LXVIII. THE EFFECT OF OXYGEN SUPPLY ON THE METABOLISM OF *BACILLUS* *COLI COMMUNIS*.

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IN work already published [Stephenson and Whetham, 1923], the respiratory exchange of a growing culture of the Timothy Grass Bacillus was studied, firstly during a period of growth on glucose and secondly during a period of starvation after the glucose had been used up. This organism is characteristically non-fermenting; that is, when grown on a synthetic medium with glucose as the sole source of carbon, and ammonia as the source of nitrogen, no intermediate products collect in the medium in any measurable quantity. Moreover, the carbon of the glucose can all be accounted for in the carbon dioxide given off by the organism, on the one hand, and in the carbon of the organism itself, on the other, with the exception of small traces of carbonaceous material in the medium, which are attributable to the autolytic products of dead bacteria.

Comparison with a fermenting organism seemed desirable, *i.e.* one which breaks down carbohydrates and other compounds in such a way that intermediate products collect in the medium. As an example of such a type of organism *Bacillus coli communis* was chosen.

The apparatus used consisted of four sections, *A*, a respiration flask, kept in a water-bath at 37°, *B* and *C*, sulphuric acid and pumice absorption tubes, *D*, potash bulbs. Dry air, free from carbon dioxide, was passed continuously through the apparatus, which was cooled and weighed at intervals. The total gain in weight over any period gave the oxygen uptake, while the gain in weight of *C* and *D* gave the carbon dioxide output. The technique is fully described in our previous communication [1923]. The application of this method to the study of a fermenting organism presents a difficulty in the possible carrying over of volatile products from the respiration flask to the absorption tubes or beyond. The products of the action of *B. coli* on glucose have been examined by a number of investigators. Grey [1914, 1917] in particular has made a careful quantitative study and has shown the presence

of lactic, succinic, acetic and formic acids, ethyl alcohol, and hydrogen. Blank experiments were accordingly made by us with formic and acetic acids and with alcohol, in concentrations exceeding anything found by Grey. No measurable amounts of these substances were carried over.

The question of hydrogen, however, remained unsettled. It will be apparent that any hydrogen produced in the respiration flask would be carried out through the absorption system and be lost, causing a loss of weight which would invalidate the figures for oxygen uptake. From Grey's results we learn that hydrogen is produced from glucose by *B. coli* in quantities not exceeding 0.5 % of the weight of glucose fermented. The total amount of glucose used by us in each experiment was 0.5 g., and any loss from hydrogen would thus probably not exceed 0.0025 g., an amount lying within our experimental error (± 0.004 g. on each weighing of the whole system). Nevertheless it appeared prudent to investigate this point. The escaping gases were passed over a red-hot platinum spiral and then through a calcium chloride absorption tube, the hydrogen being thus estimated by weight as water. The apparatus was blanked with known quantities of hydrogen and found satisfactory. In one experiment, 0.0145 g. water was produced, corresponding to 0.0016 g. of hydrogen, from 0.5 g. of glucose. Since such an amount falls within the experimental error and therefore does not affect the figures for oxygen uptake, any production of hydrogen in our experiments was neglected.

An inorganic medium of the following composition was used:

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

the hydrogen ion concentration being about p_{H} 7.6. 50 cc. of medium were introduced into the sterile respiration flask, together with 0.5 g. of the carbon compound under investigation. Organic acids were added in the form of the ammonium salt. The medium was sown with a loopful of a 24 hour broth culture of *B. coli*. Three strains of *B. coli* were used in the course of the experiments, and were found to give similar results. Subcultures were made and examined after each experiment to ensure that the culture had remained free from contamination.

EXPERIMENTS ON GLUCOSE.

Fig. 1 shows the oxygen uptake and carbon dioxide output of *B. coli* on 1 % glucose inorganic medium, bubbled continuously with air. At the end of 24 hours, though extensive growth had occurred and the medium had already become acid, no oxygen had been taken up and the carbon dioxide output was very low. Subsequently a small oxygen uptake is recorded. Fig. 2 shows the results of an identical experiment made at a different time with a different strain of *B. coli*. It will be recalled that *B. coli* is a facultative anaerobe. It would seem that in the early stages of glucose breakdown, *B. coli* behaves as an anaerobe even in the presence of air.

In an atmosphere of oxygen, on the other hand, the type of metabolism of the organism is changed. Fig. 3 shows the greatly increased oxygen uptake and carbon dioxide output of *B. coli* on a glucose medium bubbled with oxygen instead of with air. The oxygen was displaced by air before each weighing to avoid the errors consequent on the difference of density between air and oxygen. If this precaution is omitted, an error amounting to 0.03 g. may be introduced. Blank experiments prove that at least two litres of air must be passed through the system in order satisfactorily to displace the oxygen.

Fig. 1. Carbon dioxide output and oxygen uptake of *B. coli* on 1% glucose in air.

Fig. 2. Carbon dioxide output and oxygen uptake of *B. coli* on 1% glucose in air.

Fig. 3. Carbon dioxide output and oxygen uptake of *B. coli* on 1% glucose in oxygen.

The increased gaseous exchange of *B. coli* in oxygen might at first be ascribed to a speeding up of the entire metabolism of the cell. This, however, is not the whole truth. The oxygen uptake is proportionately more increased than the carbon dioxide output; moreover, if the effect of the increased pressure of oxygen were merely to push up the metabolism as a whole, it would seem likely that the carbon dioxide output and consumption of glucose would be increased by the same ratio. This is not the case. In a pair of

parallel experiments, each supplied with 0.53 g. of glucose, the organism in oxygen broke down 0.295 g. glucose and gave off 0.0598 g. carbon dioxide, while that in air utilised nearly as much glucose, namely 0.28 g., but only evolved 0.0350 g. carbon dioxide. Furthermore, in an experiment in which the disappearance of glucose was followed in air and oxygen respectively, the amount of glucose broken down in the two cases was found to be identical (Fig. 4). The longer latent period in oxygen which is apparent in Fig. 4 was a fairly constant phenomenon with experiments on glucose inorganic medium, and may perhaps be attributed to a higher mortality among the bacteria originally sown, due to the more drastic change in environment. Experiments in which glucose was added to tryptic broth [Cole and Onslow, 1916], instead of to inorganic medium, gave results closely parallel with those described above except in this one respect: with broth, there was no difference in length of latent period between cultures kept in air and those kept in oxygen.

Fig. 4. Utilisation of glucose by *B. coli* in air and in oxygen.

Verzár, Nábráczky and Szányi [1923] have shown that *B. coli* ceases to break down glucose at a p_H of 4.08. If the medium is heavily buffered, more acid accumulates before this limiting p_H is reached, and if the glucose is not too concentrated, the medium sufficiently buffered, and of a sufficiently high p_H to begin with, the whole of the glucose may be utilised. In our experiments with inorganic medium, the limiting p_H was reached when still about half the glucose was left. When broth was substituted for inorganic medium, the increase of buffer substances enabled more glucose to be utilised. The effect of oxygen now became clearer (see Fig. 5). The carbon dioxide output was not only greater in oxygen than in air, but continued for a longer time. It seemed probable, therefore, that in an atmosphere of oxygen the organism was able to remove one of the acid breakdown products of glucose to a greater extent than in air, thus deferring the appearance of the limiting p_H . In the analyses of Grey, lactic acid was the chief product of the breakdown of glucose

by *B. coli*, and we consequently studied the oxygen uptake and carbon dioxide output of *B. coli* on a medium containing 1.0 % lactic acid (as ammonium lactate) as the sole source of carbon.

Fig. 5. Carbon dioxide output of *B. coli* on 1 % glucose broth in oxygen and in air.

EXPERIMENTS ON LACTIC ACID.

The inorganic medium used was the one described above. The lactic acid was estimated at the beginning and end of the experiment by the micromethod of Meyerhof [1920, 1921]. In order to test the validity of this method for a fermentation liquid containing a variety of products, a mixture of the following composition was made up in inorganic medium: ethyl alcohol, 1 %; lactic, acetic and formic acids, each 1 %; succinic acid, 0.16 %. 50 cc. of this were

Fig. 6. Carbon dioxide output and oxygen uptake of *B. coli* on ammonium lactate in oxygen and in air.

[Note: some CO₂ fixed in the medium.]

acidified with phosphoric acid and the volatile products distilled off *in vacuo* at 60° till about 10 cc. remained in the distilling flask: this was washed out, made up to 1 litre, and 10 cc. taken for the estimation. It has been shown by many workers that succinic acid is not oxidised by permanganate under the conditions of the Meyerhof method; our experiments confirm this. For example, in one estimation, 0.0051 g. of lactic acid was present in 10 cc. of diluted solution: 0.0048 g. and 0.0049 g. were found by two estimations. For the estimation of the lactic acid remaining in the respiration flask after an experiment, the organism was removed by centrifuging the liquid after the volatile products had been distilled off and the volume of the residue made up to 500 cc.

The figures for the respiratory exchange of *B. coli* on lactic acid in oxygen and in air are recorded in Fig. 6. The medium in this case becomes more alkaline as growth proceeds, owing to the liberation of ammonia. A certain amount of carbon dioxide remains in the medium as ammonium carbonate: this was driven into the potash bulbs at the end of each experiment by aeration after the addition of about 2 cc. of syrupy phosphoric acid to the contents of the respiration flask. The amount found was 0.0362 g. in oxygen, and 0.0137 g. in air. Increase of oxygen pressure is shown to increase both oxygen uptake and carbon dioxide output on a lactic acid medium, and at the same time, the breakdown of lactic acid is also increased.

Lactic acid present at beginning of experiment	...	0.41 g.	
Lactic acid present at end of experiment in air	...	0.176 g.	used 0.234 g.
Lactic acid present at end of experiment in oxygen		0.00 g.	used 0.41 g.

The amount of growth in the two cases was compared. The contents of the respiration flask were made up to 100 cc. and centrifuged, the clear liquid was poured off and the bacteria mixed afresh with water and again centrifuged; this was repeated and the washed bacteria were transferred to a platinum dish. The water was evaporated off and the dish dried to constant weight at 105°, and weighed. The dish was then heated to redness, cooled and again weighed, the weight of combustible material (bacterial growth) was thus obtained and found to amount to 0.0258 g. in air and 0.0465 g. in oxygen.

In view of the results obtained in air and in oxygen, a sowing of *B. coli* on ammonium lactate was kept under anaerobic conditions by passing a stream of nitrogen through the respiration flask. A tower containing standard baryta solution was employed instead of potash bulbs to absorb carbon dioxide, enabling the carbon dioxide output to be followed without weighing, and making possible the determination of small amounts at frequent intervals. No growth whatever occurred, and no carbon dioxide was evolved on any occasion from an ammonium lactate medium in nitrogen. But if such a medium, after three or four days in nitrogen, was bubbled with air or oxygen, growth of the organism took place after a short latent period, and carbon dioxide was rapidly evolved. Such an experiment is shown graphically in Fig. 7.

Fig. 7. Carbon dioxide output of *B. coli* on ammonium lactate, first in nitrogen, then in oxygen.

Fig. 8. Carbon dioxide output of *B. coli* on 1 % glucose in nitrogen.

Anaerobic growth of *B. coli* on glucose is of course well known to occur. There is rapid production of carbon dioxide, falling off in about three days owing to accumulation of acid (see Fig. 8). Experiments were made with other known products of glucose as well as with lactic acid, and the observations are summarised below. The carbon dioxide was in each case estimated by baryta titrations. Absence of growth and carbon dioxide output were not regarded as certain until the experiment had lasted about five days. When no growth took place in air, the effect of oxygen was tried.

Growth of B. coli on various sources of carbon.

Inorganic medium with 1 %	Growth in nitrogen	Growth in air	Growth in oxygen
Glucose	+	+	+
Ammonium lactate	-	+	+
„ succinate	-	+	.
„ acetate	-	+	.
Glycerol	-	+	.
Ethyl alcohol	-	-	-

Observations of a similar kind have been made by Braun and Cahn-Bronner [1921], who found that *B. coli* would not grow anaerobically on a lactate medium, and by Fürth and Lieben [1922] who showed that the rate of utilisation of lactic acid by yeast was increased by bubbling the medium with oxygen.

An explanation of these results may be sought in the energy relations of

the reactions involved. If an organism is to grow anaerobically on a simple carbon compound, this compound must be able to supply it not only with the material but also with the energy for growth. The first stage in the breakdown of the compound must not involve the interposition of oxygen and must proceed with evolution of energy. Glucose can be broken down anaerobically into (say) two molecules of lactic acid with the evolution of 22·56 Calories per molecule of glucose. We might consequently expect anaerobic growth on glucose, and in fact we find it to occur. With lactic acid, on the other hand, all the likely paths of anaerobic breakdown appear to involve an absorption of energy. For example, calculating from the heats of combustion of the compounds (as recorded by Landolt and Börnstein [1923]) we have

The same is true of succinic acid:

In the case of glycerol, the heats of combustion of likely breakdown products such as hydroxyacetone and lactic aldehyde are not known: deducing them from those of compounds of similar constitution, all the reactions appear to involve absorption of energy.

From such considerations it would appear that energy cannot be obtained from the anaerobic breakdown of lactic and succinic acids and glycerol, and hence it is unlikely that any of them will serve as sole source of carbon to an organism growing anaerobically. The inability of *B. coli* to grow anaerobically on these substances may perhaps thus be explained. We wish to offer this hypothesis in a tentative manner only, but hope later to investigate the matter further in collaboration with Mr J. H. Quastel, who was led to consider the subject from this aspect by his work on *B. pyocyaneus*.

When *B. coli* and other fermenting organisms attack carbohydrates, they liberate acid products. In anaerobic conditions, these products accumulate, since the organism is probably only able to utilise them to a small extent by employing part of the energy obtained from the initial exothermic breakdown of carbohydrate. When oxygen is supplied at atmospheric pressure, the acids are slowly attacked, and poisoning of the cell by acid is delayed. When the concentration of oxygen is increased, the oxidation of the acids is hastened and the metabolism approaches more nearly that of the non-fermenting type of organism. We are reminded of Pasteur's dictum, "La fermentation est la vie sans air."

¹ The heat of combustion of lactic acid was taken at 325·72 Cal. as recently re-determined by Meyerhof [1922]

SUMMARY.

1. A technique previously applied to the study of the metabolism of a non-fermenting bacillus was extended to one of a fermenting type, *B. coli* being employed.

2. The carbon dioxide output and oxygen uptake of *B. coli* on a glucose inorganic medium were followed. The organism was found to be almost anaerobic during the early stages of glucose breakdown.

3. The effect of an atmosphere of oxygen was observed. There was an increase in carbon dioxide output and a larger increase in oxygen uptake.

4. This did not correspond, in the early stages of the experiment, to an increased utilisation of glucose. Metabolism continued longer in oxygen than in air, the limiting p_H not being reached so soon. The increase of gaseous exchange with increase of oxygen pressure was therefore attributed to an effect on some acid breakdown product of glucose.

5. Growth on lactic acid (as the ammonium salt) was studied in air and in oxygen. The disappearance of lactic acid and production of carbon dioxide were greatly increased with increased oxygen tension.

6. Growth did not occur on lactic acid in anaerobic conditions.

7. Succinic and acetic acids and glycerol were also found to be suitable sources of carbon for *B. coli* in air but not in anaerobic conditions.

8. A possible explanation of these facts may be found in the energy relations of the reactions involved.

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