XCVI. ON A POSSIBLE RÔLE OF PYRUVIC ACID IN BACTERIAL GROWTH¹.

By JUDA HIRSCH QUASTEL (1851 *Senior Exhibitioner*)

From the Biochemical Laboratory, Cambridge.

(Received June 19th, 1925.)

It is interesting to consider the various ways in which the constitution of a synthetic nutrient medium may be changed without appreciably affecting its nutritive character for any particular species of bacterium. It is well known that there are numerous compounds, any one of which, when supplied as the sole source of carbon, will support bacterial growth. A consideration of the various media upon which a particular bacterium will thrive makes it clear that the ability of the organism to grow is in some way connected with the chemical constitution of the substrate. Not all carbon compounds will supply carbon to an organism for growth, and it seems logical to suspect the existence of some relationship between molecules possessing a nutritive character.

If it is assumed from the outset that related to each nutritive molecule there exists a set of *specific* enzymes which bring about various syntheses culminating in the production of protoplasmic material whose nature remains fundamentally the same whatever the nature of the nutrient molecule, it is apparent that an investigation which seeks to determine a relationship between nutrient molecules would be pointless. This assumption, however, is merely a restatement of the fact that nutrition occurs, and since it throws no light on the connection between nutritive character and chemical constitution, and moreover postulates the existence of a great number of specific enzymes for which there is no clear evidence, it cannot be of use in predicting the nutrient character of any given molecule or in interpreting any of the steps by which nutrition occurs. It may be discarded, therefore, in favour of some suggestion which possesses, even in a slight degree, this power of prediction and of interpretation.

Experiment, so far, has failed to demonstrate any fundamental transformation in an organism (say *B. coli* or *B. pyocyaneus*), which is caused by an alteration in the constitution of the nutritive medium. Variations in the strains may occur, but the lack of any fundamental change suggests the presence in any particular organism of an orderly series of reactions which express themselves in growth, multiplication and the general properties which characterise this

¹ The views expressed in this communication are contained in a dissertation which was laid before the authorities of Trinity College, Cambridge, in August, 1924.

particular organism. There may be secondary reactions which account for variations in the strains, but the important point is the presence of a definite series of reactions which is characteristic for each bacterial species. If this view is right it is difficult to perceive how two nutrient molecules of widely differing chemical nature (say glutaminic acid and acetic acid) can each support bacterial growth and yet each produce the identical series of reactions which is characteristic of the organism. There appears to be but one explanation—that each nutrient molecule gives rise to a particular substance (which will be termed *P*) which is the first organic material to enter into the synthetic operations of the organism. Thus *P* may be regarded as the initial or primary substrate of the organism, and all other compounds must proceed through *P* before entering into protoplasmic synthesis. It may be that for any organism there exists a number of substances any one of which will act as *P*; but the suggestion which the writer wishes to offer is that this number is strictly limited and may be in many cases simply one. This suggestion does not mean that *P* cannot be the same for any two organisms, for it is clear that subsequent operations on *P* must depend on the nature and variety of enzymes present in the cell, and hence the material into which *P* is built will be characteristic not only of *P* but of these enzymes. It is possible therefore that *P* may be the same for a large number of organisms.

Only *B. coli* has been studied at all systematically from this point of view, and the evidence points to pyruvic acid (or some very closely allied substance) being the first organic molecule to enter into protoplasmic synthesis. The work of Neuberger and his colleagues gives great prominence to the part played by pyruvic acid in fermentation phenomena and the work of de Graaff and Le Fèvre [1925] supports the view of Neuberger as to the importance of this acid as an intermediate substance during bacterial fermentation. Aubel [1924] has shown that pyruvic acid is an excellent nutrient material for bacteria, and very recently Aubel and Salabartan [1925] have come to the conclusion (from the standpoint of energetics) that in the growth of *B. coli* upon glucose pyruvic acid serves as the source of the bacterial substance. In the work of Quastel, Stephenson and Whetham [1925] on the growth of *B. coli* upon lactate and nitrate, it was shown how the production of nitrite at a toxic concentration afforded an explanation of the fact that much pyruvic acid did not appear in the medium until the nitrite had reached this concentration. "Below this strength of nitrite the pyruvic acid produced from the lactate is almost entirely used for growth. As soon, however, as the nitrite reaches its toxic concentration and prevents further bacterial synthesis the pyruvic acid, which is still being produced from the lactic acid by the action of nitrate and the resting organism, is diverted from synthesis and is liberated from the medium."

The following conclusions follow from the work of Quastel, Stephenson and Whetham [1925] and from the suggestion put forward in this communication.

1. That a molecule which is activated by *B. coli*, which can give rise to pyruvic acid and which in its reactions (oxidation, etc.) can liberate energy, may serve as a source of nutrient carbon to *B. coli*. The activation is demon-

strated by the resting bacteria—methylene blue method (see Quastel and Whetham [1925] for details).

2. That a molecule which is not activated by *B. coli* but which can give rise to pyruvic acid cannot serve as a nutrient carbon supply, *e.g.* maleic acid.

3. That a molecule which is activated by *B. coli* but which cannot give rise to pyruvic acid cannot serve as a nutrient carbon supply, *e.g.* formic acid.

The following chart illustrates how various substances can give rise by known reactions to pyruvic acid. All these substances should, if they are activated, serve as nutrient supplies of carbon to *B. coli*.

For experiment a number of substances were investigated and the results are expressed in Table I.

Table I.

Substance	I	II	III	IV
Formic acid	+	0	0	0
Acetic acid	+	+	+	+
Propionic acid	+	0	0	0
Butyric acid	+	0	0	0
Lactic acid	+	+	+	+
α-Hydroxybutyric acid	+	0	0	0
Mandelic acid	+	0	0	0
β-Hydroxybutyric acid	+	0	0	0
Oxalic acid	0	0	0	0
Malonic acid	Slight	0	0	0
Succinic acid	+	+	+	+
Glutaric acid	Slight	0	0	0
Glycol	+	0	0	0
Glycerol	+	+	+	+
Erythritol	0	0	0	0
Fumaric acid	+	+	+	+
Maleic acid	0	+	0	0
Glycine	Slight	0	0	0
Alanine	+	+	+	+
Aspartic acid	+	+	+	+
Glutaminic acid	+	+	+	+
Malic acid	+	+	+	+
Tartaric acid	+	+	+	+
Glyceric acid	+	+	+	+
Glycollic acid	+	+	(+)	(+)

In column I, + shows that the substance is activated by *B. coli*, 0 indicating the absence of activation.

In column II, + shows that the substance may undergo change to pyruvic by reactions of which we are aware (*e.g.* oxidation, dehydration); 0 indicates that it is unlikely that a change to pyruvic acid occurs. In the cases of acetic acid and glycollic acid (which have both been shown by Quastel and Whetham [1925] to be hydrogen donators) it is assumed that the first products of oxidation are succinic and tartaric acids respectively. It is conceivable that propionic acid may undergo a change, similar to that in the body, to glucose or lactic acid, and thence to pyruvic acid, but since there is as yet no direct evidence for this with *B. coli* this assumption will not be made. (β -Hydroxybutyric acid may also be expected to be oxidised through acetoacetic to acetic acid and thence to pyruvic acid, but as yet there is no evidence for this.)

In column III a + sign indicates that the growth of *B. coli* on this substance, present as sole source of carbon under aerobic conditions, is expected; a zero sign has its usual significance. The sign (+) indicates only slight growth.

Column IV gives the experimental results.

For experiment the substance was made up to 0.1 *M* concentration in a solution containing the following:

0.4 % $(\text{NH}_4)_2\text{HPO}_4$; 0.1 % NaCl; 0.1 % KH_2PO_4 ; 0.07 % $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$;
0.5 % NaNO_3 .

The media after neutralising to p_{H} 7.2 were autoclaved in 5 cc. lots and inoculated with two drops of a 20-hour broth culture of *B. coli*. A slight growth generally occurred on the inorganic medium alone, this being due to the growth on the organic matter introduced in the actual inoculation. This growth was taken as a standard zero growth for the comparison of the growths of *B. coli* upon the various media. Growths were examined after 48 hours' incubation at 37°.

In no case so far examined has the experimental result contradicted the one predicted.

It should be added that pyruvic acid may not be the *only* initial or primary substrate; experiment, however, so far does not seem to indicate the existence of another substance which will act in this capacity.

REFERENCES.

- Aubel (1924). *Bull. Soc. Chim. Biol.* **6**, 288.
Aubel and Salabartan (1925). *Compt. Rend. Acad. Sci.* **180**, 1784.
De Graaff and Le Fèvre (1925). *Biochem. Z.* **155**, 313.
Quastel, Stephenson and Whetham (1925). *Biochem. J.* **19**, 304.
Quastel and Whetham (1925). *Biochem. J.* **19**, 520.