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This study was undertaken to determine the effect of pantothenic acid, in a highly concentrated form, on the growth of various species representing several genera of bacteria.

It was found, that out of forty-three species representing twenty-one genera tested, pantothenic acid was essential for the growth of species representing six genera. Under more stringently synthetic conditions, it might possibly be necessary for more.

Although β-alanine has been found to be a part of the pantothenic acid molecule, it could not fulfill the role of pantothenic acid in the metabolism of those organisms which require it.

Pantothenic acid was found to stimulate the growth of Streptococcus lactis in concentrations as low as one milligram in 250,000 liters. β-alanine does have a stimulating effect on the growth of yeast in the presence of small amounts of aspartic acid but had no effect on Streptococcus lactis. As a result of this, a new method of assay for pantothenic acid was developed using Streptococcus lactis, in which the presence of large amounts of β-alanine did not interfere.

THE EFFECT OF PANTOTHENIC ACID ON THE GROWTH OF VARIOUS GENERA OF BACTERIA

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THE EFFECT OF PANTOTHENIC ACID ON THE GROWTH OF VARIOUS GENERA OF BACTERIA

INTRODUCTION

The problem of determining the chemical nature of the substances necessary for the normal growth of bacteria has been investigated for many years. It is now well known that in addition to the common substances which supply food and energy to the cell, small amounts of other substances are necessary for normal growth and development. Many investigators in the field of bacterial nutrition have been concerned with the problem presented by these accessory growth factors, and although much progress has been made in the determination of their chemical constitution and their distribution in nature, their function in the metabolism of the cell is still a matter for conjecture. A discussion of the results of these investigations can be found in several excellent reviews by Peskett(17), Knight (5) and Koser (9).

A few of these accessory growth factors have been isolated from crude plant or animal extracts and identified. Knight (4,6) has shown that the factor obtained from yeast extract which stimulates the growth of Staphylococcus aureus may be replaced by small amounts of Vitamin B_1 and nicotinic acid or amide, and with this information he succeeded in growing this organism on a medium of known

chemical composition (2). Richardson (18) found that uracil was essential for the anaerobic growth of Staphylococcus aureus.

Mueller and coworkers have attempted to separate from a meat infusion, the factors necessary for the growth of Corynebacterium diphtheriae (10,14). From this crude tissue extract, he has isolated and identified pimelic acid (11) and shown that nicotinic acid (12) and β-alanine (13) could be substituted for two other concentrated fractions. All three compounds were necessary for the normal growth of the diphtheria bacillus. These workers have done much toward clarifying the requirements of this organism and have made many contributions to the field of bacterial nutrition in general.

Knight and Fildes (3) have reported the concentration of a "vitamin" necessary for the growth of certain Clostridium from yeast and human urine.

Vitamin B_1 was found to replace a factor obtained from yeast (and other sources) which stimulated the growth of the Propionibacterium (23). Another factor, which has not yet been identified but which is present in the acid-ether extract of yeast or potato was also necessary.

Orla-Jensen et. al. (16) stated that riboflavin and one or more other activators were necessary for the growth of the lactic acid bacteria. Snell (22) found

that two factors present in peptone appeared to be necessary for the normal growth of Lactobacillus delbrukii when riboflavin was present in a hydrolyzed casein medium containing tryptophane and a fermentable carbohydrate.

These factors mentioned above, and others, have all been obtained by separation from various plant and animal extracts. In some cases, as with pimelic acid, it was possible to obtain a pure crystalline material and identify it as some known chemical compound. In other cases, the factors were obtained in a concentrated form and certain chemical and physical characteristics led to a successful substitution by some known compound. With others, the chemical structure of the factor is still unknown or unproved, although it may have been prepared in a fairly pure concentrate. Pantothenic acid is one of these.

In 1933, Williams et. al. (25) reported the discovery and partial purification of an acidic substance which markedly stimulated the growth of the "Gebrüder Mayer" strain of Saccharomyces cerevisiae. They found, by fractional electrolysis experiments, that this substance was present in the tissues of organisms from almost every phylum and as a consequence it was called pantothenic acid (Greek, meaning from everywhere). Since the wide distribution of this substance indicated that it might be of tremendous importance, further studies on the chemical nature, concentration and physiological action were carried

out.

The concentration and purification of pantothenic acid was accompanied by considerable difficulties because of the peculiar nature of the compound. However, by a tedious procedure, numerous fractions having various potencies were obtained (30). Tests to determine the chemical constitution were carried out and pantothenic acid was found to be a relatively simple molecule with eight carbon atoms and containing one carboxyl group, two hydroxyl groups and probably a substituted amide linkage (29). Previously, Williams and Rohrman (27) had shown that in the presence of small amounts of aspartic acid, β-alanine had a stimulating effect on several strains of yeast. Latereevidence on the structure of pantothenic acid indicated that β-alanine was present as a part of the molecule and it was suggested that the organisms probably were utilizing the β-alanine to synthesize the pantothenic acid which they required for growth.

The physiological action of pantothenic acid was first noticed by its strong stimulatory effect on the G:M. strain of Saccharomyces cereviseae when grown in a synthetic medium. Later (26) it was shown to have a marked effect on several other strains of yeast, either when alone or in conjunction with inositol of Vitamin B_1.

Snell, Strong and Peterson have found that pantothenic acid is identical with the growth factor for the lactic acid bacteria which they had reported (20). Recently, they have discovered that, in addition to stimulating the growth of several lactic acid bacteria (21), pantothenic acid is the essential factor for the propionic acid bacteria previously described (23). Mueller (15) has observed that pantothenic acid can be substituted for β-alanine and was much more effective in stimulating the growth of Corynebacterium diphtheriae in his synthetic medium. These observations on the stimulating effect on yeasts and on the diphtheria bacillus have been confirmed but tests on four other organisms gave negative results (8). Salle and Dunn (19) reported that 34 out of 35 known carbohydrate fermenting organisms were stimulated by crude rice bran extract and concluded that pantothenic acid was the stimulating agent. Their tests were run in a peptone medium which may have contained appreciable amounts of pantothenic acid and therefore their results cannot be evaluated.

There have been few investigations in which the effect of one accessory growth substance was tested on a number of unrelated organisms. Therefore, the present study was undertaken to determine the effect of pantothenic acid, in a highly concentrated form, on the growth of various species representing several genera of bacteria.

EXPERIMENTAL

The organisms used in these experiments were the following:

Lactobacillus penoaceticus 118 Fred
Lactobacillus acidophilus Kopoloff
Lactobacillus casei Wis Wisconsin
Lactobacillus mannitosporus 247 Pederson
Lactobacillus casei 65

Streptococcus liquefaciens 16S Stark 805
Streptococcus lactis 125 Yauger
Streptococcus lactis L21 Stark
Streptococcus bovis 41 Stark
Streptococcus thermophilus 6 Sherman
Streptococcus zymogenes IN3 Sherman
Streptococcus durans 98A Sherman
Streptococcus salivarius 318 Sherman
Streptococcus fecalis 22S Stark 24
Streptococcus agalactae 14S Sherman 706

Leuconostoc citraverous 25 Geneva
Leuconostoc dextranicus 22 Geneva
Leuconostoc mesenteroides 5 Geneva

Staphylococcus albus B15 ICHall 1
Staphylococcus citreus B4 ATC 395
Staphylococcus aureus B2 (official)
Staphylococcus aureus 135 Brown

Sarcina lutea D3 OSC
Sarcina aurantica D2 OSC

Propionibacterium rubrum 147 Sherman
Propionibacterium shermanii D Sherman

Bacillus subtilis 10 Geneva
Bacillus megatherium 14 Stark

Serratia marcescens E22c Geneva 42

Pseudomonas fluorescens 21 I:C:Hall 548

Erwinia amylovra 163 Burkholder

Aerobacter laevens 70 Levine 238

Alkaligenes fecalis E20 Cornell 192T

Proteus vulgaris E10 Cornell 104

Salmonella aertrycke 618T OSC
Salmonella schottmullerii (para-B) E15 OSC

Elberthella typhii E18H Hopkins

Brucella abortus E41 Cornell Vet.

Corynebacterium diphtheriae F4 Park-Wms. 8

Phytomonas michiganensis 32 Burkholder

Spirillum rubrum E43 OSC

Azotobacter chroococcum B8 Burk
Rhizobium trifolii 532 U:S:D.A.

The organisms were carried in stock culture media best siuted to their nutritional demands and transferred at least once every two weeks during the course of the experiment.

A medium for growth tests was selected which would supply all knwon growth nutrients with the exception of pantothenic acid. The known instability of pantothenic acid to alkali (25) suggested the use of some nutrient extract in which the pantothenic acid had been removed by treatment with alkali. For this, the alkali-treated peptone developed by Snell, Strong and Peterson (20) and used in their medium B was used as a base. It is prepared in the following manner:

Forty grams of Difco-Bacto peptone are dissolved in 250 ml. of water. To this is added a solution of 20 grams of NaOH in 250 ml. of water. The mixture is allowed to

stand for 24 hours. The NaOH is then neutralized with glacial acetic acid and 7 grams of anhydrous sodium acetate are added. The mixture is diluted to 800 ml., which gives a solution containing 6% sodium acetate and 5 %NaOH treated peptone. (private communication, W.H. Peterson to R. J. Williams). Sixty ml. of this solution are added to each liter of medium giving a concentration of 0.3 %.

The medium had the following composition:

KH_2PO_4	0.144 gram
Na_2HPO_4	0.568 gram
$CaCl_2$	0.025 gram
$MgSO_4$	0.13 gram
$(NH_4)_2SO_4$	3.00 gram
lactoflavin	100 gamma
glucose	5.00 grams
alkali-treated peptone solution	60 ml.
$ThCl_3$	0.001 gram
H_3BO_3	0:001 gram
$ZnSO_4$	0.001 gram
$MnCl_2$	0.001 gram
$CuSO_4$	0.0001 gram
KI	0.0001 gram
$FeCl_3$	0.0005 gram
distilled water	1000 ml.

As observed by Snell et al. (20), the alkali-treated peptone must be supplemented with lactoflavin since the alkali treatment destroys it. The traces, or those salts which are present in very low concentrations, are made up in solution so that 1 ml. contains the amount required for one liter of medium. Due to the precipitating effect of some of the ions on each other, the potassium iodide and ferric chloride are made up in a separate solution. These traces were added to insure a complete medium for bacterial growth. Vitamin B_1 (Merck) was added in two experiments but found to be unnecessary.

Inoculations were made by adding 0.1 ml. of a light suspension of the cells in sterile water to 10 ml. of the above medium. The suspension was obtained by centrifuging the cells from 10 ml. of a 24 hour culture in tryptone-peptone- phosphate dextrose broth (or a loopful of cells scraped from an agar slope) washing once in sterile distilled water, recentrifuging and resuspending in sterile water to give a very slight turbidity.

The preliminary runs were read by visual inspection at the end of 24 and 48 hours and eventually, one week. For the final data, the turbidity of the cultures was read in a thermocouple turbidometer (26) in use in this laboratory for the determination of the amount of growth in yeast growth tests. This apparatus has been calibrated for

yeast growth but the amount of time which would be required to calibrate it for each organism used here was prohibitive. Instead, the apparatus was set to read zero for the blank medium and the amount of growth is reported as degree of turbidity. The exact amount of growth cannot be found from this data since it does not follow a straight line, but relative growth can be determined quickly and easily. However, in several cases, plate counts were made to determine the number of live cells present and these serve as a rough index to the amount of growth.

The pantothenic acid used for these experiments was a sample which had been prepared in this laboratory according to the method described by Williams (30). It was in the form of the calcium salt and had a "potency" of 6,000 as determined in this laboratory. As each test was set up, the amount of pantothenic acid needed was diluted from a concentrated solution of this sample and pipetted into the medium before tubing and autoclaving. Autoclaving at pH 7.0 for 20 minutes does not destroy pantothenic acid.

It has been recently proved in this laboratory that β-alanine is formed from pantothenic acid as a cleavage product upon acid or alkaline hydrolysis(31). The alkali treatment of the peptone would yield small amounts of β-alanine resulting from the hydrolysis of the pantothenic

acid present. The blank medium therefore contained some β-alanine and since this compound has been shown to have some effect upon yeast growth in a synthetic medium(27) and upon the growth of the diphtheria bacillus (13), it was decided to test β-alanine for its effect on these bacteria.

RESULTS

PART I. Determination of Favorable Concentration of Pantothenic Acid

The first problem was to determine the approximate concentration of pantothenic acid needed for maximum growth. Since preliminary experiments showed that Streptococcus lactis 125 gave no visible growth when washed cells were added to the base medium, but gave abundant growth when very small amounts of pantothenic acid were present, it was used as the test organism. Six runs, similar to the one shown in Table 1, were made.

It may be seen that at amounts above 0.16 γ per culture there is very little added stimulation upon addition of more pantothenic acid. This flattening of the curve was anticipated because growth tests with yeast give similar results. Since 0.16 γ gives the maximum growth, all future tests were run with this level of pantothenic acid.

It had previously been reported (30) that pantothenic

TABLE 1

Stimulation of Streptococcus lactis by various amounts of pantothenic acid

Amt. of pantothenic acid gammas per culture	Turbidity reading 48 hours	Cells/ml. x 10^6
8.0	5.79	74
4.0	6.00	79
0.8	5.65	104
0.4	5.65	132
0.2	5.45	98
0.16	5.38	105
0.08	4.58	106
0.04	2.89	47
0.02	1.52	32
0.008	1.65	21
0.004	1.00	12
0.0008	1.57	9
0.0004	0.46	6
0.00008	0.80	4
0.00004	0.54	4
0.0	0.00	1
0.0	0.15	1

Initial inoculum was 3,800 cells per ml.

acid could be detected in dilutions of 5 parts per ten billion by its stimulation of yeast growth. The organisms used in this test, Streptococcus lactis seemed to be even more sensitive and a test was run to determine the minimum amount of pantothenic acid which would give growth above the blank. These results are also given in Table 1 and it may be seen that pantothenic acid can be detected in amounts of 4×10^{-6} micrograms per ml. This is equivalent to one part in 250 billion. Bothe the turbidometer readings and the plate counts show stimulation at this astonishingly low figure. The only other factor reported in the literature which can be detected in amounts approaching this figure is "biotin", a crystalline compound isolated by Kogl and Tonnis (7). This compound has a stimulating effect on the growth of a strain of Saccharomyces ceriviseae in concentrations of one part per 400 billion. In these cultures there are approximately three million cells per cubic centimeter and by the following calculations it was determined that there are about 4,000 molecules per cell.

approx. mol. wt. pantothenic acid $= 200 \fallingdotseq 6.06 \times 10^{23}$ mol.

$$\frac{200}{6.06 \times 10^{23}} = \frac{4 \times 10^{-12}}{x}$$

$$x = 1.2 \times 10^{10} \text{ molecules}$$

1.2×10^{10} molecules/ 3×10^{6} cells $\fallingdotseq 4 \times 10^{3}$ molecules/cell

PART II. The Effect of Pantothenic Acid on Various Bacteria

The second part of the experimental work consisted of testing the effect of pantothenic acid on the growth of species representing many genera of bacteria. For each organism, two sets of three tubes were inoculated. Each tube contained 10 ml. of base medium. The first tube was left blank, 0.16 γ of pantothenic acid was added to the second and 5 γ of β-alanine to the third. These were inoculated as described above and incubated at 30°C. for the specified length of time and turbidity readings taken. The data obtained is given in Tables 2, 3 and 4.

The results shown in Table 2 clearly show the stimulation of growth of these organisms by pantothenic acid. In most cases there was no growth in the blank medium while the addition of 0.16γ of pantothenic acid per culture caused good growth. In no case was there stimulation by β-alanine. On the contrary, with some organisms e.g. Streptococcus salivarius β-alanine appeared to be toxic. Although these organisms cannot produce visible growth without pantothenic acid they apparently lack the ability to utilize the β-alanine to synthesize it.

It was noted with the propionic acid bacteria, which are naturally slow growing organisms, that all three tubes showed about the same growth for the first two days. However, after this time, the tubes containing the blank medium

and the β-alanine remained constant, while the tube containing pantothenic acid showed additional growth. This may be seen by the added reading taken at 7 days on Propionibacterium shermanii. This type of stimulation was typical of this organism throughout the entire experiment.

Similar results were obtained with Staphylococcus albus, Staphylococcus aureus, and Aerobacter laevens. These organisms evidently can grow well without pantothenic acid and yet the addition of it causes marked stimulation. This is most striking in the case of Staphylococcus albus B2. It is evident that here we are dealing with organisms which can synthesize pantothenic acid from β-alanine and that the rate of this synthesis appears to be a limiting factor in the amount of growth.

One complete set of experiments was run in which the bacteria were tested in the presence and absence of glucose in the medium. There was no stimulation of any organism by pantothenic acid unless sugar was present. Those organisms which can grow without sugar gave visible growth but the addition of pantothenic acid failed to have any effect. Since, in addition, pantothenic acid has been shown to stimulate only those organisms which produce lactic acid, it is possible that pantothenic acid is in some manner concerned with the carbohydrate metabolism

of the cell.

The organisms listed in Table 3 are those which grew in the base medium and showed no additional growth when either pantothenic acid or β-alanine was added to the medium. These organisms, representing eight genera, either do not require pantothenic acid for growth or have the ability to synthesize it from the β-alanine present.

Corynebacterium diphtheriae is included in this group. Although Mueller (15) has found that pantothenic acid is five times as effective as β-alanine in stimulating its growth, under the conditions here tested this stimulation is not as marked. This result shows the effect of difference in basal medium in the stimulating effect of pantothenic acid. Since, under our conditions, we could not get stimulation upon an organism which is known to require pantothenic acid, we might conclude that perhaps pantothenic acid could be proved necessary for the growth of many of the bacteria in this group if they were grown in a more stringently synthetic medium.

Table 4 gives a list of organisms which gave no visible growth in any of the three tubes. It is interesting to note that of three Staphylococci tested, one was stimulated by pantothenic acid, one gave growth without it but showed some stimulation and the third did not grow at all. This shows a marked variation in nutritional

requirements even between strains of the same species, as has been observed by several other workers.

The pathogenic organisms were tested only once and on that run, Elberthella typhii showed no visible growth in the medium containing sugar but grew in the base medium without sugar. It is possible that the slight carmelization of the dextrose in this case produced some substance which was toxic for this organism but such toxicity was not noted on any of the other bacteria tested(1). Streptococcus bovis is included in this group as it gave no growth on any of the runs. It is the only Streptococcus tested which was not stimulated by pantothenic acid. No explanation is offered for the failure of these organisms to grow but it is highly probable that there are other essential growth factors (as the factor for Rhizobium recently reported (24)) which must be present for growth to take place.

PART III. A New Method of Assay for Pantothenic Acid

The method of assay for pantothenic acid in use in this laboratory for several years has been described(28). The organism used was the "Gebrude Mayer" strain of Saccharomyces cerevisiae and the biological activity of various preparations was tested by their stimulation of the growth of this yeast in Williams synthetic medium.

TABLE 2

Organisms showing stimulation by pantothenic acid

Organism	Turbidity readings 48 hours		
	Blank	Pantothenic acid 0.16γ/culture	β-alanine 5γ/culture
Lact. penoaceticus	1.19	9.32	1.19
Lact. acidophilus	0.00	3.29	0.00
Lact. casei Wis	0.00	3.70	0.69
Lact. casei 65	0.51	6.30	0.70
Lact. mannitosporus	0.40	3.18	0.19
Strep. liquefaciens	0.00	5.69	0.00
Strep. lactis 125	0.00	6.00	0.00
Strep. thermophilus	0.75	8.88	0.44
Strep. zymogenes	0.00	5.77	0.00
Strep. durans	0.00	6.32	0.17
Strep. salivarius	1.58	6.75	0.50
Strep. lactis L21	0.15	7.25	0.10
Strep. fecalis	0.40	6.70	0.43
Strep. agalactae	0.29	3.31	0.75
Leuc. mesenteroides	0.49	3.88	0.40
Leuc. citraverous	1.87	8.30	1.14
Leuc. dextranicus	0.41	3.05	0.41
Prop. shermanii[1]	2.80	6.39	2.24
Prop. rubrum	1.59	5.70	0.70
Staph. albus	6.35	11.30	7.00
Staph. aureus 135	6.41	8.02	6.60
Aer. laevens	9.00	11.10	12.39
[1]Prop. shermanii 7 days	2.85	8.72	2.39

TABLE 3

Organisms not observed to be stimulated by pantothenic acid

Organism	Turbidity readings 48 hours		
	Blank	Pantothenic acid 0.16γ/culture	β-alanine 5γ/culture
Alk. fecalis	16.95	17.52	16.59
Sarc. aurantica	9.70	10.07	7.35
Sarc. lutea	8.00	6.30	6.00
B. megatherium	8.29	4.70	9.50
B. subtilis	1.50	2.51	2.19
Prot. vulgaris	1.50	2.00	1.55
C. diphtheriae	2.50	3.50	2.00
Ser. marcescens	0.90	1.25	0.94
Sal. aertrycke	++	++	+
Sal. achottmullerii	++	++	+
Bru. abortus	++	++	++

TABLE 4

Organisms showing no growth with or without pantothenic acid

Staph. aureus B15
Phyto. michiganensis
Sp. rubrum
Azot. chroococcum
Strep. bovis
Rhiz. trifolii
Eb. typhii

Whenever a sample is tested, a series of standards, that is flasks containing known amounts of pantothenic acid, are included in each test. A curve is made by plotting the amount of pantothenic acid against the galvanometer deflections of the thermocouple apparatus. In this manner, the amount of pantothenic acid in any sample can be determined. However, because this yeast can synthesize pantothenic acid from β-alanine, β-alanine gives some stimulation when added in a sample under these conditions. Therefore, in some cases, there is some question as to whether pantothenic acid or β-alanine is causing the growth. This uncertainty can be avoided by using Streptococcus lactis 125 as the indicator rather than yeast.

The new method follows a procedure similar to the one used with yeast tests. The medium is the one containing alkali-treated peptone and the test organism is Streptococcus lactis 125. The samples are placed in 50 ml. Erlenmeyer flasks and made up to 2 ml. with distilled water. The flasks are plugged and the samples, the medium and all apparatus are sterilized by autoclaving at 15 pounds for 20 minutes. The suspension of Streptococcus lactis to be used for seeding is prepared by centrifuging the cells from a 24 hour culture grown in tryptone broth as previously described, and then suspending in a small amount of sterile medium in concentrations to give a rather turbid suspension. The concentration is determined

in the thermocouple apparatus and enough of this is pipetted into the total volume of medium used in seeding the small flasks to give a standard initial concentration. Ten ml. of this dilute suspension are pipetted aseptically into each flasks containing the samples and in this manner a uniform seeding is obtained. The flasks are incubated at 30°C. for 16 hours and at the end of this period are shaken for five minutes on a mechanical shaker. They are then diluted with 10 ml. of chlorothymol. This is done to dilute the culture so that it can be read in the large cell of the turbidometer, and also to prevent the organism from growing during the time necessary to read the test. After dilution, the degree of turbidity is read in the turbidometer.

The data in Table 5 shows a typical test with this organism as compared to a similar yeast test. There is little or no growth in the blank medium or in the presence of amounts of β-alanine as high as 20 γ per 10 ml. culture when Streptococcus lactis is used although β-alanine gives decided stimulation in the same concentration when tested on yeast in Williams synthetic medium. By plotting the points on the pantothenic acid standard which is included in each test, against the galvanometer deflections, the amount of pantothenic acid present in other samples can be read from this curve.

Since pantothenic acid markedly stimulated growth

TABLE 5

Comparative tests on pantothenic acid assay

Pantothenic acid gammas/12 ml.	β-alanine gammas/12 ml.	Turbidity readings 16hrs. Strep. lactis	yeast
0.00	0.00	0.00	0.00
0.00	0.00	0.02	0.02
0.01		1.20	0.35
0.02		1.77	1.19
0.04		2.96	3.00
0.06		4.90	3.80
0.08		5.37	4.02
	20.0	0.22	8.20
	10.0	0.20	7.50
	5.0	0.20	5.17
	1.0	0.06	1.31
	0.75	0.00	0.70

of Streptococcus lactis, and since β-alanine does not, it is thus possible to determine the pantothenic acid potency of various preparations without the interference of stimulation by β-alanine.

CONCLUSIONS

The data presented here leads to the conclusion that pantothenic acid is essential in the growth processes of many organisms. Never before has one growth factor been shown to be necessary for so many different bacteria. From forty-three species representing twenty-one genera tested, pantothenic acid has been proved absolutely essential for the growth of species representing six genera and under more stringently synthetic conditions might possibly be necessary for more.

It has been shown that β-alanine cannot fulfill the role of pantothenic acid in the metabolism of those organisms which require it. But do those organisms which grow in the basal medium utilize the β-alanine present in order to synthesize the pantothenic acid which they need? This question cannot be answered until we can grow these organisms in a synthetic medium and test the effect of β-alanine. Yeast grown in synthetic medium produces pantothenic acid from β-alanine and it is not improbable that various other microorganisms do the same.

It had been hoped that from a survey of this type, some clue as to the role of pantothenic acid in bacterial metabolism might be found. The stimulation of Streptococcus lactis in such extremely small amounts would most logically suggest that pantothenic acid is acting as a catalyst for

some essential cell reaction. In addition to this striking stimulation, the fact that pantothenic acid stimulated only in the presence of sugar and that the organisms which are stimulated all have in common the production of lactic acid as one end product in their cell metabolism, suggests that pantothenic acid functions in the mechanism concerned with carbohydrate utilization.

SUMMARY

1. Pantothenic acid has been shown to stimulate the growth of Streptococcus lactis in concentrations of one milligram per 250,000 liters.

2. Pantothenic acid has been proved essential for the growth of species representing six genera of bacteria. It cannot be replaced by β-alanine.

3. A new method of assay for pantothenic acid has been developed using Streptococcus lactis as the test organism. β-alanine has no effect on this organism and thus does not interfere with the test.

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