168. *The Growth of Coliform Bacteria in Media containing Nitrate and Nitrite. Part IV. Some Biochemical Considerations.*

By P. R. Lewis.

The observations contained in Parts I—III (previous papers) are brought into relation with certain biochemical considerations.

The hypothesis put forward in Part II (this vol., p. 833) is entirely general in character, and in this form explains quite satisfactorily many of the observations recorded. Some amplification of this theory, particularly as regards the possible identity of XH_2, seems of interest in view of the large amount of biochemical research which has been carried out on glucose metabolism and on the mechanism of enzymic dehydrogenations. In this paper, therefore, there is set out very briefly a possible extension of the more general theory, though a detailed discussion of it would occupy too much space. What follows is somewhat speculative, and the rejection of the specific identifications would in no way affect the correctness of the more general theory.

A great deal of the work on the path by which glucose is metabolised has been carried out with tissue slices (of animal origin), and not with bacteria, but the methods used by most living cells bear a close similarity to one another. Thus, it is probable that glucose is converted into pyruvic acid (or to a phosphorylated derivative), which can be utilised by (i) reduction to lactic acid, (ii) decarboxylation to acetaldehyde which can then give products such as ethyl alcohol, (iii) combination with carbon dioxide to oxalacetic acid, which can then undergo a series of reversible reactions giving successively malic acid, fumaric acid, and succinic acid (Table I).

There is considerable evidence for the view that in muscle there exists a complete reaction cycle whereby pyruvic acid can be degraded to carbon dioxide and water (provided oxygen is present to take up the hydrogen made available). This cycle is known as Krebs's cycle or as the " four-carbon acid cycle " (abbreviated to C_4 acid cycle), and a possible form of it is given in Table II. The existence of this cycle in bacteria has not been proved and has actually been denied. But some such system for the conversion of glucose into carbon dioxide and water must exist and probably does not differ in essentials from that given in Table II.

TABLE I.

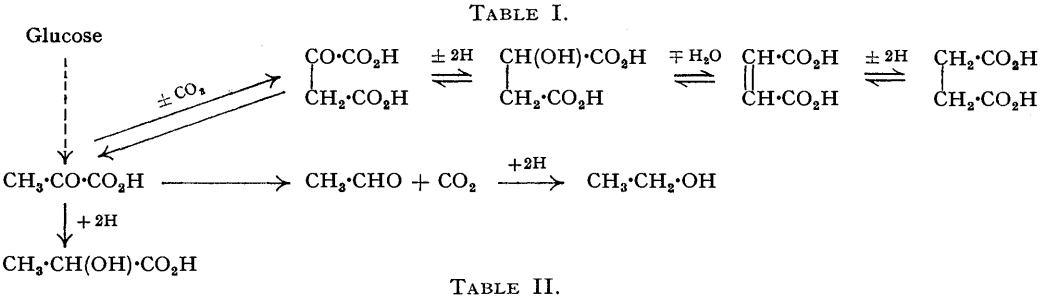
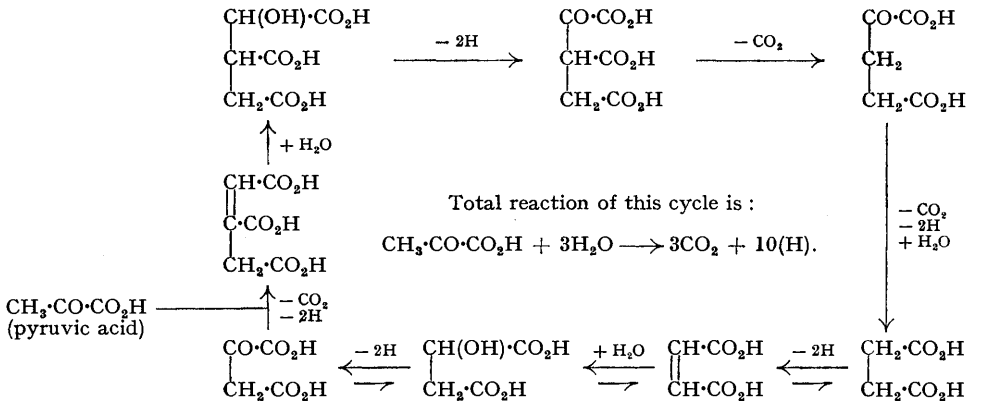


TABLE II.

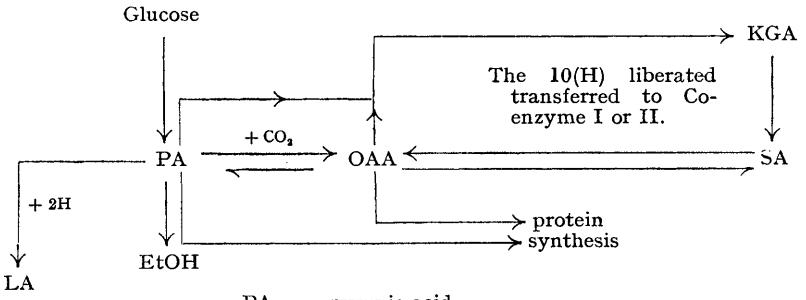


The primary reactions involved in the utilisation of ammonia by bacteria have not been definitely discovered, but several have been postulated and involve combination of ammonia or hydroxylamine with either pyruvic, fumaric, or oxalacetic acid; these three acids are in enzymic equilibrium with each other, and, for the present purpose, oxalacetic acid can be regarded as the acid needed for the utilisation of the nitrogen source. There is considerable evidence for the view that keto-acids are needed for the formation of the corresponding amino-acid residues; both alanine and aspartic acid are present in large quantities in bacterial protein, so that, during growth, large amounts of the corresponding keto-acids, pyruvic and oxalacetic, will be removed at a rate approximately proportional to the growth rate. If all the various reactions given above are brought together in one scheme as is done in Table III (in abbreviated form) it is obvious that this scheme bears a very close resemblance to the more general one put forward in Part II.

The mechanism of dehydrogenations is now fairly well known. The oxidisable substrate is adsorbed on the enzyme surface and the hydrogen is transferred to an appropriate co-enzyme. These co-enzymes are easily reducible compounds which act as carriers of hydrogen between the various enzymes. The two commonest carriers are the closely related co-enzymes I and II, and either one or other of these is involved in most, if not all, of the reactions represented in Table III. The fact that there are two carriers involved complicates the picture somewhat, but it is justifiable for the purposes of a kinetic discussion to assume that only one carrier is concerned, in which case all the available hydrogen released in the oxidations of the C_4-acid cycle goes into one " hydrogen pool " from which it is then passed on to molecular oxygen via a series of other hydrogen carriers (flavo-proteins and cytochromes). The substances X and XH_2 introduced in the general scheme may therefore well be the oxidised and reduced forms of the co-enzyme. X is converted into XH_2 by the various reactions of the glycolysis cycle, and X is re-formed by the handing on of the available hydrogen either to molecular oxygen or to some other reducible substance such as nitrate or nitrite. Obviously, if oxygen is able to take up all the available hydrogen formed, the ratio XH_2/X will be kept very low, and the rates of nitrate and nitrite reductions will be very small. If oxygen is not able to remove all the hydrogen, the ratio XH_2/X will rise until the rates of other reductions are high enough to maintain hydrogen balance.

Thus, from a synthesis of kinetic and biochemical data, we arrive at the scheme given in Table III, which provides a suggestive picture of part of the internal working of bacteria. The

TABLE III.



PA = pyruvic acid.
OAA = oxalacetic acid.
KGA = α-ketoglutaric acid.
SA = succinic acid.
LA = lactic acid.

idea of a " hydrogen pool " explains very neatly the effects of æration on the rate of nitrate and nitrite reduction and also the fact that, in the absence of oxygen, lactic acid and ethyl alcohol are formed in much larger quantities. When ammonia is added to a growing nitrate culture, the growth rate increases, oxalacetic and pyruvic acids therefore disappear more rapidly leaving less to be metabolised by the C_4-acid cycle, less hydrogen becomes available, and, if it becomes less than can be taken up by the oxygen, reduction of nitrate will cease.

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