cluded in this group since cell suspensions of all 3 species can decompose single amino acids anærobically. However, it is not yet certain that these decomposition reactions can satisfy the energy requirements for growth of these bacteria.

Recent work in this laboratory has resulted in the discovery of 2 additional obligately anærobic bacteria that derive energy by the fermentation of single amino acids. Both organisms undoubtedly represent new species.

The first organism is a spindle-shaped, spore-forming, motile, Gram-negative rod which ferments alanine, serine and threonine as well as lactate and pyruvate. Methionine, valine, cysteine and aspartate are also attacked but much more slowly. Other amino acids and carbohydrates are not fermented.

The fermentation products are very similar to those produced by the genus *Propionibacterium* since all $C_3$ substrates give rise to propionic and acetic acids approximately in accordance with the following equations:

$$3\ CH_3CHNH_2COOH + 2\ H_2O \rightarrow 3\ NH_3 + 2\ CH_3CH_2COOH + 1\ CH_3COOH + 1\ CO_2$$

$$3\ CH_2OHCHNH_2COOH + 1\ H_2O \rightarrow 3\ NH_3 + 1\ CH_3CH_2COOH + 2\ CH_3COOH + 2\ CO_2$$

$$3\ CH_3CHOHCOOH \rightarrow 2\ CH_3CH_2COOH + 1\ CH_3COOH + 1\ CO_2 + H_2O$$

$$3\ CH_3COCOOH + 1\ H_2O \rightarrow 1\ CH_3CH_2COOH + 2\ CH_3COOH + 2\ CO_2$$

Threonine, however, gives butyric instead of propionic acid as the characteristic product:

$$3\ CH_3CHOHCHNH_2COOH + H_2O \rightarrow 3\ NH_3 + 2\ CH_3CH_2CH_2COOH + 1\ CH_3COOH + 2\ CO_2$$

The second organism is a Gram-negative coccus that ferments glycine readily. Very few other amino acids are attacked and none is decomposed as rapidly as glycine. The organism may therefore prove useful for the identification and estimation of this amino acid. The fermentation of glycine proceeds approximately in accordance with the equation:

$$4\ CH_2NH_2COOH + 2\ H_2O \rightarrow 4\ NH_3 + 3\ CH_3COOH + 2\ CO_2$$

Under certain conditions, a considerable amount of hydrogen (as much as 0.35 moles per mole of glycine fermented) is also produced, while the carbon dioxide is increased and the acetic acid decreased.

Since the above reactions are carried out by growing cultures as well as by cell suspensions there can be no doubt that they provide the main energy source for these bacteria.

## 13930 P

## Preparation and Partial Analysis of a Pancreatic Protein Material of Unusual Growth-Promoting Properties.

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During the course of an investigation of the chemistry of beef pancreas, two protein materials were prepared. The experiments described in the following paper[1] indicate that one of these (P-1) has unusual growth-promoting properties as tested with white rats.

*Preparation.* Frozen beef pancreas was finely minced and treated for 24 hr with acid 90% 3A alcohol (30 gallons of alcohol and 1 liter of concentrated hydrochloric acid for each 100 lb of pancreas). The insoluble residue was centrifuged off and thoroughly washed with 70% 3A alcohol (28 gallons of alcohol for the residue from 100 lb of glands). After separation with the centrifuge, the air-dried insoluble material was

1 White, A., and Sayers, M. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 270

treated continuously with warm acetone and the treatment continued until nearly all of the lipid had been removed. Acetone was removed by air-drying or by the use of the vacuum oven. P-1a and P-1b were prepared from 2 separate lots of this acetone-treated material by successive passages through a meat grinder and a sieve (20 mesh). P-1c was passed through the meat grinder, but was not sieved. P-1d was finely powdered in a hammer mill.

TABLE I.
Partial Analysis of Preparations.

| | Analyses of P-1a, P-1b, P-1c and P-1d | | Analysis of P-2 |
|---|---|---|---|
| | Average, % | Range, % | % |
| Moisture | 9.2 | 6.6 -10.8 | 13.9 |
| Ash | 2.2 | 1.6 - 2.6 | 5.35 |
| Lipid | 1.9 | 0.05- 3.2 | 1.0 |
| Nitrogen* | 16.2 | 15.8 -16.5 | 16.7 |
| Phosphorus* | 1.56 | 1.48- 1.62 | 1.51 |
| Tryptophane* | 1.56 | 1.47- 1.62 | 1.86 |
| Tyrosine* | 3.85 | 3.64- 4.06 | 4.77 |

*Moisture-free, ash-free, lipid-free basis.

The alcoholic centrifugate and filtrate mentioned above were combined and adjusted to pH 8 with ammonium hydroxide. The insoluble material that separated was removed by filtration, thoroughly treated with hot acetone, and air-dried. This material was designated P-2. It contained some P-1 material that was not removed when the alcoholic mixture was centrifuged.

*Analysis.* Analytical figures for the various preparations are given in Table I. Tyrosine and tryptophane analyses were carried out by the method of Lugg,[2] without the use of stannite. Phosphorus was determined by the method of Benedict and Theis,[3] or by the method of Fiske and Subbarow.[4] Values found by the two were in close agreement. The Klett-Summerson photoelectric instrument was used for the colorimetric measurements. The nitrogen values were obtained by micro-Kjeldahl analysis.

P-1d was analyzed for prine nitrogen by the method of Graff and Maculla.[5] The value found was 1.31% (8.1% of the total nitrogen). The trichloracetic acid-soluble inorganic phosphorus content of this same sample was found to be 0.146% (9.9% of the total phosphorus). The purine nitrogen and phosphorus figures suggest a nucleic acid content in the neighborhood of 10-14% of the dry, lipid-free, ash-free protein. The atomic purine nitrogen:phosphorus ratio found for this sample (2.18) is fairly close to the ratios calculated from the data of Loring[6] for the nucleic acid of tobacco mosaic virus (1.96-2.16).

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2 Lugg, J. W. H., *Biochem. J.*, 1937, **31**, 1422.

3 Benedict, S. R., and Theis, R. C., *J. Biol. Chem.*, 1924, **61**, 63.

4 Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

5 Graff, S., and Maculla, A., *J. Biol. Chem.*, 1935, **110**, 71.

6 Loring, H. S., *J. Biol. Chem.*, 1939, **130**, 251.