THE EARLIER PHASES OF THE BACTERIAL CULTURE CYCLE

C.-E. A. WINSLOW

Yale School of Medicine, New Haven, Conn.

AND

HAROLD H. WALKER

University of Tennessee, Nashville

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THE BACTERIAL CULTURE CYCLE AND ITS SIGNIFICANCE

Our knowledge of this subject may be considered to have begun with recognition of the fact that bacteria transferred to a culture medium suitable for their growth exhibit a period of delayed multiplication or "lag." Specific study of the problem dates at least as far back as the observations of Müller (1895). He recognized three phases of the early bacterial culture cycle, lag, logarithmic increase and slackened growth and followed Buchner, Longard and Riedlin (1887) in computing generation times during the logarithmic phase by the formula

$$G = \frac{T \log 2}{\log b - \log a};$$

where T represents the time interval, and b and a the final and initial numbers of cells. In the same year, Ward (1895) in a brilliant and exhaustive study of *Bacillus ramosus* also determined generation times and demonstrated the three fundamental phases of slow acceleration, maximum acceleration and reduced acceleration. Furthermore, he indicated clearly the effect upon the culture cycle of temperature, of light rays from the violet end of the spectrum, of food, of oxygen, of dilution and of antiseptic substances.

The phases of the culture cycle were analyzed in a broader sense

by Rahn (1906) and Lane-Claypon (1909), who recognized four phases, lag, logarithmic increase, stationary population and decrease. McKendrick and Pai (1911) attempted to explain assumed changes in growth rate during the culture cycle as manifestations of autocatalytic reactions, the governing factors at any moment being bacterial numbers and amounts of available nutriments. Chesney (1916) made valuable contributions to our knowledge of the culture cycle, as did Buchanan (1918 and 1925) who recognized seven phases, instead of four,—initial stationary phase, lag phase, phase of logarithmic increase, phase of negative acceleration, phase of maximum stationary population, phase of accelerating mortality and phase of logarithmic mortality.

The lag period was specifically studied by Hehewerth (1901) and Whipple (1901), although the phenomenon had been noted by many earlier bacteriologists in connection with multiplication of bacteria in water samples and in relation to the "bactericidal properties of milk," (for further references, see Winslow, 1928). It was carefully analyzed by Rahn (1906), Coplans (1910) Penfold (1914), Ledingham and Penfold (1914) and Chesney (1916).

That the phenomenon of lag had a biological basis was indicated by Müller (1895) who showed that when cultures of differing ages were used for inoculation into a new medium, the generation times in the new medium differed widely. When the source culture of typhoid bacilli was $2\frac{1}{2}$ to 3 hours old, the generation time was 40 minutes in the new medium; when the same culture was $6\frac{1}{4}$ hours old the generation time was 80 to 85 minutes; when the source culture was 14 to 16 hours old, the generation time was 160 minutes. Similarly, Barber (1908) and others showed that when transfer was made from a culture in the logarithmic phase, to the same medium and under the same conditions, the new culture multiplied at once at a logarithmic rate.

It was Müller again, in 1903, who first studied the chemical activity of bacteria at various stages of the culture cycle. He did not draw any conclusions as to the ratio of end-products formed to bacterial numbers but comparison of the various tables in his paper makes it clear that the amounts of carbon dioxide and

hydrogen sulfide per cell must have been much greater in the earlier phases of the culture cycle. This phenomenon was clearly recognized at a much later date by Bayne-Jones and Rhees (1929) for heat production, by Cutler and Crump (1929) for liberation of carbon dioxide and by Stark and Stark (1929a) for acid production.

A third differential characteristic of specific phases of the culture cycle is resistance to various harmful environmental influences. Many early observations showed that very old cultures were characterized by low resistance; but this might be due merely to degenerative changes. Much more significant was the discovery by Reichenbach (1911) that young cultures in the lag and early logarithmic phase were more sensitive to heat treatment than those at the peak of their population curve and that resistance increased again in a late stage of the phase of stable maximum population. Even more striking results were reported by Schultz and Ritz (1910). These authors found that a given heat treatment (53°C. for 25 minutes) killed about 95 per cent of colon bacilli from a 20-minute culture. In slightly older cultures, resistance decreased, so that at 4 hours 100 per cent destruction occurred. Then, resistance increased again so strikingly that 7-hour to 13-hour cultures showed no reduction whatever under the same heat treatment. A markedly low resistance to harmful chemical agents was demonstrated by Sherman and Albus (1923) to be characteristic of the lag and early logarithmic phase and these authors, on the basis of their experiments, developed in a highly fruitful manner the concept of "physiological youth" as applied to the bacterial culture cycle.

A fourth characteristic of the phase of "physiological youth" had meanwhile been described by Clark and Ruehl (1919) and by Henrici (1921 to 1928). These investigators demonstrated that certain early phases of the culture cycle are characterized not only by rapid multiplication, high metabolic activity and low resistance to certain harmful environmental conditions but also by highly characteristic types of morphology, the individual cells being in general much larger than in the maturing culture. Wilson (1926) demonstrated the same phenomena by a comparison

of plate counts with measurements of the opacity of bacterial cultures.

Finally, a fifth differential characteristic was described by MacGregor (1910) and by Sherman and Albus (1923), who recorded that young cultures were more resistant than old cultures to acid agglutination. Shibley (1924) demonstrated that in the early phases, the electrophoretic charge on the cells was much less than at a later period.

The fundamental biological significance of the bacterial culture cycle was perhaps first clearly recognized by Henrici. In one of his earlier papers (Henrici, 1925a) he says:

> The cells of bacteria undergo a regular metamorphosis during the growth of a culture similar to the metamorphosis exhibited by the cells of a multicellular organism during its development, each species presenting three types of cells, a young form, an adult form and a senescent form; that these variations are dependent on the metabolic rate, as Child has found them to be in multicellular organisms, the change from one type to another occurring at the points of inflection in the growth curve. The young or embryonic type is maintained during the period of accelerating growth, the adult form appears with the phase of negative acceleration, and the senescent cells develop at the beginning of the death phase.

The same theme was developed in his later monograph (Henrici, 1928) as follows:

> The acceptance of this theory demands the acceptance of certain corollaries. If it be granted that the cells of bacteria undergo a metamorphosis of the same kind as that exhibited by multicellular organisms, then it must be granted that to this degree a population of free one-celled organisms, even though those cells have no connection other than the common nutrient fluid which bathes them, behaves like an individual. There has already been accumulated a great deal of evidence of other kinds to support the idea that there is no essential difference, that there can be drawn no hard and fast line, between populations of one-celled organisms and multicellular individuals; that a higher plant or animal is but a population of more highly differentiated cells. But there has been, in the past at least, a tendency to look upon cell differentiation in multicellular organisms as being the result of some

organizing agency peculiar to such individuals. If, however, we find in cultures of micro-organisms where no such governing agency can be supposed to exist, a differentiation of cells, even though very primitive, we are forced to conclude that such is not the case; that the high degree of organization of higher organisms is a result and not a cause of the high degree of cell differentiation.

Acceptance of the validity of this analogy between a bacterial culture cycle and a multicellular organism clarifies very greatly the long conflict between pleomorphists and monomorphists in the field of bacteriology. One group has assumed a bacterial "life cycle" governed by some inherent biological tendencies; the other group denies that a cycle of any kind exists. Both perhaps are wrong and both right. One of us (Winslow, 1935) has pointed out that variations in bacterial morphology and physiology certainly do exist but that their succession is governed by environmental and not automatic inherent factors. Furthermore, this is precisely what occurs with higher forms of life where the organism as a whole forms the environment for its individual cells.

May we not assume then, that with all living cells, the "life cycle"—so far as the individual cell is concerned—is a cycle of simple binary fission. Other phenomena involving change in cell morphology and physiology of a cyclical nature are responses to changing environmental conditions and not the result of any inherent time mechanism. If a unicellular organism shows a definite series of morphological and physiological alterations in response to certain changes in environment which are likely to occur with reasonable frequency in its natural life we may call it a "life cycle" if we wish or we may call it something else. In any case, this is the only kind of life cycle (other than binary fission) which can occur in unicellular and relatively simple multicellular forms. In this sense, the bacteria have life cycles. When we find a more complex and more regular life cycle in the higher plants and animals (relatively independent of external environment), it is because the interrelationships of the complex organism produce a cyclical change in the internal environment which is comparable with the change which takes place in a bacterial culture and which affects the individual body cell very much as the cultural environment affects the unicellular organism. (Winslow, 1935.)

The study of the bacterial life cycle is, then, the bacteriological equivalent of the study of embryology, adolescence, maturity and senescence in the higher forms. It is the purpose of the present article to review in orderly fashion some of the things we know about the earlier parts of this cycle—those included under the Buchanan phases of initial stationary, lag and logarithmic growth. No attempt will be made to cover all the literature, which would be impossible in so vast a field; but certain significant and typical data will be cited in regard to each essential point.

THE PHASE OF ADJUSTMENT

Müller, in his remarkable pioneer paper of 1895, pointed out that an inoculum from a young typhoid culture showed a much shorter generation time in a new medium than did an inoculum from an old culture. His conclusions did not deal specifically with the lag period but the length of that period obviously influenced his generation times. This is true of many data cited in the present section. Their use as illustrative of factors governing the initial stationary phase seems justified, however, since it is the length of this phase which chiefly determines early generation times. Hehewerth (1901) made similar observations. Barber (1908), working with a microscopic counting method, first showed conclusively that if transfer be made to an identical medium from a culture in the stage of logarithmic increase both stationary phase and lag disappear and multiplication continues at once in the new medium at a logarithmic rate. Lane-Claypon (1909) Penfold (1914) and Chesney (1916) demonstrated the same phenomena. Buchanan (1928) in his general review of bacterial growth curves stresses the fact that transfer from any phase of the culture cycle to an identical medium is followed by continuance of the phase which had been reached by the parent culture, cultures inoculated from either initial stationary phase, lag phase or logarithmic phase, starting in the new culture where they left off in the old one.

The term "lag phase" is commonly applied to the whole period preceding the onset of logarithmic growth; and Ledingham and

Penfold (1914) and Slator (1917) even attempted to formulate a mathematical expression to describe increase in numbers during this period. Such an analysis does not seem very profitable when one considers what radically different processes are at work. Buchanan (1918) was clearly correct in separating a primary phase of initial stationary population from that phase characterized by increase at a rate less than logarithmic. We prefer, however, to call this entire period "The Phase of Adjustment." Instead of the population being stationary during the first minutes or hours after inoculation it may often show a marked decrease. The essence of this phase of the bacterial culture cycle is the adjustment of the inoculated cells to a new medium. Its course and its length depend on the character of the inoculated cells and the nature of the inoculated medium. We agree in part with Hershey (1939) when he attributes the lag phenomenon "to initially unfavorable conditions of growth"; but we cannot agree that this phenomenon is "quite distinct from any peculiarity inherent in the cells." Whether a given condition is unfavorable or not may very clearly depend on peculiarities inherent in the cells, as shown by many observers, from Reichenbach (1911) to Sherman and Albus (1923).

We do, however, concur with Hershey in his contention that when a bacterial culture is inoculated into a *favorable* medium, an initially slow rate of increase in cell numbers cannot be interpreted as indicating "lag," in the conventional sense of low vitality. Most of the early work on this problem deals with cell numbers only; and, since we know that in the early phases of the culture cycle individual cells are of large size, a slow increase in cell numbers may not necessarily mean a slow increase in bacterial mass. Hershey and Bronfenbrenner (1938) and Hershey (1938) have shown that when source cultures of different ages are used to inoculate a highly favorable medium the rate of increase in bacterial mass and the rate of oxygen consumption per unit of mass in the secondary culture is the same for both young and old source cultures. In a more recent paper, Hershey (1939) describes even more conclusive experiments. He cultivated *Escherichia coli* in peptone beef-extract broth and for source

cultures used 3-hour and 24-hour portions of this broth culture. These portions were inoculated into the same medium and the increase in bacterial mass was measured by a photoelectric nephelometer. Simultaneously, determinations were made of bacterial nitrogen and of rate of oxygen consumption. During the first two or three hours of growth in the secondary cultures, the inocula from the young primary cultures showed a much slower rate of cell multiplication than the inocula from old primary cultures but the same rate of increase in total protoplasmic growth, as measured by either nephelometer readings, nitrogen determinations or oxygen consumption. What actually happens is that the cells from a young primary culture develop just as rapidly in total mass in the secondary culture as do the cells from the old culture; but they divide less rapidly.

Unpublished nephelometer studies made in the Department of Public Health of the Yale School of Medicine fully confirm these conclusions of Hershey, and show that the actual rate of increase of bacterial mass is nearly constant from the time growth actually begins up to the attainment of a maximum population. The "lag" in rate of cell-increase is therefore largely a result of delayed cell-division.

The "lag phase," as ordinarily defined by cell counts, may include two quite distinct phenomena, a period of adjustment, characterized by bactericidal or bacteriostatic processes and a period of normally rapid increase in mass with a low rate of cell division. In a very favorable medium, such as Hershey's, the first period disappears; in a very unfavorable medium the second period disappears. It is difficult to distinguish the two processes if cell-mass determinations are not made, and the results of Müller, Hehewerth, Barber and other investigators cited above were no doubt chiefly determined by delayed cell-division. Other data, however, clearly point to temporary bactericidal or bacteriostatic influences.

For example, injury to the inoculum before its introduction into the new medium will prolong lag, as Penfold (1914) found with prolonged chilling. Sturges (1919) noted much-delayed development of colonies on plates seeded from sewage which had

been partially disinfected by copper or sulphurous acid. Allen (1923) reported similar results (measured by generation times) following heat treatment. Recently, Hollaender and Duggar (1938) found that after exposure of *Escherichia coli* and *Serratia marcescens* to ultra-violet radiation which killed four-fifths of the cells the survivors, when inoculated into broth, showed an initial increase, followed by prolonged lag.

The phase of adjustment, as characterized by a stationary or decreasing population, was demonstrated by the early observations on bacterial changes in water samples (reviewed by Winslow 1928) where the alteration of the environment due to placing a sample in a bottle is usually sufficient to cause a temporary decrease in the mixed bacterial flora present. The fact that the bacterial count of a milk sample drawn from the udder shows a similar decrease was first pointed out by Fokker (1890); and there is an enormous literature on the "bactericidal property of milk," a problem recently discussed by Little (1937). Sherman and Curran (1924) showed that a pure culture of *Streptococcus lactis*, transferred in the stage of rapid multiplication to autoclaved milk, showed no lag but, if transferred to unautoclaved freshly drawn aseptic milk, did show a brief lag of half an hour. Sherman and Cameron (1934) found that inoculation from a peptone medium at 45° to the same medium at 10° or *vice versa* and transfer from a peptone medium to the same medium plus 5 per cent NaCl or *vice versa* all showed a considerable initial mortality in the new culture.

The results of Winslow, Walker and Sutermeister (1932) are of special interest in this connection. The strain of *Escherichia coli* studied by these investigators was cultivated in an aerated peptone medium. The addition of 0.1 M NaCl to this medium led to an initial decrease in numbers (instead of the normal lag, characteristic of the plain peptone) followed by a more rapid logarithmic rise and a higher final peak. The addition of 0.5 M NaCl caused a much greater initial decrease, followed by a delayed rise and a final peak lower than that for plain peptone. The addition of 1.25 M NaCl led to a prompt and continuing decline in numbers. Thus, we may find either a steady increase,

a steady decrease or a decrease followed by an increase, depending on the concentration of salt present.

An extremely important point affecting the adjustment of the organism to its new environment is the influence of the amount of inoculum introduced. Wildiers, as far back as 1901, showed that, even in a supposedly ideal medium, a very small inoculum of yeast failed to develop. He concluded that yeast requires for its development an unknown substance ("bios"), soluble in water, dialyzable, difficult to alter or precipitate but destroyed by incineration. Rahn (1906), working with bacteria, discussed this problem and concluded that heat-stable non-filterable substances formed by the bacteria were necessary for maximal multiplication. Penfold (1914) criticized Rahn's work (which, in fact, did not bear clearly on the lag phase on account of the long time-interval employed). Yet Penfold himself found that very small inocula did increase lag. He considered that lag was chiefly due to the lack of intermediate bodies involved in the synthesis of proteins. Chesney (1916) added the interesting observation that an inoculum of washed cells showed a greater lag than one of unwashed cells. Robertson (1923) discussed the problem from a broad biological standpoint and explained it on the ground of an autocatalytic theory of growth.

Walker (1932) demonstrated, in a particular case, that the length of the lag period can be directly controlled by concentration of carbon dioxide, which Valley and Rettger (1927) have shown to be essential to bacterial growth. Walker found that the multiplication of *Escherichia coli* in a synthetic medium could be indefinitely delayed by aeration of the culture with air free from carbon dioxide while growth could be initiated at any moment by intermitting the removal of carbon dioxide. He concludes that "the phenomenon of lag may be due largely, if not entirely, to the time it takes the culture to build up the CO_2 content of the medium or of the cells themselves to a value essential for growth." These conclusions were extended to a wide variety of bacterial species by Gladstone, Fildes and Richardson (1935).

It may be that carbon dioxide represents the "bios" of Wildiers and the "intermediate bodies" of Penfold. Such a hypothesis

might well explain the influence of the size of inoculum upon lag phenomena. We should be cautious, however, in claiming that this is necessarily the only factor at work. It is probable that it is one important factor and it certainly was the controlling one in Walker's study. Other factors may, however, be involved under other circumstances.

The nutrient value of the medium is, of course, another factor in controlling the early phases of growth. Coplans (1910), for instance, showed that lag was increased by addition of dulcitol to a peptone medium and was greater in unheated than in heated milk. Penfold and Norris (1912) showed that early generation times for *Eberthella typhosa* were increased by decreased proportions of peptone in peptone water and decreased by addition of glucose. Winslow, Walker and Sutermeister (1932) found that, when aerated with CO_2-free air, growth was easily initiated in presence of peptone while lag lasted indefinitely in a Dolloff synthetic medium.

Hydrogen-ion concentration and oxidation-reduction potential are, of course, among the most important factors governing the rate of bacterial reproduction. Cohen and Clark (1919) showed for a number of bacterial types that there is a broad zone of pH within which rates of logarithmic increase in cell numbers are fairly uniform but that on the borders of this zone, very slight changes in pH produce a marked decrease in reproductive rate. The period of "lag" is more pronounced in alkaline than in acid media. That other factors than pH may enter into the picture is indicated by the fact that the border of acid tolerance is different for acetic and for hydrochloric acid.

Fildes (1929) found that the period required for the germination of spores of *Clostridium tetani* was primarily determined by the reducing intensity of the medium. Dubos (1929) reported that growth of pneumococci, streptococci and staphylococci in meat-infusion broth was dependent on a suitable reduction potential.

Finally, the length of the stationary phase is, of course, directly related to temperature. This was shown for water samples by Whipple (1901) by Müller (1903) by Penfold (1914) and by many

others. A recent study by Anderson and Meanwell (1936) of a milk streptococcus (cultured in milk) showed a lag of half an hour at 42°, one hour at 37°, two hours at 30°, and 26°, and three hours at 20°.

Jahn (1934) has recently reviewed the problem of population growth in the Protozoa and finds essentially similar phenomena. He lists food supply, presence of waste products, pH, temperature, CO_2, oxygen tension, oxidation-reduction potential and light as the chief factors influencing development.

THE PHASE OF PHYSIOLOGICAL YOUTH

If the medium be potentially favorable, a time at last arrives when the process of adjustment between inoculum and medium is complete, either by selection of better adapted cells or by accumulation of carbon dioxide or other necessary substances. At this point the initial phase of stationary or decreasing population ceases and the phase of physiological youth begins. The change will not, of course, occur simultaneously with all the cells present in a culture. If we may visualize a given individual cell at this point it will presumably have the general characteristics of the original inoculum. We may assume that the inoculum was taken from a parent culture in the phase of maximal population, since that is the condition under which a complete life cycle is manifest. Under such conditions the culture in its early lag phase will have the following general properties.

The cells will be characterized by relatively low physiological activity (Martin, 1932; Mooney and Winslow, 1935; Huntington and Winslow, 1937); relatively small size (Bayne-Jones and Sandholzer, 1933; Clark and Ruehl, 1919; Henrici, 1928; Jensen, 1928); low multiplication rate (Rahn, 1906; Coplans, 1910; Lane-Claypon, 1909; Penfold, 1914; Ledingham and Penfold, 1914); rather high resistance to unfavorable conditions (Schultz and Ritz, 1910; Sherman and Albus, 1924; Elliker and Frazier, 1938); and relatively high electrophoretic mobility (Moyer, 1936).

As soon as the process of adjustment is completed, however, the cell in its new medium passes into a phase of physiological youth,

characterized by active metabolism and rapid increase in mass but—at first—with delayed cell-division.

The morphological and physiological manifestations of youthful activity are nearly simultaneous. Huntington and Winslow (1937), however, have shown that with *Escherichia coli*, *Salmonella pullorum* and *Salmonella gallinarum* in an aerated medium, both the initial increase and the peak of metabolic activity slightly precede the initial increase and peak in cell size. In eight sets of experiments with these three organisms in three different media, the ratio of cell size for the one-hour culture as compared with the 24-hour culture was close to unity in six instances and about 2 in the other two instances. The corresponding ratio for metabolic activity was unity in two instances and from 3 to 16 in the other six instances. Martin (1932) considers increases in cell size and metabolism to be simultaneous, but since his "simultaneous" increases represent observations at the end of a given time interval in the case of cell size and cumulative results of the entire corresponding interval in the case of metabolic activity, his results, too, fit the theory that increased metabolic activity is the first evidence of physiological youth.

Metabolic activity

The generally high metabolic activity for the early phase of the culture cycle was first indicated in the remarkable study of Müller (1903). He does not give strictly comparable figures for cell numbers and metabolic activity, but does show that carbon-dioxide production, H_2S-production and formation of products of nitrogenous decomposition all reach a high peak in the early phases of the culture cycle. If one may assume that, in these metabolic studies, the rates of multiplication were the same as those given by Müller in his other tables it is clear that metabolic activity per cell was enormous during the early lag period.

Bayne-Jones and Rhees (1929) were perhaps the first investigators to provide actual data on metabolic activity per cell per hour at different periods of the culture cycle. They studied heat production in cultures of *Escherichia coli* and *Staphylococcus*

aureus. Their most striking results were for *E. coli* in peptone broth, where the gram-calories of heat produced per cell were 60×10^{-11} at one hour, 198 at two hours, 130 at 3 hours and 75 at seven hours; but their other experiments indicated the same general relationship. With *E. coli* in plain broth, the increase was more than eightfold. Wetzel (1932) presented a formal mathematical analysis of the data of Bayne-Jones and Rhees and concluded that the formulae involved described equally well the course of heat production in a bacterial culture and in the growing human body. Schmidt and Bayne-Jones presented similar data for *E. coli* in peptone water, which yielded values of 232×10^{-11} gram-calories per cell for the first hour, 194 for the second hour, 51 for the third hour and 4 for the sixth hour.

Similar data for oxygen consumption next became available. Burk and Lineweaver (1930) showed for *Azotobacter* that rate of oxygen consumption per unit rate of increase in cell numbers was greater during the first nine hours of the culture than for the ninth to the twelfth hours. Eaton (1931), working with staphylococcus cultures, reported a higher respiration rate during the first two and a half hours. Gerard and Falk (1931) provided the first definitely quantitative data for *Sarcina lutea*. They computed a consumption of 6.5 cu.mm. of oxygen per milligram dry weight of culture for the early stages of the culture cycle as compared with 2.6 in the phase of stable maximal population. They did not, however, interpret the phenomenon as a manifestation of physiological youth but attributed it to a partial asphyxia produced in the preparation of the inoculum. Martin (1932) saw the problem in its true setting, although he did not compute rates per cell. He noted, however, that the rate of oxygen consumption reached a peak for *Escherichia coli* between 30 and 90 minutes while cell size was greatest at 60 to 120 minutes.

The fact that Hershey (1939) did not detect any change in rate of oxygen consumption at different periods of the culture cycle is not surprising since the range of ages covered in his secondary cultures was only from 1.2 to 2.3 hours. What he does show, however, is that this rate—under the conditions of his experiment—was not affected by wide variations in the age of the

primary culture from which this secondary culture was inoculated. This latter conclusion, we are quite ready to accept. As pointed out above, the physiological state of a primary culture influences the rate of growth in mass in a secondary culture chiefly through its influence on the phase of adjustment—and, in Hershey's case, the medium was so favorable that the phase of adjustment practically disappeared.

More complete information is available with regard to CO_2 production. Cutler and Crump (1929) reported that liberation of CO_2 in sands and soils was greatest per million bacteria when the numbers of bacteria were low. Walker and Winslow (1932), working with *Escherichia coli* in an aerated culture, reported 41 to 185 mg. $\times 10^{-11}$ CO_2 per cell per hour formed in the lag period against less than 2 mgm. for the close of the logarithmic phase. Walker, Winslow and Mooney (1934) studied the problem in media aerated with nitrogen instead of air and found that, under such anaerobic conditions, CO_2-production per cell per hour in a peptone medium fell from 42 mg. $\times 10^{-11}$ in the first hour to 27 in the second hour, rose to 68 in the third hour and then fell. Under anaerobic conditions in peptone-glucose the figure fell from 42 in the first to 36 in the second hour, rose to 211 in the fourth hour and then fell. The second-hour temporary decrease seems to be a peculiarity of the anaerobic state and the enormous rate recorded for peptone-glucose indicates anaerobic utilization of sugar. No increase of CO_2 production per cell was ever noted in the earlier work under aerobic conditions in sugar-containing media, as a result of the presence of the sugar, in spite of the fact that sugar was actively fermented. Walker, Winslow, Huntington and Mooney (1934) worked with *Escherichia coli* in various media and reported maximal production of CO_2 (117 to 123 mg. $\times 10^{-11}$ per cell per hour) during the second hour of the culture cycle (late lag and early logarithmic phase) falling to 16 to 22 after five hours (beyond the close of logarithmic phase). Mooney and Winslow (1935) studied *Salmonella gallinarum* and *Salmonella pullorum* as well as *E. coli*. A high peak of metabolic activity during the lag and earlier logarithmic period was always apparent. In this early phase,

79 to 145 $\times 10^{-11}$ mg. of CO_2 was formed per cell per hour as compared with 2 to 8 in the stage of maximal population. *Salmonella pullorum* in peptone-glucose had a very long lag period and the figures in table 1 give a valuable slow-motion picture of the processes involved.

Where the process is more rapid, the various stages are telescoped, so that a first-hour observation often shows a high metabolic rate; but such an experiment as that cited gives us a true picture of the processes at work.

In all the studies cited (except that of Gerard and Falk) the high metabolic rates of the early growth phases were computed

TABLE 1

AGE OF CULTURE	BACTERIA	CO_2-PRODUCTION PER CELL PER HOUR
hours	*millions per ml.*	*mg.* $\times 10^{-11}$
1	12	6
2	10	30
3	13	33
4	16	99
5	39	114
6	70	96
7	320	57
8	603	26
9	706	17
25	332	8

per cell; and these high rates of activity might be in part due to the larger size of the cells. It was clear from consideration of the magnitude of the changes involved that this could not explain the whole phenomenon; but the relationships of cell size and metabolic activity were finally clarified by Huntington and Winslow (1937). These observers combined simultaneous data on cell numbers, cell size and metabolic activity, working with *Escherichia coli*, *Salmonella gallinarum* and *Salmonella pullorum* in aerated cultures and using three different media. They computed rates of CO_2-production per cubic micron of bacterial substance and found that maximum values for the lag and early logarithmic phases ranged from 86 to 216 $\times 10^{-11}$ mg. per cubic

micron while for the phase of stationary maximum population the corresponding figures varied from 5 to 19.

A fourth measure of metabolic activity, for which similar evidence is available, is acid production. Stark and Stark (1929a) found the rate of fermentation of *Escherichia coli* to be 4.6×10^{-11} mg. per cell per hour for young cells and 0.9 for old cells. Less striking but similar relationships for fermentation by *Streptococcus lactis* have been reported by Rahn, Hegarty and Deuel (1938).

Liberation of NH_3-nitrogen is a fifth type of metabolic activity associated with physiological youth. Here, there arises the question whether lowered values for NH_3-nitrogen in later growth phases may not be due to more rapid utilization, rather than to a lessened rate of liberation of NH_3. This question cannot be categorically answered, but the close parallelism between NH_3 and CO_2 suggests that both are examples of the same phenomenon. The first suggestion of such an effect in the case of NH_3 (after the pioneer work of Müller) came from Meiklejohn (1930) who studied the relation of numbers and NH_3-production in a peptone culture of a soil bacterium. Number of cells and efficiency in NH_3-production showed an inverse relationship. Walker and Winslow (1932), in a more detailed study, determined the rate of NH_3-production for *Escherichia coli* in the lag phase in different media as 6 to 36 mg. $\times 10^{-11}$ per cell per hour while the corresponding figure for the phase of maximum population was 0.2 or less. Walker, Winslow, Huntington and Mooney (1934) reported that at $1\frac{1}{2}$ hours (late lag phase) the NH_3-nitrogen yield varied in different media from 26 to 50 mg. $\times 10^{-11}$ per cell per hour while after 5 hours (post-logarithmic phase) the values fell to 3 or less.

Hewitt (1937) reports electrode-potential curves for various organisms at various phases of the culture cycle. With hemolytic streptococci in broth the E_h begins to fall after 30 minutes and drops to a minimum in 12 hours (approximate end of the logarithmic phase). Subsequently the value rises or (with the diphtheria organism) may remain at a low level. In aerobic

glucose-broth, the streptococcus shows a sharp rise after five hours (Hewitt 1929). The phenomena involved are, however, too complex to be related with certainty to the physiological culture cycle.

Child (1929), in his striking essay on Senescence and Rejuvenescence, points out that phenomena exactly like those described above are manifest in the life history of some of the simpler invertebrates (hydroids and planaria). From the very early stages of the life of these multicellular organisms there is manifest a progressive decrease in oxygen consumption, carbon dioxide production and growth rate. Again, the analogy between a bacterial culture cycle and the life of a multicellular organism is strikingly illustrated.

Morphological changes

In parallel with the outburst of metabolic activity which characterizes the early lag period,[1] and almost—but not quite—simultaneous with it, come fundamental changes in size and other morphological characteristics of the bacterial cell.

Many of the earlier workers in bacteriology noted the presence of large cells in the initial phases of the culture cycle (see review by Ward, 1928). One of the most significant of such observations was that of Fuhrmann (1908; 1926) who observed that an organism which he called *Pseudomonas cerevisiae* exhibited a rather regular series of morphological phases in various media, beginning with small rods and passing on to large swollen cells and thread-like forms with refractive points and stainable granules. The first exhaustive study of this problem, with clear emphasis on the time-relations involved, was, however, that of Clark and Ruehl (1919).

These investigators studied 70 strains belonging to 37 species of bacteria and found that in all cases, except certain corynebacteria and the glanders organism, marked increase in size occurred

[1] These changes are commonly attributed to the *late* lag phase; but this is because under the term "lag phase" there is also included the "initial stationary phase." Using Buchanan's more penetrating analysis the characteristics discussed are associated with the very early lag phase.

in the early stages of the culture cycle. This increase generally manifested itself after 2 hours and the maximum size was as a rule noted between 4 and 6 hours. In a later review, Clark (1928) says "During the logarithmic period when maximum reproduction occurs, the cells from the young cultures of many genera of bacteria attain their maximum size, two to six times larger than the cells from the twenty-four-hour parent-cultures." During later progression, up to 18 to 24 hours, toward a stationary period the bacteria became gradually smaller in size. The large cells stain more intensely than those of normal size.

The major contribution to our knowledge of this subject came from Henrici who published a remarkable series of papers, and finally a book on the subject of Morphologic Variation and the Rate of Growth of Bacteria,[2] between 1921 and 1928. In his first paper (1921) he showed for a spore-bearing aerobe that the cells began to increase in size in the lag rather than the logarithmic phase, reached their maximum dimensions shortly after the beginning of maximum multiplication (six times those of the original inoculum), then gradually becoming shorter. *Escherichia coli* (1924a) showed maximum size at three hours (about the middle of the logarithmic phase)[3] and was down again to normal by 6 hours (end of logarithmic phase). When the cells are largest, intracellular granules disappear, the protoplasm becomes more hyaline and stains more deeply. If transferred at the moment of increasing cell size, increase proceeds; if at the moment when original size has been reached, increase begins at once; if transferred later, lag occurs (in cell size increase). The richer the medium, the longer is the period of size increase and the greater the maximum size attained (1925a). The cholera vibrio develops oval cells of less than normal curvature in the lag phase. As the logarithmic phase sets in, the cells become elongated and curved again (1925b). In Henrici's final monograph (1928) he reviews all this material with full data as to the curve of distribution of cell length and form at each stage. The phase of large

[2] Passages from this work are cited below through courtesy of Charles C. Thomas, Publisher, Springfield, Illinois.

[3] Henrici would probably have found even larger cells at a still earlier period.

cells is clearly associated with greater variability in size. The more rapid the growth, the greater is the cell size. The presence of sodium ricinoleate tends to produce very elongated cells while $CaCl_2$ (which raises surface tension) has an opposite effect. Large cells of *Escherichia coli* are more stainable and perhaps show an isoelectric point further to the acid side.

In general comment, Henrici says:

> These three correlated properties: Increased length and slenderness of the cells, indicating a greater magnitude of some axially disposed force opposing the surface tension of the medium; increased intensity of staining with basic dyes and decreased susceptibility to acid agglutination, indicating an isoelectric point of the protoplasm more on the acid side; and increased susceptibility to injurious agents, all serve to distinguish the young, actively growing cells from the resting cells, and justify our recognizing these long cells as a distinct morphologic type, as *embryonic* cells, characteristic of the growth phase of the culture.

The general significance of the phenomena involved are discussed as follows:

> It would seem from my data that the division of the growth curve into a lag phase (of accelerating growth), a logarithmic growth phase, and a resting phase, is not so significant as a division into a phase of accelerating growth and a phase of negative acceleration in growth; the so-called logarithmic growth phase when present is but a long drawn out point of inflection. For the morphologic variations which occur during the early stages of growth progress definitely to this "mid-point" (as Pearl designates it) of the growth cycle, then turn sharply in the opposite direction. The embryonic forms reach their maximum development just at the beginning of negative acceleration in growth rate, the mature forms at the end of growth.
>
> These embryonic forms will vary in their characters with different species of bacteria, but it is apparent from what has been presented here, as well as from the observations of Clark and Ruehl, that with most forms, especially the rods, they differ from the mature forms particularly in increased length and slenderness. The diphtheroid group are apparently an exception, the embryonic forms being shorter and more nearly approaching the spherical form. In all cases these embryonic forms seem to possess a higher affinity for the basic aniline

dyes. In those cases where the young cells show an increased size there is also apparent an increased variability in size. In those forms which develop intracellular granules or other structures these are lacking in the embryonic cells. In the case of the cholera vibrio, which may perhaps be taken as a type of the spiral organism, the embryonic forms are characterized particularly by straightness of their cells; they are bacillary in form.

The mature or differentiated forms, beginning to develop with negative acceleration in growth and reaching their maximum at the end of growth, are just the reverse of the embryonic forms in the characters enumerated above; . . . It is of course obvious that these cells are not differentiated in the sense that different cells show a great diversity of form and internal structure as occurs in the differentiated cells of a multicellular organism; such cannot be the case because the cells are all contained in the same environment which must be nearly uniform throughout. But the individual cells do show a differentiation in their internal structure, many forms developing within the protoplasm spores or granules of one type or another, especially volutin. Now it is just this development of internal "paraplasmatic" structures which characterizes the differentiated cells of a multicellular organism, and which are either the result or the cause of their diversified function. In this sense at least, then, there does occur differentiation in the mature cells of bacteria. (Henrici, 1928.)

It should be noted that the diphtheria bacillus seems to offer an exception to the general rules which operate with the other organisms studied. Albert (1921) recorded an early increase in size even for this organism but Clark and Ruehl (1919) found the young cells here smaller than in a later resting period. Henrici (1922), working with a chromogenic diphtheroid, confirmed Clark and Ruehl's observations but his figures (Henrici, 1928) suggest that the special tendency of this organism to form chains of streptobacilli may be the complicating factor in this case.

Meanwhile, Wilson (1926) has demonstrated increase in cell size during the earlier phases of the culture cycle by an interesting new method. He compared increasing opacity of a culture with cell-counts and found that the number of viable cells necessary to produce a given opacity was five times as great in a 26-hour culture as in a 4-hour culture of *Salmonella aertrycke*. Alper

and Sterne (1933) present similar data. The possible presence of more non-viable cells in the later phases somewhat vitiates these conclusions.

Jensen (1928) made a particularly significant study of the morphology and growth of *Escherichia coli*, with special emphasis on the history of the individual cell. His careful observations led him to the conclusion that the phase of absolute latency is one in which cell size increases without fission. He finds that, when an individual cell has reached its maximal size, multiplication at full logarithmic rate begins, so that the phase of relative latency (increase in numbers at sub-logarithmic rate) is merely a statistical characteristic of the culture as a whole. He gives us a new and very interesting type of information in regard to the proportion of individual cells in a culture which proceed to subdivide within a reasonable period of observation. At two hours (late lag or early logarithmic phase) 100 per cent of the cells exhibit prompt fission, while after four or five hours (end of logarithmic phase) the ratio falls to 10 to 54 per cent. After twelve hours, it rises once more.

The work of Clark and Ruehl and Henrici tended to show that the phase of maximum cell size and that of most rapid multiplication coincide. Jensen's studies, however, strongly suggest that increase in cell size actually precedes a rise in division rate. Thus, his colon bacilli in the first half hour were large but not yet dividing. From one to one and one-half hours (early logarithmic phase) they were large and dividing. At $3\frac{1}{2}$ hours (middle logarithmic phase) weakly-staining shadow forms began to appear. At five hours (post-logarithmic phase) the shadows had disappeared and cells were small again. These shadow forms were capable of reproduction if transferred to a new medium but apparently could not do so in the original medium.

The next important contributions to this problem came from Bayne-Jones and his associates at Rochester, using a very accurate technique based on measurement of cinematograph records. Working with *Bacillus megatherium*, Adolph and Bayne-Jones (1932), like Jensen, found that fission rates lagged behind rates of increase in total cell protoplasm. They noted

(as had Schmalhausen and Bordzilowskaja, 1926) that the rate of growth of a single cell from fission to fission is approximately constant at a given time but that the mean rate of growth in size per cell in a culture rises sharply to the end of the second hour and falls after the third hour (when an 18-hour inoculum is used). At the peak of increase in cell size the bulk of cell substance doubles every 22 minutes, and the authors compute that if such a growth rate continued for 24 hours a single filament could be produced reaching 4000 times the distance between the earth and the sun. With *Escherichia coli*, Bayne-Jones and Adolph (1932b) found that at the end of the first hour there was

TABLE 2

Time of maximum activity (hours after inoculation)

	SIZE OF CELL	RATE OF CELL MULTIPLICATION
E. coli		
Peptone	2	4
Peptone-glucose	2	3
S. gallinarum		
Peptone	3	5
Peptone-glucose	3	6
Peptone-lactose	3	7
S. pullorum		
Peptone	4	9
Peptone-glucose	4	7
Peptone-lactose	3	7

a maximum rate of growth in cell size, with no increase in numbers, while the maximum rate of reproduction occurred at the end of the second hour. Bayne-Jones and Sandholzer (1933), with *E. coli*, report that the volume of a cell of the initial inoculum was less than 1 cubic micron. At the end of the first hour this value had increased to 4 cubic micra. At the end of the logarithmic phase (160 minutes) the mean cell size was again about 1 cubic micron.

Huntington and Winslow (1937) have more recently confirmed, once more, the conclusion that increase in cell size precedes the logarithmic phase of multiplication. They studied *Escherichia coli*, *Salmonella gallinarum* and *Salmonella pullorum* in aerated

cultures using various media and found that, with eight combinations of organisms and media, the peak of reproductive activity came from one to five hours later than the peak of cell size, as indicated in table 2.

In five out of these eight instances maximum cell volume was noted during the lag phase, in the other three instances in the early logarithmic phase.

A suggestive check on these time studies is to be found in a paper by Fischer (1932) who observed the development of spreading cultures of *Escherichia coli* in soft agar. He noted in the outer zone of a spreading colony large cells (7 x 2 μ); in an inner ring, small cells (4 x 1 μ); and in the center, a region of autolysis. Here are the various time changes of Jensen, manifest at one time in those areas of a culture which are of different age.

Longworth (1938) has recently reported interesting studies of the variations in cell morphology in bacterial cultures as well as in ratios between sizes, counts and total mass as measured by a photoelectric densitometer. Hershey (1939), in the paper discussed in an earlier paragraph, expresses cell size as a ratio between nephelometric count and viable count and finds that when a 24-hour primary culture is used for inoculation the initial ratio is about 1 and increases to 3 during the first hour of the secondary culture and to 6 or 8 during the second hour. Inoculation from a 3-hour primary culture gives an initial ratio of 5 to 6, which is maintained for several hours, showing that in this respect (though not in mass-reproductive rate) the cells of a young primary culture behave differently for a time in a secondary culture from those derived from an old primary culture.

Lowered resistance to unfavorable agents

A third very important characteristic of the large actively metabolic cells of the early culture cycle is their markedly reduced resistance to various harmful chemical and physical conditions.

This phenomenon was, perhaps, first pointed out by Schultz and Ritz (1910). These investigators, using colon bacilli, exposed cultures of various ages to heat treatment at 53° for 25 minutes. In a 20-minute culture about 5 per cent survived such

treatment. In a 50-minute culture (still in the lag phase) 1 per cent survived. In 4-hour cultures (early logarithmic phase) 100 per cent were killed, and in cultures from 7 to 13 hours old (late logarithmic phase) the same treatment produced no reduction at all. These results would seem almost unbelievable if they had not been so often confirmed. Reichenbach (1911), in the very next year, reported a reduction (caused by exposure to a temperature of 47 to 51° for five minutes) of 71 to 100 per cent in 5 to 8 hour cultures and of only 3 per cent in a 28-hour culture.

The biological importance of this phenomenon was first emphasized by Sherman and Albus (1923) in their striking paper on *Physiological Youth in Bacteria.* These investigators demonstrated low resistance for *Escherichia coli* in the early culture-phases with respect to four independent conditions. They confirmed the general conclusions of Schultz and Ritz and of Reichenbach with respect to heat resistance, (although the differences were not quite so striking). They found that chilling the cultures showed a similar differential, old cultures exhibiting no reduction while young cultures did. They demonstrated the same phenomenon on transfer to a 2 per cent NaCl solution in distilled water and, finally, on exposure to 0.5 per cent phenol. In general four-hour cultures (logarithmic phase) were the ones which showed the low resistance. The results, so far as exposure to heat and NaCl was concerned, were confirmed for a *Proteus* strain. In a second communication Sherman and Albus (1924) worked out the time relations in greater detail. *E. coli* was grown at 37° in peptone-water and at intervals transferred to 5 per cent NaCl. Cells removed after one hour showed no mortality. After 90 minutes (while the parent culture was still in the lag phase) the cells showed definite susceptibility; and after 2 and $2\frac{1}{2}$ hours (when rapid increase in numbers was going on in the parent culture) mortality was greatly increased. The authors point out that this phenomenon gives clear evidence of "biologic rejuvenescence" before active reproduction begins.

The greater stainability reported by Henrici (1928) and others for the phase of increased cell size may be related to the lessened

resistance to chemical agents at this period. A later contribution in this field of resistance to chemical agents was that of Watkins and Winslow (1932) on disinfection by N/100 NaOH at 30°C. The following values for K were reported for cultures of varying age:

Age hours	K
8	.38
11	.22
14	.15
17	.14

Literature as to the Schultz and Ritz phenomena of lowered resistance to heat is voluminous. It was described by Ørskov (1925) for colon-typhoid strains and by Robertson (1927) for *Microbacterium lacticum*, *Sarcina lutea* and *Streptococcus thermophilus*. *S. lutea*, exposed to 71°, showed a 99 per cent reduction in a four-hour culture and no reduction in a post-logarithmic culture. *S. thermophilus*, heated at 63°, showed over 99 per cent reduction in a six-hour culture and only 55 per cent reduction in a 30-hour culture. Sherman, Stark and Stark (1929), and Stark and Stark (1929a; 1929b) made similar observations for streptococci and ropy-milk organisms. Fabian and Coulter (1930), Hammer and Hussong (1931), Heiberg (1932), Dorner and Thöni (1936) added more evidence along the same line. Hershey (1939) inoculated from 3-hour and 24-hour cultures into distilled water and found that while the young cells were completely eliminated after 15 minutes, half of the cells from the old culture were alive after one hour. It should be noted that, in many of these studies, the low resistance phase was contrasted with rather late periods of the culture cycle; so that it is not quite clear whether it is a contrast between youth and maturity or between maturity and old age of a culture with which we are dealing.

There are a few studies which appear to conflict with this considerable mass of evidence. The first of them, by Anderson and Meanwell (1936) does, indeed, seem hard to explain. These investigators worked with a thermoduric milk streptococcus cultivated in milk at various temperatures. At intervals tubes were removed and exposed to a temperature of 63° for 30 minutes.

When the parent culture was grown at 42° the lag period was very short and resistance to heat treatment decreased steadily. At 37° the lag period lasted about one hour and heat resistance rose at half an hour and then fell. In the cultures grown at 30°, 26° and 22°, respectively, lag was of course more and more prolonged but, in each case heat resistance was greatest in the early logarithmic phase. In other words, these results directly contradict all the earlier work of a score or more of different investigators. Somewhat similar results have recently been reported by Claydon (1937).

A third study which apparently conflicts with general experience appears, on closer analysis, not to do so but merely to bring out a very interesting new phenomenon with regard to the

TABLE 3

Parent culture at 28°

AGE	NUMBER OF BACTERIA PER ML.	PER CENT SURVIVAL WHEN HEATED AT 53°
hours		
0	25,000	6.4
1.5	33,800	34.0
3	40,350	1.4
6	297,000	.1
9	730,000	.02
36	3,200,000	5.8

culture cycle. Elliker and Frazier (1938 a and b) worked with *Escherichia coli* grown at 28° and 38° and then heat-shocked at 53° for 30 minutes. The results obtained are best illustrated by table 3.

The usual fall in resistance is shown for the late lag and early logarithmic phase; but the new phenomenon revealed is a temporary brief increase in resistance in the very early lag phase. More careful studies, at shorter intervals during the lag phase, showed that the initial increase in resistance lasted up to the first hour at 28° and from the twentieth to the fortieth minute at 38°. This suggests a new and exceedingly interesting characteristic of the early lag period.

The lowered resistance of the late lag and early logarithmic

phase has been demonstrated with respect to other physical influences than heat and cold by other investigators. Gates (1929) reports that a four-hour culture of *Staphylococcus aureus* is much more readily killed by a given intensity of ultra-violet light than is a 28-hour culture; and Kimball (1938) finds that budding of yeast is inhibited in a magnetic field only in the last half of the lag phase.

Bayne-Jones and Sandholzer (1933) reported that young cells are more readily attacked by bacteriophage than older ones.

A most interesting observation has recently been reported by Hegarty (1939), which may or may not be related to those characteristics of the youthful bacterial cell which make it susceptible to harmful agents in the environment. This investigator found that cells from a mature culture of *Streptococcus lactis* grown in glucose-tryptone broth can not attack galactose, lactose, sucrose or maltose until they have multiplied for some time in the presence of the respective carbohydrates. Cells from a 1- or 2-hour culture, however, attack these new sugars much more promptly. This is in the late lag and very early lag phase and the power of adaptation to the new carbohydrate, thereafter, decreases progressively. The power to utilize new carbohydrates is a favorable one while susceptibility to harmful agents is an unfavorable one; but it is conceivable that both may be related to some underlying property of ready responsiveness to the environment.

Acid agglutination and electrophoretic mobility

Finally, there is a fifth characteristic of the large and metabolically active cells of the youthful phase of the bacterial culture cycle, which may again be related to their low resistance to harmful chemical agents but is demonstrated by direct physical measurements. This is the property of low susceptibility to agglutination, coupled with low electrophoretic charge.

The first observation of this kind with which we are familiar was made by MacGregor (1910) who reported that meningococci from a two-day culture needed a concentration of 1:10 to 1:40 of normal serum to produce flocculation, while a four-day culture

sometimes flocculated with a concentration of 1:100 and at times even showed spontaneous agglutination. Gillespie (1914), working with pneumococci, found that cultures up to eighteen hours did not agglutinate in 20 hours without salt while, after twenty-four hours, they agglutinated in 6 hours. The reaction favorable to agglutination was less acid for the young cultures. Sherman and Albus (1923), working with *Escherichia coli*, reported that four-hour cultures were not agglutinated by an acidity of pH 3.0 while a twenty-four-hour culture agglutinated at a pH of 3.8.

Shibley (1924) made the first direct observation of electrophoretic charge (with pneumococci and paratyphoid organisms). In cultures from five to six hours old, the charge was low for both organisms. Between nine and ten hours, it rose and later fell slightly. Kahn and Schwarzkopf (1931) reported different phenomena for the tubercle bacillus. In four-day cultures and nine-day cultures, mobility was high, in twenty-day and five-week cultures, much lower. The tubercle bacillus is so different from ordinary bacteria and we know so little about its culture cycle that we cannot expect to interpret the data in analogous terms. There may well be a decrease in the charge on other bacteria in very old cultures. Buggs and Green (1935) indeed reported such a decrease in cultures of *Escherichia coli* and *Staphylococcus aureus* after 127 days; and these observers could find no difference between six-hour and ten-day cultures. Pedlow and Lisse (1936) also reported no change in electrophoretic mobility for *Escherichia coli* between three and twenty-eight hours.

Moyer (1936), in an exhaustive and extremely illuminating study, has given us the clearest and most complete picture of the phenomena involved. He worked with *Escherichia coli* in an aerated medium and demonstrated the following main conclusions. Rough and smooth strains exhibit distinctly different charges but, within each strain, results are highly consistent. Mobility is high at the start and falls during the first hour for the rough strain, remaining low for the second and part of the third hour and then rising again. The mobility of the smooth strain drops during the first and second hours and remains low during

the third and fourth hours. The low mobilities correspond to the lag and beginning of the logarithmic phases, and the subsequent rise in mobility comes toward the end of the logarithmic phase. Moyer made a peculiarly convincing experiment by mixing the large cells from a ninety-minute culture with the small cells from a sixteen- to twenty-four-hour culture when the differential mobility of the two morphological types was clearly manifest under the microscope. The large young cells moved at a mean rate of about 0.7μ/sec./v./cm. while the mean rate for the small old cells was about 0.9.

In a careful analysis of the causative factors involved, Moyer showed that the low mobility of the young cells was not due to irreversible changes in surface caused by buffer; nor to adsorption of dissolved metabolites; nor to adsorption of gaseous metabolites; nor to presence of flagella; but, probably, to a change in physical or chemical nature of the surface of the cells, perhaps associated with "expansion" of the surface and increased permeability. He demonstrated that heating at 56° for $\frac{3}{4}$ hour did not alter mobility of the 24-hour cells but greatly reduced the mobility of the 3-hour cells.

These results would suggest that all of the previous workers except Sherman and Albus and Pedlow and Lisse, began their observations at too late a period of the culture cycle to detect the phenomena in question. The failure of Pedlow and Lisse, Moyer ascribes to certain technical defects in their procedure, particularly to the use of distilled water as a suspending medium for the electrophoretic determinations.

THE PHASE OF LOGARITHMIC INCREASE AND THE COMPLETION OF THE NORMAL CULTURE CYCLE

It has been pointed out in earlier paragraphs that the fifth and final characteristic of "physiological youth" is a rapid rate of cell multiplication. This characteristic (which is the only one observed in ordinary bacteriological studies) is, however, initiated slightly later than the increase in metabolic activity and cell size, the lowered resistance and decreased electrophoretic charge, of the youth phase.

By the time the maximum rate of cell multiplication is reached —at about the middle of the logarithmic phase—most of the other characteristics of youth are on the decline.

Metabolic activity at this period has fallen substantially below its earlier maximum value, as shown by Bayne-Jones and Rhees (1929) and Schmidt and Bayne-Jones (1933) for heat production by *Escherichia coli*, by Eaton (1931) for the respiration of a staphylococcus, by Walker and Winslow (1932), Walker, Winslow, Huntington and Mooney (1934), Mooney and Winslow (1935), and Huntington and Winslow (1937) for production of CO_2 and NH_3 by *Escherichia* and two species of *Salmonella* and by Rahn, Hegarty and Deuel (1938) for fermentation by *Streptococcus lactis*. Schmidt and Bayne-Jones (1933), for example, reported 51 gram-calories $\times 10^{-11}$ per cell per hour produced in the phase of most rapid reproduction as compared with 232 for the lag period; and Mooney and Winslow (1935) reported 57 $\times$ 10^{-11} mg. of CO_2 per cell per hour at the period of most rapid reproduction against an earlier maximum of 114.

Cell size was noted as reduced at the middle of the logarithmic phase by Jensen (1928), Bayne-Jones and Adolph (1932b), Bayne-Jones and Sandholzer (1933) and Huntington and Winslow (1937) for *Escherichia coli*. This phenomenon did not appear in the studies of Henrici (1928) whose data for *Escherichia coli* and *Bacillus megatherium* show maximum cell size coincident with most rapid reproduction. All the more recent work, however, indicates decreased cell size in this phase. Thus, Bayne-Jones and Adolph (1932b) record a volume of 1 micron just after the point of maximum multiplication rate against 2.5 to 3.5 micra before that maximum rate. Huntington and Winslow (1937), in eight sets of experiments with three *Escherichia* and *Salmonella* species in various media, obtained a mean volume of 0.7 micron for the phase of most rapid cell-division against an earlier mean maximum of 1.1 micron.

Resistance to heat treatment was found by Robertson (1927) to be at a minimum at the height of logarithmic growth (when the "shadow forms" of Jensen, 1928, are most numerous). Elliker and Frazier (1938b) report a survival of under 3 per cent

of colon bacilli heated at 53° when the organisms were in the logarithmic phase as compared with survivals of 34 to 74 per cent for cells from earlier phases of the culture cycle.

The low electrophoretic mobility of the young cells, on the other hand, appears to persist well through the logarithmic phase (Moyer, 1936).

By the end of the logarithmic phase most of the characteristics of physiological youth have wholly disappeared.

Return to pre-lag values for heat-production at the end of the logarithmic phase are reported by Bayne-Jones and Rhees (1929), Mooney and Winslow (1935) and Huntington and Winslow (1937).

TABLE 4

Milligrams $\times$ 10^{-11} CO_2 *per cubic micron of bacterial substance per hour*

HOUR	PEPTONE	PEPTONE-GLUCOSE
First	11	82
Fourth	149	132
Tenth	17	11
Twenty-fifth	12	5

Table 4 from the data presented in the last-cited paper illustrates the general phenomenon, so far as metabolic activity is concerned.

In the peptone medium no cell multiplication had occurred during the first hour and the normal minimum metabolic activity was recorded; in peptone-glucose, however, multiplication began more rapidly and the first hour exhibits the phenomena of physiological youth. By the tenth hour, in both media, reproduction was still going on, though very slowly, and metabolic activity was nearly down to its minimum value.

Cell size was reported as down to its minimal values by the end of the logarithmic phase by Jensen (1928), Henrici (1928), Bayne-Jones and Sandholzer (1933) and Huntington and Winslow (1937). This phenomenon may be illustrated by the data (table 5) from Henrici (1928).

In each case the ten-hour culture was still increasing in numbers but was well past the height of the logarithmic phase.

The recovery at the end of logarithmic increase of normal resistance to harmful agents has been demonstrated clearly by many observers from Schultz and Ritz (1910) and Reichenbach (1911) to Elliker and Frazier (1938b). For example, Schultz and Ritz found that colon bacilli from a 7-hour culture, which had reached its peak population, were completely unaffected by heating to 53° for 25 minutes while a four-hour culture was completely sterilized by the same treatment. The recent data of Elliker and Frazier are almost as striking.

Moyer (1936) finds that at the close of the logarithmic period electrophoretic potential rises again sharply to its normal value.

After the culture cycle has passed through the phase of logarithmic increase, and the phase which Buchanan (1928) describes as that of "negative acceleration" it reaches the "maximum

TABLE 5

Size of B. megatherium, micra

HOURS	STRAIN 1	STRAIN 2	STRAIN 3
0	3.4	5.2	5.2
5	11.3	7.6	9.6
10	4.5	5.0	5.9
20	3.8	3.6	4.4

stationary phase" and then passes through the phases of "accelerated death" and "logarithmic death." With these latter phases we are not concerned in the present review. An optimum cycle, corresponding to the succession of generations in a multicellular organism, is reproduced by transferring to a new medium at the close of the period of active multiplication in the source culture. Such transfer would give us a picture in each new medium of a burst of physiological youth characterized by high metabolic activity, large cell size, low resistance to harmful physical and chemical agents, low electrophoretic mobility and rapid cell division; and, as the culture ages, all these evidences of youth gradually decrease to their initial levels. By transferring at earlier periods, the course of the life-cycle may be "short-circuited," as may be done with mammalian cells in tissue cultures.

The changes in a culture during the phases of accelerated and logarithmic death correspond to the senile changes in the multicellular organism after the normal reproductive period is passed. A comparative study of such senile changes would perhaps be timely; but they represent phenomena of a somewhat distinct type from those apparent in the early phases, which we have considered in the present review.

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