FACTORS LIMITING BACTERIAL GROWTH

IV. The Age of the Parent Culture and the Rate of Growth of Transplants of Escherichia Coli

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Contrary to the earlier idea that the initial lag in the multiplication of bacteria represents a period of latency, it is now recognized that actually this is a period of intense growth during which the cells are increasing in size and metabolic activity (Huntington and Winslow, 1937). Nevertheless, the new facts have been regarded as manifestations of rejuvenescence, and the description of lag as an interval of preparation for active multiplication has been retained (Topley and Wilson, 1936). Obviously, if it is to conform to the usual definitions, rejuvenescence of bacterial cells must be marked by an increased capacity for growth and metabolism which is independent of the increase in size. We have already shown to our satisfaction that when environmental conditions are held constant, the age of the source culture of *Escherichia coli* does not influence the rate of oxygen consumption per unit of bacterial substance (Hershey and Bronfenbrenner, 1938). At the same time indirect evidence was obtained that the initial rate of growth of cells transplanted from young and old cultures is the same, and this was substantially confirmed by the observation that under favorable conditions growth in broth regularly occurs without lag, if changes in the size of cells are taken into account (Hershey, 1938). The experiments to be reported at the present time are concerned with the direct measurement of the rate of increase in bulk of bacterial substance in relation to the age of the culture of *E. coli*.

The few data of this kind contained in the literature do not

bear directly on the question of age. However, certain observations on the rate of growth during the phase of lag should be referred to. Henrici (1928) constructed curves representing the growth in total cell length of microcolonies of *Bacillus megatherium*, and noted a marked shortening of the lag period as compared with curves representing the uncorrected cell count. A similar peculiarity was found by Alper and Sterne (1933) in curves obtained by photo-electric measurement of the opacity of cultures of *Salmonella gallinarum*.

Adolph and Bayne-Jones (1932) and Bayne-Jones and Adolph (1933) computed rates of volume increase of individual cells from motion picture photomicrographs of agar cultures of *B. megatherium* and *E. coli*. Occasional cells initiated growth at the maximal rate, but lag was noticeable in most cases. The considerable differences in behavior of individual cells were not clearly correlated with the age of the parent culture.

Coombs and Stephenson (1926) have described a gravimetric method for determination of bacteria, but this appears not to be applicable to measurement of initial rates of growth. A similar method has been used by Bach (1937), however, in following the development of *Staphylococcus aureus*. This author obtained curves of increase in bacterial mass which he believed to be atypical, a conclusion evidently proceeding from misinterpretation of data. No observations of the initial growth period were made.

The observation of lag has thus been the rule whatever the method of measuring rate of growth, and the inference has usually been made that the early increase in rate is a reflection of intrinsic changes in cell physiology. Our previous experiments with *E. coli* (*loc. cit.*) have failed throughout to confirm these suggestions, and the results reported below seem to give decisive support to our earlier evidence that the age (i.e., phase of growth) of cells is not one of the factors influencing the rate of growth of this organism. Lag, when it is observed, we are inclined to attribute to initially unfavorable conditions of growth quite distinct from any peculiarity inherent in the cells.

In the following experiments we have applied three different

methods to the measurement of growth in cultures of *E. coli*, each of which has proved to yield authentic values for total increase of bacterial substance. Since these methods were suited to the study of cultures in liquid media, the findings could be directly correlated with existing knowledge of bacterial multiplication. And since the rates obtained were averages exclusive of individual variations, they could be conveniently related to the age of the parent culture.

Considerable care has been exercised to obtain physiologically "young" and "old" bacteria of constant properties. The strain of *E. coli* used was a stock culture subjected to repeated plating and dried *in vacuo* in sealed ampoules by the method of Brown (1926). To insure against dissociation during the course of the work, a new transplant was made each week from the same lot of desiccated culture and maintained in the interval by daily transfer in broth. A single lot of medium, consisting of a stock solution of 10 per cent Bactopeptone, 5 per cent Difco beef extract, and M/10 phosphate buffer of the desired pH, filtered and autoclaved, and diluted to 10 volumes with sterile 0.5 per cent saline as needed, was used throughout each series of measurements. Seeded in 200 ml. amounts in one-liter Erlenmeyer flasks with 0.02 ml. (2×10^7 bacteria) of a similar culture 24 hours old, cultures yielding reproducible growth curves were obtained. For the experiments with young bacteria, cultures prepared in this manner were centrifuged approximately three hours after seeding, at which time the organisms have attained maximal size and are just entering the phase of logarithmic multiplication. For old bacteria, cultures of 24 hours or more were used.[1] Accompanying each experiment, counts of viable cells have been made to verify that the rate of multiplication corresponded with that of young or old bacteria respectively, and to compute the average size of cells. In addition a few trials were made to determine whether the organisms in the three-hour cultures would show the increased susceptibility to dele-

[1] In these, and similar unreported experiments, no significant change in growth capacity or metabolic activity has been observed in the viable cells remaining in cultures from one to seven days old.

terious influences commonly ascribed to "physiologically young" cultures (Sherman and Albus, 1923). In every case these various evidences fully justified our choice of conditions for obtaining young and old bacteria. (See tables 1, 3 and 4.)

THE ASSAY OF BACTERIAL SUBSTANCE

Total bacterial substance was estimated for measurement of growth rate by means of a photo-electric nephelometer suited to quantitative measurements (Stier, Arnold, and Stannard, 1933–4), by determination of centrifugable nitrogen (Mueller, 1935), or by a manometric method.

The first of these was found to be the most convenient, and, when used with the proper precautions, extremely accurate.

TABLE 1

Survival of young and old bacteria in distilled water

AGE OF CULTURE	DILUTION	DILUTING FLUID	NUMBERS PER ML.* AFTER			
			0 minutes	15 minutes	30 minutes	60 minutes
hours						
24	10^{-7}	Distilled water	109	113	85	55
3	10^{-4}	Distilled water	90	1	0	0
3	10^{-4}	Saline	88	90	87	79

* Average of three plates.

The recommendations of the author regarding the method of expressing optical density, and of calibration, were followed in detail. The excellent discussion of this instrument by Longsworth (1936) was also found helpful. It was convenient to make the calibration using one tube for the blank containing broth, and reserving a second for the sample of culture. In every case the reading of the latter was preceded and followed by a reading of the blank, all three readings being repeated if necessary until the first and last were in agreement. Calibration was made with dilutions in broth of a formalinized culture of *E. coli* which had been incubated 24 hours and standardized by plate count. The turbidity of formalinized cultures did not change during several hours. The instrument was suitable for

measuring populations of 10^7 to 10^9 organisms of minimum size (10^6 to 10^8 in young cultures) per milliliter, giving values reproducible within less than two per cent in the region of 10^8 per milliliter. In the present experiments, cultures were diluted, if necessary, with broth to approximate this number before measurement.

Since the optical density of bacterial suspensions may vary with factors other than the size and number of organisms, preliminary experiments were necessary to ascertain the validity of interpreting as bacterial mass the turbidity of cultures of different age in terms of a single calibration. For this purpose, suspensions of organisms washed in saline were prepared from three-

TABLE 2

Comparison of optical density with nitrogen content of suspensions of young and old cells

AGE OF SOURCE CULTURE	VIABLE* COUNT (VC)	NEPHELOMETRIC* COUNT (NC)	NITROGEN* PER ML. (N)	APPARENT SIZE (NC/VC)	RATIO (N/NC)
hours	× 10^6 *per ml.*	× 10^6 *per ml.*	*mgm.*		
24	940	835	0.025	0.9	0.30
3.5	310	739	0.025	2.4	0.34

* Cells washed twice and resuspended in sufficient saline to give a turbidity equal to 10^9 organisms per ml.

and-one-half and twenty-four hour cultures respectively, and the bacterial content of each was determined by parallel measurements of bacterial nitrogen and optical density. In addition the suspensions were counted to provide a measure of relative size.[2] Table 2 sets forth the results of this experiment, in which it can be seen that the ratio of turbidity to nitrogen is constant within expected error in spite of the three-fold difference in aver-

[2] Since measurements of size are based on viable counts, the values are valid only for suspensions of viable organisms. There is no indication that our 24-hour cultures contain appreciable numbers of non-viable cells (see, for example, an earlier experiment, Hershey, 1937). Nor is it probable that considerable numbers of cells from young susceptible cultures die during the process of washing, since in actual test cultures of this age diluted largely in saline were found to be 90 per cent viable after one hour at room temperature, in contrast to their mortality in water (table 1).

TABLE 3

Initial growth rates in broth at pH 7.4 of E. coli from cultures of different age

A. Nitrogen determinations

DATE OF EXPERIMENT	PARENT CULTURE		SUBCULTURE								
	Age	Viable count	Period of incubation	Initial* viable count	Final viable count	Initial* N/ml.	Final N/ml.	Initial N/cell	Final N/cell	Rate of multiplication	Rate of growth†
	hours	*per ml.*	*hours*	$\times 10^{8}$/*ml.*	$\times 10^{8}$/*ml.*	$\times 10^{-4}$ *mgm.*	$\times 10^{-4}$ *mgm.*	$\times 10^{-10}$ *mgm.*	$\times 10^{-10}$ *mgm.*	*gen./hr.*	*per hr.*
3/16/38	24	2.2×10^{9}	1.58	2.2	58.0	0.57	21.0	0.26	0.36	0.7	3.3
3/17/38	3	7.4×10^{6}	1.50	0.37	51.0	0.49	14.0	1.32	0.28	3.4	3.2

B. Manometric measurements

DATE OF EXPERIMENT	PARENT CULTURE		SUBCULTURE								
	Age	Viable count	Period of incubation	Initial* viable count	Final viable count	Initial* RO_2/ml.	Final RO_2/ml.	Initial RO_2/cell /hour	Final RO_2/cell /hour	Rate of multiplication	Rate of growth‡
	hours	*per ml.*	*hours*	$\times 10^{8}$/*ml.*	$\times 10^{8}$/*ml.*	*mm³./hr.*	*mm³./hr.*	$\times 10^{-7}$ *mm³.*	$\times 10^{-7}$ *mm.³*	*gen./hr.*	*per hr.*
3/20/38	24	2.0×10^{9}	1.25	20.0	39.0	4.05	35.4	2.0	9.1	0.8	2.5
4/ 5/38	36	1.5×10^{9}	1.50	1.5	5.0	0.45	9.0	3.0	16.3	1.2	2.9
3/18/38	72	1.2×10^{9}	2.33	1.2	20.0	0.21	13.5	1.8	6.8	1.7	2.6
3/18/38	3.3	2.0×10^{7}	1.17	2.0	28.0	1.35	15.6	6.8	5.6	3.3	3.0

C. Nephelometric measurements

DATE OF EXPERIMENT	PARENT CULTURE		SUBCULTURE								
	Age	Viable count	Period of incubation	Initial* viable count	Final viable count	Initial§ nephelometric count	Final§ nephelometric count	Initial size¶	Final size¶	Rate of multiplication	Rate of growth‖
	hours	*per ml.*	*hours*	$\times 10^6$*/ml.*	$\times 10^6$*/ml.*	$\times 10^6$*/ml.*	$\times 10^6$*/ml.*			*gen./hr.*	*per hr.*
2/24/38	24		3.00			0.1	79.0				3.1
3/13/38	24	1.3×10^9	2.50	0.13	4.5	0.2	41.0	1.4	9.1	2.1	3.1
3/16/38	24	2.2×10^9	1.58	2.2	4.0	2.1	58.0	1.0	6.0	0.7	3.1
4/ 5/38	36	1.5×10^9	1.50	1.5	5.5	1.7	32.0	1.2	6.0	1.2	2.8
3/18/38	72	1.2×10^9	2.33	1.2	20.0	2.0	97.0	1.6	4.8	1.7	2.4
4/ 5/38	24	1.9×10^9	0.75	9.7	9.3	7.5	34.0	0.8	3.7	0.0	2.9
3/20/38	24	2.0×10^9	1.25	20.0	39.0	16.0	18.0	0.8	4.5	0.8	2.8
3/21/38	24	5.0×10^9	0.50	50.0	41.0	47.0	80.0	0.9	2.0	0.0	2.1
3/24/38	36	1.0×10^9	0.50	26.0	26.0	28.0	64.0	1.1	2.5	0.0	2.4
3/ 3/38	3.0	1.3×10^7	2.00	0.13	17.0	0.8	80.0	6.1	4.7	3.5	3.3
3/17/38	3.0	7.4×10^6	1.50	0.37	6.8	2.9	51.0	8.0	4.5	3.4	2.8
3/18/38	3.3	2.0×10^7	1.17	2.0	28.0	9.7	110.0	4.8	3.9	3.3	3.0

* Values obtained by analysis of the parent culture, corrected for the dilution resulting when the subculture was seeded.

† Two-fold increases in bacterial nitrogen per hour.

‡ Two-fold increases in rate of O_2-use per hour.

§ Turbidity expressed in terms of calibration with 24-hour culture.

¶ Ratio of nephelometric to viable count (24-hour cells = ±1.0).

‖ Two-fold increases in turbidity per hour.

age size of cells in the two suspensions. The nephelometric method is, therefore, suitable for the estimation of bacterial mass in cultures of different age, a conclusion which might also be made from the agreement between growth rates obtained by the various methods (table 3).

The method used for determining bacterial nitrogen has already been described (Hershey, 1937). The principal source of error with this method is in centrifugation. Recentrifuging in conical tubes is essential. Populations of 10^7 per ml. or greater may be satisfactorily determined, the micro-Kjeldahl method requiring about 200 ml. of the three-hour cultures, and as little as 10 ml. (0.3 mgm. N) of cultures fully grown.

The use of the Warburg-Barcroft manometer in our hands has been referred to (Hershey and Bronfenbrenner, 1937 and 1938). Bacteria in young cultures were estimated by measuring the oxygen consumption of five ml. aliquots without added nutrient, after determining that the oxygen use under these conditions was not different from that in fresh broth. This has been found to hold substantially true for cultures in which the population has not reached 10^9 organisms per milliliter. For the estimation of bacteria in older cultures, one ml. of the uncentrifuged culture was placed in the side arm of the manometer vessel, with 4 ml. of broth on the floor. In either case, after temperature equilibration, the side-arm was tilted and the oxygen consumption at zero time obtained in the usual way. This method was applicable to measurement of 10^8 or more organisms of minimum size per milliliter. Values obtained in this way represent, of course, the bacteria present at the time of reading, rather than at the time of removal from the parent culture.

NEPHELOMETRIC MEASUREMENT OF RATE OF GROWTH

Broth in 100 ml. amounts, filtered asceptically through paper into a one-liter Erlenmeyer flask, was warmed to 37°C. in a water bath and seeded with the desired aliquot of the parent culture. The latter was prepared as previously described from a seeding of 10^5 organisms per milliliter. If the final population for the

growth rate measurement was to exceed 10^8 organisms per milliliter, aeration was practised to avoid suppression of growth due to lack of oxygen. Immediately after making the transfer, the parent culture was counted, and directly killed by addition of 0.5 per cent formalin. Turbidity of the formalinized sample was measured within 10 minutes. At the end of the incubation period the subculture was analyzed in the same way. From the values obtained for the two cultures, the rate of growth was expressed by the formula $n/t = \frac{\log b - \log B}{t \log 2}$, where n/t is the reciprocal of the apparent generation time, B is the initial and b the final number of bacteria, and t the period of incubation, these values referring to the daughter culture. Substitution into this formula of the viable counts gave the rate of multiplication in generations per hour, while the nephelometric values gave the rate of mass increment.[3] Since the turbidity of young cultures is slightly below the value expected from the nitrogen content (table 2), the tendency of error from this source is to give abnormally low values for the growth rates of old cells. The ratio of the nephelometric to the viable count was taken as the index of size. The nephelometer having been calibrated with 24-hour cultures, the index is near unity for cultures of this age and proportionately larger for the young cultures, with a maximum value approaching ten.

MEASUREMENT OF RATE OF GROWTH BY DETERMINATION OF BACTERIAL NITROGEN

This method has been used primarily as a control corroborating nephelometric experiments. The procedure described above was followed exactly, with the exception that nephelometric measure-

[3] The growth rate calculated from nephelometric data may be defined as the number of two-fold increases in turbidity per hour, and would be identical with the multiplication rate (generations per hour) in a hypothetical culture in which the average size of cells remained constant. This unit has been chosen to give values numerically comparable with the multiplication rate. Values given by the formula we have used can be readily converted to those commonly expressed by the equation $C_v = 1/t \ln b/B$ to which they are proportional, by applying the factor 0.69.

ments were supplemented by determinations of the nitrogen in the washed sediment from the formalinized cultures. Growth rates were computed in the same way as from the nephelometric data. Size was expressed as nitrogen per viable cell, which was found to be about 0.3×10^{-10} mgm. for 24 hour cultures, with a maximum of 10 times this figure for young cultures.

MANOMETRIC MEASUREMENT OF GROWTH RATE

This method provided a valuable check on the two preceding methods, since it is not subject to possible errors due to formation of non-bacterial sediments in the medium. A systematic error is introduced, however, from the fact that removal of carbon dioxide in the respirometer vessel depresses the rate of growth during the interval of equilibration of temperature. This effect tends to cancel out when young cultures are being studied, and to give somewhat low values for the growth rate of cultures seeded with old cells. The depressive effect of lack of carbon dioxide on the growth rate was found to be less marked the larger the numbers of bacteria in the vessel, and introduced no significant error under the conditions used when 10^8 or more bacteria per ml. were present.

The manometric measurement of growth rate differed in detail from the two preceding methods. Aliquots of the parent culture representing 10^8 to 10^9 bacteria each were placed in duplicate vessels of the respirometer and the rate of oxygen consumption determined after equilibration of temperature. The bacterial count was made and the seed introduced into the subculture at the moment when reading of the manometers was begun, samples being taken for this purpose from the duplicate vessel. After a suitable incubation period, as the numbers approached 10^8 bacteria per ml., five ml. aliquots of the daughter culture were placed in each of the two warmed vessels and equilibrated 20 minutes in the water bath. When the temperature was constant, the viable count was made from the duplicate vessel, and readings were begun as before. The period of incubation of the subculture was thus represented by the interval between the zero times of the respective measurements. The

growth rate was obtained by substitution of these values into the formula. Size, expressed as rate of oxygen uptake per cell, was found to be about 2.0×10^{-7} mm^3 per hour for 24-hour cultures, and five to eight times greater in young cultures. Maximal size is difficult to observe by this method, for the reason that the cells are already becoming smaller when the bacteria are numerous enough to permit of measurement, but the values conform well with indices obtained at corresponding times by the other methods.

Values for the rates of multiplication and of growth determined by the three methods, together with data on population and average size in the cultures studied, are given in tables 3 and 4. Reference to the last column of table 3A, B and C, showing the rate of growth obtained by the various methods, discloses at once the substantial agreement.

In table 5 the observed values have been averaged and submitted to statistical analysis. Transplants made from the young cultures have shown only insignificantly higher rates of growth, the odds against these differences resulting from chance being about three to one and two to one, respectively, for the two experimental series. The ratio of the difference to its standard error for the first series is 1.1, for the second 0.8, while the minimum significant value is generally taken to be 3.0. On statistical reasoning alone, therefore, neglecting the tendency of known experimental errors, the smallest difference in growth rate to be regarded as significant is 0.36 generation per hour for the second series. Thus, with the average value of 21.4 minutes for the apparent generation time of old cells, that of more rapidly growing young cells should be at most 19.0 minutes. Actually, the latter value is 20.7 minutes. It will be remembered, moreover, from the discussion of the several methods, that the known errors tend in each case to give abnormally low values to the growth rate of the elder seedings, and the presence of dead cells in these seedings undoubtedly augments this tendency. The difference actually observed, about six per cent for the totaled values, is entirely within the limits of magnitude reasonably attributable to these sources of error.

TABLE 4

Initial growth rates in broth at pH 6.8 of E. coli from cultures of different ages

Nephelometric measurements

DATE OF EXPERIMENT	PARENT CULTURE		SUBCULTURE								
	Age	Viable count	Period of incubation	Initial* viable count	Final viable count	Initial§ nephelometric count	Final§ nephelometric count	Initial size ¶	Final size ¶	Rate of multiplication	Rate of growth‖
	hours	*per ml.*	*hours*	$\times 10^6$*/ml.*	$\times 10^6$*/ml.*	$\times 10^6$*/ml.*	$\times 10^6$*/ml.*			*gen./hr.*	*per hr.*
4/ 8/38	24	1.9×10^9	2.50	0.2	5.5	0.21	35.0	1.1	6.3	1.9	3.0
4/14/38	24	9.1×10^8	2.91	0.1	8.5	0.15	44.0	1.4	5.2	2.2	2.9
4/ 9/38	24	1.9×10^9	1.40	1.9	3.0	2.1	22.0	1.1	7.3	0.5	2.5
4/15/38	24	1.2×10^9	1.40	1.2	1.4	1.4	17.0	1.2	12.1	0.2	2.6
4/16/38	24	1.1×10^9	1.66	1.1	3.1	0.9	22.0	0.8	7.1	0.9	2.8
4/16/38	48	1.6×10^9	1.78	1.6	5.4	1.8	32.0	1.1	6.0	1.0	2.4
4/11/38	12	8.5×10^8	0.66	8.5	7.3	8.8	35.0	1.0	4.8	0.3	3.0
4/13/38	12	7.9×10^8	0.66	7.9	9.6	8.8	30.0	1.1	2.5	0.4	2.6
4/12/38	24	1.3×10^9	0.33	6.5	6.5	5.7	12.0	0.9	1.8	0.0	3.2
4/12/38	24	1.3×10^9	0.66	6.5	6.8	5.7	25.0	0.9	3.7	0.1	3.2
4/13/38	24	1.1×10^9	0.33	5.4	4.7	6.6	12.0	1.2	2.6	0.0	2.6
4/14/38	24	9.6×10^8	0.43	9.6	9.0	12.0	28.0	1.2	3.1	0.0	2.8
4/12/38	3.0	5.5×10^6	3.40	0.01	1.2	0.07	14.0	6.0	11.7	2.0	2.6
4/14/38	3.2	9.3×10^6	2.75	0.03	8.8	0.15	35.0	5.3	3.9	3.0	2.9
4/13/38	3.5	3.2×10^7	2.53	0.06	7.4	0.26	42.0	4.1	5.7	2.7	2.9
4/11/38	2.5	2.2×10^6	1.33	0.22	4.2	1.55	27.0	7.0	6.5	3.2	3.1
4/17/38	2.5	3.1×10^6	1.33	0.31	5.3	2.00	29.0	6.5	5.5	3.1	2.9
4/17/38	3.0	6.6×10^6	1.33	0.33	5.2	2.00	30.0	6.1	5.8	3.0	2.9
4/15/38	3.5	2.3×10^7	1.66	0.23	3.3	1.01	20.0	4.4	6.1	2.3	2.6
4/ 9/38	3.5	3.0×10^7	0.45	3.0	7.3	12.4	36.0	4.1	5.0	2.9	3.4
4/15/38	3.5	2.3×10^7	0.66	2.3	7.0	10.1	30.0	4.4	4.3	2.4	2.4

* § ¶ ‖ See table 3-c.

If it be agreed that no significant differences exist between the growth rates of young and old bacteria, the same cannot be said of the rates of multiplication. The latter, calculated for the same cultures (tables 3 and 4), illustrate very clearly the marked differences corresponding with the age of the inoculum which are commonly observed. The paradox that old and young bacteria may exhibit identical growth rates, while differing markedly in rate of multiplication, is to be explained only by consideration of average cell-size. Growing at the same rate, the smaller bacteria from old cultures require a longer time

TABLE 5

*Effect of pH and age of culture on rate of growth of transplants of E. coli**

NUMBER OF OBSERVATIONS	pH	AGE OF PARENT CULTURE	GROWTH RATE	APPARENT GENERATION TIME	DIFFERENCE	STANDARD DEVIATION	STANDARD ERROR OF MEAN	STANDARD ERROR OF DIFFERENCE	RATIO OF DIFFERENCE TO S. E.	ODDS AGAINST DIFFERENCE DUE TO CHANCE
		hours	*per hour*	*minutes*	*per cent*					
13	7.4	12–72	2.8	21.4	10.0	0.338	0.094	0.270	1.1	3:1
5	7.4	2– 3.5	3.1	19.3		0.566	0.253			
12	6.8	12–72	2.8	21.4	3.3	0.257	0.074	0.118	0.8	2:1
9	6.8	2– 3.5	2.9	20.7		0.283	0.094			

* Analysis of data of tables 3 and 4.

("lag") to reach the size at which fission occurs in freshly seeded broth, than do young bacteria of greater initial size.

Inspection of the tables reveals no systematic errors affecting the conclusions to be drawn. The two series of experiments, using broth of pH 6.8 and 7.4 respectively (table 5), have yielded identical results. The somewhat smaller experimental variation in the latter series bespeaks the accuracy which comes with practice.

It should be observed that the experiments include a rather wide range of initial seedings, and periods of incubation, neither of which has had any effect on the rate of growth. This adds considerably to the significance of the findings, for the magnitude and kind of errors expected under the several conditions

are very different. The smaller seedings were used especially to test the argument that differences in growth rate of organisms of different age might be magnified in this case, as are differences in the rate of multiplication. On the other hand, with large seedings growth could be measured over a much shorter time period, even a fraction of a single generation time, which might be expected to consist quite neatly of the period of "rejuvenescence." No sign of lag in absolute growth rate was encountered, however, in any of the experiments, the recorded values agreeing precisely with the maximum rates of growth attained in cultures observed continuously by the same methods (unpublished data). The initial growth rates measured in these experiments represent, therefore, the maximum rate for this organism under the conditions of cultivation employed, and no "period of adjustment," either of bacteria to the new medium or the reverse, is evident. The fact that the maximum absolute rate of growth is less than three "doublings" per hour, while the rate of multiplication at its maximum may exceed four generations per hour, is explained by the circumstance that the latter is observed during the period in which size is decreasing, i.e., bacteria are actually multiplying faster than they are growing.

The bearing of these observations on the general problem of growth phases is clear: that so far as differences in rate of numerical increase are concerned, "physiological youth" of the cells in young cultures of *E. coli* is an artifact, and that changes in size, conditioned in turn by changing environmental status, and the direct effect of the latter on metabolism, sufficiently explain the course of events in the development of the culture. Experiments are being planned, directed toward further elucidation of these relationships.

SUMMARY

Initial growth rates deduced from measurements of turbidity, bacterial nitrogen, and oxygen uptake of broth transplants of *Escherichia coli* seeded from young and old cultures respectively, have given identical values, while parallel measurements of rate of multiplication revealed the expected differences. We con-

clude that the physiologic state of the cells does not influence their rate of growth, and that the lag in rate of multiplication must be attributed to changes in the factors limiting the size at which fission occurs during the period of growth.

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