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Prolongation of the Lag Phase of Irradiated *Escherichia coli*. (20992)

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Although the lethal effect of ionizing radiations on bacteria has been the subject of repeated and extensive study(1), the recovery of damaged organisms has received relatively little attention. In the course of attempts to modify the lethal effect of X rays on bacteria, we have observed a delay in the onset of multiplication of the *surviving* organisms. Because recovery from radiation injury in higher animals may well be dependent on the resumption of growth by damaged cells rather than on the revival of "killed" cells, we believed that this aspect of the problem deserved further investigation.

Materials and methods. Bacteria. A culture of *Escherichia coli*,* serially passed on blood agar plates and stored in a refrigerator, was used throughout these studies. Unless otherwise stated, all cultures were incubated aerobically at 37°C. Pour plates were made by adding one cc of culture, diluted to produce approximately 50 to 200 colonies, to 8 cc of melted tryptocase soy agar† at 40 to 44°C. Colony counts were made after an 18-hour incubation period. Reincubation of some plates from both irradiated and non-irradiated cultures for additional 24- and 48-hour periods failed to give any increase in colony counts. *Irradiation.* Cultures were irradiated in Evelyn spectrophotometer tubes with 250-kvp X rays at 15 ma with .25-mm Al filtration. The tubes were held in an ice water bath by a modification of the apparatus described by Stapleton *et al.*(2). Exposure rates, calibrated with a Victoreen thimble chamber inside of an Evelyn tube, varied from 425 to 480 r per minute. In each experiment, a single overnight culture of *E. coli* in tryptocase soy broth served as the source of both control and irradiated bacteria. Except where otherwise indicated, the original culture was diluted sufficiently in tryptocase soy broth prior to irradiation so that pour plates could be made from irradiated and control cultures without further dilution. Because the survival ratio of the strain of *E. coli* employed was approximately one in 2500 at 30,000 r, the non-irradiated cultures were diluted 10^3- and 10^4-fold further than the cultures receiving this exposure. All cultures were kept in ice water baths from the time of dilution, throughout irradiation, and until after the initial pour plates had been made after irradiation. After pour plates had been prepared from irradiated and control cultures, they were placed into a 42°C water bath and warmed for 5 minutes prior to transfer to a 37°C incubator. The control and irradiated cultures were handled identically in all respects throughout: equal volumes were moved in and out of the water baths and incubator in equal-sized tubes.

Results. The growth of irradiated and non-irradiated control cultures was studied repeatedly. Typical growth curves are shown in Fig. 1. Cultures that had been X-irradiated with 30000 r consistently showed a ¾- to 1½-hour delay in the onset of the phase of logarithmic

* Isolated from a patient and supplied to us through the kindness of Miss Dorcas Fasan, Department of Pediatrics, University of Chicago.

† Baltimore Biological Laboratory, Baltimore, Md.

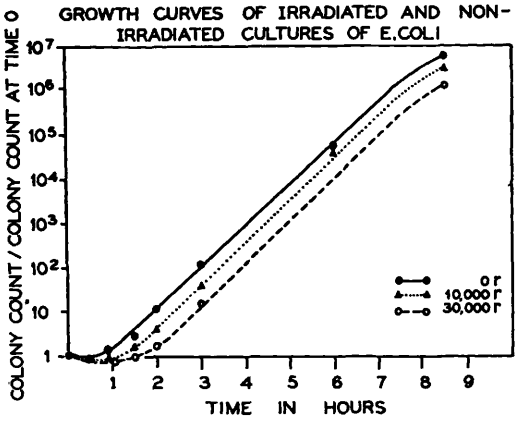

FIG. 1.

growth when compared with unirradiated controls. With 10000 r, the delay was shorter. A slight decrease in the colony count was observed frequently during the lag phase. The logarithmic growth rate was the same for irradiated and control cultures.

Because it appeared likely that the prolongation of the lag phase of irradiated cultures might be due to toxic changes induced in the culture medium, a more concentrated culture was irradiated and subsequently diluted 100-fold into fresh medium. The growth curve of irradiated bacteria thus transplanted into non-irradiated medium was identical with that plotted for growth in an irradiated medium.

Prolongation of the lag phase was increased to 2½ hours and the rate of growth in the logarithmic growth phase was considerably reduced when incubation subsequent to irradiation was carried out at 24°C.

The effect of storage at −2°C and 7°C on the lag phase of irradiated and control cultures was also studied. Because the colony counts of cultures stored at these temperatures decreased progressively, undiluted cultures were irradiated and diluted as necessary at the end of the storage period. Colony counts were made before and 2 hours after re-incubation of the stored culture. Even after a 27-day cold storage period there was no significant multiplication of irradiated cultures upon incubation. However, the non-irradiated cultures multiplied at a reduced rate after cold storage for as little as one week. This suggests that cold storage alone induces a prolongation of the lag phase. These 2 means of prolongation proved to be additive in cultures that had been stored at cold temperatures for several weeks and then X-irradiated.

The modifying effect of various substances on the X-ray-induced prolongation of the lag phase was studied to assay materials for radiation-recovery potency. In each experiment, the simultaneous growth of non-irradiated control cultures and irradiated cultures was compared. Test substances were added after irradiation, but prior to incubation. The growth at the end of 2 hours was selected as a convenient endpoint for screening purposes, colony counts being made, in general, only before and 2 hours after incubation. Media containing 7.5% freshly drawn mouse blood; 15% rabbit serum, which had been heated to 56°C for ½ hour; fresh 0.5% cysteine solution, added before as well as after irradiation; or the filtrate of an actively-growing non-irradiated *E. coli* culture all failed to reverse the radiation-induced lag phase. The addition of fresh spleen, liver, or kidney mash from unirradiated mice consistently altered the growth curve of irradiated organisms in the direction of normal. However, the addition of these tissues also increased the rate of growth of non-irradiated cultures. A mash of spleen or liver from mice given 900 r immediately prior to the removal of these organs had a less marked effect on growth.

The growth of irradiated bacteria in intimate contact with growing, non-irradiated cells was observed in 2 experiments. A streptomycin-resistant variant of the *E. coli* strain was produced by serial passage of the strain through graduated streptomycin-containing broths. This variant was irradiated and then incubated together with a vigorously-growing unmodified culture. Colony counts were made as before, but with the incorporation of streptomycin in such concentration as to completely inhibit the non-irradiated streptomycin-sensitive strain, while permitting normal colony formation by the irradiated, streptomycin-resistant strain. In this way it was possible to follow the growth of the originally irradiated bacteria in cultures in which non-irradiated bacteria predominated.

The growth of irradiated and non-irradiated bacteria was also observed in the chorio-allantois of 10-day embryonated hens' eggs. Control observations were made on eggs that had been irradiated with 1000 r on the assumption that any substances with radiation-recovery potency would be destroyed in such eggs. No increase in the growth rates of irradiated bacteria could be demonstrated in either of these experiments.

Discussion. Although the lag phase of *E. coli* cultures given a single large exposure to X radiation is prolonged, the logarithmic growth rate is identical in irradiated and control cultures. The early inhibition of multiplication is thus completely reversible and therefore does not appear to represent the development of a stable mutant. It is clearly not the result of any persistent toxic changes in the media. The rapidity of resumption of normal growth varies directly with the temperature and inversely with the size of the X-ray exposure. This phenomenon is not limited to the strain of *E. coli* used in these experiments: we have also observed it in cultures of *Diplococcus pneumoniae* and strain B/R of *E. coli*‡(3). Because it occurs in cultures held at 0° and 37°C throughout, it appears to be unrelated to the recovery from the lethal effect of irradiation that has been described by Hollaender(4,5) as occurring in certain media only at intermediate temperatures.

It is well established that irradiation delays cellular division in many species(6,7). The effect of irradiation on the multiplication of bacteria has been reported in detail for cultures undergoing continuous irradiation during growth(8,9). Lea, Coulson, and Haines (9) have suggested that the failure of the viable counts of such cultures to increase may be due to a reversible delay of cellular division, rather than growth, so that long forms that subsequently divide are formed.

Although there is considerable evidence that the spleen and certain other organs elaborate a humoral substance or substances that will enhance recovery of irradiated mammals(10), no satisfactory means has been developed for assaying such materials in laboratory animals. This lack of success may, at least in part, be ascribed to the great dilution of injected materials in body fluids and to the relatively limited number of animals available. From these points of view the use of bacteria for assay is advantageous. Changes in the mortality rates of irradiated bacteria are readily demonstrated by altering the concentration of cysteine, oxygen, alcohols, pyruvate, and numerous other substances in the suspending medium *before* and *during* irradiation(5,11). Except for studies on the recovery of bacteria at intermediate temperature(5), the efficacy of any material added *after* irradiation has not been reported.

It must be emphasized that our studies have been concerned with a *sublethal* rather than a *lethal* effect of X rays on bacteria. Since only colony counts (viable counts) have been made, the growth curves describe only the survivors. The prolongation of the lag phase differs from the lethal effect of irradiation in that it is inherently reversible. It is not an all-or-none phenomenon. Fundamental differences are also suggested by the failure of pre-treatment with cysteine to affect the prolongation of the lag phase, while the influence of this compound on the lethal effects of X radiation is well known(11). It seems quite likely, therefore, that the mechanisms of these types of injury may be totally different.

Other studies have failed to show the influence of any substance, added after irradiation, on the *lethal* effects of X rays; in this study we have measured the effect of various substances added after irradiation on *recovery* from the lag phase. Although some positive effect on growth was achieved by the addition of spleen or liver mash, this effect was not limited to irradiated cultures. Furthermore, the effect was only partially destroyed by irradiation, and an effect of equal magnitude could be obtained through the use of a mash made from mouse kidney, which does not appear to enhance recovery from irradiation in mammals. For these reasons it seems unlikely, although not impossible, that the growth-promoting activity that has been ob-

‡ Supplied to us by courtesy of G. E. Stapleton and A. Hollaender, Oak Ridge National Laboratory, Oak Ridge, Tenn.

served represents the humoral factor that has been implicated in recovery of mammals from the damaging effects of ionizing radiations.

Summary. 1. A prolongation of the lag phase in irradiated *E. coli* is described. The modifying effects of temperature during growth, storage of irradiated organisms, and the addition of mouse blood, inactivated rabbit serum, 0.5% cysteine solution, the filtrate of actively growing *E. coli* cultures, fresh spleen, liver and kidney mash, or growing non-irradiated *E. coli* cultures, have been studied. 2. Evidence that sublethal effects of X irradiation in bacteria differ from lethal effects is discussed.

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Ribonuclease Activity of the Mouse Pancreas. (20993)

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Although the activities of several enzymes have been studied in the pancreas after fasting(1-3) and after the administration of cholinergic drugs to normal animals(3-6), no data bearing on pancreatic ribonuclease could be found. Information on this enzyme is of particular interest because of the occurrence of large amounts of ribonucleic acid(7) and because the pancreas is a rich source of crystalline ribonuclease. The evidence presented in this paper indicates that ribonuclease activity of pancreatic homogenates is reduced by fasting and by the administration of pilocarpine.†

Materials and methods. Adult white mice fed Purina dog chow were used. Males were studied, with the exception of those in Exp. I, Table I. In the fasting experiments the mice received only water for the times indicated. For the pilocarpine experiments, the fasted or unfasted mice regardless of body weight were injected intravenously with 2 doses of 0.6 mg each of pilocarpine hydrochloride (total volume 0.6 ml) at 30 minute intervals. Controls were similarly injected with distilled water. Intense salivation, lacrimation and diarrheic stools followed the injections. The mice were killed by a blow on the head, and weighed. The pancreas was removed, and weighed on a torsion balance. 0.1% homogenates were prepared with chilled distilled water, and the homogenates stored at −10°C until the determinations of enzyme activity were performed, within the next 18 hours. Control and pilocarpine-treated mice from each experiment were injected and killed alternately, and determinations of enzyme activity were performed simultaneously. Comparisons are to be made only within the individual experi-

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† After completion of this paper a preliminary communication appeared(8) which claims that a reduction of the ribonuclease activity of the mouse takes place after the injection of carbamyl choline.