THE OXYGEN CONSUMPTION OF ESCHERICHIA COLI DURING THE LAG AND LOGARITHMIC PHASES OF GROWTH

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INTRODUCTION

The oxygen consumption of bacteria has been studied by various methods and by numerous observers. Many of these methods are qualitative and will not be discussed here. The quantitative methods which have been used have been adequately described by Wohlfeil (1930, *a*). In general, most of the quantitative work has been done with thick suspensions of the organisms in saline or Ringer's solution to inhibit growth and reduce the variations due to changes in the number of organisms studied. Inasmuch as rapid multiplication is one of the characteristic processes in the normal life of a bacterium, correlation of respiration and growth presents an interesting problem. Verzar and Bogel (1920), using a Barcroft differential manometer, followed the oxygen consumption and carbon dioxide output of a bouillon culture of *Escherichia coli* over a period of 4 or 5 days but did not record the rate of growth quantitatively.

Novy, Soule and their coworkers (1925) investigated the gaseous metabolism of the tubercle bacillus and other organisms over long periods of time, but growth was followed only qualitatively. Wohlfeil (1930, *b*) determining oxygen consumption by gas analysis studied the respiration of a growing culture of *E. coli* and followed the rate of growth by quantitative methods. His results will be discussed later.

Methods

Oxygen Consumption.—A microrespirometer similar to Fenn's (1927) modification of Thunberg's apparatus is admirably suited to accurate determinations of the oxygen utilization of bacterial cultures, because of its sensitivity over a wide

range of rate changes. The apparatus used in these experiments was a further modification of Fenn's respirometer by Jares.[1] It consists essentially of two flasks, *E* and *C*, (Fig. 1) connected by means of ground glass stoppers and three-way stop-cocks, *S*, to side arms, *A*, opening to the air, and to each end of an even-bored capillary tube, *T*. The respirometer differs from the one used by Jares only in the shape and size of the flasks. The control flask, *C*, may be of any shape but the experimental flask, *E*, is shaped like a short squat Erlenmeyer flask and in addition contains a "well," *W*, made of a short piece of glass tubing fused into the bottom of the vessel which serves as a container for the sodium hydroxide. The shape of the latter flask allows 5 cc. of medium to have a surface area of 25 sq. cm.

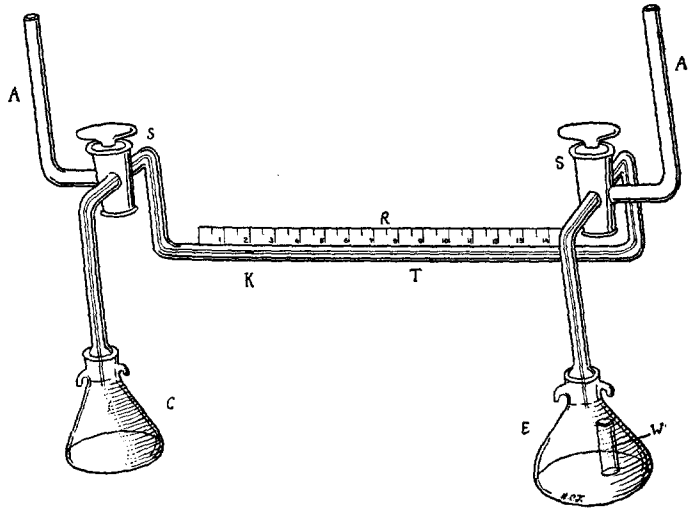

FIG. 1

Calibration of the flasks was done by filling to the level of the stop-cocks with mercury and weighing. Similarly, the capillary was calibrated by weighing the mercury which filled a measured length of the tube. The constants for the apparatus used in the following experiments were:

Vol. of experimental flask = 36.06 cc.
" " control " = 24.11 "
" " capillary tube 1 cm. in length = 8.214×10^{-3} cc.

Only the flasks were autoclaved, as it was found that contamination from the stoppers occurred only rarely, and the purity of the culture was checked hourly by plating methods. A small drop of kerosene, *K*, was placed in the capillary tube and a celluloid rule, *R*, marked in millimeters, was wired to the tube.

[1] To be published.

After placing 1 cc. of sodium hydroxide in the center well, W, of the experimental flask, 5 cc. of sterile medium was placed in this flask and 1 cc. in the control flask. The entire apparatus was then immersed to the level of the top of the stopcocks in a rapidly stirred water bath kept at a temperature of 37.5° ±0.01°C. and was shaken at a constant rate by a motor-driven shaking device. The carbon dioxide formed during the experiment was taken up by the sodium hydroxide and as oxygen was consumed by the culture the drop moved toward the experimental flask. Readings of one end of the drop were taken at various intervals by sighting along a plumb-line held near the end of the drop in order to avoid parallax. Determination of the amount of oxygen consumed by the culture was calculated from observation of the distance through which the drop had moved during a definite time interval t, according to Fenn's (1928) formula:

$$x = d\left(\frac{V_c + V_e}{V_c + d}\right)\left(\frac{p - y}{p}\right),$$

where x = oxygen consumption during time t
d = volume of capillary tube through which drop has moved during time t
V_c = volume of control flask minus volume of medium
V_e = volume of experimental flask minus volume of medium and sodium hydroxide
p = barometric pressure
y = vapor pressure in both flasks

Since d is so small in comparison with V_c, for practical purposes it has been dropped from the expression $\frac{V_c + V_e}{V_c + d}$ but of course must be retained elsewhere in the equation.

One of the disadvantages of this method lies in the fact that in order to withdraw samples of the culture for analysis, the apparatus must be removed from the water bath and the flask opened to the air, which disturbs the pressure equilibrium and increases the risk of contamination. Control experiments have shown, however, that if the sample is taken quickly and the apparatus returned to the water bath immediately, 10 minutes is sufficient time for equilibrium to be reestablished.

Medium.—A 2 per cent Difco Bacto-peptone solution in distilled water, containing 0.5 per cent NaCl, pH 7.6, was used in all experiments. As may be expected, the sterile medium *per se* used oxygen at a very slow rate. The rate of oxygen consumption is practically constant over a period of a few hours, but gradually diminishes over a period of 24 hours or longer. The greatest rate observed with this medium was 1.0×10^{-5} cc. of oxygen per minute per cc. of medium. In the early stages of the experiment, when there are comparatively few bacteria present, 50 to 60 per cent of the total oxygen consumption can be accounted for by the medium itself, but within an hour after inoculation this cor-

rection becomes almost negligible. For this reason, the medium was put into the flask 24 hours before inoculation and the apparatus placed in the water bath and shaken. Readings were taken at various intervals and the rate of oxygen consumption of the sterile medium during the hour preceding inoculation was assumed to be constant throughout the experiment, all calculations being corrected according to this figure. Even though this value may not be correct 4 to 5 hours after inoculation it may be assumed to be constant for the first hour or two, which is the only period in which the value is large enough to cause error.

Organisms.—Esch. coli (Strain S) was used in all experiments. The culture was carried along on agar slants or in peptone water until 12 to 20 hours before the beginning of the experiment, when one loopful was inoculated into a large flask of medium identical with the medium in the respirometer. This flask was kept in the incubator until 1 hour before inoculation, when it was placed in the water bath with the respirometer, so that the temperatures of the inoculum and substrate were almost identical. 0.05 cc. of this culture was the usual amount transferred to the experimental flask.

Bacterial Counts.—0.05 cc. of culture was withdrawn at 30 minute intervals throughout each experiment. Plates were streaked for the detection of contamination, and after suitable dilution of the organisms in saline, the count was made in an ordinary Helber hemocytometer. Many of the organisms observed were not separated but in chains, sometimes as many as 8 or 10 occurring in a single chain. By careful focussing, the lines of division were easily seen and the count was made assuming each unit of the chain to be a bacterium. This method of course did not indicate whether the organisms were viable or not, but over the short period of observation—4 hours, all organisms were assumed to have some degree of viability.

Measurements of Size.—A loopful of the culture was smeared on a slide, allowed to dry in air, fixed by heat, and stained with methylene blue. As pointed out by Henrici (1923) the methylene blue method is inaccurate because of distortions of form due to shrinking of the cells. The negative staining method, however, is unsuitable in media containing peptone because of precipitation of the peptone by Congo red. At first, the length and width of each bacterium was measured by a filar micrometer, but it was later found that camera lucida drawings, using an $\times$ 25 ocular could be easily and quickly made, and measurements were made from the tracings of the cell outlines. Approximately 100 cells of each smear were measured, the lengths and widths averaged and the area of the average cell calculated assuming the cell to be cylindrical in shape.

In order to prevent selection of the cells to be measured, the smears were mixed and relabeled by another person. Only after the measurements were completed and averaged, were the smears placed in the proper sequence.

Calculations.—All calculations were reduced to a basis of 1 cc. of culture. A slight correction in the formula had to be made after each withdrawal of a sample, since the total volume of the culture was reduced, and the value for V_s increased a very slight amount with every reduction in the volume of medium.

It is obvious from the above that the measurement of the rate of oxygen utilization of the culture was not continuous, because of the time allowed for equilibrium to be regained after withdrawal of the sample. For this reason the oxygen consumption per minute per bacterium had to be calculated indirectly, since the actual number of bacteria per cc. of medium was not known during the observed period.

Buchanan's (1930) formula for the determination of the amount of chemical change per single cell per unit time was used as the basis for the calculations. This formula is:

$$m = \frac{2.303\ S \log b/B}{t(b - B)}$$

where m = amount of substance produced (or oxygen consumed) per cell per unit time

S = total amount of substance produced (or oxygen consumed) per time t

B = number of bacteria present at beginning

b = number of bacteria present at time t

The formula is based on the assumptions that m is constant throughout the period of observation and that growth is logarithmic. As will be demonstrated later, m is not constant but varies with the phases of the life cycle. For the purpose of calculation m is considered constant for only the relatively short period of actual observation of the movement of the drop, and is calculated separately from the data obtained during each observation period.

Since there is no actual count of the organisms at the time of the first reading of the position of the drop, the value B must be calculated. The formula used for this was as follows:

Let a = number of organisms present at time 0

B = number of organisms present at time x (time of first reading of position of drop)

b = number of organisms present at time $x + t$ (time of second reading of position of drop)

g = generation time during interval $x + t$

$$b = a\,2^{x+t/g}$$

$$B = a\,2^{x/g}$$

Assuming g to be constant during the relatively short interval $x + t$, and solving for B —

$$\log B = \frac{x}{x + t}(\log b - \log a) + \log a$$

Placing the value for B in Buchanan's equation, m may be calculated. The calculations of the first period in Table I is presented as an example:

The experimental flask contained 5.0 cc. of sterile medium and 0.5 cc. of 1 N NaOH in the well. The control flask contained 1.0 cc. of sterile medium. After

23 hours in the water bath under the same conditions as were present during the experiment, it was found that the drop moved a distance of 0.18 cm. in 60 minutes, a rate of 0.0006 cm. per minute per cc. of medium.

The barometric pressure was 760.4. Calculating according to the formula for the respirometer:

$$0.0006 \times 8.214 \times 10^{-3} \times \left(\frac{23.11 + 30.56}{23.11}\right)\left(\frac{760.4 - 47.9}{760.4}\right)\left(\frac{273}{273 + 37.5}\right) =$$

0.0941×10^{-4} cc. of oxygen consumed per minute per cc. of sterile medium.

(Inasmuch as the CO_2 formed in the control flask was not removed by NaOH and the R.Q. was unknown, this value should more properly be called the medium correction rather than the actual oxygen consumption of the medium).

The experimental flask was then inoculated with 0.05 cc. of a 12 hour culture containing 421×10^6 organisms per cc., giving an initial concentration of 4.17×10^6 organisms per cc. in the 5.05 cc. of medium in the flask. 12 minutes after inoculation the position of one end of the drop was 5.51 on the scale, and 30 minutes after inoculation the reading was 5.70, a movement of the drop through a distance of 0.19 cm. in 18 minutes.

Calculating by the same formula (V_e now becoming 30.51 because of the increase in the volume of the medium to 5.05 cc.) the total oxygen consumption was 2.982×10^{-3} cc.

5.05 cc. of sterile medium would account for $0.855 + 10^{-3}$ cc. in 18 minutes leaving $2.127 + 10^{-3}$ cc. as the oxygen consumption of the organisms *per se*. Reducing this to oxygen consumption per cc. of culture it becomes 0.4212×10^{-3} cc.

At time of inoculation there were 4.17×10^6 organisms per cc. (a). 30 minutes later there were 5.58×10^6 organisms per cc. (b). Calculating the organisms per cc. 12 minutes after inoculation (x):

$$\log B = \frac{12}{12 + 18}(\log 5.58 \times 10^6 - \log 4.17 \times 10^6) + \log 4.17 \times 10^6$$

$$\log B = 6.6708$$

$$B = 4.686 \times 10^6$$

Substituting into Buchanan's formula

$$m = \frac{2.303 \times 0.4212 \times 10^{-3} \log \frac{5.58 \times 10^6}{4.686 \times 10^6}}{18\,(5.58 \times 10^6 - 4.686 \times 10^6)}$$

$= 4.57 \times 10^{-12}$

= the average oxygen consumption per minute per organism during the period 12 to 30 minutes from inoculation.

All subsequent periods are calculated in the same way.

Eighteen experiments were done and the measurements of the average surface area of the organisms were carried out in eight. Because of the individual variations in each experiment, the results are better considered separately rather than averaged. The tables and graphs of three representative experiments are presented.

TABLE I

Inoculum 0.05 Cc. of a 12.0 Hour Peptone Water Culture Containing 421 × 10^6 Organisms Per Cc.

(*a*)

Time from inoculation	No. of organisms per cc. × 10^6	Log No. of organisms per cc.	Average length of organisms	Average diameter of organisms	Average area
min.			*cm.* × 10^{-4}	*cm.* × 10^{-4}	*sq. cm.* × 10^{-8}
0	4.17	6.620	1.83	0.61	4.09
30	5.58	6.747	2.17	0.66	5.19
60	9.05	6.957	2.75	0.80	7.92
90	24.5	7.389	3.22	0.76	8.59
120	67.0	7.826	2.35	0.73	6.23
150	151.9	8.182	2.18	0.64	5.02
180	343.0	8.535	1.97	0.64	4.61
210	900.0	8.954	1.72	0.56	3.52
240	1850.0	9.267	1.42	0.53	2.81

(*b*)

Time from inoculation	O_2 consumption per cc. of medium during observed period	O_2 consumption per min. per cc. of medium	Log O_2 consumption per min. per cc. of medium	O_2 consumption per min. per organism
min.	*cc.* × 10^{-3}	*cc.* × 10^{-4}		*cc.* × 10^{-12}
12– 30	0.4212	0.234	$\bar{5}.369$	4.57
40– 60	0.9760	0.488	$\bar{5}.688$	6.31
70– 90	3.372	1.686	$\bar{4}.227$	9.43
100–120	7.296	3.648	$\bar{4}.562$	7.47
130–150	14.84	7.42	$\bar{4}.870$	6.44
160–180	27.00	13.50	$\bar{3}.130$	5.10
190–202	30.72	25.60	$\bar{3}.408$	4.44
204–210	17.06	28.43	$\bar{3}.454$	3.47
220–230	32.54	32.54	$\bar{3}.512$	2.52
232–240	26.24	32.80	$\bar{3}.516$	1.95
250–260	40.50	40.5	$\bar{3}.607$	

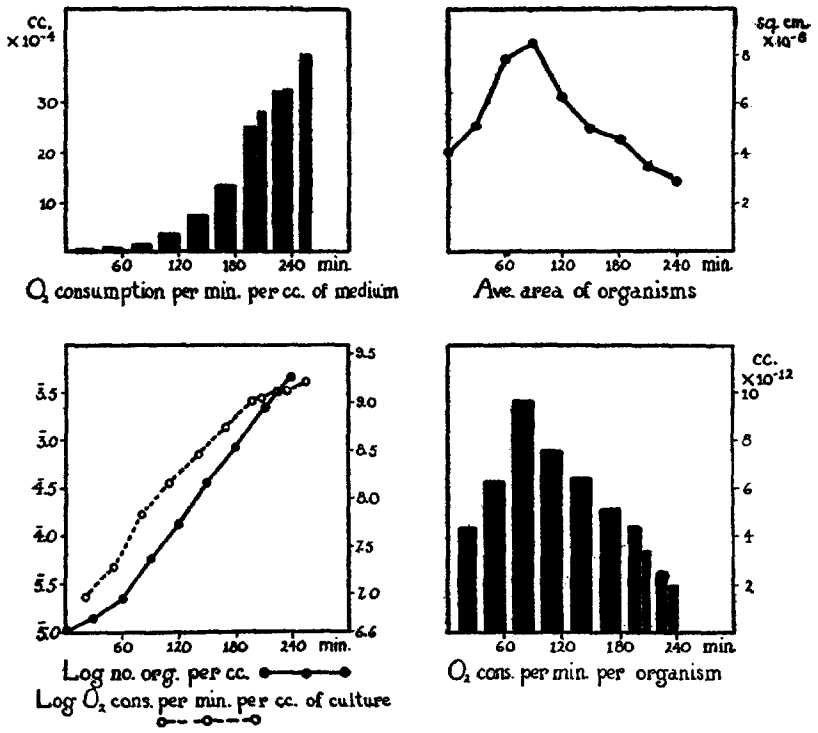

FIG. 2. Graphs of data in Table I.

TABLE II

Inoculum 0.05 Cc. of an 18.0 Hour Peptone Water Culture Containing 503 × 10^6 Organisms Per Cc.

(*a*)

Time from inoculation	No. of organisms per cc. × 10^6	Log. No. of organisms per cc.	Average length of organisms	Average diameter of organisms	Average area
min.			*cm.* × 10^{-4}	*cm.* × 10^{-4}	*sq. cm.* × 10^{-8}
0	4.98	6.697	1.95	0.47	3.23
30	9.44	6.975	2.17	0.49	3.71
60	13.85	7.141	3.15	0.65	7.10
90	53.12	7.725	2.67	0.59	5.49
120	125.6	8.099	2.39	0.59	4.98
150	285.0	8.455	2.13	0.58	4.41
180	650.0	8.813	1.87	0.55	3.71
210	1125.0	9.051			
240	2125.0	9.327			
270	3100.0	9.491			

(*b*)

Time from inoculation	O_2 consumption per cc. of medium during observed period	O_2 consumption per min. per cc. of medium	Log O_2 consumption per min. per cc. of medium	O_2 consumption per min. per organism
min.	*cc.* × 10^{-4}	*cc.* × 10^{-4}		*cc.* × 10^{-12}
10– 30	0.654	0.327	$\bar{5}.515$	4.26
40– 60	2.258	1.129	$\bar{4}.053$	9.24
70– 90	5.510	2.755	$\bar{4}.440$	7.86
100–120	9.670	4.835	$\bar{4}.684$	5.06
130–150	20.08	10.04	$\bar{3}.002$	4.56
160–180	43.34	21.67	$\bar{3}.336$	4.33
190–210	48.70	24.35	$\bar{3}.386$	2.58
220–240	54.82	27.41	$\bar{3}.438$	1.58
250–270	51.70	25.85	$\bar{3}.412$	0.94
280–300	46.92	23.46	$\bar{3}.370$	

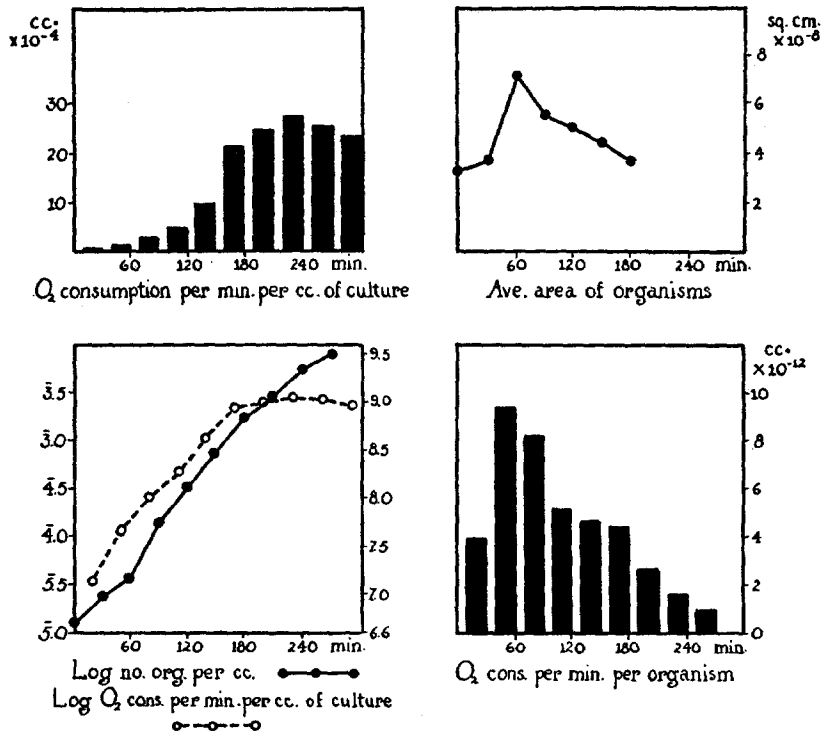

FIG. 3. Graphs of data in Table II.

TABLE III

Inoculum 0.02 Cc. of a 20.0 Hour Peptone Water Culture Containing 565 × 10^6 Organisms Per Cc.

(*a*)

Time from inoculation	No. of organisms per cc. × 10^6	Log No. of organisms per cc.	Average length of organisms	Average diameter of organisms	Average area
min.			*cm.* × 10^{-4}	*cm.* × 10^{-4}	*sq. cm.* × 10^{-8}
0	2.25	6.352			
30	2.50	6.398			
60	3.80	6.580	2.05	0.50	3.61
90	5.5	6.740	2.51	0.52	4.52
120	11.0	7.041	3.45	0.58	6.82
150	28.0	7.447	2.66	0.51	4.67
180	68.75	7.837	2.41	0.53	4.45
210	155.0	8.190	1.80	0.52	3.36

(*b*)

Time from inoculation	O_2 consumption per cc. of medium during observed period	O_2 consumption per min. per cc. of medium	Log O_2 consumption per min. per cc. of medium	O_2 consumption per min. per organism
min.	*cc.* × 10^{-3}	*cc.* × 10^{-4}		*cc.* × 10^{-12}
10– 30	0.260	0.130	$\bar{5}.114$	5.37
40– 60	0.368	0.184	$\bar{5}.265$	5.53
70– 90	0.600	0.300	$\bar{5}.477$	6.17
100–120	1.892	0.946	$\bar{5}.976$	10.74
130–150	3.848	1.924	$\bar{4}.284$	9.23
160–180	7.790	3.895	$\bar{4}.591$	7.54
190–210	14.00	7.000	$\bar{4}.845$	5.85

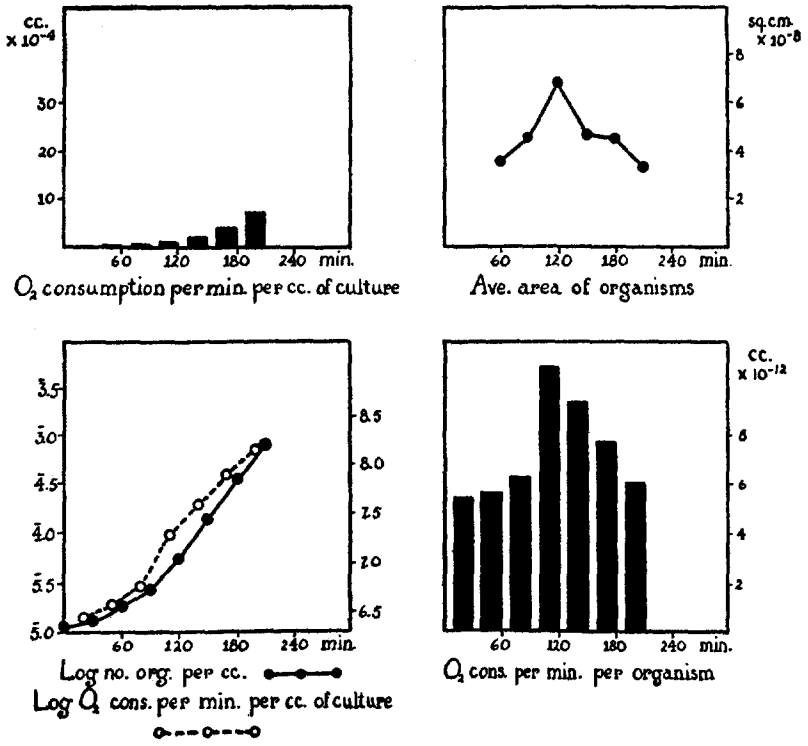

FIG. 4. Graphs of data in Table III.

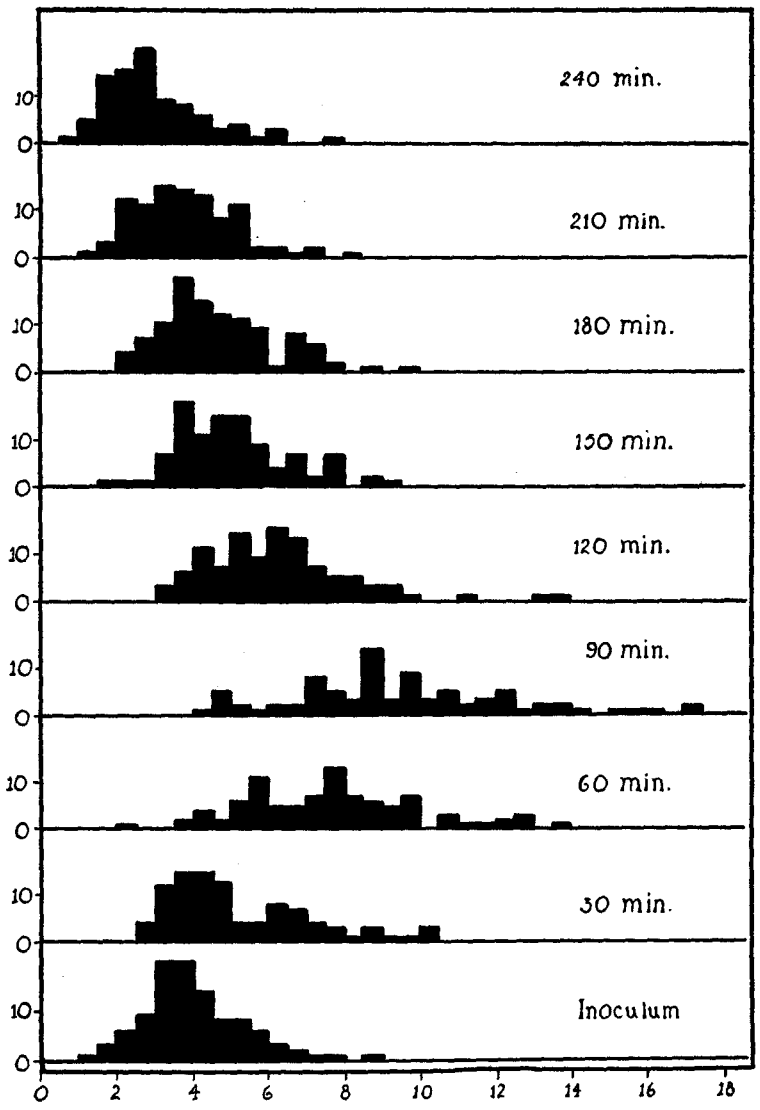
240 min.
210 min.
180 min.
150 min.
120 min.
90 min.
60 min.
30 min.
Inoculum
10
0
0 2 4 6 8 10 12 14 16 18
Area in sq. cm. x10⁻⁸

FIG. 5

DISCUSSION

Rate of Growth.—Under the conditions of these experiments, the rate of growth was usually very rapid. In one experiment (Table I, Fig. 2) an average generation time of 23.4 minutes was maintained, the culture 4 hours after inoculation attaining a concentration of 1850 $\times 10^6$ organisms per cc. This may be contrasted with the cell count of the inoculum which was 421 $\times 10^6$ organisms per cc. after 12 hours' growth in the same medium but under considerably less oxygen tension in the incubator. Whether this stimulating effect was due to the increased availability of oxygen or to the prompt removal of formed CO_2 by the NaOH was not studied. There were marked differences in the growth rates, which generally varied, as might be expected, with the age and amount of inoculum. The lag phase in these experiments lasted from 1 to $1\frac{1}{2}$ hours before the logarithmic phase began.

Oxygen Consumption.—In general, the rate of oxygen consumption of the culture was proportional to the rate of growth of the cells in the culture, highest values being obtained in those experiments in which growth was most rapid. In nearly all experiments, the rate of oxygen consumption continued to rise throughout the observed period, but in several instances (Table II, Fig. 3), there was a slight falling off in the rate of oxygen utilization near the end of the experiment.

A comparison of the logarithmic curves of the rates of growth and rates of oxygen consumption is interesting. It will be noted that the logarithmic curve of the rate of oxygen consumption becomes a straight line consistently earlier than does the logarithmic growth curve, and in about one-half the experiments (Figs. 2 and 3) this logarithmic phase is apparently present at the time of the first observation—20 minutes after inoculation. The difference between these curves becomes more apparent when the rate of oxygen consumption per organism is calculated.

A period of increased metabolic rate per organism occurred near the end of the lag phase from 1 to 2 hours after inoculation. In Table I, the maximum rate was found 80–100 minutes after inoculation, in Table II it occurred from 40–60 minutes after, and in Table III 100–120 minutes from the start of the experiment. Most of the experiments showed this phase to be during the 80–100 minute period of

observation. The protocol in Table III was from the only experiment in which the maximum was reached as late as 2 hours after inoculation. When the lag phase was prolonged the appearance time of this period of maximum rate of oxygen consumption was delayed.

Bayne-Jones and Rhees (1929) in their study of the heat production of a growing culture of *Esch. coli* noted an increased heat production per cell 2 hours after inoculation. Their figures were based on the total heat production per cell, calculating the total heat produced from the inoculation to time T. Recalculating their figures on the basis of the rate of heat production per cell modifies the shape of their curves to only a slight extent, still showing a period of markedly increased metabolic rate per cell in the early stages of growth.

Although the surface-volume ratio of the culture medium in Bayne-Jones' calorimeter was much less than that in the respirometer used in these experiments, and the rate of growth was correspondingly less, it is interesting to compare the absolute values obtained by direct calorimetry with those obtained by the indirect method.

Table 2, page 131, of the paper by Bayne-Jones and Rhees (1929) presents the data obtained on the heat production of the same strain (S) of *Esch. coli* in 2 per cent peptone water. Calculating the heat production from the 1st to 2nd hour (the period of maximum rate of heat production) it is seen that the total heat produced by the 100 cc. of culture was 7.76 gm. cals. The bacterial count at the beginning of this period was 20.5×10^6 organisms per cc. and at the end 45.4×10^6 per cc. Calculating from Buchanan's formula, the value 4.13×10^{-11} gm. cals. per minute per organism is obtained. Since the R.Q. is not known, the average R.Q. of protein oxidation (0.82) may be assumed for the purpose of calculation, which gives 4.825 as the caloric equivalent of 1 cc. of oxygen. Reducing gm. cals. to cc. oxygen, this figure becomes 8.56×10^{-12} cc. oxygen consumed per cell per minute. This is within 15 per cent of the average value obtained by indirect calorimetry during the period of maximum oxygen consumption per cell. Although the exact extent of the oxidative processes as compared with anaerobic processes taking place in the culture under these conditions has not been investigated, the close agreement between the figures obtained by these totally different methods at least suggests

that the cells derive a considerable portion of their energy from an oxidative metabolic process.

Additional evidence toward this point is brought by Walker and Winslow (1932), working on the carbon dioxide and nitrogen output of an aerated culture of bacteria of the colon group. In their abstract they state that "late in the preliminary lag period and in the phase of logarithmic increase the culture produced 40 to 100 $\times$ 10^{-11} mgm. of CO_2 per cell per hour." Reducing these figures to CO_2 per cell per minute they become 3.39 $\times$ 10^{-12} to 8.48 $\times$ 10^{-12} per minute per organism.

The similarity in magnitude of the figures obtained from direct calorimetry, carbon dioxide production, and oxygen consumption by entirely different methods and by different observers is striking.

Wohlfeil (1930, *b*) found that the oxygen consumption per cell decreased with time. When he plotted the relative oxygen consumption per cell as a function of the bacterial count he obtained the same shape of curve as he had previously found in measuring the oxygen consumption of saline suspensions of non-multiplying bacteria in varying concentrations. He concluded from this, that:

> "The bacteria respire during growth at the beginning more intensively, not because they need more oxygen for the building up of their body substance, but only because there are fewer microbes present in the unit space and because there is more oxygen available per organism."

There are several possible explanations of his failure to find a low rate of oxygen consumption per cell in the very first stages of growth as compared with the increased rate near the end of the lag phase. One source of error may have been the relatively long periods of time over which the oxygen consumption was measured; *i.e.*, the period of low initial rate per cell may have been so short that it was missed entirely. It is also possible that the failure to correct for the oxygen consumption of the medium *per se*, which has been shown can be as great as that of the bacteria during the initial stages, might have given this apparent high initial rate per cell.

Surface Area.—The variations in size of the organisms in growing cultures have been studied by Henrici (1928), and Jensen (1928). The same phenomenon was noted in these experiments, but with the

added observation that the period of maximum rate of oxygen utilization per cell coincided in every instance with the period of maximum surface area of the organisms. This is brought out in Figs. 2-4, where the maximum surface area appeared at three different time intervals, namely, 90, 60, and 120 minutes after inoculation. Calculations based on the relations of surface area and oxygen consumption have been disappointing as the variations were too great to permit drawing quantitative conclusions. Some of the difficulties faced in determining an average size for the organisms in a single smear will be apparent from a glance at a frequency curve (Fig. 5) obtained from the same experiment as that in Table I. There is a very large "spread" in the size of the cells in a single smear. This is, of course, to be expected when it is considered that a large cell just before division has almost twice the surface area one of the daughter cells will have a few moments later. This great variation prohibits drawing quantitative conclusions on the basis of surface area unless many times as many cells are measured. It is interesting to note that the average area of the cells 4 hours after inoculation was less than that of the 12 hour old inoculum. This may be related to the fact that there were more than four times as many cells per cc. at this time than there were in the inoculum.

The similarity in the shape of the area-time curve and the shape of the curve of the rate of oxygen consumption per organism suggests that the metabolism of the cell is related in some way to the surface area of the cell, but the data presented do not bear this out quantitatively. There are so many other factors of great importance in determining the rate of energy metabolism that further conclusions seem unjustified.

SUMMARY

The oxygen consumption of rapidly growing cultures of *Esch. coli* (S) have been measured by means of Fenn's respirometer.

The rate of oxygen consumption of a growing culture uniformly attains a phase of logarithmic increase before the growth curve of the organisms becomes logarithmic.

The rate of oxygen consumption per cell increases rapidly from the time of inoculation to a point of maximum respiration near the end of the lag phase of the growth curve, followed by a gradual decrease in the respiratory rate.

The surface area of the average cell when plotted against time passes through a point of maximum surface area which coincides with the point of maximum oxygen consumption per cell.

Figures obtained by different methods, CO_2 output and heat production when reduced to the same units, agree remarkably well.

CONCLUSIONS

The measurement of oxygen consumption of a growing bacterial culture is easily and accurately measured by use of Fenn's respirometer.

Esch. coli (S) in a growing culture passes through a stage of increased metabolism per cell, this stage occurring near the end of the lag period at a time when the individual cells have the greatest size and surface area.

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