NUTRITIONAL STUDIES ON STREPTOCOCCUS LACTIS

I. An Unidentified Growth Factor Found in Yeast Extract

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Received for publication April 30, 1943

The use of micro-organisms in assaying the vitamin content of food products has resulted in attempts to develop media of known composition. As the medium becomes more highly purified, growth may be retarded or may fail completely. From time to time, therefore, new substances essential or stimulating to growth have been recorded. Certain unknown "growth-promoting" factors still exist, while some have been successfully identified. As Snell and Mitchell (1941) demonstrated, adenine and thymine are essential for *Streptococcus lactis*. Stokstad (1941) discovered, for *Lactobacillus casei*, a growth factor which was a dinucleotide or a mixture of nucleotides and which could be partially replaced by a mixture of guanine and thymine. Strong, Feeny, and Earle (1941) recorded the stimulating action of asparagine on *Lactobacillus casei*. In wheat flour, Wegner, Kemmerer, and Fraps (1942) found a highly stable stimulating substance for *Lactobacillus casei*. According to Pollack and Lindner (1942), glutamine or glutamic acid is essential for the lactic-acid bacteria. Feeny and Strong (1942) identified as l-asparagine, the stimulating material found in several substances, while Bauernfeind, Sortier, and Boruff (1942) recorded the stimulating action of lecithin on *Lactobacillus casei*. Pollack and Linder (1943) studied in peptone, a substance that stimulated the growth of *Lactobacillus casei*. Although they did not identify this substance, they gave several of its chemical characteristics.

MEDIUM

The composition of the test medium was as follows: glucose 10 gm., sodium citrate 6 gm., adenine 10 mg., guanine 10 mg., xanthine 10 mg., uracil 10 mg., dl-glumatic acid 0.5 gm., d-cysteine 0.1 gm., dl-leucine 0.1 gm., dl-isoleucine 0.1 gm., l (+) histidine 0.04 gm., d-arginine 0.08 gm., dl-valine 0.16 gm., dl-lysine 0.2 gm., thiamin hydrochloride 100γ, pyridoxin hydrochloride 100γ, calcium pantothenate 100γ, riboflavin 200γ, nicotinic acid 100γ, biotin 1γ (S.M.A. concentrate ✻1000), folic acid[1] 0.005, K_2HPO_4 0.5 gm., KH_2PO_4 0.5 gm., $MgSO_4 \cdot 7H_2O$ 0.2 gm., NaCl 0.01 gm., $FeSO_4$ 0.01 gm., $MnSO_4$ 0.01 gm., distilled water 1000 ml.

The hydrogen-ion concentration was adjusted to pH 6.6–6.8. All vitamins were sterilized separately and added aseptically to the medium. Tubes contained 10 ml. and were sterilized at 15 pounds for 20 minutes.

[1] The folic acid used in this study was obtained from Dr. H. K. Mitchell, University of Texas.

ASSAY METHOD

Two strains of *Streptococcus lactis* were used in these studies with identical results except that one strain grew faster than the other. The cultures were carried in yeast glucose peptone broth. Transfer to the test medium was made by a 0.01 ml. loop directly from a 24-hour culture. Where growth resulted, at least five loop transfers were made through the test medium. The dilution resulting from this transfer would remove any contaminating material from the original medium. This method avoids any possible injury to the organism from washing with saline or water.

Growth was measured by visual turbidity and pH development.

RESULTS

The basal medium failed to support the growth of the organism. Whatever growth did occur was slow and sparse and failed upon transfer. The addition of other amino acids did not produce increased growth. The following amino acids, besides those already in the medium, were tested: dl-methionine, l-tyrosine, l-tryptophane, dl-phenylalanine, dl-threonine, l-proline, dl-serine, B alanine, glycine, aspargine, l-aspartic acid, l-hydroxy proline, l-cystine, dl (+)-alanine, and norleucine.

Conceivably, the substance required for growth might be a known vitamin. To test this possibility the following vitamins were added to the basal medium: inositol, 2-methyl-1-4-naphthoquinone, choline, and p-amino benzoic acid. Various combinations of amino acids and vitamins, as well as a complete list of both, were added to the substrate.

The addition of a yeast extract was found to stimulate the organisms, resulting in very good growth and acid development. The unknown substance appeared to be organic in nature, since ashing the extract over a bunsen flame completely destroyed the activity. An attempt was made to obtain the material in a somewhat purer state. Twenty grams of Difco yeast extract were dissolved in 250 ml. of distilled water; and excess of lead acetate was added to the mixture; and the precipitate was removed by filtration. ($AgNO_3$, $HgCl_2$, $CuSO_4$, and $ZnCl_2$ have failed to precipitate the material in question.) The metallic ion was removed by H_2S. The active material was found in the filtrate, while slight to negative activity was found in the precipitate. Fuller's earth and darco charcoal failed to adsorb the factor at pH 2.0, 3.0, 6.6, or 10.5. The addition of $Ba(OH)_2$ or of oxalic acid did not precipitate or destroy the activity. The material, though readily soluble in water, was insoluble in $CHCl_3$, $(CH_3)_2CO$, $C_2H_5—O—C_2H_5$, C_2H_5OH and CH_3OH. Attempts to concentrate the active substance from the water by adding alcohol were a failure. The active material was readily dialyzable through a cellophane membrane. Vacuum distillation at higher temperatures failed. After the substance had been placed under vacuum, the temperature was slowly raised from 100°C to 210°C. A receiving flask was placed in the system and immersed in ice water. As subsequent tests proved, the activity was destroyed by the heat. No active substance was found in the distillate or residue. Evaporation and examination under the microscope indicated a

mixture of crystals. Since many salt impurities would naturally be present, one would expect to find these various crystal structures.

The addition of $CuSO_4$ to the substance, followed by evaporation and solubility tests, indicates that a copper complex, if formed, must be classed as water-soluble and methyl-alcohol-insoluble.

Several preparations of the stimulating substance were made, but results were invariably the same. In all tests the liquid was concentrated to a volume of 20 ml., and 0.1 cc. was added to each 10 ml. of the medium.

Attempts to replace the unknown substance with known materials have failed. The addition of yeast nucleic acid had no effect. The addition of d-arabinose and/or d-galacturonic acid did not stimulate growth.

SUMMARY

An unknown substance in yeast extract is essential for the growth of certain strains of *Streptococcus lactis*. It is apparently not a known vitamin and could not be replaced by a combination of 23 amino acids tested. It is not precipitable by Pb, Ag, Hg, Cu or Zn salts. Fuller's earth or darco activated charcoal fails to adsorb it at various pH values. It is not soluble in any common lipoid solvent. Heating to 210°C. under a vacuum destroys the activity.

The author wishes to thank the members of the Chemistry Division, also Samuel Lepkovsky of the Poulty Husbandry Division, for advice and assistance; Merck and Company for certain amino acids used.

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