AN OXYGEN EFFECT ON X-IRRADIATED INTRACELLULAR BACTERIOPHAGE T2Hr

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Introduction. The inactivation by x-irradiation of bacteriophage suspended in broth is often considered to be due to direct effect (Watson, 1950; Luria, 1955). The broth is believed to compete for chemical agents produced by the x-irradiation in the water of the suspending medium, thus damping out such indirect effects on the phage. Dry phage T1 has been reported (Adams and Pollard, 1952) to have the same radiosensitivity as broth phage. The presence or absence of oxygen has been reported (Hewitt and Read, 1950) to have no effect on the x-ray sensitivity of free phage in broth. It has been observed that the hard x-ray sensitivity of the recently infected bacterium is very similar to that of the free phage irradiated in broth (Harm, 1958). Since it is reasonably certain that essentially only the phage DNA enters the bacterium (Hershey and Chase, 1952), the DNA is implicated as the radiation target. If the x-ray inactivation of phage in broth is by direct effect, the intracellular inactivation would also seem to be by direct effect. Now oxygen enhances the effectiveness of radiation-produced water free radicals (as in the ferrous sulfate dosimeter). The oxygen effect in biological systems has, by analogy, been considered to result from a similar mechanism. Thus, no oxygen effect would be expected on intracellular phage, just as with free phage in broth.

But Escherichia coli cells are more readily inactivated by x-radiation in the presence of oxygen than in its absence (Hollaender, Baker and Anderson, 1951; Flanders, 1957). It would seem not unreasonable that the DNA of the intracellular phage and the host are in a similar environment in terms of

substances competing for free radicals. Thus, if the oxygen effect is on DNA, an oxygen effect could exist on intracellular phage. Preliminary experiments designed to ascertain the existence or non-existence of an oxygen effect on x-irradiated intracellular bacteriophage have been performed.

Methods and Materials. In these experiments bacteriophage T2Hr and Escherichia coli, strain B, were used. Singly infected (1 per 2000 bacteria) phage host complexes were prepared by adsorption onto starved cells (Benzer, 1952) and irradiated in suspension, in an ice bath, while the 1 ml. aliquots were bubbled either with air or 99.9% pure nitrogen (.001% oxygen). Free phage were irradiated in Difco nutrient salt broth or in twice as concentrated salt broth (.5% NaCl). Control aliquots were similarly iced and gassed. Several replicate samples were withdrawn after appropriate doses and plated with an excess of sensitive cells in soft top agar. Plaques were scored after 18-24 hours of incubation at 37°C.

Dose was measured using ferrous sulfate, assuming a G value of 15.5 molecules per 100 ev. The x-ray source was a General Electric maxitron operated at 250 Kevp, 30 ma, with an HVL of 1 mm. Cu, and a dose rate of about 1200 rads per minute.

Results. Figure 1 shows the results from one such experiment. The graph indicates: (1) no appreciable oxygen effect on free phage, in broth; (2) no effect of the doubled broth concentration; (3) an oxygen effect on the complex of a factor of 1.8; and (4) a mean lethal dose for free phage and the aerobic complex of about 36,000 rads and about 65,000 rads for the anaerobic complex. No significant change in titre was found in the controls.

Discussion. The apparent absence of an oxygen effect on free phage irradiated in broth agrees with the work of others (Hewitt and Read, 1950). The magnitude (36,000 rads) of the mean lethal dose of broth irradiated phage T2 agrees well with the values of 39,000 roentgens (Luria and Exner, 1941) and 40,000 roentgens (Watson, 1950). One roentgen here is about 0.9 rad. The magnitude of the oxygen effect in the complexes is similar to that found in cells (Hollaender, Baker and Anderson, 1951), although this agreement may be

fortuitous. The existence of an oxygen effect on intracellular phage T2 has been independently confirmed (Flanders, 1959).

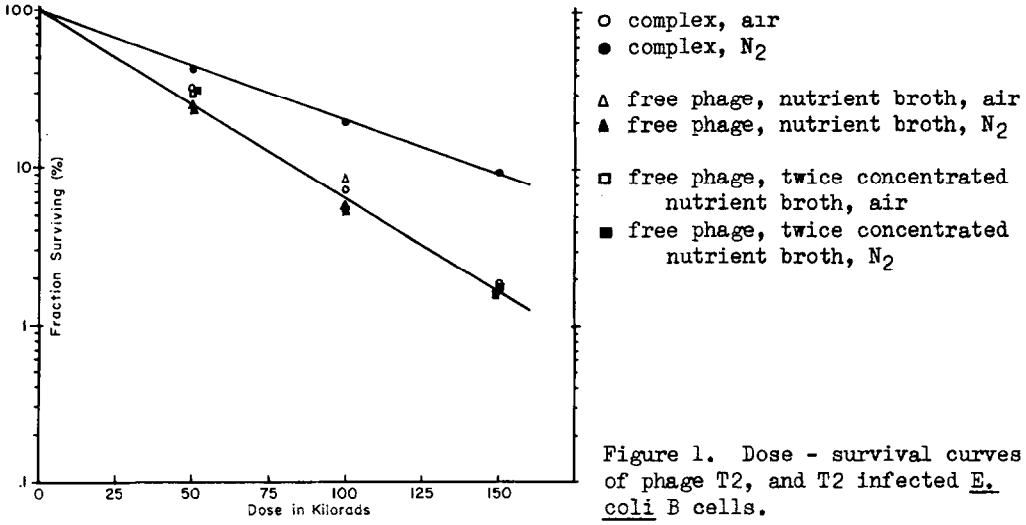

Figure 1. Dose - survival curves of phage T2, and T2 infected E. coli B cells.

The reduction in x-ray sensitivity of the intracellular phage by anaerobiosis is in addition to the reduction brought about by the addition of broth to suspensions of free phage or, presumably, by the intracellular constituents. The working hypothesis has been adopted that these oxygen-effect results are determined by two competing processes, one involving oxygen (but not water free radicals) and the other, a repair which can take place only inside the cell. If oxygen is present, this repair or restitution is blocked. It seems likely that the oxygen effect is on DNA.

We have x-irradiated free phage and host cells simultaneously, under nitrogen, followed by mixing and maintenance of the gas state for a time before assaying (15 minutes at 0°C plus 15 minutes at room temperature). The survival of phage so treated was the same as that for free phage aerobically irradiated and plated. It has been shown (Watson, 1950) that x-ray inactivated phage will still adsorb. Multiplicity reactivation (Luria, 1947) and marker rescue (Doermann et al., 1955) have been shown to be low (Watson, 1950; Harm, 1958) following extracellular irradiation of phage, but high (Weigle and Bertani, 1956; Harm, 1958) if the phage is irradiated intracellularly. The evidence seems to indicate an early step damage (Weigle and

Bertani, 1956; Harm, 1958) which may result in incomplete injection of the infecting phage's genome. Thus, although our early experiments may not have satisfied some requisite condition, it seems more likely that early step damage results in incomplete injection and hence repair cannot take place.

It must be mentioned that irradiation of the host cells alone with doses twice as large as the highest dose used in these experiments has been shown (Latarjet, 1948; Stent, 1958) to have no appreciable effect on their ability to produce at least one phage particle upon subsequent infection with phage T2.

Summary. Under the experimental conditions outlined in the text, an effect of oxygen on the x-ray inactivation of intracellular bacteriophage T2Hr has been found. Some of the possible implications of these results have been discussed.

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