TABLE II. Yields of Conjugated Bile Acids from Various Species.

Species	Yield of bile acids per 10 ml bile, mg
Rat	80–100
Rabbit	225
Chick	240
Goose	1800*
Frog	650

* Bile partially dehydrated.

bile acids leaving the free acids in solution.

We have applied this method to the bile of several species (Table II) with uniformly good results. In all cases, the isolated bile acids were chromatographed in collidine-water-NH_3(2), and compared with chromatograms of whole bile. The isolated bile acids were always chromatographically identical with the conjugated bile acids of whole bile. It should be noted, however, that since conjugated bile acids are slightly soluble in methanol-ethyl ether mixtures, this method cannot be used for the quantitative recovery of conjugated bile acids in bile.

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Received May 4, 1954. P.S.E.B.M., 1954, v86.

Observations on Effects of X-Irradiation on *Achromobacter fischerii*.* (21080)

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Bacterial luminescence offers a unique opportunity for studying the effect(s) of x-irradiation on the living cell. Due to the interaction of irradiation and cellular substance, an almost immediate visual indication of alteration in the biochemical processes of the cell is observed at proper dosage levels. The most recent review on the effect of x-irradiation on luminescent bacteria [Harvey(1)], has called attention to the need for more extensive investigation of this subject.

Lorenz and Henshaw(2) have studied the survival of *Achromobacter fischerii* after x-irradiation and Whipple(3) has reported some effects of x-rays on bacterial luminescence. Unfortunately, the experimental technics differ, and no correlative evaluation of the relative sensitivities of bacterial luminescence and survival can be made from this material. It has been the purpose of this study to investigate the effect of graded doses of x-irradiation on bacterial luminescence relative to survival of the microorganisms.

Materials and methods. The test organism used for this study was *Achromobacter fischerii*,‡ cultured in Farghaly's basal media (4) supplemented with 200 mg purified casamino acids per liter. The cells were grown at room temperature on a horizontal oscillating machine for a period of 18 hours and then harvested in the cold by centrifugation. The centrifuged cells were washed with a cold NaCl-PO_4 buffer(1), and resuspended in a suitable volume of buffer to give a cell concentration of approximately 5 million cells per milliliter. This cell concentration was used as the standard. Aliquots of the standard cell suspension were pipetted into small round glass cups and exposed to x-irradiation delivered by a 250 kv Westinghouse unit with a 15 ma output at a distance of 14 cm using 1 mm Al and 0.5 mm Cu filters. The dose rate was 545 r per minute as measured with a Victoreen r-meter The liquid depth of the exposed bacterial suspension was 5 mm. After irradiation, samples of both the test and control suspensions were taken to determine the

* Supported in part by the U. S. Atomic Energy Commission.

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‡ The original culture of this organism was kindly supplied by Dr. Frank H. Johnson, Biology Department, Princeton University.

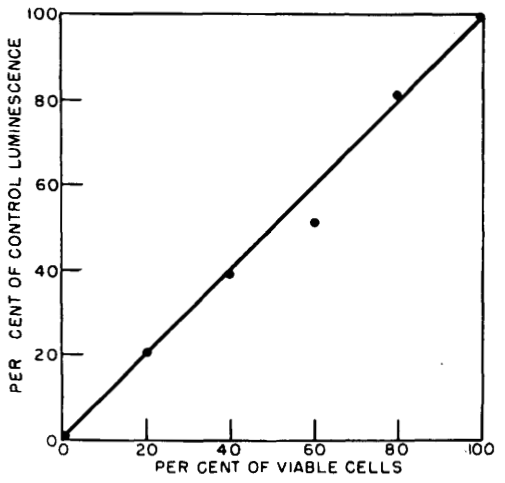

FIG. 1.

x-irradiation effects on luminescence and viability. *Luminescence* was determined on both the test and control samples after buffered 15% dextrose had been added to the samples to give a final concentration of one percent dextrose. Three aliquots of each sample were then taken for determinations. The luminescence was measured by means of a GE 931-A electron multiplier tube incorporated within a small constant temperature (26°C) apparatus which maintained a constant rate of agitation for the tubes containing the suspensions. Aeration of the suspensions was facilitated by means of a small glass bead in each tube. *Viability* was determined by taking 1 ml aliquots of both test and control cultures and diluting with buffer through a series of ten-fold steps. One-tenth ml for each dilution was placed on each of three agar plates and smeared evenly over the surface with a sterile bent glass rod. The cultures were incubated for a period of 36 hours and then counted. The survivals are listed as a log percentage of the controls. The medium for plate counts was essentially the same as described by Harvey(1) with the exception that yeast extract and $NaHCO_3$ were substituted for beef extract and $CaCO_3$ respectively, to enhance the transparency of the medium. The carbonate was added to the medium after autoclaving and just prior to pouring the plates.

Results. The effect of various percentages of dead cells in a standard bacterial suspension on the percentage luminescence of this mixed dead and live cell suspension was studied. In this manner the lethal effects of various dose levels of x-irradiation could be simulated and a quantitative relationship between luminescence and viable cell count obtained. For this purpose a suspension of formalin-heat-killed cells was used as a diluent for the standard suspension. This killed suspension first was washed 5 times with buffer and then diluted to a turbidity equal to that of the standard cell suspension. Dilutions of the viable standard cell suspension were then made with the killed cells as noted in Fig. 1. By keeping the turbidity constant, while varying the relative number of dead cells, conditions supposedly existing in x-irradiated cultures could be approximated.

To test the above results, a standard cell suspension was exposed to doses of x-irradiation ranging from 500 to 10,000 r. Aliquots of the irradiated and control suspensions were then plated out after exposure to determine the sensitivity of the organism to graded doses of x-ray. Aliquots were also taken from these samples for measurements of luminescence to find the immediate effects of the irradiation on this system. The results shown in Fig. 2 represent luminescence 2 hours after x-irradiation at which time the ratio of the test to the control had become constant in all groups. It is apparent that x-irradiation at all dose levels affected the survival of the cells, while the effect on the luminescence did not become apparent until the dose was increased to 5000 and 10000 r. A comparison of the log percentage survival and the log percentage luminescence after treatment with the higher x-ray doses suggests that a large proportion of the cells unable to multiply after x-irradiation are still capable of carrying out the necessary metabolic processes required for the production of luminescence. Using the results in Fig. 1 as a reference, the *calculated viability* was obtained, and it was noted that the luminescence persisting two hours after x-irradiation with 5000 r was equal to a survival of approximately 56% on the control suspension, while the luminescence of those exposed to 10000 r was equivalent to a 17% survival.

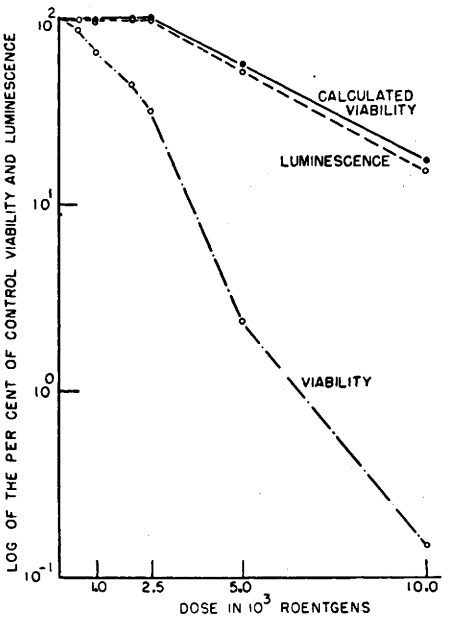

FIG. 2.

The measurements of reproductive ability of the suspensions were only 1.24 and 0.115% survivals respectively, indicating that the capacity of the cell for multiplication after exposure to x-irradiation is increasingly more sensitive to exposure than is the system for luminescence. Giese(5) has observed that although low dosages of ultra-violet light have a sterilizing effect on *A. fischerii,* such treatment does not immediately impair the respiration or luminescence. Likewise, Billen *et al.* (6) have found bacterial respiration after x-irradiation to be far greater than could be expected on the basis of viable cell count.

Inasmuch as bacterial luminescence in the intact cell is dependent on oxygen concentration(7) as well as the proper functioning of the respiratory system(1) and cell extracts require at least coenzyme I(8) and riboflavin phosphate(9), it can be assumed that a large proportion of the sterilized cells is able to carry on metabolic activities for a measurable length of time after x-irradiation.

Summary. It has been observed that the reproductive mechanism of *A. fischerii* is more sensitive to x-irradiation at all measured dosage levels than is the luminescent system of this organism. These findings indicate that cells sterilized by x-irradiation are capable of metabolic activity for a measurable length of time after exposure.

Grateful acknowledgement is made to Mr. G. Westinskow and Mr. D. Taysum for designing and assembling the electronic and mechanical equipment used in this work.

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Received May 11, 1954. P.S.E.B.M., 1954, v86.