290. *The Nucleic Acid Content of* Bact. lactis aerogenes.

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The deoxyribose nucleic acid content of individual cells of *Bact. lactis aerogenes* grown under a wide variety of conditions is found to be constant, as has been previously shown for certain nucleated cells. The ribose nucleic acid content and the cell size show marked variations.

The results suggest that the deoxyribose nucleic acid content is a major factor determining cell division in bacteria.

Measurements made with the filaments induced respectively by *m*-cresol and 2 : 8-diamino-acridine (proflavine) indicate that these substances disturb the cell morphology by different mechanisms.

The importance of the nucleic acids in cell processes is now generally conceded and has been accentuated by the recent observation (Boivin, Vendrely, and Vendrely, *Compt. rend.*, **1948, 226**, 1061; Mirsky and Ris, *Nature*, **1949, 163**, 666) that, for a given species of vertebrate, the nuclei of the somatic cells contain fixed amounts of deoxyribose nucleic acid.

For some time past, a systematic study has been made in this laboratory of the growth and metabolism of the coliform organism *Bacterium lactis aerogenes* and, as part of a wider investigation of the phosphorus metabolism, we have determined the ribose and deoxyribose nucleic acid content of cells grown under a wide range of conditions. As conditions vary, the ratio, bacterial mass : number of cells, which is a measure of the average size of the individual cells, shows considerable fluctuation. Consequently, one of our primary objects has been to find out whether, despite the alteration in cell size, the deoxyribose nucleic acid content of each cell shows anything like constancy. Information of this character should throw light on the function of nuclear material in bacteria. In particular, it might be relevant to the problem of adaptive changes, since, according to one hypothesis, cell division is attendant upon the attainment of a standard amount of certain key substances. The question arises whether these could be nucleic acids.

Cell Number and Cell Mass.—The number of cells in a culture was determined by counting in a hæmocytometer under the microscope. An estimate of the cell mass was obtained by measurement of the turbidity by means of a Spekker photoelectric absorptiometer. The turbidity has been shown by Monod ("La Croissance des Cultures Bactériennes," Paris, 1942) to be proportional to the dry weight of the cells contained in a given volume of culture. His conclusion is in general agreement with findings in this laboratory, that the turbidity is proportional to the organic-nitrogen content of the culture and that the ratio, turbidity : nitrogen content, is usually independent of the carbon source used by the cells. The absorptiometer readings are therefore converted into nitrogen contents by a calibration curve, and cell size has been expressed in terms of these quantities.

Estimation of Deoxyribose Nucleic Acid.—The deoxyribose nucleic acid was estimated by the Dische diphenylamine method (*Mikrochemie*, 1930, **8**, 4). In its original form this method is not entirely specific and certain modifications were therefore introduced. Fig 1 *A* shows how the light absorption of thymus nucleic acid (measured by the scale reading on a Spekker absorptiometer) varies when the solution is heated with the reagent at 100°. A constant intensity is reached after 10 minutes.

Fig. 1 *B* shows the colour development in a washed suspension of bacteria subjected to an identical treatment, the cell debris being removed by centrifugation before the absorption is measured. Here a rapid initial rise in the colour intensity is followed by a phase of gradual development. The initial steep rise must be due to deoxyribose nucleic acid, and the subsequent slow development to other carbohydrate components. The deoxyribose nucleic acid content of the cells can be determined by extrapolation as shown, the intercept giving the intensity due to the nucleic acid itself. The amounts are expressed in terms of nucleic-acid-phosphorus by comparison with standard solutions of thymus nucleic acid.

Estimation of Ribose Nucleic Acid.—The ribose nucleic acid was estimated by a modification of the Bial pentose reaction (*Deutsche med. Woch.*, 1902, **28**, 253). The pentose in a solution was determined by treating 4 ml. of the solution with 4 ml. of 0·1% ferric chloride solution in 10N-hydrochloric acid and 2 ml. of 30% orcinol in absolute alcohol, 2-ml. samples of the resultant solution being heated in sealed tubes at 100°. Fig. 2 *A* shows the colour development, after various periods of heating, for a solution of yeast nucleic acid. (Blanks from samples containing no pentose have been subtracted from the values shown in the figure.) Maximum colour development occurs under these conditions after 25 minutes and a calibration curve can be constructed by heating standard samples of yeast nucleic acid with the reagents for 30 minutes. This calibration is expressed in terms of the phosphorus content of the nucleic acid.

Unfortunately estimations cannot be carried out directly on washed bacterial suspensions since the cells contain substances (probably proteins) which react with the reagent to give a brown colour. They can be largely eliminated by extraction of the nucleic acids with 7·5% trichloroacetic acid at 100° for 20 minutes. The colour development with a trichloroacetic acid extract is shown in Fig. 2 *B*. It is similar to that in the diphenylamine test. The initial rise is due to the purine ribonucleotides and the colour is a vivid green (pyrimidine ribonucleotides do not react with the reagent). The slow subsequent rise is due to interfering substances, and during this phase the samples become brownish-

yellow. The contribution to the colour made by the pentose is found by extrapolation to zero time (allowance being made for a 4-minute time-lag in the heating, indicated by the broken line in Fig. 2).

Estimation of Total Phosphorus.—Estimations of the total phosphorus were made to check the nucleic acid estimations. A given quantity of cell suspension was heated with sulphuric acid, potassium sulphate, and a trace of copper sulphate. After oxidation of all the organic material, the mixture was diluted to a known volume with distilled water and the pH adjusted to about 0. The orthophosphoric acid formed was estimated by a modification of the Berenblum–Chain method (*Biochem. J.*, 1938, **32**, 295). Since these estimations showed that 80—90% of cell-phosphorus could be accounted for as nucleic acid, the methods described in the preceding sections can be regarded as reliable.

General Procedure.—About 270 ml. of culture were grown to a standard bacterial count in a sterilised cylindrical vessel at 40°. 250 Ml. were centrifuged and washed 3 times with isotonic saline solution,

FIG. 1.

Colour development in Dische diphenylamine test: A, thymus nucleic acid; B, cell suspension.

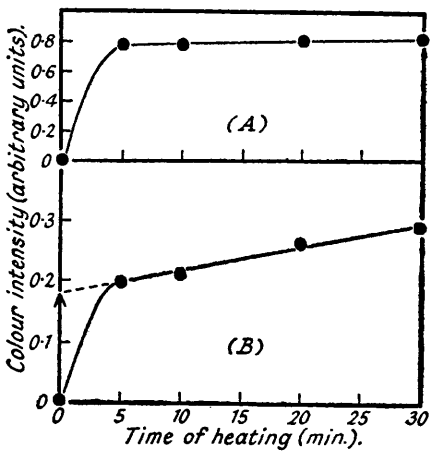
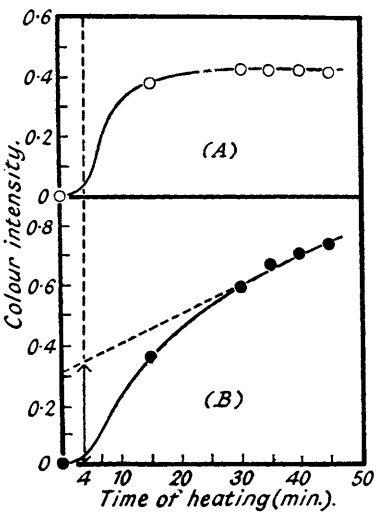


FIG. 2.

Colour development in the Bial pentose test: A, yeast nucleic acid; B, cell extract.



a pH measurement being carried out on the first batch of supernatant liquid. The cells were then made up to a final volume of 10 ml. with distilled water. 1 Ml. of cell suspension was diluted to 25 ml. for estimation of the cell count and the turbidity (the ratio, turbidity : hæmocytometer count, of the cells remained unaffected by the centrifugation). Further quantities of the suspension were then used for estimation of the deoxyribose, ribose, and total phosphorus.

Cultures Studied.—Cells were grown in a medium containing phosphate buffer, glucose, ammonium sulphate, and magnesium sulphate, aerobically and anaerobically. In some experiments veronal was added to the buffer and in others the glucose was replaced by malic or succinic acid as examples of alternative carbon sources. *m*-Cresol (0·8%), proflavine (2 : 8-diaminoacridine) (45 mg./l.), and colchicine (0·1%) were also added in some cases to the normal glucose medium, the first two because they are known, with this organism, to induce the formation of long filaments, the third because it is known to act in many cells as a mitotic poison.

DISCUSSION.

In Fig. 3 the deoxyribose nucleic acid content per cell is plotted against average cell size, expressed in terms of the amount of nitrogen per cell, for cultures grown under a variety of conditions. Fig. 4 shows a similar plot for the ribose nucleic acid content.

Fig. 3 shows that the deoxyribose nucleic acid content of *Bact. lactis aerogenes* is nearly constant despite pronounced variations in cell size. The values obtained represent an average, since the cultures examined contained cells at all stages of growth. Nevertheless, it appears that the deoxyribose nucleic acid constitutes a component which must be present in a fixed amount. It is not unreasonable, therefore, to suppose that the quantity present in an individual cell determines division.

On the other hand, the ribose nucleic acid content per cell shows marked variations (Fig. 4). Since the factors determining the actual amount of this acid are not yet clear, detailed discussion is postponed until further investigations have been completed. The present observations are, however, recorded to show the marked contrast between the two types of nucleic acid.

FIG. 3.

Deoxyribose nucleic acid content of cells grown under different conditions.

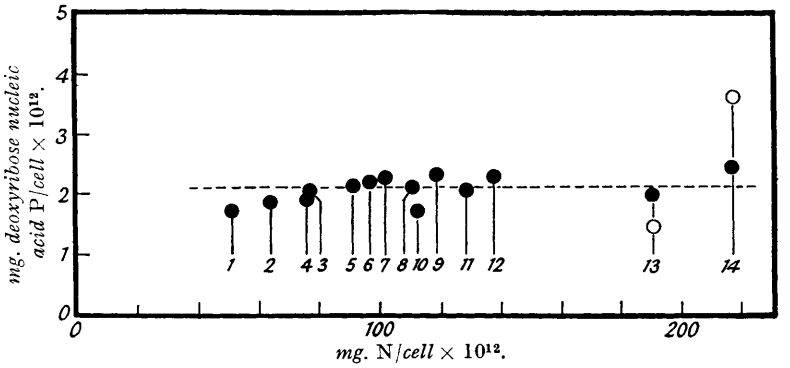
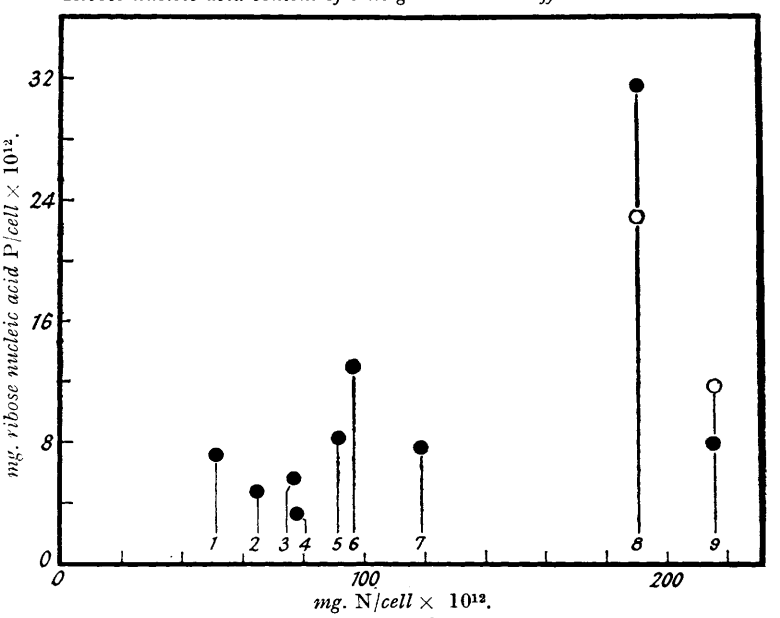


FIG. 4.

Ribose nucleic acid content of cells grown under different conditions.



KEY FOR FIGS. 3 AND 4.

Fig. 3.	Fig. 4.		Final pH.
1.	1.	*Malic acid (aerobic ; logarithmic growth)*	7·7
2.	2.	*Succinic acid (aerobic ; logarithmic growth)*	7·8
3.	3.	*Glucose (aerobic ; logarithmic growth)*	6·1
4.	4.	*Mutant, induced by the irradiation of normal cells with ultra-violet light, in glucose, in which it grows very slowly (aerobic ; logarithmic growth)*	6·4
5.	5.	*Glucose (anaerobic ; stationary phase)*	4·7
6.	6.	*Glucose* + 0·1% *of colchicine (aerobic ; logarithmic growth)*	6·4
7.		*Glucose + veronal buffer ; phosphorus-deficient (aerobic ; stationary phase)*	4·4
8.		*Glucose (aerobic ; stationary phase)*	5·1
9.	7.	*Glucose + veronal buffer (aerobic ; logarithmic growth)*	5·7
10.		*Glucose + veronal buffer ; phosphorus-deficient (aerobic ; stationary phase)*	4·4
11.		*Glucose (anaerobic ; stationary phase)*	4·7
12.		*Glucose (anaerobic ; stationary phase)*	5·0
13.	8.	*Glucose + proflavine* (45 *mg./l.*) *(aerobic ; logarithmic growth). The shaded circle shows the deoxyribose nucleic acid per cell, each filament being regarded as a single cell. The open circle shows the content per cell, each filament being regarded as the equivalent number of cells of normal size*	5·4
14.	9.	*Glucose* + 0·8% *of* m-*cresol (aerobic ; logarithmic growth). The open circle shows the deoxyribose nucleic acid per cell, each filament being regarded as a single cell. The shaded circle shows the content per cell, each filament being regarded as the equivalent number of cells of normal size*	6·0

The effect of colchicine on the deoxyribose content will next be considered. Colchicine acts on nucleated cells as a mitotic poison, inducing polyploid forms. It has no obvious toxic effects on *Bact. lactis aerogenes* as judged from lag and growth rate, but, since this organism is now shown to resemble the nucleated cells in respect of its deoxyribose nucleic acid content, it seemed possible that an effect of colchicine might be revealed by changes in this quantity. In fact no such change is produced, though an increase in the ribose nucleic acid content occurs.

m-Cresol is a much less specific mitotic poison than colchicine. In 0·8% concentration in growing bacterial cultures it causes the formation of large numbers of long filamentous cells. If the deoxyribose content of these cells is expressed as the amount per cell, the value obtained is considerably higher than normal. If, however, each long filament is counted in the calculation as the equivalent number of cells of normal length, the deoxyribose content has the usual value. This suggests that *m*-cresol inhibits the cell-division process at a stage which lies between the completion of the deoxyribose structure and the actual fission. A *m*-cresol-induced filament might, in a sense, be called a polyploid bacterial cell. This character, however, is not permanent, and usually disappears when the cells are grown in the absence of *m*-cresol.

Proflavine also gives rises to filamentous cells. These appear when unadapted cells undergo their first sub-culture at a proflavine concentration just below that required for bacteriostasis. Large numbers of cells of about twice the normal size appear, together with a few filaments of greater length. With a culture of this type the result is complementary to that obtained with *m*-cresol. If the filaments are counted as single cells, the deoxyribose content is normal. If, on the other hand, they are counted as the equivalent larger number of single cells, the deoxyribose content is below the constant level. This suggests that, unlike *m*-cresol, proflavine produces filaments by interfering with the duplication of the deoxyribose structure. It is worth noting that rapid adaptation to proflavine takes place and the filaments disappear. No adaptation, however, is observed with *m*-cresol.

The work described in this paper, while in no way indicating the existence of a discrete nucleus in bacteria, has shown that the deoxyribose nucleic acid, which is a constant component of various other cells, can in fact be a constant component of the bacterial cell. Furthermore, there is a strong suggestion that the function of this component is in certain respects identical with that in other cells.

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