large, growth was very slow and appeared to occur chiefly at the surface. The contents of the bottle were rotated frequently during a period of 72 hours. Despite the obviously aerobic conditions, a yield of 62% of lactic acid was obtained. The yield of volatile products was similar to that obtained in aerated cultures of *Diplococcus pneumoniae*.[1] Neither *Oidium lactis* nor *B. subtilis* produced the maximum of lactic acid from sugar by growth in this medium. The yield of 89% of lactic acid in a culture of *Lactobacillus helveticus* (*casei* ε, v. Freudenreich) perhaps represents the maximum which could be obtained by the growth of microörganisms in this batch of culture medium.

Summary. *Oidium lactis* and *B. subtilis* were inoculated into culture media prepared from 0.3% solution of meat extract or an infusion of beef muscle, to which were added 1% of Witte peptone, 1 to 1.8% of $Na_2HPO_4 \cdot 12\ H_2O$ and 0.9% of dextrose. Both microorganisms grew rapidly at 37.5° and both yielded about 70% of lactic acid on the basis of the sugar consumed. Approximately one-fifth of the C_3-intermediates from the sugar was further metabolized into 2 moles of formic acid and 1 mole each of acetic acid and ethyl alcohol. Their metabolism in the same culture medium is compared with that of *Eberthella typhosa, Shigella paradysenteriae, Lactobacillus helveticus (casei ε)* and *Corynebacterium diphtheriae.*

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Pantothenic Acid and Nicotinic Acid as Essential Growth Substances for Morgan's Bacillus (*Proteus morganii*).

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Following the announcement by Fildes[1] that nicotinic acid was found to be an essential accessory nutrient for 10 strains of *Proteus,* an investigation was undertaken in this laboratory to determine the nutritional requirements of some 240 strains of *Proteus vulgaris* and other species of the same genus. During the course of the study[2] it was observed that all of the *Proteus morganii* strains exhibited a

1 Fildes, P., *Brit. J. Exp. Path.*, 1938, **19**, 239.

2 Pelczar, M. J., and Porter, J. R., *J. Bact.*, in press.

marked variation from the other *Proteus* cultures. This difference was apparent when the organisms were subcultured in a simple synthetic medium composed of inorganic salts, lactate, and nicotinic acid. None of the Morgan's bacilli was capable of serial subculture in the medium, while practically all of the remaining *Proteus* strains responded with good growth. When this simple medium was supplemented with some 16 amino acids in various combinations the situation was not altered. The absence of some essential growth substance (or substances) appeared to be the likely explanation for the failure of the strains to grow.

This paper is a preliminary report on the nutrition of *Proteus morganii* and the ability of pantothenic acid and nicotinic acid to serve as the essential accessory substances for the growth of the organism.

*Experimental.** Thirty-five strains of *Proteus morganii* constituted the test organisms. Twenty-four-hour cultures on meat infusion agar slants were used as the source of inoculum. The basal medium used throughout was made up of inorganic salts [KH_2PO_4, $(NH_4)_2SO_4$, NH_4Cl, Fe $(NH_4)_2$ $(SO_4)_2 \cdot 6H_2O$, $MgSO_4 \cdot 7H_2O$], glucose, distilled water, and the following amino acids which were incorporated to insure an adequate supply of nitrogenous compounds: dl-alanine, dl-valine, dl-leucine, s-glycine, l-proline, dl-hydroxyproline, dl-aspartic acid, dl-glutamic acid, dl-methionine, dl-phenylalanine, l-tyrosine, d-arginine, l-histidine, dl-lysine, l-tryptophane and l-cystine.

Ten substances which have been identified as growth accessory factors for various organisms were tested alone and in various combinations in the amino acid basal medium. These included: vitamin B_1,† vitamin B_6,† riboflavin,† β-alanine, pimelic acid, nicotinic acid, inositol, cocarboxylase,† glutamine, and cozymase.† Growth of the Morgan's strains failed to occur on subsequent serial transfers in the same medium when one or all of these substances were present in the basic medium.

At this time it was observed that very small amounts of an alcoholic extract of wheat or rice bran stimulated the growth of *Proteus morganii* when added to an otherwise synthetic medium. This led us to believe that panthothenic acid might be an essential growth substance for this organism.

Through the kindness of Professor R. J. Williams, we were able to

* The details of methods and media employed will be reported in a subsequent paper.

† We are indebted to Merck and Company, Inc., for the vitamin B_1 (Betabion), riboflavin, and vitamin B_6, and to Dr. Henry Tauber for the cocarboxylase and cozymase.

test the biological activity of 2 preparations of barium pantothenate, one labeled as 20% pure and the other as 88% pure. When either of these 2 pantothenate preparations was added in quantities of one μg to 10 ml of the basal amino acid medium plus all 10 of the above growth substances, a luxuriant growth occurred within 24 hours after inoculation and continued through 5 subsequent transfers on a similar medium. Further experiments showed that the pantothenate preparation alone would not support growth. Consequently several media were prepared using various combinations of the 10 previously mentioned growth factors along with the pantothenate. By a process of elimination it was observed that when nicotinic acid and pantothenate or cozymase and pantothenate were present in the basal medium, the resulting growth was good. Pantothenate in combination with the other growth substances gave negative results. It is not surprising that cozymase could be substituted for nicotinic acid since it is generally accepted that the nicotinic acid molecule is used in the synthesis of cozymase. A quantitative comparison of the biological activity of the pantothenate preparations is given in Table I.

Although neither sample is pure, we are informed[3] that the impurities present are physiologically inactive. It will be observed that the activity of the 88% pure preparation of barium pantothenate (II) is roughly 4 times that of the 20% preparation (I). The former was easily detectable at 0.01 μg per 10 ml of medium. This fact together with the effectiveness of the material in such an infinitesimal concentration seems to be sufficient evidence for pantothenate to be classed as a growth factor for *Proteus morganii*.

To our knowledge, this is the fourth instance in which pantothenic

TABLE I.
Quantitative Comparison of Biological Activity of 20% and 88% Pure Barium Pantothenate with *Proteus morganii* as Test Organism.

20% barium pantothenate (I)		88% barium pantothenate (II)	
μg added per 10 ml of medium*	Visible growth	μg added per 10 ml of medium	Visible growth
.0	0	.0	0
.01	0	.0025	0
.04	++	.01	+
.1	++	.025	++
.4	+++	.10	+++
1.0	+++	.25	+++

+++, good growth; ++, moderate growth; +, poor growth.
* Medium: Nicotinic acid, 16 amino acids, inorganic salts, glucose and distilled water.

[3] Williams, R. J., personal communication.

acid has been demonstrated to serve as a growth factor for certain bacteria. It has previously been reported as an essential growth factor for *Corynebacterium diphtheriae* (Mueller and Klotz,[4] Evans, Handley and Happold[5]), for the lactic and propionic acid bacteria (Snell, Strong and Peterson[6]) and for the hemolytic streptococcus (Subbarow and Rane,[7] and McIlwain[8]).

Summary. Evidence has been presented which indicates that pantothenic acid and nicotinic acid are the essential growth factors for *Proteus morganii.*

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Urea-Treated Virus as a Vaccine against Rabies.*

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Urea and allied compounds have been studied with regard to their denaturing action on proteins[1, 2] and their inactivating effect on viruses.[3, 4] MacKay and Schroeder mixed rabies-fixed virus and poliomyelitis virus with concentrated urea solutions; complete inactivation of these viruses and loss of antigenicity resulted. The work here reported concerns the action of urea solutions on rabies-fixed virus.

In the first experiment fresh rabbit-fixed virus was ground with a saturated (room temperature) solution of urea in normal saline to make a 50% suspension of tissue. The resulting mixture was smooth

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2 Greenstein, J. P., *J. Biol. Chem.*, 1938, **125**, 501.

3 Stanley, W. M., and Lauffer, M. A., *Science*, 1939, **89**, 345.

4 MacKay, E. M., and Schroeder, C. R., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 74.