614. *The Ribose Nucleic Acid Content and Cell Growth of* Bact. lactis ærogenes.

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The amount of ribose nucleic acid in *Bact. lactis ærogenes* is shown to be approximately proportional to the rate at which the cells have grown. This applies to cultures taken from various nutrient media, with and without inhibitors, and to slow-growing mutants produced by ultra-violet irradiation.

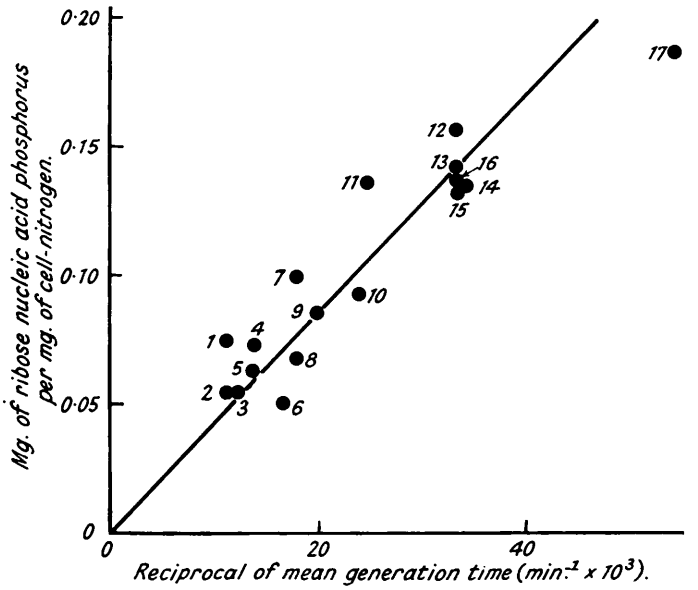
During growth little of the nucleic acid phosphorus is exchanged with the medium, as shown by the use of ^{32}P.

In a previous communication (*J.*, 1950, 1415) observations on the nucleic acid content of cells of *Bact. lactis ærogenes* were described. These showed that the amount of deoxyribose nucleic acid per cell remained approximately constant even if the organisms were grown in a wide range of conditions. The ribose nucleic acid content, on the other hand, showed considerable variations.

There is some evidence, due mainly to the work of Caspersson and his school, indicating that the rate of synthesis of protein in cells is in some way connected with the quantity of ribose nucleic acid which they contain. (See for instance, Caspersson, " The Relations between Nucleic Acid and Protein Synthesis," *Soc. Exp. Biol. Symposium* No. 1, p. 127.) Furthermore Malmgren and Hyden (*Acta Path. Microbiol. Scand.*, 1947, **24**, 448) have studied by ultra-violet

absorption methods the nucleic acid content of various species of bacteria during growth in broth, and consider that their results show a relationship between the nucleic acid content and the rate of growth of the cells at various stages of the growth cycle. In the work to be described in this paper an attempt has been made to correlate the ribose nucleic acid content of cells of *Bact. lactis ærogenes* grown under rather widely differing conditions with the corresponding rate of growth.

FIG. 1.



1. *Phenol* 0·09%. 2. *Phenol* 0·06%. 3. m-*Cresol* 0·08%. 4. *Succinic acid.* 5. *Proflavine* 120 *mg./l.** 6. *Proflavine* 80 *mg./l.** 7. *Decyl alcohol* (12% *of a saturated aqueous solution*). 8. *Veronal* 9·23 *mg./l.* 9. *Proflavine* 40 *mg./l.** 10. *Glucose medium, anærobic conditions.* 11. *Malic acid.* 12, 13, 14, 15. *Normal glucose medium.* (*It will be noted that these values are somewhat higher than that published in a previous paper. The earlier result is now considered to be untypical since the culture upon which the measurement was made was stored for a time at* 0° *in circumstances which would allow it to grow. Since growth at this temperature is very much slower than at* 40° *some alteration of the ribose nucleic acid content would probably have taken place.*) 16. *Colchicine* 0·1%. 17. *Broth.*

* These values were obtained for cells trained to these concentrations. The result reported previously applied to untrained cells undergoing their first subculture in the presence of proflavine.

The study falls into three parts. In the first, cells of a normal strain of *Bact. lactis ærogenes* have been examined after growth in different media, or in the presence of drugs. In the second, slow growing "mutants," produced by irradiation of the cells with ultra-violet light are investigated. These mutants show a wide range of mean generation times in the normal glucose medium and it is of interest to look for some connection between the rate of growth and the ribose nucleic acid content, since this might yield information about the nature of the damage which the radiation has inflected on the cells. Finally, the rate of exchange of the organic phosphorus under different conditions has been investigated in the hope that experiments of this type might provide a clue to the mechanisms involved in the functioning of nucleic acids.

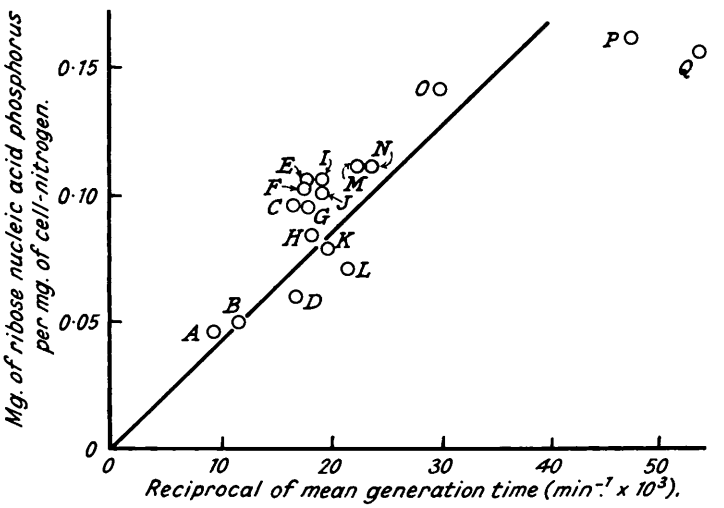
For the nucleic acid estimations cells were usually grown in a medium containing phosphate buffer, glucose, ammonium sulphate, and magnesium sulphate, both ærobic and anærobic conditions being employed. In certain experiments various toxic agents such as *m*-cresol (0·8%), proflavine (40, 80, and 120 mg./l.), phenol (0·6 and 0·9%), decyl alcohol (12% of a saturated aqueous solution), and veronal (9·3 mg./l.) were added. In others, different carbon sources, such as malic acid and succinic acid, were substituted for glucose. In one experiment cells which had been grown in broth were studied.

The ultra-violet mutants were usually examined after growth in the normal glucose culture medium under ærobic conditions. On two occasions they were investigated after growth in broth.

Ribose Nucleic Acid and Growth Rate.—The results of the experiments on growth rate and

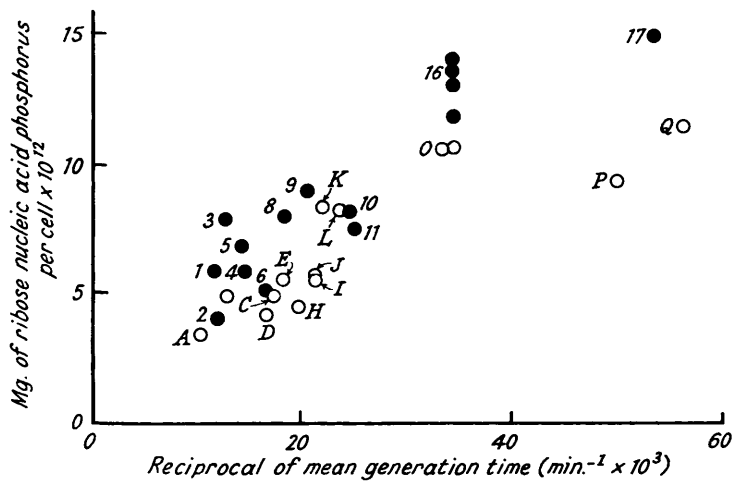
nucleic acid content are summarised in Figs. 1, 2, and 3. Fig. 1 shows the ribose nucleic acid of normal cells (expressed in terms of cell *mass*), from a variety of media, plotted against the reciprocal of the mean generation time (*i.e.*, a quantity proportional to the rate of growth). A roughly linear relation exists between these two quantities.

FIG. 2



A, B, C, D, E, F, G, H, I, J, K, L, M, N. *Different ultra-violet mutants in normal glucose medium.* O. *Mutant* A, *after a period of training in glucose during which it had regained a mean generation time of* 30 *mins.* P. *Mutant* F *in broth.* Q. *Re-trained mutant* O *in broth.*

FIG. 3



The numbers and letters are as in Figs. 1 *and* 2.

A similar plot for the ultra-violet mutants is given in Fig. 2 which shows that a similar relation holds. The values obtained with various mutants, each growing at a different rate, are compared with those obtained for normal cells growing in the normal glucose medium.

In Fig. 3 the ribose nucleic acid *per cell* is plotted against the reciprocal of the mean generation time. Here the values for normal cells and mutants are given on the one diagram. There is not much to choose in respect of linearity between Fig. 3 and Figs. 1 and 2.

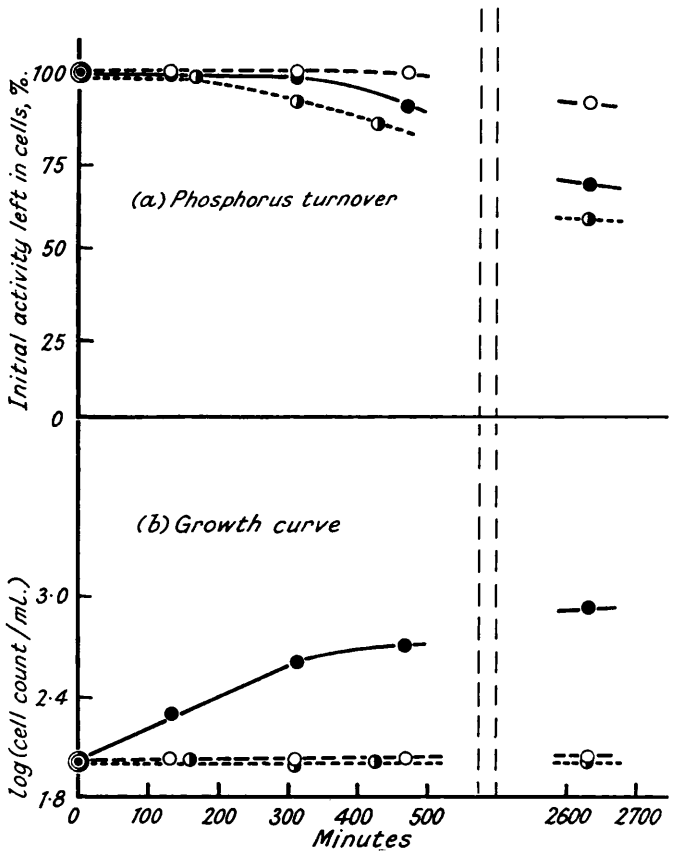
The general conclusion to be drawn from these experiments is that the ribose nucleic acid content of the cells is approximately proportional to the rate at which they have actually been growing in the culture from which they are taken. It would appear, therefore, to reflect the

circumstances prevailing in the culture upon which the measurement was made rather than to give any indication of future capabilities of the cells.

This results suggests, however, a close relation between the rate at which the cells can grow and the rate of synthesis of ribose nucleic acid. An attractive explanation for the slow growth of the ultra-violet mutants might have been that the action of the radiation resulted in the destruction of some of the ribose nucleic acid of the cell, and that they were therefore incapable of growing at the normal rate. If this were so, the slow growth observed in the normal glucose medium should persist when the mutant is cultured in broth, the ribose nucleic acid level being unaltered. Two values obtained with mutants growing in broth are shown in Fig. 2, from which it is seen that they grow at the normal rate, and that their ribose nucleic acid content is

FIG. 4.

Phosphorus exchange.



○ *Resting cells.* ● *Cells in full growth medium.* ◑ *Cells fermenting glucose.*

very much higher than in the synthetic medium. It seems likely, therefore, that the radiation has damaged some other component of the cell, and that the ribose nucleic acid content assumes a value characteristic of the rate of growth of which the injured cell is capable.

These experiments have shown a hyperbolic relationship between mean generation time and ribose nucleic acid content. Malmgren and Hyden (*loc. cit.*) found a relationship of this form between the nucleic acid newly formed per cell and the time elapsing between two divisions, the measurements being made at different stages of the growth cycle in a single medium (broth). Their measurements, while indicating the type of relationship which might be expected, did not indicate the extent to which the two different types of nucleic acid contribute towards it.

If the results given in this paper are combined with those of the previous paper and referred to in the introduction, the following general conclusions can be drawn :

1. The deoxyribose nucleic acid forms an approximately constant component of the cell and probably causes the onset of division.

2. The ribose nucleic acid content of the cells is approximately proportional to the rate at which they are growing and is probably closely concerned in autosynthesis.

Exhange of Organic Phosphorus during Growth (Experiments made in Collaboration with Dr. C. J. Danby).—An example typical of the results obtained in exchange experiments with ^{32}P is shown in Fig. 4. During the phase of logarithmic growth, when protein synthesis is at its height, not more than 10% of the organic phosphorus which was originally present is exchanged. These experiments suggest, therefore, that while the nucleic acid is actually participating in the synthesis of protein, little of its phosphorus undergoes exchange, which means that the molecules probably remain polymerised during this process.

During the stationary phase, however, a considerable loss of the active phosphorus takes place from the cells. A similar result is found for cells which are fermenting glucose in the absence of a nitrogen source, a large decline in activity taking place but only after the pH has fallen below 5·5. This loss probably arises from the hydrolysis of nucleic acid rather than from a turnover of its phosphorus. In the control experiment in the veronal–saline buffer containing no phosphate, where a decline in the activity of the cells can only take place as a result of a direct loss of phosphorus, exchange being impossible, very little alteration is observed, even after 48 hours.

Experimental.

The methods used for estimating cell number and cell mass, ribose nucleic acid, and the general procedure for the growth of the cultures were described in the previous paper (*J.*, 1950, 1415).

Measurement of Mean Generation Time.—During the growth of the culture, 2-ml. samples were removed at intervals and treated with formaldehyde. The organic nitrogen content of each was measured turbidimetrically and the mean generation time was calculated from these determinations. In most of the examples studied, the mean generation time was measured with the actual culture upon which the determination of ribose nucleic acid was carried out. In certain instances, however, the two determinations were made with separate cultures.

Preparation of the Ultra-violet Mutants.—These were prepared by Mr. A. Dean, who obtained them by irradiation of a suspension of normal cells in saline with ultra-violet light. The mutants were selected after irradiation (2540—2800 A.) by the normal plating technique (cf. Lederberg and Tatum, *J. Biol. Chem.*, 1946, **165**, 381).

Exchange Experiments with ^{32}P.—The cells used in these experiments were taken from cultures grown in glucose–veronal media which contained radioactive phosphorus. These media were so constituted that exhaustion of the phosphorus was the factor limiting growth. This precaution was observed to ensure that most of the cell phosphorus would be in the form of polymerised nucleic acid rather than in the form of phosphorylated nucleic acid intermediates.

These radioactive cells were then suspended in three different media: a standard glucose–veronal medium containing inactive phosphate; a glucose–veronal medium lacking nitrogen but containing inactive phosphate; a veronal–saline buffer containing no phosphorus. The quantities of inactive phosphorus present in the first two media were about 100 times greater than the active phosphorus in the cells. Complete equilibration of the cell phosphorus with that in the medium would reduce the activity in the cells to 1% of its original value.

At intervals after inoculation, two samples were withdrawn from each culture. The first (2 ml.) was treated with formaldehyde and later used for estimations of cell count. The second (20 ml.) was centrifuged and the cell mass was transferred to an aluminium dish and dried. The activities of the samples were then measured. (In certain experiments where the number of cells in the sample was small, a quantity of a culture which had been grown in inactive phosphate was added as a carrier before the centrifuging.) pH determinations were occasionally made on a third sample of the culture.

The percentage of the original radioactive phosphorus which remains in the cells at different times is given by the expression:

$$\% = \frac{100 \times \text{Activity present in the cells in 20 ml. of culture at the time of sampling}}{\text{Activity present in the cells in 20 ml. of culture at the beginning of the experiment}}$$

This expression is independent of the actual number of cells present at the time of sampling and gives an exact estimate of the phosphorus turnover.

Since about 80% of the cell phosphorus has been shown to be nucleic acid, any large decrease in the percentage of active phosphorus should reflect an exchange or loss of nucleic acid phosphorus.

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