the mechanism of these reactions will be found and means of avoiding them described. Meanwhile, in cases where toxic symptoms do occur, penicillin must be administered judiciously and stopped if necessary.

THE ORAL ADMINISTRATION OF PENICILLIN

The difficulties encountered in the administration of penicillin by the parenteral route are well known to those who have ever administered this drug. To receive intramuscular or intravenous penicillin therapy the patient must be hospitalized. Once he is in the hospital, he must receive frequent doses in order that adequate levels of penicillin are established in his blood. This requires the time and skill of some attendant, and even with regular doses there is often a gap of one half to one hour (12) during which effective blood levels are not maintained. Also, there is a certain amount of discomfort to the patient associated with the injection procedure, and it has been pointed out (72) that thromboses occur with relative frequency in continuous intravenous therapy.

These and other inconveniences of injecting penicillin have stimulated a search for some other, more practical means of administering the drug. Obviously, the oral route has been the logical consideration. However, all early investigation was seriously hampered by the lack of penicillin available for experimental purposes. This made the study of preparations for oral use doubly

difficult because most of the orally administered dose was found to be destroyed by the acidity of the stomach, (3)(89) and as a result it was considered impractical at that time to try to evaluate a route of administration which obviously involved a waste of the valuable material.

Nevertheless, the small amounts of penicillin recovered from the urine, together with the demonstration of even a limited activity in the blood, indicated that some absorption did take place, (3)(53)(89) and since then, as more penicillin has become available, more experimental work has been done. With the present supply of penicillin so greatly increased, it now seems logical that a re-evaluation of the oral route of administration should be made.

Problems Faced by Investigators

Research on any problem is directed toward the answering of one or more specific questions. The investigation of the oral administration of penicillin has been no exception to this, and the following is a list of questions which have been considered and answered, wholly or in part, by those workers in this field.

(1) Can penicillin be protected against inactivation by the hydrochloric acid of the stomach?

(2) Can penicillin be absorbed into the blood from the gastro-intestinal tract if it escapes destruction by gastric acidity?

(3) If so, can it be absorbed in sufficient amounts to establish and maintain adequate blood levels?

(4) Is penicillin toxic in the large doses required for oral therapy?

(5) Is the cost of penicillin prohibitive when the drug is given by mouth?

(6) What are the advantages and disadvantages of the oral administration of penicillin?

An attempt is made in this paper to present in considerable detail the experimental work done by those interested in oral penicillin. Following this there is a summary and some speculation as to the present answers to the preceding questions.

Laboratory and Clinical Studies

First Use of an Antacid (sodium bicarbonate) and an Enteric Coating

Abraham and his co-workers (3) made the first attempt to give penicillin orally. They found that the substance was rapidly destroyed by the hydrochloric acid of the stomach, and they were not particularly successful in raising the pH of the gastric contents by using sodium bicarbonate. However, although they did not obtain adequate concentrations in the blood after oral administration, they did recover satisfactory antibacterial amounts in the urine, as was proven clinically by the successful treatment of a urinary tract infection in a six month old infant. These workers also suggested the use of capsules to protect penicillin from gastric acid, but abandoned this method after unsuccessful attempts with phenyl salicylate coated vehicles.

In 1942 Herrell, Heilman and Williams (53) stated that penicillin was destroyed by the acid of the stomach but that some absorption occurred when sodium bicarbonate was given conjointly. Staphylococcic infections in mice, however, were cured by penicillin given in combination with sodium bicarbonate. (85)

Free et al. (42) in 1944 pointed out that less antibiotic activity was demonstrable in the urine after penicillin was given orally together with sodium bicarbonate than when it was given without the bicarbonate. This indicated to these workers that less penicillin was excreted due to a decrease in absorption. The latter was not definitely explained. They mentioned that perhaps the sodium bicarbonate decreased the emptying time enough so that the penicillin was destroyed. They suggested also that the alkaline urine which was excreted following the ingestion of sodium bicarbonate might have caused a destruction of the penicillin while the urine was in the bladder. This, of course, might have been eliminated as a possibility if a retention catheter had been used. Little and Lumb (71) gave the same dose of bicarbonate to a patient with achlorhydria to see the effect of over-alkalinization and found that there was a normal excretion of penicillin, indicating that the alkaline pH did not destroy the penicillin.

Penicillin in Tap Water

Two reports by Rammelkamp and his associates (89)(91) in 1943 confirmed the impression that oral administration was unsatisfactory. These workers administered penicil-

lin by the oral, duodenal and rectal routes. For their experiments they used a solution of 200 cc. of tap water containing 10,000 to 20,000 units of penicillin. Three subjects were given this solution orally after a twelve hour fast, one of them being given 4 grams of sodium bicarbonate ten minutes prior to the ingestion of the penicillin solution. Their results lead them to the conclusion that after oral administration of penicillin absorption was poor. The results after intra-duodenal administration were better, approximating those levels obtained after intra-muscular administration. Rectal administration was unsatisfactory, probably due to the inactivation of penicillin by Escherichia coli in the colon. (89)

Achlorhydria and Oral Penicillin

These same investigators gave a solution containing 1,000 Oxford units of penicillin per cubic centimeter orally to two patients with achlorhydria associated with pernicious anemia, and found that the blood levels rose to heights corresponding to those obtained after intramuscular or intra-duodenal administration. They indicated also that penicillin was not inactivated by saliva, bile or succus entericus.

Cellulose Hydrogen Acetate Phthalate

Cellulose hydrogen acetate phthalate was tested as an enteric coating by the Floreys, (36) but it was found that reliable therapeutic levels could not be assured, even though penicillin could sometimes be demonstrated in the blood. These workers showed further that administration by means of the duodenal tube was unreliable.

Penicillin in Oil

Early in 1945, Libby (70) made solutions of penicillin in various oils, the best of which was cottonseed oil, in which a concentration of 7,500-10,000 units of penicillin per cc. of oil was obtained. These solutions were unstable so he prepared dispensions or dispersions of salts of penicillin in various oils and found them stable at room temperature for two to three months without the loss of any penicillin activity. These results agreed with those of Romansky. (94)

To test the effectiveness of such a dispersion, Libby (70) administered 90,000 units of sodium penicillin dispersed in cottonseed oil in a single dose to an 86 kilogram man, and took urine and blood samples at intervals to determine the respective levels of

penicillin. The first urine sample taken 25 minutes after administration of the penicillin contained 0.4 units of penicillin per cc. This indicates a fairly rapid passage of penicillin in oil through the stomach and absorption from the intestine. Maximum amounts of penicillin were found in the urine during the first two hours, decreasing to 1.8 units per cc. eight hours after administration. Blood levels, determined by a method of turbidimetric analysis developed by Libby, (70) were found to be 0.05, 0.04, 0.04, 0.02, and zero units per cc. from the 1, 2, 4, 6, and 8 hour bleedings respectively. In cases where subsequent doses of 20,000 units were given at three and six hours after the initial oral dose of 90,000 units, levels between .03 and .07 units per cc. serum were maintained for 7 hours, and only slightly less than the therapeutic level at 8 hours.

These workers were assuming that the blood levels of from 0.03 to 0.06 units of penicillin per cc. of blood, as indicated by other investigators,(55)(91) were sufficient for therapeutic purposes. On this basis, their work showed that a single dose of 90,000 units given orally in their dispersion in cottonseed oil would produce therapeutic levels in the blood. When compared with the blood levels obtained by in-

tramuscular injection, (91) it is easily seen that, when using doses of 20,000 units of an aqueous solution of penicillin, two or three, or possibly more injections would be required to maintain a comparable blood concentration over the same period.

Penicillin Altered in Passing Through the Body

To determine whether there was any change in the sensitivity of penicillin to pH and to heat by passage through the human body, Little and Lumb (71) devised an experiment in which the urines of twenty-five patients receiving intramuscular injections of penicillin were divided into aliquot portions and diluted so that each one contained a certain amount of penicillin. These were then compared with similar samples of the original penicillin solution with the result that showed the antibacterial substance in the urine to be more resistant to changes in pH and to heat than the unaltered penicillin.

Penicillin in Raw Egg

On the basis of the above experiment, these investigators (71) thought that perhaps they could stabilize penicillin outside the body. A former associate, Lt. Colonel R. J. V. Pulvertaft, had previously rendered penicillin salts more stable to heat by dissolving

them in various chemical mixtures, and had suspected sulfur to be the important element, so these men tried eggs and milk, having no suitable pure chemicals available.

They first determined the range of activity of penicillin at various pH levels and found the maximum activity between pH 4.6 and pH 8.0. Previous workers (38)(40) had found an optimum pH of 5.8 for penicillin activity. Following this, penicillin was mixed with various diluents and the pH was altered to determine the activity at various levels. Also, the solutions were incubated at different temperatures to learn the effect of heat. Finally the solutions were titred, and it was shown that the least damage was suffered by the penicillin dissolved in raw egg, indicating that some substance present in the egg protected the penicillin against the acid medium.

When volunteers were given a dose of alkali, followed by penicillin in egg, the bacteriostatic activity of their blood rose to satisfactory levels. The serum levels were not quoted in the report made by these men, but they presented a graph showing the relation of intramuscular and oral administration by comparing the bacteriostatic activity of the serum after each of the two

methods. This graph showed a typical sudden rise in the activity with a peak at about 15 minutes after administration with the intramuscular dose of 15,000 units. After oral administration, the bacteriostatic activity rose steadily to a peak two thirds as high as that obtained by the intramuscular penicillin at 30 minutes, and continued at about this same level for at least three hours. This was tried clinically in a few cases of tonsillitis, and the oral method of administration was found successful.

Magnesium Trisilicate Buffer

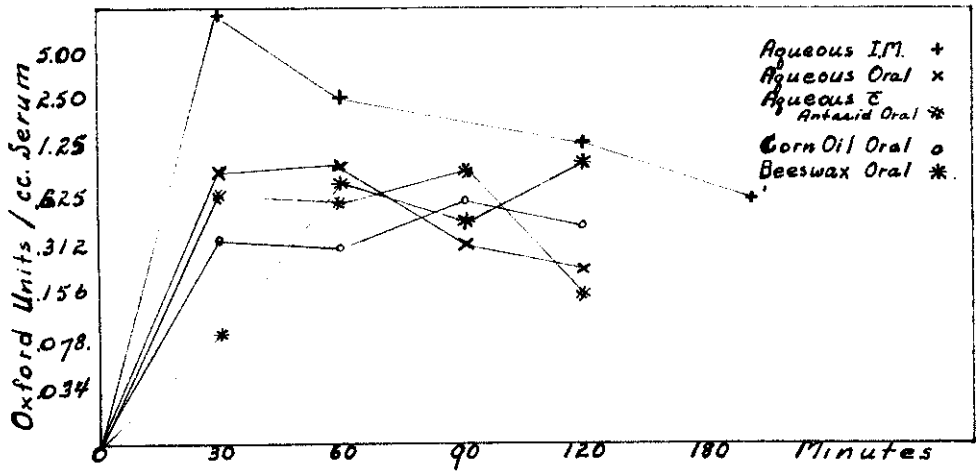
McDermott, Bunn et al. (73) used a 4 gram dose of magnesium trisilicate as a buffering agent and gave it just preceding the ingestion of penicillin in the following mixtures:

Penicillin in corn oil.
Penicillin in water.
Penicillin in water, preceded by a buffer.
Penicillin in peanut oil and 4 per cent beeswax.

All patients were kept in a fasting state during the period of observation and assays were made by the Rammelkamp method. Doses of penicillin were 315,000 units.

The results of these experiments are indicated by the following graph taken from the report given by these workers. It shows a comparison between oral

and intravenous methods of administration, and also the different levels obtained by the various mixtures ingested by the subjects.



Oral Administration of Penicillin
Comparison of Four Methods

The serum concentrations ranged from .312 to 1.25 units of penicillin per cc. of serum at 30 and 60 minutes respectively, and it is interesting to note that about the same concentrations were obtained regardless of whether the penicillin was given in one vehicle or another. The height of the penicillin concentration at two hours after the ingestion of the oil and oil in beeswax suggested to these investigators that the duration of penicillin action may be prolonged by the use of these vehicles by mouth in a manner similar to the prolongation which Romansky and Rittman have shown follows the administration of penicillin in oil and

and beeswax by the intramuscular route. (94)

High Concentrations

Using high concentrations of penicillin, 20,000 units per cc., Moses (81) obtained therapeutic effects by the oral route, but estimations of the blood levels were not made.

Trisodium Citrate

Trisodium citrate was found by Charney, Alburn and Bernhart (16) to be a suitable buffer for the administration of penicillin by mouth. The work of Rammelkamp and Keefer (91) had indicated that only very samll amounts of penicillin can be detected in the urine after oral administration, and it had been shown (89) that sodium bicarbonate given with penicillin would increase the urine levels very little. Therefore, after Charney et al. (16) had shown that there was a considerable increase in the urine levels after using trisodium citrate with the penicillin, Gyorgy and his co-workers (45) proceeded to make clinical trials to see the actual effectiveness of this preparation.

Since the rapid cure of gonorrhea by injected penicillin gave a reliable basis of comparison, this disease offered the best approach for the therapeutic evaluation of penicillin when given by mouth. For this

reason, 18 male adults and 5 children were treated with oral doses varying from 10,000 units every three to four hours in children to 15,000 to 40,000 units every three hours in adults. This dosage was maintained for two to three days in combination with trisodium citrate, 1 to 5 grams per dose. Cure was achieved in all these cases in one to three days, but in three girls, 2 to 5 years, 10,000 units of penicillin given every four hours with 1 or 2 grams of the buffer gave only temporary clinical cure of gonorrhea. These cases were promptly and permanently cured by the same dosage of penicillin given intramuscularly after the oral procedure had been repeated twice. These failures were thought to have been caused by too low doses, or by too short a duration of treatment. (Their total dose orally was 200,000 to 300,000 units.)

The highest blood level obtained by Gyorgy et al. (45) was 0.2 unit per cubic centimeter after one hour, while the lowest was 0.02 unit per cubic centimeter. The dose given was 40,000 units. As a rule, considerably higher blood levels were obtained in those patients who received trisodium citrate with the penicillin. These workers did not present figures for the urine levels obtained in their investigations, but such figures would likely have been relatively high. In this con-

nection, it might be mentioned that Charney and his associates (16) obtained urinary excretions of 18 per cent of the dose administered using trisodium citrate, Free and his co-workers (42) found 8 to 33 per cent of the dose in the urine without using a buffer, and an average excretion of 8.6 per cent was reported by Rammelkamp (89) who also found no significant increase in urinary excretion by the use of sodium bicarbonate. Gyorgy et al. (45) stated that it is known that penicillin is sensitive not only to acid but also to alkaline reaction, and emphasized the importance of having a buffer salt with a buffering range not reaching alkaline pH values.

Double, Treated Capsules

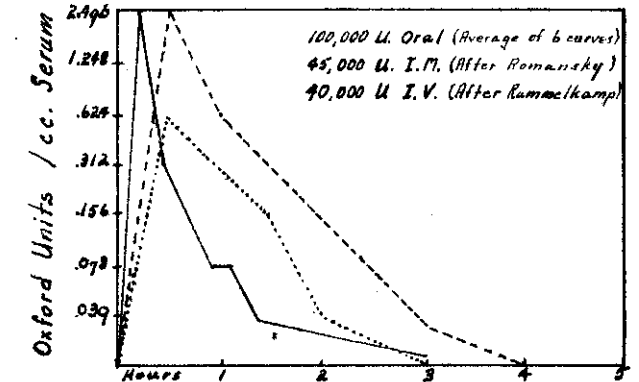
Burke, Ross and Strauss (12) placed powdered penicillin in specially treated capsules to be given orally. The powder was used instead of saline solution because the latter caused disintegration of the capsule. The capsule was subjected to a preliminary hardening by immersion in a solution of formaldehyde (USP diluted 1:20) and alcohol (95%), and were shown to be stable for one to two hours in aspirated gastric contents. In addition a double capsule was used. 100,000 units of sodium penicillin was placed in the

capsule. To further decrease the influence of gastric acidity, two tablets of aluminum hydroxide were swallowed by each subject one-half hour prior to taking the penicillin capsules.

In a series of experiments carried out by these men, a characteristic common in all instances of penicillin given by mouth was the rapid rise in the blood level during the first half hour. They concluded that this was due to rapid absorption from the duodenum. In two experiments when 200,000 units of penicillin was ingested, initial levels at the end of a half hour were 9.984 units and 2.496 units, the penicillin having been taken in the first instance two hours before a meal and in the second case one-half hour after a meal. Subsequent levels taken at hourly intervals showed a moderately rapid fall closely paralleling each other in these two cases. Considering these results, it seems that it would be advisable to give penicillin orally at suitable intervals before meals. These men emphasized this point by stating that the capsules probably reach the duodenum more quickly when passing through an empty stomach, and that a high fluid intake and a low fat diet might well be given to further passage of the penicillin through the stomach.

A similar initial rapid rise during the first half hour was found in six other cases in which 100,000 units of penicillin in capsules was taken. Therapeutic levels of penicillin were assayable at the end of two hours in all cases as compared with therapeutic levels present after five and one-half hours and four hours in the two cases given 200,000 units as previously mentioned.

In their report, Burke, Ross and Strauss compared the levels of penicillin in the blood with those levels reported by Romansky, (94) who experimented with intramuscular injection, and with the levels obtained by Rammelkamp (91) after intravenous administration. The chart presented below is taken directly from the report of Burke et al.



Comparison of Penicillin Blood Levels after Oral and Parenteral Administration

Rammelkamp (91) found a rapid rise in blood level, the

highest point being reached within the first 15 minutes. This was followed shortly by a sharp fall, only a trace being detectable after two hours. An intramuscular dose of 45,000 units usually produced the highest level about one-half hour after administration, with detectable levels present at two to three hours.

Regarding therapeutic blood levels, it was found by Rammelkamp and Keefer (90) that the degree of antibacterial action was directly related to the concentration of penicillin in the serum. Against Streptococcus hemolyticus, the maximum effects were found to be in concentrations from 0.019 to 0.156 units per cc. of serum. However, at least 0.156 units were required for a maximum effect against Staphylococcus aureus. Burke et al. concluded after their experiments that the concentration and prolongation of penicillin in the blood is more or less directly proportional to the oral dose employed, and that 100,000 units administered every three hours in capsules as they gave it would insure adequate levels in the blood for most susceptible infections. They stated that they believed it questionable whether concentrations above 0.312 units per cubic centimeter would have any added antibacterial value. In contrast to McDermott (73) who recommended an oral dose five times as large as the amount used in intramuscular therapy, these men found an

oral dose of only twice as much as that required for intramuscular administration. They thought this was due to the mechanical protection afforded the penicillin by the double capsule, and they believed that the effect of the antacid which was given with these capsules could not be evaluated from these experiments. It seems, however, that this antacid, aluminum hydroxide, may have had more to do with protecting the penicillin from the hydrochloric acid than was indicated in the report of this work. This probability seems even more real when the work of Welch, Price and Chandler (100) and that of Howard and Scott (60) are considered.

At any rate a clinical trial of this method of protecting and administering penicillin in 10 children with gonorrhea, 2 with pneumonia and 2 with cellulitis resulted in prompt recovery. (12) These trials were presented in a later report by these same men, and in the same report it was shown quite definitely that the administration of a 100,000 unit penicillin capsule every three hours provided constant therapeutic penicillin levels in the blood.

Aluminum or Magnesium Hydroxide

As suggested previously, Welch, Price and Chandler (100) administered by mouth penicillin adsorbed on either

aluminum or magnesium hydroxide. The preparation was made by dissolving the sodium salt in 20 cc. of water to which had been added dropwise with constant agitation, 30 cc. of U.S.P. aluminum hydroxide. This dose was given to 11 normal subjects two to three hours after breakfast and an adequate blood level (0.3 units per cc. serum) was obtained 30 minutes after ingestion. This level, however, fell rapidly after 30 minutes.

Succeeding this, four equal doses of 25,000 units each of penicillin-aluminum hydroxide were administered to 21 subjects at two hour intervals. One-half hour blood levels after each ingestion ranged from 0.147 to 0.27 units per cc. of serum, and following each dose there was a pronounced increase in blood concentration over the previous level. Moreover, blood levels of from 0.03 to 0.19 units per cc. were maintained for as long as twenty-four hours after the administration of 100,000 units in four doses of 25,000 units each using the penicillin-aluminum hydroxide combination.

The average total urinary excretion over a twenty-four hour period following four oral doses of 25,000 units of penicillin with aluminum hydroxide in 11 persons was 7.2 per cent. This indicates that the penicillin is largely inactivated within the body. These workers suggested also, that there seemed to be some absorption

of penicillin directly from the stomach into the blood stream. Then, following this, the penicillin-aluminum hydroxide passes into the upper intestine, where penicillin is released slowly from the aluminum hydroxide and adsorbed through the walls of this organ, thus reaching the blood stream via the portal system and the liver.

This work was corroborated by Howard and Scott (60) who gave 100,000 units of penicillin mixed with aluminum hydroxide in four doses of 25,000 units each at intervals of two hours to a normal subject. Levels of 0.25, 0.50, and 1.00 units per cc. of serum were obtained at 1, 1½, and 3 hours after ingestion. These investigators assigned a therapeutic level at 0.3 units per cc. of serum.

Using penicillin buffered with sodium citrate, these same authors found that an oral dose of 20-40,000 units did not produce a blood level over 0.2 units per cc. of serum, but that when the dose was increased to 60,000 units an adequate blood concentration was reached (more than .29 units per cc. of serum). Comparing the two methods of administering penicillin, however, they concluded that the aluminum hydroxide provided better levels than did the sodium citrate. They further recommended that the penicillin be given 1-3 hours after meals.

Basic Aluminum Aminoacetate

A new antacid, basic aluminum aminoacetate was studied by Krantz, Evans and McAlpine (68) who found that when they treated artificial gastric juice with this substance they could protect the activity of penicillin placed in the same juice. When penicillin was treated with this artificial gastric juice without the benefit of the antacid, it was completely destroyed, while it retained 50 to 70 per cent of its original activity when the juice was treated with the antacid. To test this clinically, 12 persons were given 100,000 units of penicillin mixed with 3 grams of basic aluminum aminoacetate, suspended in 100 to 150 cc. of water. The dose was given in the morning on a fasting stomach and serum levels were determined at 2, 3, 5 and 7 hours after administration using the method of assay proposed by Rake and Jones. (87) Levels of .39, .68, .37, and .17 units per cc. serum were attained as average figures. Both these workers, and Paul and his co-workers, (83) successfully cured gonorrhea with 100,000 units of penicillin using aluminum dihydroxyaminoacetate as an antacid.

Phenyl Salicylate

Moldavsky and Hesselbrock, (78) also using 100,000 unit doses, produced blood levels of 0.75 unit per cc.

after coating the penicillin capsules with phenyl salicylate. This contrasts with the failure of phenyl salicylate reported previously by Abraham et al. (3)

Triisopropanolamine, Trisodium Citrate Sodium Carbonate

In a very recent report, Cutting et al. (23) made extensive trials of administering penicillin by mouth with various enteric coatings and adjuvants, with the thought of either protecting penicillin or of promoting its absorption. They found that mixtures of penicillin with triisopropanolamine, trisodium citrate or sodium carbonate enclosed in a resin-cellulose plastic enteric coating were useful combinations. By using a dose of 50,000 units, administered every two hours for ten doses, they produced penicillin blood concentrations of from 0.02 to 0.05 unit per cubic centimeter. They were able to cure 38 out of 53 cases of acute gonorrhea with several of their most promising combinations, and concluded that if a total dose of a half million units were prepared in one of the three superior mixtures, it might well be expected to cure probably 90 per cent of cases of acute gonorrhea in the male.

In an earlier experiment, (22) these men had coated capsules with a mixture of "Enterab" and cellulose

hydrogen acetate phthalate containing 50,000 units of penicillin, mixed with the following different agents. These capsules were given every two hours for 20 hours and the following blood concentrations determined:

1 gram acetylsalicylic acid plus 1 gram sodium bicarbonate	0.02 on alternate hours.
8 cc. triisopropanolamine	.0045 u/cc
2.5 cc. ethyl alcohol	.02 u/cc
2.5 cc. acetone	.03 u/cc

One patient was cured of gonorrhea with each of these four.

Comparison of Several Buffers and Stabilizers

It has been reported by Finland and his associates (29) that ordinary commercial penicillin powder when given in saline solution before a meal gives levels which are at least as high and as well sustained as the various preparations tested which contained buffers and stabilizers in tablets and capsules. He used penicillin combined with aluminum hydroxide gel, penicillin mixed with shellac, beeswax and cottonseed oil in a capsule, calcium penicillin in corn oil placed in a capsule, a tablet containing penicillin with a highly soluble binder and another capsule in which were placed 25,000 units of sodium penicillin alone.

After single doses of 90,000 units of penicillin, Finland (29) found levels of from .22 to .45 units per cc. of serum when the saline solution was given before breakfast. Using the other preparations, he obtained levels of from .11 to .22 units per cc. of serum. When he gave the saline solution one-half hour after breakfast the levels ranged from .02 to .06 units per cc. of serum, while they were found to be from less than .02 to .11 units per cc. of serum with the other preparations. Penicillin in aluminum hydroxide gel produced the best results in the doses given after the meal.

For their experiments in serum levels during repeated oral administration, these investigators (29) gave a large priming dose just before breakfast, then gave a sustaining dose one hour later, followed by four more doses at two hour intervals. By this procedure, they gave an initial dose of 300,000 units and followed, as outlined above, with succeeding doses of 60,000 units. This produced levels of from .03 to .22 for a period of ten hours. An initial dose of 120,000 units followed by 60,000 units every two hours produced levels which were less than .03 units per cc. of serum. On all dosage schedules above this, however, the levels reached were comparable with those seen when penicillin

was given intramuscularly in a dose of 20,000 units every three hours.

In either single or repeated doses, the relationship of the meal to the time of administration is important, as may be seen from the results given above. Serum levels obtained when single doses were given before a meal were high and regular, but when given after the meal they were irregular and unpredictable. In persons with achlorhydria, the serum levels were higher and better sustained both before and after a meal than they were in normal persons. Not quite so much influence of the meal was seen in those instances where repeated doses of penicillin were given, but even so it was not possible to predict the level at any time in any one subject as long as food was being taken during the treatment.

A total of 61 patients with acute gonorrhea were treated with various preparations and dosage schedules, as were 7 cases of typical lobar pneumonia. The results in these cases suggested to these workers (29) that oral penicillin is a feasible therapy in these infections, and that it should prove effective in other infections in which low doses of parenteral penicillin have proved adequate. They found that a concentration of 0.03 unit per

cubic centimeter was sufficient in vitro to sterilize cultures of all strains of gonococcus and group A hemolytic streptococcus, the great majority of strains of pneumococcus and Streptococcus viridans, but only one half or less of the strains of meningococcus and Staphylococcus aureus. From this they concluded that oral penicillin therapy may be expected to prove effective in gonococcic, hemolytic streptococcic and pneumococcic infections, but that it may fail in many cases of subacute bacterial endocarditis.

Powdered Penicillin in Capsules

Other cases of pneumococcic pneumonia were treated with oral doses of penicillin by Bunn et al. (11) who encountered only one death and one serious complication in a series of forty-five patients. In nine of these patients, the penicillin concentrations in the blood were measured during the acute illness. No attention was paid as to whether the patients had recently ingested food when the determinations were made, and the figures represent random determinations, some of which were thought by the authors to reflect a cumulative effect.

After a dose of 50,000 units of the sodium salt of penicillin in the usual powdered form enclosed in

a gelatin capsule, there was a blood level of .04 units per cc.; in some instances this was higher. In only two patients were the levels lower than this at 30 or 60 minutes after administration. It is interesting to note that the blood levels in these pneumonia patients were equal or superior to those commonly observed following the administration of similar amounts of penicillin to normal subjects.

Here again approximately four or five times as much penicillin was used orally as would have been required to produce the same concentrations in the blood. The authors stated that the initial oral dose of 200,000 units is the approximate equivalent of 40,000 units given intramuscularly and the subsequent 50,000 unit dose is the equivalent of 10,000 by the latter route.

Regarding the dosage regimen, these investigators (11) used an initial administration of 200,000 units of penicillin followed by 50,000 unit doses at two hour intervals throughout the twenty-four hour period. Twenty-four to thirty-six hours after the appearance of a crisis the administration throughout the night was discontinued. From this point on the 50,000 unit doses were given from 8 a.m. to 10 p.m. inclusive.

Corn Oil and Lanolin

Pearlstein, Kluener and Liebmann (84) mixed penicillin with corn oil and lanolin and put it in a gelatin capsule in amounts of 55,000 units. They planned to thus protect penicillin from the effect of gastric acidity and make it available for absorption from the small intestine, where most of the fat digestion occurs. One of these capsules taken by the subjects after an overnight fast caused the urinary excretion of relatively large amounts of penicillin in the urine, 14 to 16 per cent of the original dose being recovered during a twenty-four hour period.

They found penicillin in measurable quantities in the urine for 24 hours in some individuals and for more than 42 hours in others. This delayed excretion was attributed to the effect of the slow degradation of both the corn oil and lanolin, and it was about five times higher than the excretion found after the oral administration of penicillin in saline solution.

As has been mentioned previously in this paper, (16)(42)(91)(100) excretions of from 8.0 to 35.0 per cent of the original oral dose of penicillin have been recovered in the urine. In contrast, when given by intravenous or intramuscular injection, urine recoveries

of penicillin have been reported to average approximately 60 per cent. (3)(91) This leaves about 40 per cent of the dose unaccounted for, and suggests that penicillin is inactivated in the body in some manner other than by the influence of gastric acid or by the enzyme penicillinase produced by E. coli in the gut. The reason for this has as yet not been explained.

SUMMARY AND COMMENT

To facilitate quick reference, and to allow for some discussion of the problems of administering penicillin orally, the following summary is presented. The questions concerning this mode of administration of penicillin will be considered in much the same order as they were previously outlined, namely, inactivation, absorption and excretion, toxicity, cost, advantages and disadvantages.

INACTIVATION

(a) Gastric Acidity

If penicillin could be transported through the stomach without being subjected to the action of the gastric acid, the concentration in the blood could quickly be raised to a high level. In an effort to solve this problem, numerous antacids and buffers, capsules, oils, enteric coatings and other substances to increase absorption of the drug have been used.

Antacids and Buffers

1. Sodium bicarbonate has been said to be unsatisfactory because penicillin is subject to attack by excessive alkali as well as by excessive acid. (3)(14)(42)(45)

2. Volunteers given a dose of alkali followed

by penicillin mixed in raw egg showed satisfactory therapeutic blood levels. (71) This was successful in the treatment of tonsillitis cases.

3. Trisodium citrate was used as an antacid to protect penicillin by neutralizing gastric acidity. (16)(23) The result was that 40,000 units of penicillin produced blood levels of .02 to .2 units per cc. serum at one hour. (16) A dose of 60,000 units elevated serum levels to more than .29 units per cc.

4. Basic aluminum aminoacetate used as an antacid with 100,000 units of penicillin was sufficiently absorbed to produce serum concentrations of .39, .68, .37, and .17 units per cc. at 2, 3, 5, and 7 hours after administration. (68) Gonorrhea was successfully cured with this preparation. (68)(83)

5. Four equal doses of 25,000 units of a penicillin-aluminum hydroxide mixture, given at two-hour intervals, was found to give blood levels of from .147 to .27 units per cc. serum at one-half hour after each ingestion. Concentrations ranging from .03 to .19 units per cc. were maintained for as long as 24 hours. (100) Other workers, using the same antacid and dosage regimen, found serum levels of .25, .50 and 1.00 units per cc. (60)

Oils

1. Using magnesium trisilicate for a buffer preceding the administration of 315,000 units of penicillin in water and in oils, McDermott et al. (73) found serum concentrations ranging from .312 to 1.25 units per cc. at 30 and 60 minutes respectively. One preparation, peanut oil in beeswax, produced high levels at two hours after ingestion, which suggests that this might be a way to prolong the action of penicillin similar to the prolongation seen when it is used in intramuscular injections.

2. By the use of cottonseed oil as a vehicle for the drug, Libby (70) obtained a blood level of .05 units per cc. serum at one hour, with .04 unit present at the end of four hours after the subjects ingested 90,000 units of penicillin. He gave subsequent doses of 20,000 units after the initial dose of 90,000 units in some patients and maintained levels of from .03 to .07 units per cc. for seven hours. This procedure required two to three times as much penicillin as would have been needed for intramuscular injection.

3. Corn oil and lanolin mixed with penicillin in a 55,000 unit capsule and given to subjects who had fasted overnight, was effective in producing higher and more prolonged urinary excretion of penicillin. (84) This indicates a more gradual absorption of penicillin

from the gut without destruction of its activity before absorption, and it is due to the degradation of the corn oil in the gut instead of in the stomach, plus the slow decomposition of lanolin.

Capsules and Enteric Coatings

1. To afford a mechanical conveyor for the penicillin, Burke, Ross and Strauss (12) placed powdered penicillin in a specially-treated, double capsule. They obtained concentrations in the blood of 9.984 and 2.496 units per cc. serum in two cases where 200,000 units of penicillin were given before and after a meal respectively. They suggested the administration of penicillin before meals, and also a high fluid intake plus a low fat diet as means of increasing blood concentrations of the drug. Cases of gonorrhea, pneumonia and cellulitis were cured by the method reported by these workers.

2. Phenyl salicylate coating of 100,000 unit penicillin capsules produced blood levels of .75 units per cc. serum (78) as had been suggested earlier by Abraham, Florey et al. (3)

3. Powdered penicillin in gelatin capsules was used by Bunn et al. and produced a level of .04 units per cc. in patients with penumonia.(11) Excellent therapeutic results were obtained by administering 200,000

units of penicillin, followed by 50,000 units at two-hour intervals until a crisis appeared. After the crisis, 50,000 units was given every two hours from 8 a.m. to 10 p.m. inclusive, with no penicillin being given at night. The therapy was continued until the temperature decreased and remained normal, or, at least 7 days.

4. Triisopropanolamine, trisodium citrate and sodium carbonate enclosed in resin-cellulose plastic enteric coating were found useful by Cutting and his co-workers. (23) Penicillin in 50,000 unit doses every two hours for ten doses, with one of these adjuvants, produced blood concentrations of from .02 to .05 units per cc., and many cases of gonorrhea were cured by these methods.

5. Finland (29) found levels of from .22 to .45 units per cc. serum when single doses of 90,000 units of penicillin were given in saline solution to subjects in fasting condition. He used other preparations containing buffers and stabilizers in tablets or capsules, but they were no better than the saline preparation except when given after meals. In the latter instance, aluminum hydroxide gel was the best adjuvant. He emphasized the importance of administering penicillin before meals. An initial dose of 300,000 units in saline, followed by 60,000 units at two hour intervals for four

doses, produced levels of .03 to .22 unit per cc. for a period of ten hours.

It is evident from a review of the work done that the investigators are not in agreement as to the best adjuvant to be used in giving penicillin by mouth. Relatively high serum concentrations have been obtained by all of the penicillin-adjuvant combinations, which proves that penicillin can be protected from the action of gastric acidity. The highest levels were reported by Burke, Ross and Strauss (12) who used powdered penicillin placed in a double, specially-treated, capsule. However, they used higher dosages than were reported by most other investigators. Bunn et al. (11) obtained good therapeutic results without any adjuvant except the gelatin capsule to protect the penicillin as did Finland and his associates. (29)

Naturally, if adequate blood concentrations can be obtained by the use of only a capsule, the expense of oral preparations of penicillin can be decreased. However, the very fact that so many other adjuvants have been tried indicates that there is much more to be desired from the oral administration of penicillin. The experiments reported have varied greatly as to the dosage used and the time of administration in relation to the meal.

A carefully planned experiment, in which the dosages could be varied with each of the various adjuvants, and in which a definite dosage regimen could be worked out for each combination, as determined by the actual serum concentrations using a standard method of assay, would be the most accurate way to determine which method is the most efficient, and thereby most economical way to give penicillin by mouth. This would be too great an undertaking for any one laboratory, but several groups of investigators could do it in much the same manner as the early work on penicillin by other means of administration was done.

Without the aid of such a series of planned investigations, it will likely be some time yet before the problem of the oral administration of penicillin is settled. The convenience of giving penicillin orally will maintain interest in this problem, and if the cost of the drug is decreased in the future, further interest in its administration by the oral route will be stimulated.

(b) Penicillinase

It was shown by Abraham and Chain (1) that Escherichia coli, commonly present in the colon, produces an enzyme which inactivates penicillin. This was thought to be a large part of the reason that the rectal route

of administering penicillin would not work satisfactorily. (89) Cutting and his associates (23) stated, however, that the flora in the upper intestine is probably sparse, which makes the factor of destruction of penicillin by E. coli less important than the other variables for peroral administration.

Of course, if penicillin were carried down into the lower intestine by means of enteric coatings or some other slowly-decomposing adjuvant, the chances for destruction by the above-mentioned organism would be increased.

ABSORPTION AND EXCRETION

Because penicillin is so rapidly excreted from the body by the kidneys, it is important that it be very quickly absorbed from the intestine if a high concentration is to be built up in the blood stream. Intra-duodenal administration of penicillin has produced relatively high levels in the blood (89) and other studies (29)(89) have shown that in cases of achlorhydria the penicillin is absorbed promptly into the blood. Furthermore, a common finding by most investigators has been a rapid rise of blood concentration to a peak level within the first thirty to sixty minutes after oral administration with various antacids, oils, etc. (12)(16)(45)(70)(71)(73)(84)(100)

These findings indicate that absorption from the upper intestine is rapid and is not a major cause for the loss of penicillin in the body, although it indirectly aids the rapid excretion by allowing penicillin to enter the blood in a short time.

Comparison of Blood Concentrations after Parenteral and Oral Administration

To properly evaluate the blood levels obtained after oral administration of penicillin, a comparison should be made with the levels seen after intravenous or intramuscular injections. A very rapid rise in the blood concentration to the highest peak within the first ten to fifteen minutes, was followed by a sharp fall during the first hour, with only a trace being detectable after two hours following intravenous administration by Rammelkamp. (91) The intramuscular injection of 45,000 units by Romansky and Rittman (94) produced the highest level in one-half hour, followed by a more slow drop during the first hour with detectable levels remaining at two to three hours.

After oral administration, the highest level is reached usually in about one-half hour, followed by a moderately rapid fall, with assayable levels still present after three or four hours. The height of the

blood concentration depends upon the amount of the oral dose. According to Burke et al., (12) who achieved outstanding levels by the use of a double capsule , the concentration and prolongation of the blood level is more or less directly proportional to the oral dose employed. Cutting and his associates, (23) however, state that this is not the case since they found that increasing the dose did not increase the serum concentration by the same relative amount.

In general it may be said that from two to five times the amount of penicillin necessary for parenteral injection is required to produce equal levels when given orally. Individual differences in absorption, as well as variations in the adjuvant and the time of administration will naturally vary this. It is possible, however, to produce and maintain adequate serum levels of penicillin by giving the drug orally.

TOXICITY OF PENICILLIN ADMINISTERED ORALLY

All the previously mentioned reactions to penicillin (see section on toxicity) have occurred after some means of administration other than the oral route. However, it is interesting to note that toxic reactions reported after the administration of penicillin by mouth are conspicuous only by their absence in the literature.

Bunn et al. (11) reported that out of 45 patients receiving penicillin orally, "no instances of nausea, vomiting, diarrhea or other gastro-intestinal disturbances occurred. One patient developed mild generalized urticaria, which subsided promptly after the commercial brand of penicillin was changed". These were patients with pneumonia who received from 0.3 to 7.0 million units of penicillin over periods of time ranging from 6 hours to 11 days.

Burke, Ross and Strauss (12) reported that "no objective or subjective symptoms of toxicity were observed after single doses of 100,000 and 200,000 units of penicillin orally". In another article, Ross and associates (95) stated that in one case a total of 11,500,000 units of penicillin was given over a period of fourteen days without toxic effect.

Numerous other studies on oral penicillin reported in the literature make no mention whatever of any toxic reactions encountered. No more serious toxic symptom than the one mentioned by Bunn (11) has been reported in the work considered for this paper. This makes it seem fair to conclude that, although the literature on the subject is very limited, relatively speaking, the oral preparations of penicillin are less likely to

produce toxic reactions than are those preparations administered by parenteral routes. Since it was found by Little and Lumb (71) that penicillin is altered by passage through the body, and since it was thought by Cormia et al. (21) that the vascular bed is the primary shock tissue in the body, it may be logical to assume that the process of absorption of the penicillin from the intestine into the blood stream, when the drug is given orally, by some manner eliminates the toxic impurities which accompany the penicillin in commercial preparations. If this is the case, then it should theoretically be possible to avoid reactions to penicillin by administering it in oral forms, particularly in those cases where the individual is found to be allergic to the parenteral preparations. This would, of course, require experimental proof, which is lacking at present.

LIMITED SUPPLY AND HIGH COST OF PENICILLIN

The supply of penicillin now has nearly reached a sufficiently high level to allow most any expenditure of the drug for therapeutic or experimental purposes. Hertofore, however, the drug was very limited and oral administration, in which there is used an amount of penicillin two to five times as great as would be required for parenteral administration, was not undertaken.

With the increase in production, there has been a decrease in the cost of penicillin, and it is very probable that the cost will decrease further as production methods are made more efficient and economical. The cost to the patient has already come down from $20.00 (19) to about $2.00 per 100,000 units. As Cutting pointed out (23) with the ordinarily effective dose of one-half million units, given orally, expense ceases to be an important factor in limiting oral treatment of gonorrhea, or other diseases where the ordinary blood concentrations obtained by oral administration are high enough to combat the infecting organism. The cost, however, is still high and is definitely a limiting factor in the administration of penicillin by the oral route.

NEW DOSAGE FORMS OF PENICILLIN

The following is a report of recommendations and statements released in August, 1945 by the Committee on Chemotherapeutics and Ohter Agents of the National Research Council and the Committee on Medical Research of the Office of Scientific Research and Development. (64) This report is based on a review of scientific evidence and on information gained through consultation with experts in the field of chemotherapy and the medical and

scientific staffs of interested manufacturers. It will perhaps give some evidence as to what is being "recommended" in the way of Oral Penicillin Products.

"Tablets Buffered Penicillin, Capsules Buffered Penicillin

"Tablets buffered penicillin and capsules buffered penicillin contain one or more of the buffer substances sodium citrate, citric acid, aluminum hydroxide, calcium carbonate, magnesium oxide and aluminum-dihydroxyamino acetate. The potency of each tablet or capsule is not less than 20,000 units, and the number of tablets or capsules in each single package is such that the total number of units therein is not less than 300,000.

"Capsules Penicillin in Oil

"Capsules penicillin in oil is a suspension of sodium penicillin or calcium penicillin in refined vegetable food oil. The potency of each capsule is not less than 20,000 units, and the number of capsules in each single package is such that the total number of units therein is not less than 300,000.

"Penicillin with Aluminum Hydroxide Gel

"Penicillin with aluminum hydroxide gel is a combination package of sodium penicillin or calcium penicillin and aluminum hydroxide gel. The quantity of aluminum hydroxide gel packaged with the penicillin shall be 30 cc.

for each 100,000 units.

"Indications and Dosage.--Oral penicillin preparations should be administered on a fasting stomach, not less than thirty minutes before or not less than one and one-half to two hours after eating.

"Gonorrhea.--Combined Therapy: A single injection of 100,000 units followed not later than two or three hours by oral doses of 40,000 to 50,000 units each every two or three hours for six doses per day for one or two days.

"Oral Therapy: Forty thousand to 50,000 units every two or three hours for six doses per day for one or two days.

"In complications such as arthritis, endocarditis and epididymitis, administer penicillin parenterally. Concurrent infections of gonorrhea and syphilis should always be considered. Treatment of gonorrhea under the foregoing dosage schedule may prevent the appearance of the chancre and thus mask the first evidence of syphilis. The masking of the chancre should not be construed as a beneficial effect. Blood serologic tests should be made once a month for a minimum of three months.

"Pneumococcic, Streptococcic and Staphylococcic

Infections.---A minimum of 20,000 to 40,000 units of penicillin parenterally every three hours. After the acute phase and when the temperature has been reduced, treatment may be continued with oral administration. Treat with a dosage of 40,000 to 50,000 units each two or three hours (day and night) for at least forty-eight hours after the temperature has returned to normal. If the condition is not controlled by oral penicillin return to parenteral administration.

"Prophylaxis.--Against secondary infections after tonsillectomy or tooth extraction in cases with a history of rheumatic fever or rheumatic heart disease, congenital heart disease and other conditions in which secondary infections may occur (infected teeth or tonsils), administer 100,000 to 200,000 units daily in divided doses from one day before to three or four days after surgery.

"Precautions.--In meningitis, endocarditis and peritonitis, administer penicillin only parenterally. In acute infection with bacteremia or septicemia, parenteral administration of penicillin should be continued until the blood cultures become negative and the acute condition is controlled. Do not use in those conditions in which penicillin is of questionable value or is ineffective."

ADVANTAGES AND DISADVANTAGES

The advantages of the oral route of administering penicillin are obvious. Patients who for some reason cannot be hospitalized can now receive this drug at home without the aid of a trained attendant. In the hospital frequent injections, which require time, skill and sterile equipment, are eliminated by giving the drug by mouth. Toxic reactions seem to occur more infrequently after oral administration of penicillin, and it appears that a less highly purified product would be suitable for oral use. In brief, penicillin is rendered a much more practical drug, from the standpoint of both the doctor and his patient, when it is given orally.

The disadvantages of oral administration are few. In the event that a patient is too sick or too young to swallow oral preparations of penicillin this route is of little value, and the parenteral methods of administration must be used. Also, the cost of preparations for oral use is still high, and may in some cases pronibit their use. Finally, any other disadvantages which might be incurred with the oral administration of any drug apply to such preparations of penicillin.

CONCLUSIONS

(1) Penicillin can be protected against inactivation by the hydrochloric acid of the stomach. This is accomplished by the use of various adjuvants in the form of antacids or buffers, oil suspensions, capsules or enteric coatings. At present, no one of these adjuvants has been shown to be consistently better than the others, but therapeutic blood levels have been obtained with each of those discussed.

(2) Oral penicillin is more efficient when given on a fasting stomach. Hence it should be ingested with the patient in a fasting condition, if possible , or at least one-half to one hour before meals.

(3) Absorption of penicillin from the intestine after oral administration is rapid if the drug is protected from destruction by gastric acid. Inactivation by penicillinase in the intestine is not important in oral administration.

(4) Therapeutic blood levels are established rapidly and maintained for a longer time after the administration of penicillin by mouth in spite of rapid excretion by the kidneys. However, even with the use of a suitable adjuvant, from two to five times as much penicillin is required to produce any particular blood level by the oral route of administration as would

be required if parenteral injection were used.

(5) The occurrence of toxic reactions after the oral administration of penicillin appears to be surprisingly infrequent, even though huge doses are used. This may be due to the lack of absorption of toxic impurities into the blood from the intestine.

(6) The cost of penicillin is a limiting factor in its oral administration, but it is not prohibitive in most cases. Hospitalization can be avoided in many cases by oral administration and expense to the patient is thereby decreased.

(7) With good evidence that blood concentrations of from .019 to .312 units of penicillin are sufficient to combat most susceptible organisms, it is logical to conclude that many infections can be successfully treated with penicillin administered by mouth. Some of the infections which have been very satisfactorily treated by this method are gonorrhea, pneumococcic pneumonia and cellulitis.

(8) Penicillin administered orally is very well suited for treating patients in the convalescent stage, and for maintaining blood levels after initial therapy by parenteral injection. Also it finds a large range of practicability in the home therapy of patients who can not be hospitalized.

(9) Whatever vehicle or adjuvant is used, and regardless of the relation of the oral dose to the meals, there are a great many individual variations in serum concentration and efficacy of the oral administration of penicillin among different patients, and in the same patient at different times. As is true of other drugs, penicillin therapy must be planned individually for each patient if best results are to be obtained.

SELECTIVE BIBLIOGRAPHY

1. Abraham, E. P. and Chain, E.: An enzyme from bacteria able to destroy penicillin, Nature 146: 837, (Dec. 28) '40. Quoted by Burke, F. G., Ross, Sidney and Strauss, Clifton: Oral administration of penicillin, J.A.M.A. 128:83-87, (May 12) '45.

2. Abraham, E. P. and Chain, E.: Purification and some physical and chemical properties of penicillin; with a note on the spectrographic examination of penicillin preparations by E. R. Holiday, Brit. J. Exper. Path. 23:103-115, (June) '42.

3. Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A. and Florey, H. W.: Further observations on penicillin, Lancet 2:177-178, (Aug. 16) '41.

4. Anon: Current Comment, J.A.M.A. 127:653, (Mar. 17) '45.

5. Anon: Editorial: Shortage of penicillin, J.A.M.A. 129:877, (Nov. 24) '45.

6. Anon: Organization Section, J.A.M.A. 127:864, (Mar. 31) '45.

7. Anon: Progress with penicillin, Editorial, Lancet 2:546, (Oct. 30) '43.

8. Barker, A.N.: Allergic reactions to penicillin, Lancet 1:177-178, (Feb. 10) '45.

9. Binkley, G. W. and Brockmole, A.: Dermatitis from penicillin, Arch. Dermat. & Syph. 50:326-327, (Nov.) '44.

10. Bornstein, Siegbert: Action of penicillin on enterococci and other streptococci, J. Bact. 39:383-387, (Apr.) '40.

11. Bunn, P. A., McDermott, Walsh, Hadley, S. J. and Carter, A. C.: The treatment of pneumococcic pneumonia with orally administered penicillin, J.A.M.A. 129:320-327, (Sept. 29) '45.

12. Burke, F. G., Ross, Sidney, and Strauss, Clifton: Oral administration of penicillin, J.A.M.A. 128: 83-87, (May 12) '45.

13. Catch, J. R., Cook, A. H. and Heilbrow, I. M.: Purification and chemistry of penicillin, Nature 150:633-634, (Nov. 28) '42.

14. Chain, E.: Mode of action of chemotherapeutic agents; Discussion, Biochem. J. 36:4-5, (Feb.) '42.

15. Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J. and Sanders, A. G.: Penicillin as a chemotherapeutic agent, Lancet 2:226-228, (Aug. 24) '40.

16. Charney, J., Auburn, H. E. and Bernhart, F. W.: Urinary excretion of penicillin in man after oral administration with gastric antacids, Science 101: 251-253, (Mar. 9) '45.

17. Clark, A. M., Colebrook, Leonard, Gibson, Thomas and Thomson, M. L.: Penicillin and propamidine in burns; elimination of hemolytic streptococci and staphylococci, Lancet 1:605-609, (May 15) '43.

18. Clutterbuck, P. W., Lovell, R. and Raistrick, H.: Studies in the biochemistry of micro-organisms. XXVI. The formation from glucose by members of the Penicillin chrysogenum series of a pigment, an alkali-soluble protein and penicillin--the antibacterial substance of Fleming, Biochem. J. 26:1907-1918, '32.

19. Coghill, R. D.: Penicillin, science's cinderella, Chem. Eng. News 22:588-593, (Apr. 25) '44.

20. Cooke, J. V.: A simple clinical method for the assay of penicillin in body fluids, J.A.M.A. 127: 445-449, (Feb. 24) '45.

21. Cormia, F. E., Jacobsen, L. Y. and Smith, E. L.: Reactions to penicillin, Bull. U. S. Army M. Dept. 4:694-702, (Dec.) '45.

22. Cutting, W. C., Halpern, R. M., Sultan, S. H. and Armstrong, C. A.: Oral penicillin, Federation Proc. 4:115-116, (Mar.) '45.

23. Cutting, W. C., Halpern, R. M., Sultan, E. H., Armstrong, C. D. and Collins, C. L.: Administration of penicillin by mouth, with results in the treatment of gonorrhea, J. A.M.A. 129:425-432, (Oct. 6) '45.

24. Dubos, R. J.: Studies on a bactericidal agent extracted from a soil bacillus. I. Preparation of the agent; its activity in vitro, J. Exper. Med. 70:1-10, (July) '39.

25. Dubos, R. J.: Studies on a bactericidal agent extracted from a soil bacillus. II. Protective effect of the bactericidal agent against experimental pneumococcus infections in mice, J. Exper. Med. 70:11-17, (July) '39.

26. Elder, A. L.: Penicillin, Scientific Monthly 58:405-409, (June) '44.

27. Emmerich, R. and Loew, O.: Z. Hyg. InfektKr. 31:1, 1899. Quoted by Chain, E. and Florey, H. W.: The discovery of the chemotherapeutic properties of penicillin, Brit. Med. Bull. 2:5-7, No. 1, '44.

28. Fineberg, S. M.: Penicillin allergy, J. Allergy 15:271-273, (July) '44.

29. Finland, Maxwell, Meads, Manson and Ory, E.M.: Oral penicillin, J.A.M.A. 129:315-320, (Sept. 29) '45.

30. Fleming, Alexander: On the antibacterial action of cultures of Penicillium, with special reference to their use in the isolation of B. influenzae, Brit. J. Exp. Path. 10:226-236, (June) '29.

31. Fleming, Alexander: On the specific antibacterial properties of penicillin and potassium tellurite; incorporating a method of demonstrating some bacterial antagonisms, J. Path. Bact. 35:831-842, (Nov.) '32.

32. Fleming, Alexander: Streptococcal meningitis treated with penicillin; measurement of bacteriostatic power of blood and cerebrospinal fluid, Lancet 2:434-438, (Oct. 9) '43.

33. Fleming, Alexander and Allison, V. D.: Bacteriolytic substance ("lysozyme") found in secretions and tissues, Brit. J. Exper. Path. 3:252-253, (Oct.) '22.

34. Florey, H. W.: Penicillin: Its development for medical uses, Nature 153:40-42, (Jan. 8) '44.

35. Florey, H. W. and Jennings, M. A.: Some biological properties of highly purified penicillin, Brit. J. Exper. Path. 23:120-123, (June) '42.

36. Florey, M. E. and Florey, H. W.: General and local administration of penicillin, Lancet 1:387-397, (Mar. 27) '43.

37. Foster, J. W.: Quantitative estimation of penicillin, J. Biol. Chem. 144:285-286, (June) '42.

38. Foster, J. W. and Wilker, B. L.: Microbiological aspects of penicillin. II. Turbidimetric studies on penicillin inhibition, J. Bact. 46:377-389, (Oct.) '43.

39. Foster, J. W. and Woodruff, H. B.: Improvements in the cup assay for penicillin, J.Biol. Chem. 148: 723, (June) '43.

40. Foster, J. W. and Woodruff, H. B.: Microbiological aspects of penicillin. I. Methods of assay, J. Bact. 46:187-202, (Aug.) '43.

41. Foster, J. W. and Woodruff, H. B.: Microbiological aspects of penicillin. VI. Procedure for the cup assay for penicillin, J. Bact. 47:43-58, (Jan.) '44.

42. Free, A. H., Leonards, J. R., McCullough, D. R. and Biro, B. E.: The urinary excretion of penicillin after oral administration to normal human subjects, Science 100:431-432, (Nov. 10) '44.

43. Graves, W. N., Carpenter, C. C. and Unangst, R. W.: Recurrent vesicular eruptions appearing during administration of penicillin, Arch. Dermat. & Syph. 50:6-7, (July) '44.

44. Gyorgy, Paul and Elmes, P. C.: Experiments on the toxicity of the calcium salt of penicillin, Proc. Soc. Exper. Biol. & Med. 55:76-77, (Jan.) '44.

45. Gyorgy, Paul, Vandegrift, H. N., Elias, William, Colio, L. G., Barry, F. M. and Pilcher, J. D.: Administration of penicillin by mouth, preliminary report, J.A.M.A. 127:639-642, (Mar. 17) '45.

46. Hamre, D. M., Rake, G., McKee, C. M. and MacPhillamy, H. B.: The toxicity of penicillin as prepared for clinical use, Amer. J. Med. Sc. 206:642-652, (Nov.) '43.

47. Heatley, N. G.: A method for the assay of penicillin, Biochem. J. 38:61-65, (No. 1) '44.

48. Heilman, D. H.: A method for standardizing penicillin, Amer. J. Med. Sc. 207:477-483, (Apr.) '44.

49. Heilman, D. H. and Herrell, W. E.: The use of Fleming's modification of the Wright slide cell technic for determining penicillin in body fluids, Am. J. Clin. Path. 15:7-9, (Jan.) '45.

50. Herrell, W. E.: Penicillin and other antibiotic agents. Philadelphia, W. B. Saunders Co., 1945.

51. Herrell, W. E.: The clinical use of penicillin; an antibacterial agent of biologic origin, J.A.M.A. 124:622-627, (Mar. 4) '44.

52. Herrell, W. E. and Heilman, D. H.: Tissue culture studies on cytotoxicity of bactericidal agents. I. Effects of gramicidin, tyrocidine and penicillin on cultures of mammalian lymph node, Amer. J. Med. Sc. 205:157-162, (Feb.) '43.

53. Herrell, W. E., Heilman, D. H. and Williams, H. L.: The clinical use of penicillin, Proc. Staff Meet. Mayo Clin. 17:609-616, (Dec. 30) '42.

54. Herrell, W. E. and Nichols, D. R.: The calcium salt of penicillin, Proc. Staff Meet. Mayo Clin. 18:513-519, (Sept. 8) '43.

55. Herrell, W. E., Nichols, D. R. and Heilman, D. H.: Penicillin; its usefulness, limitations, diffusion and detection, with analysis of 150 cases in which it was employed, J.A.M.A. 125:1003-1010, (Aug. 12) '44.

56. Hobby, G. L., Meyer, Karl and Chaffee, Eleanor: Activity of penicillin in vitro, Proc. Soc. Exper. Biol. & Med. 50:277-280, (June) '42.

57. Holmes, L. F. and Lockwood, J. S.: Studies on bioassay of penicillin, Amer. J. Med. Sc. 207: 267-268, (Feb.) '44.

58. Hotchkiss, R. D. and Dubos, R. J.: Bactericidal fractions from an aerobic sporulating bacillus, J. Biol. Chem. 136:803-804, (Dec.) '40.

59. Hotchkiss, R. D. and Dubos, R. J.: Fractionation of the bactericidal agent from cultures of a soil bacillus, J. Biol. Chem. 132:791-792, (Feb.) '40.

60. Howard, J. W. and Scott, E. G.: Oral penicillin; a review of the literature and preliminary report, Delaware State Medical J. 17:171-173, (Sept.) '45.

61. Joslyn, D. A.: Penicillin assay; outline of four-hour turbidimetric method, Science 99:21-22, (Jan. 7) '44.

62. Kakavas, J. C.: Quoted by Howard, J. W. and Scott, E. G.: Oral penicillin; a review of the literature and preliminary report, Delaware State Medical J. 17:171-173, (Sept.) '45.

63. Keefer, C. S., Blake, F. G., Marshall, E. K., Jr., Lockwood, J. S. and Wood, W. B., Jr.: Penicillin in the treatment of infections. A report of 500 cases. Statement by the Committee on Chemotherapeutic and Other Agents, Division of Medical Sciences, National Research Council, J.A.M.A. 122:1217-1224, (Aug. 28) '43.

64. Keefer, C. S., Herwick, R. P., Van Winkle, Walton, Jr., and Putnam, L. E.: New dosage forms of penicillin; statement concerning certifiable penicillin products, including recommended indications, dosages and precautions, J.A.M.A. 128:1161-1164, (Aug. 18) '45.

65. Keyes, J. E. L.: Penicillin in ophthalmology, J.A.M.A. 126:610-615, (Nov. 4) '44.

66. Kirby, W. M. M.: Stability of penicillin solutions and room and incubator temperatures, J.A.M.A. 125: 628-629, (July 1) '44.

67. Kirby, W. M. M. and Rantz, L. A.: Methods of measuring penicillin concentrations in body fluids, J. Bact. 48:603-608, (Nov.) '44.

68. Krantz, J. C., Jr., Evans, W. E. and McAlpine, J. G.: Oral penicillin with basic aluminum aminoacetate, Science 101:618-619, (June 15) '45.

69. Lee, S. W., Foley, E. J., Epstein, J. A. and Wallace, J. H., Jr.: Improvements in the turbidimetric assay for penicillin, J. Biol. Chem. 152: 485-486, (Feb.) '44.

70. Libby, R. L.: Oral administration of penicillin in oil, Science 101:178-180, (Feb. 16) '45.

71. Little, C. J. H. and Lumb, George: Penicillin by mouth, Lancet 1:203-206, (Feb. 17) '45.

72. Lyons, C.: Penicillin therapy of surgical infections in the U. S. Army, J.A.M.A. 123:1007-1018, (Dec. 18) '43.

73. McDermott, Walsh, Bunn, P. A., Benoit, Maria, Dubois, Rebeckah and Haynes, Willetta: Oral penicillin, Science 101:228-229, (Mar. 2) '45.

74. McMahan, J. R.: An improved short time turbidimetric assay for penicillin, J. Biol. Chem. 153:249-258, (Apr.) '44.

75. Meyer, Karl, Chaffee, Eleanor, Hobby, G. L., Dawson, M. H., Schwenk, Erwin and Fleischer, G.: On penicillin, Science 96:20-21, (July 3) '42.

76. Meyer, Karl, Hobby, G. L. and Chaffee, Eleanor: On esters of penicillin, Science 97:205-206, (Feb. 26) '43.

77. Meyer, Karl, Hobby, G. L. and Dawson, M. H.: The chemotherapeutic effects of esters of penicillin, Proc. Soc. Exper. Biol. & Med. 53:100-104, (June) '43.

78. Moldavsky, L. F. and Hesselbrock, W. D.: Oral penicillin, Bull. U. S. Army M. Dept. No. 86: 54-55, (Mar.) '45.

79. Moore, J. E., Mahoney, J. F., Schwartz, W. H., Sternberg, C. T. and Wood, W. B.: The treatment of eary syphilis with penicillin, J.A.M.A. 126: 67-73, (Sept. 9) '44.

80. Morris, C. E. and Downing, John G.: Bullous dermatitis (dermatitis medicamentosa) from penicillin, J.A.M.A. 127:711, (Mar. 24) '45.

81. Moses, Campbell: Oral penicillin, Correspondence, J.A.M.A. 128:52, (May 5) '45.

82. Pasteur, Louis and Joubert, J. F.: C. R. Acad. Sci. 85:101, 1877. Quoted by Chain, E.and Florey, H. W.: The discovery of the chemotherapeutic properties of penicillin, Brit. M. Bull. 2:5-7, No. 1, '44.

83. Paul, W. D., Rhomberg, C., McKee, A. P. and Pichette, J. W.: Administration of penicillin by mouth in combination with aluminum dihydroxyaminoacetate, J. Iowa M. Soc. 36:219-221, (June) '45.

84. Pearlstein, D., Kluener, R. G., Liebmann, A. J. and Dorrell, J.: Oral administration of penicillin in corn oil and lanolin, Science 102:66-67, (July 20) '45.

85. Powell, H. M. and Jamieson, W. A.: Penicillin chemotherapy of mice infected with Staphylococcus aureus, J. Indiana M. A. 35:361-362, (July) '42.

86. Pyle, H. D. and Rattner, H.: Contact dermatitis from penicillin, J.A.M.A. 125:903, (July 29) '44.

87. Rake, Geoffrey and Jones, Helen: A rapid method for estimation of penicillin, Proc. Soc. Exper. Biol. & Med. 54:189-190, (Nov.) '43.

88. Rammelkamp, C. H.: A method of determining the concentration of penicillin in body fluids and exudates, Proc. Soc. Exper. Biol. & Med. 51: 95-97, (Oct.) '42.

89. Rammelkamp, C. H. and Helm, J. C., Jr.: Studies on the absorption of penicillin from the stomach, Proc. Soc. Exper. Biol. & Med. 54:324-327, (Dec.) '43.

90. Rammelkamp, C. H. and Keefer, C. S.: Penicillin; its antibacterial effect in the whole blood and serum for the hemolytic streptococcus and Staphylococcus aureus, J. Clin. Investigation 22:649-657, (Sept.) '43.

91. Rammelkamp, C. H. and Keefer, C. S.: The absorption, excretion and distribution of penicillin, J. Clin. Investigation 22:425-437, (May) '43.

92. Reid, R. D.: Some properties of a bacterial-inhibitory substance produced by a mold, J. Bact. 29:215-221, (Feb.) '35.

93. Robinson, H. J.: Toxicity and efficacy of penicillin, J. Pharmacol. & Exper. Therap. 77:70-79, (Jan.) '43.

94. Romansky, M. J. and Rittman, G. E.: Penicillin; Prolonged action of beeswax-peanut oil mixture, Bull. U. S. Army M. Dept. No. 81:43, (Oct.) '44.

95. Ross, Sidney, Burke, F. G. and McLendon, P. A.: Penicillin by mouth; report of a clinical trial, J.A.M.A. 129:327-332, (Sept. 29) '45.

96. Silvers, S. H.: Contact dermatitis from amorphous sodium penicillin, Arch. Dermat. & Syph. 50: 328-329, (Nov.) '44.

97. Stokes, J. H., Sternberg, C. T., Schwartz, W. H., Mahoney, J. F., Moore, J. E. and Wood, W. B.: The action of penicillin in late syphilis, J.A.M.A. 126:73-80, (Sept. 9) '44.

98. Veldee, M. V., Herwick, R. P. and Coghill, R. D.: Recommendations of the international conference on penicillin, Reports, Science 101:42, (Jan. 12) '45.

99. Vincent, J. G. and Vincent, H. W.: Filter paper disc modification of the Oxford cup penicillin determination, Proc. Soc. Exper. Biol. & Med. 55:162-164, (Mar.) '44.

100. Welch, Henry, Price, C. W. and Chandler, V. L.: Prolonged blood concentrations after oral administration of modified penicillin, J.A.M.A. 128:845-847, (July 21) '45.

101. Welch, Henry and Rostenberg, A., Jr.: Hypersensitivity of the tuberculin type to crystalline penicillin sodium, J.A.M.A. 126:10-12, (Sept. 2) '44.