XCIX. THE RESPIRATION OF *B. COLI COMMUNIS.*

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Cook, Haldane and Mapson [1931] investigated the oxygen-uptake of toluene-treated *B. coli* in presence of succinate, lactate, and formate. The toluene treatment restricts the oxidation of the first two substrates to a single stage, and thus renders the interpretation of the results easy. But it might be objected that the bacteria had been damaged by the toluene. For example the conclusion was drawn that the oxygenases concerned in the oxidation of the above substrates differed in their sensitivities to CO and HCN. This might conceivably have been due to the fact that, for reasons depending on cell structure, some were more accessible than others to toluene, and therefore more altered by it. We have therefore investigated the oxidation of several substances by the intact organism, besides making a few more observations in presence of toluene. The technique used was the same as that of Cook, Haldane and Mapson. Our N.T.C. strain of bacteria was used in $M/15$ phosphate buffer[1] at p_H 6·3 for formate oxidation, 7·6 for other substrates. All substrates were present in $M/60$ concentration, which is sufficient to give a maximum rate of oxidation in each case. Barcroft manometers were used throughout, and all experiments were carried out at 16°. When substrates were added, a bacterial suspension without substrate was placed in the left-hand cup. All experiments were done in duplicate.

Kinetics of oxygen-uptake.

In the absence of added substrate the rate of oxygen-uptake usually fell off during the first hour, and then became constant. (Fig. 1.)

In the presence of formate the curves obtained were very similar to those found in presence of toluene (Fig. 2). After 30 minutes the rate of oxygen-uptake became constant. If the experiments were continued long enough it fell off, but never increased. But in presence of glucose, succinate, and lactate, the curves obtained were concave upwards, *i.e.* the rate of oxygen-uptake increased with time, and ultimately became steady (Figs. 3, 4, 5). The reason for this is quite clear. The lactate, to take one example, is oxidised to pyruvate. This is not further oxidised in presence of toluene, so that the rate of oxygen

[1] The concentration of phosphate was incorrectly given in our former paper. 1·0 cc. of 0·2 *M* buffer solution was present in 3·0 cc. of mixture.

consumption does not rise. But the normal organism oxidises it. However, the amount of pyruvate produced in the first half hour or so at 16° is not nearly sufficient to saturate the catalysts concerned in its oxidation, so it, and prob-

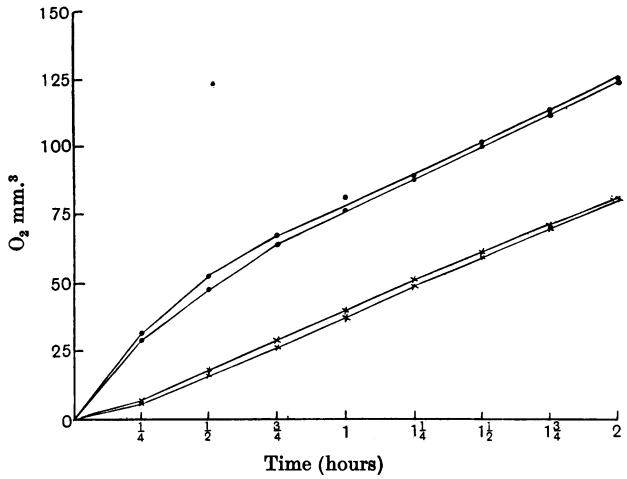

Fig. 1. O_2 consumption of washed *B. coli* (2·86 mg. N) in presence of air (upper curves) and a mixture containing 9 volumes of CO to 1 volume of O_2 (lower curves). p_H 7·6, 16°.

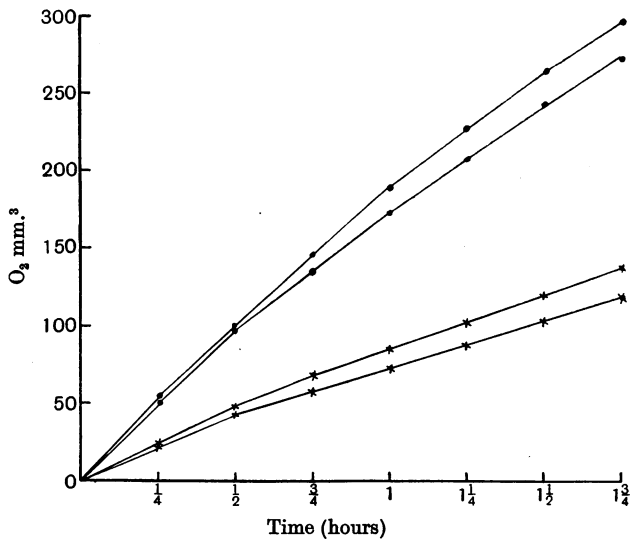

Fig. 2. O_2 consumption of *B. coli* (0·36 mg. N) + formate in presence of air (upper curves) and a mixture containing 3·88 volumes of CO to 1 volume of O_2 (lower curves). p_H 6·3, 16°.

ably further products of oxidation, continue to accumulate until, after an hour or so, a steady state is reached. At 40° the final steady state is reached in the first half hour or less, and no initial slow period is obvious.

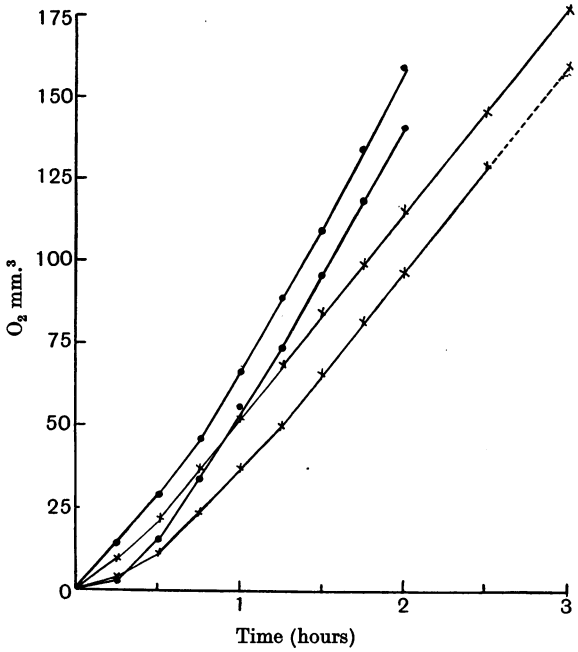

Fig. 3. O_2 consumption of *B. coli* (1·43 mg. N) + glucose in presence of air (upper curves) and a mixture containing 8·83 volumes of CO to 1 volume of O_2 (lower curves). p_H 7·6, 16°. Over the period represented by the dotted line the cup was strongly illuminated.

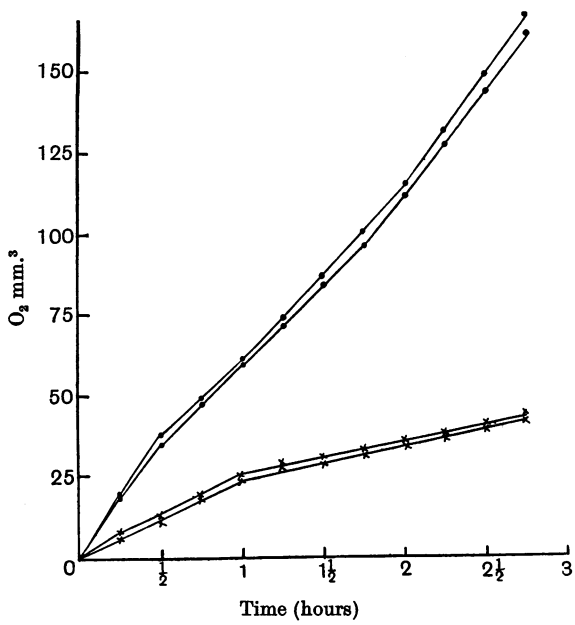

Fig. 4. O_2 consumption of *B. coli* (0·72 mg. N) + succinate in the presence of air (upper curves) and a mixture containing 9 volumes of CO to 1 volume of O_2 (lower curves). p_H 7·6, 16°.

The following calculation may make the matter clearer. Consider a substrate oxidised with constant velocity A, giving rise to a product in concentration x, and oxidised according to Henri's equation with velocity $\frac{Bx}{x+K}$. Then if v be the total velocity,

$$v = A + \frac{Bx}{x+K}, \text{ and } \frac{dx}{dt} = A - \frac{Bx}{x+K},$$

while $x = 0$ when $t = 0$. Eliminating x, and integrating,

$$\frac{(A-B)^2 t}{BK} = \frac{(A-B)(v-A)}{B(A+B-v)} + \log_e \frac{A(A+B-v)}{B(2A-v)}.$$

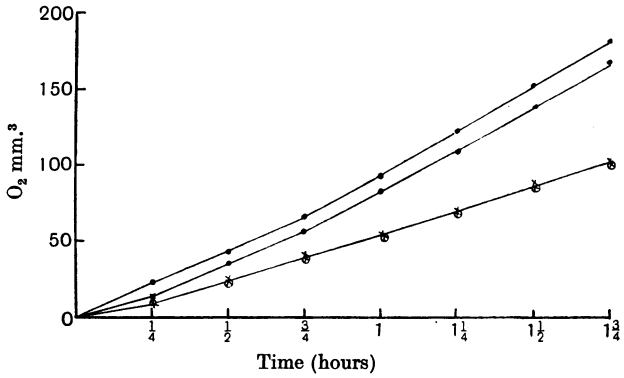

Fig. 5. O_2 consumption of *B. coli* (0·72 mg. N) + lactate in presence of air (upper curves) and a mixture containing 9 volumes of CO to 1 volume of O_2. p_H 7·6, 16°.

Hence if $A > B$, the final value of B is $A + B$, the concentration x of the intermediate increasing indefinitely. If $B > A$, the final value of v is $2A$, the final value of x being $\frac{AK}{B-A}$. In the cases here treated it appears that $A > B$. A similar calculation can be made if there are more than two steps in the oxidation.

We find that in the oxidation of glucose and lactate it is impossible to measure the initial velocity A with any great accuracy. Results are generally irregular in the first half hour, and the velocity is already increasing at the end of an hour. On the other hand with succinate no increase occurs until nearly two hours have elapsed. This is explained by the fact that fumarate, the immediate product of oxidation, must be hydrated to malate by fumarase before it can be further oxidised. There must be an accumulation, first of fumarate, then of malate. Only when enough of the latter has been formed will the rate of oxygen-uptake increase appreciably. In each case the final rate of oxidation exceeds the initial by 30–40 %.

Comparative rates of oxidation of different substrates.

Table I gives the final rates reached after two hours by the organism when washed free of substrate, and in presence of the four substrates employed. The results are expressed in mm.^{3} O_2 (reduced to 0° and 760 mm.) per mg.

Table I.

Substrate	None	Glucose	Succinate	Lactate	Formate
	16 (p_H 7·6)	72	95	158	472
	20 (p_H 6·3)	69	116	140	486
				131	
Average	18	71	106	143	479
Q_{O_2}	−217	−855	−1277	−1723	−5771

bacterial N per hour, and refer to a culture which had been kept for some weeks at 0° after washing. As these bacteria contain about 8·3 % of their dry weight of N, Warburg's Q_{O_2} is obtained on dividing by − 0·083. It will be seen that these values are very much larger than those found with yeast or mammalian tissues, where Q_{O_2} values of − 60 and − 20 respectively are normal. The contrast would have been still greater had our experiments, like most on yeast and tissues, been performed at 25° or 37°, instead of 16°. With fresh bacteria the rates of glucose and lactate oxidation are about the same. Thus in two experiments, one three days after the other, with a fresh suspension, we found O_2-uptakes per hour per mg. N of 162 and 124 mm.^{3} in presence of glucose, 170 and 126 in presence of lactate. It would seem probable that the enzymes concerned in the breakdown of glucose to more readily oxidised substances are less stable than the oxidising enzymes.

Some light is thrown on the nature of these products by experiments on fresh suspensions where glucose and lactate were oxidised alone and in mixture. The rates of O_2 consumption in mm.^{3} were:

Glucose	Lactate	Sum of rates	Glucose-lactate mixture
172·5	180·6	353·1	272·9
132·0	134·4	266·4	195·2

It will be seen that the oxygen consumption in presence of the mixture was 77 % and 73 % respectively of the sum of the rates in presence of glucose and lactate alone. On the other hand Quastel and Wooldridge [1927] found that the rate of methylene blue reduction by a glucose-lactate mixture was, if anything, slower than by lactate alone, which, as in our case, was a speedier reducer than glucose. The most obvious interpretation of our results is that some, but not all, of the glucose is transformed into lactic acid before oxidation. As the lactic dehydrogenase is already saturated with its substrate, the transformation of glucose into lactic acid does not increase the oxidation rate of the mixture. But about half the glucose appears to be converted into another substance (perhaps methylglyoxal) or substances, which are oxidised by different enzymes. This fraction cannot be calculated exactly, because substances may unite with enzymes which cannot activate them, thus inhibiting the oxidation of the proper substrate. It is conceivable that glucose may slow down the action of lactic dehydrogenase in this way. A comparison of our results with those of Quastel and Wooldridge suggests that the intermediary substances other than lactic acid reduce oxygen more readily than methylene blue in presence of *B. coli*.

Oxidations in presence of CO.

The oxygen-uptake of the starving organism during its first hour at 16° is considerably inhibited by the presence of 9 volumes of CO per volume of O_2 (Fig. 1). But the subsequent slow oxygen-uptake is hardly affected even when the ratio is raised to 15·2. Actually the duplicate uptakes in an hour without CO were 62·6 and 61·7 mm.^{3}, and with CO 57·5 and 57·6, giving a value of 190 for K, the apparent ratio of the affinities for CO and O_2. This agrees with Warburg's [1928] results with starved yeast, whose O_2 consumption is unaffected by CO.

Cook, Haldane and Mapson found that the toluene-treated bacteria gave very different values for K (the ratio of CO to O_2 giving 50 % inhibition) according to the substrate oxidised. Comparable results have been obtained with the untreated organism and are given in Table II.

Table II.

Substrate	K (toluene)	K (no toluene)
Glucose	—	19·9, 27·6
Succinate	6·3	5·8, 6·0
Lactate	9·7	9·5, 11·2, 13·7
Formate	2·3	2·4, 3·3

The method of calculation was the same as that given by Cook, Haldane and Mapson. With the exception of the value of 11·2 for lactate, all the above figures have a considerable probable error owing to inconstancy of oxidation rates, and occasionally to disagreement of duplicates. Little difference was found between the values during the initial phase of oxidation and the final, more rapid, phase. Thus the figure 19·9 for glucose is the mean of 19·0, obtained from the readings between 30 and 60 minutes from the beginning of the experiment, and 20·8, obtained from the readings between 90 and 210 minutes, over which period the rate of oxygen-uptake was nearly constant. It is clear that the values of K are, within the limits of experimental error, unaffected by toluene treatment. The oxidation of glucose is clearly less sensitive to CO than that of the other substrates, the small inhibitions obtained rendering an exact evaluation of K impossible. The slight inhibition is explained if the glucose is largely converted into lactic acid before oxidation. As the system is not saturated with lactate, the effect of CO is lessened, for, as Warburg points out, it is no longer the limiting factor.

Just as in the case of the toluene-treated organism, the inhibition by CO was practically unaffected by illumination with a fairly powerful lamp.

Oxidations in presence of phenylurethane.

When the 3 cc. of suspension included 0·5 cc. of water saturated with phenylurethane, we obtained, just as with the toluene-treated organism, only a slight inhibition. This amounted to 19 % in the case of glucose. When however CO was also present, its effect was greatly augmented. The value of K for

formate was lowered from 2·9 to 0·56. In the case of the toluene-treated organism it fell from 2·3 to 0·21. The effect without toluene was therefore comparable, though less marked.

Oxidations in presence of cyanide and other inhibitors.

The widely different sensitivity to cyanide of lactate and formate oxidation found in presence of toluene is equally characteristic of the normal bacteria. In 0·001 *M* KCN the rate of oxidation of formate was reduced to 4·6 % of the normal, that of lactate to 44 %. The corresponding figures in presence of toluene are 1·6 % and 39 %. In each case the figures for formate are a little uncertain, owing to the very slow rates, which could hardly be measured exactly. The great difference in susceptibility of the two oxygenases is evident.

In view of the fact that 2-aminophenol-4-sulphonic acid forms a complex with iron, and has been used by Krah [1930] to inhibit biological reactions believed to be catalysed by that element, it was thought possible that it might inhibit oxygenase activity. However 0·5 % of this reagent only reduced the rate of oxidation of formate by 12 %, which may not have been a specific effect. This does not of course disprove the presence of iron in oxygenase, but suggests that it is firmly bound there.

It was shown by Cook, Haldane, and Mapson that methylene blue could replace oxygenase as an intermediary between the dehydrogenases of the

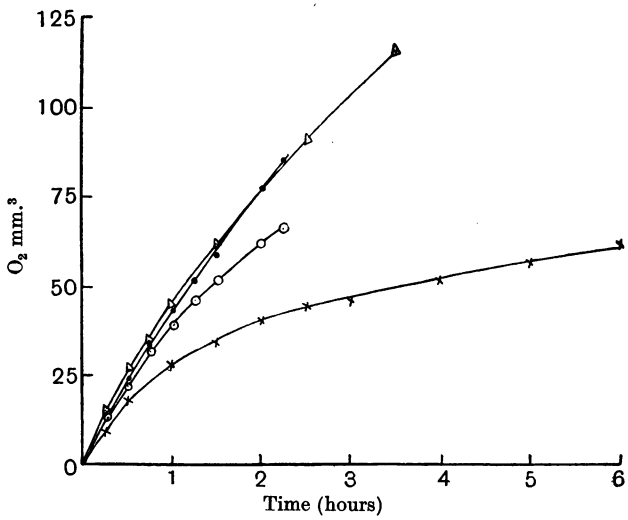

Fig. 6. O_2 consumption of *B. coli* (about 0·7 mg. N) + lactate, alone, and in mixtures containing cyanide, methylene blue and 8-hydroxyquinolinesulphonic acid (Q). p_H 7·6, 16°. Duplicate experiments agreed, but are omitted.

•—•—• Lactate control. △—△—△ Lactate + *M*/1000 KCN + *M*/750 M.b.
⊙—⊙—⊙ Lactate + 1/250 Q. ×—×—× Lactate + Q + M.b. + KCN.

toluene-treated organism and oxygen, when the oxygenase had been inhibited by KCN. The autoxidation of the methylene blue, which is catalysed by a

metal, can then be prevented by CO. We find that 8-hydroxyquinoline-sulphonic acid has a similar effect. Fig. 6 shows that this substance has little effect on the oxidation of lactate. But when, in presence of cyanide, oxygenase is replaced by methylene blue, the re-oxidation of leucomethylene blue, and hence the oxygen-uptake, is greatly slowed down by 8-hydroxyquinoline-sulphonic acid, the blue colour fading considerably. In the experiment here recorded the rate fell to 14 % of that in presence of methylene blue + cyanide. 8-Hydroxyquinolinesulphonic acid unites with copper, manganese, and nickel, but not iron. Hence our experiment confirms the view of Reid [1930] that the re-oxidation of leucomethylene blue is due to copper catalysis. It may also be regarded as a "model" illustrating the experiments of Hecht and Eichholtz [1929] in which tumour glycolysis, on the ground of its inhibition by various substances, was shown probably to include a catalysis by copper.

SUMMARY.

1. When *B. coli* oxidises glucose, succinate, and lactate (but not formate) at 16° the velocity of oxidation does not reach a maximum till more than an hour has elapsed, owing to the gradual production of further metabolites.

2. The rates of oxidation, especially of formate, are much greater than those caused by yeast or mammalian tissues.

3. The effects of CO and HCN on these oxidations differ greatly, as with the toluene-treated organism, thus confirming the view that at least three different oxygenases are present in the cell.

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