70. The Influence of the Presence of Glucose during Growth on the Enzymic Activities of *Escherichia coli*: Comparison of the Effect with that Produced by Fermentation Acids

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Kendall & Farmer [1912; 1913] showed that in growing cultures of a number of different bacterial species the ammonia liberated from protein digests decreases or even disappears when carbohydrate is present. Kendall [1922] suggested that this effect was due to the protein-sparing action of carbohydrate. Berman & Rettger [1918] showed that the excretion of protease by *P. vulgaris* is also inhibited by the presence of carbohydrate in the medium but since the effect is less marked with an organism such as *B. subtilis* which produces little acid from glucose, they suggested that the effect was due to the production of acid from fermentation of the carbohydrate. The addition of buffer to the medium decreased the inhibitory effect of glucose and this supports the theory that the effect is one of *p*H. Raistrick & Clark [1921] pointed out that the growth of many bacterial species is much heavier in the presence of glucose in the medium and suggested that the greater yield of cell-N accounts for some of the missing ammonia-N, but this factor is insufficient to explain the discrepancy in most cases. Happold & Hoyle [1935] made a non-viable preparation of 'tryptophanase' which would bring about the aerobic degradation of tryptophan to indole and they reported [1936] that cultures of *E. coli* grown in the presence of glucose contained no tryptophanase. Fildes [1938] reported that the tryptophanase of *E. coli* can be divided into a small constitutive portion and a large adaptive portion, and that the presence of glucose during growth inhibits the formation of the adaptive portion of the enzyme. Evans, Handley & Happold [1941] claim that tryptophanase is purely adaptive and that the presence of glucose in the growth medium completely inhibits its formation as long as tyrosine or phenylalanine is also present; in the absence of these amino-acids the inhibitory effect of glucose is not complete, a result explaining the findings of Fildes [1938].

Stephenson & Gale [1937 *a*] found that the addition of glucose to washed suspensions of *E. coli* grown in the absence of glucose has no significant effect on the activity of the glycine, alanine and glutamic acid deaminases, but if the organism is grown in the presence of 2% glucose, the resulting suspensions have only 10–20% of the deaminase activities of those grown in its absence. The effect of glucose is therefore not on the course of the enzyme action after growth is complete but inhibits the formation of the deaminases during growth. This result was later obtained with *dl*-serine deaminase [Gale & Stephenson, 1938] and aspartase [Gale, 1938]. In order to check the *p*H effect of the fermentation of glucose during growth, chalk was mixed with the medium and constantly stirred throughout the growth period, without effect on the inhibitory action of glucose on the deaminase formation. The effect was not due to anaerobiosis produced by fermentation gases, for bubbling the medium with oxygen did not affect the inhibition. Gale [1940; 1941], investigating the conditions under which bacteria produce amino-acid decarboxylases, found that the action of glucose in the medium in promoting the formation of these enzymes could be explained entirely by the low *p*H produced by fermentation during growth. This raised the question whether the addition of chalk to the medium in the

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previous work was an efficient method of controlling the medium *p*H, particularly within the fermenting cell. The present communication is an attempt to settle this problem of whether the effect of glucose in the medium can be attributed to the action of the acidity formed during its fermentation.

Most of the investigations of this glucose effect have been restricted in the past to enzymes concerned in the metabolism of amino-acids, probably owing to the emphasis placed on the 'protein-sparing' hypothesis, and as we were determining the action of growth *p*H on the enzymic activities in general [Gale & Epps, 1942] we decided to extend the observations to include cultures grown in the presence of glucose.

Methods. The methods of estimating the various activities dealt with below have all been outlined in the previous paper by Gale & Epps [1942]. Growth took place in tryptic digest of casein adjusted initially to *p*H 7 and to 5 (the final *p*H attained by *E. coli* in 2% glucose broth) as described before and, in addition, in broth containing 2% glucose and adjusted initially to *p*H 7. Growth took place at 27° and the final *p*H was estimated in all cases.

Experimental

Table 1 gives the activities of cultures of *E. coli* grown in plain broth at *p*H 7 and 5 and in 2% glucose broth in which the *p*H altered from 7 to 5·2 (average) during growth. The variation of activity with *p*H during growth has already been dealt with in the previous paper. If the effect of the glucose in the medium is due to the *p*H produced by its fermentation, then the activities of the cultures grown in glucose should approximate to those of cultures grown at *p*H 5. Table 1 shows that in certain cases such as those of hydrogenase and catalase, and arginine, lysine and histidine decarboxylases the difference in the activities of the *p*H 7 and glucose cultures can be ascribed to the alteration in *p*H during growth. In all the other cases quoted, with the exception of glucozymase, glucose has an inhibitory effect on the activity greater than any effect that can be ascribed to *p*H. This is particularly the case with formic and alcohol dehydrogenases where an acid growth medium produces an increased potential activity compared with growth at *p*H 7 while the action of glucose is markedly inhibitory. Glucozymase is a special case in that the presence of glucose increases the formation of this enzyme while acid growth conditions decrease it; thus the adaptive increase in glucozymase noted by Stephenson & Gale [1937*b*] is a true substrate effect.

Table 1. *Potential activities of* E. coli *when grown in casein digest adjusted to* p*H 7 or 5, and containing 2% glucose*

Final *p*H in glucose medium after growth = 5·2 (average).

Enzyme	*Q* unit	Potential activity in medium			Glucose effect
		At *p*H 7	At *p*H 5	2% glucose	
Hydrogenase	M.B.	240	136	146	None
Catalase	O_2	4200	6360	6310	,,
Arginine decarboxylase	CO_2	2	338	272	,,
Lysine decarboxylase	CO_2	53	194	198	,,
Histidine decarboxylase	CO_2	3	26	33	,,
Ornithine decarboxylase	CO_2	47	560	48	Inhibitory
Alanine deaminase	NH_3	32	4	1·7	,,
Glutamic acid deaminase	NH_3	12	3	1·2	,,
Aspartase (total)	NH_3	127	247	15	,,
Serine deaminase	NH_3	855	656	167	,,
Tryptophanase	Indole	5·4	1·6	0·25	,,
Alcohol dehydrogenase	M.B.	52	179	44	,,
Succinic dehydrogenase	M.B.	43	23	9	,,
Formic dehydrogenase	M.B.	110	138	58	,,
Formic hydrogenlyase	H_2	75	>200	139	,,
Glucozymase	Glucose	38·5	31	77	Stimulatory

Table 1 shows that the inhibitory action of glucose on the formation of enzymes is not confined to amino-acid deaminases, although the effect is marked with these systems, particularly in the case of aspartase where the culture grown at *p*H 5 has approximately double the activity of that grown at *p*H 7 while the glucose culture has about 1/9 of that activity. To confirm that the glucose effect cannot be explained in terms of *p*H, a culture of *E. coli* was grown in 2% glucose broth adjusted to *p*H 7 and the *p*H was maintained at this value throughout the growth period by the addition of sterile NaOH solution to neutralize fermentation acids. The *p*H was measured by means of a hydrogen electrode immersed in the medium, the whole apparatus being the same as that described by Gale & van Heyningen [1942]. The activities of three cultures (1) in plain broth at *p*H 7, (2) in 2% glucose broth maintained at *p*H 7 and (3) in 2% glucose broth with *p*H uncontrolled, were then determined and the results set out in Table 2 show that the inhibitory action of glucose in the medium is the same, for the deaminases tested, whether the fermentation acidity be neutralized or not.

Table 2. *Potential activities of* E. coli *when grown in glucose*

Enzyme	*Q* unit	Potential activity when grown in: No glucose at *p*H 7	2% glucose *p*H uncontrolled	2% glucose *p*H 7 maintained
Alanine deaminase	NH_3	43	12	15
Glutamic deaminase	NH_3	13	5	5
Aspartase (total)	NH_3	127	15	9
Aspartase (toluene-treated)	NH_3	45	18	19
Dry weight *coli*/ml. medium		0·27 mg.	0·71 mg.	1·31 mg.

The results so far described have been obtained after a single subcultivation in the presence of glucose. Next we tested whether it is possible to train the deaminase activities to maintain the lower level by serial subcultivation in glucose broth. 100 ml. of plain broth were inoculated with *E. coli* and incubated overnight. Then 100 ml. 2% glucose broth were inoculated from this culture and the remainder spun off for the determination of the aspartase activity of the washed suspension. This culture was then subcultivated five times in 2% glucose broth and the activity determined after each incubation. The fifth glucose culture was then subcultured into plain broth. Table 3 shows that there is

Table 3. *Effect of repeated subcultivation in 2% glucose broth*

Growth medium	Hr. of growth	Q_{NH_3} (aspartic)	Growth medium	Hr. of growth	Q_{NH_3} (aspartic)
Plain broth	16	143	2% glucose broth (4)	7	33
2% glucose broth (1)	16	24	2% glucose broth (5)	16	24
2% glucose broth (2)	7	32	Plain broth (1)	12	135
2% glucose broth (3)	16	25			

no progressive loss of activity on consecutive 'passages' through glucose broth and that the organism regains its normal aspartase activity immediately on cultivation in the absence of glucose.

Results with Micrococcus lysodeikticus

Quastel [1937] investigated the effect of the presence of glucose in the growth medium upon certain enzymic activities of *M. lysodeikticus*. He claimed that glucose stimulates the formation of urease and suppresses that of catalase and fumarase, the estimations being carried out on lysed suspensions. Quastel grew his cultures on the surface of peptone-agar; we have grown our cultures in casein-digest broth as described previously but have obtained different results under these conditions. When *M. lysodeikticus* is grown in liquid medium, the addition of 1% glucose does not cause any alteration in the *p*H during

growth and we have been unable to find any significant activity towards glucose of washed suspensions prepared from organisms so cultivated. The addition of glucose to the suspension does not cause any significant increase in the O_2 consumption measured manometrically and the anaerobic $Q_{glucose}$ measured as described for glucozymase [Gale & Epps, 1942] is less than 2. When the dry weight of the suspensions and the activities of these enzymes are determined by the methods described in the previous paper [Gale & Epps, 1942] we get results of the order shown in Table 4 showing no significant effect upon the catalase, urease and fumarase activities due to growth in the presence of glucose, whether the activities are determined with intact organism or lysed suspensions. The difference between our findings and those of Quastel may be explained in the case of catalase by the much longer experimental period used by him: we have shown that the enzyme is poisoned within 15–20 min. of the addition of the H_2O_2 and that a rate of enzymic decomposition can only be measured during the first 4–6 min. after mixing at room temperature, so that Quastel's estimation of the O_2 evolved after 2 hr. at 38° is no measure of the enzymic activity. Also we have found that the urease activity of this organism is largely destroyed by lysis unless the lysis takes place in the presence of urea; it is not possible to calculate the Q_{CO_2} from Quastel's figures, since the weight of organism is not given, but the volumes of CO_2 quoted (87–176 μl. in 2 hr.) suggest that the activity measured is the activity which remains left after lysis in the absence of substrate. Likewise we have found that the urease activity of *M. lysodeikticus* can vary greatly unless growth takes place in the presence of urea.

Table 4. *Effect of growth in glucose on activities of* M. lysodeikticus

			Potential activity when grown in	
Enzyme	*Q* unit	Organism	Broth at *p*H 7	1% glucose broth at *p*H 7
Catalase	O_2	Intact	51,000	50,000
		Lysed	330,000	320,000
Urease	CO_2	Intact	300	298
		Lysed	193	194
Fumarase	Malate	Lysed	1,420	1,490

Addition of glucose has no significant effect on *p*H of medium during growth.

Summary

1. The presence of glucose in the medium during the growth of *E. coli* suppresses the formation of certain enzymes; the degree of inhibition is greater than, or bears no relation to, the effect produced by growth in a medium adjusted to the final *p*H produced in the glucose medium by fermentation acids.

2. Neutralization of fermentation acids during growth in glucose does not alter the degree of inhibition of deaminase formation produced by the glucose.

3. The reduction of the activity of certain enzymes as a result of growth in glucose is not a permanent change in the enzyme constitution of the cell as it is removed immediately growth takes place in the absence of fermentable carbohydrate.

4. The presence of glucose in the liquid growth medium has no effect upon the urease, catalase and fumarase activities of *M. lysodeikticus*.

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