circulating blood platelets. Decrease in clot retraction observed in these animals was consistent with reduced platelet counts. Gonadectomy had no effect on post-operative platelet counts or clot retraction.

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Influence of X Irradiation on Potassium Retentivity by *Escherichia coli.** (24169)

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Hevesy and Zerahn (quoted by Hevesy (1)) observed that the K^+ efflux from yeast increased after X irradiation. Bruce and Stannard(2,3) made a detailed study of the influence of radiation on K^+ efflux and influx in yeast. The present study is an extension of their work to bacteria. Although a number of different species were tested, this report will be concerned only with *Escherichia coli.* Strain B and its radiation-resistant mutant B/r are compared, and some other factors that influence K^+ retentivity by *E. coli* are discussed.

Methods. Escherichia coli was cultivated in broth of the following percentage composition: glucose, 1; $(NH_4)_2HPO_4$, 0.4; KH_2PO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.07; and sodium citrate, 0.05. Glucose and ammonium phosphate were autoclaved separately as 20 and 8% solutions, respectively, then added to the autoclaved and cooled basal solution. A 12-hour, 500-ml culture was decanted into 5 liters of fresh broth contained within a 12-liter round-bottom flask in 37°C water bath. The resulting mixture was continuously aerated through a 4-mm bore glass tube at 2½ liters of air per minute. After 12-14 hours incubation, the cells were harvested in a Sharples centrifuge, then mixed with enough distilled water to form about 40 ml of thick suspension. This will be designated as stock suspension. Cells were washed in centrifuge once with distilled water, twice with 0.05 *M* sodium phosphate (pH 6.2) and, after the last centrifugation, were made up to 200 ml in this buffer. The suspension was divided in half and each 100-ml portion was placed

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in a Petroff flask of this capacity. One flask was affixed to a General Electric Maxitron X-ray apparatus as described by Bruce(3); the other flask served as control. Both were oxygenated through a 2-mm bore glass tube at 1 liter of oxygen per minute. This served as a means of agitation as well as maintaining high oxygen tension. Irradiation of test suspension was begun after 15 minutes of oxygenation, and oxygenation of both suspensions was continued during the irradiation period. The G. E. unit was operated at 250 kvp and 30 ma with 1 mm of added aluminum between target and test suspension; the unit delivered X-rays at 4000 r per minute. Temperature of test and control suspensions was maintained at 22°-25°C by water baths. After various doses of radiation, 10-ml aliquots of test suspension were transferred from Petroff flask to 15-ml centrifuge tubes. These aliquots were centrifuged at 4000 rpm for 10 minutes and the clear supernatants carefully decanted into test tubes. The cell pellets were immediately resuspended to volume (10 ml) with 0.02 *M* ammonium phosphate (pH 5.2); they were again centrifuged, and supernatants collected as before. In this manner, potassium ions were extracted from the bacteria. Each cycle of events required 20 minutes—10 minutes for centrifugation and decanting, and 10 minutes for resuspension and holding prior to subsequent centrifugation. The 20-minute centrifugation cycles were continued for a minimum of 3 hours; thus at least 10 supernatants were collected from each aliquot. An aliquot from control suspension was manipulated in like manner. The viable-cell count did not change appreciably as a result of these manipulations. Operations were carried out at room temperature, about 22°C. The series of supernatants in test tubes were analyzed for K^+ content by a flame photometer (Baird Associates) with suitable correction for Na^+ in the first supernatant and for NH_4^+ in subsequent supernatants. Ammonium ions, however, affected photometer readings very little. Summation of K^+ content of successive supernatants permitted construction of efflux-time curves. In these curves, amount of K^+ effluxed (mmoles) per given wet weight (kg) of cells as plotted *vs.* time (hours). Cell concentration will be expressed as mg (wet weight) of cells/ml of suspension. Cell concentration of stock suspension was determined by hematocrit reading on a diluted ½-ml aliquot, with suitable corrections for dilution. (Specific gravity of cells was assumed to be unity.) Cell concentration of this stock was always about 200 mg/ml, equivalent to a viable count of 1.5×10^{11} cells/ml. To avoid introducing cell concentration as a variable during efflux measurement and to ensure concentration of K^+ sufficiently high for flame photometry, the cell concentration during efflux was always set to 40 mg/ml. When, as outlined above, the entire stock was used, the cell concentration during irradiation was 40 mg/ml and aliquots from Petroff flasks were used directly for efflux measurement. However, when lower cell concentrations were used during irradiation it was, of course, necessary to concentrate the cells prior to efflux.

Results. We discovered that little or no potassium efflux occurs in the presence of Na^+ but efflux will occur readily in the presence of NH_4^+. Thus we used sodium phosphate as buffer during irradiation to minimize efflux during this interval and substituted ammonium phosphate thereafter (when efflux was to be measured). The first points in Fig. 1 (0 hours) represent quantity of K^+ in 0.05 *M* sodium phosphate (first supernatants). Only a very small amount of potassium has effluxed, whereas the efflux begins immediately upon replacement with ammonium phosphate. During the first hour the slope fluctuates, but after 1½ hours, the curves become linear and a numerical expression for efflux rates may be obtained.

The control efflux rate of *E. coli* B obtained in different experiments varied from 0.17 to 0.28 mmole of K^+/kg of cells/hour, but since the efflux rates after a given dose of radiation varied by a corresponding amount, data from different experiments could be compared, if the results were expressed as percentage of normal K^+ retentivity. (This expression is obtained by dividing control efflux

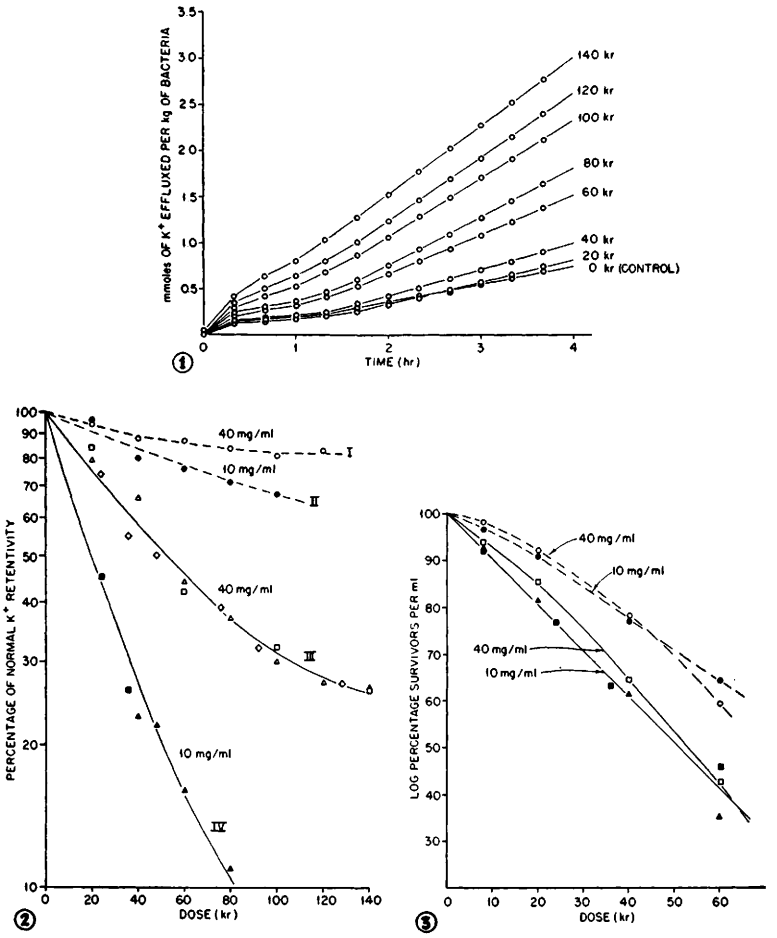

FIG. 1. Effect of X-irradiation on potassium efflux from *Escherichia coli* B. Cell concentration during irradiation was 40 mg/ml. For the 140-kr curve, efflux rate was 0.74 mmole/kg/hr; 120-kr curve, 0.71 mmole/kg/hr; 100-kr curve, 0.63 mmole/kg/hr; 80-kr curve, 0.52 mmole/kg/hr; 60-kr curve, 0.43 mmole/kg/hr; 40-kr curve, 0.29 mmole/kg/hr; 0-kr curve (control), 0.19 mmole/kg/hr.

FIG. 2. Effect of X-irradiation on potassium retentivity by *Escherichia coli* B and B/r at different cell concentrations during irradiation. Solid line, *E. coli* B; broken line, *E. coli* B/r.

FIG. 3. Effect of X-irradiation on survival of *Escherichia coli* B and B/r at different cell concentrations. 40 mg/ml equivalent to initial viable cell count of 3×10^{10}/ml; 10 mg/ml equivalent to initial viable cell count of 7×10^{9}/ml. Solid line, *E. coli* B; broken line, *E. coli* B/r.

rate by the efflux rate at a given dose and multiplying by 100; Bruce and Stannard (2).) The data from Fig. 1 have been expressed in this manner and have been plotted semilogarithmically in Fig. 2 (curve III, triangles); squares and diamonds represent corresponding data from 2 other experiments. Good reproducibility was obtained in experiments in which cell concentration was set at 40 mg/ml. Control efflux rate at 10 mg/ml varied from 0.04 to 0.09 mmole/kg/hour; this low rate of efflux was difficult to measure with the flame photometer and rendered the corresponding values for percentage normal retentivity less accurate. (All cell concentrations were those used during irradiation; as has been pointed out, cell concentration during efflux was always 40 mg/ml.) *E. coli* B/r had relative high control efflux rates; at 40 mg/ml about 0.60 mmole/kg/hour, and at 10 mg/ml 0.67 to 0.98 mmole/kg/hour. Good reproducibility in potassium retention

calculations was obtained at both cell concentrations. Since X irradiation of B/r increased efflux rate very little, the decrease in percentage normal retentivity with increasing dose was less pronounced than with strain B (compare curves I and II with curves III and IV, Fig. 2). It may also be noted in Fig. 2 that magnitude of radiation damage varies inversely to cell concentration in the irradiated suspension and, lastly, that the slope of retentivity curves decreases at higher doses.

The increase in K^+ efflux brought about by irradiation is not caused by gross cell lysis, because (1) diluted aliquots from the irradiated suspensions were no less turbid than similarly diluted aliquots from control suspensions, and (2) supernatants from irradiated suspensions contained no more 2600 A absorbing material than supernatants from control suspensions.

The influence of radiation and cell concentration on viability is presented in Fig. 3. (Use of same symbols on a curve in Fig. 3 as in Fig. 2 indicates that the data are from a common experiment.) The influence of cell concentration on K^+ efflux noted in Fig. 2 apparently has no equivalent effect on viability, although at lower cell concentration survival curves are more nearly exponential. Experiments by Hollaender *et al.*(4) demonstrated that the shape of such curves can be influenced by oxygen tension. Thus the downward concavity of curves at high cell concentration may be caused by oxygenation's insufficiency to meet the oxygen demand of this large number of cells.

The present study does not permit conclusions as to site of potassium retention damage. Possibly, damage is to the cell membrane, as Bruce(3) considered to be the case with yeast; or the damage may be to an intracellular metal-organic complex responsible for binding potassium, denaturation inducing an irreversible loss of the metal ion. Indeed, the researches with bacteria by Cowie (summarized by Roberts *et al.*(5)) and Eddy and Hinshelwood(6) make the latter idea especially attractive.

Summary. 1) Potassium efflux from stationary-phase cells of *Escherichia coli* takes place readily in the presence of ammonium ions. Hence, through the use of ammonium phosphate buffer, K^+ efflux was studied at a set value of pH. Sodium ions, on the other hand, retard K^+ efflux, a finding that has been used to advantage when sodium phosphate is used as buffer in delaying efflux measurement. 2) Potassium efflux from *E. coli* increases as a result of X irradiation. Amount of X-ray damage to K^+ retention mechanism varies inversely with cell concentration during irradiation. *E. coli* strain B is much more susceptible to this radiation damage than is its mutant B/r.

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