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Growth of *Lactobacillus acidophilus* in the Absence of Folic Acid

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ABSTRACT

SOŠKA, JIŘÍ (Kansas State University, Manhattan). Growth of *Lactobacillus acidophilus* in the absence of folic acid. J. Bacteriol. **91**:1840–1847. 1966.—A chemically defined medium, containing no folic acid, was used for the cultivation of *Lactobacillus acidophilus* R-26. In such a medium, the organism required thymine in addition to a deoxyriboside, purines, pyrimidines, and most amino acids. If thymine was present in this medium, an unlimited exponential growth was possible. The influence of the components of this medium on the growth is described. The concentration and type of adenine compounds in this medium were most important. Adenine and adenosine inhibited utilization of thymine, but not of thymidine, whereas adenylic acid inhibited recovery from amino acid starvation. In the absence of thymine or deoxyribosides, cells continued to grow in length, and after 3 hr a slow decline in viable count ensued.

Lactobacillus acidophilus R-26 which requires deoxyribosides for growth (4) can be used as an experimental organism for studies of nucleic acid or protein metabolism (7, 9). In the present study, a requirement for thymine was produced in this organism by omitting folic acid from the medium. Because growth in the media of Siedler et al. (10) and of Lovtrup and Roos (5) was not satisfactory in the absence of folic acid, the medium composition was re-evaluated. The conditions of thymine utilization have been studied, and the conditions for experiments on nucleic acid metabolism are described.

MATERIALS AND METHODS

Organism. A culture of *L. acidophilus* R-26 was obtained from E. Hoff-Jorgensen in 1957. Stock cultures were maintained on agar slants containing 2% agar in a solution of 3.5% *Lactobacillus* broth (Difco). Daily serial transfers were made in the liquid medium described by Seidler et al. (10) or in the medium described below.

Estimation of cell number. A 0.1-ml amount of a culture was diluted into 25 ml of Abbot saline (0.9% NaCl) and was counted in a Coulter Counter, model B. A 30-μ orifice was used with a current setting of 1, a lower threshold of 10, and maximal gain setting. A 0.05-ml amount of this dilution was counted. A microscopic examination showed that nearly 50% of "cells" were groups of two or more cells. Actual cell counts were thus about 50% higher than determined by the Coulter counter.

Colony counts. Bacterial cultures were diluted in a 2.5% KCl solution in which *L. acidophilus* retained its viability, and 0.2 ml amounts of the dilutions were spread on the surface of agar plates, containing 2% agar and 3.5% *Lactobacillus* broth (Difco). After 24 hr of incubation at 39 C, the colonies were counted. In growing cultures, the colony count equaled the cell count obtained with the Coulter counter.

Estimation of culture density. The absorbance of the culture was measured at 650 mμ in a Zeiss spectrophotometer with the use of 0.5-ml microcuvettes with a 1-cm light path. Cultures with densities exceeding an absorbance of 0.5 units were diluted with the medium.

Transfer of bacteria into different media. Cultures of *L. acidophilus* to be transferred into media of different composition were collected on a membrane filter (Bac-t-flex, B6; Schleicher & Schuell Co., Keene, N.H.). The cells were washed with prewarmed medium of the type to which they were to be transferred, and then were resuspended by washing the filter into the new medium.

Bacterial growth. Exponentially growing bacteria at a density of approximately 0.5×10^8 to 10^8 cells per milliliter were used as inocula. These were washed, diluted, and suspended in an incomplete medium, and then were transferred into test tubes containing the components in question. Usually 4 ml of culture was used. The cell density at the start of the experiment was usually about 5×10^6 cells per milliliter, although in some experiments more dilute inocula were used (in the range of 500 cells per milliliter). The cultures

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were incubated at 39 C, and growth curves in the region of cell concentration between 10^7 and 10^8 cells per milliliter were obtained with the Coulter counter. The final yields were measured as cell numbers and as absorbance at 650 mμ.

Use of radioactive isotopes. Tritiated adenine obtained from the New England Nuclear Corp., Boston, Mass., was used. Its incorporation by a culture was measured by collecting on membrane filters duplicate samples of cells and washing them on filters with trichloroacetic acid (5%). The incorporated activities of dried samples were measured in a Packard Tricarb scintillation spectrometer, with the use of an organic scintillation fluid: 3 g of 2,5-diphenyloxazole and 300 mg of 1,4-bis(4-methyl-5-phenyloxazolyl)benzene in 1 liter of toluene.

Ribonucleic acid (RNA) determinations. The RNA contents of samples of culture were estimated by the method of Schneider (8).

Composition of the medium. The composition of the medium is given in Table 1. This medium was prepared from the frozen concentrated stock solutions also shown in Table 1. The solutions of adenylic acid, cytidylic acid, potassium phosphate, and amino acids were neutralized with KOH to *p*H 7.0. The solutions of inorganic salts and of guanine were slightly acidified with hydrochloric acid. If the solution of amino acids precipitated, it was brought into solution by heating.

The medium was usually prepared in two steps. In the first step, the so-called "basal medium" was prepared, with the use of the indicated volumes of acetate, guanine, dextrose, adenylic acid, cytidylic acid, vitamins, spermidine, Tween, mineral salts, phosphate, and citrate. To prevent precipitation, citrate was added to the mixture before mineral salts or phosphate. In the second step, the amino acid solution, cysteine, thioglycolic acid, uracil, thymine, and deoxyriboside were added, and the volume was adjusted with water. Frequently, compounds of interest were omitted or replaced. Thioglycolic acid and cysteine solutions were renewed frequently because they undergo oxidation. The *p*H of the basal medium and of the complete medium was 6.9.

RESULTS

L. acidophilus grew well in the medium of Siedler et al. (10), with a generation time of 40 min. When folic acid was omitted and replaced by thymine (4 μg/ml), the growth was not satisfactory, the generation time was about 2 hr, and the yield of cells was low. In addition, clumping of cells was frequently observed. It was found that adenine inhibited growth in the absence of folic acid, but that it could be replaced with adenylic acid (adenosine-5′-phosphate), resulting in improved growth. The cell clumping could be prevented by lowering the concentration of mineral salts or by adding a small amount of citrate. The medium described in Table 1 avoided these problems. Growth in this medium in the absence of folic acid is shown in Fig. 1. It may be seen that in this medium *L. acidophilus* grows with a generation time of 50 min to reach an eventual

TABLE 1. *Composition of medium for Lactobacillus acidophilus*

Compound	Final content in 1 liter of medium	Concn in stock solution	Vol of stock solution to make 1 liter medium*
	mg	*mg/ml*	*ml*
Potassium acetate	15,000	150	100
Dextrose	15,000	150	100
Guanine·HCl	30	0.2	150
Adenylic acid (adenosine-5′-phosphate)	16	2.5	7.5
Cytidylic acid (cytidine-(2′,3′)-phosphate	50	5.0	10
Uracil	10	1.0	10
L-Asparagine	600	6	100
L-Glutamic acid	500	5	
Glycine	300	3	
L-Leucine	200	2	
DL-Alanine	200	2	
L-Serine	100	1	
L-Valine	100	1	
L-Arginine·HCl	100	1	
L-Tyrosine	100	1	
L-Lysine·HCl	50	0.5	
L-Threonine	50	0.5	
L-Methionine	50	0.5	
L-Isoleucine	50	0.5	
L-Histidine·HCl	50	0.5	
L-Tryptophan	50	0.5	
L-Proline	40	0.4	
L-Phenylalanine	40	0.4	
L-Cysteine·HCl	200	20	10
Riboflavine	0.5	0.1	5
Nicotinamide	0.5	0.1	
Calcium pantothenate	1.0	0.2	
Pyridoxine	0.5	0.1	
Spermidine phosphate·$6H_2O$	5	0.5	10
Tween 80	1,000	100	10
Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	220	294	0.75
KH_2PO_4	2,000	200	10
KOH (5 N solution) added, *p*H adjusted to 7.0)			
$MgSO \cdot 7H_2O$	165	25	6.5
$MnSO_4 \cdot 4H_2O$	22	3.6	
$FeSo_4 \cdot 7H_2O$	12	2.0	
Sodium thioglycolate	500	50	10
Thymine	4	0.4	10
Deoxyguanosine	8	0.4	20
Folic acid (if used)	0.1	0.01	10

* The addition of 420 ml of water completes the medium.

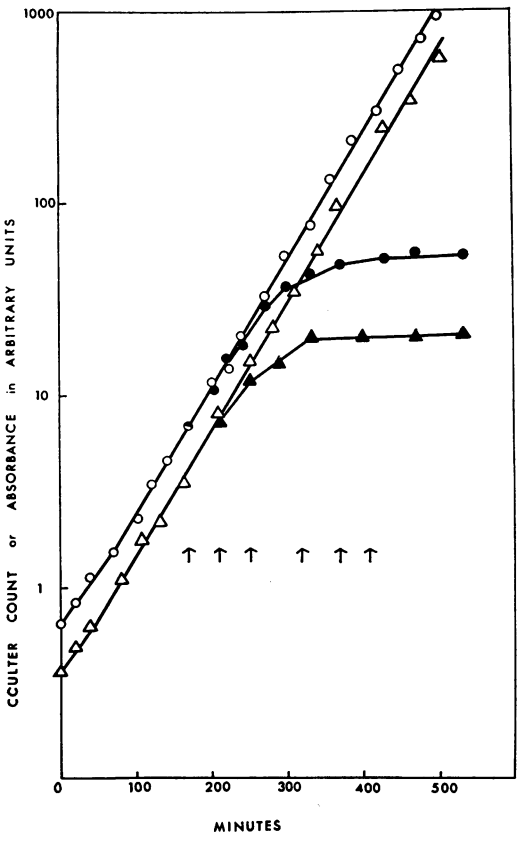

FIG. 1 *Growth curves of Lactobacillus acidophilus. A growing culture (2 × 10⁸ cells per milliliter) was diluted to a concentration of 6 × 10⁶ cells per milliliter with fresh medium and incubated at 39 C. Samples for Coulter counts and absorbancy measurement were taken at intervals. The culture was split into two parts, one of which was repeatedly diluted to keep the cell density within the limits of 2 × 10⁷ to 8 × 10⁷. Two-fold dilutions are indicated by the arrows on the abscissa. The values for the diluted culture have been corrected by these dilutions. The other part was allowed to grow to the stationary phase. Diluted culture: ○, Coulter counts; △, optical densities. Culture grown without dilutions: ●, Coulter counts; ▲, optical densities. A value of one on the ordinate corresponds to a Coulter count of 10⁷ cells per milliliter and an absorbancy of 0.100.*

cell density of about 5 × 10⁸. Balanced growth may be achieved by dilutions which maintain the culture density in the range 2 × 10⁷ to 8 × 10⁷ cells per milliliter. This is indicated by the parallel slopes of the absorbance and cell-count curves. Previous experiments (Soska and Lark, Biochim. Biophys. Acta, *in press*) have shown that such cultures synthesize deoxyribonucleic acid (DNA) at the same rate as they divide.

Unless otherwise stated, growth in this medium was carried out without folic acid and without aeration. The effects of changing the components of the new medium were checked.

Acetate was found to be obligatory for growth. Changing its concentration affected the cell size; the cells were bigger when higher concentrations of acetate were used.

Dextrose could not be omitted from the medium. Changes of its concentration within wide limits (5 to 30 g per liter), however, had no apparent effect on growth. Replacement by other sugars was not attempted.

Guanine was found to be essential for growth. To obtain a higher yield of cells, the present concentration was increased over that used by Siedler et al. (10). Guanylic acid (guanosine-2′,3′-phosphate) lowered the growth rate and gave rise to the formation of irregular, long cells. This effect was observed even in the presence of guanine. Guanosine could not replace guanine, although deoxyguanosine could.

Adenine (or adenylic acid, adenosine, deoxyadenosine) was not essential for growth. However, in its (their) presence the growth rate and yield was improved. The details of the effect of adenine will be presented below.

Cytidylic acid (cytidine-2′,3′-phosphate) was not essential for growth. However, in its absence the generation time was 2 to 3 hr. Concentrations of cytidylic acid higher than used by Siedler et al. (1957) extended the exponential character of growth into the range of 10⁸ cells per milliliter and resulted in a higher yield of cells. Cytidylic acid could be replaced by cytosine or deoxycytidine, but not by cytidine, uracil, orotic acid, or 5-methyl cytosine.

Uracil. The requirement for uracil could not be established unequivocally. There was no continued growth of the culture in the absence of uracil. However, a residual (30 to 100%) increase of cell count and absorbancy was usually observed. Synthesis of RNA in the absence of uracil was tested in two experiments.

In the first experiment, the cells were washed into a medium free from deoxyribosides and uracil—the absence of deoxyribosides prevents the synthesis of DNA (7; Soska and Lark, *in press*)—and then incubated at 38 C in tubes containing H^3-adenine (1 μc per 7 μg/ml) and (A) uracil and thioglycolate (control); (B) no uracil, but thioglycolate; (C) no uracil, no thioglycolate; or (D) no uracil, no thioglycolate, and the culture was aerated. As shown in Table 2, there was no difference between aerobic and anaerobic conditions of growth. The significance of aeration will be discussed below. Some residual RNA synthesis continued in the absence of uracil.

In the second experiment, the bacteria were

TABLE 2. *RNA synthesis in the absence of uracil*

Medium used (all media lacking deoxyribosides)	H^3-adenine incorporation (count/min)		
	0 min	20 min	60 min
(A) Control	78	948	3,156
(B) Medium lacking uracil	—	458	740
(C) Medium lacking uracil and thioglycolate	—	452	771
(D) Medium lacking uracil and thioglycolate; aerated	—	409	831

washed into a medium free from uracil and cytidylate, and were distributed into tubes containing (A) all components (control), (B) no uracil, (C) no uracil and the culture was aerated, (D) no uracil but folic acid was added (0.1 μg/ml); and, finally, (E) a tube containing no uracil and no cytidylate. The content of RNA was determined by means of the orcinol reaction (Table 3). Again, an increase in RNA content in the absence of uracil was observed, and there was no effect of folic acid, oxygen, or cytidylate.

In general, no lasting growth could be observed in the absence of uracil. Uracil could be replaced with orotic acid, uridylic acid (uridine-2′,3′-phosphate), deoxycytidylic acid, and deoxyuridine, but not with uridine, cytosine, cytidylic acid, or thymine.

Mineral salts. The effects of changing the concentration of magnesium, manganese, and iron were tested by use of a solution containing a mixture of all of these salts. At lower salt concentrations, the growth was slow and the microscopic appearance of the cells changed: under phase contrast the cells appeared swollen and transparent. At higher salt concentrations, the cells tended to be smaller, but they clumped and sedimented to the bottom of the tube.

Citrate. The small amount of citrate present in the medium prevented the clumping caused by the mineral salts. Citrate also proved to be useful in getting cells into suspension from membrane filters to which they would stick after washing with trichloroacetic acid or distilled water.

Phosphate. The phosphate concentration in this medium is excessive to obtain a better buffered system. But even at one-fortieth of this concentration the growth was normal, although the cells were only one-third to one-half as large.

Vitamins were also used at a higher concentration than necessary. A concentration one-tenth of that specified permitted normal growth. Again, however, lower vitamin concentrations led to a decrease in cell size. With the exception of pyridoxine, all the vitamins were required.

TABLE 3. *Increase in RNA in cultures of Lactobacillus acidophilus incubated in the absence of uracil*

Medium used	Increase over initial RNA content (orcinol method)	
	1 hr	3 hr
	%	%
(A) Full medium	129	510
(B) Lacking uracil	42	74
(C) Lacking uracil; aerated	49	73
(D) Lacking uracil; with folic acid added, 0.1 μg/ml	56	76
(E) Lacking uracil and cytidylate	63	78

Pyridoxine could be replaced by the lysine and D-alanine present in the medium, although the growth rate was reduced to about one-half of that observed when the vitamin was present. Cells grown in the absence of pyridoxine were fragile.

Spermidine was found to improve the growth slightly.

Thioglycolic acid and aeration. Sodium thioglycolic acid was added to the medium to get a higher yield. It did not change the growth rate significantly. Aeration lowered the yield without affecting the growth rate.

Tween 80. The presence of Tween 80 was essential for growth. At concentrations lower than 500 μg/ml, the growth rate and yield declined. An attempt to replace it with oleic acid was unsuccessful.

Amino acids. Changing the concentration of amino acids together or individually had no significant effect on the growth rate, but the yield was generally higher with higher concentrations of amino acids.

The requirement for individual amino acids was tested in an experiment in which a culture of *L. acidophilus* was transferred into medium lacking amino acids, and then into test tubes containing all of the amino acids but one. Optical densities and Coulter counts were measured at intervals. The results of this experiment are presented in Table 4. Most of the amino acids were required, but some growth occurred in the absence of lysine, cysteine, alanine, methionine, and isoleucine. The slow growth in the absence of these amino acids was confirmed repeatedly. In the absence of other amino acids, only a 10 to 20% increase of absorbancy, but not of cell count, was observed. The aspartic acid and glutamine requirement of *L. acidophilus* could be satisfied by asparagine and glutamic acid, respectively.

TABLE 4. *Growth of Lactobacillus acidophilus in the absence of different amino acids**

Amino acid omitted	Coulter count ($\times 10^{-7}$)	Optical density
Control (none omitted)..	15.4	0.83
L-Asparagine....	1.14	0.198
L-Glutamate.....	1.12	0.150
Glycine.....	1.09	0.186
L-Leucine.....	1.10	0.208
DL-Alanine.....	4.70	0.390
D-Alanine.....	11.20	0.84
L-Serine.....	1.02	0.197
L-Valine.....	1.00	0.188
L-Arginine.....	1.07	0.225
L-Tyrosine.....	1.11	0.210
L-Lysine.....	2.79	0.362
L-Threonine.....	1.03	0.198
L-Methionine.....	1.23	0.220
L-Isoleucine.....	1.64	0.231
L-Histidine.....	1.03	0.204
L-Tryptophan.....	1.04	0.202
L-Proline.....	1.13	0.194
L-Phenylalanine.....	1.04	0.190
L-Cysteine.....	2.19	0.289

* Results were determined after incubation for 3 hr. In the control, before incubation the Coulter count was 1.06×10^{-7} and the optical density was 0.180.

Recovery from amino acid starvation. Cultures of *L. acidophilus* recommenced growth very slowly after amino acid starvation. This was found to be connected with the presence of adenylic acid. If adenine, adenosine, or adenine-deoxyriboside was substituted for adenylic acid, the recovery was much more rapid. Recovery was also rapid in the absence of any adenine derivatives.

Deoxyribosides. L. acidophilus grew well in the presence of any of the natural deoxyribosides. Usually guanine deoxyriboside was used. At a concentration of 4 μg/ml, optimal growth was attained, and higher concentrations only improved the yield slightly. As a standard concentration, 8 μg/ml was used. A concentration of 1 μg/ml allowed a yield of 16×10^7 cells per milliliter, which corresponds to a synthesis of about 0.8×10^{-14} g of DNA per cell. The behavior of cultures in the absence of deoxyribosides is described in the next paragraph, together with effects of thymine starvation.

Thymine. Thymine was required for growth if folic acid was not present. The thymine requirement could be satisfied with folic acid, thymine, or thymidylic acid but only poorly with 5-methyl cytosine. Previous experiments (Soska and Lark, *in press*) demonstrated that deoxyadenosine was not incorporated by cells growing in the presence of actinomycin D and in the absence of thymine. Addition of thymine allowed DNA synthesis and deoxyadenosine incorporation. It could be determined that less than 5% DNA synthesis occurred in the absence of thymine. Under favorable conditions, 10 mμmoles/ml (about 2 μg) of thymine was necessary to obtain regular growth. Only 5 to 10 mμmoles/ml (1 to 2 μg) of thymidine or the same equivalent of thymidylic acid was necessary for the same growth rate and yield. At a concentration of 0.2 μg of thymidine per ml, 14×10^7 cells per milliliter could be grown. This corresponds to a synthesis of 0.8×10^{-14} g of DNA per cell.

If a culture of *L. acidophilus* was transferred into a medium lacking thymine or deoxyribosides, or both, the DNA content did not increase, as shown previously (Soska and Lark, *in press*). However, the cells grew in length and their number increased two to four times. The viable count started to decrease after 3 hr of incubation at a rate similar to that for deoxyriboside or thymine starvation and similar to that observed in overnight stationary cultures.

The amount of thymine necessary to provide good growth was found to depend largely on the presence of adenine and its derivatives. An experiment showing this is presented in Table 5. A suspension of growing bacteria was diluted (10^{-4}) and incubated in tubes in the usual medium containing 4 μg of thymine and 8 μg of deoxyguanosine per ml, but different concentrations of adenine or its derivatives instead of adenosine-5-phosphate. At a time when most of the tubes were showing visible turbidity, samples were taken and the cell counts were estimated. Low concentrations of adenine or adenine derivatives increased the growth over the level observed in the absence of adenine. In the presence of higher concentrations of adenine, growth was inhibited. Cells growing in the presence of 10 μg/ml or more of adenine were large thread-like forms similar to those seen in the absence of sufficient amount of deoxyribosides or thymine. Although an excess of deoxyriboside did not reverse this inhibition, it was reversed if thymidine or folic acid or an excess of thymine was added. In Table 6, the effects of thymine and thymidine on this inhibition of growth conditioned by adenine are shown in more detail.

In another similar experiment, adenosine had the same inhibitory effect as adenine. The nature of the deoxyriboside was also of importance for thymine utilization. If a low concentration of thymine (1 μg/ml) was used, growth was stimulated by the following in order of increasing activity: deoxycytidine, deoxyguanosine, deoxyadenosine, and deoxyinosine.

TABLE 5. *Effect of adenine on thymine utilization*

Adenine derivative	Concn of adenine or its derivative (μg/ml as adenine)				
	2.65	5.3	10.6	21.2	42.4
Adenosine-5′-phosphate	9.4*	23.0	22.4	28.3	28.8
Adenosine-(2′,3′)-phosphate	25.0	28.8	21.5	8.7	6.0
Deoxyadenosine	11.6	33.1	37.7	38.9	34.6
Adenine	10.2	13.1	8.1	0.0	0.0
Adenine + thymidine (2 μg/ml)	—	—	—	—	26.6
Adenine + folic acid (0.1 μg/ml)	—	—	—	—	27.5
Adenine + deoxyguanosine (80 μg/ml)	—	—	—	—	0.0
Adenine + thymine (100 μg/ml)	—	—	—	—	34.4

* Cell count × 10^{-7}. A *Lactobacillus acidophilus* culture was diluted 10^{-4} and incubated for 11 hr in media containing various adenine derivatives in concentrations as indicated. Thymine and deoxyguanosine were present at 4 and 8 μg/ml, respectively, unless otherwise stated. The control (no adenine derivative) value was 6.6 × 10^{-7} cells.

TABLE 6. *Effect of thymine and thymidine on the inhibition of growth conditioned by adenine*

Concn (μg/ml)		Cell count × 10^{-7}*	Concn (μg/ml)			Cell count × 10^{-7}*
Adenine	Thymine		Adenine	Thymine	Thymidine	
0	4	9.8	100	4	0	0
2	4	18.3	100	8	0	0
5	4	21.5	100	12	0	0.23
8	4	14.8	100	24	0	8.42
12	4	9.67	100	50	0	26.7
20	4	1.30	100	100	0	30.4
40	4	0	100	4	1	26.6
100	4	0	100	4	4	29.6

* Footnote as in Table 5.

DISCUSSION

The present composition of the medium is suitable for a continued cultivation of *L. acidophilus* in the absence of folic acid. It differs from that of Siedler et al. (10) in this respect: folic acid was omitted and was replaced with thymine which becomes a requirement under this condition. Enzymatic hydrolysate of caseine was omitted. Guanylic acid was omitted because of its unfavorable influence on cell growth and cell morphology, but the concentration of guanine was increased. Adenine was usually omitted because of its unfavorable effects on thymine utilization, and adenylic acid was used instead. As a source of deoxyribose, thymidine was replaced by other deoxyribosides which allowed independent studies of thymine and deoxyriboside starvation. Spermidine was introduced into the medium to promote growth, but growth without thymine, as described by Turner and Lansford (13), could not be detected with this lactobacillus. In addition, the concentrations of the components of the medium were changed in many cases to obtain better yields.

The requirements for pyrimidine components correspond largely to the findings of Lovtrup and Shugar (6) obtained in the presence of folic acid, with the exception that thymine could not replace uracil. This is in agreement with the work of Biswas and Broquist (1). However, the residual RNA synthesis observed in the absence of uracil remains unexplained. The possibility of an effect of CO_2 as observed by Siedler et al. (10) is not very great in this experimental condition, since no CO_2 or carbonate was introduced into the medium. Okazaki and Okazaki (7) observed growth in the absence of uracil under conditions of partial anaerobiosis. In this study, there was no effect of aerobic or anaerobic growth. Cytosine does not appear to be the source for RNA-uracil synthesis.

Inability of ribonucleosides to support the growth of *L. acidophilus* (with the possible exception of adenosine) suggests the absence of a ribonucleoside-phosphorylase. The replacement of acetate by mevalonic acid (12) was not tried in this study, but it could be useful in media where the cell-enlarging effect of acetate should be eliminated. The induction of a thymine requirement by omitting folic acid is similar to the situation in other lactic acid bacteria (11).

The requirement for thymine in the absence of folic acid is shown by the following results. There was no reproduction of *L. acidophilus* in the absence of both thymine and folic acid except for cell enlargement, and this was nearly identical to that seen in deoxyriboside starvation where the absolute requirement is well established. Furthermore, it was found, by use of H^3-deoxyadenosine incorporation as an indicator, that there was no DNA synthesis in the absence of

thymine, whereas synthesis in the presence of thymine occurred. Actinomycin D was used in these experiments to prevent the incorporation of the adenine moiety of deoxyadenosine into RNA (Soska and Lark, *in press*).

The experiments show that adenine inhibits growth in the presence of thymine, but not of thymidine. Thymine is thus in some way prevented from entering the metabolism of the cell. Adenine perhaps inhibits the entry of thymine into the cells or the conversion of thymine into thymidine nucleotides. An inhibition on the level of DNA synthesis is very unlikely, because there is no inhibition by adenine if even a small amount of thymidine is present. Large amounts of thymine are required to release the inhibition. An interference of adenine in a transglycosidation reaction of the type:

$$\text{deoxyadenosine} + \text{thymine} \rightleftarrows \text{thymidine} + \text{adenine}$$

is considered as most probable. The increased utilization of thymine in the presence of deoxyadenosine or deoxyinosine is similar to the effects of deoxyadenosine on thymine-thymidine interconversions in *Escherichia coli*, observed by Boyce and Setlow (2).

The behavior of *L. acidophilus* in the absence of thymine or deoxyribosides differs from that of thymineless mutants of *E. coli* or *Bacillus subtilis* under conditions of thymine starvation. The low death rate of *L. acidophilus* when subjected to both thymine and deoxyriboside starvation makes it improbable that *L. acidophilus* undergoes a true thymineless death. Further work in this direction is needed.

Some implications concerning the use of Lactobacillus acidophilus R-26 as an experimental organism. For a long time, *L. acidophilus* R-26 has been considered a suitable experimental organism for the study of DNA synthesis because it cannot synthesize deoxyribosides. Until now, experiments have been hindered by the limitations of available media. The present results have indicated that:

(i) The thymine requirement permits labeling of nuclear material with radioactive thymine and the study of differences between thymine and deoxyriboside starvation. In experiments where utilization of thymine is of importance, adenine or adenosine should not be used, but rather adenosine-5-phosphate or deoxyadenosine.

(ii) The requirement for amino acids makes it possible to use this organism in experiments similar to those conducted with amino acid-requiring mutants of *E. coli*, like those used, for example, by Goldstein et al. (3). An advantage of *L. acidophilus* is that the effect of starvation for a number of different amino acids can be studied in the same organism. In such work, adenylic acids should be omitted because of their adverse effects on subsequent recovery from amino acid starvation. The nature of this effect remains unexplained.

(iii) The requirement for uracil makes possible the detection of RNA synthesis by use of labeled uracil or orotic acid. However, the uracil requirement is not sufficiently stringent to be used to stop RNA synthesis immediately. On the other hand, RNA synthesis can be easily inhibited by use of actinomycin D, which causes a complete inhibition of RNA synthesis at a concentration of 2 μg/ml (Soska and Lark, *in press*).

(iv) The requirement for deoxyribosides and the relative absence of deoxyribosideless death are useful for studies of processes taking place in the absence of DNA synthesis. Similarly, the formation of large cells in the absence of deoxyribose or thymine may be useful for cytological studies.

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