CELL SIZE AND METABOLIC ACTIVITY AT VARIOUS PHASES OF THE BACTERIAL CULTURE CYCLE[1]

EVELYN HUNTINGTON AND C.-E. A. WINSLOW

Department of Public Health, Yale School of Medicine

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OBJECTIVES OF PRESENT STUDY

Experimental studies on the morphology and physiology of unicellular organisms have provided convincing evidence that the cells of early generations developing in a fresh, favorable medium are quite different from those of generations developing after the period of maximum multiplication. That bacteria during the lag or early logarithmic growth phases are less resistant than cells of subsequent population periods to such inimical agents as heat, cold, 2 per cent NaCl, 0.5 per cent phenol and dilute crystal violet has been demonstrated by Schultz and Ritz (1910), Reichenbach (1911), Sherman and Albus (1923, 1924), Sherman and Cameron (1934), Stark and Stark (1929), Heiberg (1932) and Salter (1919). Research in the field of quantitative metabolism indicates that the onset of the period of maximum reproduction is accompanied by a marked increase in metabolic activity as measured by such indices as heat production (Bayne-Jones and Rhees, 1928; Schmidt and Bayne-Jones, 1933), oxygen consumption (Eaton, 1931; Gerard and Falk, 1931; Martin, 1932), carbon dioxide and ammonia production (Cutler and Crump, 1929; Meiklejohn, 1930; Walker and Winslow, 1932; Walker, Winslow, Huntington and Mooney, 1934; Mooney and Winslow, 1935). Metabolic activity apparently declines before multiplication ceases to take place at the maximal rate. These phenomena have come to be regarded as manifestations of a

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period of physiological youth comparable to that exhibited in the life cycle of a multicellular organism.

Measurements of bacterial cells indicate that individuals of a large number of species increase in size during the early hours of culture growth, but soon decrease to approximately the original level. Thirty-five of the thirty-seven species studied by Clark and Ruehl (1919) showed such a size cycle. For the majority of these species a maximum was reported at four to six hours after transplantation, when the cultures were in the logarithmic growth phase.

Wilson (1926) observed that suspensions of four-hour and twenty-six-hour cultures of *Salmonella aertrycke* containing the same number of organisms were dissimilar in opacity and concluded that the cell volume of the younger organisms was five times as great as that of the twenty-six-hour cells.

Data on cell size of *Bacillus megatherium* and *Escherichia coli*, as well as of other species, have been accumulated by Henrici (1928) from the projected images or photomicrographs of organisms treated with Congo red and HCl. Cells of *Escherichia coli* grown on agar increased in length from 1.5 to 4 micra in the first three hours after inoculation and subsequently declined in size. Jensen (1928) reported an increase in size of the cells of *Escherichia coli* during the first hour of growth on agar as well as in broth and a decrease after the third hour of culture development.

Bayne-Jones and Adolph (1932) obtained consecutive records of changes in the size and rate of growth of individual cells of *Escherichia coli* and *Bacillus megatherium* cultivated on agar, by means of motion photomicrography. With *E. coli* it was found that the mean rate of growth in volume of individual cells reached a maximum sixty minutes after inoculation of the medium and then declined rapidly, whereas the mean rate of reproduction did not reach a peak until two hours after inoculation. Maximum cell volume (prior to fission) was greatest at ninety minutes and thereafter diminished progressively, the last cells observed being only one-fifth the size of the first cells.

An investigation of the effect of agitation on bacterial growth

was made by Mudge and Smith (1933). Their data indicate that, although agitated and unagitated cultures of *Escherichia coli* increase to a similar maximum cell size in the early logarithmic growth phase, cells of the agitated cultures are only one-third as long as cells of unagitated cultures after the close of this period.

Using a photo-electric cell in measuring the opacity of cultures of *Salmonella gallinarum*, Alper and Sterne (1933) found that the organisms increased in size for three hours following inoculation and then decreased until the seventh hour when the cells were similar in size to those from twenty-four-hour cultures.

In considering these various observations it is obvious that the increased metabolic activity characteristic of the phase of physiological youth might be accounted for by the larger size of the individual cells at this period, since metabolic activity has ordinarily been computed on a cell-per-hour basis. Walker, Winslow, Huntington and Mooney (1934) made a preliminary attempt to analyze this problem by using data for metabolic activity of *E. coli* obtained in this laboratory, on heat production reported by Bayne-Jones and Rhees (1928) and on oxygen consumption reported by Martin (1932) in comparison with cell measurements reported by Bayne-Jones and Sandholtzer (1933) for the same organism. They concluded that size changes could only in part account for increased metabolic activity. To date, however, the only report citing direct simultaneous observations of size and metabolic activity is that of Martin (1932) which dealt with the oxygen consumption of *Escherichia coli* per cell per minute during the lag and logarithmic growth phases. The rate of oxygen consumption reached a maximum near the end of the lag phase and then declined gradually. Cell measurements showed a rise and subsequent fall in cell size with a two-fold increase in surface area of the largest over the initial cells. The absolute point of maximum surface area varied with separate experiments, but in relation to the growth curve it also coincided in almost every instance with the end of the lag phase.

The problem seemed of sufficient importance to warrant a more intensive simultaneous study of cell size and metabolic

activity in several different types of organisms and in several media. The primary purpose of the investigation was to determine how nearly changes in cell size parallel changes in the hourly rates per cell of carbon dioxide production and whether computations of metabolic activity per unit volume of bacterial substance vary significantly at different phases of population development, when size measurements and data on metabolic activity are simultaneously determined. Observations on *Escherichia coli* were made to check previous data on carbon dioxide production and obtain figures for cell size under the cultural conditions employed in this laboratory. *Salmonella gallinarum* and *Salmonella pullorum* were also included in the study to discover whether these species, which differ in fermentative capacity from *E. coli*, show changes in cell size and metabolic activity similar to or distinct from those exhibited by the colon bacillus, in the presence and in the absence of carbohydrates.

METHODS

Samples for determinations of cell size were taken hourly from cultures growing at 37°C. in fluid media, continuously aerated with air freed from carbon dioxide and ammonia. Amounts of carbon dioxide produced, cell counts and rates of carbon dioxide production per cell per hour for the same cultures (which were used for a joint study) have been reported by Mooney and Winslow (1935).

Since the methods of determining carbon dioxide production and cell counts have been described in that report as well as by Walker and Winslow (1932), and Walker *et al.* (1934), it is sufficient to state that both the carbon dioxide in the medium and that carried off by aeration were measured for successive hourly periods and samples for cell counts and size measurements taken, from inoculation to the end of the logarithmic growth phase; a period of two or three hours between the twenty-second and twenty-eighth hour was also observed. Bacterial numbers were counted by the dilution and pour-plate method.

The experimental conditions of this study were such that it was necessary to make determinations of cell size on material

which had been fixed and stained. Consequently smears were prepared in the usual manner on glass slides which were then stored until measurements could be made. The apparatus used for measuring the bacteria consisted of a Spencer filar micrometer with a 10× ocular, a monocular microscope equipped with a mechanical stage and a Zeiss oil immersion, apochromatic objective magnifying 90×. We found a 10 per cent aqueous solution of Ziehl's carbol-fuchsin applied for three minutes the most satisfactory stain for this work since it was more effective than methylene blue or safranin in dyeing the cells during the later hours of culture growth.[2]

Measurements of two-, three-, and five-hour cells of *Escherichia coli* stained in this manner were compared with those of cells observed unstained in a drop of 1 per cent agar under a cover glass. The mean of 100 or more unstained cells was 4.2 by 0.9, 3.7 by 0.8, and 2.2 by 0.8 micra at two, three and five hours respectively. The corresponding figures for stained cells from the same cultures were 3.1 by 0.7, 2.7 by 0.7 and 1.6 by 0.65 micra, thus showing that the unstained organisms appeared 20 to 25 per cent larger than the stained forms. Although the processes of fixing and staining undoubtedly result in some cell shrinkage, it seems likely that the optical properties of the unstained preparation may give values in excess of the true dimensions—which perhaps lie between the two extremes. In any case, the employment throughout the work of one technique for fixing, staining and measuring should make evident any relative changes in cell size.

To determine the number of cell measurements necessary for obtaining a representative sample of the bacterial population at each observation point a statistical analysis was made of deviations obtained by counting and averaging various numbers of individual cells. This study indicated that a sample of twenty-five or thirty organisms is adequate for obtaining a reasonably

[2] The precipitation of Congo Red by peptone recorded by some workers has not been observed in this laboratory. The negative staining procedure of Benians might therefore be used and would perhaps give sizes closer to those of unstained cells.

representative mean. (See table 1 for a typical example of actual standard deviations.) Throughout our later work therefore thirty organisms from each culture have been measured at each observation point. The validity of this procedure has been demonstrated by the agreement between means when it has been necessary after a lapse of several months to measure a second or third group of thirty organisms from one slide.

TABLE 1

Mean cell volume of individual cultures of E. coli in peptone medium (cubic micra)

EXPERIMENT NUMBER	AGE (HOURS)									
	0	1	2	3	4	5	6	7	23	25
63	.43	.65	1.18	.76	.61	.36	.32	.30	.27	.31
64	.44	.57	.84	1.03	.60	.49	.24	.29	.24	.24
65	.39	.45	.86	.65	.70	.46	.31	.24	.22	.22
66	.44	.64	.92	.92	.67	.42	.35		.25	.28
67	.36	.52	1.24	1.03	.65	.58	.41	.36	.26	.25
68	.29	.59	1.18	1.09	.88	.80	.50	.37	.30	.31
Mean........	.39	.57	1.04	.91	.69	.52	.36	.31	.26	.27
Standard deviation..	±.07	±.07	±.17	±.18	±.04	±.13	±.06	±.06	±.03	±.02

In computing mean cell volume the formula

$$V = \pi\left(\frac{\text{width}}{2}\right)^2 \times (\text{length minus width}) + \frac{4\pi}{3}\left(\frac{\text{width}}{2}\right)^3$$

was used, assuming that the average cell could be considered as a cylinder bounded on each end by a hemisphere.

It was essential that the size of cell aggregates rather than of individual cells be used in computing rates of carbon dioxide production per unit volume of bacterial substance because bacterial numbers were determined by plate counts. Therefore, all values given refer to the size of cell aggregates. Aggregate and individual cell length differed most widely with *Salmonella gallinarum*, the former being 12 to 15 per cent greater than the latter at three, four and five hours, while the mean aggregate cell length of *Escherichia coli* was only 5 per cent greater than the mean cell length of individual cells.

Rates of carbon dioxide production per unit volume of bacterial substance per hour were calculated by the Buchanan formula as used by Walker and Winslow (1932) for determining the fermentative capacity of a single cell. Values for reproductive rate per hour were computed by the usual formula.

Eight series of experiments were made as follows: *Escherichia coli* in 1 per cent peptone and in 1 per cent peptone with 0.5 per cent glucose; *Salmonella gallinarum* in 1 per cent peptone, 1 per cent peptone plus 0.5 per cent glucose, and 1 per cent peptone plus 0.5 per cent lactose; *Salmonella pullorum* in 1 per cent peptone, 1 per cent peptone plus 0.5 per cent glucose plus 0.5 per cent NaCl,[3] and 1 per cent peptone plus 0.5 per cent lactose. For each of these experimental conditions six duplicate cultures were observed, except for *S. gallinarum* in peptone-glucose of which seven cultures were observed and for *S. pullorum* in peptone of which only four cultures were included. Two duplicate cultures and one uninoculated control were kept under simultaneous observation on a given day in order to minimize potential variations in the rate of aeration, water-bath temperature density of the inoculum and other environmental factors. However, in spite of the fact that every effort was made to keep the experimental conditions constant and to repeat without alteration each essential manipulation, individual cultures of each series showed a considerable degree of variation. Therefore, cell counts and amounts of carbon dioxide produced in all six or seven duplicate experiments under each of the eight experimental conditions were separately averaged as by Mooney and Winslow and the resultant hypothetical mean values compared. In the same way values for mean cell size of each culture at each observation point have been averaged in the present study for computing cell volume of the mean culture and calculating hourly rates of carbon dioxide production per cubic micron of bacterial substance. Data for cell volume of the mean culture of *E. coli* in peptone water as well as of the six individual cultures on which this mean is based appear in table 1 and are shown

[3] Mooney and Winslow (1935) found that the addition of NaCl was essential for the growth of this organism under the conditions of the experiment.

graphically in figure 1. This group of experiments was selected at random for demonstrating variation in cell size of individual cultures from the mean. Judging by the original work sheets, the six duplicate experiments varied more under this condition than under any other. The variation observed may be in part a fault of the technique used for measuring cells (particularly since values for width are difficult to measure accurately), and in part a result of the relatively long time interval between observation points (which involves variation in the exact phase of the culture cycle observed). The influence of such variation

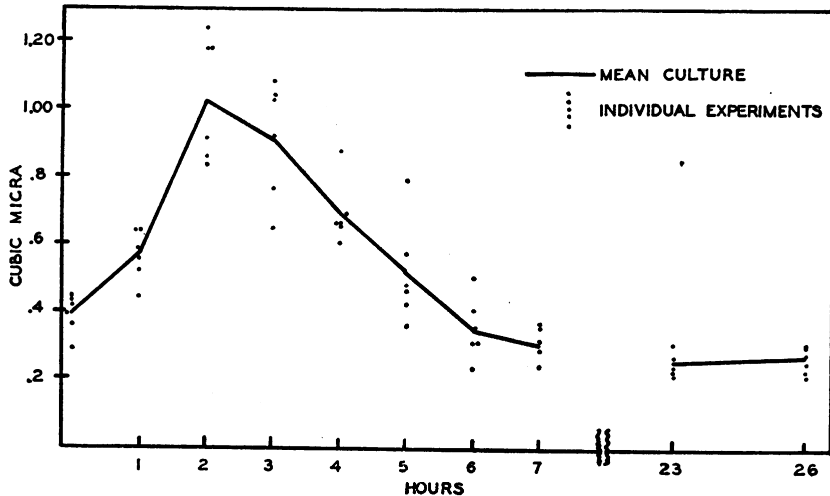

FIG. 1. CELL VOLUME OF E. COLI IN PEPTONE WATER BY HOURS

on cell numbers and cell size has, however, we believe been largely eliminated by averaging individual cultures and determining mean culture performance. Our results as presented are based on the actual measurement of over 13,000 individual cells.

EXPERIMENTAL RESULTS ON CELL SIZE

Our mean results for each organism and each medium are presented in tables 2 to 4. Curves of average cell volume appear in figure 2 where each panel contains the data for one species.

Comparison of these graphs and tables brings out four points of interest.

First, all the organisms in all the media exhibit the same general phenomena—an increase in cell volume followed by a decrease. The initial cell volumes vary only from 0.31 to 0.46 cubic micron and average 0.39 cubic micron. The final cell volumes (except in peptone-glucose) vary from 0.26 to 0.42 cubic micron, and average 0.34 cubic micron. Thus, we may consider the normal volume of all these organisms as between 0.3 and 0.4 cubic micron. At the maximum (excepting for *S. gallinarum* in peptone-lactose) the size varies from 0.76 to 1.28 cubic micra and averages 1.02 cubic micra, a remarkably close check considering that our hourly examinations could not always catch the cultures at the point of maximum size. Thus, in general the cell volume at the peak of physiological youth is three times that of the normal stable phase.

TABLE 2

Reproductive rate, cell volume and metabolic activity of E. coli

(Maximum value in heavy type)

TIME*	PEPTONE			PEPTONE-GLUCOSE		
	Metabolic activity†	Cell volume‡	Reproductive rate§	Metabolic activity†	Cell volume‡	Reproductive rate§
0		.39			.41	
1	116	.57	.02	78	.77	−.13
2	**135**	**1.04**	.40	**86**	**1.03**	1.04
3	90	.91	.92	79	.93	**1.41**
4	100	.69	**1.17**	59	.91	.64
5	95	.52	.56	31	.81	.42
6	68	.36	.94	24	.84	.04
7	43	.31	.45	19	.89	.18
23		.26			.76	
25	8	.27	−.60	7	.75	.05

* Interval after inoculation in hours.

† Milligrams $\times 10^{-11}$ of CO_2 per cubic micron per hour for preceding hour.

‡ Mean cell volume in cubic micra at stated hour.

§ Reproductive rate during preceding hour. Computed from formula,

$$\frac{1}{t} \times ln\frac{b}{B}$$

where B and b equal initial and final numbers.

The first exception to this general rule is the very great increase of size for *S. gallinarum* in peptone-lactose. Its maximum volume is 1.81 cubic micra, or nearly six times the size of the stable phase. This phenomenon can be explained only in part by a tendency of this organism to form chains in this medium.

TABLE 3

Reproductive rate, cell volume and metabolic activity of S. gallinarum

(Maximum value in heavy type)

TIME*	PEPTONE			PEPTONE-GLUCOSE			PEPTONE-LACTOSE		
	Metabolic activity†	Cell volume‡	Reproductive rate§	Metabolic activity†	Cell volume‡	Reproductive rate§	Metabolic activity†	Cell volume‡	Reproductive rate§
0		.31			.38			.42	
1	11	.35	−.04	82	.37	.09	53	.53	.01
2	61	.60	.03	102	.78	.24	70	1.07	−.25
3	113	**.98**	.09	85	**1.28**	.12	120	**1.81**	.05
4	**149**	.95	.58	**132**	.97	.56	122	1.47	.11
5	76	.85	**1.14**	117	.83	1.11	**191**	1.37	.69
6	55	.53	.92	61	.72	**1.28**	114	.77	1.32
7	43	.51	.49	42	.64	.24	42	.63	**1.45**
8	31	.44	.70	28	.69	.36	21	.53	.53
9	25	.44	.12	15	.69	.08			
10	17	.42	.19	11	.70	.07			
23		.41			.65			.33	
25	12	.41	−.57	5	.64	−.29	19	.32	−.56

* Interval after inoculation in hours.

† Milligrams × 10^{-11} of CO_2 per cubic micron per hour for preceding hour.

‡ Mean cell volume in cubic micra at stated hour.

§ Reproductive rate during preceding hour. Computed from formula,

$$\frac{1}{t} \times ln \frac{b}{B}$$

where B and b equal initial and final numbers.

Individual cells were also distinctly larger in peptone-lactose than in peptone.

The second exception to the usual course of the observed phenomena is the interesting fact that cells grown in peptone-glucose tend with all organisms to remain larger throughout the later hours of the population cycle than is the case in other

media. The phenomenon is most marked in the case of *E. coli* but comparing initial values in this medium we find for all three organisms an initial range between 0.35 and 0.41, and a final range between 0.48 and 0.75 in the presence of glucose.

TABLE 4

Reproductive rate, cell volume and metabolic activity of S. pullorum

(Maximum value in heavy type)

TIME*	PEPTONE			PEPTONE-GLUCOSE			PEPTONE-LACTOSE		
	Metabolic activity†	Cell volume‡	Reproductive rate§	Metabolic activity†	Cell volume‡	Reproductive rate§	Metabolic activity†	Cell volume‡	Reproductive rate§
0		.46			.35			.41	
1	170	.46	.16	17	.34	−.30	96	.41	.30
2	**216**	.73	.07	70	.56	−.17	59	.60	.17
3	178	.96	.05	52	.73	.27	**110**	**.93**	.18
4	128	**1.13**	.42	133	**.76**	.22	85	.81	.52
5	108	.81	.55	**178**	.56	.88	67	.71	.53
6	115	.90	.32	171	.56	.59	78	.58	.56
7	114	.78	.76	119	.43	**1.52**	71	.55	**.78**
8	85	.82	.85	61	.42	.64	44	.46	.47
9	40	.63	**1.08**	36	.56	.16			
10	37	.60	.30						
23		.42			.48			.30	
25	15	.42	.07	18	.48	−.04	13	.34	−.21

* Interval after inoculation in hours.

† Milligrams $\times 10^{-11}$ of CO_2 per cubic micron per hour for preceding hour.

‡ Mean cell volume in cubic micra at stated hour.

§ Reproductive rate during preceding hour. Computed from formula,

$$\frac{1}{t} \times ln \frac{b}{B}$$

where B and b equal initial and final numbers.

A clue to the problem of why cells remain larger in peptone-glucose than in peptone might be sought in accordance with observations by Stearn and Stearn (1933) in differences in the hydrogen-ion concentration of the two types of cultures. At the first hour the pH of all cultures lay between 6.7 and 7.0; as growth progressed the pH of the peptone cultures rose to values of 7.5 to 7.8 at seven hours, while the pH of peptone-glucose

cultures fell to 5.4 to 5.9. On the second day of culture growth the differences in pH were even greater, with 8.0 the value for peptone cultures and 4.6, 4.7 and 5.0 the respective values for *E. coli*, *S. gallinarum* and *S. pullorum* in peptone-glucose.

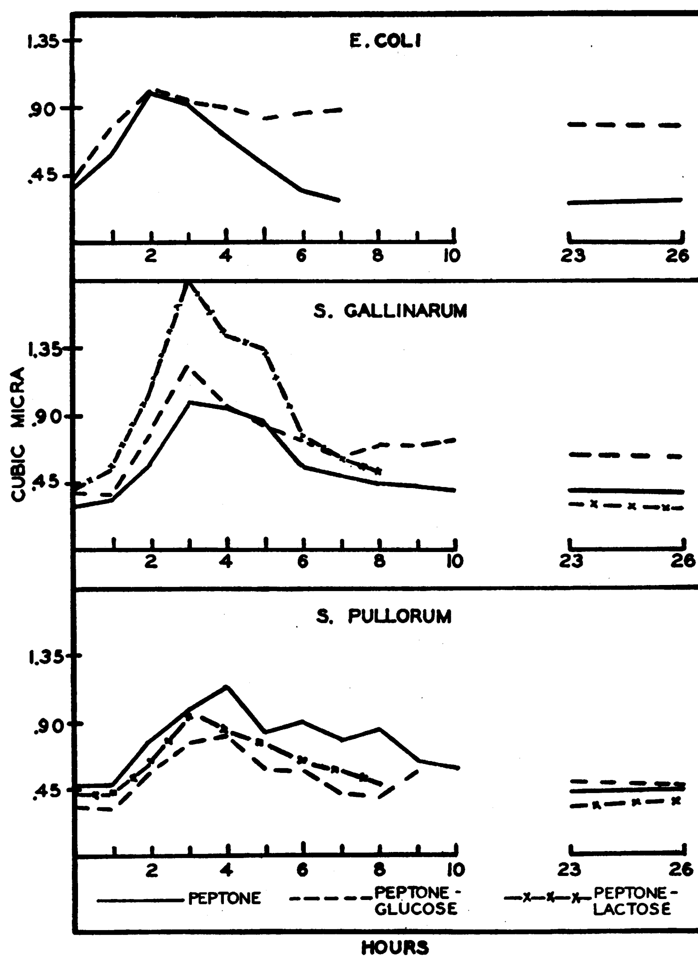

FIG. 2. AVERAGE CELL VOLUME OF THREE ORGANISMS BY HOURS

On the other hand, the increased nutrient concentration resulting from the addition of 0.5 per cent glucose to the medium might have been held responsible for alterations in mean cell

size. In a medium containing only 1 per cent peptone the decrease in cell size which occurred during and after the logarithmic growth period might reflect cellular adaptation to an environment containing a diminishing food supply. In such an environment the foodstuff would be more readily available if the bacterial culture were made up of small cells than if the culture were composed of larger cells, since in the latter case the surface-volume ratio is less. In a peptone medium to which 0.5 per cent glucose had been added the available food supply might have been such that the essential balance of assimilation of nutrients and excretion of metabolites could be maintained while cell size remained at a higher level than in peptone. Cell size figures given by Henrici show that *B. megatherium* did not increase to as great a maximum length in various dilutions of nutrient agar as in full strength nutrient agar, although the organisms did in every instance rise to a peak and then decrease to lengths less than those of the parent cells.

The enormous increase in cell size of *S.gallinarum* in peptone-lactose cannot, of course, be accounted for on either supposition since the sugar is not fermented and the medium remains alkaline.

Finally, it is of interest to note that the period of maximum cell size varies with the species of organism, coinciding with the second hour for *E. coli*, coming in the third hour for *S. gallinarum* and in the third or fourth hour for *S. pullorum*. It precedes the period of maximum reproductive rate by from one to five hours.

The point at which maximum cell volume occurred in relation to the growth curve of all the cultures reported in this study differed to some extent from that at which Henrici (1928) observed his longest cells. He concluded that *E. coli* cells reached maximum size during the logarithmic phase whereas we have found that cells were largest just before this period. However, Henrici reported no data on cell size between inoculation and the end of the third hour. During this period cell length very probably reached a higher value than that observed for cells three hours old. Other factors which may have been instrumental in yielding a different size-to-number relationship are the

composition of the medium, the density of the inoculum and lack of aeration. Since curves of cell lengths and cell numbers of *Bacillus megatherium* exhibited by Henrici show that the lag in cell length is shorter than the lag in reproduction our findings are essentially in agreement with his data, as well as with those of Martin (1932).

The bacterial cultures and the methods and intervals of observation of this study are not strictly comparable with those employed by Bayne-Jones and Adolph (1932) and Adolph and Bayne-Jones (1932) in measuring the growth in size of *E. coli* and *B. megatherium*. Although there was no way of determining the degree of maturity or the rate of growth of individual cells by the technique used in the present study, the fact remains that for each culture, maximum mean cell volume preceded the beginning of the maximal rate of reproduction and that cell size diminished during the logarithmic period of increase in numbers. In these respects our findings are in accord with those of Bayne-Jones and Adolph who observed that the maximum adult cell volume of *E. coli* was attained by the original cells inoculated on fresh agar and that, although the maximal growth rate of individual cells occurred one hour after inoculation, fission did not take place at the maximal rate until two hours after inoculation. (With *B. megatherium* the figures of Adolph and Bayne-Jones suggest that maximum cell volume appeared *after* the maximal rate of reproduction had been established.)

RATES OF CARBON DIOXIDE PRODUCTION ON A VOLUME BASIS

Data on rates of carbon dioxide production per cubic micron of bacterial substance per hour have been grouped by species in figure 3. Here again it is seen that, as growth in cell numbers and size vary at various phases of the culture cycle, so do rates of metabolic activity per unit volume of bacterial substance vary. All organisms in all media show a rise to a peak and a final fall to a point at, or below, the initial value. Thus, even when increased cell size is taken into consideration the late lag and early logarithmic growth periods are characterized by accelerated

metabolic activity. However, the excess in activity at these periods as compared with later phases is not as great when carbon

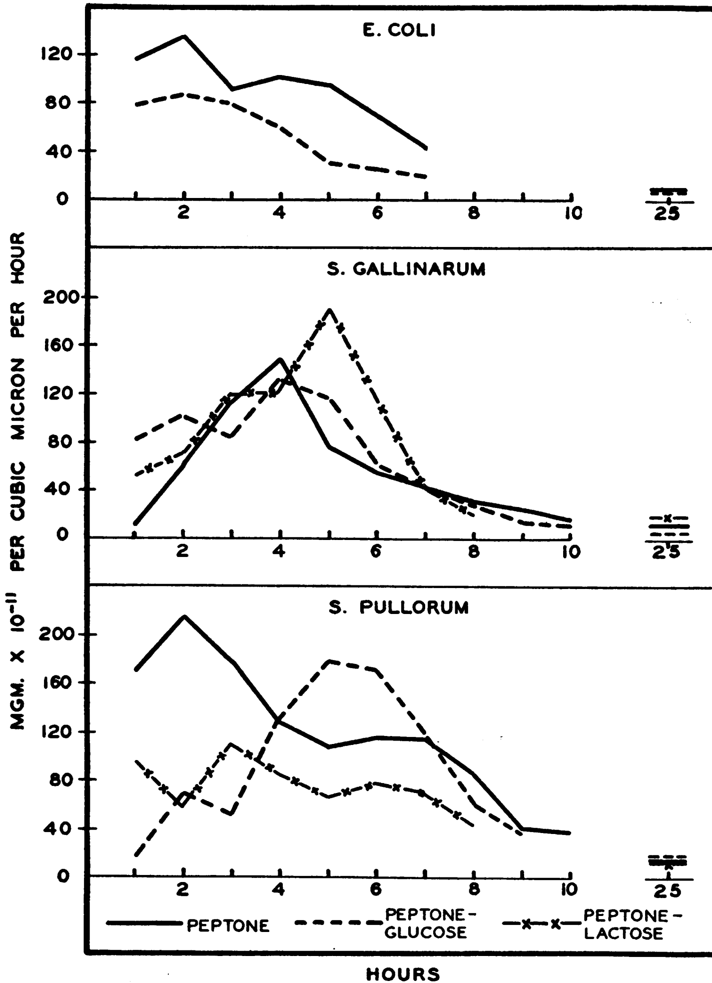

FIG. 3. HOURLY RATES OF CO_2 PRODUCTION PER CUBIC MICRON OF BACTERIAL SUBSTANCE OF THREE ORGANISMS BY HOURS

dioxide production is computed as a rate per unit mass of bacterial substance as when the product is calculated on a cell-per-hour basis.

Under the conditions of continuous aeration employed in these experiments, the presence of fermentable carbohydrates in the medium has no marked effect (with two exceptions which will be discussed later), upon metabolic activity as measured by yield of carbon dioxide. In the case of *E. coli* the amount of carbon dioxide produced in peptone-glucose per cubic micron was even less than that produced in peptone. With *S. gallinarum*, both maximal and final rates of carbon dioxide production in peptone-glucose were lower than in peptone although the sugar was actively attacked as demonstrated by the shift in pH from 7.0 to 5.3. With *S. pullorum* maximal yields were higher in peptone than in either peptone-glucose or peptone-lactose.

The failure of *E. coli* and *S. gallinarum* to liberate greater amounts of carbon dioxide per cell per hour in media containing available sugar has been reported in earlier studies made in this laboratory (Walker and Winslow, Mooney and Winslow). It was suggested that the apparently inconsistent effect of glucose in accelerating growth without increasing the rates per cell of carbon dioxide production would be reconciled by finding that cells were smaller in peptone-glucose than in peptone. Our measurements now show that such is not the case and that cells from peptone-glucose cultures are actually larger than cells from peptone cultures, thereby accentuating the difference between rates of carbon dioxide production by *E. coli* in the two media.[4]

Two exceptionally high rates of carbon dioxide production are indicated in figure 3, for *S. pullorum* in peptone (216 mgm. $\times$ 10^{-11} per cubic micron per hour) and for *S. gallinarum* in peptone-lactose (191 mgm. $\times$ 10^{-11}). For the first of these high rates we have no special explanation. The high rate for *S. gallinarum* in peptone-lactose may in part be explained by the fact that Dr. H. H. Walker in this laboratory has found plate counts of *S. gallinarum* in peptone-lactose to be abnormally low during the early hours in comparison with direct cell counts. This discrepancy between visible and viable cells was only in part the result

[4] A member of the JOURNAL editorial board has made the interesting suggestion that lower CO_2 yield and greater cell size in presence of fermentable carbohydrate might be related to increased formation of capsular carbohydrate material.

of relatively large numbers of chains of organisms. There was apparently some additional characteristic of lactose as it was used in these experiments which markedly reduced the ability of this organism to withstand dilution and plating on extract agar. In view of this phenomenon, and of the additional observation that the experimental cultures increased in turbidity even though plate counts remained low it seems likely that the actual number of viable cells present was greater than that recorded by us.

In general, it is obvious that during the phase of positive growth acceleration the presence in the experimental cultures of viable cells which fail to develop on plates might tend to lower our estimate of bacterial numbers and volume of bacterial substance and thus to make our estimates of metabolic activity too high. Dr. Walker's studies of *E. coli* and *S. gallinarum* by direct microscopic counting methods indicate, however, that this error is not serious except in the case mentioned. The direct count would, of course, yield results considerably too high in the later phases of the culture cycle when cells are dying out. Therefore the plate count is the soundest basis for comparative studies.

The summary presented below indicates that in both peptone and peptone-glucose media *E. coli* is the least active and *S. pullorum* is the most active, metabolically, of the three species studied. In peptone-lactose, on the other hand, *S. gallinarum* seems to be more active than *S. pullorum*. This latter difference may, however, be due to the presence of viable but non-cultivable cells, to which reference has been made above.

Milligrams × 10^{-11} of CO_2 per cubic micron per hour

	PEPTONE	PEPTONE-GLUCOSE	PEPTONE-LACTOSE
Maximal:			
E. coli	135	86	
S. gallinarum	149	132	191
S. pullorum	216	178	110
Final:			
E. coli	8	7	
S. gallinarum	12	5	19
S. pullorum	15	18	13

From the standpoint of the general physiological characteristics of the culture cycle, it is of marked interest to consider the relative pace at which metabolic activity, cell volume and reproductive rate, increase. The periods at which maximum activity is attained in each of these respects is indicated below.

Period of maximum activity (hour after inoculation)

	SIZE OF CELLS	METABOLIC ACTIVITY	REPRODUCTIVE RATE
E. coli:			
Peptone	2	2	4
Peptone-glucose	2	2	3
S. gallinarum:			
Peptone	3	4	5
Peptone-glucose	3	4	6
Peptone-lactose	3	5	7
S. pullorum:			
Peptone	4	2	9
Peptone-glucose	4	5	7
Peptone-lactose	3	3	7

Cell size reaches a maximum first in four instances and metabolic activity once, while they are simultaneous in three cases. Cell multiplication is the last of the three characteristics to reach its peak in every instance.

There is a possible fallacy involved in this interpretation since cell size measured at a given hour obviously represents conditions at the end of that hour while the rates of cell multiplication and of metabolic activity recorded at the same time represents the mean of growth and metabolism during the preceding hour. The differences are, however, so marked as to leave no doubt of the fact that the increases of both cell volume and metabolic rate precede by a considerable interval increase in rate of cell division.

The relation between metabolic activity and cell size is less clear. The maximum of cell size, as noted above, is reached (except with *S. pullorum* in peptone) before, or simultaneously with, maximum metabolic activity. If, however, we assume that the metabolic activity and cell size after twenty-four hours

may be taken as a norm, it is evident that increase in metabolic activity is *initiated* long before increase in cell size. Taking the results at the end of the first hour we have the following ratios for cell size and metabolic activity, as compared with the corresponding data for the stable period on the second day.

Ratio of one-hour values to twenty-four-hour values

	E. COLI		S. GALLINARUM			S. PULLORUM		
	Peptone	Peptone-glucose	Peptone	Peptone-glucose	Peptone-lactose	Peptone	Peptone-glucose	Peptone-lactose
Cell volume..............	2	2	1	<1	1	1	1	1
Metabolic activity........	14	11	1	16	3	11	1	7

In no case has the cell volume increased more than two-fold by the end of the first hour while in all but two instances the metabolic rate during the first hour is markedly above that for the stable period (three to fourteen-fold). In the two exceptional cases (*S. gallinarum* in peptone and *S. pullorum* in peptone-glucose) the cycle is delayed and the whole of the first hour is in the lag phase. For *S. gallinarum* in peptone the ratio of cell volume at the end of the second hour to that of the final phase was less than 2 and the corresponding ratio for metabolic activity was 5; for *S. pullorum* in peptone-glucose the second-hour ratios were 1 and 4, respectively. Clearly, metabolic activity begins to increase first, followed by cell volume and, finally, by reproductive rate.

Comparison of the three different organisms shows that *E. coli*, which exhibits the shortest lag in cell size, also reaches its maximum metabolic activity and its maximum reproductive rate most promptly. *S. gallinarum*, reaching its maximum cell size after *E. coli*, also attains maximum metabolic rate and maximum reproductive rate, more slowly than *E. coli*. *S. pullorum* attains maximum cell size and maximum reproductive rate last of the three organisms but shows (except in peptone-glucose) an earlier peak of metabolic activity than does *S. gallinarum*. For *Escherichia coli* in the two media, for *Salmonella gallinarum* in

all three media and for *Salmonella pullorum* in peptone-glucose, the first hour of the logarithmic growth period was characterized by maximal rates of carbon dioxide production. For *Salmonella pullorum* in peptone- and in peptone-lactose, maximum metabolic activity appeared before the period of logarithmic growth was even initiated.

SUMMARY OF CONCLUSIONS

1. Measurements of fixed and stained samples of continuously aerated, fluid cultures of *Escherichia coli*, *Salmonella gallinarum* and *Salmonella pullorum* indicate that mean cell volume increases during the lag period of culture development, reaching a maximum substantially before multiplication occurs at the maximal rate. During the logarithmic phase of rapid multiplication mean cell volume diminishes. The cells of the three organisms studied have a volume of about one-third of a cubic micron in their stable phase and increase to maximum sizes of about one cubic micron (in the case of *Salmonella gallinarum* in peptone-lactose to nearly two cubic micra).

2. When the amounts of carbon dioxide produced by these cultures are used to compute hourly rates of metabolic activity per cubic micron of bacterial substance, it appears that increased cell volume does not adequately account for the relatively greater amounts of carbon dioxide produced during the lag period and early part of the logarithmic growth phase. Although allowance for variations in cell volume eliminates a portion of the discrepancy between metabolic activity in the early and later hours of culture growth, the highest hourly rates of carbon dioxide production per cubic micron, which generally coincide with the first hour of logarithmic increase in cell numbers, vary from 86 to 216 mgm. $\times 10^{-11}$ of CO_2 per cubic micron per hour. They are from ten to twenty times greater than rates computed for later periods of negligible population change. This necessitates the conclusion that the cells appearing at the end of the lag period and the beginning of the phase of logarithmic reproduction are distinct in metabolic activity, as well as in size, from cells developing thereafter. Taken in conjunction with the observations of

earlier workers, the data reported in the present study appear to give additional support to the hypothesis that bacterial cultures exhibit a period of physiological youth closely resembling that of more highly differentiated multicellular organisms.

3. The three characteristics of physiological youth studied by us exhibit a definite and orderly relationship, metabolic activity increasing first, followed by cell volume and, in turn, by cell division rate. After the peak has been reached, cell volume and metabolic activity decrease rather rapidly while cell division rate continues high for several hours.

4. Each of the three species of bacteria exhibits a characteristic time cyle in all these relationships, *Escherichia coli* reaching its phase of physiological youth most rapidly, *Salmonella pullorum* least rapidly.

5. The presence of a fermentable sugar, glucose, seems to stimulate more rapid cell-division in the early phase of the culture cycle than is observed in plain peptone media with *Escherichia coli* and *Salmonella pullorum*. The stimulating effect of glucose is not, however, accompanied by any increase in yield of carbon dioxide per cubic micron of bacterial substance. In the presence of glucose both *Escherichia coli* and *Salmonella gallinarum* maintain larger cell sizes during the later phases of the culture cycle.

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