inary trials of sodium and barium hydroxide digestion of casein gave no striking differences, the latter seemed preferable and was investigated further. It seemed possible that hydrolysis might be conducted under conditions allowing complete racemization of tryptophane without appreciable destruction. A study of the effect of time and concentration of alkali indicated that hydrolysis of casein with 20 volumes of 5 N $Ba(OH)_2$ for about 5 hours in an autoclave at 15 lb pressure was a suitable treatment. After removal of Ba as $BaSO_4$ at a pH of 4.0 and extraction with ether the hydrolysate was prepared for assay by adjustment to a pH of 6.8 and a concentration of casein of 5 mg/cc. Assays of such hydrolysates against *l*-tryptophane standards yielded recovery values about one-half those obtained from pancreatic hydrolysates of casein and on the assumption of complete racemization of tryptophane during alkaline hydrolysis indicated close agreement between the two methods (Table II). It had been found earlier that pure *l*-tryptophane yielded a value close to 50% when assayed after a similar treatment with baryta.

This microbiological assay procedure for tryptophane appears to compare favorably in sensitivity and reproducibility with similar methods employed for the assay of the B-complex vitamins. A report of its application to a variety of proteins and foods will appear in a later publication.

Summary. Evidence was obtained which indicates that *L. arabinosus* 17-5 may be used for the assay of *l*-tryptophane. Of other related substances tested only anthranilic acid and indole produced a growth response and these are removed by ether extraction.

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Studies on the Absorption of Penicillin from the Stomach.*

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In a previous study,[1] penicillin was shown to be absorbed poorly when it was administered by mouth. In view of the fact that penicillin must be injected parenterally at such frequent intervals in the treatment of certain infections caused by gram-positive organisms, it seemed desirable to make further observations on the absorption of this antibacterial agent from the gastro-intestinal tract.

Methods. The sodium salt of penicillin† was dissolved in 0.85% sodium chloride in a concentration of 1,000 Oxford units per cubic centimeter. This standard solution was used in all experiments.

When penicillin was administered by mouth, 20,000 Oxford units were given in 200 cc of tap water. Frequent samples of blood were withdrawn from the anticubital vein and the serum then separated by centrifugalization. All urine passed for a period of 24 hours was stored as individual specimens at 5°C. The concentration of penicillin in these samples was determined by a method described previously.[2] Gastric juice, bile, and succus entericus were obtained by a Miller-Abbott tube; saliva was collected in a sterile container. When these substances were tested for their destructive effect upon penicillin, strains

* Supported by a grant from the Johnson Research Foundation, New Brunswick, New Jersey.

† Penicillin used in this study was supplied through the courtesy of Dr. George A. Harrop, Squibb Institute for Medical Research, New Brunswick, New Jersey.

1 Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 425.

2 Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 95.

of both Lancefield group A, beta hemolytic streptococcus and *Staphylococcus aureus* were used as the test organism.

Procedure and Results. It was first necessary to determine the effect of the various gastro-intestinal secretions on the activity of penicillin. The pH of the samples of saliva, gastric juice, bile, and succus entericus was determined and a known amount of penicillin was added to 10 cc of each sample. The tubes were incubated at 37°C for a period of 24 hours, during which time frequent determinations of the concentration of the antibacterial substance were made. Gastric juice was found to inactivate the penicillin rather rapidly, whereas saliva, bile, and succus entericus exhibited no such effect. In general, those specimens of gastric juice with the lowest pH destroyed the penicillin most readily.

It was next necessary to determine what factor in gastric juice destroyed the antibacterial action of penicillin. Pepsin was added to veal infusion broth at a pH of 5 so that the final concentration was 0.2%. When a known amount of penicillin was added to such samples and to tubes containing broth at a pH of 5, the rates of destruction of the penicillin in the two tubes were equal.

The effect of acid on the antibacterial action of penicillin was determined by adding it to samples of veal infusion broth with the pH adjusted to 2, 3, 4, 5, and 7.3 with dilute hydrochloric acid. Penicillin was added to 10 cc samples of each so that the final concentration in each instance was 0.625 Oxford units per cubic centimeter. After the penicillin had been added, half of the sample was refrigerated at 5°C and the other half incubated at 37°C. For a period of 24 hours the concentration of penicillin in these samples was followed closely. A portion of one of these experiments is shown in Fig. 1 and it is at once apparent that at a pH of 4, or lower, there is a rapid loss of penicillin activity at 37°C whereas at icebox temperature the destruction is somewhat delayed. In all experiments a pH of 7.3 was associated with only a slight inactivating effect during the 24 hours of incubation.

As a result of the above observations, it

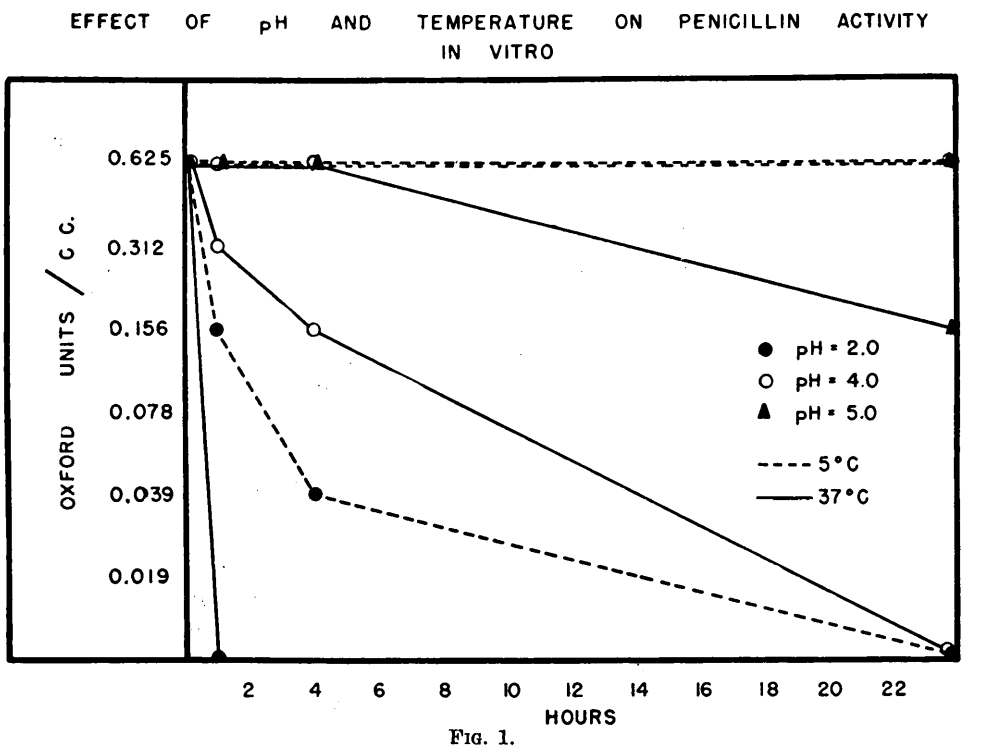

FIG. 1.

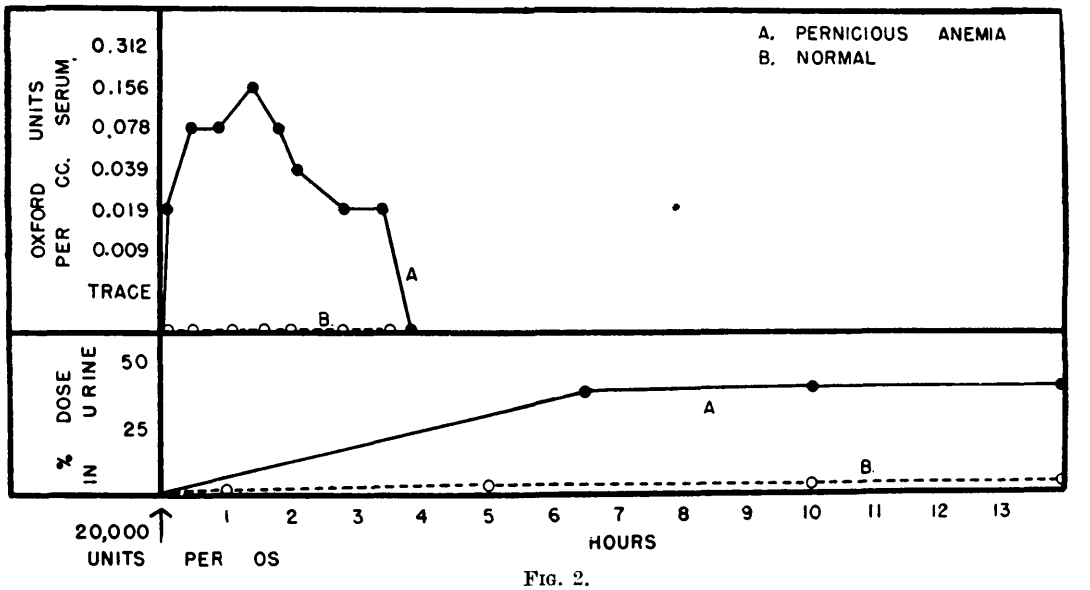

FIG. 2.

seemed apparent that destruction by the acid of the gastric juice was responsible for the apparent poor absorption of penicillin when administered by mouth. If this were true, then, penicillin should be absorbed readily when administered to subjects with achlorhydria. Two patients with the clinical and laboratory findings of classical pernicious anemia were given 20,000 Oxford units of penicillin by mouth. Following the ingestion of this amount in subject A (Fig. 2), the concentration of penicillin in the serum rose rather rapidly to a peak of 0.156 Oxford unit per cubic centimeter. It is of interest that within 10 minutes the serum contained 0.019 Oxford unit per cubic centimeter. At 225 minutes the blood stream showed no antibacterial activity as determined by the test. The first sample of urine, passed 390 minutes after the antibiotic substance was given, was found to contain 7,880 Oxford units. Further specimens yielded only an additional 44 Oxford units.

The results obtained in subject A are in distinct contrast to the observations made in a normal subject (subject B, Fig. 2). In this instance no penicillin was detected in the circulating blood and only 3% of the administered dose was excreted in the urine.

In a second subject with pernicious anemia the total amount excreted in the urine was 4,535 Oxford units after an oral dose of 20,000 units. No blood was obtained for determination of the serum penicillin concentration.

Discussion. Penicillin is absorbed poorly when administered by mouth to normal subjects but if it is administered directly into the duodenum a relatively large amount is absorbed.[1] In one normal subject who received 20,000 Oxford units by mouth both with and without a previous dose of sodium bicarbonate, the concentration of penicillin in the serum and the amount of antibacterial substance excreted in the urine suggested that the acidity of the gastric juice was responsible for the poor absorption observed.[1]

It is well known that acid destroys the activity of penicillin,[3] and these observations have been confirmed by the present study. The inactivating effect of hydrochloric acid was especially marked at a pH of 4 and below, although it may be inhibited somewhat by low temperatures. The specimens of gastric

[3] Abraham, E. P., Florey, H. W., and Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., and Jennings, M. A., *Lancet*, 1941, **2**, 177.

juice studied *in vitro* showed that the lower the pH of the specimen, the greater the inactivating effect. That pepsin was not responsible for the destruction of the penicillin was shown by its failure to cause a decrease in the concentration of the active substance in concentrations of 0.2%.

These studies suggested, then, that penicillin should be absorbed when administered by mouth to subjects with achlorhydria. In 2 patients with pernicious anemia reported herein, the concentration obtained in the serum and the amount excreted in the urine showed that penicillin was absorbed readily. The curve of the plasma concentrations in subject A was not unlike that obtained after intramuscular and intraduodenal administration.[1]

Saliva, bile, and succus entericus did not exhibit any inactivating effect on penicillin, suggesting that oral administration of penicillin might be accomplished effectively by the administration of large amounts of alkali or by the use of enteric coated capsules. The latter method is not likely to prove effective since unless the capsule is dissolved in the upper gut the bacteria in the lower intestines may inactivate the penicillin.[1]

Conclusion. Saliva, bile, and succus entericus obtained from man do not inactivate penicillin. Gastric juice destroys this antibacterial substance rapidly especially at body temperature. Hydrochloric acid, and not pepsin, is apparently responsible for this action of gastric juice.

In 2 subjects with pernicious anemia the absorption of penicillin, when administered by mouth, was greater than that observed in normal subjects.

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Vascular Fragility and Permeability. II. Influence of Fasting and Bile Salts on Induced Pulmonary Hemorrhage.*

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When normal mice on a regular laboratory diet are subjected to sudden and extreme reduction of atmospheric pressure, they die, and their lungs show extensive hemorrhage. This fact was utilized in testing the ability of certain mixtures derived from lemon or orange peel to decrease capillary fragility.[1]

While comparing the effects of several of these mixtures, some of the mice were left for 24 hours to observe any delayed effect they might exhibit. It was noted, however, that the mice in one lot refused food. When these were killed and post mortem examination revealed absence of hemorrhage, it appeared that the "protection" might be due to fasting. Accordingly, one lot of mice was set aside and deprived of food, but allowed free access to water. At the end of 24 hours they were subjected to the partial vacuum, along with others which had received the regular diet. The fed mice showed the usual hemorrhage of the lungs, but the fasted mice showed much less. By extending the fasting time to 48 hours, the difference between the fasting and fed animals was increased.

The fact that the animals which had been taking food showed hemorrhage, suggested that the effect might be associated with the process of digestion, and prompted an investigation of the role which bile salts might play.

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1 Majovski, George J., Lesser, A. J., Lawson, Homer C., Carne, Herbert O., and Thienes, C. H., *J. Pharm. and Exp. Therap.*, in press.