elution with hot 60% ethanol solution; (3) continuous extraction by diethyl ether from acid aqueous solution; (4) partition of the brucine salt between chloroform and water, the activity appearing in the aqueous phase; (5) conversion of the brucine salt into the calcium salt; (6) fractionation of the latter by the procedures of Williams and co-workers [(THIS JOURNAL, **60,** 2719 (1938)].

By these means 510 mg. of white, varnish-like calcium salt (corresponding to Williams' fraction "C") was obtained from an extract derived from 160 kg. of liver. This material was fed in amounts averaging 8 mg. per week to each of six albino rats receiving a vitamin B-free diet supplemented by thiamin, flavin and the alkali-hydrolyzed eluate from fuller's earth adsorbate of liver extract. The average gain in weight for each week was as follows: (1) 13.4 g., (2) 19.1 g., (3) 18.8 g. The animals of a control group receiving the same basal diet and supplements, but without the calcium salt preparation, gained on the average as follows: (1) 6.5 g., (2) 4.5 g., (3) 4.2 g. The calcium salt preparation therefore actively stimulates rat growth.

Through the kindness of Dr. Leo Rane the calcium salt preparations were tested for their ability to stimulate the growth of *Streptococcus hemolyticus* and the diphtheria bacillus. They were found to behave like pantothenic acid preparations in stimulating the growth of both microorganisms. For these reasons it appears likely that pantothenic acid is one of the substances, in liver extracts, which are necessary for rat growth.

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RECEIVED APRIL 24, 1939

PANTOTHENIC ACID AS A GROWTH FACTOR FOR THE DOCHEZ NY5 STRAIN OF HEMOLYTIC STREPTOCOCCUS

Sir:

A medium composed of gelatin hydrolyzate, amino acids, inorganic salts, glucose plus such accessory factors as glutathione, thiochrome, nicotinic acid, betaine, flavin, and glucosamine in the presence of a calcium-alcoholic precipitate of a highly purified liver extract provides almost optimum conditions for the growth of the Dochez NY5 strain of hemolytic streptococcus [L. Rane, and Y. Subbarow, *Proc. Soc. Exp. Biol. Med.*, **38,** 837–839 (1938)]. We have found that the further addition of uracil, guanylic acid, xanthine, hypoxanthine, nicotinic acid amide in place of nicotinic acid, and a fraction of liver extract as yet unidentified may also be of significance in the growth of this strain of hemolytic streptococcus.

Certain similarities in the isolation and properties of the unknown factor in the liver extract and pantothenic acid suggested the possibility of substitution. Pantothenic acid "U-6000, *ca.* 50%," kindly supplied by Dr. R. J. Williams, has been tried. Pantothenic acid is active in the growth of the Dochez NY5 strain of hemolytic streptococcus, as indicated in the table. The amount of growth was equal to that obtained with the calcium-alcoholic precipitate of liver extract as described in our previous publication.

Pantothenic acid per 10 cc. basal medium, γ	100	50	25	10	5	2.5	1	0.5
Nephelometer reading (cf. L. Rane, and Y. Subbarow, —*loc. cit.*)	2.9	2.9	2.8	2.8	2.8	2.9	3.5	>4.7
Control, growth of organism in meat infusion broth	2.3							

It is of additional interest that a product synthesized in collaboration with G. H. Hitchings of the Harvard School of Public Health is able to replace pantothenic acid in an otherwise chemically-defined medium. The compound was made by the conjugation of β-alanine ethyl ester with the acyl chloride of acetylated α,δ-dihydroxyvaleric acid. The dihydroxyvaleric acid was obtained by the deaminization of *d*-ornithine. However, the material so prepared was needed in larger amounts than was pantothenic acid.

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RECEIVED APRIL 24, 1939

REACTION OF NEOPENTYL CHLORIDE WITH SODIUM

Sir:

We have isolated from the reaction of one mole of neopentyl chloride and sodium, a 13% yield of 2,2,5,5-tetramethylhexane, b. p. 135° at 736 mm., $n^{20}D$ 1.4049, a 36% yield of neopentane, f. p. −19 to −20°, b. p. 8.3° at 720 mm., and 17.6 g. of a