LXIX. THE OXYGEN UPTAKE OF BACTERIA.

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THERE appears to be no reference in the literature to the basal metabolism of bacteria, that is the respiration of bacteria which have been washed free from culture medium and are therefore unable to grow, but are able to oxidise the material of their own cells. Differences in the oxygen uptake of different types of bacteria would indicate differences in constitution of the cells. In the present investigation, the oxygen uptake of a number of bacteria, aerobic and anaerobic, has been measured, and it appears to be fairly constant for each strain, but reveals many striking points of difference in different species of bacteria. Interesting results have also been obtained by using bacteria which have been dried in a vacuum desiccator over sulphuric acid. The dried bacteria readily yield a fine powder which can be weighed out, emulsified in buffer solution and examined for oxygen uptake. Judging by the work of Shattock and Dudgeon [1912] it is unlikely that bacteria are killed by the rapid drying process used in these experiments. Throughout the paper when the oxygen uptake of "dried" bacteria is mentioned, it must be understood that the dried powder is suspended in buffer solution.

METHOD.

The apparatus used was Barcroft's micro-respirometer with flat-bottomed bottles, specially designed for the respiration of tissue. Caustic soda was placed in the small cups to absorb CO_2 and the oxygen uptake at 22° was measured.

Bacteria were grown on agar plates or in Roux bottles at 37° for 18 hours, except in the case of slowly growing organisms which were grown for two or three days. The medium was tryptic agar or tryptic broth, with or without the addition of 1% glucose.

Broth cultures were centrifugalised, the clear liquid removed, and the bacteria washed twice with distilled water to remove traces of broth, the final deposit being emulsified in water or buffer and used for the determinations. In the case of agar cultures, the growth was carefully removed from the surface of the agar and washed twice with distilled water.

Cultures of the anaerobe *B. sporogenes* had to be treated in a special way because the large amount of gas (H_2 , H_2S , etc.) produced during growth was not removed by washing, and interfered with the measurement of oxygen uptake. The following technique was adopted to avoid this difficulty. The washed bacteria, suspended in distilled water, were cooled in ice for an hour or two, during which time a stream of sterile nitrogen was passed through. The suspension was then centrifuged, washed with distilled water and used for the Barcroft experiment.

For experiments on dried bacteria, the washed deposit was placed in a small dish and dried in a vacuum desiccator over sulphuric acid for 18 hours. The resulting dried mass was powdered finely and 0.1 g. was weighed out for each experiment. Before drying, it is more difficult to take a known weight, as it is unsatisfactory to weigh the washed bacteria. The following procedure, which gives the approximate weight (expressed as dry weight), was therefore used throughout. The washed bacteria were carefully emulsified in 8 cc. buffer solution, 3 cc. being placed in the Barcroft bottle, and an equivalent 3 cc. in a tared centrifuge tube. The latter was centrifuged, the buffer solution poured away, the deposit washed with distilled water and again centrifuged. The water was decanted off and the tube dried in a vacuum desiccator and weighed, thus giving the approximate dry weight of the bacteria placed in the Barcroft bottle. All results were calculated as cc. of oxygen per g. of dried bacteria.

Effect of hydrogen ion concentration of the buffer solution.

Since the hydrogen ion concentration is of great importance in certain oxidations it was considered advisable to find the oxygen uptake of bacteria in buffers of different p_H . Sterile buffer solutions [p_H 6.6, 7.6 and 8.6] were prepared from a basal solution containing 1 % KH_2PO_4 . *B. pyocyaneus* was grown for 18 hours in three Roux bottles of broth, then centrifuged and washed twice with distilled water. The deposit was divided into three portions:

1. Emulsified in 8 cc. buffer of p_H 6.6, 3 cc. being taken for the Barcroft bottle and 3 cc. for the weight determination.
2. Emulsified in buffer of p_H 7.6.
3. Emulsified in buffer of p_H 8.6.

The results of the Barcroft experiments were then calculated as cc. per g. (dry weight). Fig. 1 shows that the three experiments show remarkable agreement, considering that in each case the weight of bacteria used was determined separately.

This experiment was repeated with dried *B. pyocyaneus*, also with *B. prodigiosus* and *B. proteus* with similar results, thus showing that changes in p_H between 6.6 and 8.6 have practically no effect on the oxygen uptake of these bacteria.

The effect of replacing buffer solution by distilled water.

This effect was investigated in order to see whether the phosphate buffer either accelerated or inhibited oxygen uptake.

Fig. 1. *B. pyocyaneus* Fig. 2. *B. pyocyaneus*. Fig. 3. *B. prodigiosus* Fig. 4. *B. pyocyaneus*.

Fig. 2 shows that the oxygen uptake of *B. pyocyaneus* in sterile 1 % phosphate buffer at p_H 7.6 is identical with that in sterile distilled water. This experiment has been repeated with *B. alcaligenes*, *B. sporogenes* and *Streptococcus acidi lactici*, and in each case the oxygen uptake was practically the same in buffer as in distilled water. These three organisms were also dried and emulsified in Clark and Lubs' phosphate buffer (sterile) and borate buffer, and here again the results were almost identical with those obtained with distilled water.

For all subsequent experiments a sterile 1 % phosphate buffer, p_H 7.6, was used.

The effect of growing bacteria on different media.

As some of the bacteria examined grew better in glucose broth than in plain broth, it was important to see whether this change in the medium

affected the oxygen uptake. *B. prodigiosus* was therefore grown for 18 hours in broth and in glucose broth, and Fig. 3 shows that the oxygen uptake was the same in each case. Fig. 4 shows the same similarity in oxygen uptake in the case of *B. pyocyaneus* when grown on agar and in broth.

The effect of autoclaving bacteria.

After being autoclaved at 120° for 15 minutes, *B. alcaligenes* and *S. acidilactici* were unable to take up any oxygen, in an experiment lasting three hours.

Fig. 5.

B. pyocyaneus. Duplicate Experiments.

Fig. 6.

B. fluorescens. Duplicate Experiments.

THE OXYGEN UPTAKE OF AEROBIC AND ANAEROBIC BACTERIA.

The following experiments deal with the oxygen uptake of 13 species of bacteria, 12 aerobes and 1 anaerobe, both before and after having been dried *in vacuo*.

The results of duplicate experiments (*A* and *B*) are given graphically so that the amount of variation in each organism can be seen. Cultures were grown in tryptic broth for 18 hours at 37° unless otherwise stated. Bacteria were in every case washed twice with distilled water and emulsified finally in sterile buffer, p_H 7.6.

I. *Aerobic Bacilli without spores.*

1. *B. pyocyaneus*. Fig. 5 shows that the results of the duplicate experiments *A* and *B* are almost identical. In two hours, *B. pyocyaneus* emulsified in buffer takes up 22 cc. of oxygen per g. of dry weight, but, when the bacillus

has been dried and then again emulsified in buffer, the uptake is reduced to 2 cc. Both before and after drying, the oxygen uptake is quite regular, and can be represented graphically by straight lines. Subsequent experiments show that this linear uptake is characteristic of most aerobic bacteria.

2. *B. fluorescens*. The oxygen uptake of *B. fluorescens* (see Fig. 6), like that of *B. pyocyaneus*, is much reduced when the organism is dried, and here

Fig. 7.

B. prodigiosus. Duplicate experiments.

Fig. 8.

B. alcaligenes. Duplicate experiments.

Fig. 9.

B. proteus. Duplicate experiments.

Fig. 10.

B. coli. Duplicate experiments.

Fig. 11.

Timothy Grass Bacillus

Fig. 12.

Timothy Grass Bacillus

again the rate of uptake shows no falling off either in the normal or dried bacteria.

3. *B. prodigiosus* (Fig. 7) shows a certain amount of reduction in the oxygen uptake in the dried bacteria, but not as much as do *B. pyocyaneus* and *B. fluorescens*.

4. *B. alcaligenes* (Fig. 8) is remarkable because it takes up almost as much oxygen after drying as before.

5. *B. proteus* (Fig. 9). 6. *B. coli* (Fig. 10). These two organisms resemble *B. prodigiosus* in that they are somewhat affected by drying.

7. *Timothy Grass Bacillus* was grown for three days in glucose broth in order to obtain good growth. The results obtained (Figs. 11 and 12) are of special interest as the process of drying has no effect on the oxygen uptake. This is probably due to the protective action of the fatty envelope of this acid-fast organism.

Fig. 14.

B. subtilis. Duplicate experiments.

Fig. 13.

B. megatherium. Duplicate experiments.

Fig. 15.

B. subtilis.

II. Aerobic spore-bearing Bacilli.

8. *B. megatherium* was grown for 18 hours at 37°. The culture consisted almost entirely of bacilli without spores. Fig. 13 shows that with this organism there is a considerable reduction of oxygen uptake after drying.

9. *B. subtilis* was grown for 18 hours at 37°, and the culture consisted almost entirely of bacilli without spores. Fig. 14 shows the very large uptake of *B. subtilis*, which is a strict aerobe, incapable of growing anaerobically. On drying, the oxygen uptake is greatly reduced, as with *B. pyocyaneus*. An older sporing culture gave much lower results: 7.5 cc. per g. in two hours, and 1.0 cc. per g. in two hours after being dried. However, it is impossible to say that this reduction was entirely due to spore formation as, in order to obtain a culture composed chiefly of spores, growth was continued for four

days, and other experiments have shown that four day cultures of non-sporing bacilli take up less oxygen than 18 hour cultures of the same organism. For example, a culture of *B. alcaligenes*, which took up 11 cc. oxygen per g. in two hours when 18 hours old, only took up 7 cc. in the same time when four days old. The same culture dried took up 8 cc. when 18 hours old and 4 cc. when four days old.

Fig. 15 shows the oxygen uptake of an 18 hour culture of *B. subtilis* continued over a period of six hours, during which time the rate of uptake remains quite regular.

Fig. 16.

B. sporogenes Metchnikoff.

Fig. 20.

Streptococcus acidilactici.

Fig. 17.

Staphylococcus aureus. Duplicate experiments.

Fig. 18.

Sarcina aurantiaca. Duplicate experiments.

Fig. 19.

Sarcina aurantiaca.

III. *Anaerobic spore-bearing Bacillus.*

10. *B. sporogenes* was grown anaerobically for two days in glucose broth, about 3 litres of medium being required for each experiment. The culture consisted almost entirely of bacilli without spores. Both before and after drying the oxygen uptake of *B. sporogenes* is so minute that the graph had to be drawn to a much larger scale than has been used for all the previous work (Fig. 16). It will be noticed that the initial rate rapidly falls off so that, instead of the usual straight line, a curve is obtained. Drying has little or no effect, and in both cases the results are practically negligible when compared to the morphologically similar aerobic spore-bearers *B. subtilis* and *B. megatherium*. Duplicate results of *B. sporogenes* are seen in the controls of Figs. 23 and 24.

IV. *Aerobic Cocci.*

11. *Staphylococcus aureus* was grown in six Roux bottles of broth for two days. Fig. 17 shows the large uptake of oxygen of *Staph. aureus*, which is only slightly affected by drying.

12. *Sarcina aurantiaca* was grown on agar plates for three days. Fig. 18 shows the oxygen uptake, which is somewhat reduced on drying. Fig. 19 shows the prolonged steady oxygen uptake of this organism, no diminution in the rate appearing during a period of 10 hours.

13. *Streptococcus acidi lactici* was grown for two days in 18 Roux bottles of glucose broth. Fig. 20, which is drawn to the scale used for *B. sporogenes* (Fig. 16), shows that in *S. acidi lactici* the oxygen uptake is almost negligible. Duplicate results are to be found in the controls of Figs. 21 and 22.

It is most striking to compare this result with those of the other aerobic cocci, *Staphylococcus* and *Sarcina*, and to find that, instead of resembling these organisms, *S. acidi lactici* gives an oxygen uptake that is almost exactly equal to that of the anaerobic *B. sporogenes*. In addition to the fact that *S. acidi lactici* and *B. sporogenes* both take up only a minute amount of oxygen, these organisms also both show rapid reduction in the rate of oxygen uptake, unlike all the other bacteria investigated.

EFFECT OF ADDITION OF CATALASE ON OXYGEN UPTAKE OF
BACTERIA WHICH LACK CATALASE.

Previous investigations [Callow, 1923] had revealed the fact that *S. acidi lactici*, like the anaerobes, contained no catalase, whereas all the other aerobes mentioned in this paper contained catalase. On this account it was thought that possibly the addition of catalase to *S. acidi lactici* or to *B. sporogenes* might lead to an increase in oxygen uptake, or at any rate to a reduction in the falling-off that is so characteristic of the rate of uptake of these two organisms.

Preparation of Sterile Catalase. Ox liver was minced finely, ground up with distilled water and filtered. Alcohol was added to the clear filtrate until no more precipitation occurred. The precipitate was filtered off, washed with absolute alcohol and dried *in vacuo*. The dry powder was dissolved in distilled water, filtered and passed through a bacteriological filter. The resulting clear liquid contained active catalase but gave no peroxidase reaction with benzidine and either H_2O_2 or ethyl hydroperoxide.

For control experiments the enzyme was inactivated by boiling for five minutes.

S. acidi lactici was washed as usual and emulsified in buffer but, instead of the usual 3 cc., 2.5 cc. were placed in each of three Barcroft bottles. To one was added 0.5 cc. sterile catalase solution, to another 0.5 cc. inactivated "catalase" solution, and to the third 0.5 cc. sterile distilled water to act as a control.

Fig. 21.

S. acidi lactici with addition of catalase.

Fig. 22.

Dried *S. acidi lactici* with addition of catalase.

Fig. 23.

B. sporogenes with addition of catalase.

Fig. 24.

Dried *B. sporogenes* with addition of catalase.

Fig. 21 shows that, before the organism was dried, the liver extract supplied a certain amount of readily oxidisable material which caused a slight increase in the oxygen uptake, but clearly the catalase had no specific effect, as the inactivated enzyme gave exactly the same result. Fig. 22 shows that in the case of the dried *Streptococcus* there was no increase in oxygen uptake with the addition of either catalase or inactivated "catalase."

Figs. 23 and 24 show that *B. sporogenes*, like *S. acidi lactici*, is unable to distinguish between boiled and unboiled liver extract, thus showing that under these conditions the addition of catalase has no effect on the oxygen uptake.

DISCUSSION OF RESULTS.

Setting aside for the moment the exceptional case of *S. acidi lactici*, it must be remarked that the oxygen uptake of aerobic bacteria is large when compared with that of mammalian tissue. The bacteria examined took up 5–25 cc. oxygen per hour per g. of dry weight. Meyerhof [1919] found that 1 g. of intact frog muscle at 22° took up 30–48 c.mm. oxygen in one hour. Assuming that the frog muscle contained 70–75 % water, the oxygen uptake per g. of dry weight would be at most 152 c.mm. as against 5–25 cc. for the same weight of bacteria in the same time. After cutting up the frog muscle very finely, Meyerhof found that the oxygen uptake was increased to 400–540 c.mm. which would give at most 2.16 cc. per hour per g. of dry weight, which is less than half the amount of oxygen taken up by the least efficient (considered from this point of view) bacterium. The investigation of the oxygen uptake of bacteria after drying and then emulsifying in buffer yielded several interesting points. The *Timothy Grass Bacillus*, an acid-fast organism which is characterised by its fatty envelope, actually gave the same oxygen uptake as before. Several other organisms, such as *B. alcaligenes* and *Staph. aureus*, were hardly affected by the process of drying while others, such as *B. pyocyaneus*, showed considerably reduced uptake of oxygen after having been dried. This point is of great interest in connexion with the work of Shattock and Dudgeon [1912]. These authors dried certain non-sporing bacteria in a charcoal liquid air vacuum, allowing controls to dry in the air for the same length of time. They found that, in the case of *B. coli* and *B. typhosus*, the bacteria were dead after 4–6 days in the vacuum tubes, and the air dried controls were dead on the fourth day, that is to say the presence of air had little effect on the viability. *Staph. aureus* was found to be more resistant to desiccation both in the vacuum tubes and in air. *B. pyocyaneus* however was exceptional as it survived for more than six months if kept in a vacuum tube, while the air control was never alive after the ninth day. The authors suggest that the bacillus may be killed “by a recurrent process of oxidation.” It must be remembered that the process of drying used in the present investigation (in vacuum desiccator over H_2SO_4) does not exclude the presence of air, so that the dried *B. pyocyaneus* in this case would correspond to the air-dried control of Shattock and Dudgeon, which they found to be rapidly affected. This fits in exactly with the results of oxygen uptake, which were much lower on drying in the case of *B. pyocyaneus*, pointing to the fact that this organism has a high content of readily oxidisable material which is not protected against the action of atmospheric oxygen.

Unlike the majority of aerobic bacteria, which take up large amounts of oxygen at a rate which shows no diminution even after 10 hours, the anaerobe *B. sporogenes* only takes up a minute quantity of oxygen, and this at a rapidly decreasing rate. The same small and rapidly decreasing uptake is actually found in the aerobe *S. acidi lactici*, which in this respect behaves like an

anaerobe. This organism in fact appears to bridge the gulf between aerobes and anaerobes. Like an aerobe it can grow aerobically on the surface of an agar slope, an action that no anaerobe can perform, and yet like an anaerobe it has no catalase and is only capable of taking up small quantities of oxygen. The oxygen uptake of bacteria is presumably due to the action of enzymes on the oxidisable material present, and in the case of those bacteria which have only a small oxygen uptake there appear to be three possibilities; either there is lack of suitable enzymes, or lack of available oxidisable material, or some product of oxidation rapidly inhibits the reaction, as for example the accumulation of H_2O_2 in the non-catalase bacteria [see McLeod, 1922]. This last possibility has been investigated in the case of *B. sporogenes* and *S. acidi lactici*, both of which lack catalase and are only able to take up a very small amount of oxygen, at a rapidly decreasing rate. A sterile preparation of liver catalase was added to each of these organisms before and after drying, but in no case was there any increase in oxygen uptake other than that given by addition of the control inactivated "catalase," which provided a small amount of readily oxidisable material. Of course this negative result may merely mean that these bacterial cells are not permeable to liver catalase, and that this enzyme cannot be utilised unless it enters the cell. However, this and other observations mentioned in a former paper [Callow, 1923] point to the fact that possibly some factor other than catalase is of importance here, either in connexion with catalase or alone. It is of course possible that the lack of catalase in these organisms is not connected with the remarkably low oxygen uptake. Further work on this point is in progress.

In conclusion, the author gladly takes this opportunity of thanking Prof. F. Gowland Hopkins for his interest during the progress of this work.

SUMMARY.

1. Bacteria were washed free from culture medium, emulsified in sterile buffer solution, p_H 7.6, and investigated for oxygen uptake at 22° in Barcroft's micro-respirometer.

2. Variations in p_H of the buffer solution, 6.6 to 8.6, had no appreciable effect on the oxygen uptake, nor had the replacement of the phosphate buffer solution by distilled water.

3. Bacteria grown on agar or glucose tryptic broth gave the same oxygen uptake as when grown on tryptic broth.

4. *B. pyocyaneus*, *B. fluorescens*, *B. prodigiosus*, *B. alcaligenes*, *B. proteus*, *B. coli*, *Timothy Grass Bacillus*, *B. megatherium*, *B. subtilis*, *Staph. aureus* and *Sarcina aurantiaca* took up large amounts of oxygen (5 to 25 cc. per hour per g. of dry weight) at a steady rate.

5. Bacteria were dried *in vacuo*, emulsified in buffer and examined for oxygen uptake. *B. alcaligenes* and *Staph. aureus* took up nearly as much

oxygen after drying as before, and the acid-fast *Timothy Grass Bacillus* gave the same result dried as normal. In *B. pyocyaneus* the oxygen uptake was very much reduced on drying.

6. The aerobe, *S. acidi lactici*, resembled the anaerobe, *B. sporogenes*, in taking up only a minute amount of oxygen at a rapidly decreasing rate (less than 0.5 cc. per g. of dry weight in the first hour, and practically none in the succeeding hours).

7. Addition of sterile catalase solution had no effect on the oxygen uptake of *S. acidi lactici* or *B. sporogenes*.

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