XLV.—The Rate of Multiplication of Micro-organisms: A Mathematical Study.

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The problem of the rate of multiplication of micro-organisms is one which has often been attacked, but which has not, to our knowledge, been reduced to a simple law.

If there be an unlimited supply of nutriment, an organism reproduces itself by compound interest: in a geometrical progression—*i.e.* 1, 2, 4, 8, etc. That is to say, that the rate of growth under unimpeded conditions is proportional to the number present, at any moment, or

$$\frac{dy}{dt}=by. \tag{1}$$

In test-tube experiments, however, this simple state of affairs is complicated by the fact that the supply of nutriment is limited, and consequently, as time goes on, the rate of multiplication falls off.

Every living organism employs the nutriment which it has absorbed for two objects: first, the maintenance of the individual; and, second, its reproduction. As, however, in the case of those micro-organisms with which we shall deal, the rate of multiplication is very fast, we may, for all practical purposes, consider that the amount of food-stuff utilised for their upkeep is negligible, and assume that the whole of it is employed in reproduction.

If we accept this simplifying assumption we may say that organisms in a test-tube multiply, by a simple conversion of the available food-stuff, into other organisms, and that the rate of multiplication is proportional to the concentration of that food-stuff.

If a be the original concentration of food-stuff, the concentration at the time t will be $(a-y)$.

Introducing this factor into equation (1), we have

$$\frac{dy}{dt}=by(a-y), \tag{2}$$

which means that the *rate of increase of fast-growing organisms is proportional to the number of organisms present, and to the concentration of the food-stuff.*

(*b*) In curve 2. The broth was heated to 37° C. prior to inoculation. Tube placed in the air of the incubator. No external precautions taken.

(*c*) In curves 3, 4, 5, as in (*b*), but in addition the tubes were placed in a water bath in the incubator, and during manipulation, bath and tubes were removed, the tubes being kept as much as possible in the water bath.

(*d*) In curves 6 and 7. As in (*c*), but in this instance two water baths were used, one in the incubator, the other outside of it. The tubes were manipulated in the second water bath, which was also kept at 37° C.

(*e*) In curve 8. The same precautions were taken as in (*d*): the culture used for inoculation had been incubated for fourteen days.

We found, then, that by adopting the procedure as stated in (*d*), and by using for inoculation only actively growing organisms from very young cultures, the latent period was entirely eliminated (as is shown in curves 6 and 7).

The figures of curves 3, 4, 5, 6, 7 are given on the opposite page.

[*Note.*—It will be noted that in curves 4 and 5 the latent period was still in evidence.]

We return to the mathematical theory.

Equation (2) $$\frac{dy}{dt} = by(a-y)$$

becomes on integration

(3) $$y = \frac{a}{1 + \frac{a - y_0}{y_0} e^{-abt}}$$

where y_0 is the value of y at the time $t=0$, *i.e.* the number of organisms originally inoculated, and a and b are constants to be determined.

When $\frac{dy}{dt} = 0$ (*i.e.* when multiplication has ceased)

$$y = a.$$

Enumerations beyond this point give irregular results; the samples taken are uneven, probably on account of clumping.

From a study of the manner in which the curves flatten after eight or nine hours' growth, a suitable value of a may be inferred in each case.

	Curve 3.		Curve 4.		Curve 5.		Curve 6.		Curve 7.	
Time in hours.	y	$\log_{10}y$	y	$\log_{10}y$	y	$\log_{10}y$	y	$\log_{10}y$	y	$\log_{10}y$
0	1,760 1,760	3·246 3·246	19,000 14,070	4·279 4·148	176,000 110,200	5·246 5·042	2,850 2,850	3·455 3·455	64,250 64,250	4·808 4·808
½	4,020 4,483	3·604 3·652	35,900 35,833	4·555 4·554	280,000 280,390	5·447 5·448	7,500 7,080	3·875 3·850	165,000 163,550	5·217 5·214
1	... 11,419	... 4·058	88,000 91,243	4·944 4·960	608,500 712,200	5·784 5·852	17,500 17,587	4·243 4·245	357,500 415,960	5·553 5·619
2	72,000 74,057	4·857 4·870	482,000 590,100	5·683 5·770	3,870,000 4,506,000	6·588 6·654	105,000 108,440	5·021 5·035	2,625,000 2,660,900	6·419 6·425
3	380,000 479,240	5·580 5·680	3,400,000 3,749,900	6·531 6·574	28,200,000 25,194,000	7·450 7·401	625,000 664,050	5·796 5·822	16,250,000 15,820,600	7·211 7·199
4	2,600,000 3,057,200	6·415 6·485	26,300,000 21,462,000	7·419 7·332	74,200,000 86,131,000	7·870 7·935	2,250,000 3,971,200	6·352 6·599	45,375,000 66,542,000	7·657 7·823
5	19,750,000 17,877,000	7·296 7·252	71,600,000 78,903,000	7·855 7·897	127,000,000 137,320,000	8·104 8·138	17,750,000 20,333,000	7·249 7·308	122,125,000 131,520,000	8·087 8·119
6	77,000,000 72,718,000	7·886 7·862	118,000,000 134,299,000	8·072 8·128	150,000,000 151,170,000	8·176 8·179	50,000,000 61,169,000	7·699 7·787	158,500,000 154,834,000	8·200 8·190
7	120,000,000 130,420,000	8·079 8·115	... 150,590,000	... 8·178	149,000,000 153,550,000	8·173 8·186	97,500,000 90,672,000	7·989 7·957	194,500,000 159,181,000	8·289 8·202
8	106,000,000 149,830,000	8·025 8·175	135,000,000 153,460,000	8·130 8·186	154,000,000 153,900,000	8·188 8·187	...	...	...	...
Quantity of broth.	25 c.c.	...	25 c.c.	...	25 c.c.	...	12 c.c.	...	12 c.c.	...
Age of culture inoculated.	1½ hours	...	1½ hours	...	1½ hours	...	1 hour	...	1 hour	...
Constants.	$a=154$	$ab=1·87$	$a=154$	$ab=1·87$	$a=154$	$ab=1·87$	$a=100$	$ab=1·82$	$a=160$	$ab=1·87$

Upper figures are observed. Lower figures are calculated.

The following figures illustrate this maximum value:—

y_0.	a after fourteen hours.
22,800	147,000,000
32,150	151,000,000
100,550	167,000,000

From a practical point of view this is of extreme importance, as obviously error in the number inoculated has little or no effect on the total number attained to. Provided that the number planted be comparatively small, it is unnecessary to enumerate each flask, in the preparation of vaccines in large quantities. Indeed, with a suitable and accurately based system (concentration of broth, etc., being kept constant) vaccines could be prepared month after month at standard strength without any enumerations being made. B. coli, in the quantities of broth which we have used, reaches a maximum in from twelve to fifteen hours.

When $t=0$

$$y=y_0,$$

and $\frac{dy_0}{dt}$ (the initial rate of increase) $=by_0(a-y_0)$,

i.e.

$$\frac{d\log y_0}{dt}=b(a-y_0).$$

As in our experiments a is measured in hundred millions and y_0 rarely exceeds a hundred thousand, we may consider

$$\frac{d\log y_0}{dt}=ab.$$

That is to say, with log y as ordinate and t as abscissa the slope of the curve at the commencement is equal to ab. This value can be obtained from observed results, and its value substituted in equation (3). The values of ab so obtained are entered on the Table of Numbers given above.

[*Note.*—ab is measured in logarithms to base e, whereas in the accompanying tables logarithms to base 10 are employed. Log y to base 10 multiplied by $2{\cdot}3026=\log y$ to base e.]

Since the constant a denotes the original concentration of food-stuff, and b depends on the ability of the organism to acquire its food (modified by such accelerating or retarding influences as temperature, degree of alkalinity, presence of medicaments, etc.), it might have been hoped that in the relation

$\frac{d \log y_0}{dt} = ab$ lay a method of estimating nutritive values and possibly even of determining whether a particular substance acted as a food or as a mere accelerator. But it is at this point that the simplifying assumption breaks down, for obviously an extreme degree of concentration of food-stuff cannot cause an infinite rate of multiplication. The simplifying assumption ascribes to the organism the business of obtaining its food; it presupposes a lightning rapidity of assimilation after it has come in touch with its food, and a lightning rate of multiplication after sufficient nutriment has been assimilated. These conceptions are impossible, and it is only where the period of a generation is large in comparison with the times required for assimilation and division that the simplifying assumption holds good. We have, in fact, applied molecular physics to molar vital phenomena, and we meet with necessary limitations.

The rate of growth (ab) may, however, be applied to a comparison of bacteria as to their multiplying properties; and it may also be most advantageously employed in the comparative investigation of fluid media (bouillon, sugar, etc.) with a view to their standardisation and improvement.

The period of a generation can be deduced as follows:—One generation corresponds to a multiplication of the original number by 2; or to an addition of 0·301 to its logarithm to base 10. But ab is the rate of change of $\log_{10} y_0$, or change per unit time. Hence $\frac{ab}{0 \cdot 301}$ = number of generations per unit time. In curves 3, 4, 5, $ab = 0 \cdot 812$, and unit time is one hour; consequently the period of a generation is 22·27 minutes.

Conclusions.

1. The rate of multiplication of fast-growing micro-organisms is proportional to the number of organisms, and to the concentration of food-stuff.

2. The initial rate of multiplication affords a factor of comparison both of efficiency for media and of reproductive properties of organisms.

3. Vaccines may be prepared in large quantities on the basis that a maximum number of organisms is attained to, this maximum being dependent on the concentration of nutriment, and independent of the amount of culture inoculated.

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