A NOTE ON THE LAG-PHASE IN THE GROWTH OF MICRO-ORGANISMS.

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(With one Chart.)

THE mechanics of the growth of micro-organisms in nutrient solutions has of late years received considerable attention. The logarithmic law governing the phase of unrestricted growth is now well established (Lane-Claypon (1909), Penfold and Norris (1913), Slator (1913), (1916) and others). Considerable information regarding another period of growth is also available. When a suitable nutrient medium is seeded with bacteria, there is usually a period during which the bacteria grow at a slower rate than is the case later when the logarithmic law holds good. This period is called the lag-phase of growth. The laws governing such growths have been carefully and successfully worked out by Penfold (1914) and Ledingham and Penfold (1914). *Bacillus coli* was the organism employed in their experiments. In the paper by Ledingham and Penfold on "The mathematical analysis of the lag-phase in bacterial growth" the authors have shown that the relationship between the number of bacteria and the time can be represented by an equation involving two constants both of which vary in different experiments, but remain of the same value throughout each single experiment. It has apparently escaped notice that there is a relationship between the two constants of such a nature that one of them can be replaced by a third which remains of the same value throughout the whole series of experiments. If use is made of this new constant further information regarding the lag-phase in growth can be obtained.

The notation employed in this paper is essentially the same as that used by Ledingham and Penfold. Log = logarithm to base 10, *ln* = natural logarithm to base *e*, G.T. = generation time.

Ledingham and Penfold show that the growth of bacteria during the lag-phase is accurately represented by the equation

$$X^n = k \log Y \quad \text{.............................(1)},$$

where Y is the number of bacteria after a time X, the initial seeding being one. n and k are constants. They find the following values for n and k in eight separate experiments.

TABLE I.

Experiment	n	k	$\frac{k}{n}$	$\log \frac{k}{n}$	$\frac{\log k/n}{n}$
1	1·88	10988	5840	3·766	2·00
2	1·77	6322	3570	3·553	2·01
3	1·56	2329	1490	3·173	2·03
4	1·56	2465	1580	3·199	2·06
5	1·97	16732	8490	3·929	1·99
6	1·74	5483	3150	3·498	2·01
7	2·01	23020	11450	4·059	2·02
8	2·7	1045000	387000	5·588	2·07
					Average = 2·024 = A

It is clear from this table that there is a relationship between n and k of such a kind that $\frac{\log k/n}{n} = A$, a constant for the whole series of experiments.

Now let $10^A = \frac{1}{K_1}$, then $k = n10^{nA} = \frac{n}{K_1{}^n}$ ....(2).

Equation (1) then becomes

$$X^n = \frac{n}{K_1{}^n} \log Y \text{ or } X^n K_1{}^n = \log Y^n \quad \text{..............(3)}.$$

For this series of experiments A averages 2·024. Therefore

$$K_1 = 10^{-2\cdot024} = 0\cdot00945.$$

The equation of the lag-phase of growth reads therefore

$$X^n (0\cdot00945)^n = \log Y^n.$$

Differentiating equation (3) we have

$$nX^{n-1} K^n dX = \frac{n}{Y} \log e dY,$$

or

$$\frac{\frac{dY}{dX}}{Y} = \frac{K_1{}^n X^{n-1}}{0\cdot4343}.$$

This value $\frac{dY}{dX}\Big/ Y$ is the "constant" of growth at any time X and can be called Z.

Therefore $$0{\cdot}4343Z = K_1{}^n X^{n-1} \quad \text{.......................(4).}$$

The equations of unrestricted growth corresponding to (3) and (4) are

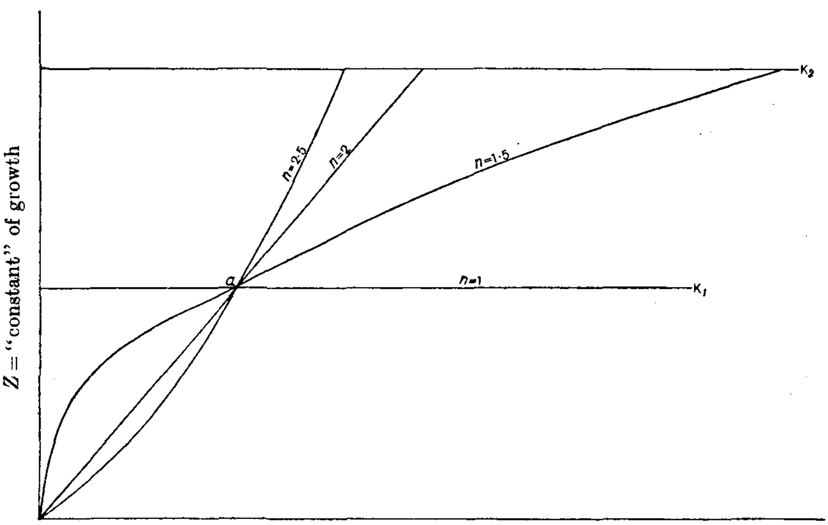
$$XK_2 = \log Y \quad \text{.............................(5),}$$

$$0{\cdot}4343Z = K_2 \quad \text{.................................(6),}$$

G.T. $= \frac{\log 2}{0{\cdot}4343Z}$ in both cases.

The minimum generation-time found by Ledingham and Penfold is usually 18—20 mins., though in some cases values 16·6 and 17·1 mins. have been observed (Experiments 5 and 8, pp. 253 et seq.). The lowest value 16·6 mins. corresponds to a value of $K_2 = 0{\cdot}0182$. K_2 is therefore approximately equal to $2K_1$. The constant $K_1 = 0{\cdot}00945$ corresponds to a G.T. of 32 mins.

The peculiarities of the lag-phase can be conveniently discussed by the aid of a $Z - X$ diagram. Four of the family of curves given by the general equation $0{\cdot}4343Z = K^n X^{n-1}$ are shown in the chart. They



are the particular cases when $n = 1$, $n = 1{\cdot}5$, $n = 2$ and $n = 2{\cdot}5$ and they illustrate the general shape of the curves with varying n. The following relationships are evident. All curves pass through a point a.

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When $n = 2$ the curve is a straight line joining the origin to the point a. When $n = 1$ the curve is a straight line passing through a parallel to the time axis. When $n = 2{\cdot}5$ the curve rises slowly at first and then rapidly. When $n = 1{\cdot}5$ the curve rises rapidly and then slowly.

All these curves theoretically can be continued indefinitely but in practice they end abruptly when they cut the horizontal line corresponding to the minimum G.T., that is $0{\cdot}4343Z = K_2$. When $n = 1$ the lag is infinitely great. The equation of unrestricted growth is evidently a limiting case where $n = 1$, but the value of K is about twice as great as in the lag-phase ($K_2 = 2K_1$). If there exist growths of bacteria which exhibit values of n approaching 1 the minimum G.T. will be reached only after a long time, and growths will be obtained which give apparently constant values of G.T. higher than the true minimum value. An average value $K_1 = 0{\cdot}00945$ corresponds to a point $1/0{\cdot}00945$ mins. (106 mins.) from the beginning of the experiment. All growths therefore (no matter what n is) should give a G.T. 32 mins. at a time 106 mins. Ledingham and Penfold's figures, given on pp. 252 and 253, show this to be approximately the case. Closer agreement would be obtained if the figures were adjusted to an extrapolated time origin when $Z = 0$ (G.T. $= \infty$). Ledingham and Penfold's results show another peculiarity which can be pointed out in the following way. When yeast cells are placed in suitable sugar solution, the rate of fermentation is proportional to the number of yeast cells present. This result can be expressed by means of the equation $qt =$ constant, where q is the quantity of yeast and t the time to bring about a given amount of fermentation. Arrhenius, in his book, *Quantitative Laws in Biological Chemistry*, shows that this qt-rule holds good for a large number of reactions brought about by cells and enzymes. In the case of a reaction which takes place in the cell itself it is a result which would be anticipated and deviations from the qt-rule are usually more interesting than agreement with it. We have such a deviation in Ledingham and Penfold's results. If the qt-rule held good n would be constant when the medium and condition of the seeding were the same, independently of the amount of seeding. Thus the equation $X^nK^n = \log Y^n$ takes no note of the seeding only of ratios between the seeding and number of cells at time X. Ledingham and Penfold find however that n varies with the seeding. The probable explanation is that n is influenced by a substance present in small concentration in the medium and that the amount is not great compared with the seeding used.

A knowledge of the factors which influence n would no doubt help to determine the cause of lag and the physical meaning of n.

Possibly growth depends on a substance present in the cell and is at all times proportional to its concentration z. For the convenience of having a name for this substance it has been called the "enzyme of growth." If z were initially zero and increase with the time during the lag-phase according to the equation $z = at^b$ where a and b are constants ($b = n - 1$) an explanation of the lag-phase would be obtained.

No doubt both the medium in which development takes place and the condition of the cell play their part in determining n.

It is possible that cultures of micro-organisms can be obtained which grow in one medium but not in another, although they grow readily in the latter medium if care is taken to get rid of lag. Irregularities in the growth of yeast in certain nutrient solutions have given rise to the idea that a certain mysterious "Bios" is necessary for yeast growth. The experiments on which the idea of "Bios" is founded can be readily explained by peculiarities in the lag-phase of growth. Text books by Bayliss (1915) and by Sykes and Ling (1907) give accounts of these experiments.

It is difficult to understand why the relationship shown in Table I holds good only for logarithms to the base 10. If natural logarithms are used another constant has to be introduced which cannot be eliminated by giving K another value.

Using natural logarithms equations (3) and (4) read

$$X^n a K^n = ln Y^n \quad \text{.............................(7)},$$

$$Z = aK^n X^{n-1} \quad \text{.......................(8)}.$$

There are certain advantages to be gained by using equation (8) as the fundamental equation of growth.

Let us consider the case when n is less than 1. By keeping K and a constant and by varying n we get a family of hyperbola-like curves all passing through a given point. The possibility that such curves represent rates of retarded growth of micro-organisms is worth investigating. When a culture of bacteria or yeast develop in a suitable medium after the logarithmic phase there occurs a period of retarded growth brought about by changes in the medium or by scarcity of food. The retarding influences finally become so great that growth ceases. The laws governing growth under such conditions are not easy to determine for the retarding factors do not remain constant and usually more than one is at work. It should however be possible to devise experiments in which the retarding influence remains constant and to determine the $Z - X$ curve under these circumstances.

In the special case where $n = 0$ and $a = 1$, $ZX = 1$ or the $Z - X$ curve is a hyperbola.

Further $Z = \frac{dY}{dX}\Big/Y$. Therefore $\frac{dX}{X} = \frac{dY}{Y}$ or $Y = cX$, where c is a constant. The curve of growth is therefore a straight line.

The interest in this calculation lies in the fact that rectilinear curves of yeast growth have been observed by H. T. Brown (1914). Brown explains these curves on the assumption that yeast growth requires oxygen and that the lack of oxygen under the conditions of the experiments has a retarding effect on the growth just sufficient to change the logarithmic curve to a rectilinear one. There is no doubt that if oxygen acts in the manner he describes the $X - Y$ curve becomes a straight line. On the other hand if the influences retarding growth are such that no increase in the amount of "enzyme of growth" takes place then rectilinear curves of growth would be obtained, and there is no difficulty in explaining Brown's results on such lines.

Whatever the true explanation is, rectilinear curves of growth can be expected if equation (8) represents the growth when retarding influences come into play.

Another phase in the existence of growths of micro-organisms is the final one when they gradually die. The curve of disappearance of living cells has been shown to be logarithmic in character[1] and may

[1] Though the logarithmic curve of the dying of cells and organisms seems to be followed in a surprisingly large number of cases it is not difficult to find conditions under which such a law would not hold. Thus for instance the natural death rate of a number of men all of the same age is not of a logarithmic character. This is shown in Table II, where

Y = the number of men surviving at various ages. The figures are taken from a table on the "Expectation of Life" based on the mortality for the 10 years 1891—1900. (See *Whitaker's Almanack*, 1917, 452).

Z = "constant" of decrease and is calculated over a short period of time (1 year) at various ages. Z is assumed to be constant over this short period and is measured by the difference between the logarithms of Y at the beginning and end of the year.

Z increases rapidly with the age of the man. By subtracting a constant from Z we get a series of figures $(Z - 0{\cdot}0011)$ which are approximately in geometrical progression. This is shown in the last column where A is calculated from the equation

$$A = \frac{1}{x} \log \frac{Z_0}{Z_x},$$

where $Z_0 = Z - 0{\cdot}0011$ at age 20 and $Z_x = Z - 0{\cdot}0011$ at age x.

The constancy of A shows that the decrease in the number can be calculated by means of an equation of the type $Z = a + e^{kx}$.

The death rate is apparently determined mainly by two factors, the one a constant

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therefore be considered a special case of the curve given by the general equation.

The equation

$$Z = aK^n X^{n-1},$$

covers therefore the main phases of growth of micro-organisms developing in a nutrient medium. By giving n values greater than 1 and keeping a and K constant curves representing the lag-phase of growth are obtained. When $n = 1$ and a is suitably adjusted the logarithmic period of growth is obtained. When $n = 0$, $a = 1$, special rectilinear curves of retarded growth are obtained. When $n = 1$ and a is given a suitable negative value the curve of disappearance of living cells is obtained.

It is evidently possible that if the constants are adjusted for each phase of growth the equation will hold good throughout the whole life period of a growth of micro-organisms. The period of retarded growth

independent of age (if this were the only factor the logarithmic law would apply), the other a factor increasing with the age, becoming twice as effective after each period of about 9½ years. Between 20—30 deaths are due about one half to the one factor and one half to the other; in later periods of life the second factor far outweighs the first.

This equation does not hold good for the earlier periods of life, when doubtless other factors are of importance.

TABLE II.

Age	Y	Z	Z − 0·00110	A
20 21	711714 708463	0·00199	(Z_0) 0·00089	—
30 31	673200 668682	0·00292	0·00182	0·311
40 41	615964 608632	0·00520	0·00410	0·332
50 51	530888 520608	0·00849	0·00739	0·306
60 61	409518 394793	0·0159	0·0148	0·305
70 71	246630 228844	0·0325	0·0314	0·309
80 81	82298 69789	0·0716	0·0705	0·317
90 91	7724 5470	0·150	0·149	0·318
100 101	68 36	0·276	0·275	0·311
			Average	0·314

especially in cases when the $X - Y$ curve is not a straight line requires investigation, and overlapping periods when for example growth and dying off take place simultaneously also deserve attention.

SUMMARY.

Ledingham and Penfold have shown that during the lag-phase of growth of *B. coli* in a nutrient medium the time and bacilli are connected by an equation of the form $X^n = k \log Y$ where n and k are constants (the initial seeding = 1). It has been pointed out in this communication that there is a relationship between n and k, and that the above equation can be put in the form

$$X^n K^n = \log Y^n.$$

The advantage of this new equation is that K remains of the same value throughout the whole series of experiments.

In the case investigated by Ledingham and Penfold the constant of unrestricted growth is approximately equal to $2K$.

The equation can also be put in the form

$$Z = aK^n X^{n-1},$$

where Z is the "constant" of growth $\left(\frac{dY}{dX}\Big/ Y\right)$ at any time X. By suitably adjusting n and a this equation can be made to represent not only the lag-phase of growth but also the logarithmic phase, and the special phase of retarded growth when the $X - Y$ curve is rectilinear. When cell-death occurs the bacteria usually perish at such a rate that the $X - Y$ curve is logarithmic; the general equation therefore also covers this case.

REFERENCES.

ARRHENIUS, S. (1915). *Quantitative Laws in Biological Chemistry.*

BAYLISS, W. M. (1915). *Principles of General Physiology.*

BROWN, H. T. (1914). Studies on Yeast. *Annals of Botany,* XXVIII. 197.

LANE-CLAYPON, J. E. (1909). Multiplication of Bacteria. *Journ. of Hygiene,* IX. 239.

LEDINGHAM, J. C. G. and PENFOLD, W. J. (1914). Mathematical Analysis of the Lag-phase in bacterial growth. *Journ. of Hygiene,* XIV. 242.

Penfold, W. J. (1914). On the Nature of bacterial Lag. *Journ. of Hygiene*, xiv. 215.

Penfold, W. J. and Norris, D. (1913). The Relation of Concentration of Food Supply to the Generation-time of Bacteria. *Journ. of Hygiene*, xii. 527.

Slator, A. (1913). The Rate of Fermentation by growing Yeast cells. *Biochem. Journ.* vii. 197.

—— (1916). The Rate of Growth of Bacteria, *Trans. Chem. Soc.* cix. 2.

Sykes, W. J. and Ling, A. R. (1907). *The Principles and Practice of Brewing.*

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