dized phospholipid was extracted by ether and the experiments carried out as before. The material had now markedly oxidizing properties. Moreover it was soluble in water to a clear solution giving a phospholipid + buffer solution which transmitted light without absorption. A typical reading (liver cephalin 10 mg.) is given in Table II.

TABLE II.
Oxidized Phospholipid.

	Readings	Absorption
	mm.	
Buffer alone	200	
" + phospholipid	200	0
" + red. m. blue	115	85
Total absorption by reagent		85
Buffer + phospholipid + red. m. blue	28	172
Difference in absorption due to oxidation		+87

Lecithin gave similar but somewhat lower values. As before, the pH of the solution in the range 5 to 8 had no effect.

Summary. A buffered suspension of the phospholipid from liver and muscle had little or no oxidizing effect on a sensitive preparation of reduced methylene blue. After exposure to air for 24-48 hours, the phospholipid became soluble in water to a clear solution and had marked oxidizing effect on the reduced methylene blue. pH changes of from 5 to 8 were found not to affect the oxidation.

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Growth-Factors for Propionic and Lactic Acid Bacteria.

H. G. WOOD, A. A. ANDERSON AND C. H. WERKMAN.*

From the Bacteriology Section, Iowa Agricultural Experiment Station, Ames, Iowa

Wood, *et al.*,[1] and Tatum, *et al.*,[2] showed that an ether-soluble acid (obtained from yeast-extract, potato-extract or corn-steep) and vitamin B_1 stimulate growth of the propionic acid bacteria. Snell, *et al.*,[3] have shown that ether-extract of potato stimulates *Lactobacillus delbrückii* but an additional factor present in peptone is

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1 Wood, H. G., Tatum, E. L., and Peterson, W. H., *J. Bact.*, 1937, **33**, 227.

2 Tatum, E. L., Wood, H. G., ad Peterson, W. H., *Biochem. J.*, 1936, **30**, 1898.

3 Snell, E. E., Tatum, E. L., and Peterson, W. H., *J. Bact.*, 1937, **33**, 207.

essential for their growth in hydrolyzed casein, tryptophane and glucose medium.

In the present investigation we have continued this study, particularly in determining the effect of lactoflavin on growth. Lava, *et al.*,[4] fractionated yeast-extract and found the portion which contained B_2 was the most active. They suggested that flavin or B_2 might be a stimulant for the propionic acid bacteria but were unable to prove this because of the impurity of their B_2 preparation and lack of a basal medium free of B_2.

We have tested the activity of lactoflavin by adding it to a basal medium which contained per 100 cc. (1) ether-extract of 3 gm. of yeast-extract, (2) 1.0 gamma B_1 (Merck's crystalline), (3) 0.3 gm. $(NH_4)_2SO_4$, (4) 0.6 gm. NaOAc, (5) 1.0 gm. glucose, (6) inorganic salts. In this medium most propionic acid bacteria failed to grow after the 4th transfer. Addition of hydrolyzed casein or a mixture of purified amino acids gave consistently good growth throughout numerous transfers with all cultures tested (11W, 34W, 39W, 52W, 53W, 49W).

Crystalline lactoflavin was added to the basal medium in concentration equivalent to 0.00, 0.01, 0.05, and 0.20 mg. per 100 cc. of medium; 5 cc. amounts of medium were inoculated with one drop of a suspension of cells grown on yeast-extract medium. Transfers were made after 3 days' growth at 30°C. and the acid was titrated after incubation for 5 days. The stimulation produced by lactoflavin was evident on the first transfer but by repeated transfer the results were more distinct. With lactoflavin there was a shorter lag phase. Culture 34W produced the following quantities of N acid per 100 cc. of medium when no flavin was added, 2.2, 1.9, 2.3, 0.8, 1.2, 0.4, 0.9 and with 0.1 mg. of flavin, 3.6, 3.4, 4.4, 3.1, 4.0, 4.1, 4.4 respectively on the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th transfers. Certain cultures failed to grow on repeated transfers and apparently require amino acids even in the presence of lactoflavin which is non-essential when amino acids are supplied.

The lactic acid cultures were not able to grow on the basal $(NH_4)_2SO_4$ medium excepting one unidentified homofermentative-lactic strain. It does not require amino acids or lactoflavin but ether-extract of yeast-extract is indispensable for its growth on the medium and vitamin B_1 acts as a further stimulant. Its simple growth-requirements imply that it may be of considerable economic importance.

With other homofermentative-lactic bacteria, *L. delbrückii* and

[4] Lava, V. G., Ross, R., and Blanchard, K. C., *Philippine J. Sci.*, 1936, **59**, 493.

L. helveticum, no growth has been observed when hydrolyzed casein, tryptophane and lactoflavin are added to the basal medium. Addition of an alcohol-soluble fraction from malt-sprouts stimulates growth.

The heterofermentative-lactic bacteria are somewhat different in their requirements. If 0.15 gm. of hydrolyzed purified casein is added to 100 cc. of the basal medium, culture L4, *L. pentoaceticus,* does not grow vigorously but when tryptophane (10 mg.) is added as well, growth is abundant on repeated transfer. Tryptophane is undoubtedly a beneficial if not an essential amino acid for *L. pentoaceticus.* Snell, *et al.,*[3] have shown the same to be true for certain homofermentative-lactic bacteria but Orla-Jensen, *et al.,*[5] state that tryptophane is not essential for the lactic acid bacteria. Since their basal medium contained whey, it is doubtful whether the conclusion is justifiable. When purified amino acids (glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, cystine, methionine, tryptophane, proline, hydroxy-proline, aspartic acid, glutamic acid, arginine, lysine, histidine) were substituted for the hydrolyzed casein, growth was very poor. Apparently some additional amino acid is essential. Cultures L2 *L. mannitopoeus,* L5 *L. lycopersici,* and S9 *Streptococcus paracitrovorous* grow very poorly on the basal medium plus hydrolyzed casein and tryptophane but when lactoflavin is added the growth is more luxuriant and the cultures may be carried through repeated transfer. This observation of the activity of lactoflavin in lactic fermentation is similar to that made by Orla-Jensen, *et al.*[5] Since pantothentic acid is ether insoluble, their conclusion that it is essential to the lactic acid bacteria is questionable. The only unknown factor in our medium other than hydrolyzed casein is the ether-soluble factor.

[5] Orla-Jensen, S., Otle, N. C., and Snog-Kjer, Agnete, *Kgl. Danske Vidensk. Selskav,* Copenhagen, 1936, ser. 9, **6**, 1.