

doxine triacetate is inactive although the diacetate is almost as active as pyridoxine itself.¹⁹ The slow initial growth with biotin methyl ester indicates that this ester also, is used with some difficulty.

¹⁷ Unna, K., and Mushett, C. W., *Am. J. Physiol.*, 1942, **135**, 267.

¹⁸ Snell, E. E., and Strong, F. M., *Enzymologia*, 1939, **6**, 186.

¹⁹ Bohonos, N., Hutchings, B. L., and Peterson, W. H., *J. Bact.*, 1942, **44**, 479.

Since the retardation in growth and acid formation of *L. casei* with biotin methyl ester as compared to biotin, may still be evident after 3 days incubation, it is preferable to use biotin as a standard or vitamin supplement in assay methods involving this organism.

Summary. In common with other organisms which require biotin for growth, *L. casei* can utilize the methyl ester of biotin. However growth and fermentation are slower than with free biotin.

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Excretion of Penicillin in Bile.*

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Florey demonstrated in animals that bile contained penicillin after the intravenous exhibition of the antibacterial substance.¹ The present study was undertaken in order to determine whether it is excreted similarly in the bile in man.

A solution containing 1,000 Oxford units of the sodium salt of penicillin[†] per cubic centimeter in 0.85% sodium chloride was injected intravenously in all patients studied. In 5 patients a double lumened tube was passed under fluoroscopic guidance so that gastric and duodenal contents (bile) could be collected separately by means of constant suction by the method of Agren and Lagerloff.² Specimens were obtained every 15 minutes and placed immediately in the refrigerator. Samples of blood were withdrawn

several times and the serum separated by centrifugalization.

The concentration of penicillin in the serum was determined by a method described previously.³ The pH of the gastric juice and bile was obtained just prior to the determination of the concentration of penicillin. The penicillin in the bile and gastric juice was assayed by the same method as was used with the serum except that in these samples *Staphylococcus aureus* was used as the organism since hemolytic streptococci did not grow satisfactorily. The smallest amount that could be detected was about 0.078 Oxford units per cubic centimeter of bile or gastric juice.

Results. Fig. 1 shows the concentration of penicillin in the blood and in the bile in 3 of the 5 patients studied after an intravenous injection of 20,000 Oxford units. The concentration of penicillin in the serum one minute after the injection varied from 0.625 to 2.5 Oxford units per cubic centimeter. There then followed a rather rapid decrease so that 120 minutes later only small amounts of the bactericidal substance could be de-

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

¹ Abraham, E. P., Florey, H. W., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., and Jennings, M. A., *Lancet*, 1941, **2**, 177.

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

² Agren, G., and Lagerloff, H., *Acta Med. Scandinav.*, 1936, **90**, 1.

³ Rammelkamp, C. H., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 95.

EXCRETION OF PENICILLIN IN THE BILE OF NORMAL SUBJECTS

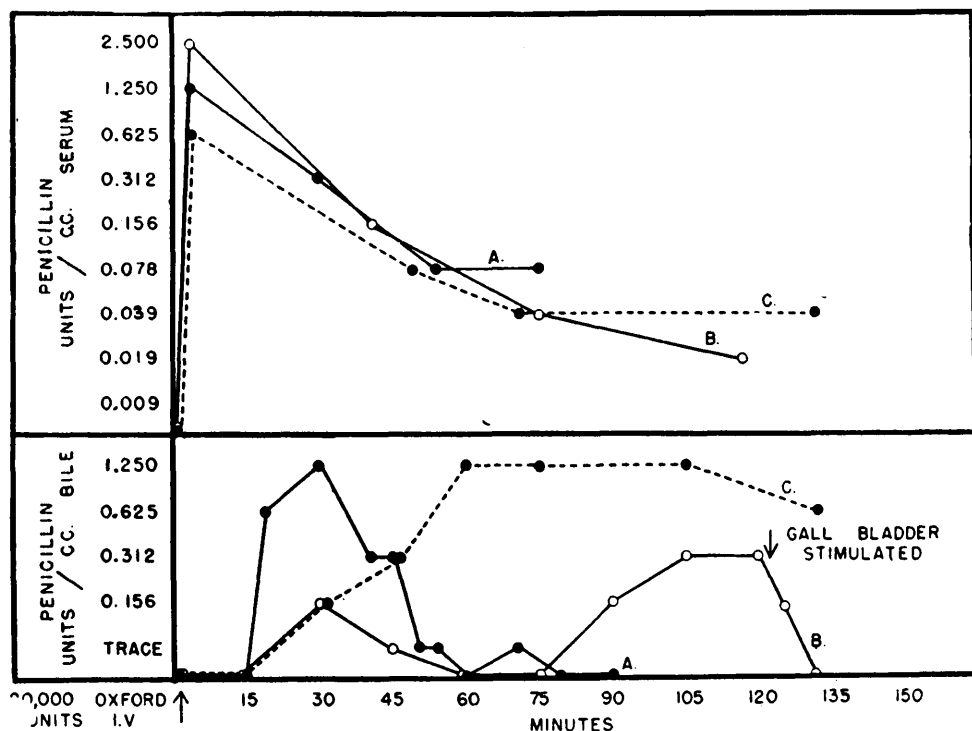


FIG. 1.

tested in the serum.

Gastric juice aspirated every 15 minutes failed to show any penicillin as determined by the tests described, but the bile did contain considerable amounts of the substance. It is to be noted that the first specimen obtained 15 minutes after the administration of penicillin exhibited no antibacterial activity. Specimens obtained at 20 and 30 minutes contained significant amounts of penicillin.

Of interest is the variation in the curves of the concentration of penicillin in the bile in these three subjects. Subject A reached a maximum level at 30 minutes, and then fell rather rapidly. In contrast is curve B which reached its highest level of 0.312 Oxford unit per cubic centimeter 105 minutes after the injection. At 120 minutes, following the administration of a small amount of magnesium sulfate through the tube, the concentration of penicillin in the bile diminished rapidly.

In subject A the pH of the bile at 30

minutes was 7.2, whereas the remainder of the specimens of this bile showed a pH varying from 3 to 6. In the 2 subjects not shown in Fig. 1 the bile contained no penicillin. This may be explained by the observation that the duodenal contents of one subject were white suggesting that no bile was obtained, while in the second subject the test for penicillin was unsatisfactory because of contamination of the cultures.

In view of the fact that the 5 patients had intact, functioning gall bladders, 20,000 Oxford units were given intravenously to a patient recovering from a cholecystectomy, the bile being collected by means of a T-tube in the common bile duct. Fig. 2 shows the concentrations of penicillin in the serum and bile of this subject. The first sample of bile obtained 15 minutes after the injection contained 1.25 Oxford units per cubic centimeter, whereas the serum at 5 minutes contained only 0.625 Oxford unit. The concentration of penicillin in the bile fell gradually

EXCRETION OF PENICILLIN THROUGH A BILIARY FISTULA

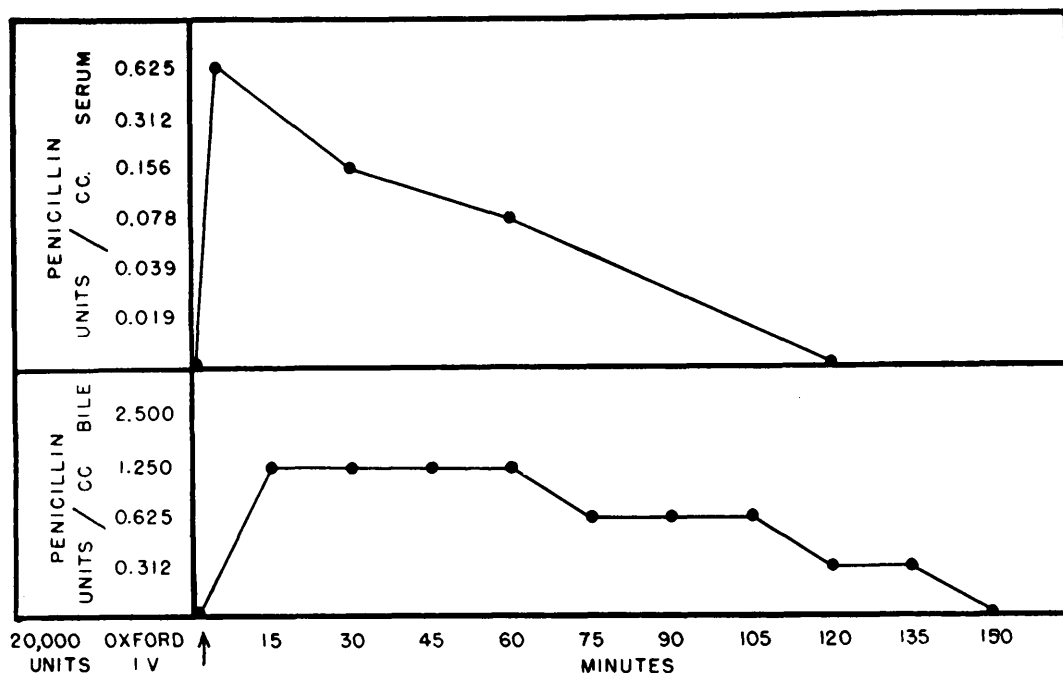


FIG. 2.

and this is in contrast to the results obtained in those subjects with normal gall bladders. The total amount of penicillin excreted in the bile in this subject was 64 Oxford units.

Discussion. It has been demonstrated that saliva, bile and succus entericus do not inactivate penicillin, whereas gastric juice obtained from normal subjects rapidly destroys its antibacterial properties.⁴ The acid in the gastric juice is apparently responsible for the inactivating effect.⁴ In the present study the administration of penicillin to normal subjects resulted in the excretion of the substance in the bile, but not in the gastric juice. In subject A, it was noted that somewhat irregular levels of penicillin were obtained in the bile and this was thought to be due to the inadvertent passage of gastric juice into the duodenum thus lowering the pH and destroying the penicillin. Further, the irregular curves of the concentration of penicillin in the bile might be accounted for by the

periodic emptying of the gall bladder, resulting in dilution of the common duct bile. That this may play a role is suggested by the rapid lowering of the concentration of penicillin in subject B after the stimulation of the gall bladder.

When penicillin was given to a subject without a gall bladder, it appeared in the first specimen of bile obtained at 15 minutes and thereafter the concentration diminished gradually. It is of some interest that the concentration of penicillin was greater in the bile than in the serum in several instances, suggesting that the material was concentrated by the liver.

It is now well recognized that considerable amounts of penicillin are destroyed in the body but the explanation for this loss is not quite clear. The fact that a subject with complete diversion of the bile excreted only 64 Oxford units makes it seem unlikely that loss of penicillin by this route can account for an appreciable amount of that destroyed.

Since penicillin appears in the bile in con-

⁴ Rammelkamp, C. H., and Helm, J. D., Jr., submitted for publication.

centrations which have been shown to be effective against gram-positive organisms,⁷ it would seem that this substance should prove useful in the treatment of certain infections of the biliary system.

Conclusions. The administration of peni-

⁵ Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, to be published.

cillin in man results in the excretion of the substance by the liver in somewhat higher concentrations than are present in the blood stream. No penicillin could be demonstrated in the gastric juice.

Marjorie Jewell, Thelma Maxon and Grace Helm assisted in this work.

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Production of Pulmonary Edema by Thiourea in the Rat, and Its Relation to Age.*

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In earlier communications^{1,2,3} we reported that the oral administration of certain sulfonamides, and of thiourea and its derivatives produces hyperplastic enlargement of the thyroid gland, concomitant with a marked drop in the B.M.R. in weanling and adult rats. Thyroidectomy cells were also found in the anterior pituitary. All of these changes were abolished by the parenteral administration of thyroxin. Iodine was of no avail in the prevention of this "thyroid-metabolic" effect. The hypothesis was advanced that the syndrome is due to an inhibition of thyroxin formation. A consequence of this inhibition is increased thyrotropic hormone formation by the pituitary with the resultant cellular stimulation of the thyroid. Astwood *et al.*⁴ have published similar observations, and furthermore have met with success in the treatment of hyperthyroidism with thiourea and thiouracil.⁵ Kennedy⁶ reported that allyl thiourea will cause hyperplasia of the

rat thyroid, together with changes in the cells of the anterior pituitary, an observation in agreement with our findings with respect to both thiourea and allyl thiourea.

Richter and Clisby⁷ reported that 1-2 mg doses of phenyl thiourea usually proved to be lethal for their rats. Adult animals weighing between 164 and 350 g when given from 1-10 mg of this substance by various routes, showed signs of acute respiratory distress, accompanied by a marked drop in body temperature in less than 30 minutes. Their survival period varied from 2-18 hours. The only detectable pathologic signs were marked edema of the lungs and pleural effusion. These workers suggested that the depression in body temperature might be an indirect effect of the lowered body metabolism, resulting from the pleural effusion and respiratory distress. If the phenyl thiourea was administered in *sub-lethal doses* for a few days, and then gradually increased, the adult animals developed a tolerance for this substance. Adult rats, treated for several months in this

* Supported by a grant from the Rockefeller Foundation.

¹ Mackenzie, J. B., Mackenzie, C. G., and McCollum, E. V., *Science*, 1941, **94**, 518.

² Mackenzie, J. B., and Mackenzie, C. G., *Federation Proc.*, 1942, **1**, 122.

³ Mackenzie, C. G., and Mackenzie, J. B., *Endocrinology*, 1943, **32**, 185.

⁴ Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R., *Endocrinology*, 1943, **32**, 210.

⁵ Astwood, E. B., *J. A. M. A.*, 1943, **122**, 74.

⁶ Kennedy, T. H., *Nature*, 1942, **150**, 233.

⁷ Richter, C. P., and Clisby, K. H., *Arch. Path.*, 1942, **33**, 46.