level of the popliteal space; 8 by crushing.

Measurements of the loss of water were made periodically from the normal and denervated sides. In the series denervated by section and suture, 15 to 30 weeks elapsed before the first sign of response was observed. The amount of the response thereafter increased gradually. After an average of 41 weeks the rate of water output reached an average of 42% of that put out by the control side.

In the animals in which denervation had been accomplished by crushing, the return of the response was quicker and more complete. It commenced an average of 15 weeks after operation and in the nineteenth week had risen to 69% of that of the control side. In 4 of the 8 animals, complete return of function was attained; the others showed rapid increases in function with each succeeding week.

Comments. The amount of the return of the response of the sweat glands on heating the body of the cats measures, we believe, the amount of recovery of the sympathetic fibers. After the crushing operation, almost twice the function returned in less than half the time than when section and suture were employed. The possible role of atrophy of the sweat glands as a contributing factor in the result cannot be fully measured, but the complete return of function in 4 animals suggests that the glands retain their ability to respond as soon as innervation is restored.

Crushing the nerve does less permanent harm, obviously, than cutting it. This result resembles what has been found in somatic nerves and probably on the same basis—less interruption to the pathways over which regeneration takes place.[4] The present method of estimating the amount of return of function in peripheral sympathetic nerves may be useful in forecasting the rate and amount of recovery after injuries to peripheral nerves in human beings. The method may, of course, have value in deciding which procedures are best in facilitating the regeneration of injured nerves[5] and may have application in the clinical study of central nervous lesions and of drugs affecting the secretion of sweat.

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[5] Craig, W. M., *U. S. Naval Medical Bulletin*, 1943, **41**, 613.

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Utilization of Biotin and Biotin Methyl Ester by *Lactobacillus casei.*

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Kögl noted that biotin and its methyl ester are equally active for the growth of yeast (*Saccharomyces cerevisiae*, strain M).[1] Subsequently, it has been shown that biotin methyl ester can be utilized by a number of bacteria which require biotin for growth: *S. aureus*,[2] *B. radicicola*,[3] *Cl. butylicum*,[4] *Streptobacterium plantarum*[5] and a hemolytic streptococcus (C203S);[6] also by various

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[5] Möller, E. F., *Z. physiol. Chem.*, 1939, **260**, 246.

[6] Hottle, G. A., Lampen, J. O., and Pappenheimer, A. M., *J. Biol. Chem.*, 1941, **137**, 457.

TABLE I.

Availability of Biotin and Biotin Methyl Ester for *L. casei*.

2.0 mg of biotin methyl ester in 20 ml of methanol was diluted with water to .0005 γ in 5 ml. Biotin was similarly diluted from a stock solution of 48.88 γ per ml of 20% ethanol. 5 ml of each solution was added to an equal volume of double-strength medium.

Substance	Treatment	Assay medium of	
		Landy and Dicken	Shull *et al.*
		ml 0.1 N acid formed	
Biotin methyl ester (No. 1)	Autoclaved	10.4	4.0
" " " "	Seitz filtered	8.8	3.2
" " " (No. 2)	Autoclaved	9.1	3.6
" " " "	Seitz filtered	10.0	4.1
Biotin	Autoclaved	8.8	4.5
Distilled water (5 ml)	Seitz filtered	1.5	0.9
" " "	Autoclaved	1.6	0.4

fungi,[7,8,9] pea embryos,[10] the rat[11] and the chick.[12] It is presumed that these biological systems can convert the methyl ester to biotin-free acid. The ester is readily hydrolyzed by mild treatment with acid[1] or alkali.[1,13]

In contrast to the equal activities of biotin and biotin methyl ester for yeast, the latter is only about 10% as potent as biotin for *S. plantarum*.[5] The rat, also, may not utilize the ester as efficiently as the free acid.[14]

Recently it was reported that *Lactobacillus casei* cannot utilize biotin methyl ester and must be supplied with the free acid for growth.[15] However, data obtained by us repeatedly under carefully controlled conditions, indicate that *L. casei* can utilize the methyl ester, although growth is somewhat slower than with the free acid. The reason for the difference in results reported from the two laboratories is not apparent.

Methods. The general procedure and medium described by Landy and Dicken[16] in their assay method for biotin was used. Folic acid was kindly supplied by Dr. R. J. Williams.

Unless otherwise indicated, the experiments were made with a strain of *L. casei* originally obtained from Dr. Williams and used for the past two years for the assay of riboflavin and pantothenic acid. Two other strains were also used, one employed by Shull *et al.*[15] and obtained from those investigators and the other from the American Type Culture Collection (No. 7469).

Crystalline biotin and biotin methyl ester were used. The latter was added to the medium within 2 to 3 hours after solution in methyl alcohol to minimize possible hydrolysis and a fresh solution of the crystalline material was prepared for each experiment. A stock solution of biotin in 20% ethanol was prepared and used throughout. The ester was sterilized either by autoclaving it in the medium or by filtration through a Seitz E-K pad or a sintered glass filter, after which it was added aseptically to the sterilized medium. Biotin was autoclaved in the medium.

Experiments. Table I shows that essentially maximum acid formation by *L. casei* occurs with two different preparations of the methyl ester of biotin as well as with biotin. There is no significant difference in the re-

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[16] Landy, M., and Dicken, D. M., *J. Lab. Clin. Med.*, 1942, **27**, 1086.

TABLE II.
Rate of Growth and Acid Formation of *L. casei* with Biotin Methyl Ester and Biotin.*

	1 day		2 days		3 days	
	% transmissible light†	ml 0.1 N acid	% transmissible light	ml 0.1 N acid	% transmissible light	ml 0.1 N acid
Biotin methyl ester	85	1.0	47	6.2	39	9.6
	89	1.1	44	6.1	41	9.8
	85	1.0	48	6.1	39	9.2
Biotin	56	3.1	37	9.4	32	10.3
	56	3.2	36	9.3	33	10.1
	55	3.3	37	9.2	33	10.1
No biotin	99	0.4	94	0.5	94	0.7

* Both compounds (.001 γ/10 ml of medium) were autoclaved in the medium.
† Determined with an Evelyn photometer.

sults with autoclaved and Seitz filtered solutions of the methyl ester thus eliminating the possibility that growth of the lactobacillus is due to thermal hydrolysis of the ester to biotin. For some unknown reason, the amount of acid formed in the medium of

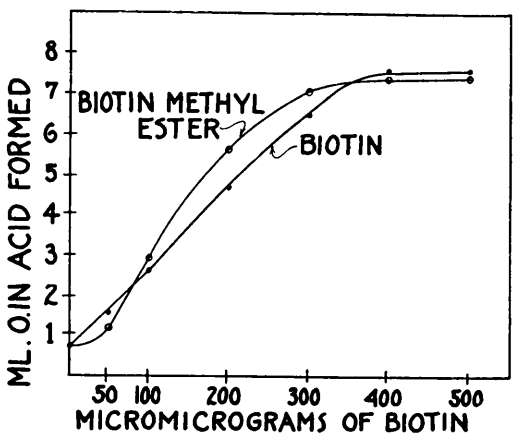

FIG. 1.
Formation of acid by *L. casei* with biotin and biotin methyl ester.

Shull *et al.* was only about half that to be expected. However, the trend of the results follows that noted in Landy's medium.

The initial pH of the medium (pH 5.8 to 7.6) does not affect the ability of *L. casei* to develop with biotin methyl ester.

The Wisconsin and A.T.C.C. strains of *L. casei* also developed abundantly in Landy's medium containing biotin methyl ester and formed as much lactic acid (7 to 8 ml, N/10) as with biotin.

Fig. 1 shows that, quantitatively, biotin and its ester* are equally available to the lactobacillus as judged by titratable acidity of the cultures after 3 days incubation. However, when the course of growth and fermentation is followed periodically, it is noted that the cultures develop more slowly with the ester than with the free acid (Table II). Very little growth is evident with the ester after 1 day, and 3 days are required for maximum acid formation as compared to 2 days with biotin. This lag may be a measure of the time required for conversion of the ester to the physiologically active free acid. The sequence of initial slight growth followed by abundant growth in biotin methyl ester medium is not due to gradual hydrolysis of the ester by the medium itself, since the same sequence occurs if the medium is stored at 37° for as long as five days prior to inoculation.

Nine serial subcultures of *L. casei* in biotin methyl ester medium did not increase its rate of growth.

Discussion. Esters of vitamins required by *L. casei* for normal development are generally inactive or nearly so for this organism: ethyl monacetyl pantothenate and ethyl pantothenate are only slightly active;[17] riboflavin tetraacetate is inactive;[18] and pyri-

* Sterilized by filtration through a sintered glass filter since Seitz pads may adsorb appreciable quantities of the growth factor especially from dilute solutions.

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.

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[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.