MATHEMATICAL ANALYSIS OF THE LAG-PHASE IN BACTERIAL GROWTH.

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(With 9 Charts and 1 Text-figure.)

Of the large number of experiments recorded by Penfold (1914) in connexion with the question of lag in bacterial growth, a certain proportion have been carried out in such detail as to render them eminently suitable for mathematical analysis. In all work of this kind, which is intended to throw light on the numerical aspect of bacterial growth, it is desirable to take observations at frequent and, if possible, regular intervals, during the period of lag, and to count a sufficiently large number of colonies on the plates. To secure this last and most important desideratum, orientating experiments must be performed, from the results of which one is enabled to calculate what dilution of the culture at any given period of growth will yield a tolerably large and accurately countable plate population.

The experiments which Penfold has performed on the influence exerted on lag by variations in the initial seeding, lend themselves admirably to mathematical analysis, and we propose here to confine our mathematical treatment solely to the two parallel series of experiments dealing with this point. The literature of the subject has been fully dealt with in the paper by Penfold (*loc. cit.*).

The term "lag" has been used in different senses by different writers on the subject. By some, it is understood as a definite period

during which growth is apparently in abeyance. More usually, it is taken to mean that period which elapses between the time of seeding and the point at which the velocity of reproduction attains its maximal level or, in other words, the point at which the generation-time becomes minimal.

The attainment of this minimal level of generation-time is the prelude to what is known as the second or logarithmic phase of bacterial growth during which the generation-time remains at this constant minimum.

In most recorded work on the multiplication of bacteria, analysis of the lag-phase has received scanty attention, whereas the second or logarithmic phase has been very fully dealt with. There can be no doubt, however, that the phenomenon of lag is of profound significance in the life-history of the organism, and in the following analysis we hope to show that this phase proceeds in an orderly fashion and according to a perfectly definite law, until the logarithmic phase commences. In other words, we shall hope to show that the generation-time diminishes steadily and regularly from the commencement of seeding till a minimal length is reached.

What the minimal generation-time is to which a *B. coli* culture can attain, is difficult to state with exactness, but, in practice, it has not been possible to demonstrate a generation-time of less than 18–20 minutes. That the interval between two successive divisions cannot be reduced below a certain minimum, is an important fact in the mechanics of bacterial growth, and has to be reckoned with in any theory which attempts to explain growth in a nutrient medium as a continuous or discontinuous process. This point will be discussed later. At present, there are no data covering the whole period of growth from the commencement of seeding to the period when growth ceases altogether and cell-death comes into play. Ample observations are available on the logarithmic phase or phase of constant generation-time, but only a few isolated observations have been made on the subsequent phase during which the generation-time increases till it probably again becomes infinite as at the commencement of seeding. Accurate and sufficient data on these phases, as also on that of cell-death, will, it is hoped, soon be forthcoming.

Analysis of experiments on lag.

Series A. Experiments 1—4.

In these four experiments, the initial seedings, calculated from the plates, were respectively 217·5, 59·4, 20·2, and 2·4 bacilli per drop, or roughly as 100 : 25 : 10 : 1.

Incubation at 37° C.

Experiment 1.

Time (X)	Bacilli (Y)	Log X	Log Y
0	217·5	—	2·3374
45	287	1·653	2·4578
60	345	1·778	2·5378
80	470	1·903	2·6721
100	718	2	2·8561
120	1362	2·079	3·1341
150	2535	2·176	3·4039
180	7610	2·255	3·8813

Transfer the origin from (0, 0) to (0, 2·3374).

Then log Y or Y' becomes

0
0·1204
0·2004
0·3347
0·5187
0·7967
1·0665
1·5439

The values of log X and of log Y were plotted out in the usual way and from the shape of the smooth curve so obtained, it was conjectured that an equation of the form $X^n = kY'$ would fit most closely the observed data. If this proved to be the case, then the points obtained by plotting log X against log Y', *i.e.* against log (log Y) ought to lie on a straight line.

The values of log (log Y) are as follows:

$-\infty$
$\bar{1}$·0806
$\bar{1}$·3016
$\bar{1}$·5246
$\bar{1}$·7149
$\bar{1}$·9012
0·0277
0·1886

These values were then plotted against the corresponding values of log X and it was found that the points so obtained lay very closely along a straight line the tangent of whose inclination to the axis of X was 1·88.

This value of n, viz. 1·88, gives the following values for log k the other constant in the equation $X^n = k \log Y$.

4·027
4·039
4·053
4·045
4·007
4·063
4·050

These values indicate a quite satisfactory constancy of k.

The average value of k is found to be 10988.

Transfer back to the old origin and we find that the following equation

$$X^{1\cdot88}=10988 \log \frac{Y}{217\cdot5}$$

will fit most closely the experimental data.

Experiments 2, 3, and 4 were treated similarly and the following values for the exponent n were obtained, 1·77, 1·56 and 1·56 respectively.

These values for n give the following values for log k', log k'' and log k''' resp.

Exp. 2	Exp. 3	Exp. 4
$n=1\cdot77$ Log k'	$n=1\cdot56$ Log k''	$n=1\cdot56$ Log k'''
3·814	3·343	3·364
3·750	3·391	3·337
3·795	3·382	3·402
3·837	3·371	3·447
3·790	3·367	3·433
3·801	3·365	3·387
3·811	3·346	3·358

The average values for k', k'', and k''' are 6322, 2329, and 2465 resp.

The following equations, therefore, will fit most closely the experimental data in Experiments 2, 3, and 4.

$$X^{1\cdot77}=6322 \log \frac{Y}{59\cdot4},$$

$$X^{1\cdot56}=2329 \log \frac{Y}{20\cdot2},$$

$$X^{1\cdot56}=2465 \log \frac{Y}{2\cdot4},$$

Series B. Experiments 5—8.

In this parallel series, the initial seedings were 144, 35·7, 14, and 1·7 bacilli per drop resp. or roughly as 100 : 25 : 10 : 1.

The data from these experiments were dealt with in a similar way and the following values for the exponent n were obtained, viz. 1·97, 1·74, 2·01 and 2·7 resp.

These values for n gave the following values for log k, log k', log k'', and log k''' resp.

Exp. 5	Exp. 6	Exp. 7	Exp. 8
$n=1\cdot97$ Log k	$n=1\cdot74$ Log k'	$n=2\cdot01$ Log k''	$n=2\cdot7$ Log k'''
4·298	3·565	4·667	6·225
4·228	3·762	4·366	6·160
4·184	3·723	4·374	6·052
4·185	3·730	4·351	5·985
4·178	3·744	4·343	6·017
4·239	3·736	4·359	6·025
4·236	3·742	4·379	6·020

It will be observed that the constancy obtained for log k, log k', log k'', and log k''' in these four experiments is very satisfactory. The following equations will, therefore, fit very closely the experimental data.

$$X^{1\cdot97}=16732\log\frac{Y}{144},$$

$$X^{1\cdot74}=5483\log\frac{Y}{35\cdot7},$$

$$X^{2\cdot01}=23020\log\frac{Y}{14},$$

$$X^{2\cdot7}=1045000\log\frac{Y}{1\cdot7}.$$

In the following tables the observed values for Y (Bacilli per drop) are compared in parallel columns with those calculated from the theoretical equations.

Series A.

Exp. 1.

Equation: $X^{1\cdot88}=10988\log\frac{Y}{217\cdot5}$.

X (time)	Y (observed)	Y (theoretical)
0	217·5	217·5
45	287	284·49
60	345	344·95
80	470	480·67
100	718	726·88
120	1362	1188·2
150	2535	2888·4
180	7610	8288·9

Exp. 2.

Equation: $X^{1\cdot77}=6322\log\frac{Y}{59\cdot4}$.

X (time)	Y (observed)	Y (theoretical)
0	59·4	59·4
45	80	80·72
60	105·5	99·01
80	140·5	138·99
100	189·5	210·03
120	354	339·4
150	789	790·6
180	1950	2112·8

Exp. 3.

Equation: $X^{1\cdot56}=2329\log\frac{Y}{20\cdot2}$.

X (time)	Y (observed)	Y (theoretical)
0	20·2	20·2
45	30	29·37
60	35·2	36·33
80	49·1	50·64
100	73·8	74·37
120	114·4	113·86
150	240	234·72
180	621	525·40

Exp. 4.

Equation: $X^{1\cdot56} = 2465 \log \frac{Y}{2\cdot4}$.

X (time)	Y (observed)	Y (theoretical)
0	2·4	2·4
45	3·5	3·42
60	4·5	4·18
80	5·6	5·72
100	7·1	8·22
120	10·6	12·31
150	25	24·35
180	67	52·03

Series B.

Exp. 5.

Equation: $X^{1\cdot97} = 16732 \log \frac{Y}{144}$.

X (time)	Y (observed)	Y (theoretical)
0	144	144
40	170	175·39
60	222	223·2
80	335	311·61
100	533	477·5
120	966	800·49
150	1885	2067·84
180	5840	6537·6

Exp. 6.

Equation: $X^{1\cdot74} = 5483 \log \frac{Y}{35\cdot7}$.

X (time)	Y (observed)	Y (theoretical)
0	35·7	35·7
40	52·4	46·19
60	58·5	60·11
80	87	84·50
100	130	126·84
120	199	203·73
150	472	465·52
180	1194	1215·22

Exp. 7.

Equation: $X^{2\cdot01} = 23020 \log \frac{Y}{14}$.

X (time)	Y (observed)	Y (theoretical)
0	14	14
40	15·2	16·52
60	20·3	20·37
80	26·8	27·31
100	41	39·91
120	67·8	63·18
150	151·3	148·96
180	371	422·80

Exp. 8.

Equation: $X^{2\cdot7} = 1045000 \log \frac{Y}{1\cdot7}$.

X (time)	Y (observed)	*Y* (theoretical)
0	1·7	1·7
40	1·75	1·78
60	1·88	1·95
80	2·25	2·30
100	3·09	2·95
120	4·22	4·19
150	8·66	8·87
180	25·16	25·43

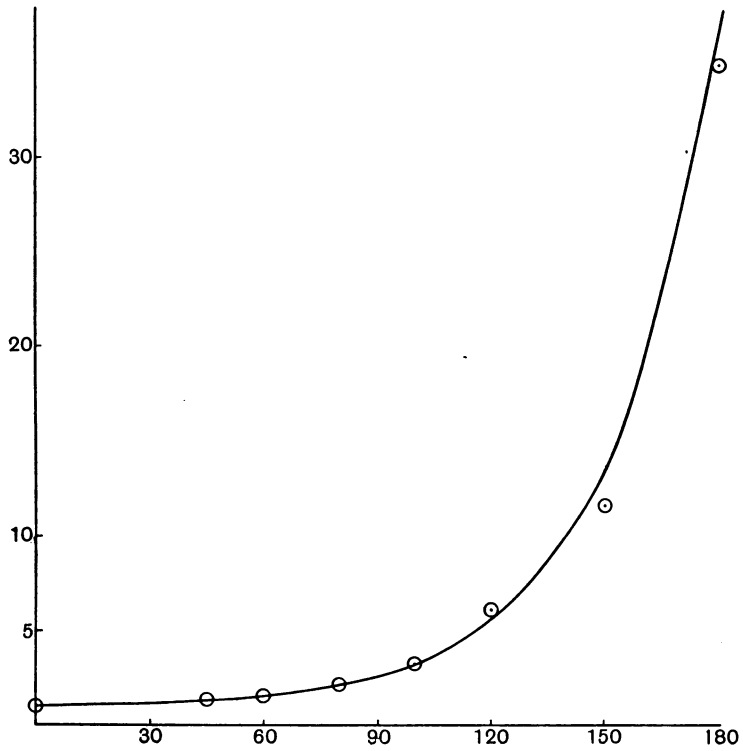


Chart I. Series A. Exp. 1. Ordinates. Bacilli. (Initial seeding taken as 1.) Abscissae. Time (minutes).

In Charts I—VIII the theoretical curves have been drawn to scale and the observed points have been inserted as circles. The closeness of fit is, in most cases, very satisfactory.

Summarising the results of these eight experiments, we find that during the whole lag-phase, growth takes place in a perfectly regular

fashion, the bacterial content (Y) at any time (X) being very satisfactorily determined from the general equation

$$(X^n = k \log Y/s),$$

where n and k are constants and s is the initial seeding.

The value of the exponent n falls slightly as the seeding is reduced except in the last two experiments of Series B.

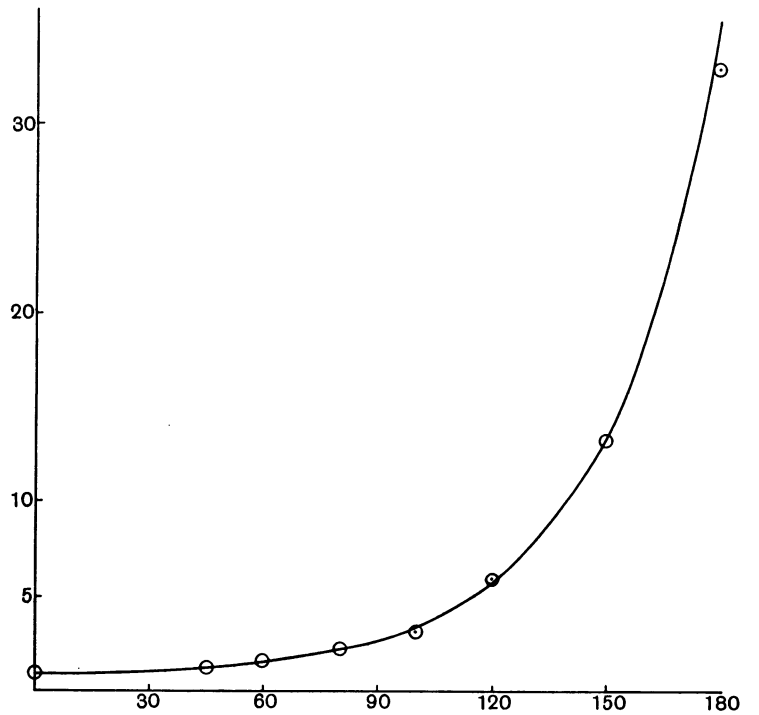


Chart II. Series A. Exp. 2.

The figures may be conveniently tabulated here:

Seeding	Series A	Series B
100	1·88	1·97
25	1·77	1·74
10	1·56	2·01
1	1·56	2·7

The two discrepant values for n in Series B will receive more full consideration when we come to discuss the question of generation-time during lag, but it would appear that in the case of very small

initial seedings (as in Experiments 7 and 8 of Series B) where the number of bacilli per drop were only 14, and 1·7 respectively, the latter part of the lag-phase may be characterised by a very marked acceleration of the growth-rate. This will appear when the generation-times have been calculated.

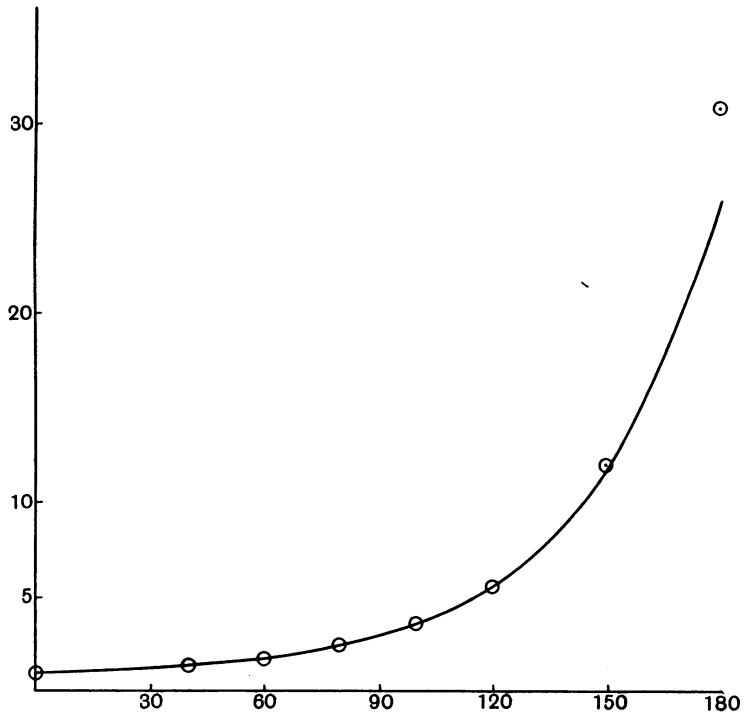


Chart III. Series A. Exp. 3.

The question of generation-time.

The generation-time or the period which elapses between two successive divisions of an organism is usually calculated from the bacterial contents at the beginning and end of a certain arbitrary period. It represents essentially the mean generation-time during the period in question. If short and equal periods are chosen, a very fair impression can be obtained of the changes which this function undergoes in the course of growth. In practice, however, this is not always feasible but it is always possible to get an approximation to the value of the generation-time at any point, by determining the

inclination of the tangent to the log-curve at that point. From the mathematical expressions which we have obtained for the course of the lag-phase in the above series of experiments, it is possible to calculate with exactness the generation-time at any point in the course of the lag.

Let P be any point (x, y) on the curve and P' and P'' two points on either side of P whose co-ordinates are $(x - \delta x, y - \delta y)$ and $(x + \delta x, y + \delta y)$ respectively, where δx and δy are very small. If $r =$ the number of generations between the bacterial contents corresponding to $y + \delta y$ and $y - \delta y$, we must have $r \log 2 = \log (y + \delta y) - \log (y - \delta y)$.

The whole period is $2\delta x$.

Therefore one generation-time equals $\frac{2\delta x \log 2}{\log (y + \delta y) - \log (y - \delta y)}$ *.

Let $y' = \log y$, then $dy' = \frac{1}{y} dy$.

The expression for the generation-time becomes

$$\frac{2\delta x \log 2}{y' + \delta y' - y' + \delta y'} = \frac{\delta x \log 2}{\delta y'}.$$

In the limit, when δx and δy are infinitely small, the value for the generation-time becomes $y \log 2 \frac{dx}{dy}$. Now $\frac{dx}{dy}$ can be obtained directly from the equation $x^n = k \log y/s$. We have $nx^{n-1} \frac{dx}{dy} = \frac{k}{y}$. Therefore the expression for the generation-time at the point P becomes

$$\frac{ky \log 2}{nyx^{n-1}} = \frac{k \log 2}{nx^{n-1}}.$$

* The expression $\frac{2\delta x \log 2}{\log (y + \delta y) - \log (y - \delta y)}$ may be otherwise reduced thus:

$\log (y + \delta y)$ [expanded by Taylor's Theorem] $= \log y + \frac{\delta y}{y} - \frac{1}{2}\frac{\delta y^2}{y^2} + \frac{1}{3}\frac{\delta y^3}{y^3} -$ &c.,

$\log (y - \delta y)$ [do. do.] $= \log y - \frac{\delta y}{y} - \frac{1}{2}\frac{\delta y^2}{y^2} - \frac{1}{3}\frac{\delta y^3}{y^3}$ &c.,

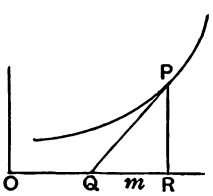
$$\therefore \log (y + \delta y) - \log (y - \delta y) = 2\frac{\delta y}{y} + \frac{2}{3}\frac{\delta y^3}{y^3} \text{ \&c.}$$

The expression for G.T. then becomes

$$\frac{2\delta x \log 2}{2\frac{\delta y}{y} + \frac{2}{3}\frac{\delta y^3}{y^3} \text{ \&c.}}.$$

In the limit, powers of δy may be neglected and G. T. becomes

$$y \log 2 \frac{dx}{dy} \text{ (as before).}$$



If the tangent at the point P cuts the axis of X at Q and the ordinate of P at R, then if the intercept between these two points be denoted by m, the G. T. at the point P is equal to $m \log 2$.

We can therefore determine the generation-time at any moment (x) after the commencement of growth, by substituting in this expression the value of x and the values determined for k and n.

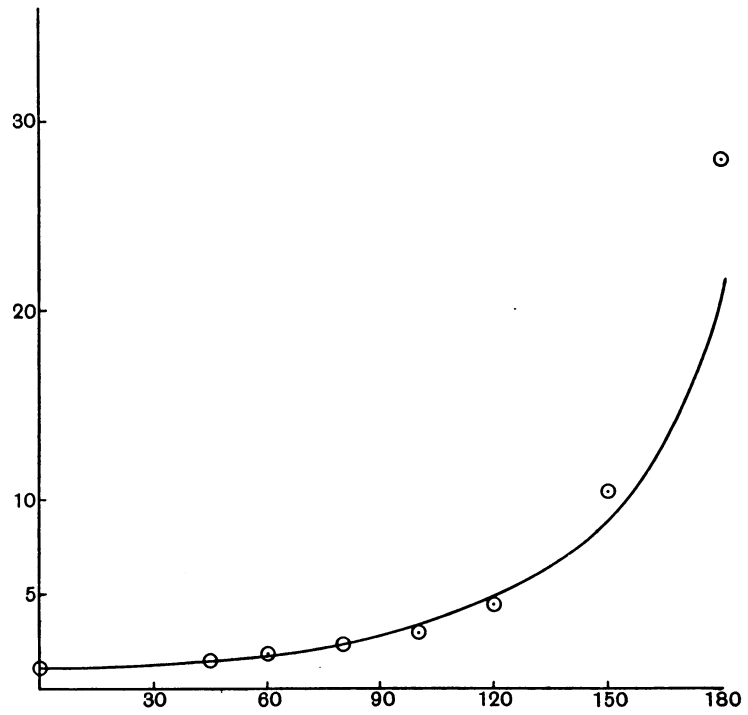


Chart IV. Series A. Exp. 4.

This determination has been made at half-hourly periods during the lag-phase of each of the eight experiments with the following results :

Series A.

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Time	G. T.	G. T.	G. T.	G. T.
30	88·19	78·36	66·90	70·81
60	47·92	45·95	45·38	48·03
90	33·54	33·62	36·16	38·27
120	26·04	26·94	30·78	32·58
150	21·40	22·69	27·17	28·75
180	18·23	19·72	24·53	25·96

Series B.

Time	Exp. 5 G. T.	Exp. 6 G. T.	Exp. 7 G. T.	Exp. 8 G. T.
30	103·4	76·5	111·0	359·1
60	48·14	45·82	55·09	110·5
90	32·55	33·97	36·56	55·47
120	24·63	27·48	27·35	33·97
150	19·80	23·29	21·88	23·29
180	16·62	20·33	18·20	17·07

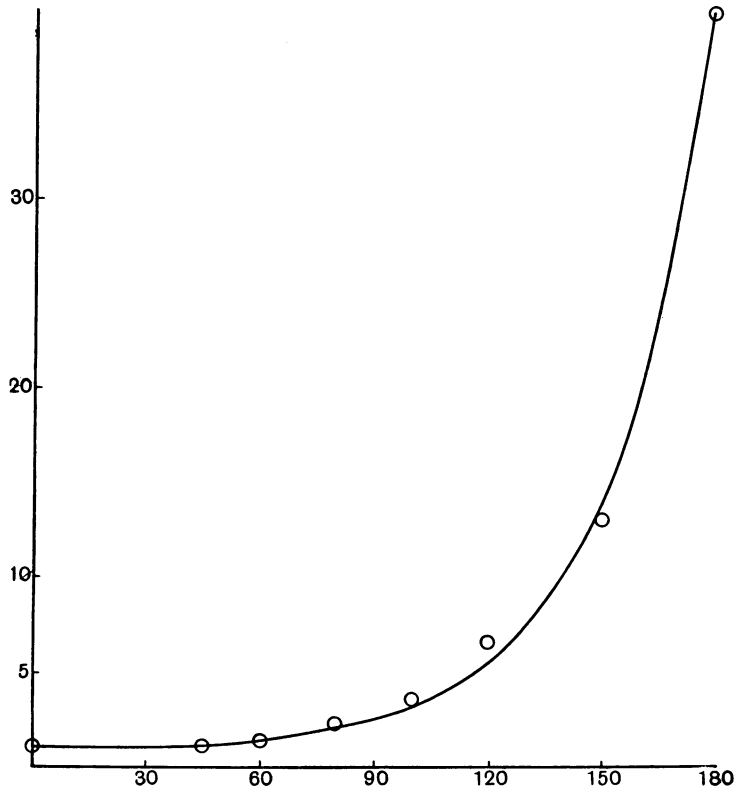


Chart V. Series B. Exp. 5.

Analysis of Series A. (Generation Times.)

In Exps. 1 and 2, in which the seedings were 217·5 and 59·4 bacilli per drop respectively, the generation-times attained at each half-hourly period are practically identical and at the end of the 3-hour period the minimum generation-time of 18–19 minutes has been reached.

The influence of reduction in the initial seeding is however very apparent in Exps. 3 and 4. Here the seedings were respectively 20·2 and 2·4 bacilli per drop. The value of the exponent n was in both cases 1·56 and consequently there is practically no difference between the generation-times attained at the half-hourly periods. In both experiments, however, the end of the lag period has not been reached in three hours, the final recorded generation-time being 24–25 minutes as compared with 18–19 minutes in Exps. 1 and 2.

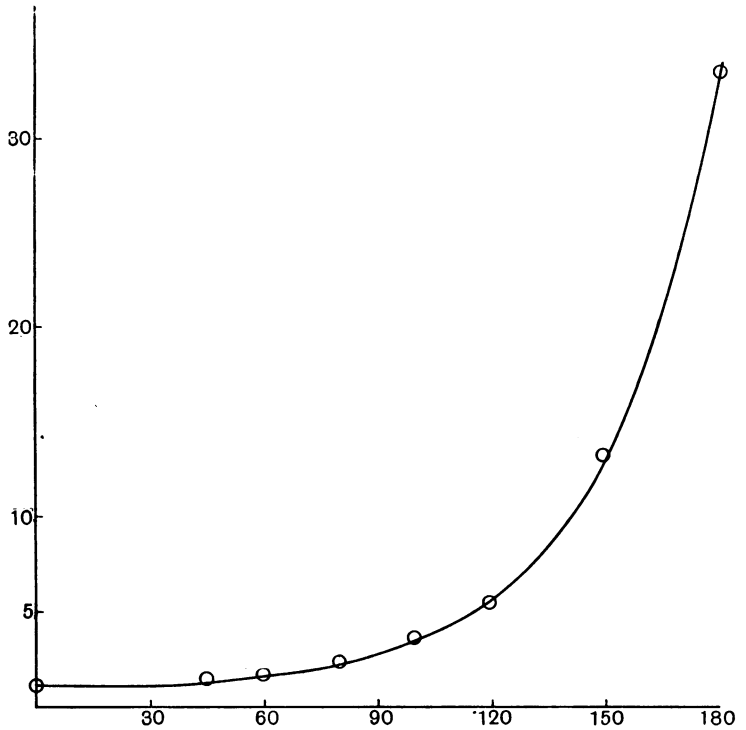


Chart VI. Series B. Exp. 6.

At each half-hourly period (after the 1st hour) the generation-times are distinctly longer by 3–6 minutes than the corresponding times in Exps. 1 and 2.

Series B.

In this series all have reached the minimum generation-time in three hours so that the total period of lag is practically the same in all cases. It will be observed, however, that at 90 minutes the generation-time of

Exp. 7 is appreciably behind those of Exps. 5 and 6 while that of Exp. 8 is very far behind. At 120 minutes the generation-time of Exp. 8 is still behind the others, but at the end of the next half-hour (at 150 minutes) the generation-time of Exp. 8 has reached the level of the others. The remarkable acceleration in rate of growth which has occurred in Exps. 7 and 8, during the latter period of the lag is of course simply an expression of the enhanced value of the

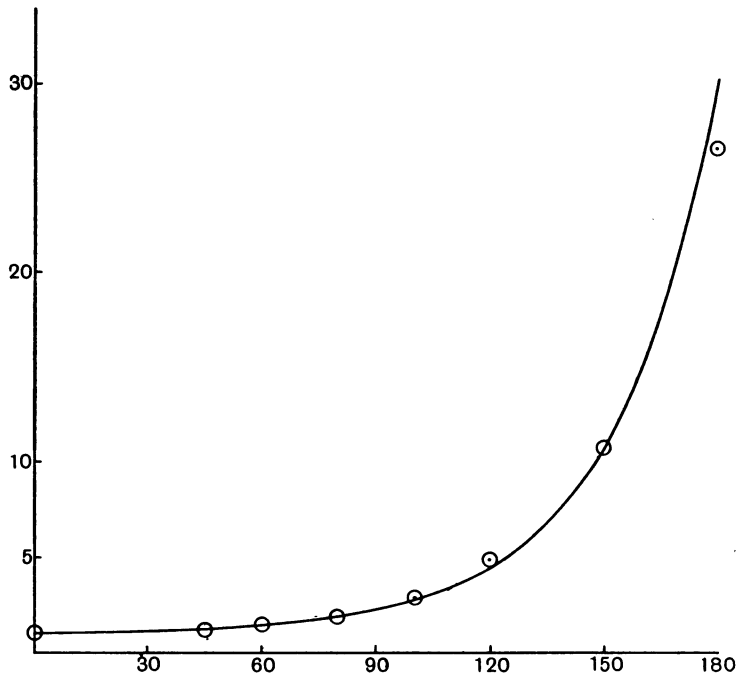


Chart VII. Series B. Exp. 7.

exponent n in the derived equations and at present we have no satisfactory explanation to offer for this fact. It may possibly find an explanation when the factors that enter into the causation of lag are more thoroughly understood.

The later phases of Growth.

It is not our purpose here to discuss in any detail the later phases of growth succeeding the lag. The second or logarithmic phase has been very thoroughly investigated by Lane-Claypon (1909) and others who find that during this phase the generation-time remains at a constant minimum.

Time and bacilli are related to each other by the simple equation $X = k \log Y$ so that, when the logs of the bacilli are plotted against time, the points so obtained lie on straight lines. The exponent of X is unity during this phase. Its duration varies with the medium in which the organisms are growing and with the temperature. In peptone-water, at 37° C., it lasts about three to six hours according to the size of seeding after which time the 3rd phase or phase of rising (*i.e.* slower) generation-time ensues. This phase proceeds till growth ceases and cell-death begins. Whether a plateau occurs during which the bacillary content remains at a constant maximum for a certain

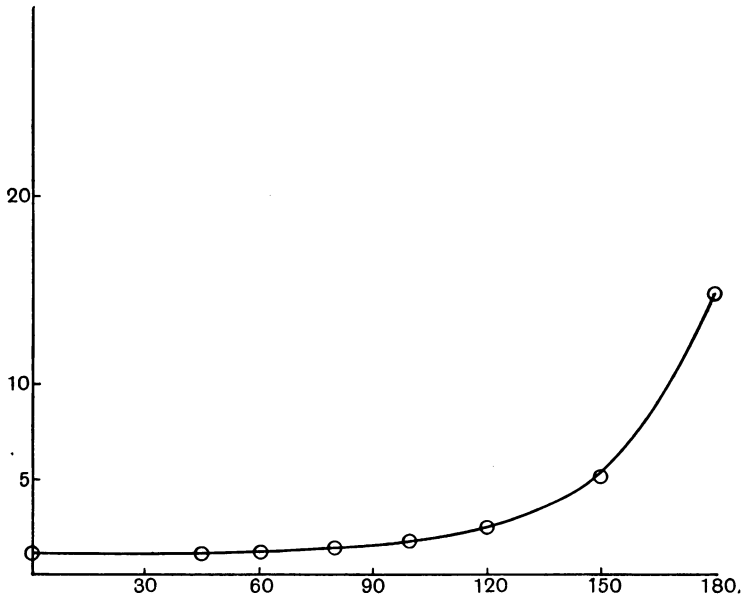


Chart VIII. Series B. Exp. 8.

time is not definitely established though there is some evidence in support of this occurrence. No complete data from a single experiment are available from the period of seeding onwards to the commencement of cell-death, but by a fortunate circumstance we are in a position to state the most probable course taken by the growth curve over a period including the lag, the 2nd phase, and a large portion of the 3rd phase.

An experiment was performed in which the initial seeding was 37·3 bacilli per drop and the first observation was made at the 3rd hour and at hourly intervals thereafter.

The figures obtained were as follows:

Time, hrs.	Bacilli per drop
0	37·3
3	1,692·5
4	7,833·3
5	46,000
6	231,000
7	553,888
8	974,166
9	1,166,666
10	1,643,333
11	2,112,222
12	2,396,470

Now, it will be noted that in Exp. 6 (Series B) the initial seeding was 35·7 bacilli per drop and the figure reached at three hours was 1194. We can therefore legitimately employ the figures for the lag-phase of Exp. 6 to complete the data in this new experiment where the lag-phase was not observed. From the equation for Exp. 6 it was calculated that a figure of 1692 bacilli per drop would have been reached in 186 minutes. The logarithmic phase of the new experiment lasted about 3 hours at a constant generation-time of about 25 minutes. At the following times therefore (increments of 25 minutes) the following figures would be reached:

Time, mins.	Bacilli per drop	
186	1,692	
211	3,384	
236	6,768	
261	13,536	
286	27,072	
311	54,144	
336	108,288	
361	216,576	(Actual figure obtained at
421	553,888	360 mins. was 231,000.)
481	974,166	
601	1,643,333	
661	2,112,222	
721	2,396,470	

The above figures and times along with those for the lag-phase of Exp. 6 have been plotted in Chart IX.

The actual course of the growth curve is indicated by the successive divisions *OA* (lag), *AB* (logarithmic phase), *BC* (3rd phase during which the curve passes through a point of inflexion and becomes concave to the axis of *X*). On the scale employed in Chart IX it is possible to exhibit the lag-phase *OA*, as a straight line only.

Had growth continued according to the law maintained during the lag, the growth curve would have followed the route AA'.

Had the logarithmic phase continued, the curve would have followed the route BB' instead of the actual route BC.

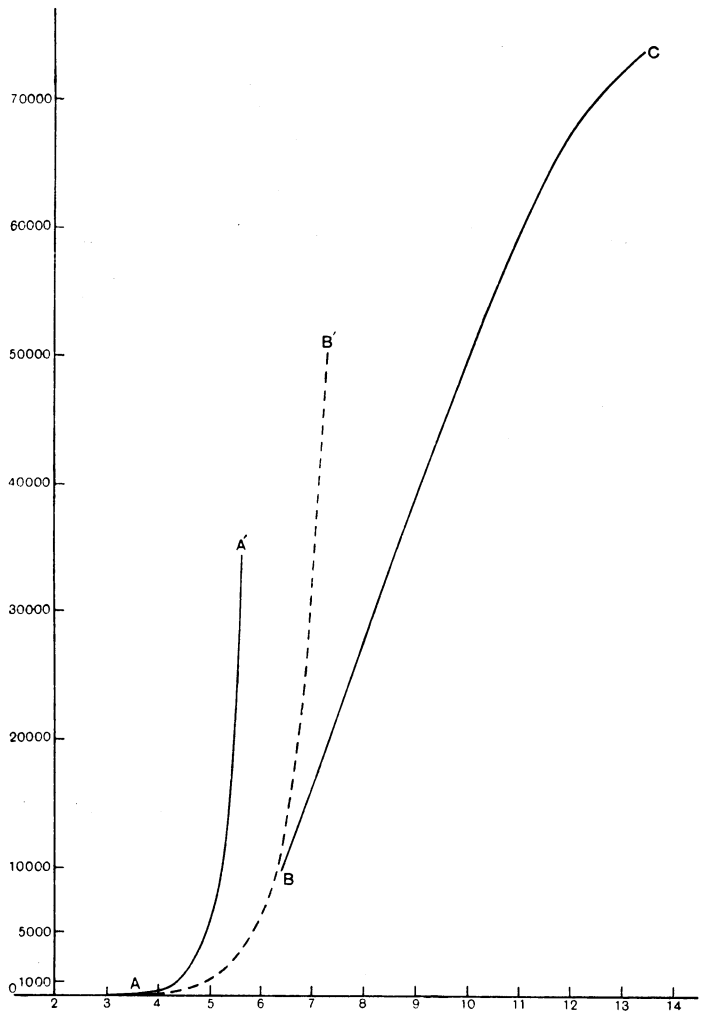


Chart IX. Ordinates. Bacilli. (Initial seeding taken as 1.)
Abscissae. Time in hours.

We see therefore that growth proceeds in those phases during each of which a definite law is maintained. Sufficient data are not at present

available to establish the law which holds during the 3rd phase, and consequently the point at which a maximum bacterial population would be reached.

The general form of the complete curve *OABC* suggests that possibly a frequency-curve might be got to fit all the data from the seeding onwards. This possibility, however, cannot be seriously entertained at present owing to the lack of data.

GENERAL CONCLUSIONS.

1. The phase of bacterial lag is a perfectly definite one, during which growth proceeds regularly from the period of seeding to the attainment of a minimum generation-time. During this phase time and bacilli are connected by an equation of the form $X^n = k \log \frac{Y}{s}$, where n and k are constants and s is the initial seeding.

2. At the close of the lag an entirely different law begins to hold and is maintained for another period of variable duration. During this phase the law $X = k \log Y$ holds and the generation-time remains constant throughout.

3. The logarithmic or 2nd phase is succeeded by the 3rd phase during which the generation-time gradually lengthens till it finally becomes infinite and no further growth occurs.

4. Growth is probably discontinuous in the sense that it conforms to different laws in the successive phases and it remains to be decided what theory of lag will most adequately accord with the numerical data and the mathematical laws derived therefrom. Various theories of the causation of lag have been discussed by one of us (Penfold, 1914) and special prominence has been given to two, viz. (1) based on variation of the bacterial cell and (2) based on a purely chemical analogy with ferment action generally and the importance of intermediate products in particular. Which of these two theories would best accord with the fact of discontinuous growth-laws cannot at present be decided in the absence of further experimental evidence of a crucial nature. In the meantime one cannot fail to be impressed by the analogy that exists between recently ascertained facts with regard to bacterial variation in a sugar-containing medium and the discontinuous growth-phases that occur in a medium of simple constitution. It may, for example, be assumed that during the lag-phase an organism is being selected out which can propagate itself with a constant minimum generation-time.

This selected strain holds the field during the second or logarithmic phase just as the selected dulcite-fermenting *B. typhosus* variant holds the field after the initial period of selection is over. In the 3rd phase we again have competing strains, but here the mean result is of an inverse character and the mean generation-time progressively lengthens. (Phase of reversion.)

The hypothesis that variation processes are at work receives further support from the fact that a seeding taken during the lag-phase grows with diminished lag, while one taken during the 2nd phase proceeds to grow with practically no lag.

It is possible that more frequent observations at the boundary zones of these phases would shed further light on this problem.

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