INFLUENCE OF GLUCOSE ON THE GROWTH FACTOR REQUIREMENT OF A MUTANT OF ESCHERICHIA COLI[1]

H. EDWIN UMBARGER

Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts

Received for publication March 6, 1953

Many reports can be found in the literature concerning the effect of glucose in the growth medium on the metabolism of bacterial cells. Typical of these reports are those of Gale (1940) who noted decreased deaminase activities as a result of growing *Escherichia coli* in a glucose medium or that of Forbes and Sevag (1951) who found that a strain of *Staphylococcus aureus* was unable to utilize glutamic acid to replace its proline requirement in the presence of glucose. Maas (1952) reported that propionate inhibited pantothenate synthesis in *E. coli* only in the presence of glucose and this effect of glucose was demonstrable with as little as one microgram of glucose per ml.

Such observations have remained unexplained. More recently, however, Boyd and Lichstein (1951) have presented evidence that, in the case of the deaminases of aspartic acid, serine, and threonine, glucose exerted its effect not on the apoenzyme but on the coenzyme. They suggested that an increased destruction of the coenzyme might be responsible rather than a decreased synthesis. To date, their observation probably comes nearest to explaining any of the reported inhibitory effects of glucose on the metabolic activities of bacteria.

Recently, a mutant of *E. coli* has been studied in this laboratory which grows approximately as well as the parent strain on a minimal medium containing acetate as the sole carbon source, whereas it requires the addition of a member of the citric acid cycle or a closely related amino acid for growth on a glucose mineral salts medium. Since no reports of any similar mutants have been found, this paper is devoted to a description of its nutritional requirements as well as preliminary observations on its metabolic activities.

[1] This investigation was supported by a research grant from the National Institutes of Health, Public Health Service, and by funds from the Eugene Higgins Trust.

MATERIALS AND METHODS

The mutant used in this study was strain B-42 of *Escherichia coli*. It was obtained from strain K-12 by ultraviolet irradiation and selection by means of penicillin (Davis, 1949) by Dr. I. Tsung Cheng in this department and kindly supplied by him.

The medium employed was that of Davis and Mingioli (1950). In some experiments, the sodium citrate was omitted without influencing the results reported here.

Growth experiments were performed in 125 ml Erlenmeyer flasks containing 10.0 ml of medium. The flasks were shaken for 24 hours on a Camp shaker at 37 C, and turbidities were measured at 590 mμ on a Coleman Universal spectrophotometer, model 11. The inoculum was prepared by inoculating the organism from a nutrient agar slant into 5.0 ml of basal medium enriched with 0.2 per cent enzymatically digested casein ("N-Z-Case") and 0.2 per cent glucose in a 50 ml Erlenmeyer flask. After incubating for 18 hours with shaking at 37 C, the culture was centrifuged and the sediment resuspended in 5.0 ml of sterile distilled water. One-tenth ml of this suspension was used to inoculate each of the experimental flasks.

The amino acids used were obtained from Nutritional Biochemicals Company. The acid hydrolyzed casein was casamino acids, technical (Difco).

For the respiration experiments the usual manometric procedures were employed. The cells were harvested by centrifugation after incubating for 17 hours at 37 C. The cells were washed twice in one-half the original volume of M/120 phosphate buffer at pH 7.0 and finally resuspended in distilled water for use. The cell concentration was determined from the turbidity of the suspension and reference to a curve previously calibrated with respect to dry weight. Dry weight was determined after heating an aliquot of the suspension overnight at 100 to 110 C.

RESULTS

Preliminary observations. When strain B-42 was seeded in minimal agar plates containing glucose as the carbon source, very faint growth zones could be seen around the points where loopfuls of either 1 per cent L-proline or 1 per cent L-glutamic acid had been applied. However, very heavy growth was found in the vicinity of an application of an acid hydrolyzate of casein. In liquid medium, only slight growth was permitted by the addition of as much as 300 micrograms of L-glutamic acid. An equal quantity of L-aspartic acid in addition produced little improvement. The four carbon dicarboxylic acids were even less effective than L-glutamic acid. An enrichment of 0.05 per cent casein hydrolyzate permitted fairly good growth of the mutant in liquid medium. Such a growth level was chosen as a standard to be achieved when defined media were employed.

It was observed quite early in the study that, if acetate served as the carbon source, the growth of strain B-42 was similar to that of the parent strain. However, the addition of glucose to an acetate-mineral salts medium was inhibitory to the mutant. The inhibition was reversed partially by the dicarboxylic acids. This effect is shown in table 1. It is interesting that the mutant tolerated the higher concentrations of sodium acetate better than did strain K-12.

This inhibitory effect on growth in acetate medium was not a specific one of glucose. Arabinose, gluconic acid, glycerol, and pyruvic acid, chosen as representative carbon sources, have been tested and all were found to inhibit growth of the mutant when added in the concentration tested (0.2 per cent) to the acetate-mineral salts medium. In the case of glucose, at least, only small amounts were required to inhibit, with one microgram per ml suppressing the growth from small inocula.

Growth of strain B-42 in an amino acid medium. Since the amount of growth obtained could be raised to almost any desired density by using increasing amounts of casein hydrolyzate as supplement to the glucose minimal medium, it was of interest to establish which of the component amino acids were responsible. It was observed that of the amino acids known to be present in acid hydrolyzed casein only L-glutamic acid, L-aspartic acid, L-proline, and L-lysine showed any significant influence when added to the basal medium.

When it was desired to employ a defined medium, these amino acids were employed in the form of medium B, which contained L-glutamic acid, 40 mg; L-aspartic acid, 75 mg; L-proline, 12.5 mg; and L-lysine, 12 mg per liter of the 0.2 per cent glucose minimal medium. Such a medium permitted roughly the same amount of growth as did a medium containing 0.05 per cent casein hydrolyzate and 0.2 per cent glucose. As is shown in table 2, L-aspartic acid and L-lysine were the more important constituents of the medium with L-glutamic acid and L-proline being less stimulatory.

TABLE 1

Effect of glucose on growth in acetate medium

Medium, sodium acetate	Other additions	Optical density	
		Strain B-42	Strain K-12
%			
0.2	None	0.252	0.284
0.4	None	0.367	0.252
0.2	Succinate	0.444	0.469
0.4	Succinate	0.378	0.328
0.2	Glucose	0.027	1.155
0.2	Glucose, succinate	0.198	1.187

Succinate, where added, 0.05 per cent; glucose, where added, 0.2 per cent. For other conditions see text.

TABLE 2

Influence of various components of medium B

Medium	Optical density
Medium B (see text)	0.570
L-Aspartic omitted	0.029
L-Lysine omitted	0.227
L-Glutamic omitted	0.403
L-Proline omitted	0.513

For culture conditions, see text.

Although the concentrations of amino acids in medium B have been chosen in an effort to minimize any amino acid antagonism, the best possible balance of components may not have been selected. It was observed that the L-aspartic acid concentration must be kept high in order to avoid what is apparently an interference with its

utilization by L-glutamic acid. It may be that the relative requirement for L-lysine is related somehow to the high concentrations of acidic amino acids in the medium although attempts to show this have not been successful.

Oxidation of acetate and glucose by mutant and wild type cells. Since no clues were obtained from the growth requirement studies as to the location of the metabolic block in strain B-42, the oxidative attack on acetate and glucose of the mutant was compared with that of the wild strain. Preliminary experiments were performed employing cells grown in the medium containing 0.05 per cent casein hydrolyzate and 0.2 per cent glucose. It was observed that the wild strain, when grown in this medium, oxidized acetate with an uptake of 1.5 moles of oxygen per mole of substrate (75 per cent of theory) and oxidized glucose with an uptake of about 3 moles of oxygen per mole of substrate (50 per cent of theory). In contrast, the mutant grown on the same medium could not oxidize acetate and exhibited an oxygen uptake with glucose of only 1.5 moles per mole of substrate.

The extent of oxidation of acetate and glucose by the wild strain was about that expected under conditions such as these permitting assimilation. Since mutant strain B-42 was unable to oxidize acetate, it seems quite possible that the low oxygen uptake on glucose might be accounted for by the accumulation of acetate. That this is probably true was shown in an experiment in which mutant cells were allowed to oxidize glucose until the rate of oxygen uptake levelled off to that of the endogenous. Using the direct method for carbon dioxide measurement, it was observed that 1.6 moles of oxygen were consumed and 1.6 moles of carbon dioxide were evolved per mole of substrate (80 per cent of theory for acetate formation). At the same time 1.7 moles of volatile acid per mole of substrate were formed (85 per cent of theory). It has been observed that pyruvate also is oxidized only to acetate.

When mutant cells were grown as described above, they could oxidize acetate only if a dicarboxylic acid (e.g., fumarate) was present as a sparking agent. If, however, the amount of casein hydrolyzate in the growth medium was increased to 0.2 per cent, cells were obtained which oxidized both glucose and acetate to the same extent as did the wild strain K-12.

Influence of the growth medium on acetate oxidation. Since mutant cells of two types in regard to their ability to oxidize acetate could be obtained by using either a 0.05 per cent or 0.2 per cent casein hydrolyzate enrichment with the 0.2 per cent glucose-mineral salts medium, an attempt was made to obtain similar results with media of known composition. Medium B described above yielded cells which were unable to oxidize acetate in the absence of fumarate. Cells grown on acetate, as would be expected, could oxidize acetate. Cells which could oxidize acetate without the addition of fumarate also were obtained by using an enrichment of 15 amino acids equivalent to 0.2 per cent casein hydrolyzate. No attempts have been made to attribute their shift in metabolism to any particular amino acid.

A clue as to the controlling factor in the medium was obtained when the mutant was grown in a medium containing 0.2 per cent casein hydrolyzate and 1.0 per cent glucose. The cells obtained were unable to oxidize acetate unless fumarate was added. Other combinations were tested, and it was recognized that the acetate oxidizing system was only formed if, at the time of harvesting the cells or perhaps a few hours before, all the glucose had disappeared from the medium. Thus, in a medium containing 0.05 per cent casein hydrolyzate and 0.2 per cent glucose, growth stops fairly early so that glucose is remaining at the time of harvest. With 0.2 per cent casein hydrolyzate there is a considerably larger cell crop, and by the end of the 17 hour incubation period, that amount of glucose has been utilized. If 1.0 per cent glucose is present with 0.2 per cent casein hydrolyzate, about half of the glucose remains at the time of harvest. More recently, whenever mutant cells requiring fumarate for acetate oxidation were desired, the latter medium has been employed. If acetate oxidizing cells were desired, the glucose was omitted.

Only with the mutant does glucose cause cells to be formed which require fumarate for acetate oxidation, but there is still another effect of glucose on the wild strain as well as on the mutant. It can be seen in figure 1 that the acetate oxidation by cells of both mutant and parent strains grown in a high glucose medium proceeds at a slow rate. The effect of fumarate is to be seen most clearly in the case of the mutant although in strain K-12 there is often a slight, transient increase in rate. Omission of glucose

or its incorporation in much smaller amounts results in a two- to threefold increase in the rate of acetate oxidation of both strains and the elimination of the fumarate requirement of the mutant.

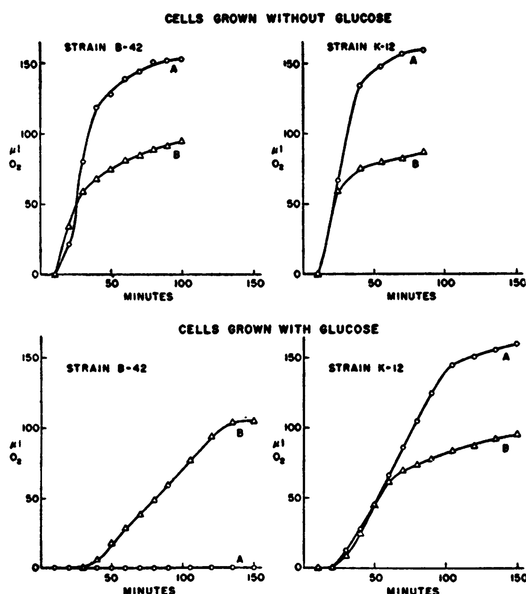

Figure 1. Influence of growth medium on acetate oxidation by strains B-42 and K-12. All vessels contained phosphate buffer 0.067 M (pH 7.0) 2.5 mg (dry weight) of cells per flask, and substrate in a total volume of 3.0 ml. Curve A, 5 μM acetate; curve B, 2.5 μM acetate and 1 μM fumarate. Substrates tipped after 10 minutes. Temperature 37 C, gas phase air, KOH in center well. Endogenous respiration subtracted.

DISCUSSION

The results obtained thus far in this study do not permit one to determine the nature of the metabolic block in mutant strain B-42. The observations, however, do serve to define the differences between it and the wild strain as well as to establish the conditions necessary for their demonstration. Since the wild strain K-12 has been subjected to the same manipulations as the mutant, some of the observations are perhaps of considerable consequence to those studying the pathways of acetate oxidation in microorganisms.

The lowered acetate oxidizing ability of cells grown in a medium containing glucose in comparison to cells grown in an acetate medium has been recognized by Ajl (1950). The finding reported here that growth on an amino acid medium also yields cells capable of rapid oxidation of acetate suggests that energy is obtained from amino acids largely through the oxidation of two-carbon fragments originating from amino acid breakdown.

The question whether or not acetate oxidation in *E. coli* proceeds via the citric acid cycle has been raised often. In this study, it was observed that cells of mutant strain B-42 grown in the high glucose medium required the addition of fumarate as a sparking agent in order to oxidize acetate not unlike the cyclophorase preparations of Green *et al.* (1948) which required the addition of a member of the tricarboxylic acid cycle for the complete oxidation of pyruvate. This finding suggests that, under these conditions, the mutant oxidized acetate via the citric acid cycle. That the cells of the parent strain grown under these conditions oxidized acetate at virtually the same rate suggests that they, too, employed the citric acid cycle, even though the sparking agent was not required. It may be that the mutant differs from the parent strain in having lost the ability to synthesize or store a dicarboxylic acid essential for acetate oxidation.

On the other hand, when harvested from a glucose-free medium, the cells of both strains oxidized acetate much more rapidly and those of the mutant did not require a sparking agent. The difference in rate of the two types of acetate oxidation might well be explained by differences in the amounts of the appropriate enzymes depending upon the conditions employed for growth. In the case of the mutant, rapid acetate oxidation without the addition of fumarate might be explained by the ability of cells to synthesize or store a sparking agent only when grown in the absence of glucose or by the utilization of a pathway other than the citric acid cycle.

In this regard, it should be mentioned that the rapid acetate oxidation of both strains is much more sensitive to fluoroacetate inhibition than is the slow acetate oxidation of glucose grown cells. The possibility that this difference is due to separate pathways is being investigated.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the technical assistance of Mrs. Martha Wallace and Miss Barbara Brown.

SUMMARY

A mutant of *Escherichia coli*, strain B-42, has been examined. It has been characterized as requiring an amino acid supplement of L-aspartic

acid, L-lysine, L-glutamic acid, and L-proline when glucose serves as the major carbon source. in contrast, in an acetate-mineral salts medium it grows as well as the wild strain, K-12.

When grown in an amino acid medium with no glucose, both the mutant and parent strains oxidized acetate rapidly. When grown in a similar medium containing in addition 1 per cent glucose, the mutant could oxidize acetate only slowly and only in the presence of a dicarboxylic acid (e.g., fumarate). Strain K-12 when grown in 1 per cent glucose also oxidized acetate slowly, but fumarate was not needed.

It was concluded that the slow type of acetate oxidation proceeds via the citric acid cycle. As yet there is insufficient evidence to suggest whether rapid acetate oxidation proceeds via this pathway or some other.

REFERENCES

AJL, S. 1950 Acetic acid oxidation by *Escherichia coli* and *Aerobacter aerogenes*. J. Bact., **59,** 499–507.

BOYD, W. L., AND LICHSTEIN, H. C. 1951 The inhibitory effect of glucose on certain amino acid deaminases. J. Bact., **62,** 711–715.

DAVIS, B. D. 1949 The isolation of biochemically deficient mutants by means of penicillin. Proc. Natl. Acad. Sci. U. S., **35,** 1–10.

DAVIS, B. D., AND MINGIOLI, E. 1950 Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bact., **60,** 17–28.

FORBES, M., AND SEVAG, M. G. 1951 Inhibition by glucose of the synthesis of proline from glutamic acid. Arch. Biochem., **31,** 406–415.

GALE, E. F. 1940 Enzymes concerned in the primary utilization of amino acids by bacteria. Bact. Revs., **4,** 135–176.

GREEN, D. E., LOOMIS, W. F., AND AUERBACH, V. H. 1948 Studies on the cyclophorase system. I. The complete oxidation of pyruvate to carbon dioxide and water. J. Biol. Chem., **172,** 389–403.

MAAS, W. 1952 Studies on the mechanism of resistance to propionate inhibition. Bact. Proc., **1952,** 150.