AN OXYGEN EFFECT ON THE EFFICIENCY OF INACTIVATION OF ESCHERICHIA COLI BY INCORPORATED RADIOPHOSPHORUS

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Introduction. Oxygen increases the inactivating effect of X-radiation on cells of Escherichia coli (Hollaender, Baker, and Anderson, 1951; Flanders, 1957) and on intracellular bacteriophage (Van Dyke, 1959; Flanders, 1960). Deoxyribonucleic acid (DNA) is implicated as the radiation target (Latarjet, 1948; Hershey and Chase, 1952; Hershey, 1955; Harm, 1958) and as the site of the oxygen effect (Van Dyke, 1959).

Radioactive phosphorus incorporated into E. coli or its phages has been shown to inactivate them with a constant efficiency. At 4°C about one of ten disintegrations per unit of DNA is lethal (Hershey, Kamen, Kennedy, and Gest, 1951; Stent, 1953; Thomas, 1959). The efficiency of inactivation is too high to be accounted for on the basis of the energy absorbed from the beta particles emitted (Hershey, Kamen, Kennedy, and Gest, 1951). Also, it has been shown that inactivation of E. coli by incorporated radiophosphorus is efficient only when the radiophosphorus is incorporated into DNA, although DNA phosphorus is only some 12% of the total cell phosphorus (Fuerst and Stent, 1957).

The inactivation kinetics for X-radiation and radiophosphorus are very similar and both seem to exert a cutting action (Stent and Fuerst, 1955) on DNA molecules as evidenced by 1) the apparent inability of X-ray and P^{32} inactivated phage to inject its entire genome inferred from cross reactivation and multiplicity reactivation experiments and possibly by the absence of the oxygen effect on free phage (Luria, 1947; Watson, 1950; Doermann and Chase,

1955; Harm, 1958; Van Dyke, 1959); 2) the similar transfer sequence of the genetic characters in mating E. coli K12 obtained by mechanical breakage and the action of P^{32} (Wollman and Jacob, 1955; Jacob and Wollman, 1955; Fuerst, Jacob, and Wollman, 1956); and 3) by fragmentation of DNA in solutions (Taylor, Greenstein, and Hollaender, 1948; Conway, Gilbert, Butler, 1950; Alexander and Stacey, 1956). A review on these matters has been published (Stent, 1958). Because of the above similarities between inactivation of phages and cells by X-radiation and incorporated radiophosphorus, the effect of oxygen on inactivation by incorporated radiophosphorus has been examined.

Methods and Materials.

Organisms. Escherichia coli strain B/r (courtesy of Dr. G. S. Stent). Experiments were also done with a B strain (courtesy of Dr. M. Demerec) and another B/r strain (courtesy of Dr. R. F. Hill).

Media. The plating medium was Difco nutrient agar containing .5% sodium chloride. H medium (Stent and Fuerst, 1955) was used to grow the cells. It was made up doubly concentrated and an equal volume of either the radiophosphorus solution or distilled water was added in making up the growth tubes. Carrier phosphorus was added as sodium acid phosphate. The storage medium was a phosphate buffer containing a small amount of gelatin (Krieg, 1959).

Phosphorus Content. The total phosphorus content of the cultures was measured by the method of Fiske and Subbarrow (1925).

Radioactive Isotope. The radiophosphorus was obtained from Oak Ridge National Laboratory, via a pharmaceutical company, as phosphoric acid, carrier free and sterile at a specific activity of 1000 mc/mg.

Radioactivity Measurements. Samples (.01 ml) of the radioactive culture and the radioactive cells resuspended in storage medium were dried on planchets and counted by means of a shielded, thin window, geiger counter and a scaler. The amounts of P^{32} present were obtained by comparison counts of a C^{14} source which had been calibrated with a standard P^{32} source.

Assay Method. Ordinary dilution and plating procedures were followed, and three plates were used per dilution. Plates were scored after

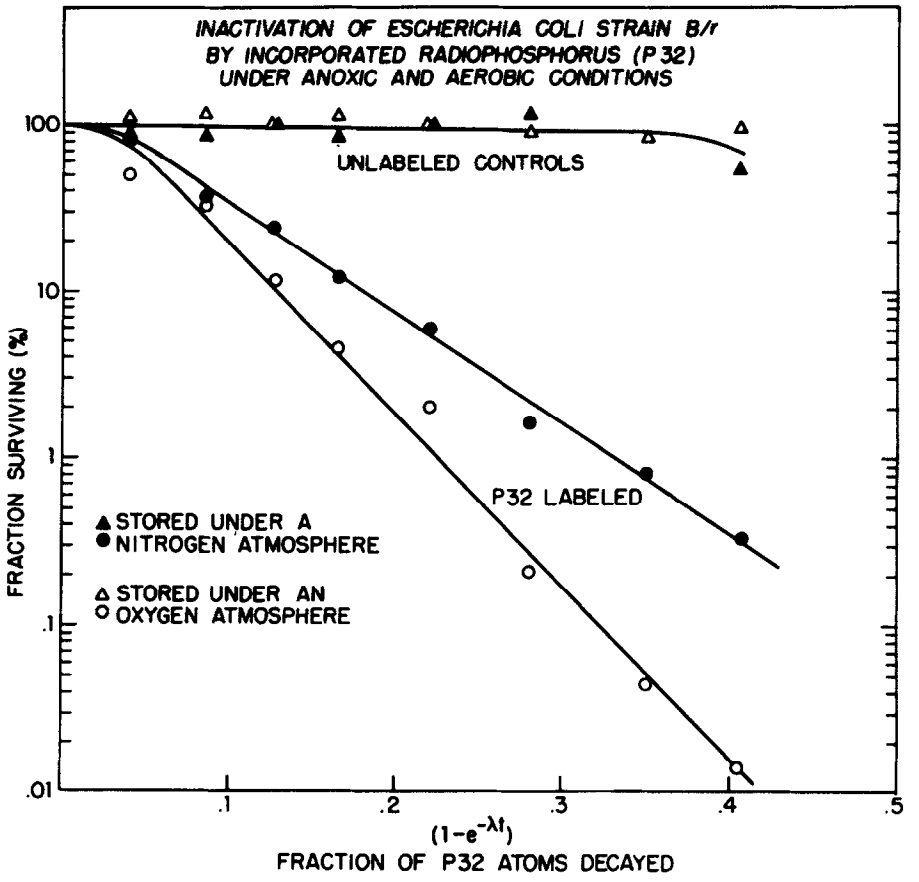
18-24 hours of incubation at 37°C.

Ampules and Storage. Samples (approximately 1 ml) were stored in small glass ampules which had been acid cleaned, rinsed repeatedly with distilled water, and dried before use. The sealed ampules were placed in beakers containing a small amount of water and were stored at 1-3°C in a refrigerator.

Gassing Method. The samples in the glass ampules, which had tips drawn out to a small diameter, were bubbled for 10 minutes with pure oxygen or nitrogen ($<$ .001% oxygen) by means of a piece of glass tubing, one end of which had been drawn to a long thin tip.

Experimental Procedure. A culture tube containing 10 ml of H medium was inoculated by means of a wire loop and incubated overnight at 37°C with aeration. In the morning, two new cultures, each containing 10 ml of H medium, one with radioactive phosphorus, one without, and both with enough carrier added to make the culture contain about 40 gamma of phosphorus per ml, were inoculated with 0.1 ml each from the overnight culture. The cultures were incubated 4.75 hours at 37°C with aeration. During the incubation period there was about a fifty-fold increase in the number of bacteria per ml. Three ml from each culture was then set aside for a total phosphorus assay. The remainder of each culture was then centrifuged for 10 minutes, the supernatant discarded, and the cells resuspended in cold storage medium and refrigerated. A sample of the radioactive suspension was counted and used to determine the dilution necessary to reduce the background of beta radiation to one microcurie per ml. Then both suspensions were diluted equally and radioactive phosphorus was added to the non-radioactive bacterial suspension to make it have one microcurie per ml, approximately. These suspensions were then dispensed by means of a capillary pipette into the ampules in about 1 ml amounts, gassed, sealed, and stored as described above. The suspensions were assayed initially, and each day four ampules, one for each condition, were opened and assayed for viable bacteria.

Results. The results of a representative experiment are shown in Figure 1. There was almost no inactivation in the controls up to the end of the



experiment at 11 days. Obviously the labelled cells stored under oxygen were inactivated more efficiently than those stored under nitrogen. The total phosphorus content was 40 gamma per ml and the initial specific activity was 4.6 mc/mg. The efficiency of inactivation was .12 under oxygen assuming 1.2×10^7 phosphorus atoms per nucleus (Fuerst and Stent, 1957). Under nitrogen the sensitivity to P^{32} inactivation was reduced by a factor of 1.7.

Very similar results were obtained in continuous bubbling experiments using strains B (Van Dyke and Leskowitz, 1960) and B/r (Hill), although the ratio of the slopes of the two curves was somewhat larger.

Discussion. There is a striking resemblance between these oxygen effect results and those obtained with X-rays. The recently reported (Matheson and Thomas, 1960) protective effect of AET (2-aminoethyl isothiouronium bro-

mide H Br) on P^{32} inactivation of phage T4 may well be a closely related phenomenon. The temperature dependence of the P^{32} inactivation efficiency that has been reported (Stent, 1953) may be due, at least in part, to a temperature dependence of the oxygen effect.

Phage ØX174 has been reported to be inactivated by incorporated P^{32} with an efficiency of 1.0 (Tessman, 1959). We are currently doing experiments to ascertain whether the efficiency of inactivation of ØX174 (also T2) is reduced by the absence of oxygen for either the free or intracellular phage. Thus, it may be possible to determine whether oxygen increases the number of single chain breaks or only the number of double chain breaks.

It is interesting to note that not only may phosphorus (phosphate) occupy a strategic position in the DNA structure, but that it has a larger Compton and photoelectric cross section than any other element in DNA. Note also that the inactivation kinetics are single hit, indicating that only one event is effective (X-rays and P^{32}). It will be a most curious result if it is established that X-rays and the disintegrations of incorporated P^{32} both produced inactivation primarily by causing a DNA phosphorus atom to break with or leave the chain, and that oxygen reacts directly with DNA to block a restitution (Van Dyke, 1959). The production of a lethal effect with a two-strand DNA virus or cell probably depends on the formation of two breaks (Stent and Fuerst, 1955) side by side, and this might be accomplished either by another ionization in the same track, or by energy transfer from the recoiling nucleus.

Summary. An oxygen effect on the efficiency of inactivation of E. coli cells by incorporated radiophosphorus has been found.

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