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FORMATION OF VALINE BY STREPTOMYCIN-DEPENDENT *ESCHERICHIA COLI*

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ABSTRACT

BRAGG, P. D. (University of British Columbia, Vancouver, British Columbia, Canada), AND W. J. POLGLASE. Formation of valine by streptomycin-dependent *Escherichia coli*. J. Bacteriol. 88:1006-1009. 1964.—The primary extracellular products of the aerobic catabolism of glucose in streptomycin-dependent *Escherichia coli* were found to be carbon dioxide and acetate, the ratio of these two products being dependent on the rate of aeration but independent of antibiotic. Secondary extracellular products of dependent *E. coli* were valine and lactic acid; the former was produced in the presence of antibiotic under aerobic conditions, and the latter metabolite was formed under conditions of either antibiotic depletion or oxygen deprivation. The fact that the same products were formed when either streptomycin or oxygen was limiting supports the hypothesis that the antibiotic is required to complete hydrogen transport in dependent *E. coli*. The formation of valine appears to represent an anomalous pathway of aerobic glucose catabolism, whereby a neutral, relatively highly reduced end product is excreted.

Tirunarayanan, Vischer, and Renner (1962) and Bragg and Polglase (1962) reported that streptomycin-dependent *Escherichia coli* growing on glucose-salts medium excreted relatively large amounts of the amino acid L-valine into the growth medium. A further observation (Bragg and Polglase, 1962) was that streptomycin-resistant *E. coli* produced lactic acid from glucose when grown in medium containing antibiotic, but failed to produce lactic acid in the absence of antibiotic. These results were interpreted (Bragg and Polglase, 1962) to signify that streptomycin-resistant and dependent *E. coli* utilized anaerobic or "quasi-anaerobic" pathways when grown in the presence of antibiotic. Recently, Grant and Hinshelwood (1964) arrived at a similar conclusion as a result of investigations on the balance of enzyme activities in streptomycin-sensitive and -resistant *Aerobacter aerogenes*. These authors stated that "training to streptomycin increases enzymes concerned in breakdown of the carbon source, i.e., catabolic enzymes, which would occur if drug adaptation led to a transfer of metabolism to anaerobic routes."

The resistance to streptomycin of anaerobes, and of facultative organisms growing anaerobically, has been noted frequently (*see* Lightbown, 1957). Hirano (1954) reported an increased resistance to streptomycin in antibiotic-sensitive *E. coli* growing anaerobically. He observed also that a streptomycin-dependent mutant would grow anaerobically without added antibiotic. Thus, the evidence from various reports indicates that there is probably a direct relationship between streptomycin sensitivity and aerobic metabolism.

The present report consists of further observations on the formation of L-valine by streptomycin-dependent *E. coli*. The earlier observation, (Bragg and Polglase, 1963*b*) that streptomycin-dependent *E. coli* produced lactic acid when depleted of antibiotic has now been extended to show that valine and lactic acid represent alternate products of the metabolism of glucose by dependent cells. This report thus provides further support to the view (Bragg and Polglase, 1963*a*) that streptomycin has an important site of action in the electron transport system of *E. coli*.

MATERIALS AND METHODS

Cultures. Two streptomycin-dependent strains were used in this work, one of which (DA) was described in previous publications from this laboratory (Bragg and Polglase, 1962). The other culture (DC) was a dependent mutant isolated from a strain of streptomycin-sensitive *E. coli* (SC) obtained from the culture collection of the Department of Bacteriology and Immunology of this University. Both of these strains required a concentration of 1,000 units per ml to achieve

maximal growth rates in glucose-salts medium. In all experiments reported here, the dihydro derivative of streptomycin was used.

Medium. The following medium, adjusted to pH 7.2, was used: 0.7% K_2HPO_4, 0.3% KH_2PO_4, 0.05% sodium citrate, 0.01% $MgSO_4$, 0.1% $(NH_4)_2SO_4$, 0.4% glucose, and 1,000 units per ml of dihydrostreptomycin (when present) as the sulfate.

Conditions of growth. The technique used for growing streptomycin-sensitive cells was described in an earlier report (Bragg and Polglase, 1962). Streptomycin-dependent cells were of three types, as described previously (Bragg and Polglase, 1963*b*): "normal" (grown with an optimal concentration of antibiotic), "depleted" (grown in streptomycin-free medium to a state of antibiotic starvation), and "supplemented" with antibiotic (after depletion). Cells were grown with three roughly defined levels of aeration corresponding to "slow," "moderate," or "fast." No attempt was made to control the rate of aeration with precision, since this was believed to be unnecessary for the achievement of the objectives of the experiments. The temperature was maintained at 37 C in all experiments.

Analysis for extracellular metabolites. Valine and alanine were determined chromatographically by the method of Bode (1955). Lactic acid was determined colorimetrically (Barker and Summerson, 1941) and also chromatographically, together with other acidic metabolites, as follows. The acids were absorbed from the culture medium with Dowex 1-x8 (carbonate), and were eluted with 10% ammonium carbonate solution. The eluates were concentrated under diminished pressure at a temperature not exceeding 40 C. The acids were then fractionated by chromatography on Celite Analytical Filter-Aid (Johns-Manville, New York, N.Y.), with either chloroform–*n*-butanol (9:1) saturated with 0.5 N sulfuric acid or ether saturated with 0.5 N sulfuric acid used as solvents (Phares et al., 1952). The acids thus separated were identified on paper chromatograms, as described by Isherwood and Hanes (1953), and were estimated quantitatively by titration. Keto acids were also determined by the method of El Hawary and Thompson (1953). Glucose was determined by the method of Montgomery (1957). The carbon dioxide evolved was determined as barium carbonate.

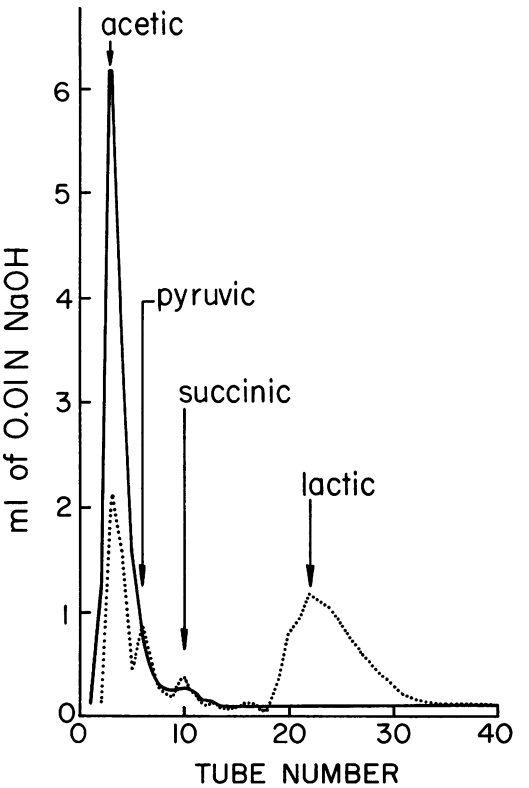

FIG. 1. *Acids produced by streptomycin-dependent Escherichia coli DA grown on glucose-salts medium. Solid line: supplemented with 1,000 units per ml of dihydrostreptomycin; dotted line: depleted of antibiotic. Solvent was ether saturated with 0.5 N sulfuric acid.*

RESULTS

Figure 1 shows the results of analysis of strain DA (dependent) for organic acids. When supplemented with dihydrostreptomycin, the dependent organism produced acetic acid from glucose but no lactic acid. On the other hand, the depleted cells produced considerable lactic acid as well as some acetic acid. These results were obtained under conditions of moderate aeration.

Table 1 gives results from a number of experiments with streptomycin-sensitive *E. coli* and with normal, depleted, and supplemented, streptomycin-dependent *E. coli*. Acetate was the primary product of cells when slow aeration was used. Carbon dioxide was the primary product with fast aeration. Moderate aeration produced a mixture of acetate and carbon dioxide. Lactic

acid (but very little valine) was produced as a secondary product by depleted dependent cells, whereas valine (but negligible lactic acid) was produced as a secondary product of supplemented or normal dependent cells.

Table 2 shows the results of analyses performed on normal DA grown in air and in nitrogen. Valine (but no lactic acid or alanine) was produced in air, whereas lactic acid and alanine (but only a small quantity of valine) were produced in nitrogen.

DISCUSSION

The aeration conditions used in these experiments were not defined precisely. However, the results appear to have justified the technique, since the rate of aeration appeared to affect only the relative amounts of the two primary products, acetate and carbon dioxide. The technique used for depletion of streptomycin-dependent *E. coli* (Bragg and Polglase, 1963*b*) was somewhat arbitrary, since the final level of residual antibiotic retained by the cells probably varied considerably in different experiments. This could explain the variability in the amount of lactic acid produced by different preparations of depleted cells. However, even with this variability, the results clearly showed that lactic acid production was a property of antibiotic-depleted cells, whereas valine production was a property of dihydrostreptomycin-supplemented cells. Small amounts of alanine were formed simultaneously with lactic acid (Table 2), presumably from pyruvate by transamination.

When streptomycin-dependent cells were grown in a nitrogen atmosphere, their metabolism resembled that of aerated, depleted cells, even though the concentration of dihydrostreptomycin was optimal (at least for aerobic growth). These results imply that in streptomycin-dependent *E. coli* there is a close relationship between aerobic metabolism and the requirement for the antibiotic. This relationship is summarized, diagrammatically, in Fig. 2. The primary products of the aerobic catabolism of glucose in streptomycin-dependent *E. coli* are carbon dioxide or acetate. The secondary products are valine (aerobic, with antibiotic) or lactic acid plus alanine (anaerobic, or without antibiotic).

The formation of L-valine by streptomycin-dependent *E. coli* DA accounted for over 10% of the glucose carbon (Table 2). The fact that the production of valine by dependent cells tends to decrease during depletion of antibiotic, and that lactic acid is formed in its place, suggests

TABLE 1. *Products of metabolism of glucose by streptomycin-sensitive and -dependent Escherichia coli*

Culture*	Aeration condition	Percentage of glucose carbon in product				
		Carbon dioxide	Acetate	Valine	Lactate	Alanine
SC	Slow	4.4				
	Slow	12.9	60.0		2.1	
	Moderate		6.3	0	0	0
	Fast	70.0				
DC (depleted)	Slow	9.5		0.4	2.5	
	Fast	50.8		0.2	16.1	
	Fast	75.0		0.08	3.5	
DA (depleted)	Moderate			0.5	14.5	1.6
	Moderate		5.3		20.4	
	Moderate				5.0	
	Moderate				11.8	
DC (supplemented)	Moderate	24.8				
	Moderate		32.0	2.2	0	0.1
	Fast	77.7				
DA (supplemented)	Moderate			5.6	0	0
	Moderate				0	
	Moderate				0.4	

* SC is streptomycin-sensitive. DC and DA are streptomycin-dependent *E. coli*, depleted or supplemented (with 1,000 units per ml of dihydrostreptomycin) as described.

TABLE 2. *Products of metabolism of streptomycin-dependent Escherichia coli DA grown under air and nitrogen**

Product†	Grown in air	Grown under nitrogen
	μmoles/100 ml	*μmoles/100 ml*
Total keto acid	1.9 (0.1)‡	6.1 (0.3)‡
Valine	133 (10.4)	20 (1.5)
Lactic acid	0	90 (4.2)
Alanine	0	55 (2.5)

* Glucose-salts medium containing dihydrostreptomycin (1,000 units per ml).

† Quantity of product was determined after glucose had been consumed (11 hr of growth).

‡ Figures in parentheses refer to percentage of glucose carbon in product.

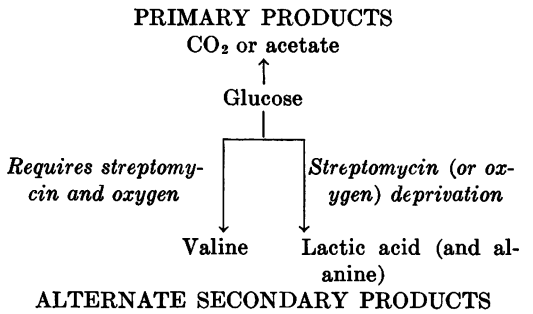

FIG. 2. *Products of catabolism of glucose by streptomycin-dependent Escherichia coli.*

that valine and lactic acid are alternate secondary products of glucose metabolism. Anaerobiosis produced an effect similar to streptomycin deprivation. The formation of valine thus appears to be a secondary, aerobic pathway of glucose metabolism established in the streptomycin-dependent organism. Previous studies in this laboratory on the effect of dihydrostreptomycin on electron transport in streptomycin-dependent *E. coli* (Bragg and Polglase, 1963*b*) suggested that valine may be playing the role of a neutral hydrogen acceptor in carbohydrate metabolism. If this were the case, the role of streptomycin (or dihydrostreptomycin) in streptomycin-dependent *E. coli* would be to participate in the transfer of hydrogen from reduced coenzymes to valine.

If these suggestions are correct, it must be concluded that the role of L-valine in the catabolism of glucose by streptomycin-dependent *E. coli* is both unique and incongruous, since valine is a rather highly reduced metabolite to have resulted from oxidative reactions. A study of enzyme activities of extracts of streptomycin-dependent *E. coli* in relation to the excretion of valine and lactic acid is now under way, and will form the subject of another report.

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