THE INHIBITORY EFFECT OF GLUCOSE ON CERTAIN AMINO ACID DEAMINASES[1]

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The incorporation of glucose into growth media was observed as early as 1912 (Kendall and Farmer) to decrease markedly the production of ammonia by bacteria. Since that time, it has been learned from studies in which bacteria were grown in the presence of this carbohydrate (Epps and Gale, 1942) that a variety of deaminases are either not produced or are formed in low concentration. These findings have been interpreted as an inhibition of enzyme formation during glycolysis.

The present work was undertaken because of our general interest in the deaminases of aspartic acid, serine, and threonine, and the results obtained suggest that the glucose effect is not primarily on apoenzyme production.

METHODS

Bacterium cadaveris, Gale's strain, and *Escherichia coli*, strain 86G, were employed. The former was grown in a medium composed of 1 per cent each of peptone and yeast extract and 0.5 per cent K_2HPO_4; the latter was cultured in a synthetic mineral salts medium to which 0.5 per cent acid-hydrolyzed vitamin-free casein was added (Billen and Lichstein, 1950). The cells in the foregoing media, with and without from 0.2 to 2 per cent glucose, were incubated for a period of from 16 to 18 hours at 30 C, harvested by centrifugation, washed once, and suspended in distilled water to give a cell concentration of 0.5 to 1 mg of bacterial nitrogen per ml. The deamination experiments were performed at pH 7 in M/20 phosphate buffer at 37 C, using adequate controls without added amino acids. These blank values for 60 minutes' incubation time varied between 0 and 5 μg ammonia nitrogen for the no additions series or in the presence of biotin and adenylic acid, to 5 to 15 μg in the presence of yeast or liver extract. The values given in the tables have been corrected for these blanks. Ammonia was determined by Nesslerization and color read in a Klett-Summerson photoelectric colorimeter.

EXPERIMENTAL RESULTS

It can be seen from the data given in table 1 that a general reduction of deaminase activity occurs when cells are harvested from a glucose medium. These results confirm the findings of Epps and Gale (1942). It was learned from further experiments (table 2) that a variety of agents known to be involved in the deami-

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TABLE 1

Effect of the presence of glucose in growth medium on deaminase activity

ORGANISM	AMINO ACID	μG NH_3 NITROGEN PRODUCED	
		Without glucose	With glucose
Bacterium cadaveris	DL-Aspartic acid	25.8	2.6
		16.1	5.7
	DL-Serine	20.2	3.0
	DL-Threonine	38.9	1.3
	DL-Alanine	19.7	8.0
Escherichia coli	DL-Aspartic acid	21.5	16.0
		27.0	17.1
		24.7	0.9
		10.7	0
	DL-Serine	40.0	14.8
	DL-Threonine	10.2	7.3
	DL-Alanine	66.7	17.4

Amino acids added at 0.005 M final concentration, pH 7, 60 min, 0.1 to 0.2 mg bacterial nitrogen per tube.

TABLE 2

Effect of various agents on the deaminase activity of glucose-grown cells

		μH NH_3 NITROGEN PRODUCED									
		No additions		+biotin		+AA		+B + AA		+YE or LE	
		Without glucose	With glucose	Without glucose	With glucose	Without glucose	With glucose	Without glucose	With glucose	Without glucose	With glucose
Bacterium cadaveris	DL-Aspartic acid	65.5	38.5					92.1	68.4	94.2	96.7
		66.0	22.2	70.7	27.0	66.0	34.4	76.4	51.1	66.3	58.7
		25.8	2.6	26.4	2.6					48.8	37.1
	DL-Serine	20.2	3.0	21.1	4.2	18.3	7.6	21.8	4.0	38.0	27.6
	DL-Threonine	38.9	1.3	37.8	20.0	57.9	17.7	62.7	19.6	50.7	24.3
Escherichia coli	DL-Aspartic acid	24.7	0.9	26.4	8.4	30.7	11.3	24.9	7.0	42.3	36.3
		10.7	0	14.8	13.9	2.3	1.0	21.6	7.5	23.8	18.9
		33.9	2.3	27.6	12.5					45.4	36.5
		21.5	16.0	22.9	20.6	21.2	26.7	31.6	20.6	33.1	30.7

Conditions as for table 1.
Conc of additions: biotin 0.005 μg/ml
adenylic acid (AA) 50 μg/ml
yeast extract (YE) or liver extract (LE) 0.5 mg/ml.

nases of aspartic acid, serine, and threonine (Lichstein and Umbreit, 1947; Lichstein and Christman, 1948; Lichstein, 1949) increase the activity of the glucose grown cells.

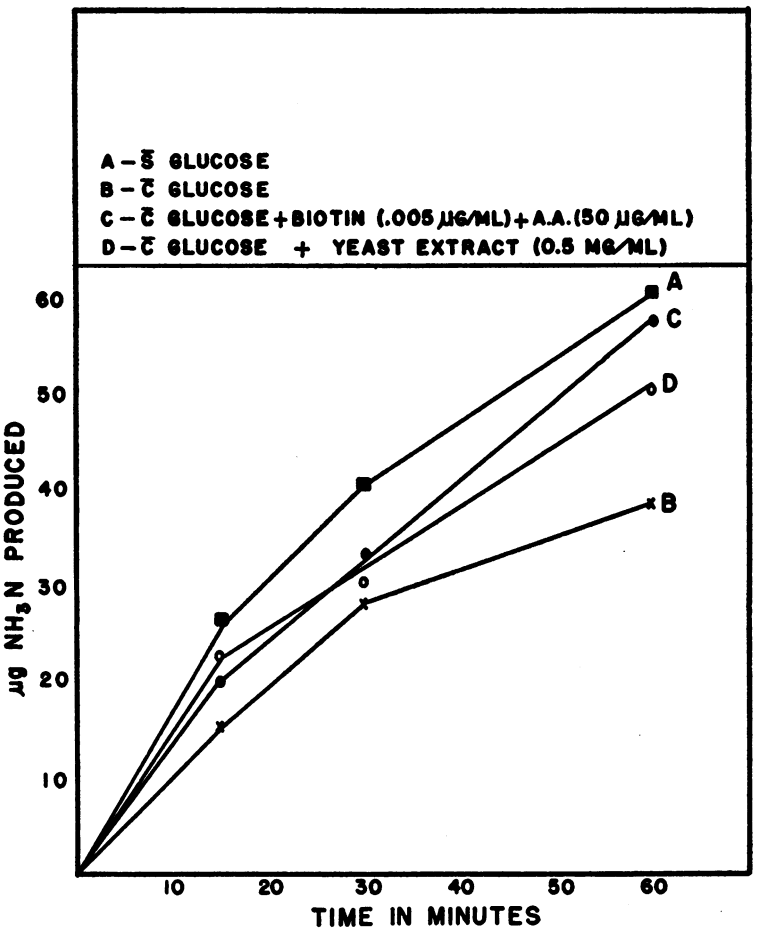

Figure 1. Effect of biotin, adenylic acid, and yeast extract on the aspartic acid deaminase activity of *Bacterium cadaveris*. Conditions as for table 1.

TABLE 3

Effect of incorporation of various materials into the growth medium on aspartic acid deaminase activity of Escherichia coli

GROWTH CONDITIONS	µG NH_3 NITROGEN PRODUCED						
	1	2	3	4	5	6	7
Without glucose	55.4	30.5	17.4	20.3	18.6	16.7	26.7
With glucose	33.6	13.1	4.6	17.4	9.3	4.9	3.2
With glucose + biotin	42.6	14.5	9.3	27.0			
With glucose + biotin + AA		19.1		25.5			
With glucose + YE 10%					17.7	11.9	11.3
With glucose + YE 5%						8.1	
With glucose + YE 1%					10.7	2.3	
With glucose + YE 0.1%					9.0		

Conditions as for table 1.
Biotin = 30 µg/ml.
Adenylic acid = 100 µg/ml.

Although only rarely does this recovery equal the activity of cells grown in the absence of glucose, the results are consistent with an interpretation that the presence of glucose during growth somehow either prevents the formation of the coenzyme of these deaminases or causes its destruction. That the glucose effect is not primarily on apoenzyme production may be seen from the data given in figure 1. Since stimulation of activity occurs almost immediately, it is probable that preformed apoenzyme was present in the glucose-grown cells.

In view of these findings it was deemed advisable to ascertain whether or not this glucose effect could be overcome by the incorporation of coenzyme or of coenzyme precursors into growth medium of known glucose content. The results of such experiments are given in table 3. Although none of the agents employed is capable of completely counteracting the effect of glucose, the agents consistently exhibit significant activity, suggesting that a large excess of preformed coenzyme or coenzyme precursors may be able, at least partially, to replace that amount which is either destroyed or not produced.

DISCUSSION

Since it is known that a bacterial cell can lose preformed folic acid under suitable conditions, and that concurrent glycolysis accelerates this phenomenon (Nimmo-Smith *et al.*, 1948; Lichstein and White, 1950), it is perhaps not surprising that a similar phenomenon should exist with biotin.

That the presence of glucose in the growth medium affects primarily the concentration of coenzyme may be inferred from the fact that stimulation by biotin or other agents is in most cases almost instantaneous. It is improbable that these early stimulations could be due to synthesis of protein (apoenzyme). Furthermore, the large amount of preformed coenzyme required in the growth medium to overcome even partially the effect of glucose (table 3, especially numbers 5 and 6) suggests that destruction of coenzyme may be the mechanism.

SUMMARY

The data presented are interpreted as evidence that the coenzyme of aspartic acid, serine, and threonine deaminases is resolved during glycolysis. Activity can be restored significantly by the addition of preformed coenzyme, or of coenzyme precursors, to resting cell suspensions, or by growing the cells in the presence of high concentrations of these agents in the presence of glucose.

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