33. *The Phosphorus Metabolism of* Bact. lactis aerogenes.

By P. C. Caldwell and Sir Cyril Hinshelwood.

A survey has been made of certain aspects of the phosphorus metabolism of the coliform organism *Bact. lactis aerogenes*. The cells are found to have a constant phosphorus requirement during the period of logarithmic growth, but under adverse conditions division can continue without further uptake of phosphorus from the medium.

No significant lowering of the growth rate can be detected at phosphate concentrations as low as 10^{-6}M.

In glucose media, buffered with veronal, phosphorylated intermediates, which probably contain a ribose-3 phosphate linkage, are built up inside the cells during the lag phase, reach a steady level during the logarithmic period, and decline towards the onset of the stationary phase. Intermediates of this type are not, however, detected during growth in glycerol–veronal media. The bearing of the results on the question of nucleic acid synthesis is discussed.

The cells utilise free inorganic phosphate to the exclusion of the nucleotide guanylic acid supplied in the medium—as shown by experiments with ^{32}P.

The work described in this paper forms part of a general study of the phosphorus metabolism of *Bact. lactis aerogenes*. Phosphorus, which is normally supplied in the synthetic culture medium as inorganic phosphate, is required for the production of a variety of substances. Some of these are involved in intermediary metabolism whereas others form an integral part of the cell structure.

The first problem to be dealt with is the relationship between the assimilation of phosphorus from the culture medium and the growth of the organism. Next the influence of the phosphate-ion concentration on the rate of growth is discussed, and it is shown that, even at the lowest concentrations accessible to experiment, the uptake of phosphorus has not yet become a rate-controlling step. The variations in the phosphorus content of the cells during the growth cycle are then considered, and alterations in the amount of various phosphorylated intermediates are described. In certain circumstances, these appear to be formed more rapidly than they are removed, and they show systematic rises and falls during the growth cycle. Finally, since substances of the nucleotide type must constitute necessary intermediates, the effect of guanylic acid on the growth of the cells is investigated.

Assimilation of Phosphorus from the Culture Medium.—The usual culture medium contains glucose (or some alternative carbon source), ammonium sulphate, magnesium sulphate, and a mixture of alkaline phosphates which serves both as a supply of phosphorus and as a buffer. Since the concentrations of phosphate required for adequate buffering are much greater than the amounts assimilated by the cells, chemical estimation of the removal of phosphorus from the normal culture medium is not practicable. It was necessary, therefore, to provide an alternative buffer, and veronal (sodium diethylbarbiturate) was used for this purpose. It interferes to some extent with growth, which is a disadvantage, but after a number of subcultures the cells show partial adaptation (though the mean generation time never drops to the value found in the normal culture medium).

Measurements of phosphorus uptake during the logarithmic phase show that the amount assimilated at any time is given by the relation :

$dx/dt = kx$, where x is the amount of phosphorus assimilated and k the growth rate constant.

This is of the same form as the equation which governs the increase in the number of cells :

$$dn/dt = kn, \text{ where } n \text{ is the number of cells.}$$

A plot of log x against time is linear, and a typical example is shown in Fig. 1, where it is compared with the corresponding growth curve.

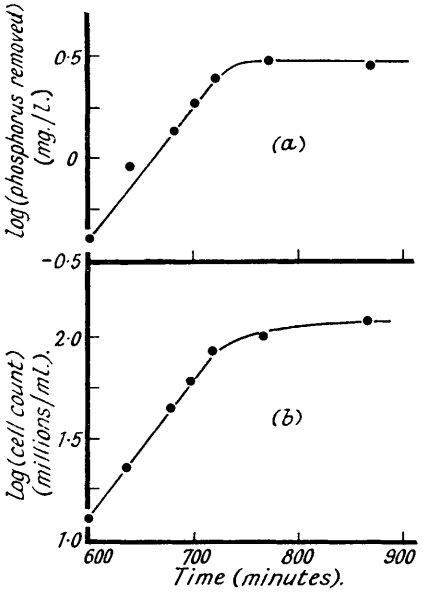
The fact that the assimilation is represented by an equation, identical in form with that for the multiplication of the cells, suggests that during the logarithmic period the requirement for phosphorus is steady. (This conclusion is not, however, valid for the lag and the stationary phase, as will appear.)

The relative amounts of phosphorus and nitrogen in cells can be found if the nitrogen content of a culture is compared with the quantity of phosphorus which it has assimilated. The ratio

phosphorus/nitrogen is found to be 0·29 for cells growing in the presence of veronal. This compares with the value of 0·20 found by Taylor (*J. Biol. Chem.*, 1946, **165**, 271) from analysis of the material of *Bact. coli* grown in a synthetic medium.

FIG. 1.

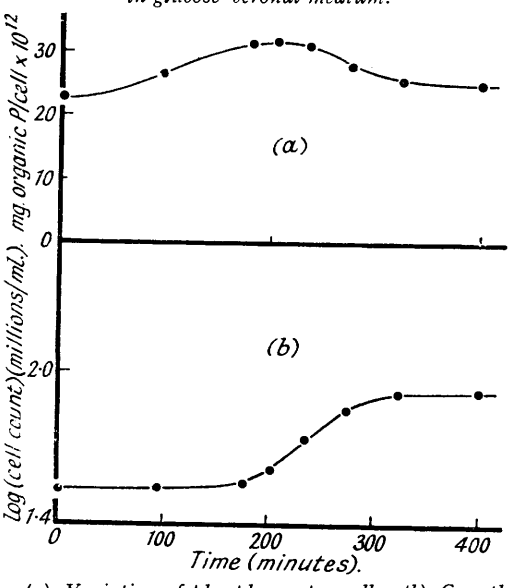
Assimilation of phosphorus during growth.



(*a*) *Removal of phosphorus from medium.* (*b*) *Growth curve. Initial phosphate concentration,* 25 *mg./l.*

FIG. 2.

Variation of total organic phosphorus during growth in glucose–veronal medium.



(*a*) *Variation of phosphorus per cell.* (*b*) *Growth curve. The two time scales are identical.*

This figure is constructed by the superposition of two sets of experimental results—one set taken to the end of lag, the other carried into the stationary phase.

The results of these experiments are summarised in Table I where values of the mean generation time obtained from the rate of assimilation of phosphorus are compared with those obtained from the rate of increase of cell numbers (mean generation time = $k/\ln 2$).

TABLE I.

	Mean generation time (min.).			Mean generation time (min.).	
Initial phosphate concn., mg./l.	From cell count.	From phosphorus assimilated.	Initial phosphate concn., mg./l.	From cell count.	From phosphorus assimilated.
50	60	60	10	70	60
25	45	48	5	62	55
20	48	52	1	72	—
15	50	45	0·1	75	—

Phosphate-ion Concentration and Growth Rate.—Table I shows that, within the range of conditions studied, very little, if any, systematic variation in growth rate can be detected over a 500-fold range of concentration, the mean generation time showing only slight increases of doubtful significance at phosphorus concentrations as low as 10^{-6}M. Chemical estimation shows that the inorganic phosphate in the medium must become almost completely exhausted before any serious effect is observed. The rate of growth cannot be measured in the normal way at phosphate concentrations below 10^{-6}M. because the cell numbers involved are too small, but it is very likely that a decrease would eventually be found. This investigation merely sets a limit above which no important fall occurs.

A somewhat different state of affairs is encountered if the concentration of the carbohydrate component of the medium is lowered. Various investigators, in particular Monod ("La Croissance des Cultures Bactériennes," Paris, 1942), have shown that considerable decreases in the rate of growth occur when the carbohydrate concentration is reduced to about 10^{-5}M.

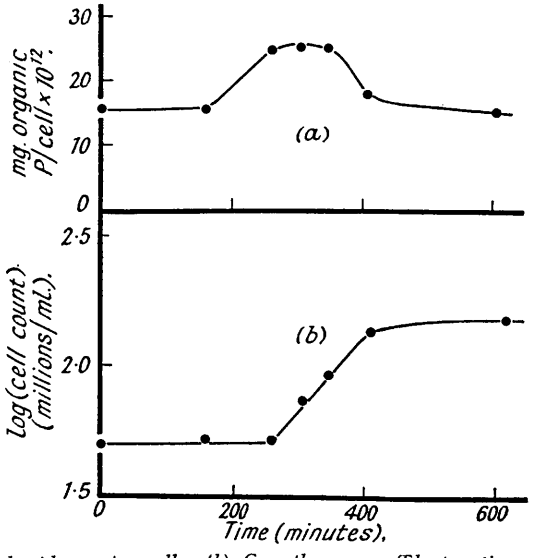
In most reactions involving enzymes, the dependence of the rate, r, on the substrate concentration, c, can be expressed by an equation of the form :

$$r = k_\infty c/(c + K_m)$$

where k_∞ is the limiting rate which is attained at high substrate concentrations and K_m is the concentration at which the rate is half the maximum value. This relation can be interpreted either in terms of an enzyme-substrate complex (Michaelis–Menten equation) or in terms of a surface reaction, the adsorption of molecules on the enzyme being governed by the Langmuir isotherm. In general, the growth constant for the cells, k, will be proportional to r. The region of substrate concentration at which a marked lowering of the rate of growth takes place will be determined by the value of K_m. The magnitude of this depends on the tightness with which the substrate is bound to the enzyme, the larger the value the looser being the binding.

FIG. 3.

Variation of total organic phosphorus during growth in glycerol–veronal medium.



(*a*) *Variation of phosphorus per cell.* (*b*) *Growth curve. The two time scales are identical.*

The experimental results suggest that the value of K_m governing the processes responsible for the assimilation of carbohydrates must be considerably larger than that for the process involved in the uptake of phosphate. This means that the binding of phosphate ions to the relevant enzymes must be tighter than that of carbohydrates, as is quite understandable, since with phosphate an electrostatic interaction is superimposed on the van der Waals binding. With carbohydrate molecules there is no ionic interaction, and they are less tightly held. It is of interest that Lewis and Hinshelwood (*J.*, 1948, 841) were unable to detect any decrease in the rate of growth when the concentration of ammonium ions in the medium was reduced, which indicates that these, like phosphate, are more firmly bound than carbohydrate molecules as a result of an ionic interaction.

Variations in the Cell Phosphorus during the Growth Cycle.—Fig. 2 shows the variations in the total organic phosphorus per cell during growth in the glucose–veronal medium. During the lag period the amount rises and reaches a steady value at the beginning of the logarithmic-growth phase. As the stationary phase is approached it declines to its original level. Fig. 3 shows that the same sequence of events takes place in a veronal medium in which the glucose is replaced by glycerol, and a similar state of affairs is observed under anaërobic conditions in a glucose–veronal and in a (−)-D-arabinose–veronal medium.

In these particular examples the primary factor which limits growth is the exhaustion of the phosphate provided in the medium. The effects observed in media where the breakdown of the buffer is the limiting factor are, however, similar.

The deoxyribose nucleic acid content per cell of *Bact. lactis aerogenes* has been shown to be

approximately constant, even under conditions of phosphorus deficiency (*J.*, 1950, 1415). Changes in the amount of total organic phosphorus per cell during the growth cycle should represent, therefore, variations in the remainder of the cell phosphorus relative to the deoxyribose nucleic acid. In the lag phase, the total phosphorus in the cell increases at the expense of the phosphate in the medium. At the end of the growth phase, on the other hand, a certain amount of cell division takes place after the conversion of phosphate into the organic form has virtually ceased, the cells becoming smaller in the process. Since an increase in the number of cells must, by the previous result, involve an increase in the deoxyribose nucleic acid in the culture as a whole, the synthesis of this can presumably occur at the expense of the other organic phosphates. (This explains the observation that the total population supported by a medium does not fall off linearly as the inorganic phosphate concentration drops towards zero.)

Estimations have been carried out of the amounts of ribose and deoxyribose nucleic acid in cells growing in a variety of media and have been reported previously (*J.*, 1950, 1415). The determinations on cells growing in the glucose–veronal medium are summarised in Table II, which gives the percentage of the total phosphorus present in the various forms.

TABLE II.

	%.
Deoxyribose nucleic acid	18·1
Ribose nucleic acid (including free pentose nucleotides)	61·3
Total nucleic acid	79·4
Other organic phosphorus	20·6
Total organic phosphorus	100·0

This shows that most of the cell phosphorus, other than deoxyribose nucleic acid, is in the form either of ribose nucleic acid or free pentose nucleotides. The changes in the amount of organic phosphorus per cell may, therefore, be due to variations in this group of substances, and it is these which are probably converted into deoxyribose nucleic acid at the end of logarithmic growth.

These observations bear some relation to those of Malmgren and Hyden (*Acta Path. Microbiol. Scand.*, 1947, **24**, 448). These authors have studied the ultra-violet absorption of a number of bacterial species during the growth cycle in broth. Their results show that, in *Bact. lactis aerogenes* and other Gram-negative organisms, substances showing the absorption of nucleic acids are built up in the cells during the lag and reach a maximum just before the onset of active growth, after which they decline. Although these experiments were carried out with a different medium, a comparison with those reported here is probably legitimate. There is a difference in that the absorption measurements show a maximum to be reached at the end of the lag, whereas the present work shows certain substances to rise in amount during the lag to a value which is maintained during much of the logarithmic phase. The ultra-violet absorption is not, however, specific for the nucleic acids themselves, but may include contributions from free purines, nucleosides, and nucleotides. The two sets of observations become compatible if part of the increase in absorption which is observed during the lag is attributed to an accumulation of free purines and nucleosides. Towards the end of the lag, conversion of these into nucleotides and nucleic acid takes place, the phosphorus content of the cells increases, and, when this has reached an optimum level, growth begins. At this stage the rate of removal of free purines and pyrimidines becomes equal to their rate of formation and, as the cells divide, their total purine content (and hence the intensity of their absorption in the ultra-violet) decreases.

The Later Stages of Phosphorus Metabolism.—The foregoing discussion has raised the question of the occurrence of metabolic intermediates in the cell. Between the assimilation of inorganic phosphate and the final steps leading to the formation of nucleic acid there exists an intricate series of reactions. The progress of most of these cannot, at the moment, be demonstrated experimentally, but it has proved possible, under certain conditions, to detect the formation and disappearance of substances which may be the immediate precursors of the polymerised nucleic acids.

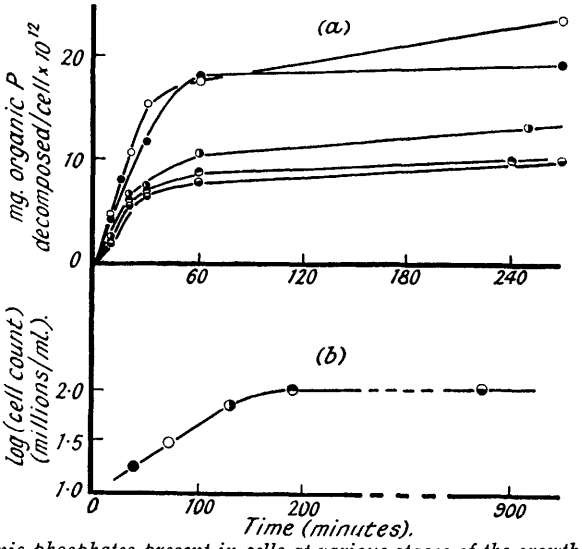
Some differentiation of the various types of organic phosphate present in the cells can be made by examination of the rates of hydrolysis of the ester linkages by N-sulphuric acid at 100°. Under these conditions the organisms are lysed in a few minutes and their contents dispersed into solution where they become exposed to the action of the acid.

Fig. 4 shows, for cultures at various stages of growth in the glucose–veronal medium, the

amounts of inorganic phosphate formed per cell after different periods of heating. The marking of the curves in the upper part of the diagram corresponds to that in the lower part which shows the stage of the growth cycle at which the cultures were sampled. The form of the curves shows

FIG. 4.

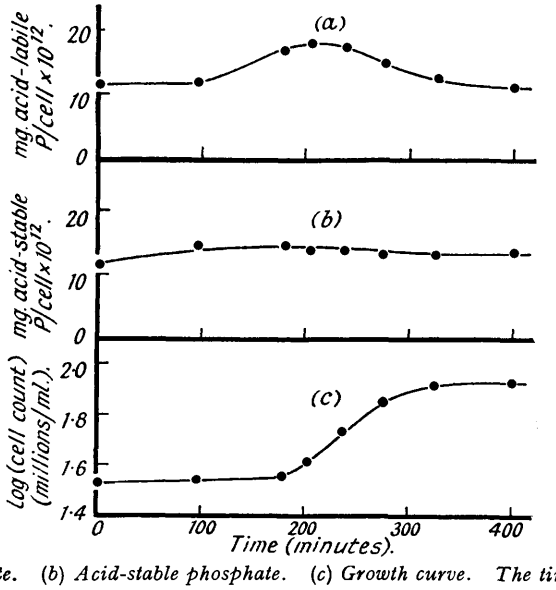
Hydrolysis of organic phosphates in the cell by N-*sulphuric acid at* 100°.



(*a*) *Hydrolysis of organic phosphates present in cells at various stages of the growth cycle.* (*b*) *Composite growth curve in which the marking of the points corresponds to the separate curves in* (*a*).

FIG. 5.

Variation of components of cell phosphorus during growth in glucose–veronal medium.



(*a*) *Acid-labile phosphate.* (*b*) *Acid-stable phosphate.* (*c*) *Growth curve. The time scales are identical.*

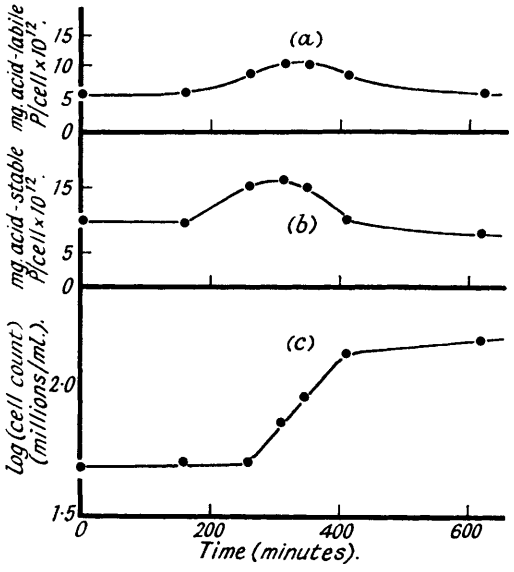
that one part of the organic phosphate in the cells is completely decomposed after 60 minutes. This acid-labile fraction probably consists of ribose-3 phosphate, adenosine-3 phosphate, and guanosine-3 phosphate (see following paper), and it will be seen that a considerably larger quantity of it is present during the logarithmic period than is present in the stationary phase. A

reasonably good estimate of the amounts of phosphorus in the acid-labile and acid-stable fractions, respectively, is obtainable from the proportion of the total hydrolysis which takes place in 60 minutes.

Fig. 5 shows results obtained for growth in a glucose–veronal medium. The relative amounts per cell of these two fractions are plotted against time, the corresponding growth curve being shown for comparison. During the lag, ribose-3 phosphate and purine nucleotides containing a ribose-3 phosphate linkage are built up to a steady level which is attained just before the beginning of active growth. They remain at this higher level during much of the logarithmic period, and show a sharp decline at the approach of the stationary phase. The acid-stable group of phosphates on the other hand remains nearly constant. The rise in the labile group at the end of the lag takes place entirely at the expense of the inorganic phosphate in the medium.

FIG. 6.

Variation of the components of the cell phosphorus during growth in glycerol–veronal medium.



(*a*) *Acid-labile phosphate.* (*b*) *Acid-stable phosphate.* (*c*) *Growth curve. The time scales are identical.*

During the decline at the end of the logarithmic phase, however, little inorganic phosphate is converted into the organic form, and synthesis of the acid-stable group continues for a time largely at the expense of the labile group. This interconversion is reflected in the relative percentages of the total phosphorus corresponding to the two groups in the logarithmic phase and in the stationary phase, respectively. The figures in Table III are typical.

TABLE III.

	Early logarithmic phase.	Late logarithmic phase.	Early stationary phase.	Late stationary phase.
% of total P present as the labile group	56	53	45	47
% of total P present as the stable group	44	47	55	53

These changes are also observed in growth under anaërobic conditions in the glucose–veronal medium. In the medium containing glycerol as carbon source, however, the unstable intermediates which are convertible into other substances under unfavourable conditions of growth do not appear. The sequence of events in this medium is represented in Fig. 6 which shows that both groups of phosphate increase during the lag, reach a maximum value during the logarithmic period, and decline to a comparable extent in the stationary phase. That no large interconversion of the two groups occurs at the end of growth is shown by the values given in Table IV.

TABLE IV.

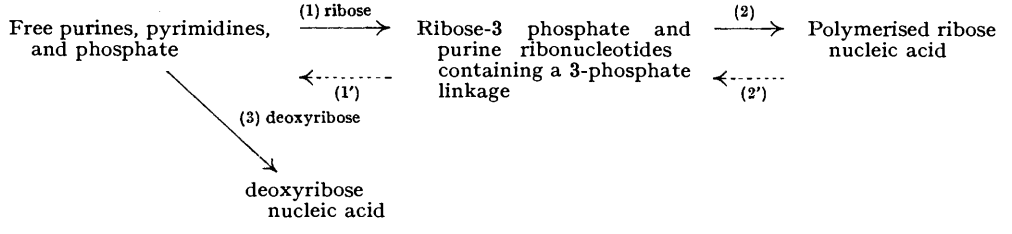
	Early logarithmic phase.	Late logarithmic phase.	Early stationary phase.	Late stationary phase.
% of total P present as the labile group	41	43	43	40
% of total P present as the stable group	59	57	57	60

The fact that labile intermediates cannot be detected during growth in glycerol suggests that in this medium they may be formed with greater difficulty and therefore consumed as rapidly as they are formed.

These experiments show that, in conditions of phosphorus shortage in the glucose–veronal medium, part of that needed for the synthesis of deoxyribose nucleic acid may be derived from free ribose nucleotides. In similar circumstances in glycerol, however, this synthesis must presumably take place at the expense of a breakdown of ribose nucleic acid itself.

The formation of ribose-3 phosphate intermediates during growth in the glucose–veronal medium may to some extent be an accident of the conditions of growth. Veronal, as mentioned earlier, is an inhibitor. Barbiturates are known to inhibit the metabolism of uracil which suggests that they act by blocking the enzymes responsible for the utilisation of this substance. Since these enzymes may well be instrumental in the production of uridylic acid from uracil and ribose-3 phosphate, interference with their normal action may lead to an accumulation of the latter which would not occur under normal conditions. Whether any accumulation takes place in the normal glucose culture medium is as yet uncertain.

The observations which have just been discussed suggest as possible the following scheme for the events leading to the synthesis of nucleic acid.



The dotted arrows indicate the probable route by which deoxyribose nucleic acid is formed at the expense of ribose nucleic acid, since a direct interconversion is highly unlikely. In general, the process (1) will be associated with the transition from the lag to the logarithmic period, processes (2) and (3) with logarithmic growth, and the processes (1') and (2') come into operation to feed (3) at the end of growth.

Utilisation of Inorganic Phosphate by the Cells in Preference to Guanylic Acid (work carried out in collaboration with Dr. C. J. Danby).—Purine nucleotides are probably intermediates in the formation of nucleic acid, and it is of interest to discover whether these substances are assimilated in preference to inorganic phosphate when they are provided in the culture medium. For this purpose two cultures were grown in glucose–veronal media containing enough radioactive inorganic phosphate to cover the requirements of the cells. To one was added also a quantity of inactive guanosine-3 phosphate (guanylic acid) equivalent in phosphorus content to the active inorganic phosphate.

TABLE V.

Culture with guanylic acid.		Control.	
Number of cells (10^6/ml.).	Activity (Counts/mg. cell-N).	Number of cells (10^6/ml.).	Activity (Counts/mg. cell-N).
82	447	64	444
273	433	181	436
435	371	441	395

If the guanylic acid were taken up preferentially, the activity, per unit amount of nitrogen, of cells growing in its presence, should be lower than that of the cells in the control experiment. Table V shows that the activities are the same for both cultures, and thus indicates that none of the cell phosphorus is derived from guanylic acid.

Evidently the bacteria prefer to synthesise nucleotides in their own way or are, perhaps, compelled to do so if for some reason guanylic acid is unable to penetrate the cell wall.

Experimental.

Growth Media.—The media used had the composition: carbon source (glucose 50 g./l.; glycerol, (−)-D-arabinose 10 g./l.), 100 ml.; veronal buffer (sodium diethylbarbiturate 9·3 g./l.; pH 7·25), 100 ml.; sodium and potassium phosphates (180 mg. of phosphate per l.), x ml.; sterile water, $(100 - x)$ ml.; ammonium sulphate (5 g./l.), 50 ml.; magnesium sulphate (1 g./l.), 10 ml. (The relative amounts of phosphate and distilled water depended on the concentration required in the final medium.) The mean generation time in the veronal medium is usually about 60 minutes.

Growth of Cultures.—All the experiments were carried out on cultures growing at 40·0° in sterile cylinders. Losses of liquid by evaporation from aerated cultures were corrected when necessary.

Measurement of Cell Numbers and Cell Nitrogen.—For this purpose a 2-ml. sample of the culture was taken and treated with 1 drop of 40% formalin, the number of cells being determined by hæmocytometer. The amount of cell nitrogen was measured turbidimetrically (*J.*, 1950, 1415).

Measurement of Inorganic Phosphorus.—Two methods were employed, the Fiske–Subbarow method (*J. Biol Chem.*, 1925, **66**, 375) and the Berenblum–Chain method (*Biochem. J.*, 1938, **32**, 295). In the former, a period of 60 minutes was allowed to elapse between the addition of the reagents and determination of the colour intensity, but otherwise the method was used in its original form. A number of modifications were, however, introduced into the latter, which was carried out as follows. The reagents employed were: stock stannous chloride solution, 10 g. of the salt in 25 ml. of concentrated hydrochloric acid; stannous chloride solution for reduction, 0·5 ml. of the stock solution in 5 ml. of N-hydrochloric acid; approximately N-hydrochloric acid; 11N-sulphuric acid; ammonium molybdate solution, 10 g. of the salt in 100 ml. of distilled water; *iso*butanol.

Quantities of the diluted stannous chloride reagent, N-hydrochloric acid, and ammonium molybdate solution were cooled to 0° and saturated with *iso*butanol. A quantity of *iso*butanol was also cooled to 0° and saturated with water. 10 Ml. of the solution under examination were treated with 1 ml. of 11N-sulphuric acid and then cooled to 0°. 2 Ml. of *iso*butanol were added, and the aqueous layer was saturated with this by shaking. 10 Ml. of the saturated layer were placed in a separating funnel, 1 ml. of the ammonium molybdate solution and 5 ml. of the *iso*butanol were added, and the contents of the funnel were shaken for 60 seconds, after which the aqueous layer was run off. The *iso*butanol layer, into which all the phosphomolybdate had passed, was washed twice with the N-hydrochloric acid, and the *iso*butanol layer was then treated with 5 ml. of the reducing solution whereupon the molybdenum-blue colour was formed. A sample of this layer was transferred to the absorptiometer cell and allowed to reach room temperature; the colour intensity was then compared with a standard.

The main differences between this procedure and that suggested by Berenblum and Chain are the following: (1) The reducing solution is made up in hydrochloric acid instead of sulphuric acid, since a solution of stannous chloride in the former was found to keep its activity for a longer period. (2) All the solutions are saturated with *iso*butanol, and the *iso*butanol with water. This eliminates any significant changes in the volume of the *iso*butanol layer during the measurements, and obviates the necessity for dilution to a standard volume before the colour intensity is measured. Furthermore, it eliminates errors due to possible loss of part of the *iso*butanol layer when one of the aqueous layers is removed. (3) By operation at 0° a rise to room temperature is provided for, and this removes water droplets in the *iso*butanol layer which would otherwise interfere with the estimation of the colour intensity. The need for adding ethyl alcohol with its attendant complications is thereby avoided.

Experiments on the Assimilation of Inorganic Phosphate.—At intervals during growth two samples were taken, the first for determination of the mean generation time from the hæmocytometer count, the second for phosphate determination. If the concentration of phosphate lay between 50 mg./l. and 10 mg./l., 25 ml. were taken and treated with 5 ml. of 10% trichloroacetic acid. The cells were removed by centrifugation, and the free inorganic phosphate in 25 ml. of the supernatant liquid was determined by the Fiske–Subbarow method. If the concentration was below 10 mg./l., a 10-ml. sample was taken, treated with 2 ml. of 10% trichloroacetic acid and centrifuged, the phosphate determination being carried out on 10 ml. of the supernatant liquid by the modified Berenblum–Chain method. (At very low concentrations a 25-ml. sample was used for this determination.)

The Effect of Phosphate-ion Concentration on Growth Rate.—The relevant results were obtained mainly from the experiments described above. All the mean generation times were derived from the measurement of cell numbers with a hæmocytometer.

Experiments on the Variations in the Organic-Phosphorus Content of the Cells during the Growth Cycle.—Samples were removed from the culture at intervals, one of 2 ml. for measurement of the cell number, the other of 10 ml. being treated with 1 ml. of sulphuric acid (to stop growth) and centrifuged. 10 Ml. of the supernatant liquid were then analysed by the Berenblum–Chain method. Since, as other experiments (cf. following paper) have shown that nearly all the organic phosphate in a culture is located inside the cells, the amount of inorganic phosphate which has disappeared after a given time may be taken as equivalent to their organic-phosphorus content.

Experiments on the Occurrence of Phosphorylated Metabolic Intermediates in the Cells.—The first series of experiments under this heading involved a determination under standard conditions of the rate of hydrolysis of the phosphate esters contained in the cells. A culture was inoculated, and at the appropriate stage of growth 250 ml. were centrifuged, the cells being then suspended in distilled water to a total volume of 250 ml. 10-Ml. samples were pipetted into glass tubes and treated with 1 ml. of 11N sulphuric acid. The tubes were sealed and heated for varying times at 100° after which the inorganic phosphate was determined in 10 ml. of the sample by the Berenblum–Chain method. The cells disintegrated after about 5 minutes, so that their structure had little effect on the rate of hydrolysis of the esters. In samples which had been heated for less than 10 minutes, however, sufficient cell debris remained to

interfere with the separation of the *iso*butanol layer from the aqueous layer in the phosphate analysis, and it was necessary to centrifuge these before the determination. The counts of the suspensions were also measured.

The second series of experiments was made as follows. Three samples were withdrawn from a culture at intervals. The first of these (2 ml.) was used for the measurement of cell numbers. The other two were both of 10 ml., and 1 ml. of 11N-sulphuric acid was added to each. One was centrifuged immediately and the total organic phosphate in the culture at the time of sampling was determined from the difference between the free inorganic phosphate remaining and that initially present. The second was placed in a sealed tube which was heated for 60 minutes at 100°, after which the inorganic phosphate was determined by the Berenblum–Chain method. Hence it was possible to find the amounts of " acid-labile " and " acid-stable " phosphate in the cells.

Experiments on the Ability of the Cells to use Guanylic Acid as a Source of Phosphorus.—These experiments were carried out with the collaboration of Dr. Danby whose counting equipment was used in measurements of the activities of the cells.

At stages during the growth of the two cultures a 2-ml. sample, for a determination of nitrogen content and a 10-ml. sample, for the measurement of the activity of the cells, were taken simultaneously. The latter was centrifuged and the cells were transferred to an aluminium disc. After they had been dried their activity was measured.

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