Gravity, Radiation and Growth

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All living things have evolved and continue to evolve in the presence of the forces of gravity and the electromagnetic spectrum. In a previous communication the authors have emphasized the effects of increased gravity on the growth and ultrastructural characteristics of a phage free strain of *Escherichia coli* B.[3, 4] When these cells were maintained in an ultracentrifuge at 110,000 X G for 24 hours their growth characteristics showed the following deviations from the controls: (a) an increased log phase, (b) a prolonged generation time, (c) a decreased maximal concentration. At the end of the 24 hour period of centrifugation these cells were studied by means of electron microscopy. These observations indicated that the centrifuged cells were larger than the control cells and that this enlargement was due to an enlargement of the entire cell and an enlargement of its intracellular organelles. These changes were considered to explain the reduction in the growth characteristics on the basis that gravity had disassociated cell growth from cell division. This disassociation produced giant cells by suppressing cell division more than cell growth. A similar biologic phenomenon has been reported by one of us in tissue cultures of Chang liver cells exposed to low doses of X-radiation.[2]

In view of these observations it seemed of interest to determine whether or not similar growth and ultrastructural changes could be induced in the same phage free strain of *E. coli* by continuously exposing them to X-radiation. Accordingly, the following experiments were performed.

METHODS

Cultures of this phage free strain of *E. coli* were grown overnight in nutrient broth plus 5 gms. of NaCl/L and used as inoculum. Duplicate sets of cultures in the same medium were prepared containing approximately 5 x 10^3 cells per milliliter. One set was continuously exposed at room temperature to a radiation source containing 15 curies of cobalt 60, from which they received a dose of 55.8 roentgens per hour. The control set was shielded and placed adjacent to the irradiation facility where they were maintained at the same temperature for the same time intervals as the tests. A test and a control culture were each removed at the end of 1, 2, 3, 4, 6, 7½, 12½, 24, 27 and 30 hours and assayed for viable count. The results were then plotted on a graph.

For studies with the electron microscope the irradiated *E. coli* cultures were placed in the cobalt 60 bomb for periods of 16, 20, 24, 26, 30, 55 and 72 hours. The controls were shielded and maintained at room temperature as before. At the end of each time, a test and a control culture were centrifuged and rinsed with water. They were then fixed in osmium tetroxide according to Palade's technique, dehydrated with alcohols and embedded in methacrylate (4:1). Ultra-thin sections were obtained with a Porter Blum ultramicrotome and the specimens were examined and photographed with the Hitachi HU-11A electron microscope.

A total of six experiments of each of these types was performed.

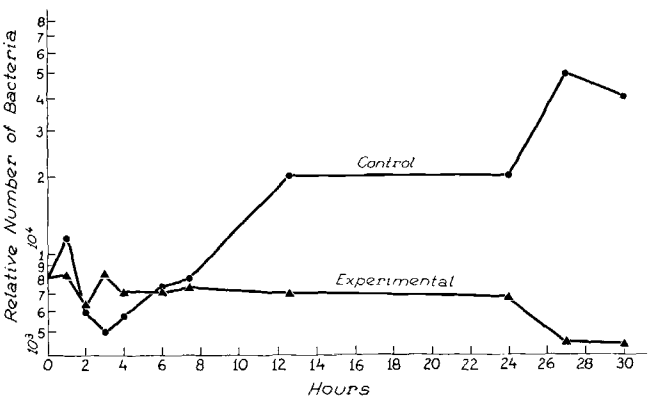

Fig. 1. Growth curves of normal and continuously X-irradiated E. coli B from zero to 30 hours.

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RESULTS

The results of the growth studies are given in the graph in Figure 1. A study of this graph demonstrates that the irradiated bacteria show a marked depression of their growth characteristics when compared to the controls. Since the radiation source was in an unheated room its temperature reflected the variations in the outside temperature. It may be observed that neither the controls nor the irradiated cells show evidence of growth from the 12th to the 24th hour. At one point in this period the temperature reached a low of 12°C.

Figure 2 is an electron micrograph of a typical control *E. coli* cell. This electron micrograph illustrates the ultrastructural features of all the control *E. coli* cells. Care was taken to insure that significant variations from these features did not occur as a result of normal variations with respect to the time of sampling during the various phases of the cell's growth. In this electron micrograph one can see the fine small electron dense granules within the cell which are considered to be ribosomes, the centrally located fine fibrillar threads which are considered to be DNA [1] and associated with these are occasional small electron dense masses of an as yet undetermined nature. Figure 3 is an electron micrograph at the same magnification of an *E. coli* cell after 30 hours of exposure to the cobalt 60 source. This cell received a total of 1,674 R at the rate of 55.8 R per hour. This cell is quite evidently larger than the typical control cell. This increase in size is a generalized one and includes an enlargement of the intracellular components. Thus the cell wall encloses a larger volume of material. The small electron dense granules considered to be ribosomes appear to be enlarged. The centrally located fine fibrillar threads considered to be DNA are coarser and enlarged as compared with those within the control cell. The centrally located round or oval electron dense bodies associated with these threads are enlarged and they now form an outstanding feature of the ultrastructural characteristics of these continuously irradiated cells. The intimate association of these latter bodies with the fibrillar material considered to be DNA and their obvious enlargement in circumstances accompanying a growth disturbance has led the authors to designate these bodies "nucleoloids" by analogy with irradiated mammalian cells.[2] Cells identical to these began to appear in the irradiated cell cultures at the end

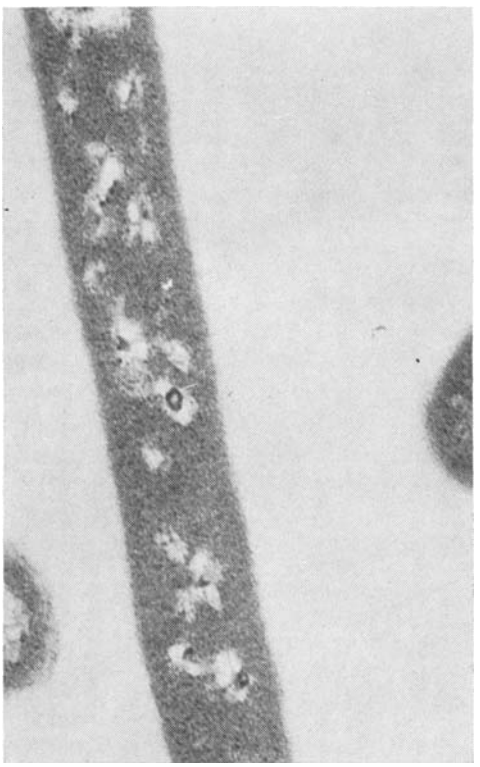

Fig. 2. Election micrograph at 50,000 X of a typical normal E. coli B bacterial cell.

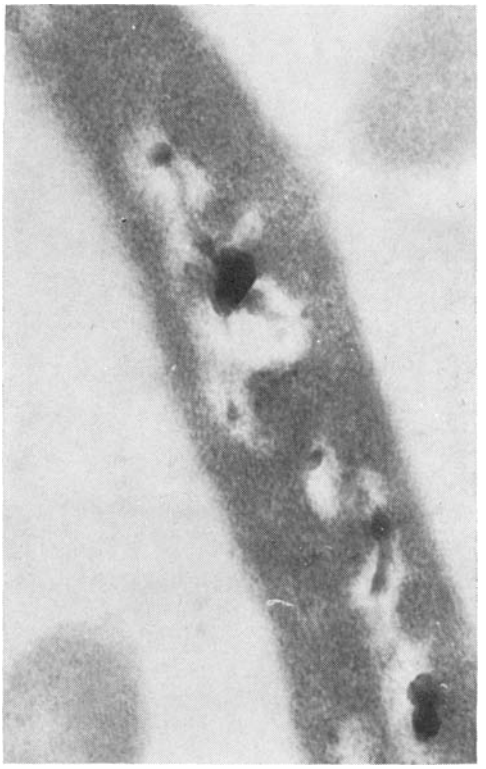

Fig. 3. Electron micrograph at 50,000 X of a continuously X-irradiated E. coli B bacterial cell which received 1,674 R over a 30 hour period.

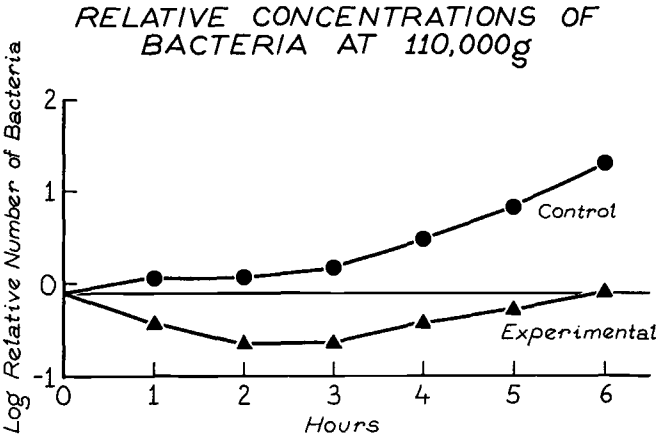

Fig. 4. Growth curves of normal and continuously certrifuged E. coli B bacteria from zero to 6 hours.

of 16 hours. They reached their maximum concentration in the irradiated cultures between 26 and 30 hours of irradiation. At this time approximately 75 per cent of all the cells were of this type. After 30 and 72 hours these large cells were still present in all of the cultures but by the time the cells had received irradiation for 72 hours the majority of them appeared dead.

DISCUSSION

Figure 4 is a graph of the growth characteristics of this strain of *E. coli* B cells when they are maintained in an ultracentrifuge at 110,000 X G. By comparing this graph with the graph given in Figure 1 it is apparent that continuous exposure to gravity or to X-radiation will result in a reduction in the growth rate of these bacterial cells. The measure of growth in this instance is the measure of the increase in the number of cells per unit time and does not indicate or measure any other aspects of growth such as bacterial mass.

Figure 5 is an electron micrograph of an *E. coli* B

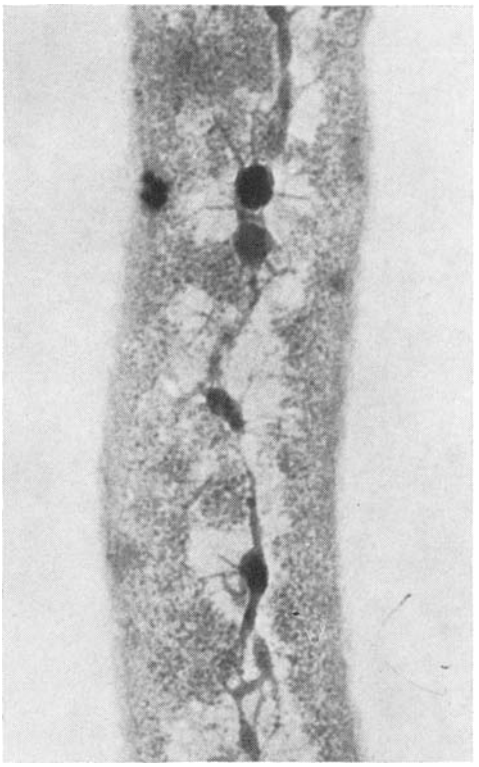

Fig. 5. Electron micrograph at 50,000 X of an E. coli B bacterial cell after continuous centrifugation for 24 hours at 110,000 X G.

cell after centrifugation for 24 hours at 110,000 X G. A comparison of this electron micrograph with the electron micrograph of the control *E. coli* B cell in Figure 2 and with the electron micrograph of the X-radiated *E. coli* B cell in Figure 3 demonstrates the similarity of the morphological effects of continuous centrifugation and continuous X-radiation on these bacterial cells. In each instance the experimentally treated cells are larger than the controls. This cellular enlargement includes a striking hypertrophy of the central oval or round bodies which we have designated "nucleoloids" and of the fibrillar threads associated with them. The numerous small electron dense granules of the cytoplasm thought to be ribosomes are enlarged although this enlargement is not so striking. The generalized growth of these bacterial cells under each of these circumstances is considered to result from the disassociation of division from growth. This disassociation has the effect of suppressing cell division more than cell growth. The result is a large bacterial cell with hypertrophy of the intracellular morphologic components.

The fact that continuous exposure to increased gravity and to increased irradiation produce the same growth alterations and the same ultrastructural morphologic alterations in these bacterial cells suggests that they have a common action on similar structures within the organism. The nature or location of such an action site or sites is unknown. From these studies it seems evident that exposure to high gravity and X-radiation have in common a major biological effect, namely, the unequal suppression of division and growth.

Perhaps the most outstanding biological effect of X-radiation is its ability to influence the genetic apparatus and to thereby produce permanent alterations of a mutant nature in living specimens. Studies in this laboratory are now in progress to determine whether or not these ultrastructural alterations induced in *E. coli* B cells by increased gravity and X-radiation are partly and wholly genetic alterations. Preliminary observations indicate that many of these ultrastructural alterations are transmissible for at least short periods of time in a normal environment.

SUMMARY

Phage free *E. coli* B cells were exposed to continuous ionizing radiation from a cobalt 60 source which delivered 55.8 R per hour.

Cells so treated show a depression of their growth curves when compared to unirradiated control cells.

Ultrastructural observations indicate that these irradiated cells continue to enlarge despite their failure to divide. This enlargement involves the entire cell and its intracellular ultrastructural components.

A comparison of these effects of X-radiation and the effects of increased gravity on these cells was made. It is apparent that increased gravity and increased X-radiation produce similar disturbances in the growth curves and in ultrastructural characteristics of *E. coli* B cells. The possibility that these alterations may be of a genetic nature is considered.

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