Influence of Biotin on Protein Formation in 2 Lactobacilli. (25060)

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The role which biotin plays in metabolism has been investigated extensively. In some instances participation of vitamin in specific chemical transformations has been well established. It has been shown that a deficiency of biotin in the diet of the rat results in decrease in rate of fatty acid synthesis(1). It has been postulated that in the mechanism of this process biotin functions in carboxylation of acetyl-coenzyme A to form malonyl-coenzyme A. A biotin deficiency in chicks has been reported to result in sharp decrease in tissue amylase activity and in albumin synthesis(2). These effects were demonstrated closely related to interference in some of the various reactions involved in the Krebs Cycle in which biotin is known to take part rather than to protein formation as such. Konikova *et al.*(3) reported that excess biotin ingested by biotin-deficient rats resulted in increased protein formation. A more recent investigation by these authors(4) led them to conclude that biotin increased rate of S^{35} methionine incorporation into body proteins. In this connection it seemed worthwhile to ascertain whether or not biotin functioned similarly in growth of certain microorganisms.

Methods. The studies were carried out in an attempt to relate biotin concentration in media to protein formation in cells of 2 microorganisms (*L. casei* and *L. arabinosus*). Cultures of the 2 microorganisms were grown in presence of S^{35} labeled methionine added to a Difco assay medium. Biotin was incorporated in this medium in varying concentrations ranging from 0.00004 μg to 0.016 μg/ml, the lowest level being minimum concentration of vitamin required to support cell growth. Inoculated cultures were incubated at 37°C and growth of the organisms terminated during the log phase. Yield of cells from each condition of growth was harvested, and the cells washed and dried. Total nitrogen/unit weight of dry cells was determined by micro-Kjeldahl procedure and the S^{35} count of hydrolyzed cells measured by automatic scaler.

Results. Typical results are presented in Table I. It appears that biotin incorporated in the medium in excess of that required for optimal growth of the 2 organisms did not result in an increase in nitrogen content or in radioactivity of the harvested cells. In other words, our data do not indicate an increase in protein formation with increased biotin concentration in the medium. While the 2 microorganisms employed responded somewhat differently with respect to range of biotin concentrations required to sustain optimal growth, their general performance was similar. Because of this similarity in response, only typical data for *L. casei* are presented. Nitrogen concentration/unit weight of dry washed cells decreased within the range of 400-fold increase in biotin concentration as indicated by increase in ratio of non-nitrogenous material, whereas radioactivity/unit weight of dry cells remained essentially constant. Results of studies with *L. arabinosus* showed essentially the same trends with somewhat less increase in non-nitrogenous material to nitrogenous material ratio.

TABLE I. Influence of Biotin Concentration in Medium on Ratio of Non-Nitrogenous to Nitrogenous Materials (Non-N/N) and on Ratio of S^{35} Activity (Counts/Min.) to Nitrogen Content (S^{35}/N) of *L. casei* Cells Grown Thereon.

Biotin conc. (μg/ml medium)	Non-nitrogen-nitrogen ratio	S^{35}/nitrogen ratio
.00004	10.8	700
.00008	11.4	660
.00016	12.1	690
.0016	13.1	650
.016	12.9	700

Summary. The data obtained do not indicate that biotin concentration of the medium in excess of that required for optimum growth of organism exerts any positive effect on protein formation in either *L. casei* or *L. arabinosus*. On the contrary, a slight negative effect was noted, particularly when *L. casei* was used as test organism. The results appear to support the observations that lipide formation is preferentially affected by biotin concentration.

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Analyses of a Toxic Factor, Lethal to Paramecium Present in Non-Glass-Distilled Water. (25061)

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While working on the effect of proteolytic enzymes on *Paramecium aurelia* (1) we became aware of an unknown substance in our distilled water which caused the death of this protozoan within 20-30 minutes. The same water, after redistillation in Pyrex glass (to be referred to as doubly glass-distilled), was no longer toxic for these organisms. The toxicity pattern is as follows: First, the ciliate's locomotion is affected, causing it to move erratically in all directions and to spin around on its long axis. Body deformation then sets in usually accompanied by ectoplasmic extrusion, or "blistering" (Fig. 1). Finally, the afflicted organism slows down and succumbs. After this, some degree of disintegration of the cytoplasm of the dead organism occurs (Fig. 2) seldom involving the nucleus. According to Heilbrunn (2) toxic action of distilled water can be attributed in certain instances to presence of poisonous impurities which may presumably represent contamination products from the conventional types of metallic distillation apparatus. It is also known (*loc. cit.*) that a trace of calcium is capable of neutralizing the action of these substances. But the toxic agent or agents were not identified, nor was the nature of their action sufficiently elucidated. The purpose of this study was to use *Paramecium* as the test organism in an attempt to analyze the toxic factor or factors in our distilled water.

Materials and methods. A kappaless stock of *Paramecium aurelia*† was cultured in a medium containing boiled oats. The culture reached peak growth in about 2 weeks, stayed luxuriant for a few more days, then rapidly declined. Doubly glass-distilled water‡ was used exclusively for culturing, making solutions as well as washing glassware. The experimental procedure was as follows: The Paramecia were transferred with a micropipette from an actively growing culture at its peak growth to a depression slide (3″ x 1¾″) with a central 1⅜″ diam. depression. Two drops (40 drops equal approximately 1 ml) of culture medium, containing no less than 100 paramecia, were added to 1½ ml of the test solution. Observations were made at room temperature with both stereoscopic and compound microscopes. A moisture-saturated Petri dish was utilized for maintenance of test cultures for lengthy periods of observation. Stock solutions (0.001 M) were prepared from which appropriate dilutions or combinations were made. Routine pH determinations were made with a Beckman pH meter on all test solutions. 35 mm Kodachrome slides were made with the aid of an AO Spencer 20X dark phase contrast objective. The 3 slides used as illustrations were printed from negatives taken from the original Kodachrome transparencies. A threshold concentration is specified as one which most closely duplicates the toxicity of the distilled water in question, *i.e.*, death of all or nearly all paramecia in 20-30 minutes, preceded by morphological

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