A COMPARISON OF THE METABOLIC ACTIVITIES OF AEROBACTER AEROGENES, EBERTHELLA TYPHI AND ESCHERICHIA COLI

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The rate of oxygen consumption per cell in actively growing broth cultures of *Escherichia coli* has been reported by Martin (1932) to reach a maximum value near the end of the lag period of growth at a time when the individual cells have the greatest volume and surface area. Walker and Winslow (1932) reported similar observations on carbon dioxide and ammonia production in peptone and lactose-peptone cultures of *E. coli*, maximum metabolic rates per cell being observed towards the end of the lag period of growth. Walker, Winslow, Huntington and Mooney (1934) concluded that the high rates of carbon dioxide and ammonia production observed in the late lag period of growth could only in part be explained by the increased cell size observed during the same period. Comparison of their results with studies on cell size reported by Henrici (1928), Martin (1932) and Bayne-Jones and Adolph (1932) suggested that while the size of the cells at their maximum near the end of the lag period was not over ten times that at their minimum during the latter phases of growth, yet the rates of carbon dioxide and ammonia production per cell were fifty to one hundred times higher than those observed during the maximum stable period of growth. These observations, together with studies by Mooney and Winslow (1935) on carbon dioxide and ammonia production in cultures of *Salmonella pullorum* and *Salmonella gallinarum* suggested that there is a period of "physiological youth" in bacterial cultures as postulated by Sherman and Albus (1923)

which is characterized not only by larger cells but also by cells that are much more active metabolically per unit of living matter.

Wohlfeil (1930) from his studies on oxygen consumption concluded that the rate of bacterial respiration is higher during the early phases of growth because there are fewer cells present per unit volume and therefore more oxygen is available per organism. An hypothesis similar to that advanced by Wohlfeil was presented by Clifton, Cleary and Beard (1934) in their studies on oxidation-reduction potentials and ferricyanide reducing activities in peptone cultures and suspensions of *E. coli*. Clifton (1936), in a preliminary report, presented additional evidence that the concentrations of the reactants must be considered in any interpretation of the rates of metabolic activity in bacterial cultures.

Since the concentrations of reactants apparently influence the rate of metabolic activity in bacterial cultures as suggested by the studies on oxidation-reduction potentials and ferricyanide-reducing activities in cultures and suspensions of *E. coli*, the studies were extended to include oxygen consumption and carbon dioxide production with the same organism and in the same medium. The studies to be reported here also represent a comparison of these metabolic activities of *E. coli*, and factors influencing their values, with those observed in cultures and in suspensions of *Aerobacter aerogenes* and *Eberthella typhi*.

EXPERIMENTAL

Time-potential relationships

The oxidation-reduction potentials, viable populations and hydrogen ion concentrations developed in aerobic cultures of *Aerobacter aerogenes* (A-5), *Eberthella typhi* (I-4) and *Escherichia coli* (K-12) in a 1.0 per cent Difco peptone, 0.5 per cent sodium chloride medium (pH 7.0) at 37.5°C. were determined by the methods described by Clifton, Cleary and Beard (1934). Typical time-potential, time-population and time-pH relationships observed in stationary and in continuous flow (250 ml. daily) cultures of these bacteria are presented in figure 1.

Ferricyanide reduction and oxygen consumption by samples of stationary and continuous flow cultures

Nine and one-half milliliter samples of the cultures described above were placed in the reduction tubes described by Clifton, Cleary and Beard (1934) and deaerated with nitrogen for one-half hour before the addition of 0.5 ml. of a freshly prepared solution consisting of three parts of 0.1 molar potassium ferricyanide and one part of 0.1 molar potassium ferrocyanide. The rate of

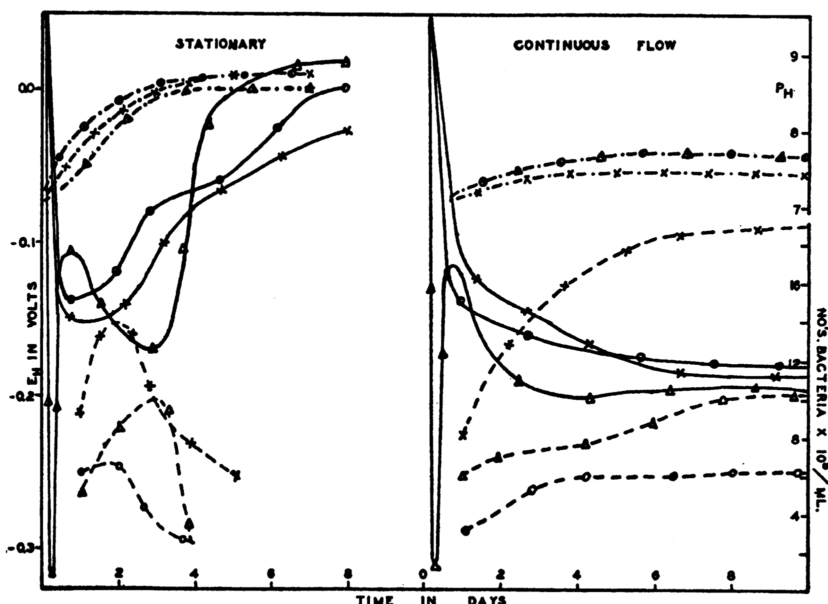

FIG. 1. Time-potential (————), time-population (- - - - - -), and time-pH (.-.-.-) relationships observed in aerobic peptone cultures of *A. aerogenes* (△), *E. typhi* (○) and *E. coli* (×) at 37.5°C.

reduction of the ferricyanide was calculated from the time required to reduce the concentration of ferricyanide from 0.003 to 0.002 M, the ratio of ferricyanide to ferrocyanide and consequently the amount of ferricyanide present at any time being calculated directly from the observed potentials.

Two milliliter samples of the same cultures were placed in Warburg flasks and the oxygen consumption was determined by the usual Warburg technic (1931), the carbon dioxide pro-

duced being absorbed by 5.0 per cent potassium hydroxide. These flasks were shaken for 5 minutes at 37.5° at a rate of 110 cycles per minute with an amplitude of 4 cm. The stopcocks on the manometers were then closed and the vessels shaken for an additional 10 minutes before the first readings were taken. The oxygen consumption was then determined at intervals of 20 minutes. A marked tendency was noted for the oxygen consump-

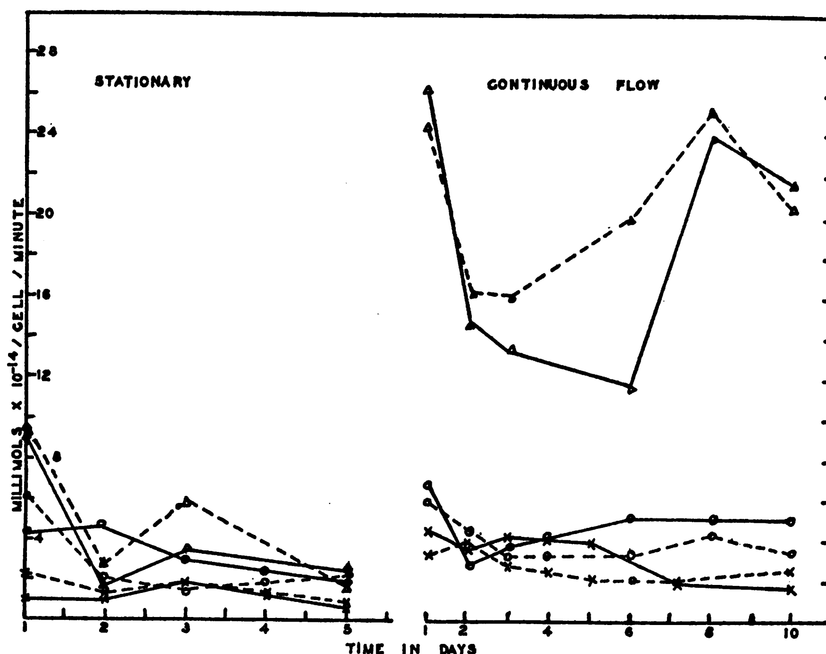

FIG. 2. Millimols $\times$ 10^{-14} of oxygen consumed (————) and of ferricyanide reduced (----------) per cell per minute by samples from stationary and continuous flow cultures of *A. aerogenes* (△), *E. typhi* (○) and *E. coli* (×) in a 1.0 per cent peptone, 0.5 per cent sodium chloride medium at 37.5°C.

tion of the samples to decrease with time, this decrease being most pronounced in the samples from the older cultures. A marked increase in oxygen consumption was observed when concentrated peptone was added to the cultures, this increase in oxygen consumption of the cultures being generally accompanied by growth of the organisms.

Typical results comparing the influence of the age of the

cultures on the rate of oxygen consumption per cell per minute for the first 20-minute period and the rate of ferricyanide reduction are recorded in figure 2. The oxygen consumption or ferricyanide reduction by filtrates of the cultures was negligible during the test periods.

The results presented in figure 2 indicate that the amounts of oxygen consumed or of ferricyanide reduced per cell by a given culture are of the same order of magnitude. Both the rate of oxygen consumption and of ferricyanide reduction tend to decrease as the average age of the cells increases in the stationary cultures. The oxygen consumption or ferricyanide reduction by stationary cultures older than five days was negligible. As the age of the stationary cultures increased the observed oxidation-reduction potentials also shifted to more positive values than those observed during the period of marked metabolic activity. The potentials in the continuous flow cultures were maintained at a higher reducing intensity and the rates of oxygen consumption and of ferricyanide reduction per cell were in general higher than in the stationary cultures.

Metabolic activity of suspensions of "resting" bacteria

Forty-eight hour cultures of *A. aerogenes*, *E. typhi* and *E. coli*, grown at 37.5°C. on nutrient agar in Kolle flasks, were suspended in physiological saline and washed three times by centrifugation. The cultures were then suspended in mixtures of equal parts of M/15 phosphate buffer, pH 7.4, and physiological saline to give suspensions of approximately the same turbidity. These suspensions were then incubated for 2 hours at 37.5°C. in shallow sterile dishes in order that the cells might utilize any residual foodstuffs.

Measured volumes of these suspensions were introduced into the reduction tubes and appropriate amounts of buffer, saline and substrate added. The concentrations of phosphate buffer and of saline were maintained constant. These suspensions were deaerated with nitrogen for one-half hour before the addition of the 0.075 molar potassium ferricyanide, and 0.025 molar potassium ferrocyanide solution employed in these tests. The results

obtained with typical suspensions of *A. aerogenes*, *E. typhi* and *E. coli*, illustrating the influence of different concentrations of peptone, ferricyanide and viable cells on the rate of ferricyanide reduction are recorded in table 1. The rate of reduction of the ferricyanide was calculated from the time required to reduce the concentration of ferricyanide from 0.003 to 0.002 M.

TABLE 1

Influence of the concentration of peptone, oxidant and organisms on the rate of reduction of $K_3Fe(CN)_6$ at 37.5°C. by suspensions of "resting" bacteria, pH 7.4

Millimols × 10^{-14} $K_3Fe(CN)_6$ reduced per cell per minute

VIABLE COUNT	A. AEROGENES (13 × 10^9)	E. TYPHI (13 × 10^9)	E. COLI (16 × 10^9)
	Influence of peptone concentration†		
4.0 per cent	11.1	5.8	5.2
2.0 per cent	11.5	5.3	5.2
1.0 per cent	11.5	4.8	5.1
0.5 per cent	8.7	4.3	4.1
0.1 per cent	5.1		1.7
	Influence of initial ferricyanide concentration*		
0.225 millimols	11.9	5.6	5.6
0.150 millimols	11.5	4.8	5.1
0.075 millimols	9.5	1.7	2.8
	Influence of bacterial concentration*†		
1.5 × viable count above	8.6	4.3	4.6
1.0 × viable count above	11.5	4.8	5.1
0.5 × viable count above	13.5	5.4	6.3

* Peptone concentration, 1.0 per cent.

† Initial ferricyanide concentration 0.150 millimols per 20 ml.

Measured volumes of a suspension of well-washed bacteria in the phosphate buffer-saline solution were placed in the Warburg flasks and appropriate amounts of phosphate-buffer saline solution and peptone were added. The concentrations of buffer and saline were maintained constant during the studies on the influence of the concentration of bacteria, peptone or oxygen on

the oxygen consumption of the cells. All results were corrected for oxygen consumption by the medium and by the washed cells.

TABLE 2

Influence of the concentration of peptone on oxygen consumption of a suspension of E. coli during five consecutive twenty-minute periods

Cubic millimeters of oxygen consumed per 20 minutes per 2 ml. of suspension

TIME INTERVAL	CONCENTRATION OF PEPTONE					
	2.0	1.0	0.5	0.25	0.12	0.06
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0–20	37.2	36.7	34.7	31.4	23.8	12.8
20–40	59.1	60.0	41.7	33.1	10.6	6.5
40–60	94.4	63.5	52.8	20.0	9.0	2.8
60–80	96.5	63.8	38.8	13.1	3.5	1.4
80–100	114.5	77.6	19.3	8.0	3.5	

TABLE 3

Influence of the initial concentration of bacteria in suspensions of E. coli on oxygen consumption during eight consecutive twenty-minute intervals

Peptone concentration 1.0 per cent

Cubic millimeters of oxygen consumed per 20 minutes per 2 ml. of suspension

TIME INTERVAL	RELATIVE INITIAL NUMBER OF CELLS				
	12	8	4	2	1
minutes					
0–20	77.1	54.9	25.3	15.6	8.3
	(99.6)*	(66.4)	(33.2)	(16.6)	
20–40	76.1	80.3	51.6	27.5	15.8
40–60	109.8	84.2	64.3	35.3	26.5
60–80	126.1	120.6	74.2	43.9	40.8
80–100	137.2	136.0	112.7	68.9	57.8
100–120	86.0	128.8	137.2	103.7	98.8
120–140	75.8	76.8	73.5	83.6	143.3
140–160	38.7	53.2	68.0	44.8	76.6

* Represents amount of oxygen consumed per unit number of cells multiplied by the relative number of cells in the suspension.

The influence of peptone concentration on the amount of oxygen consumed is well illustrated in table 2 in which the

amounts of oxygen consumed in 5 consecutive twenty-minute periods are recorded, the initial numbers of "resting" bacteria being the same in each dilution of peptone.

The influence of the initial concentration of bacteria on the amount of oxygen consumed is illustrated in table 3, the initial concentration of peptone in each flask being 1.0 per cent.

Increasing the concentration of oxygen increased to a slight extent the amount of oxygen consumed per unit time. The amount of oxygen consumed was practically independent of the pH over the range studied, 6.4 to 8.4. The results presented in tables 1 to 3, together with similar results on oxygen consumption obtained with suspensions of *A. aerogenes* and *E. typhi*, illustrate the marked influence of the concentration of peptone and of bacteria on ferricyanide reduction or oxygen consumption by suspensions of these bacteria. The results on oxygen consumption are complicated by the fact that marked growth of the cells occurred in concentrations of peptone greater than 0.25 per cent.

The marked influence of concentrations of peptone less than 0.5 per cent on both the rate of ferricyanide reduction and the rate of oxygen consumption and the influence of peptone concentration on the total oxygen consumption in a 100-minute period by suspensions of these bacteria suggest that the decrease in the rate of metabolic activity of bacteria observed as the age of the culture increases may in part be due to depletion of readily available foodstuffs. The rate of oxygen consumption by suspensions of *A. aerogenes*, *E. typhi* and *E. coli* in filtrates of their respective cultures (adjusted to pH 7.0) was considerably less than in fresh 1.0 per cent peptone and decreased during successive time intervals in a manner analogous to that reported in table 3 for suspensions of *E. coli* in the lower concentrations of peptone. Little or no multiplication of these organisms occurred after inoculation of filtrates of 48-hour or older peptone cultures under the conditions prevailing in the Warburg vessels (low CO_2 tension, marked tendency for pH to shift to more alkaline values) although growth occurred under ordinary test tube conditions.

Marked growth and oxygen consumption was observed in the same filtrates following the addition of small amounts of a concentrated peptone solution. These observations suggested that a more detailed study of the influence of the concentration of peptone on the growth and metabolic activities of these bacteria might aid in explaining the different rates of metabolic activity observed at different phases of the growth cycle.

GROWTH AND OXYGEN CONSUMPTION OF THE ORGANISMS AS INFLUENCED BY DIFFERENT CONCENTRATIONS OF THE REACTANTS

The oxygen consumption of cultures of *A. aerogenes, E. typhi* and *E. coli* in different concentrations of peptone was determined by the ordinary Warburg technic. The peptone solutions were inoculated with the test organism and 2-ml. samples were immediately placed in the Warburg vessels which were then attached to the manometers and placed in the water bath at 37.5°C. The gas mixtures in the flasks were replaced with CO_2-free air at frequent intervals during these tests in order to maintain a fairly uniform concentration of oxygen. The flasks were shaken at a rate of 130 cycles per minute with an amplitude of 4 cm. Control tests indicated that a rate of shaking greater than 130 cycles per minute did not appreciably increase the rate of oxygen consumption. Four-tenths of a milliliter of a 20 per cent potassium hydroxide solution was employed in the central cups of the vessels to absorb the carbon dioxide, the absorption of this gas being further facilitated by placing a roll of No. 40 Whatman filter paper in the hydroxide solution. The hydroxide solution was renewed at frequent intervals during the tests. Plate counts were made from duplicate control cultures at one-hour intervals during the period of rapid growth and at longer intervals as the age of the cultures increased. The pH of the cultures rapidly shifted to more alkaline values (pH 8.4 or higher) during the course of these experiments.

The oxygen consumption per cell per minute during the period of rapid growth was computed by the formula of Buchanan (1930) which may be represented as follows:

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

where m = oxygen consumed per cell per minute;
t = duration of time interval in minutes;
S = oxygen consumed by culture during time t;
B = number of bacteria present at start of time t, and
b = number of bacteria present at end of time t.

Since this equation is valid only during the logarithmic growth phase the arithmetical average of the number of cells present during a given interval of time was employed in calculating the rate of oxygen consumption per cell during the later phases of growth. All observed values for oxygen consumption were corrected for thermo-barometric changes and for oxygen consumption by the sterile media during the test periods.

Typical time-growth, time-oxygen consumption and time-oxygen consumption per cell per minute relationships are recorded in figure 3 for 1.0 per cent Difco peptone, 0.5 per cent sodium chloride cultures of *A. aerogenes*, *E. typhi* and *E. coli*. Similar time-oxygen consumption relationships were observed when the same organisms were grown on a nutrient agar surface in the Warburg flasks.

The oxygen consumption in cultures older than 20 hours (under the conditions of these tests) was of a very low order of magnitude but marked increases were observed following the addition of 0.1 ml. of a 10 or 20 per cent peptone solution to the cultures. This suggests that depletion of readily available foodstuffs may be a limiting factor for growth under the conditions of these tests along with the marked shift in hydrogen ion concentration of the cultures to pH values of 8.5 or higher.

The studies were extended to include the influence of a wide range of peptone concentrations (0.1 to 10.0 per cent), of filtrates of these cultures and of oxygen on the oxygen consumption of these organisms. Typical results obtained with *E. coli* as the test organism are presented in figure 4, together with a summary of the results obtained with *A. aerogenes* and *E. typhi*. All tests reported in figure 4 were conducted in the presence of air with

the exception of one culture in 5.0 per cent peptone in an atmosphere of oxygen.

The rate of growth of the bacteria increased slightly as the concentration of peptone or of oxygen was increased (same initial numbers of bacteria) while the total crop in 24 hours increased approximately four times as the concentration of

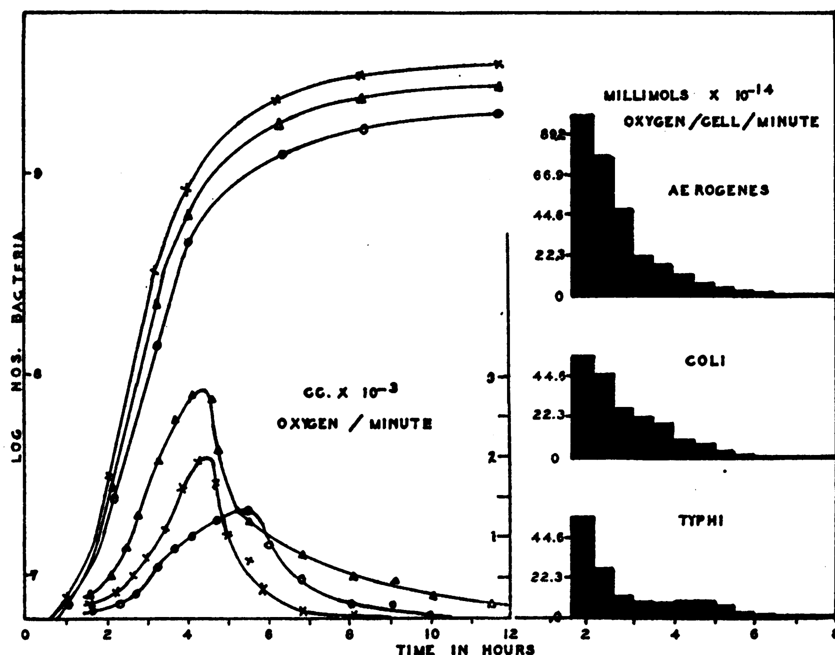

FIG. 3. Time-growth, time-oxygen consumption per minute per milliliter of culture and time-oxygen consumption per cell per minute relationships observed during the growth of *A. aerogenes* (△), *E. typhi* (○), and *E. coli* (×) in a 1.0 per cent peptone solution at 37.5°C. (44.6×10^{-14} millimols oxygen = 1.0×10^{-11} cc.).

peptone was increased from 1.0 to 10.0 per cent. The substitution of oxygen for air in the flasks markedly increased the oxygen consumption in the early phases of growth. However, the oxygen consumption fell to a low level earlier in the growth curve in the presence of oxygen as compared with air.

The total amounts of oxygen consumed by the three test organisms during the first ten hours in 1.0, 5.0 and 10.0 per cent

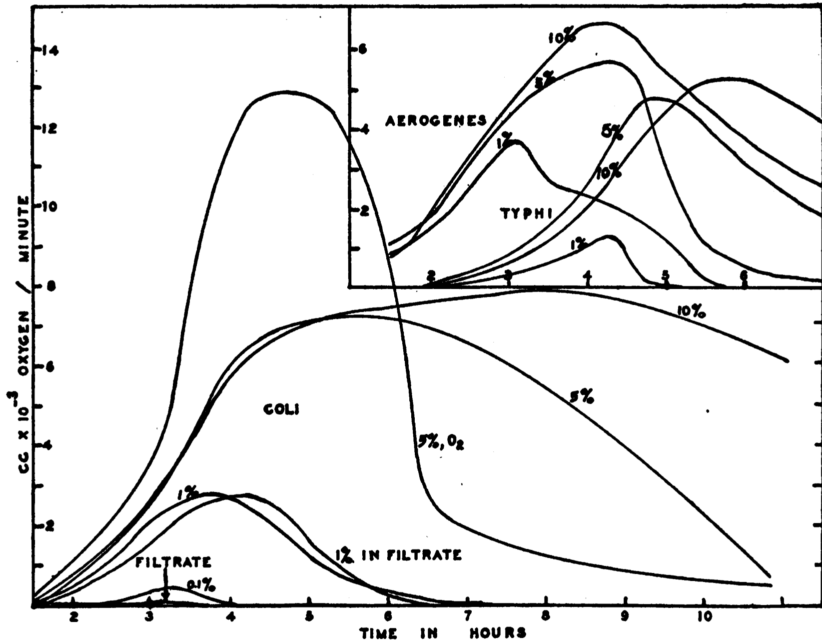

FIG. 4. The influence of peptone concentration, of a 48-hour filtrate (adjusted to pH 7.0) of a 1.0 per cent peptone culture of *E. coli* and of oxygen on the oxygen consumption per minute per milliliter by cultures of *E. coli*. The influence of peptone concentration on the oxygen consumption of *A. aerogenes* and of *E. typhi*.

TABLE 4

The influence of peptone concentration on the amounts of oxygen consumed per milliliter of cultures of A. aerogenes, E. typhi and E. coli during the first ten hours of growth in Warburg vessels at 37.5°C.

Cubic millimeters of oxygen consumed per milliliter of culture

ORGANISM	CONCENTRATION OF PEPTONE IN PER CENT		
	1.0	5.0	10.0
A. aerogenes	510	1,300	1,500
E. typhi	180	800	1,400
E. coli	430	2,400	3,300

peptone cultures were estimated from the graphs. The amounts of oxygen consumed per milliliter of the various cultures during the first ten hours are summarized in table 4.

In addition to the studies on oxygen consumption, the carbon

dioxide production of cultures of *E. coli* at 37.5°C. in 1.0, 5.0 and 10.0 per cent peptone was determined by the methods described by Walker (1932). Typical results are presented in figure 5.

The studies on the rate of carbon dioxide production by cultures of *E. coli* in peptone solutions of different concentrations show the same general time-activity relationships as observed in the studies on oxygen consumption and again illustrate the

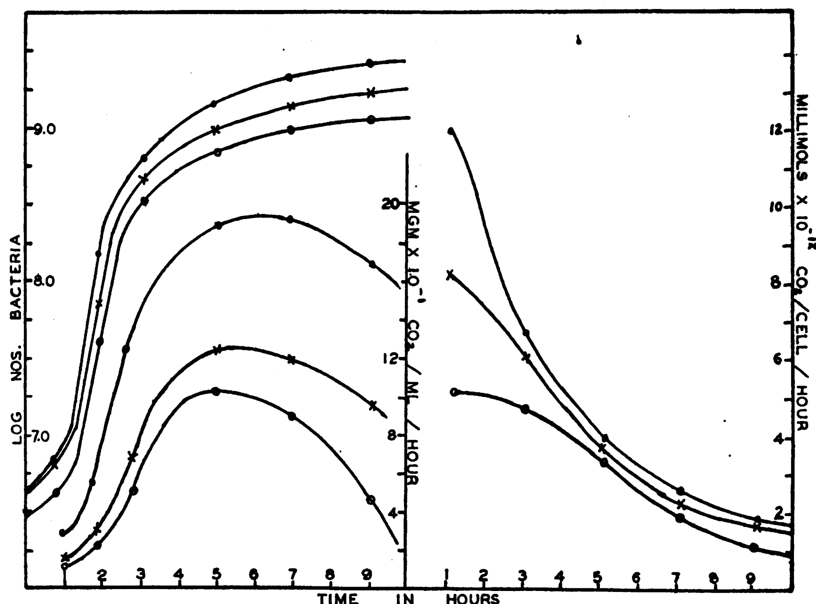

FIG. 5. GROWTH AND CARBON DIOXIDE PRODUCTION IN AERATED PEPTONE CULTURES OF *E. coli* AT 37.5°C.
1 per cent peptone (O——O), 5 per cent peptone (X——X) and 10 per cent peptone (●——●)

marked influence of concentration of foodstuffs on the metabolic activities of bacteria. The carbon dioxide production in 24-hour, 1.0 per cent peptone cultures of *E. coli* was markedly higher than the oxygen consumption in similar cultures as determined by the Warburg technic. However, the pH was only 7.5 in the 24-hour aerated culture employed in the carbon dioxide production tests as compared with 8.5 or higher in the oxygen consumption tests.

DISCUSSION

The time-potential relationships observed in ordinary and continuous flow cultures of *E. typhi* and *A. aerogenes* are similar to those previously reported for *E. coli*, with the exception of the high reducing conditions (-0.320 *v*) temporarily developed during the early stages of growth in cultures of *A. aerogenes*. These cultures also show the same general tendency to develop lower reducing intensities as the numbers of viable bacteria decrease in the stationary cultures and to maintain the reducing intensity quite constant and at a relatively high level in the continuous flow cultures.

The general tendency for the rate of reduction of ferricyanide per cell to decrease with increasing age of the cells, as previously reported for *E. coli*, is also observed in cultures of *A. aerogenes* and *E. typhi*. The highest rate of reduction in the cultures tested is observed in cultures of *A. aerogenes*. The rate of reduction per cell in cultures of *E. typhi* is higher than in similar cultures of *E. coli* but the studies with "resting" bacteria indicate that the order would be reversed if the actual concentrations of viable cells were considered.

The rate of reduction of ferricyanide per cell increases as the concentration of peptone or of ferricyanide is increased. This increase is not proportional to the concentration of peptone or of ferricyanide and holds only within certain limits, due to an apparent inhibitory action of high concentrations of peptone or of ferricyanide. The rate of reduction of ferricyanide per cell decreases as the concentration of bacteria is increased, within the range studied, other factors being maintained constant.

The rates of oxygen consumption per cell in samples of the stationary cultures are approximately equal to the rates of ferricyanide reduction and both tend to decrease with increasing age of the cells. The rates of oxygen consumption per cell are in general higher in the continuous flow cultures than in the stationary cultures and tend to remain at a fairly constant level after maximum growth is established.

The rate of oxygen consumption per cell in suspensions of the test organisms increases as the concentration or peptone is

increased, this increase being most marked when the concentration of peptone is less than 0.25 per cent, while the rate of oxygen consumption per cell tends to decrease as the concentration of bacteria is increased. These results, while somewhat complicated by growth of the cells during the tests, lend further support to the theory that the rate of the metabolic activities per cell in a given culture under favorable conditions is controlled by the concentrations and nature of the foodstuffs, oxidants and bacteria and that these factors play closely connected rôles in regulating growth and oxidation-reduction potentials in bacterial cultures.

The results of these studies on oxygen consumption and carbon dioxide production by cultures of *A. aerogenes*, *E. typhi* and *E. coli* are in general agreement with those reported by Martin (1932) and by Winslow and his coworkers (1932–1935) for cultures of *E. coli.* The rate of oxygen consumption or of carbon dioxide production per cell increases rapidly from the time of inoculation to a point of maximum activity near the end of the lag phase or early in the logarithmic period of the growth curve. A limited number of observations indicate that these bacteria also have a maximum size near this time. A marked decrease in the metabolic rates per cell is observed as the age of the cultures increases. However, the maximum rate of oxygen consumption or of carbon dioxide production per unit volume of the cultures is observed early in the negative acceleration in growth phase of the growth curve in 1.0 per cent peptone and later in the growth curve when the concentration of peptone is increased to 5.0 or 10.0 per cent.

Attempts to explain the observed differences in the rate of metabolic activity at various phases of the growth cycle on the basis of changes in cell size or in physiological activity of the cells have not been completely satisfactory. The first factor undoubtedly plays an important rôle in controlling the rates of metabolic activity per cell but the concentrations of the reactants must also be considered in any interpretation of studies on the rates of metabolic activity in bacterial cultures. As the numbers of bacteria increase in a culture, the total amount of oxygen consumed, of carbon dioxide produced or of ferricyanide

reduced per unit time increases to a maximum value determined by the concentrations of the reactants, the nature and size of the organisms and the physical and chemical influence of the environment on the cells. The decrease, increasing with age of the culture, in the rate of metabolic activity per cell may be interpreted on a probability basis. As the number of bacteria increases the concentration gradient of foodstuffs and of oxidants between the cell and its environment decreases. Therefore, the probability of sufficient materials being available per cell per unit time to provide, upon interreaction, for the maximum requirements of the cells decreases. The addition of concentrated foodstuffs to a culture in the later phases of growth increases the foodstuff concentration gradient per cell and produces an increase in the metabolic activities of the cells. This increase, however, is only of limited duration due to the high total energy demand of the large number of cells in the cultures.

The results obtained in the studies on oxygen consumption indicate the marked influence of the concentration of peptone, of bacteria and of oxygen on the metabolic activities of the cells and suggest that, within limits, an increase in concentration of foodstuff or of oxidant, increases the probability of sufficient materials being available per cell per unit time to meet the maximum requirements of the cell. The rapid decrease in the observed oxidation-reduction potentials in the cultures during the early phases of growth together with the maximum reducing intensity developed in or near the maximum stationary growth phase also suggest the marked influence of concentrations of reactants on the metabolic activities of the cells. Longsworth and MacInnes (1936) have presented evidence that there is a correlation between the observed oxidation-reduction potentials and the rate of metabolic activity (acid production at constant pH) in cultures of *Lactobacillus acidophilus*. The rate of acid production per cell in their cultures decreased markedly during the growth period, the generation time tended to increase and the observed potentials rapidly fell to more negative values. An increase in the initial concentration of sugar markedly increased the rate of acid production.

These various observations suggest that growth may be primarily controlled by the probability relationships discussed above (see also Cleary, Beard and Clifton, 1935). The growth rate decreases as the concentration per cell of materials essential for growth decreases in cultures of bacteria in which a relatively high population has been established. These results also suggest that the maximum population developed under favorable conditions, particularly pH and concentration of waste products, may be primarily controlled by the concentration relationships.

SUMMARY AND CONCLUSIONS

Growth, oxidation-reduction potentials, ferricyanide reduction, oxygen consumption and carbon dioxide production have been studied in cultures and in suspensions of *Aerobacter aerogenes*, *Eberthella typhi* and *Escherichia coli.*

The concentrations of peptone, organisms and oxidant play closely connected rôles in controlling the metabolic activities of the cells, as measured by the metabolic indices mentioned above.

The various observations suggest that the rate of bacterial metabolism per cell is highest during the early phases of growth because of the increased size of the cells and the higher concentration gradient of foodstuffs between the cells and their environment.

Additional support is presented for the hypothesis that the growth rate and the maximum population developed in a favorable environment may be primarily controlled by the probability of sufficient materials being available per cell per unit time to provide for the metabolic requirements of the cell.

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