been adequately described for this organism; however, a previously described medium[4] in which the phosphate buffer was replaced by sodium acetate and which was supplemented with an oleic acid source, "Tween 80," 10 mg. per 10 cc., enzymatic digest of casein,[5] 10 mg. per 10 cc., and Wilson's liver fraction LR, 100γ per 10 cc., supports good growth of the organism in the presence of liver extracts containing anti-pernicious anemia principles and can be used successfully as an assay medium. The enzymatic digest of casein replaces clarified tomato juice.[3] Tests were incubated for twenty-four hours at 37–38°.

With the above medium or one containing clarified tomato juice (0.5 cc. per 10 cc.) in place of the enzymatic digest of casein, thymidine adequately replaced the liver extracts containing anti-pernicious anemia principles. Half-maximum stimulation of growth was obtained at a concentration of 1–3 γ of thymidine per 10 cc. Thymine was inactive at concentrations as high as 100 γ per 10 cc.

When the medium containing tomato juice was utilized, as little as 1 cc. of sterile, aerated distilled water added aseptically to 10 cc. of medium replaced the liver extract, and this effect was enhanced by aseptic addition of ascorbic acid. However, when the enzymatic digest of casein was used in place of tomato juice, the aerated water was inactive, but ascorbic acid (1 mg. in 1 cc. of sterile, aerated water per 10 cc. of medium) added aseptically still adequately replaced the liver extracts containing anti-pernicious anemia principles for the nutrition of this organism. The function of ascorbic acid in replacing the liver extract will be reported separately.

Since thymidine adequately replaces vitamin B_{12} in the nutrition of *Lactobacillus lactis* Dorner, it appears probable that vitamin B_{12} functions in the biosynthesis of thymidine.

(4) Guirard, *et al.*, *Arch. Biochem.*, **9**, 361 (1946).
(5) Roberts and Snell, *J. Biol. Chem.*, **163**, 499 (1946).

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A NEW COLOR TEST FOR TRYPTOPHAN

Sir:

It has been observed that at room temperature perchloric acid converts trytophan to a fluorescent yellowish green compound. Fluorescence is particularly strong in ultraviolet light. Tryptophan may readily be identified in untreated proteins by this test. This reaction is not given by other amino acids and biologic substances with which it is usually associated. Indol acetic acid, however, gives a slight pink color and slight fluorescence under the conditions of the test.

The Test.—One-half of 1 cc. of water containing 0.5 mg. of tryptophan, or about 10 mg. of albumen (egg powder) or any other tryptophan-containing protein, is placed in a test-tube. The protein does not have to be in solution. Three cc. of perchloric acid (C. P. 70–72%) is added and the contents of the tube are well mixed. A quite stable, intense greenish-yellow color develops within a few minutes attaining maximum intensity in about ten minutes. Upon the addition of 0.1 cc. of a 1% ferric chloride solution, the greenish-yellow color becomes reddish-orange. If the ferric chloride solution is added to the tryptophan-containing solution before the perchloric acid, the reddish-orange color is formed instantaneously. For the detection of minute amounts of tryptophan, ultraviolet light and perchloric acid without ferric chloride should be employed.

The following tryptophan-containing materials gave the reaction: casein, albumen (egg powder), human blood serum, pepsin and crystalline soybean trypsin inhibitor.

The following amino acids did not give the reaction: glycine, alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, cysteine, cystine, methionine, threonine, proline, hydroxyproline, histidine, arginine, lysine, serine, aspartic acid, glutamic acid, and *p*-aminobenzoic acid.

S. S. Cohen (*J. Biol. Chem.*, **156**, 691 (1944)) made the interesting observation that when carbohydrates and tryptophan were heated for ten minutes at 100° in 30% perchloric acid colored condensation products form. In Cohen's reaction boiling is an essential factor. The green fluorescent compound described in the present communication, however, forms readily at room temperature, carbohydrates do not interact and this reaction does not take place in 30% perchloric acid.

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