missions began to receive reinforcements in the form of a number of competent botanists. The survey work in the trackless and precipitous Andean forests of Ecuador just outlined would never have been possible without the help of W. H. Camp, W. B. Drew, G. W. Prescott and Ira L. Wiggins.

Although the best wild cinchona ever known, from which came the cultivated "Ledger" varieties, was discovered in Bolivia, the delicate diplomatic situation prevented the establishment of an official cinchona mission there. However, the best Bolivian barks, like those of the classic Loja region of southern Ecuador and northern Peru, had been exterminated through a destructive exploitation lasting two centuries. The highest quality bark still remaining in Bolivia and much of Peru is so inaccessible that its exploitation in any quantity is almost impossible. Consequently, the great volume of cinchona bark resulting from our work during more than two years has originated primarily in Colombia and Ecuador, even though it is of somewhat inferior quality.

The single factor of greatest importance in our work, to which I should attribute much of its success, has been our ability to obtain prompt and accurate analyses of our field samples of bark. No praise can be too high for the chemists who pioneered the cinchona mission laboratories now operating in Bogotá, Quito, Lima and La Paz. In cinchona "booms" of previous centuries, analyses could be made only after the shipment reached Europe, months after the bark had been bought and the proceeds spent. This situation led not only to fantastic speculation on good bark, but to excessive traffic in worthless barks, and one still hears reminiscences of fortunes made or lost overnight in the "quina" or "cascarilla" business. Through prompt analyses we were able to stop the harvest of poor barks and to encourage the production of good ones. Many species of Remijia, Ladenbergia and other rubiaceous genera closely resemble cinchona to the untrained eye, and our technical aid, both botanical and chemical, has saved many thousands of dollars which dealers would otherwise have "invested."

The quotas of cinchona bark set up in 1942 (which I am not free to mention) have been greatly exceeded by the supplies already harvested, and the cinchona mission botanists are returning one by one to the United States. As a consequence, we may expect publication of extraordinarily important researches on cinchona and related genera, to augment the very few publications which have already appeared on current work.[1, 2, 3] The availability of analyses has brought to light physiological distinctions between species, varieties and forms not heretofore suspected, and has provided an important new approach to the interpretation of a complex and badly misunderstood genus. From Dr. W. H. Camp and Dr. F. R. Fosberg, especially, we may certainly anticipate much enlightenment on the taxonomy of this difficult group of plants.

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SPECIAL ARTICLES

ORAL ADMINISTRATION OF PENICILLIN IN OIL

IT has been generally accepted that the various salts of penicillin in an aqueous media can not be administered orally[1, 2, 3] due to its rapid inactivation by gastric acidity. However, all the penicillin activity is not lost in the stomach. This was demonstrated by some preliminary experiments in which mice were completely protected against a thousand or more lethal doses of a virulent culture of hemolytic streptococci by mixing the dry sodium salt of penicillin in their regular diet. Approximately five to ten times the equivalent of a dosage of 100,000 units of penicillin per day in human beings was required for complete protection. This as well as data[2, 4, 5] that have appeared in the literature indicates that penicillin can be absorbed from the small intestine.

It seemed reasonable to believe that if penicillin could be protected from the gastric acidity and yet be available for absorption from the small intestines it could be administered successfully by mouth. Accordingly a series of experiments was undertaken in this regard. Enteric coated penicillin tablets appeared to be one possible method of accomplishing this; however, it was found in agreement with others[6] that consistent blood levels of penicillin in dogs could not be obtained. This was probably due to the variability in the time and the location at which the enteric coating disintegrated in the gastro-intestinal tract. Next the

[1] E. P. Abraham, H. W. Florey *et al.*, *Lancet*, 2: 177–188, 1941.
[2] C. H. Rammelkamp and C. S. Keefer, *Jour. Clin. Invest.*, XXII: 425–437, 1943.
[3] F. J. Thompson, *Jour. Am. Med. Asn.*, 126: 403–407, 1944.
[4] H. M. Powell and W. A. Jamieson, *Jour. Ind. State Med. Assoc.*, 35: 361–362, 1942.
[5] C. H. Rammelkamp and J. D. Helm, *Proc. Soc. Exp. Biol. and Med.*, 54: 324–327, 1943.
[1] W. H. Hodge, *Jour. N. Y. Bot. Gard.*, 45: 32–43, 1944.
[2] F. Rosengarten, "History of the Cinchona Project of Merck and Co., Inc., and Experimental Plantations, Inc. 1934–1943." 45 pp., 82 figs. Rahway, N. J. 1944.
[3] W. C. Steere, *Flora (Revista Inst. Ecuat. Cienc. Nat.)* 4: 1–9, 1944.
[6] Personal communication to B. W. Carey at Lederle Laboratories, Inc., Pearl River, N. Y. from C. S. Keefer, Boston, Mass.

simultaneous administration of penicillin and an antiacid was considered but discarded probably as being impracticable because of various adverse reports which have appeared in the literature.[2, 3] Finally it appeared it might be practical to utilize the fact that little if any fat-splitting takes place under the acid conditions of the stomach and that most of the digestion and breakdown of fats occurs in the small intestine. It is the purpose of this report to present our experience in the preparation and stability of and the human and animal experiments carried out with oil or fat solutions or dispersions of penicillin.[7]

Experience with various extraction and purification procedures for penicillin indicated that it was relatively stable in many organic solvents. Solutions of penicillin acid in an extensive list of naturally occurring fats and oils were prepared by the addition of an ether solution of penicillin acid to the fat or oil. The ether was removed subsequently under vacuum. It was found that the solubility of 300 to 400 units per mgm penicillin acid varied considerably between different oils and fats. One of the best in our series was cottonseed oil in which it was possible to obtain a concentration of 7,500 to 10,000 units of penicillin per ml of oil.

Stability tests on the solutions of penicillin acid in oils, with and without the addition of antioxidants such as α-tocopherol, were disappointing. The half-life of the penicillin activity at room temperature of even the best preparations was from a few days to a week. Because of the apparent instability of penicillin acid in oils or fats no animal tests appeared warranted.

Finally suspensions or dispersions of the Na, Ca, Mg and NH_3 salts of penicillin in various oils were prepared. Many of these preparations are very stable and have been kept at room temperatures for periods of two or three months without any apparent loss in activity. These results are in agreement with those reported by Romansky.[8]

For the trials on oral administration of penicillin in oil suspensions of the Na and Ca salts of penicillin, 150 to 300 units per mgm were prepared in cottonseed oil. These suspensions were dispensed in gelatin capsules made up to contain either 10,000, 25,000 or 50,000 units per capsule. Fig. 1 shows a typical example of the blood and urinary levels of penicillin over an eight-hour period obtained by the oral administration as a single dose of approximately 90,000 units of Na penicillin dispersed in cottonseed oil to an 86 kilogram man. As shown the first urine sample taken 25 minutes after administration contained about

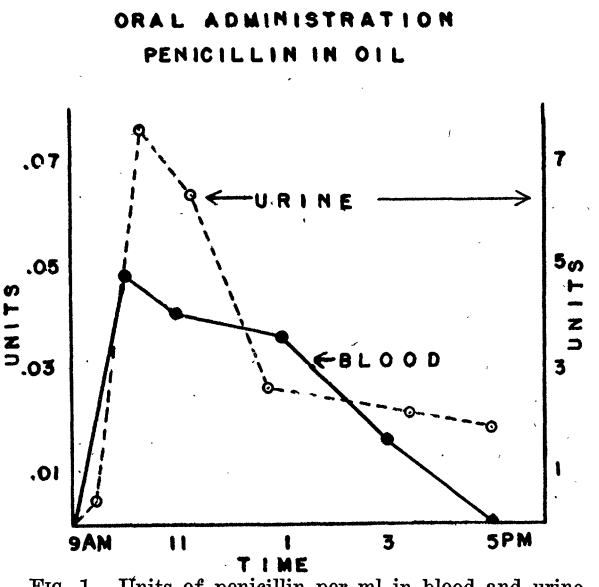

FIG. 1. Units of penicillin per ml in blood and urine after a single oral administration of 90,000 units of penicillin in oil.

0.4 units[9] of penicillin per ml. This would indicate a fairly rapid passage of the 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 finally decreasing to a level of 1.8 units per ml eight hours after administration. Blood levels of approximately 0.05, 0.04, 0.04, 0.02 and zero units[10] of penicillin per ml were obtained from the 1, 2, 4, 6 and 8 hour bleedings. This as well as other trials in human beings and dogs indicate that detectable blood levels of penicillin may be maintained for considerable periods of time after the oral administration of a single dose of penicillin in oil.

It is obvious that a therapeutic blood level of penicillin will vary in different diseases and with different individuals. However, an analysis of the available data in the literature indicates that in actual clinical practice levels of between 0.03 and 0.06 units of penicillin per ml of blood are usually maintained.[2, 11] If this range is accepted as a mean value then a single oral dose of 90,000 units of penicillin in oil will maintain a fairly uniform therapeutic blood level for a period of at least four hours.

If the results presented in Fig. 1 are compared with the blood levels[2] obtained after the intramuscular injection of 20,000 units of an aqueous solution of peni-

[7] The penicillin used in this work was supplied by the Lederle Laboratories, Inc., Pearl River, N. Y.

[8] M. J. Romansky and F. E. Rittman, SCIENCE, 100: 196–198, 1944.

[9] Cup plate assay: J. W. Foster and H. B. Woodruff, *Jour. Bact.*, 47: 43–58, 1944.

[10] The assay method for blood levels was developed in this laboratory and is based upon the turbidimetric measurement of the inhibition of growth of a staphylococcus culture in blood serum-broth mixtures. The results are double checked by means of plate counts. This method will be described elsewhere.

[11] W. E. Herrell, D. R. Nichols and D. H. Heilman, *Jour. Am. Med. Asn.*, 125: 1003–1011, 1944.

cillin it is apparent that 2 or 3 or possibly more injections would be required to maintain a comparable blood concentration over the same period.

Fig. 2 shows the units of penicillin per ml of blood

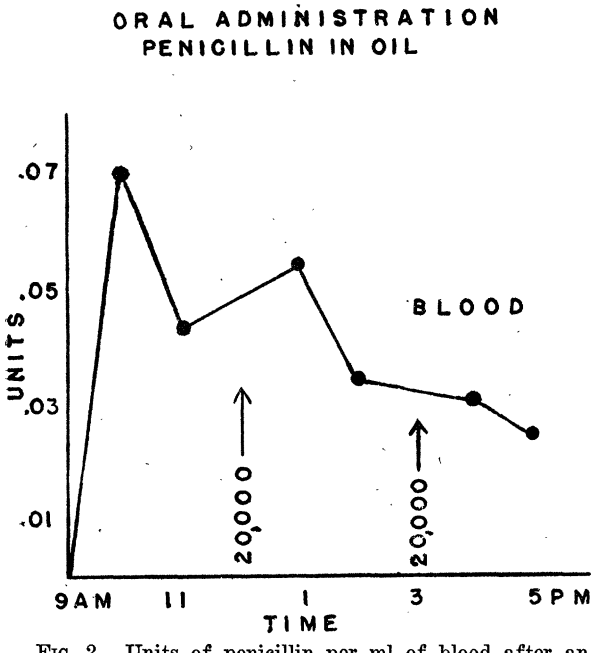

FIG. 2. Units of penicillin per ml of blood after an initial oral dose of 90,000 units and two subsequent doses of 20,000 units of penicillin in oil given three and six hours later.

obtained after the oral administration of 90,000 units of penicillin in oil and two subsequent doses of 20,000 units each given 3 and 6 hours later to a human being. As indicated, a therapeutic blood level was maintained for a period of at least seven hours and only slightly less than a therapeutic level as previously defined was found after eight hours.

It is apparent that even with the oral administration of penicillin in oil a portion is inactivated probably by the gastric acidity, so that one would expect that optimum blood levels would be obtained if the dose were given on an empty stomach. This has been found to be true in dogs and is illustrated in a human in Fig. 2. The first subsequent dose of 20,000 units was given on an empty stomach with a consequent 25 per cent. increase in the penicillin blood level. The second dose of 20,000 units was given approximately one hour after a heavy meal, no apparent increase in the penicillin blood level being observed.

It should be noted that although these preliminary results indicate that a greater amount of penicillin might be required if administered orally than if by intramuscular injection, this increased use of penicillin will probably be offset by several factors. In this regard certainly the greater ease of administration both from the point of view of the doctor as well as the patient must be considered. Furthermore, for oral use less highly refined penicillin should be entirely satisfactory, thus simplifying the present procedures for the production of suitable material.

The author would like to express his appreciation to Dr. R. Bowling Barnes, director of the Physics Division, and to the various members of the staffs of the Stamford Laboratories and of the Lederle Laboratories, Inc., for their helpful advice and assistance.

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RIBOFLAVIN PRODUCTION BY CANDIDA SPECIES

THE synthesis of relatively large quantities of riboflavin (vitamin B_2) by the yeast *Candida guilliermondia* (Northern Regional Research Laboratory strain No. 488) during cultivation in a synthetic medium has been reported by Burkholder.[1] In a second communication[2] the same investigator has described the influence on riboflavin formation of such factors as the concentration and type of carbohydrate, nitrogen and inorganic salts in the medium. For example, it was noted that whereas a riboflavin yield of 75 μg per ml was obtained in the basal medium containing 2 per cent. glucose, the use of maltose, xylose or galactose in place of glucose resulted in a marked inhibition of riboflavin synthesis, although growth was abundant. Similarly, it was found that urea, when employed at a concentration of 0.5 g per liter, enhanced riboflavin production, while in larger concentrations (2.0 to 4.0 g per liter) it was inhibitory to the synthesis of this vitamin. Yeast extract also was shown to promote growth while inhibiting riboflavin production.

These results were confirmed in our laboratory, where it was found that riboflavin formation was extremely variable, ranging from 2 to 80 μg per ml under seemingly identical conditions; whereupon a thorough study was undertaken of the influence of each of the constituents of the medium upon vitamin synthesis. From these studies it was learned that riboflavin production was greater and that higher yields were obtained more consistently, when the trace elements (B, Mn, Zn, Cu, Mo and Fe) were omitted from Burkholder's medium. When the medium was treated with 8-hydroxyquinoline in the manner described by Waring and Werkman[3] to remove Fe, Cu, Zn, Mn, etc., higher riboflavin yields resulted. However, when each of the latter elements was added to a medium extracted with 8-hydroxyquinoline, only

[1] P. R. Burkholder, *Proc. Nat. Acad. Sci.*, 29: 166, 1943.
[2] *Idem., Arch. Biochem.*, 4: 217, 1944.
[3] W. S. Waring and C. H. Werkman, *Arch. Biochem.*, 1: 303, 1943.