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SYNTHESIS OF VITAMINS BY INTESTINAL BACTERIA

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Recent studies on growth factors in microörganisms have contributed much to our general knowledge of nutrition and have provided a basis for devising quantitative microbiological assays for vitamins occurring in both plant and animal materials. Deficiencies of special growth factors for yeasts, molds and bacteria have received much attention recently, but the synthesis of vitamins by these non-green plants, of equally great significance, has not been extensively investigated.

The extent to which bacteria living normally in the alimentary tract of an animal may synthesize growth factors and contribute directly to the vitamin requirements of the animal constitutes a problem of some importance. The present paper represents an attempt to determine the approximate amounts of certain B vitamins produced by species of intestinal bacteria grown as pure cultures in a chemically defined medium.

Synthesis of vitamins has already been reported for a considerable number of bacteria. Snell and Strong[1] demonstrated synthesis of riboflavin by lactic acid bacteria. Silverman and Werkman[2] showed that certain propionic acid bacteria make thiamine or its intermediates. Some strains of dysentery bacilli[3] are able to form thiamine, and also Coenzyme I or II, riboflavin and perhaps biotin. It has been reported that a strain of diphtherial organisms[4] can make thiamine, Coenzyme I or II and riboflavin.

The evidence obtained by several investigators indicates that bacteria normally living in the rumina of herbivores, such as sheep and cattle, produce considerable quantities of vitamins. Thus it has been found that the common *Bacillus vulgatus* living in the intestines of herbivores is capable of synthesizing thiamine.[5] Recently it has been shown[6] that considerable amounts of riboflavin, pyridoxine and the antihemorrhagic vitamin are formed in the rumina of sheep and cows fed on diets low in these vitamins, and the source of the vitamins is assumed to be commensal microörganisms. Almquist, *et al.*,[7] demonstrated that common bacteria, such as *Bacillus subtilis* and *Escherichia coli*, can synthesize vitamin K. The phenomenon of refection which gives protection against certain vitamin deficiencies in labo-

ratory animals indicates that the normal intestinal flora of mammals may synthesize appreciable amounts of vitamins.

The intestinal bacteria employed in the present study were kindly supplied by Dr. George Valley, Department of Bacteriology, Yale University. The organisms, *Escherichia coli*, *Proteus vulgaris*, *Bacterium aerogenes*, *Alcaligenes fecalis*, *Bacillus mesentericus* and *B. vulgatus*, were grown as stock cultures on Difco nutrient agar. A special liquid medium prepared for the studies of growth factor production was made up as follows: K_2HPO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.1 gm.; NaCl, 5.0 gm.; $CaCl_2$, 0.005 gm.; glucose, 10.0 gm.; recrystallized asparagine, 2.6 gm.; l-tryptophane, 0.1 gm.; l-cystine, 0.05 gm.; redistilled water, 1 liter. Small measured amounts of the following trace elements were added: Fe, Mn, B, Zn, Cu

TABLE 1

VITAMIN CONTENT OF BACTERIAL CULTURES GROWN FOR 48 HOURS AT 36°C. AND INOCULATED MEDIUM KEPT AT −2°C.

	BIOTIN		RIBOFLAVIN		THIAMINE		NICOTINIC ACID	
SPECIES OF BACTERIA	MILLI-GAMMA PER ML. OF MEDIUM	MILLI-GAMMA PER ML. OF CULTURE	GAMMA PER ML. OF MEDIUM	GAMMA PER ML. OF CULTURE	GAMMA PER ML. OF MEDIUM	GAMMA PER ML. OF CULTURE	GAMMA PER ML. OF MEDIUM	GAMMA PER ML. OF CULTURE
Escherichia coli	0	1.050	0	0.048	0.023	0.075	0.004	0.028
Proteus vulgaris	0	2.385	0.001	0.044	0.023	0.104	.....	Required
Bacterium aerogenes	0.015	2.370	0.004	0.140	0.005	0.148	0.005	0.300
Alkaligenes fecalis	0.023	0.446	0	0.067	0.033	0.146	0	0.066
Bacillus mesentericus	.....	Required	0	0.023	0.015	0.103	0	0.339
Bacillus vulgatus	0.028	1.365	0	0.136	0.031	0.150	0	1.181

and Mo. The medium was adjusted to pH 6.8 and sterilized by autoclaving at 15 lb. for 15 minutes. The inoculum was prepared by transferring a small amount of the organisms growing on agar to 5 ml. of physiological salt solution in test tubes. With a sterile pipette 0.1 ml. of the saline suspension was inoculated into 20 ml. of sterile culture liquid contained in small Erlenmeyer flasks. One set of inoculated flasks was kept as a control with growth inhibited in a room at −2°C., while the other was maintained in an incubator at +36°C. The period of growth was 48 hours unless stated otherwise.

Proteus vulgaris appears unable to synthesize nicotinic acid and *B. mesentericus* is deficient in biotin. It was found necessary, therefore, to add the deficient vitamin to the basal medium for these species in order to obtain growth so that tests could be made for the other vitamins which might be synthesized.

The methods used in assaying for riboflavin, biotin and nicotinic acid involved the use of *Lactobacillus casei* ϵ, *Saccharomyces cerevisiae F. B.* and *Lactobacillus arabinosus* as indicators in microbiological tests described by Williams and others.[8] Thiamine activity was tested by the *Phycomyces* assay method.[9] Growth of the bacteria to be tested was measured with a turbidimeter, and the fresh weight of the cells in each culture was calculated by reference to standard fresh weight turbidity graphs prepared for all the species studied. At the end of the growth period, all cultures were acidified with sufficient concentrated H_2SO_4 to make the liquid approximately 1 *N*. The acidified cultures were autoclaved at 15 lb. pressure for 30 minutes to effect hydrolysis of the cells. The samples were cooled, brought to pH 5.0 with NaOH and diluted to standard volume. The amounts of the solutions to be used in making the tests were determined by preliminary trials, and

TABLE 2

SYNTHESIZED VITAMIN RESIDUES IN CULTURES GROWN FOR 48 HOURS AT 36°C. VALUES EXPRESSED AS GAMMA PER GRAM OF FRESH CELLS

SPECIES	BIOTIN	RIBOFLAVIN	THIAMINE	NICOTINIC ACID
E. coli	2.3	106	115	62
P. vulgaris	3.2	57	95	None ?
B. aerogenes	1.1	41	43	89
A. fecalis	0.5	78	132	77
B. mesentericus	None ?	14	53	204
B. vulgatus	0.8	82	72	709

appropriate aliquots were employed at two concentration levels for each vitamin assay so that growth of the indicator organism would fall within a suitable range of response.

Some of the results obtained with vitamin assays performed on six species of bacteria are shown in table 1. Each value in the table represents the average of four or six determinations. The whole series of assays were repeated at different times on different cultures. In actual practice the test organisms gave satisfactorily consistent responses both to varied amounts of synthetic growth factors and to additions of bacterial extracts.

As indicated in table 1, the cultures which had grown at 36°C. for 48 hours showed a higher content of the four vitamins per ml. of fluid than did the inoculated medium in which growth was inhibited by low temperature. The results are taken to mean that under the conditions of the experiment these species of bacteria synthesize B vitamins in greater amounts than are used in their metabolism, and the residues accumulate in the cultures. The biotin, nicotinic acid, riboflavin and thiamine accumulated by the growing organisms were calculated as gamma per gram of fresh bacterial cells. These data are shown in table 2. The quantity of biotin was much lower

than the other growth factors found in the cultures. It is generally known, of course, that biotin exhibits biological activity in exceedingly small amounts. What the influence of different cultural conditions might be upon production of vitamins by bacteria would be worth further study.

An important question is how much of the total growth factors produced may be liberated from the cells into their environment. The few tests which have been made on filtrates and whole cultures of *E. coli* and *B. aerogenes* indicate that from 1 to 15% of the total biotin and nicotinic acid present in 48-hour cultures may occur outside the cells, while somewhat larger portions of the riboflavin and thiamine appear to be leached from the bacteria. In cultures of *B. aerogenes* which had grown for 111 hours, about 30% of the biotin, thiamine and riboflavin and 40% of the nicotinic acid produced by the bacteria were found in the filtrate. Age of the cultures and other factors appear to be important in determining the distribution of these water-soluble vitamins between the microörganisms and the medium in which they live.

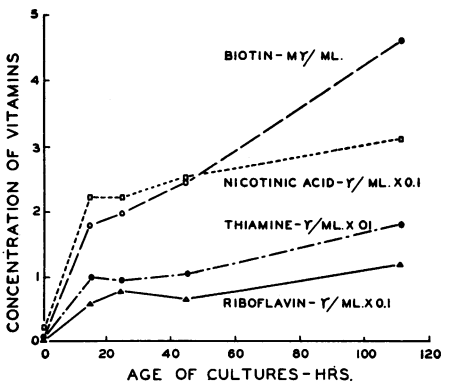

FIGURE 1

Concentration of vitamins in cultures of *B. aerogenes* grown in a chemically defined medium for different periods of time. Maximum values per ml. of culture suspension were as follows: biotin, 4.6 mγ; nicotinic acid, 0.31 γ; thiamine, 0.18 γ; riboflavin, 0.12 γ.

A study was made concerning the vitamins occurring in cultures of *B. aerogenes* at different periods of time up to 111 hours after inoculation of the basal medium. The results of this study are shown in the accompanying figure 1. It appears that the bacteria synthesized comparatively large amounts of vitamins during the early stages of growth. At $14^1/_2$ hours the thiamine, nicotinic acid and riboflavin content of the cultures attained values almost as great as those reached subsequently up to 48 hours. The biotin content increased continuously throughout the entire period. From the standpoint of utilization of growth factors by the bacteria, it is significant that synthesis of the vitamins occurred early in the period of growth. Information of this kind should be valuable also in any attempt to estimate possible uses which may be made of the vitamins synthesized by bacteria.

In recent times the synthetic powers of microörganisms have been steadily assuming greater significance in relation to human welfare, as, for example, in the employment of bacteria and molds in the dairy and chemical industries and the use of yeast for the sake of its B vitamins as a supple-

ment in the diet of man. The extent to which microörganisms living normally in the alimentary tract of an animal may synthesize vitamins and contribute directly to the vitamin requirements of that animal constitutes a problem which has not yet received adequate attention. Demonstration of the synthesis of vitamins by intestinal bacteria, as presented in this report, should have considerable significance in connection with further investigations on the nutritional relationships existing among microörganisms, animals and man.

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THEORY OF THE EFFECTS OF LIGHT INTENSITY AND DURATION IN DETERMINING VISUAL RESPONSES

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In this paper are presented some applications to the phenomena of brightness discrimination and absolute threshold measurements of a theory based partly on the familiar differential equation proposed by Hecht[2] to account for some of the phenomena of the sensory process. A generalized form of this equation is

$$\frac{dx}{dt} = \sum_{i=1}^{N} \left[k_{1i} I \left(a_0 - x \right)^{m_i} - k_{2i} x^{n_i} \right],$$

where, for vision, I represents the intensity of the exciting light, x the concentration of photoproducts broken down from the original concentration a_0 of the light-sensitive substance, t represents time, m_i and n_i are integral exponents indicating the order of the reactions and k_{1i} and k_{2i} are dimen-