The Breakdown and Biosynthesis of Glutamic Acid

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Glutamic acid is important in the carbon and nitrogen metabolism of micro-organisms; it participates in the structure of the folic acid coenzymes, and is a component of the bacterial cell wall. Although a number of micro-organisms, including some of the lactic acid bacteria, require glutamate as an essential amino acid, most micro-organisms synthesize glutamate from simple carbon sources.

The biosynthesis of glutamic acid has been investigated by isotopic tracer techniques, by the use of auxotrophic mutants, and by the study of the enzymes involved in the biosynthetic pathway. The isotope technique was applied to a number of micro-organisms using differentially labelled acetate $^{13}\text{CH}_3\text{.}^{14}\text{COOH}$ by Ehrensvärd and co-workers (Cutinelli *et al.* 1951*a*; Ehrensvärd *et al.* 1951; Anderson-Kottö *et al.* 1954). The glutamate was not degraded completely and unequivocally. Nevertheless, the results with the aerobic micro-organisms they examined were consistent with the incorporation of acetate into glutamate via the tricarboxylic acid cycle: that is through the successive formation of citrate, aconitate, isocitrate, oxalosuccinate and α -oxoglutarate. Wang, Christensen & Cheldelin (1953) examined the incorporation of $[1\text{-}^{14}\text{C}]$ -acetate and $[2\text{-}^{14}\text{C}]$ -pyruvate into glutamate by *Saccharomyces cerevisiae* with results consistent with the tricarboxylic acid cycle. This technique has also been applied to glutamate biosynthesis in higher plants and animals (Bilinski & McConnell, 1957; Koeppe & Hill, 1955; Black & Kleiber, 1957; Black *et al.* 1957).

Ehrensvärd and co-workers extended their studies to the anaerobic photosynthetic bacterium *Rhodospirillum rubrum* using $^{13}\text{CH}_3\text{.}^{14}\text{COOH}$ and $\text{NaH}^{14}\text{CO}_3$ (Cutinelli *et al.* 1951*b*). Again the isolated glutamate was not completely and unequivocally degraded although the results were different from those found with the aerobic bacteria. Alanine was also isolated and degraded and was found to be specifically labelled in a manner different from that found in aerobes (Table 1). Assuming the alanine to be derived directly from pyruvate, the glutamate degradation data were consistent with its formation via the following series of reactions including part of the tricarboxylic acid cycle:

The final step in the above reaction sequence could be carried out by glutamic dehydrogenase or transaminases. Hug & Werkman (1957) showed that *R. rubrum* possessed transaminases capable of catalysing the final step in the reaction sequence.

The isotope technique was extensively used in studies of amino acid biosynthesis in *Escherichia coli* by Roberts *et al.* (1955), and their successful application of the 'isotopic competition' method showed that glutamic acid, formed via the tri-

carboxylic acid cycle, was a precursor of other amino acids including proline and arginine; results which were consistent with earlier studies on the biosynthesis of these amino acids in micro-organisms (notably work with auxotrophic mutants of *Neurospora crassa*). Similarly, aspartic acid was shown to be a precursor of threonine, isoleucine, methionine and lysine. It was thus clear that the tricarboxylic acid cycle in *E. coli* was an important source of cell constituents, especially amino acids. These findings supported the earlier conclusions of Krebs, Gurin & Eggleston (1952) as a result of their studies on acetate metabolism in yeast. However, it yet remained to be established that the tricarboxylic acid cycle was the only significant source of glutamate in micro-organisms. This was demonstrated very clearly by Gilvarg & Davis (1956) with auxotrophic mutants of *E. coli* and *Aerobacter aerogenes*. These mutants were blocked at the citrate condensing enzyme and had an absolute requirement for glutamate, and were unable to oxidize acetate.

A number of bacteria and fungi accumulate α -oxoglutarate, particularly when grown with nitrogen as the growth-limiting constituent (Walker, Hall & Hopton, 1951; Hockenhull, Wilkin & Winder, 1951; Dagley, Fewster & Happold, 1952). These results suggest that α -oxoglutarate is the precursor of glutamate in these micro-organisms. α -Oxoglutarate accumulation has been found with species of *Pseudomonas* (Lockwood & Stodola, 1946; Kogut & Podoski, 1953) for which there is independent evidence for the operation of the tricarboxylic acid cycle which could give rise to α -oxoglutarate (Clarke & Meadow, 1959).

The tricarboxylic acid cycle may not be involved in α -oxoglutarate synthesis, and hence glutamate formation, in all aerobes: α -oxoglutarate can be formed from D-arabinose by a route independent of the tricarboxylic acid cycle in *Pseudomonas saccharophila* (Weimberg & Doudoroff, 1955). Some species of acetic acid bacteria, including *Acetobacter suboxydans* and *A. melanogenus*, can synthesize glutamic acid although they appear to be deficient in key enzymes of the tricarboxylic acid cycle (Rao, 1957). Glutamate biosynthesis in *A. suboxydans* has recently been investigated by Sekizawa *et al.* (1962) and a novel mechanism for its biosynthesis has been proposed. Kinetic studies with the green alga *Chlorella pyrenoidosa* (Bassham & Kirk, 1960; Smith, Bassham & Kirk, 1961) suggest that glutamate may not be synthesized via citrate. It was found that the rate of formation of citrate in an actively photosynthesizing cell suspension was considerably less than the rate of formation of glutamate.

There is now little doubt that novel mechanisms of amino acid synthesis operate in some strict anaerobes. This was first clearly demonstrated for the biosynthesis of alanine and glutamic acid in *Clostridium kluyveri* (Tomlinson, 1954*a, b*). Recent studies on rumen anaerobes, e.g. *Ruminococcus flavefaciens*, confirm novel pathways for the biosynthesis of valine and leucine (Allison, Bryant & Doetsch, 1962). The glutamate formed in *C. kluyveri* from [1- 14 C]-acetate and from NaH 14 CO $_3$ was degraded unequivocally and the isotope distribution was not consistent with any known metabolic reaction sequence (Table 1). As yet no enzyme studies have been carried out to elucidate the mechanism of glutamate biosynthesis in *C. kluyveri*. A common feature of the amino acids synthesized by the above anaerobes and by *R. rubrum* is the specific incorporation of bicarbonate into a carboxyl group.

Recent studies on the photoassimilation of acetate by *Rhodospirillum rubrum* stimulated a re-investigation of the isotope incorporation of acetate into glutamate

in this organism ((Hoare, 1962*a*, *b*). In short exposure experiments in which washed cell suspensions of *R. rubrum* were exposed in the light to [1-¹⁴C]- or [2-¹⁴C]-acetate in the presence of bicarbonate, one of the earliest detectable products of photoassimilation was found to be glutamic acid. This appeared to be formed before one could detect intermediates of the tricarboxylic acid cycle. The failure to detect the latter at the shortest exposures might have been due to their small pool size, although this seemed unlikely since they were readily detected when Na₂¹⁴CO₃ was assimilated in the light in the presence of acetate. The glutamate formed under the above conditions was degraded and the isotope distribution differed from that found in *Clostridium kluyveri* and from that found in aerobic organisms in which glutamate is formed via the tricarboxylic acid cycle (Table 1).

Table 1. Isotope incorporation into alanine and glutamic acid

	Alanine CH ₃ .CH(NH ₂).COOH			Glutamic acid HOOC.CH(NH ₂).CH ₂ CH ₂ .COOH				Reference
	m	m	b	b	m	m	c	
Aerobes*								Ehrensward (1955)
<i>Rhodospirillum rubrum</i>	m	c	b	b (m		c	m)	Cutinelli <i>et al.</i> (1951 <i>b</i>) Hoare (1962 <i>b</i>)
<i>Clostridium kluyveri</i>	m	c	b	c	m	c	m	Tomlinson (1954 <i>a</i> , <i>b</i>)

* Assuming the biosynthesis proceeds via the tricarboxylic acid cycle.

Studies of Cutinelli *et al.* (1951*b*) did not establish an unequivocal distribution of carbon atoms 2, 3 and 4 of glutamic acid.

In aerobic bacteria it is generally assumed that the breakdown of glutamic acid proceeds via α -oxoglutarate and the tricarboxylic acid cycle. The wide distribution of transaminases and of glutamic dehydrogenase is in accord with this view. A number of anaerobic bacteria degrade glutamic acid to carbon dioxide and volatile fatty acids including mainly acetate and butyrate. Conversion of glutamate to acetate via the tricarboxylic acid cycle should result in the formation of three moles of CO₂ per mole of glutamate. Several strict anaerobes, including *Clostridium tetani* (Clifton, 1942), *C. saccharobutyricum* (Cohen, Nisman & Cohen-Bazire, 1948), *C. tetanomorphum* (Woods & Clifton, 1937; Barker, 1937), *Micrococcus aerogenes* (Whiteley, 1957) and *Fusobacterium nucleatum* (Jackins & Barker, 1951), ferment glutamate with the formation of only 1 mole of CO₂. The fermentation of glutamate has been studied very thoroughly with *C. tetanomorphum*. Isotopic tracer studies showed that the CO₂ was derived exclusively from C-5 of glutamic acid, acetate mainly from C-1 and C-2 and butyrate from C-3 and C-4 (Wachsman & Barker, 1955; Wachsman, 1956). The enzyme steps involved in the degradation of glutamate by *C. tetanomorphum* have now been identified and studied in some detail. The initial enzyme step catalysing the isomerization of glutamic acid to β -methyl-aspartic acid was found to involve a coenzyme derivative of vitamin B₁₂ (Barker, Weissbach & Smyth, 1958). It is not yet known whether glutamate fermentation by *C. tetani*, *C. saccharobutyricum* and *F. nucleatum* proceeds by a similar mechanism to that in *C. tetanomorphum*.

Clearly the anaerobic bacteria merit a more extensive investigation with the possibilities of finding new pathways in the biosynthesis and degradation of amino acids. It is by no means established that the tricarboxylic acid cycle functions in strict anaerobes and, since such organisms may not depend upon it for a source of reducing power, they may prove to have evolved some alternative mechanism for providing intermediates for amino acid biosynthesis. A number of strict anaerobes, in addition to *Clostridium kluyveri* (which is the only one to be examined so far), use two carbon compounds as sole carbon sources and an examination of glutamate biosynthesis in these organisms would be of particular interest. Some of the methane bacteria and several organisms fermenting nitrogenous compounds (*Streptococcus allantoicus*, *Peptococcus glycinophilus*, *Clostridium cylindrosporum* and *Clostridium acidi-urici*) fall in this category.

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