Technical Papers

Effects of Cell Division Inhibition on Phosphorus Metabolism of *Escherichia coli*

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Apropos a recent article by Webb (*1*) concerning the phosphorus and nitrogen metabolism of filamentous forms of bacteria, which were induced by controlled magnesium deficiencies, we are reporting the results (*2*) obtained from a series of experiments initiated a few years ago to elucidate the mechanisms of cell division. An effort was made to correlate cell division inhibition in *Escherichia coli* with possible changes in the phosphorus compounds of the bacterium.

Filamentous forms of *E. coli* can be induced, under conditions that inhibit cell division but not growth, by treatment with α-, x-, and γ-radiations as well as by treatment with certain organic compounds (*3–5*).

Cultures of *E. coli* strain B were grown as previously described (*4, 5*). Cells were treated during the logarithmic stages of growth and division with an appropriate dose of a cell division-inhibiting agent and growth was continued for 1½ hr during which time the control cells maintained logarithmic rates of division and growth and the treated cells maintained only a logarithmic rate of growth (*5*). The treated, filamentous cells were 3 to 4 times the length of the control cells. Such treated cultures are strikingly uniform in appearance in that less than 5 percent of the cells are as small as the controls (*6*). At the end of the growth period cells were harvested and subjected to chemical analysis.

Samples weighing approximately 600 mg were fractionated by the methods of Morse and Carter (*7*). and Schmidt and Thannhauser (*8*). All phosphorus in the desoxyribosenucleic acid (DNA) fraction could be accounted for as DNA phosphorus by comparisons with a purified DNA standard of the 260 mμ absorption E_p (*9*) as well as by the colorimetric methods of Dische (*10*) and Stumpf (*11*). Similarly, all the phosphorus of the ribosenucleic acid (RNA) fraction could be attributed to RNA phosphorus by the 260 mμ absorption E_p and by its ribose content by colorimetric analyses (*12*) and comparisons with ribose and purified RNA.

Table 1 summarizes the results obtained when 5-diazouracil is used as the cell division inhibitor. With the exceptions noted for α-radiation and acriflavine, the results obtained for 5-diazouracil, x-radiation, and triethylenemelamine followed the same general pattern (*2*). With α-radiation a small but statistically significant decrease was observed in the stable organic phosphorus fraction (10 to 20 percent). However, this may be a radiation effect peculiar to the α-radiation (*15*) and may be in no way a result of cell division inhibition. Such an effect may be related to the adenosine triphosphate leakage found by Billen *et al.* (*16*) when *E. coli* was subjected to lethal doses of x-rays. Treatment with acriflavine caused a statistically significant decrease in the DNA phosphorus fraction. However, the 95 percent confidence interval indicates rather substantial experimental variation which makes the data for this fraction suspect.

Apart from the aforementioned effects, all other phosphorus fractions of the filamentous *E. coli* showed no change in intracellular phosphorus when compared with the normal cells. These results are similar to those reported by Webb (*1*) for the filamentous bacteria formed in a magnesium deficient medium in that no changes in intracellular phosphorus were found in their experiments.

The fact that for any fraction the values for the controls and experimentals are identical (total culture masses for controls and experimentals are also equal) within experimental error might indicate, as a working hypothesis, that the filamentous forms are made up of the same number of "units" as are found in the controls. Microscopic observations of stained cells have shown that the filamentous cells are multinucleated; however septa have never been observed in filamentous cells (*17*).

It is interesting to note that a specific biological function, namely cell division, may be inhibited by a variety of techniques such as subjecting them to a deficiency of substances required for growth (Mg^{++}),

Table 1. Effect of 5-diazouracil (concentration 0.3 μg/ml) on phosphorus fractions in *E. coli*.

Fractions*	Control†	Experimental†	Deviation	95% Confidence interval of the true mean‡
Ortho-P	0.158	0.164	−0.006	−0.013 → 0.001
Labile-P	.033	.033	.000	− .003 → .003
Stable-P	.157	.153	.004	− .009 → .017
Lipid-P	.129	.144	− .014	− .032 → .004
RNA-P	1.30	1.35	− .05	− .013 → .03
DNA-P	0.307	0.269	.038	− .007 → .083

* Phosphorus was determined by a colorimetric method (*13*); ortho-P by direct estimation of the acid extract; labile-P = [P (10 min at 100°C in 1*N* HCl)] − [ortha-P]; stable-P = [total phosphorus of acid extract] − [P (10 min)]; other fractions as (total − P).

† In mg of phosphorus per 100 mg of dry weight.

‡ The results of five experiments were statistically analyzed (*14*) by computing the experimental error from the variation in the differences between pairs of runs made on the same day, and the 95 percent confidence interval was then computed for the true mean difference between controls and experimentals.

to radiation, or to certain organic compounds. In all probability there is more than one mechanism involved when the inhibition is produced by these several means, even though the end result is the same, that is, cell division is inhibited and, where phosphorus analyses have been made, no changes in phosphorus metabolism have been noted. Such reasoning, however, does not preclude the possibility that *ultimately* a single sensitive locus might be affected in order to form filamentous cells.

It is clear from the data available here and in the literature (*1*) that interference with the synthesis of DNA or of RNA is not the causative mechanism responsible for filament formation. It is pertinent to make this point clear because the results of earlier investigators leave the impression that nucleic acid inhibition is involved in cell division inhibition. This seems true only when growth and division are inhibited and does not hold under conditions where only division inhibition occurs.

References and Notes

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Humidity Responsive Organics

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The electric resistance of strips cut from thin sheets of gelatin, agar, pectin, polyvinyl alcohol, and so forth, depends on the relative humidity of the surrounding atmosphere in the way shown in Fig. 1.

Strips used in obtaining data were made by cutting rectangles 2 by 0.5 cm from sheets 0.075 mm thick. Each end of a rectangle was slid between adjacent turns of a spiral of thin tinned copper wire that served as an electrode. To improve electric contact and hold the wires mechanically to the strip at each end, the wire-to-strip junction was painted on both sides with conducting paint (*1*). The sensitive region of the assembly was then 1 by 0.5 cm on each of its two sides. The charge flowed in the long direction.

Sheets were made by mixing a quantity of solid material with water, heating it in a double boiler until the liquid was clear, pouring it on a level paraffin-coated glass plate, and allowing it to dry at room temperature. All resistances were measured at 27°C by charging a condenser through the prepared strip, using a potential difference of 255 v, and measuring the time required to charge the condenser to 180 v (*2*). The circuit was calibrated with Victoreen resistors (*3*) and frequently tested to prove that leakage resistance across the supports was negligible. All materials tested have negative temperature coefficients of resistance. The atmospheres at various values of relative humidity were obtained by using saturated solutions of inorganic salts (*4*).

The graph of a unit made from a half-and-half mixture (by dry weight) of different materials is about halfway between those of the two components, as is shown in Fig. 1 for gelatin and pectin. Figure 1 also shows that a relatively small quantity of glycerol added to gelatin tends to move its graph to the left.

If a unit that has reached equilibrium in a chamber having a certain constant humidity is suddenly changed to one having a different humidity, a new state of equilibrium will be attained in about the same time that would be required for a hair hygrometer.

In view of the chemical complexity of most of the materials and the difficulty of making units having the same dimensions, reproducibility from unit to unit is not great.

Units made from various materials have been used successfully for the control of humidity of chambers

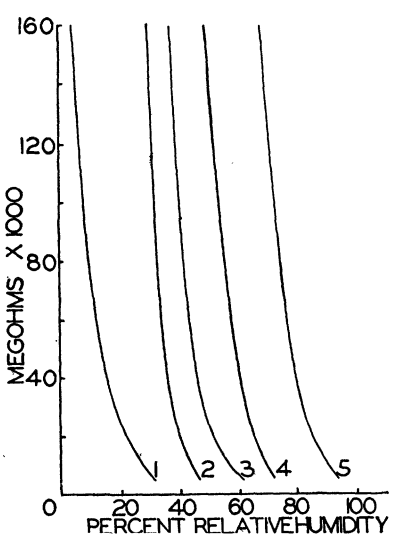

Fig. 1. Electric resistance under equilibrium conditions *vs.* relative humidity for strips of: *1*, gelatin and glycerol; *2*, pectin; *3*, agar; *4*, pectin and gelatin; and *5*, gelatin.