THE PHYSIOLOGICAL YOUTH OF A BACTERIAL CULTURE AS EVIDENCED BY CELL METABOLISM[1]

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OBJECTIVES

In an earlier contribution from this laboratory (Walker and Winslow, 1932) it was shown that toward the end of the initial lag period in either peptone or lactose-peptone water, there is manifest an enormous increase in metabolic activity, particularly with respect to ammonia production. Formation of CO_2 per cell per hour is increased thirty to seventy fold and formation of NH_3 nitrogen fifty to one-hundred-and-fifty fold as compared with the peak stability rates. There is, in these increases, a very clear demonstration of the physiological youth of the bacterial cells as postulated by Sherman and Albus (1923).

These phenomena seemed so important as to warrant further and more detailed study under different growth conditions and with observations made at more frequent intervals during the course of the growth cycle.

METHODS

The culture employed was the same strain of *Esch. coli* used in previous studies in this laboratory. The media for the growth and metabolism experiments were peptone-water (1 per cent Difco Bacto peptone) and glucose-peptone water (0.5 per cent Baker's c.p. glucose plus 1 per cent Difco Bacto peptone). Starting at a

[1] This study was assisted by a grant from the Research Fund of the Yale School of Medicine. The data on ammonia production are from an essay presented by Evelyn Huntington in partial fulfilment of requirements for the Certificate in Public Health of Yale University.

pH of 6.9 to 7.2 one series of studies on CO_2 production and one on ammonia production was conducted with each of these media under continuous aeration with air which had been freed from CO_2 and NH_3. The aeration train used for this purpose was the one described by Walker (1932). A fifth series of experiments on NH_3 production was made without aeration, in the first medium (peptone water) only.

Prior to inoculation of each test medium the organism was washed off from twelve to eighteen hour agar slants with sterile water, filtered through paper to remove clumps, diluted appropriately and added to the desired medium in Dreschel bottles so as to give an average initial count of 10 to 20 million organisms per cubic centimeter. Two duplicate culture bottles and a third uninoculated control bottle were placed in a water bath at 37°C. and aerated for at least thirty minutes to ensure stable conditions before inoculation. The duration of periodic observations after inoculation was five hours, which carried the culture well toward, or slightly past, the end of the phase of logarithmic increase. In the NH_3 experiments the culture was plated for cell counts and chemical determinations made every half hour (in the CO_2 studies, every hour).

In the three series of NH_3 studies, NH_3—N formed in the menstrum was determined by analysis of the medium only, since our earlier work had shown that the amount of ammonia carried over in the aeration train is negligible in the early phases of the life cycle (Walker and Winslow, 1932). The NH_3—N was determined by the Van Slyke and Cullen modification of Folin's aeration method with the minor changes suggested by Walker (Walker and Winslow, 1932).

In the two series of studies of carbon dioxide, we determined both the carbon dioxide in the medium and that carried off in the aeration train, the former figure being obtained by the use of the fine-bore blood gas apparatus described by VanSlyke and Stadie (1921), the latter by absorption in standard $Ba(OH)_2$ in a modified form of the Brady-Meyer[2] tube used by Walker and Winslow (1932). All

[2] Changes had to be made in the Brady-Meyer absorption tubes in the present studies in order to accomplish reasonably complete detention of the small amounts

quantitative analytical determinations for CO_2 and NH_3 throughout all analytical sampling periods were applied simultaneously to the cultures and to the uninoculated control medium and the yields reported represent the excess over the mean of all the control determinations of a given experiment.

The yield of ammonia or carbon dioxide per cell per hour during a given period was computed by the formula of Buchanan (1918) as used by Walker and Winslow (1932).

$$r = \frac{P\,(\log b - \log B)}{0.434\,(b - B)\,t}$$

where r = the amount of product per cell per hour
t = duration of period in hours
P = total product formed during time t
B = number of bacteria at start of time t
b = number of bacteria at end of time t

This formula was designed to estimate the effective population during the period of logarithmic increase and is more accurate for that period than a formula based on the mean of initial and final

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of CO_2 aerated off in single early hours. To this end, special tubes were constructed with the bulbs considerably smaller than those of the ordinary Brady-Meyer tube. The stem was also lengthened to fit into a 8 x 1⅛ inch test tube instead of the usual Erlenmeyer flask. For about 5 to 6 cm. from the bottom this stem was enlarged into a cylindrical chamber of 1 cm. diameter, with slightly increased bulbous portions at top and bottom and a small hole at the bottom. This stem was not pushed to the extreme end of the enclosing test tube. Dimensions of the whole assembly were then such that when 20 cc. was the volume of standard $Ba(OH)_2$ employed, about one third of the absorbent remained in the bottom of the test tube, one third occupied the cylindrical enlargement of the Brady-Meyer stem and the remaining third passed up into the succession of small bulbs making up the true Brady-Meyer part of the apparatus. The inlet from the culture to the absorption assembly was allowed to dip into the third of the $Ba(OH)_2$ remaining in the bottom of the large test tube. Thus CO_2 in the air current from the culture was subjected to a first absorption as it bubbled from the inlet up through the absorbent in the test tube, a second absorption as it passed along the surface of this fluid in order to enter the stem of the Brady-Meyer tube, a third absorption as it passed up the cylindrical chamber of the stem, and a final absorption as it was scrubbed through the bulbs of the Brady-Meyer tube proper. Hence, with this set-up, four serial extractions were achieved with a total absorbent of only 20 cc., which was easily rinsed down into and titrated in the single large test tube after use.

population. For the lag period it gives—so far as our data are concerned—results essentially identical with those of the simpler method of using the mean between initial and final count, and has therefore been employed throughout all the hourly or half-hourly observation periods.

GENERAL RESULTS

The general results of our experiments are summarized in tables 1 to 5. Each figure in each of these tables is the average of eight independent duplicate experiments. These duplicate experiments showed, in general, a reasonably satisfactory degree of uniformity, for phenomena of the type studied. The probable errors of the means, as cited in the tables for bacterial counts, are less than one-fourth of the recorded mean values in all but two instances and are generally far below this proportion, which is by no means bad for independent duplicate growth curves. The probable errors of the mean determinations of carbon dioxide are relatively lower still, (one sixth or less of the mean) except in the case of the results for the first hour. The ammonia data are

TABLE 1

Growth and ammonia yield in aerated peptone water

AGE	BACTERIA, MILLIONS PER CUBIC CENTIMETER		NH_3 N YIELD		
	Actual	Log	Increment	Cumulative total	Rate per cell per hour during previous interval Mgm. $\times 10^{-11}$
hours			*mgm. per 100 cc.*	*mgm. per 100 cc.*	
0	14.5 ± 2.8	1.16			
0.5	15.2 ± 2.6	1.18	0.06 ±0.04	0.06	7.9
1.0	15.2 ± 2.6	1.18	0.09 ±0.05	0.15	11.8
1.5	16.3 ± 4.2	1.21	0.40 ±0.06	0.55	50.0
2.0	22.8 ± 6.5	1.36	0.10 ±0.10	0.65	10.6
2.5	55.1 ± 14.2	1.74	0.10 ±0.14	0.75	4.0
3.0	92.3 ± 23.3	1.97	0.21 ±0.12	0.96	6.0
3.5	216.0 ± 54.2	2.33	0.14 ±0.12	1.10	1.9
4.0	333.0 ± 73.3	2.52	0.34 ±0.11	1.44	2.5
4.5	582.0 ±141.9	2.76	0.46 ±0.08	1.90	2.0
5.0	727.0 ±173.7	2.86	0.50 ±0.26	2.40	1.6

TABLE 2

Growth and ammonia yield in aerated glucose-peptone water

AGE	BACTERIA, MILLIONS PER CUBIC CENTIMETER		NH_3 N YIELD		
	Actual	Log	Increment	Cumulative total	Rate per cell per hour during previous interval mgm. $\times 10^{-11}$
hours			*mgm. per 100 cc.*	*mgm. per 100 cc.*	
0	10.4 ± 1.8	1.02			
0.5	10.1 ± 1.5	1.00	0.04 ±0.01	0.04	5.9
1.0	13.6 ± 3.0	1.13	0.02 ±0.00	0.06	5.1
1.5	15.7 ± 3.9	1.20	0.17 ±0.02	0.23	26.2
2.0	30.9 ±12.4	1.49	0.27 ±0.06	0.50	24.6
2.5	70.8 ±16.7	1.85	0.16 ±0.06	0.66	6.2
3.0	145.0 ±41.5	2.16	0.01 ±0.03	0.67	0.4
3.5	287.0 ±66.8	2.46	0.04 ±0.03	0.71	0.3
4.0	538.0 ±43.0	2.73	0.08 ±0.05	0.79	0.3
4.5	703.0 ±71.5	2.85	0.11 ±0.07	0.90	0.4
5.0	890.0 ±51.3	2.95	0.23 ±0.03	1.13	0.6

TABLE 3

Growth and ammonia yield in unaerated peptone water

AGE	BACTERIA, MILLIONS PER CUBIC CENTIMETER		NH_3 N YIELD		
	Actual	Log	Increment	Cumulative total	Rate per cell per hour during previous interval mgm. $\times 10^{-11}$
hours			*mgm. per 100 cc.*	*mgm. per 100 cc.*	
0	10.9 ± 3.8	1.04			
0.5	10.4 ± 1.2	1.01	0.02 ±0.00	0.03	5.8
1.0	10.0 ± 1.6	1.00	0.05 ±0.00	0.07	7.8
1.5	9.7 ± 0.9	0.99	0.16 ±0.05	0.23	31.9
2.0	16.0 ± 3.3	1.20	0.34 ±0.11	0.57	54.0
2.5	34.0 ± 4.4	1.53	0.10 ±0.03	0.67	8.4
3.0	62.8 ± 7.2	1.80	0.09 ±0.02	0.76	3.4
3.5	87.5 ± 8.0	1.94	0.10 ±0.09	0.86	2.7
4.0	103.0 ±11.9	2.01	0.31 ±0.11	1.17	7.3
4.5	109.0 ±12.3	2.04	0.23 ±0.09	1.40	4.3
5.0	166.0 ± 5.9	2.22	0.20 ±0.05	1.60	3.0

much more variable, the probable error sometimes equalling the mean value. This is to be expected on account of the very small

amounts involved. In any case, these random errors do not affect certain very large differences involved. The smoothness of the curves and the close check of the curves for various media give evidence of the validity of the general relationships deduced.

TABLE 4

Growth and carbon dioxide yield in aerated peptone water

AGE	BACTERIA, MILLIONS PER CUBIC CENTIMETER		CO_2 YIELD		
	Actual	Log	Increment	Cumulative total	Rate per cell per hour during previous interval mgm. $\times 10^{-11}$
hours			*mgm. per 100 cc.*	*mgm. per 100 cc.*	
0	15.9 ± 2.2	1.20			
1	14.5 ± 2.1	1.16	0.54 ±0.24	0.54	37
2	22.9 ± 3.5	1.36	2.16 ±0.34	2.70	123
3	85.3 ±13.8	1.93	3.32 ±0.15	6.02	73
4	236.0 ±32.8	2.37	7.10 ±0.64	13.12	50
5	655.0 ±80.3	2.82	9.13 ±1.14	22.25	22

TABLE 5

Growth and carbon dioxide yield in aerated glucose-peptone water

AGE	BACTERIA, MILLIONS PER CUBIC CENTIMETER		CO_2 YIELD		
	Actual	Log	Increment	Cumulative total	Rate per cell per hour during previous interval mgm. $\times 10^{-11}$
hours			*mgm. per 100 cc.*	*mgm. per 100 cc.*	
0	17.3 ± 2.6	1.24			
1	17.1 ± 2.4	1.23	0.72 ±0.34	0.72	43
2	44.5 ± 9.6	1.65	3.29 ±0.54	4.01	117
3	172.0 ± 22.7	2.24	5.76 ±0.45	9.77	63
4	585.0 ± 40.7	2.77	11.81 ±0.44	21.58	35
5	861.0 ±101.8	2.94	11.07 ±0.28	32.65	16

GROWTH CURVES

The results, so far as bacterial growth and total cumulative yield of ammonia and carbon dioxide are concerned (columns 2 and 5 in the tables), are presented graphically in figures 1 and 2. It will be noted that the initial numbers of bacteria varied from

10.4 to 17.3 in millions per cubic centimeter; that these numbers generally dropped slightly during the first hour and then rose slightly during the next hour. In the three series with peptone

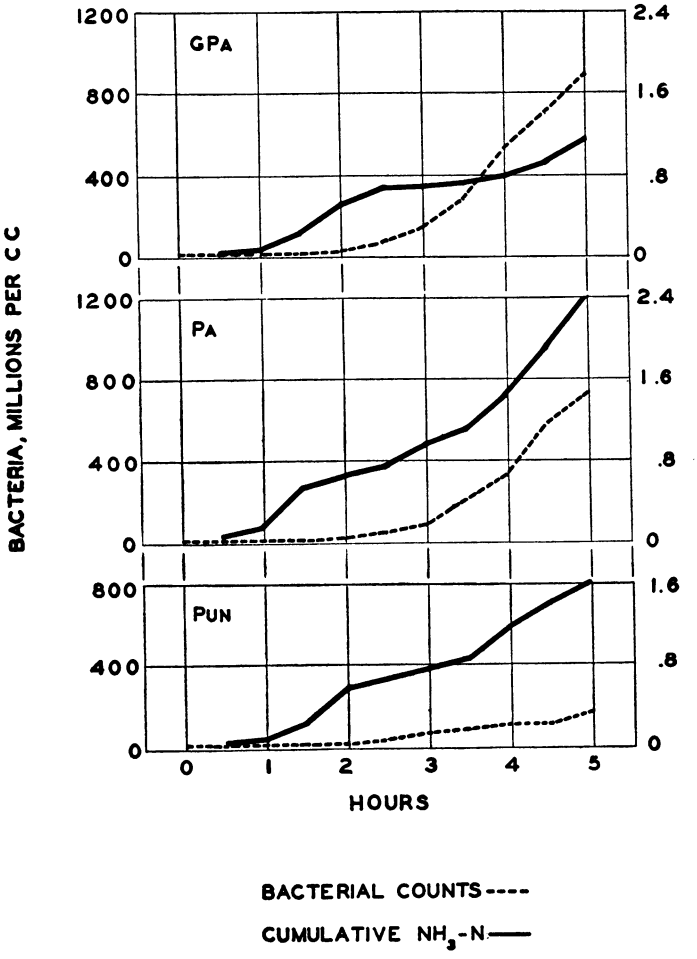

FIG. 1. BACTERIAL COUNTS AND CUMULATIVE YIELD OF AMMONIA NITROGEN
GPA = Glucose-Peptone Medium, aerated; PA = Peptone Water Medium, aerated; PUN = Peptone Water Medium, unaerated.

medium the lag period extended further, the two-hour count being less than 200 per cent of the initial count. In the glucose

peptone medium on the other hand the period of logarithmic increase began after 1.5 hours (see table 2). From the second to the third hour the population increased four-fold. This approximates most closely the true phase of logarithmic increase. After the third hour in the unaerated peptone medium and after the fourth hour in the other media, the rate of increase slackened.

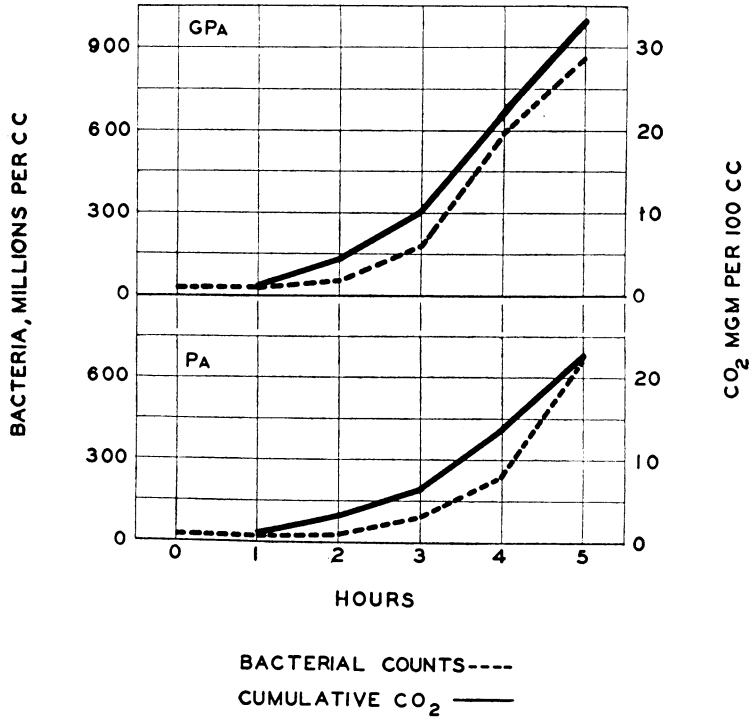

FIG. 2. BACTERIAL COUNTS AND CUMULATIVE YIELD OF CARBON DIOXIDE

GPA = Glucose-Peptone Medium, aerated; PA = Peptone Water Medium, aerated.

The final counts varied from 655 to 890 million per cubic centimeter, except in the case of the unaerated peptone medium where the number reached only 166 million. The effect of aeration in a peptone medium was, then, to prolong the period of logarithmic growth and to increase the final count. The effect of adding glucose to an aerated peptone medium was to initiate logarithmic

growth more promptly, without greatly affecting the ultimate population. The relative duration of the lag and logarithmic

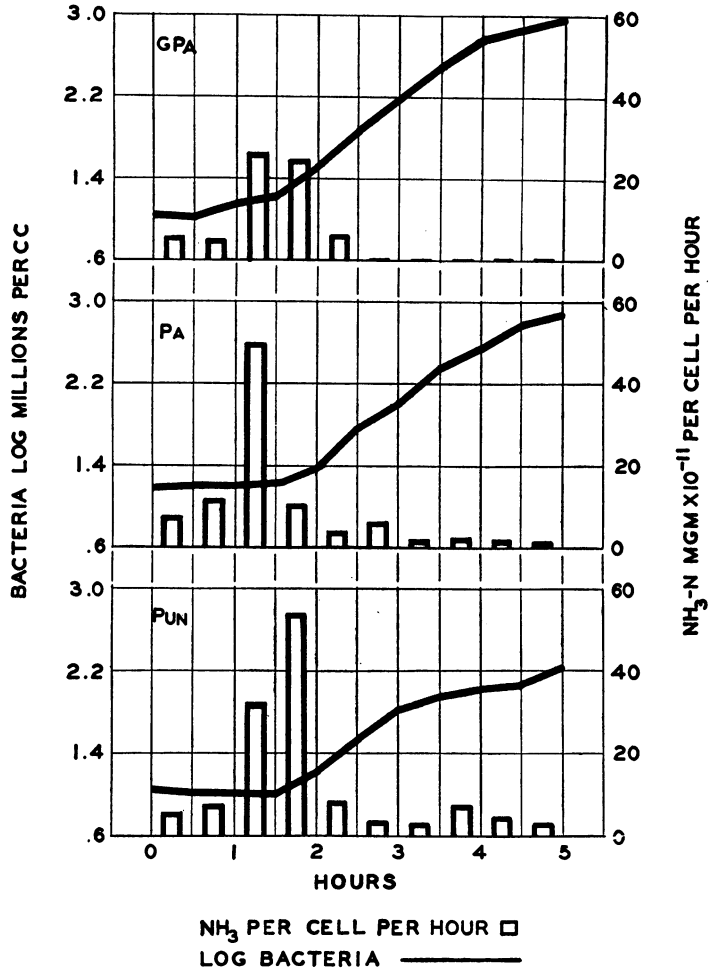

FIG. 3. LOGS OF BACTERIAL COUNTS AND YIELD OF AMMONIA NITROGEN COMPUTED PER CELL PER HOUR

GPA = Glucose-Peptone Medium, aerated; PA = Peptone Water Medium, aerated; PUN = Peptone Water Medium, unaerated.

periods and the characteristics and velocities of growth in the several experimental series may easily be compared in the logarithmic population curves of figures 3 and 4.

AMMONIA YIELD

It will be noted from figure 1 that the curve for the total ammonia nitrogen added to the medium by the bacteria goes up very sharply during the second hour—that is, just prior to the initiation of rapid logarithmic growth. The curve then flattens out some-

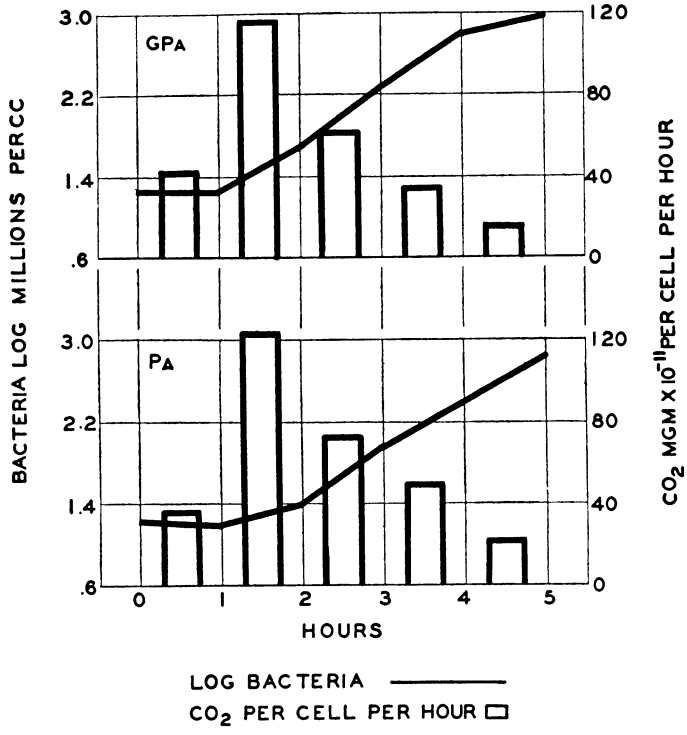

FIG. 4. LOGS OF BACTERIAL COUNTS AND YIELD OF CARBON DIOXIDE COMPUTED PER CELL PER HOUR

GPA = Glucose-Peptone Medium, aerated; PA = Peptone Water Medium, aerated.

what and later rises as the total bacterial population rises. It is obvious, however, even from these linear graphs, that something has happened to change the rate of bacterial metabolism during the late lag phase.

In figure 3 the course of the bacterial population has been plotted on a logarithmic basis, to bring out more clearly the

points of inflexion and relative rates of increase; and the ammonia yield has been indicated as a rate per cell per hour. The ammonia yield is greatest in the unaerated peptone medium, averaging for the whole five hours, 12.9 mgm. $\times 10^{-11}$ per cell per hour. It is somewhat lower in the aerated peptone, averaging 9.8 mgm. $\times 10^{-11}$ per cell per hour and is still lower in the aerated glucose-peptone medium, the value falling here to 7.0 mgm. $\times 10^{-11}$ per cell per hour. This last drop is, of course, consistent with the familiar sparing action of carbohydrates.

In all media, however, the burst of adolescent activity at the close of the lag period and the beginning of the period of logarithmic increase is very clearly shown. During the first hour the values for all three cultivation conditions were between 5.1 and 11.8. During the second hour they rose to averages of over 25 for the aerated glucose-peptone medium, over 30 for the aerated peptone medium and over 40 for the unaerated peptone medium. The sharpest peak occurred during the third half-hour in the aerated peptone and during the fourth half hour in the unaerated peptone medium; while it was divided equally between the third and fourth half hours in the aerated glucose-peptone medium. In the aerated peptone the peak came at the end of the lag period, in the unaerated peptone at the end of lag and the beginning of the logarithmic phases, in the aerated glucose-peptone medium at the beginning of the logarithmic period.

After the second hour (during the course of the logarithmic period) the values fell sharply again to rates between 0.3 and 8.4 mgm. $\times 10^{-11}$ per cell per hour.

This subsequent decline does not, of course, necessarily imply a correspondingly reduced production of ammonia per cell per hour. It is quite possible that, as the bacteria undergo more rapid multiplication, they may utilize in the synthesis of new protoplasm some of the already formed ammonia-nitrogen in the medium at a rate sufficient to mask the further destruction of peptone. The initial burst of activity is, of course, obvious; and we know from our earlier studies that a very low balance of ammonia yield is manifest in the stable phase of the population cycle. Just how fast the curve of actual ammonia production falls during the logarithmic growth phase is, however, uncertain.

CARBON DIOXIDE

Corresponding data with regard to carbon dioxide yields and rates are presented in figures 2 and 4, except that no tests were made without aeration, since with our technique the carbon dioxide could not be determined with accuracy without the use of an aeration train.

The average carbon dioxide yield for the whole five hours was 55 mgm. $\times 10^{-11}$ per cell per hour for the glucose-peptone medium and 61 mgm. for the plain peptone water. The average hourly rates and the ratios of the products are summarized in table 6. The rates for hourly ammonia do not correspond entirely to the averages of the

TABLE 6

Average hourly yield of ammonia and carbon dioxide in peptone and glucose peptone media (aerated)

HOURS	NH_3 N PER CELL PER HOUR MGM. $\times 10^{-11}$		CO_2 PER CELL PER HOUR MGM. $\times 10^{-11}$		RATIO CO_2/NH_3 N			
	Peptone	Glucose-peptone	Peptone	Glucose-peptone	Peptone	Glucose-peptone	Peptone	Glucose-peptone
					mgm.	*mgm.*	*mm.*	*mm.*
0–1	10.4	5.0	37	43	3.6	8.6	1.1	2.7
1–2	26.7	21.3	123	117	4.6	5.5	1.5	1.7
2–3	6.2	2.3	73	63	11.8	27.4	3.8	8.7
3–4	2.6	0.3	50	35	19.2	116.7	6.1	37.2
4–5	1.9	0.5	22	16	11.6	32.0	3.7	10.2

half-hourly figures in tables 1 and 2 since they have been here computed (for comparability with carbon dioxide) by applying the Buchanan formula to hourly increments and periods. In spite of the lower rate of ammonia production in the presence of glucose, the carbon dioxide yield is not only no greater but is actually less when sugar is present. During each of the last four hours the rate of carbon dioxide yield per cell was actually lower in the glucose-peptone medium than in peptone water. The average of the five periods is 61.0 mgm. $\times 10^{-11}$ per cell per hour in peptone water as compared with 54.8 mgm. in the glucose-peptone. In the study of Walker and Winslow (1932) the rate of carbon dioxide production was approximately the same in peptone and in lactose-

peptone media, likewise indicating failure of the cell to show increased CO_2 output in the presence of a fermentable carbohydrate.

As in our earlier work, the molar ratio of CO_2 yield to NH_3—N yield approximates one in the lag period and rises to much higher values in the logarithmic phase. This phenomenon, like others we have noted, may possibly be related to withdrawal of ammonia nitrogen from the medium by the rapidly-growing cells.

Figure 4 gives graphic emphasis to the fact that the bacteria during the first hour (lag period) produce carbon dioxide at a rate of 37–43 mgm. $\times 10^{-11}$ per cell per hour; while during the second hour the rate rises sharply to 117 to 123 mgm. $\times 10^{-11}$; and thereafter the rate of yield falls steadily to 16 to 22 mgm. $\times 10^{-11}$ for the fifth hour.

DISCUSSION OF GENERAL PHENOMENA

These results confirm those obtained by Walker and Winslow (1932) but bring out the wider differences which take place and which were masked by the longer time intervals first studied. The new data show very sharply the burst of adolescent energy which is associated with the last half hour of the lag period and the first half hour of the phase of logarithmic increase.

This phenomenon is clearly related to that described by Sherman and Albus (1923) in their paper on "Physiological Youth of Bacteria." Sherman and Albus found that young cultures of bacteria were more sensitive to the action of distilled water at 2°C., to 2 per cent NaCl, to a temperature of 50 to 53°C., and to 0.5 per cent phenol—and were less responsive to acid agglutination—than were older cultures. Their "young" cultures, however, were three and one-half to four and one-half hours old and their "old" cultures twenty-one hours to twelve days old. They were, therefore, probably comparing the logarithmic period with a much later stable phase, rather than the lag period with the logarithmic phase.

In a second contribution, however, Sherman and Albus (1924) showed that this state of high susceptibility was manifest after one and one-half hours, or during the latter part of the lag period. Thus, it does represent a change initiated before the period of

logarithmic increase but one which apparently continues throughout that period.

The work of Clark and Ruehl (1919) and of Henrici (1928) presents somewhat similar phenomena. Clark and Ruehl found that of thirty-seven different species of bacteria whose morphology was studied at various phases of the growth cycle all but the diphtheria bacillus showed very much larger cells in four to nine-hour cultures than in twenty to twenty-four-hour cultures. In the case of the diphtheria bacillus the cells in young cultures were distinctly smaller than in old cultures. In general, the largest cells were observed between four and six hours at the height of logarithmic increase but with *Esch. coli*, it is interesting to note, the height of the curve was reached at 2 hours. Henrici (1928) confirmed these results and found that with *Esch. coli* and *B. megatherium* young cultures showed longer and more slender cells than old cultures. In both instances the largest cells were observed during the course of the period of logarithmic increase.

The observations of Bayne-Jones and Rhees (1929) on heat production by young cells are closely in line with our CO_2 and NH_3 rate data. These investigators measured, in an apparatus specially designed for bacterial calorimetry, the heat production per cell in a unit interval of time and recorded a very sharp peak of heat production during the second hour of incubation. Their results check very perfectly with our own and point with equal clearness to a period of marked metabolic activity at the beginning of the phase of logarithmic increase and not at its height. Criticism of the methods by which Bayne-Jones and Rhees computed their rates per cell per hour does not affect the general results, as shown by the re-calculations of Rahn (1930). An important theoretical analysis of the energetics of the processes involved in such phenomena has recently been offered by Wetzel (1932) using Bayne-Jones' data and applying a formula which he believes applicable to growth and metabolism throughout the biological field.

Burk and Lineweaver (1930) in a study of the "respiration rate" of azotobacter found that the ratio of growth increase to increase in oxygen consumption rose as the cultures aged, a phenomenon

which obviously involves a higher metabolic rate in the earlier hours. Cutler and Crump (1929) give data on carbon dioxide production in soils by pure cultures of soil organisms, which show a high rate of production during young active growth, and Meiklejohn (1930) presents results on ammonia production by soil bacteria which appear to illustrate the same phenomenon. Boyd and Reed (1931) describe a sharp fall in oxidation-reduction potential in cultures of *Esch. coli* at the onset of the logarithmic growth period, which slackened somewhat in the later hours of this phase, when no sugar was present. Eaton (1931) gives curves which show quite clearly that the "respiration rate" of a staphylococcus culture goes up faster than the growth curve at first and more slowly at a later period. The author states that as the culture ages, the rate of respiration gradually decreases. Gerard and Falk (1931) noted in aqueous and glucose suspensions of *Sarcina lutea* an initial high rate of oxygen consumption, which declined during later hours, although the organisms were undergoing active growth.

Martin (1932) in Bayne-Jones' laboratory has demonstrated a clearly defined period of intense physiological activity per cell following the close of the lag period as measured by the oxygen consumption of *Esch. coli* and by the surface area of the average organism. Furthermore, he compares our earlier results and those of Bayne-Jones and Rhees from a quantitative standpoint and shows that they are closely related with each other and with his own.

In connection with this work, Martin presented data on the size of the cells of *Esch. coli* at one-half hour intervals. The largest cells were noted at 1, 1½ or 2 hours after inoculation, which points fall early in his logarithmic growth periods.

Finally, Bayne-Jones and Adolph (1932) and Bayne-Jones and Sandholzer (1933) have confirmed and extended the findings of Henrici and Martin on the cell size of *Esch. coli* at various phases of the life cycle. We shall refer again to these results in a later paragraph.

From the foregoing discussion it is apparent that a number of workers have presented evidence in terms of several metabolic

indices—oxygen consumption, heat production, carbon dioxide production, ammonia yield, and the like—reflecting a period of high activity per cell at the close of the lag and the beginning of the logarithmic growth phases. The agreement between these various researches is on the whole most striking.

INTERPRETATION

All this material seemed to us, at first, as it has seemed to others, to demonstrate beyond question the existence of a period

TABLE 7

Comparison of various characteristics at different phases of the life cycle

	HOURS			
	0–1	1–2	2–3	3–4
Oxygen consumption* per cell per hour, mgm. $\times 10^{-11}$	46.7	72.5	49.5	26.6
Heat production,† gram calories per cell per hour, $\times 10^{-11}$	87.5	247.7	92.6	25.7
Carbon dioxide production‡ per cell per hour, mgm. $\times 10^{-11}$	36.5	123.0	73.0	50.0
Ammonia nitrogen‡ per cell per hour, mgm. $\times 10^{-11}$	10.4	26.7	6.2	2.6
Average volume of cells in cubic micra§	1.9	3.1	1.0	0.8

* Computed from Martin (1932), table 1, p. 697.

† Data of Bayne-Jones and Rhees (1929). Recalculated from table 2, p. 131, using Buchanan formula.

‡ Data from present paper, table 6.

§ Computed from data used by Bayne-Jones and Sandholzer (1933) in preparing upper curve of table 1, p. 284. Courteously furnished by Dr. Bayne-Jones.

of physiological youth at the end of the lag phase of the culture cycle. It then occurred to us, however, that the variations in cell-size, recorded by Clark and Ruehl, Henrici, Martin and Bayne-Jones might have a more direct relation to metabolic activity than had been pointed out. From the data of Bayne-Jones and Rhees and Martin and from our own, we therefore made the suggestive computations summarized in tables 7 and 8.

It will be noted by reference to table 8 that the difference in cell volume between the second and the fourth hour is substan-

tially greater than the differences in oxygen consumption and in production of carbon dioxide. Thus, the higher metabolic activity per cell, as measured by these two factors, could be more than accounted for by the greater volume of the individual cells. The greater excess of ammonia production during the second hour might be due in considerable part to re-utilization during later

TABLE 8

Comparison of data in table 7 computed as ratios of values for fourth hour

	HOURS			
	0-1	1-2	2-3	3-4
Oxygen consumption	1.8	2.7	1.9	1.0
Heat production	3.4	9.6	3.6	1.0
Carbon dioxide production	0.7	2.5	1.5	1.0
Ammonia nitrogen production	4.0	10.3	2.4	1.0
Cell volume	2.4	3.9	1.3	1.0

TABLE 9

Heat production per cubic micron of bacterial substance

(Gram calories $\times 10^{-11}$ per hour)

	HOURS			
	0-1	1-2	2-3	3-4
Observed*	46	80	93	32
Computed from oxygen consumption†	74	70	148	100
Computed from carbon dioxide production‡	42	87	159	136

* Recalculated from data of Bayne-Jones and Rhees (1929), table 2, p. 131.

† Computed from data of Martin (1932), table 1, p. 697.

‡ Computed from our own data. Cell volumes used were in all cases those provided by Bayne-Jones (see footnote to Table 7).

phases of ammonia nitrogen liberated from easily disrupted peptone fractions during the first hours. Only the excess of heat production remains abnormally high during the first hours.

Following the example of Martin in attempting to discover relationship between the O_2, CO_2 and heat data, we have computed in table 9 the heat production per hour per cubic micron of bacterial substance (from Bayne-Jones and Rhees) and compared

it with the heat production which might be expected to correspond to the oxygen consumed (Martin) and to the carbon dioxide produced (our own data). Despite the many assumptions involved it is interesting to note that the values are of the same general order of magnitude, with somewhat better agreement between heat predicted by O_2 and by CO_2 than between either of these and heat observed.

We recognize that such comparisons can only be rough and suggestive, since O_2, CO_2, and calories were each obtained by separate workers, with no absolute consistency among all three as to strain of organisms, medium composition, temperature control, degree of aeration, cell-counting methods, and the like. In general, correspondence among these factors in the work of Martin and Bayne-Jones and Rhees was close, but the specific figures used in table 9 from their work are based on single experiments. Cultivation conditions in our CO_2 experiments differed somewhat from the others and the figures used are averaged from eight cultures. Moreover, rates per cubic micron for all the data have been made dependent on bacterial dimensions supplied only by Bayne-Jones, and involve the assumption that all cells of *Esch. coli* were of the same average size during each hour. Because of all the inescapable variables involved, we have here preferred not to complicate calculations by the assumption of a respiratory quotient or by too severe refinement of a factor representing the caloric equivalent of one mgm. of O_2 or CO_2. The figure chosen (3 calories per milligram O_2; 2.2 calories per milligram CO_2) was based on values commonly given in handbooks for the combustion of carbon; it also agrees approximately with values calculated long ago by Rubner for oxidation of proteins, when allowances were made for heat of solution and heat of combination by the carbonic acid.

As a further suggestive cross-check, we have calculated on the basis of average hourly CO_2 rates per cubic micron, and average hourly O_2 rates per cubic micron, (computed from Martin's data), the approximate respiratory quotients (R.Q. = vol. CO_2/vol. O_2) for each hour. The figures obtained were respectively, for successive hours after inoculation, 0.57, 1.23, 1.07 and 1.37. We place no emphasis on these as absolute values and attach no signifi-

cance to the variations; the astonishing thing is that they come out anywhere near the well-known values published for various kinds of resting or growing aerobic or anaerobic bacteria, whose published R. Q.'s have usually lain between 0.7 and 1.3. Values above 0.82 in our sugar-free media might readily be attributable to differences between Martin and ourselves in the cell-counting technic on which the rates were originally based, or to differences between average hourly cell sizes in our cultures and in his, or to increased aeration leading to increased O_2 consumption by our cultures; or apart from differences in technic, slightly high R.Q.'s might be due to occurrence of some partially anaerobic activities by the cultures.

It will be noted that when computations are made, as in table 9, on a basis of cell volume, it is the third rather than the second hour which shows maximum metabolic activity.

SUMMARY

1. This study indicates that when *Esch. coli* is grown in a peptone-water medium, with or without continuous aeration with CO_2-free, NH_3-free air, there is a burst of metabolic activity (estimated on a cell-per-hour basis) at the close of the lag period and before the phase of logarithmic increase has much more than begun. The NH_3—N yielded up to the medium per cell per hour is at this time (third or fourth half hour) five times what it is during the first hour and more than ten times what it is toward the end of the logarithmic phase, *i.e.*, after the third or fourth hour. The yield of CO_2 in an aerated medium shows a similar maximum during the second hour of three times the first-hour value and over five times the fifth-hour value.

2. The effect upon growth and metabolism of adding glucose to the peptone water is surprisingly slight in a medium continuously aerated with CO_2-free air. The logarithmic phase is initiated somewhat more promptly but the ultimate level of growth is much the same. There is a definite sparing action shown by a lessened yield of NH_3—N (an average reduction of about 33 per cent) but the yield of CO_2 is also reduced about 10 per cent. In a medium continuously aerated with nitrogen instead of air, so that defi-

nitely anaerobic conditions are produced, the effect of glucose is very different, as we have shown recently (Walker, Winslow and Mooney (1934)).

This study adds further evidence to the demonstration of a period of marked adolescent activity beginning in the late lag period of a bacterial culture cycle and including an increase in the size of the individual cell (Clark and Ruehl, Henrici, Martin, Bayne-Jones and Adolph), increased susceptibility to antiseptic agents (Sherman and Albus), decreased oxidation-reduction potential (Boyd and Reed), increased oxygen consumption (Martin) and increased heat production (Bayne-Jones and Rhees), as well as an increased yield of carbon dioxide and ammonia nitrogen.

4. It appears, however, that a considerable part of this apparent increase in physiological activity per cell per hour, as recorded by Bayne-Jones and Rhees, by Martin, and by ourselves, can be directly accounted for by the greater size of the bacterial cells at this period. If computed on the basis of unit volumes of bacterial substance, the differences in oxygen consumption and carbon dioxide production between the second and fourth hours would disappear and the third hour would be the period of highest metabolic activity.

5. The phenomenon of physiological youth, if we define this phenomenon as a rapid increase in cell size and in metabolic activity at the close of the lag phase and in the early logarithmic phase seems fully substantiated; but the ensuing fall in the middle of the phase of logarithmic increase and towards its end appears to be demonstrated only for cell size and heat formation. The apparent decrease in oxygen consumption and CO_2 production per cell during the course of the logarithmic phase seems primarily to be a function of the decreasing volume of bacterial substance or of surface area per cell; and even the decrease in rate of ammonia yield may perhaps be explained by decreasing cell size plus utilization of previously liberated ammonia.

6. It should be clearly understood, however, that while allowance for variations in cell-size may wipe out differences between the early lag period and the late logarithmic period on the one hand and the late lag and early logarithmic period on the other, it cannot account for the high metabolic activity of all these early

phases as compared with the stable period which follows the logarithmic phase. The metabolic activity per cell during the second hour is two to four times that of the first hour and two to ten times that of the fifth hour. This difference may perhaps be largely accounted for by cell size since Bayne-Jones and Adolph (1932) show that at fifty-five to sixty-five minutes of culture age the cell volume of *Esch. coli* may be ten times what it is at four hours. Variations in cell size cannot, however, explain the fact that during the later phase of stable population (twenty-three to twenty-eight hours) the production of ammonia per cell is only one one-hundredth and that of carbon dioxide only one-fiftieth what it is at its maximum. (Walker and Winslow, 1932.) The measurements of Henrici, Martin, and Bayne-Jones and Adolph all agree in showing that the colon bacillus has a size of about 1.5 to 2.0 $\times$ 0.6 to 0.7 micra in the early lag and late logarithmic phase as compared with a size of 4.0 to 5.0 $\times$ 0.9 to 1.1 micra in the early logarithmic phase; and Henrici carried his cultures on for 96 hours with no reduction below the 1.5 $\times$ 0.6 figure. Thus, the cells at their maximum are not over ten times as large as at their minimum; so that the fifty fold to one hundred fold excess in yield of CO_2 or NH_3 per cell during adolescence as compared with the stable period must involve a real difference in metabolic activity.

7. The physiological constants which may be derived from these various studies seem of some interest. It appears from approximate calculations that one cubic micron of bacterial substance (*Esch. coli*) during the first four hours after inoculation, may consume 20 to 70 mgm. $\times 10^{-11}$ of carbon dioxide, and may release 3 to 7 mgm. $\times 10^{-11}$ of ammonia nitrogen per hour, liberating in the process 30 to 90 calories of heat energy.

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