THE EFFECT OF SODIUM ON THE FERMENTATION OF GLUTAMIC ACID BY PEPTOCOCCUS AEROGENES[1]

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The anaerobe Peptococcus aerogenes degrades glutamic acid to carbon dioxide, acetic and butyric acids and ammonia (Whiteley 1957). Horler, Westlake and McConnell (1966a) have shown using specifically labelled glutamic acid-14C that carbon five is converted to carbon dioxide and that butyric acid can be formed from the first four carbons of glutamic acid without passing through two carbon units.

Washed cells of P. aerogenes, when suspended in a potassium phosphate buffer system at pH 7, readily fermented sodium glutamate; when glutamate was added as the free acid such preparations metabolized only about 10% of the added substrate. The addition of sodium ions, e.g. sodium sulphate, resulted in rapid and complete utilization of the glutamic acid. This paper describes the affect of sodium ions on the metabolism of glutamic and other amino acids by P. aerogenes.

MATERIALS AND METHODS

The techniques used for growing P. aerogenes, recovering the cells, purifying specifically labelled glutamic acid and for separating the volatile organic acids were as described by Horler, Westlake and McConnell (1966a). Amino acid utilization was followed by the ninhydrin technique. Glutaconic acid was identified by the method described by Horler, McConnell and Westlake (1966).

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Radioactivity was measured with a Scintillation Counter (Nuclear Chicago, Mark I). All reactions were buffered with potassium phosphate (pH 7.0).

RESULTS AND DISCUSSIONS

Sodium is required for the complete and rapid metabolism of both D- and L- glutamic acid, Table I.

TABLE I

INFLUENCE OF SODIUM IONS ON AMINO ACID METABOLISM BY P. AEROGENES CELLS

SUBSTRATE (6.5 mM)	% Decrease in Ninydrin Value SODIUM SULPHATE (mM) 0	6.5
Glutamic acid, L-	33	100
Glutamic acid, D-	7	92
Histidine, L-	29	100
Histidine, D-	34	100
Serine, L-	100	100
Threonine, L-	72	100

These results also indicate that D- and L- histidine metabolism requires sodium. Thin-layer chromotography of sodium-deficient histidine mixtures revealed the presence of glutamic acid. Thus the high ninhydrin values obtained under sodium deficient conditions were a result of the accumulation of glutamic acid as this amino acid was not detected when sodium ions were present. It can be concluded that sodium is not required for the metabolism of histidine *per se* by *P. aerogenes*. The accumulation of glutamic acid is consistant with our observation that this organism ferments histidine by way of the urocanic acid pathway. (McConnell, Horler and Westlake, 1967). The utilization of the L- isomer of serine and threonine is not dependent upon the presence of sodium ions although the rate of utilization of threonine was slightly faster when sodium was present. The D-isomers of these hydroxy-amino acids were not metabolized under the conditions used in these experiments. The stimulatory effect of sodium on the fermentation of glutamic acid was not replaced by ammonium, potassium, calcium or magnesium cations.

The influence of sodium ions on the distribution of radioactivity in

the steam-volatile acids, produced from the fermentation of specifically labelled glutamic acids, is shown in Table II.

TABLE II

INFLUENCE OF SODIUM IONS ON C^{14} -DISTRIBUTION IN STEAM VOLATILE PRODUCTS

GLUTAMIC ACID -C^{14} (12.5 mM)	% C^{14} Recovered in Steam Volatiles SODIUM SULPHATE (mM)	
	0	6.5
1	0.1	73
2	7.7	67
3,4	4.6	74
5	8.2	0.3

This data substantiates our observation that sodium is required for the complete and rapid utilization of glutamic acid by P. aerogenes.

The supernatants from radioactive experiments were fractionated into steam-volatile fraction (acetic and butyric acids), an ether-soluble non-volatile fraction (glutaconic acid) and an aqueous residue (glutamic acid), Table III. The distribution of C^{14} in the fractions when glutamic acid-1-C^{14} was fermented in the presence of sodium ions is consistent with our previous observations. In the absence of added sodium, the bulk of the tracer supplied is present as glutamic acid in the aqueous residue. Similar results were obtained with glutamic acid 5-C^{14}. The increase in radioactivity found in the ether-soluble fraction when fermentations were carried out in the absence of sodium suggests that sodium is involved in the metabolism of glutaconic acid Figure 1.

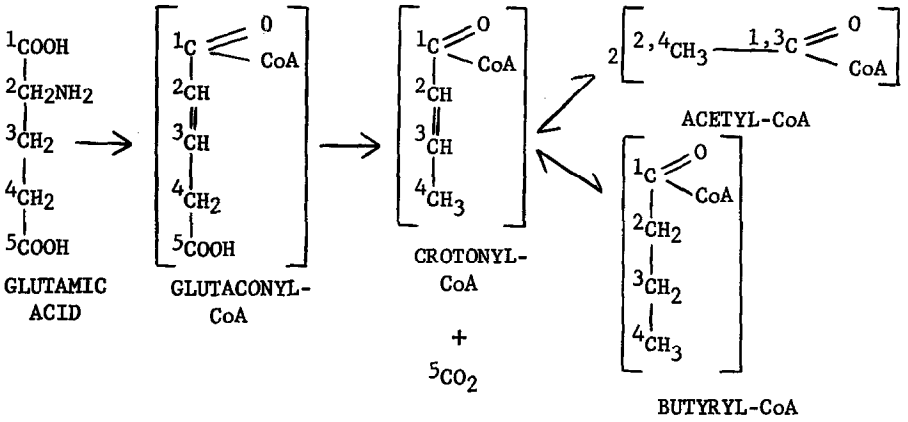
TABLE III

SODIUM AND THE METABOLISM OF CARBONS 1 AND 5 OF GLUTAMIC ACID

Fraction	Glutamic -1-C^{14} Na_2SO_4(mM)		Glutamic -5-C^{14} Na_2SO_4 (mM)	
	0	6.5	0	6.5
	mμc C^{14}	mμc C^{14}	mμc C^{14}	mμc C^{14}
Reaction Supernatant	312	398	308	55
Steam-Volatile acids	40	299	1	19
Ether-Soluble, non-volatile	30	3	43	10
Aqueous residue	250	31	222	33
Per Cent of C^{14} Recovered	84	86	87	113

FIGURE 1

HYPOTHETICAL PATHWAY FOR THE FERMENTATION OF GLUTAMIC ACID BY P. AEROGENES



The data suggesting the involvement of glutaconic acid, as the coenzyme-A derivative, in the conversion of glutamic acid to steam volatile acids have been reported previously (Horler, McConnell and Westlake, 1966a). The increased yields of glutaconic acid reported here in the absence of sodium ions lends further support to this hypothesis.

Table IV summarizes the results of five experiments in which the incorporations of carbons 1 and 5 of glutamic acid into acetic and butyric

TABLE IV

THE EFFECT OF SODIUM ON THE SPECIFIC ACTIVITY OF BUTYRIC AND ACETIC ACIDS

Tracer	Product	Relative Specific Activity* No Additions	Na_2SO_4(6.5mM)
Glutamic acid-1-C^{14}	Butyric acid	29 ± 7	100
Glutamic acid-5-C^{14}	Butyric acid	0.2 ± 0.1	3 ± 3
Glutamic acid-1-C^{14}	Acetic acid	23 ± 7	7.0 ± 5
Glutamic acid-5-C^{14}	Acetic acid	1 ± 1	6 ± 2

acids were measured both in the presence and absence of sodium. Relative values are reported as the data is a composite of experiments done with tracers of different specific activities and at slightly different concentrations. Although considerable variation was observed, the major effect of sodium deficiency was to reduce the specific activity of both acetic and butyric acids. The data

in Table II indicated a significant incorporation of carbon five of glutamic acids into volatile acids. This suggested that acetic and butyric acids might be formed by different pathways in the presence and absence of sodium and the results presented in Table IV support this view. If two or more pathways are involved, it may be that the relative contribution of each is affected by the concentration of sodium present in reaction mixtures.

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