363. *Physicochemical Aspects of Bacterial Growth. Part I. Dependence of Growth of* Bact. Lactis Aerogenes *on Concentration of Medium.*

By S. Dagley and C. N. Hinshelwood.

When *Bact. lactis aerogenes* grows in an artificial glucose–phosphate–ammonium sulphate medium, the final concentration of organisms reached varies with the concentration of the foodstuffs in such a way as to show that exhaustion of the latter, rather than the accumulation of toxic products, is over a wide range the factor limiting growth. Over this same range the mean generation time of the organisms is nearly independent of the concentration of the foodstuffs.

From the physicochemical point of view a bacterium is a colloidal particle endowed with the property of growth and division as a result of synthetic processes performed on materials

absorbed from the surrounding medium. The growth of a bacterial culture shows three stages: a lag phase, not always observed, during which division is abnormally slow, a logarithmic phase during which the number of organisms increases exponentially with time, followed by a rapid fall in growth rate to a stationary phase where the total number has reached a limit which may be called the stationary population or stationary concentration. In the logarithmic phase the rate of growth is directly proportional to the number of organisms at any instant: $dn/dt = kn$, whence $\log n/n_0 = kt$. The rate of growth is conveniently measured by the "mean generation time," which is the time required for the number of organisms to double.

The present investigation deals from the physicochemical point of view with factors determining the mean generation time and the stationary concentration in various media of the organism *Bact. lactis aerogenes*.

Technique.—Growth curves were obtained as follows. A sample from the growing culture was taken with a Pasteur pipette, and a drop of formalin was added to kill the organisms, the time being noted. The cells were then stained with methylene-blue and counted, using a Thoma hæmocytometer with a chamber 0·02 mm. deep, and a ruled grating of which 16 small squares, each $\frac{1}{400}$ sq. mm. in area, covered the microscope field. The counts recorded below are averages for several fields, and can be converted into numbers per c.c. by multiplying by the factor $1{\cdot}25 \times 10^6$.

Inocula were of two kinds. When "loop" inocula were used, a small sterilised loop of wire, capable of holding a film of about $0{\cdot}6 \times 10^{-2}$ c.c. of culture, was dipped first into a stock culture of the organisms in bouillon and then into the medium to be inoculated. A "pipette" inoculum was larger and consisted of about 0·1 c.c. of a culture which had just attained its stationary concentration. The small pipette, constructed from a drawn-out capillary tube, was calibrated, so that, the stationary concentration of the inoculating medium being known, the initial count in the fresh culture could be calculated.

The medium for the experiments of Part III was double strength veal bouillon (p_H 7·6), but in Parts I and II synthetic media were used (Gladstone, Fildes, and Richardson, *Brit. J. Exp. Path.*, 1935, **16**, 335) consisting of mixtures of the following solutions:

Solution 1: 4·5 g. of KH_2PO_4 and 1 g. of $(NH_4)_2SO_4$ } Buffered with 26 c.c. of M-NaOH and made up to 500 c.c. with sterile water.
Solution 2: 0·4% $MgSO_4,7H_2O$
Solution 3: 10% glucose solution.

All cultures were incubated in a thermostat at 40·0°.

The Stationary Phase.—There are several theories to account for the cessation of growth in this phase. One obvious possibility is exhaustion of foodstuff, but since some investigators have stated that the organisms of a natural medium can be filtered off and the sterile filtrate used to support the growth of a fresh culture, the phenomenon cannot be due always to this exhaustion. According to Bail (cf. Topley and Wilson, "Principles of Bacteriology and Immunity," 1936, p. 81), the cells have filled all the available "biological space"; other authors assume that the cells have consumed all the oxygen in the solution or that the accumulated toxic products of metabolism are responsible for the rapid fall in growth rate. These hypotheses lead to different consequences which should be verifiable by experiment. We may first consider the effect of the waste products on the rate of growth. The toxic action of a substance is some function of its concentration, and in a simple case, such as that exemplified in Part III, the expression for the growth rate, $dn/dt = kn$, may become of the form $dn/dt = kn(1 - \alpha c)$. If, on the average, an organism produces the toxic substance at a rate r, then $dc/dt = nr$, whence

$$c = r\int_0^t n \,.\, dt$$

and therefore

$$dn/dt = kn\left(1 - \alpha r\int_0^t n \,.\, dt\right)$$

The assignment of correct values to α and r will only enable the rate of growth to be predicted if the area beneath the growth curve is known. The stationary concentration is reached when $dn/dt = 0$. Determined in this way, it should not be decreased by diluting the medium. As appears later, dilution over a considerable range does not alter the actual rate of growth appreciably ; therefore at the end of a given time the same number of organisms would *ceteris paribus* have grown. They will in this time have produced a concentration of toxic product which at least will not be greater than in the more concentrated medium. The stationary concentration will therefore not be decreased.

On the other hand, if growth ceases in the stationary phase because all the foodstuff has been consumed, the problem is simple, since the equation $dn/dt = kn$ remains unmodified. If g is the concentration of a foodstuff at an instant t, and if the average rate of consumption is f per organism, $-dg/dt = fn$, and

$$g = g_0 - f\int_0^t n \,.\, dt$$

When $g = 0$, $n = n_s$, and neglecting n_0, the initial count, we have $n = g_0 k/f$, so that $n_s \propto g_0$, *i.e.*, the stationary concentration attained is proportional to the amount of foodstuff available.

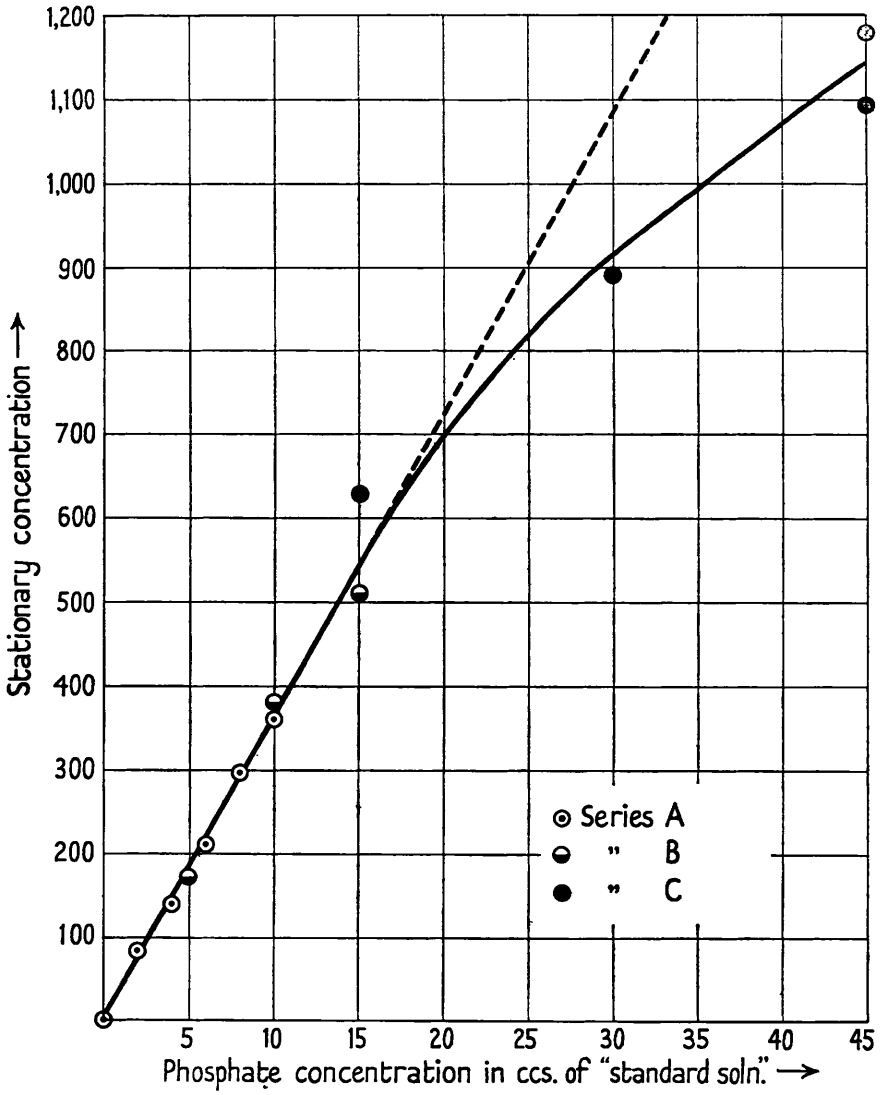
The first part of the investigation was therefore to vary the amounts of glucose and of phosphate in the medium and determine whether the stationary concentration would remain nearly unchanged as the first hypothesis requires, or would obey the linear relationship deduced from the second.

This relation is, in fact, found to hold up to stationary concentrations of about 500 million bacteria per c.c., as is seen from Fig. 1, in which the concentration of phosphate solution (soln. 1, above) is plotted against stationary concentration. At higher concentrations, departure from linearity occurs either because toxic products have accumulated or because the supply of dissolved oxygen has been exhausted by the large number of cells in the culture. In Fig. 1 the points referred to as set A were determined from growth curves of solutions containing 10 c.c. of glucose (soln. 3), 0·3 c.c. of magnesium sulphate (soln. 2), p c.c. of the phosphate mixture (soln. 1) and $(10 - p)$ c.c. of sterile water, p being varied from 0 to 10 c.c. Set B was for mixtures of 5 c.c. of soln. 3 (which provides excess glucose, as will be shown), 0·3 c.c. of soln. 2, p c.c. of soln. 1, and $(15 - p)$ c.c. of sterile water, where p was in turn 5, 10, and 15 c.c. The growth curves of all these cultures are shown in Fig. 2, the inoculum for set A being two loops of a stock bouillon culture which showed a lag phase, whilst that used for set B was a pipette inoculum of cells which had just reached the stationary phase in a synthetic medium and consequently showed no lag. The value of the stationary concentration evidently depends only on the amount of foodstuff present and, unlike the growth rate, is independent of the past history of the inoculum. The amount of phosphate solution necessary to enable the organisms of a loop inoculum to grow to the number in a pipette inoculum is very small : it is equivalent to a stationary concentration of about 3 ; so that a negligible error arises by changing the mode of inoculation in this way. The solutions of Set C for high phosphate concentrations contained 10 c.c. of soln. 3, 0·3 c.c. of soln. 2, P c.c. of a phosphate solution five times stronger than normal, and $(10 - P)$ c.c. of sterile water : these phosphate concentrations are multiplied by 5 in Fig. 1 to convert them into the standard c.c. of phosphate.

In another series of experiments the amount of phosphate was kept constant at 10 c.c., 0·3 c.c. of soln. 2 was added, and the glucose concentration was varied. The 10% glucose solution used previously was shown to be relatively far stronger than the phosphate, and was hence diluted 20-fold. Fig. 3 shows that the stationary concentration at first increases linearly with the glucose concentration and then tends to become independent of it. The reason for the bending over of the curve proves, however, to be a secondary one, and due to the exhaustion of the phosphate by the growths which the larger glucose concentrations can support. When additional phosphate is provided, the curve continues along the dotted line as indicated. This is, in fact, already evident from Fig. 1, where, for example, increase

in glucose from 5 to 10 c.c. changes the stationary concentration from 380 to 360, the phosphate being 10 c.c. If, however, the phosphate is increased to the equivalent of 30 c.c. for the 10 c.c. of glucose, the stationary concentration rises to 890. It appears that quite a small amount of glucose is enough to allow the consumption of large amounts of phosphate, and over a wide range of concentration the exhaustion of the phosphate continues to be the factor determining the stationary concentration ultimately reached. It is remarkable

FIG. 1.



that when the curve of Fig. 3 bends round it does not remain absolutely parallel to the axis. The stationary concentration increases from about 210 at 3 c.c. to 250 at 10 c.c. of 0·5% glucose, and the increase continues to 360 when the same amount of phosphate with 5 c.c. of 10% glucose is present. With 10 c.c. of 10% glucose, however, the stationary concentration is still about 360, showing that the curve has become quite parallel to the axis at these higher glucose concentrations. This would indicate that, if the cells are provided with a given amount of phosphate solution, they can grow to a greater stationary

concentration, up to a certain limit, the greater the excess of glucose present : in some way they are able to utilise one foodstuff more efficiently if an excess of another is provided. The stationary concentrations are recorded in Tables I and II.

FIG. 2.

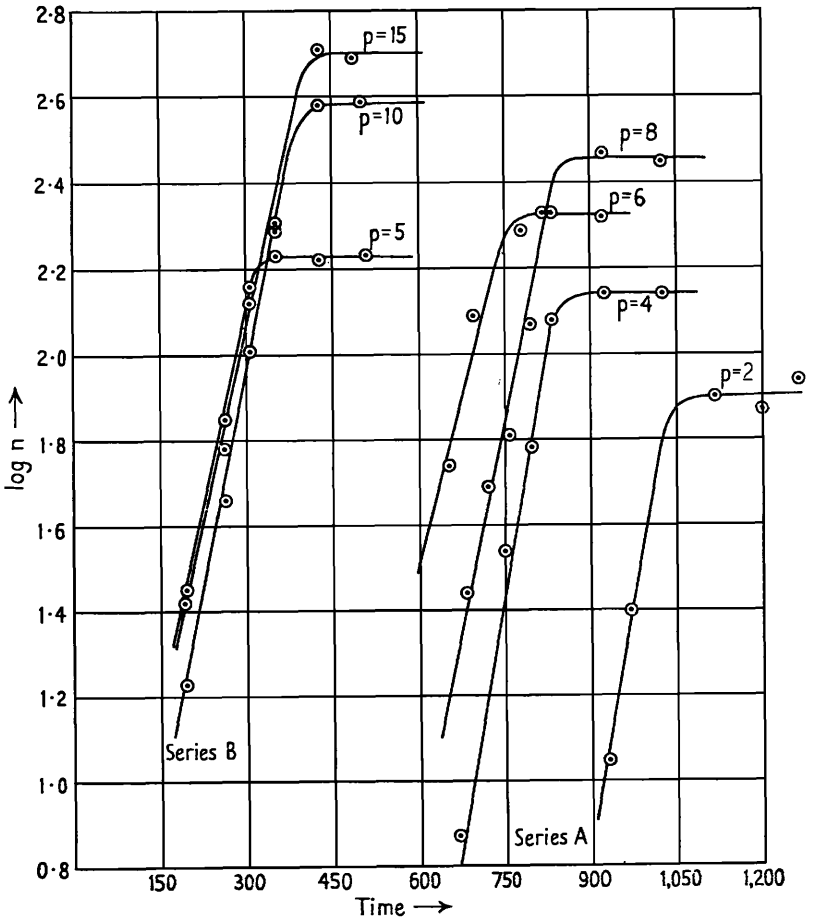


TABLE I.

Stationary concentrations at various concentrations of phosphate.

(The phosphate concentration is expressed in c.c. of " standard " phosphate–ammonium sulphate mixture.)

Phosphate concn. (c.c.)	2	4	5	6	8	10	15	30	45
Stationary concn.	82	140	170	210	295	360, 380	510, 630	890	1100, 1180

TABLE II.

Stationary concentrations at various concentrations of glucose.

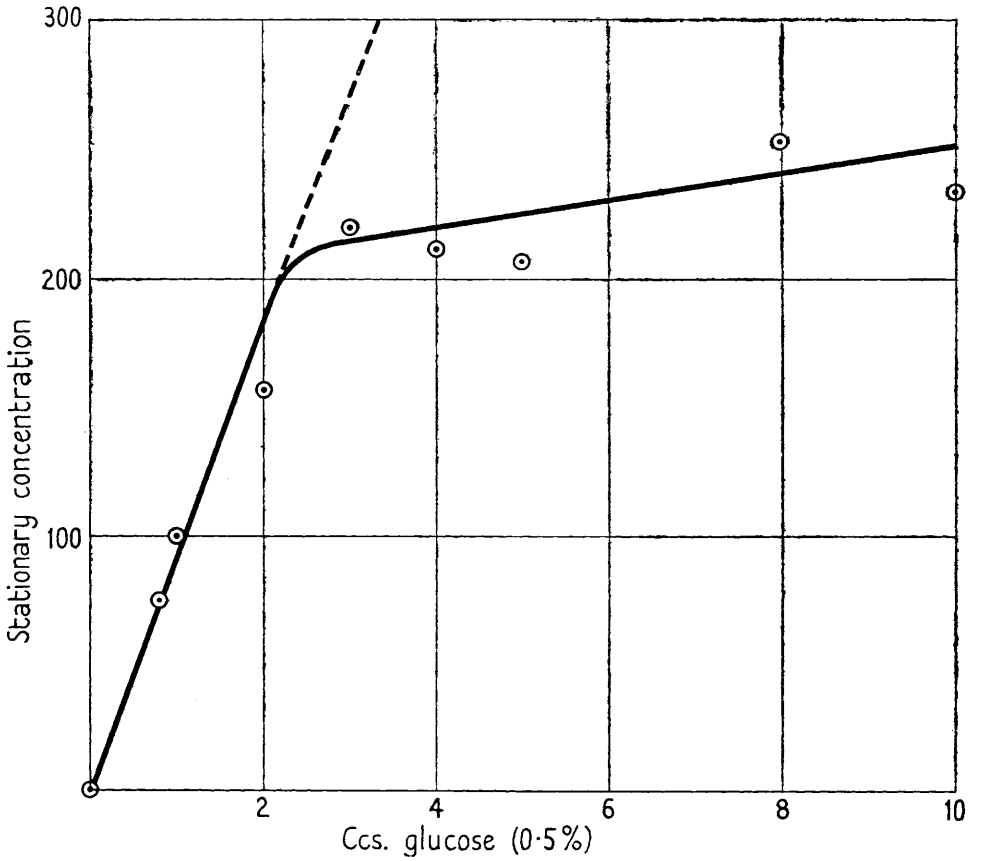
(The glucose concentration is expressed in c.c. of 0·5% glucose.)

Glucose concn. (c.c.)	0·8	1	2	3	4	5	8	10
Stationary concn.	74	100	156	220	212	206	254	233

The influence of hydrogen-ion concentration on the growth rates and on the stationary counts is itself an interesting problem which is being investigated. Although acid is produced during growth, changes of p_H are not relevant to any of the conclusions in Parts

I, II, and III. In Part I, the argument that cessation of growth is not due to the accumulation of a toxic product applies even though the potentially toxic product be regarded as hydrions themselves. In Part II, the carbon dioxide concentration is varied with all other factors constant : and Fildes and collaborators have shown that the carbon dioxide effect is not in itself a p_H effect at all. In Part III, again, all conditions are constant except the concentration of the alcohols : the medium is, in fact, a buffer solution, and the logarithmic growth curves, some of which are reproduced in the paper, show that there is a constant mean generation time over a large range of growth. Consequently, acid production by the organism can hardly be supposed to influence the results.

FIG. 3.



Growth Rates.—The growth curves for the organisms at various concentrations of foodstuffs did not reveal a dependence of growth rate on foodstuff concentration corresponding to the relationship existing for stationary concentrations. Table III summarises the results. At the time when these experiments were made, the method of controlling inoculum and gas atmosphere had not been developed. With this (Part II), much more consistent results could have been obtained, but repetition of the experiments was not considered worth while in the present instance since even when the glucose concentration was varied 250-fold, the changes in growth rate were purely random. It may be concluded that even at the lowest concentrations of glucose used there is sufficient of it in the solution to provide for complete saturation of the active centres; it is continually supplied at a rate sufficient for maximum growth rate until all of it is consumed. Glucose solutions more dilute than those listed in Table III cannot be investigated by the present technique, since the stationary concentrations corresponding to them will be so low : growth will have ceased before a satisfactory count can be obtained in the hæmocytometer. The following

TABLE III.

The Effect of Variation of Foodstuff Concentration on Growth Rate.

1. *Glucose.* In the media there were the following solutions : 10 c.c. of phosphate, 0·3 c.c. of magnesium sulphate, and g c.c. of glucose, the whole being made up in each case to 20·3 c.c. by the addition of $(10 - g)$ c.c. of sterile water.

g	0·04	0·06	0·08	0·10	0·12	0·14	0·19	0·20	0·30
Mean generation time (mins.)	68	58	59	60	72	68	68	60	60
g	0·5	1·0	2·0	3·0	4·0	5·0	8·0	10·0	
Mean generation time (mins.)	56·5	42·5	51	28	36·5	52	41	38	

2. *Phosphate.* The same procedure was adopted in this case, the glucose being kept constant at 10 c.c., and the phosphate concentration varied.

Phosphate (c.c.)	2	4	6	8
Mean generation time (mins.)	44	44·5	50	49

3. *Magnesium sulphate.* Glucose and phosphate concentrations constant at 10 c.c. each.

$MgSO_4$ (c.c.)	0	0	0·2	0·3	0·5
Mean generation time (mins.)	43·5	53·5	64·5	46	51

The inocula in all cases were two loopfuls of stock bouillon cultures.

considerations confirm the view that changes in growth rate would only occur at such low concentrations.

Suppose the bacteria grow to a stationary concentration n_s in a medium containing initially a concentration of foodstuff c_s, n_s being of such magnitude that all the food is consumed when the stationary phase is reached and complications due to oxygen exhaustion or the accumulation of toxic products do not arise. The amount of foodstuff, c, which has been consumed by the organisms in growing to a count n at an instant t will be that required to support a stationary concentration of n and can be read off from curves such as Figs. 1 and 3. We can thus relate $c_s - c$, the amount of foodstuff still unused at the instant t, with the mean generation time; the latter will remain constant almost to the point at which the stationary phase is reached, since the curve is linear nearly to that point. The abruptness of attainment of the stationary phase is an indication of the extent to which a solution must be diluted before the growth rate is affected, and the range of concentration over which this may occur can be estimated by plotting the slopes of the tangents to the curve in this region where food is rapidly becoming exhausted against the values of $c_s - c$ obtained in the manner indicated. This was done for a carefully determined curve for a medium in which phosphate exhaustion was the limiting factor : with 2 c.c. of phosphate solution present, the mean generation time was 44; when the phosphate concentration had fallen to 0·3 c.c. it was 85, after which it fell rapidly, and when 0·1 c.c. of phosphate was left it was 230, and at 0·05 c.c., 330. Thus no considerable change in mean generation time would occur until the concentration of phosphate solution was 0·3 c.c. : this corresponds to a stationary concentration of about 8, and hence the growth curve could barely be determined by using our present technique.

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