THE INACTIVATION OF BACTERIOPHAGES BY X-RAYS—INFLUENCE OF THE MEDIUM

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Several studies on the inactivation of bacteriophages by x-rays have recently been published.[1] Qualitative experiments of Wollman and Lacassagne have shown that the x-ray sensitivity of different phages suspended in broth increases with their particle size as determined by ultrafiltration and centrifugation.[2] Holweck, Luria and Wollman have analyzed the inactivation of C16 phage by x-rays and alpha-particles, and interpreted their results in terms of the "hit theory." Further discussion of the same data has been given by Lea.[3]

We have carried out experiments with x-rays on a series of bacteriophages (3K, C16, C13, P28), some of which have been measured by Elford.[2] In all experiments the concentration of phage particles was determined by the plaque count method. Samples were irradiated in celluloid tubes with careful attention to x-ray dosage measurement. The same x-ray generator was used for all exposures. Technical details will be described in a complete account to be published later.

1. When bacteriophages are irradiated in the original filtrate or in broth dilutions the results (Figs. 1 and 2) can be summarized as follows:

(*a*) The curve of inactivation (percentage of remaining phage as a function of the dose of x-rays measured in *r*) is exponential in the first part of its path (straight line in semi-logarithmic plot). When the dosage becomes very high (Fig. 2) the rate of inactivation shows an increase, which however, is so slight as to be detectable only because of the high precision obtained in the phage assay. This increase in inactivation rate with high dosage had also been found in the experiments of Exner and Zaytzeff-Jern[1] and is probably due to superposition on the principal inactivation process of some effect of a different kind. We hope to determine the nature of this effect.

We have followed the inactivation to a diminution in titer by a factor of 2×10^{-7} and have always found consistently reproducible results. The inactivation is a function of the total dose of x-rays, and not of the dosage rate (no "time factor"). The fractional inactivation produced by a given dose is independent of the concentration of the phage suspension during irradiation.

(*b*) For x-ray tube voltages between 200 and 1000 kv. the inactivation rate has been found independent of the quantum energy (wave-length) of the x-rays. This statement refers to x-ray dosage measurements based on a Victoreen thimble chamber (wall thickness increased for measurements at highest voltage). It is of interest to point out the difference between this result and that obtained with tobacco mosaic virus by Gowen,[4] who finds a definite wave-length effect.

(*c*) The x-ray sensitivity of each bacteriophage is a highly reproducible property of that strain. The inactivation curve for phage C16 obtained a year ago by Holweck, Luria and Wollman in Paris coincides exactly with the curve established by us on the same phage. The two samples, though of common origin, had been kept many years in different surroundings and grown on bacterial hosts of different species (Dysentery Y6R and Coli 234).

(*d*) The slope of the inactivation curve is different for the different phages (Fig. 1). Phages of smaller particle size are more resistant. In table 1 are shown the dimensions of the "sensitive volumes" as calculated

TABLE 1

BACTERIOPHAGE	SUSCEPTIBLE ORGANISM	INACTIVATION DOSE* IN ROENTGENS	TARGET DIAMETER, MILLIMICRONS	PARTICLE SIZE (ELFORD) MILLIMICRONS
3K	Staphylococcus "K"	45,000	48	50–70
C16	Coli 234 or Dysentery Y6R	40,000	50	50–75
C13	Coli 234	115,000	32	25–30
P28	Coli "PC"	90,000	36	.....

* Dose giving inactivation ratio $N/N_o = 1/e$.

according to Lea's method,[3] and the size of the particles as given by Elford.[2] It is evident that the two series of values fall close to one another, in agreement with the hypothesis (Holweck, Luria and Wollman) that in the case of bacteriophages the sensitive volume corresponds to the phage particle itself. The small particle size of P28 phage seems to be confirmed by centrifugation experiments now in progress. The utility of the radiation method as a means of gaining information about the size of ultramicroscopic structures seems to be well borne out by these results.

2. We wish now to call attention to a new aspect of the inactivation of bacteriophages by x-rays, which becomes evident when phages are suspended in different media.

If a bacteriophage filtrate is diluted in distilled water, instead of broth, so that less than 1 per cent of the original filtrate is present, *its inactivation by x-rays is found to be greatly increased.* A dose of x-rays that leaves 40 per cent active phage in broth inactivates more than 99 per cent of phage diluted 10^{-3} in distilled water. This effect and the other facts related below have been found to hold for all four phages so far investigated, although

the most thorough of our studies have been made on the 3K and C16 phages. We have established the following facts:

(*a*) The effect of distilled water as a medium does not depend on difference of pH as compared to broth. Broth and water suspensions with pH adjusted to different values between 6.8 and 8.0 were irradiated. All broth suspensions gave closely the same inactivation rate. On the other hand, the water suspensions all showed the higher order of sensitivity found with distilled water.

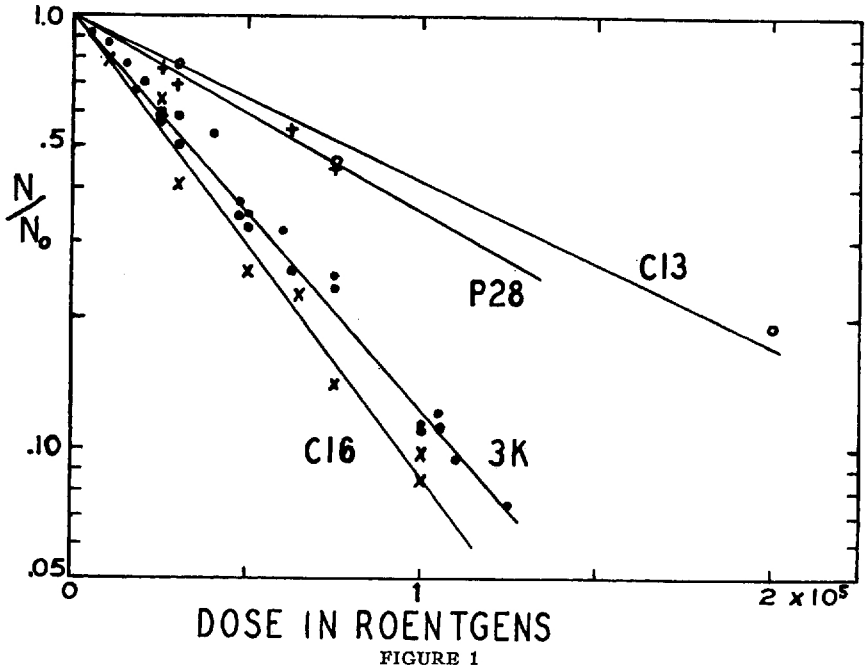

FIGURE 1

Inactivation of four bacteriophages suspended in broth. Abscissas: dose of x-rays. Ordinates: proportion of phage particles remaining active after irradiation.

(*b*) Suspensions of phage in different saline solutions (Na, K, Ca and Mg in various proportions) and in phosphate buffer show sensitivities of the same order as that of water suspensions.

(*c*) Suspensions of phage in aqueous solutions of ordinary gelatin or of Eastman Purified Gelatin behave like broth suspensions whenever gelatin is present in concentrations above 10^{-4} gm. per cm.[3]

(*d*) Protection against x-ray inactivation, similar to that exerted by gelatin and broth, though less complete, was found when solutions of egg albumin and serum albumin were used as media.

(*e*) No protection was given by either reducing or non-reducing sugars (dextrose, sucrose).

(*f*) Oxidizing (H_2O_2) and reducing agents (Sodium Thioglycolate and Cysteine Hydrochloride) added to un-irradiated phage suspensions inactivated the phage. Similar observations have been reported by other

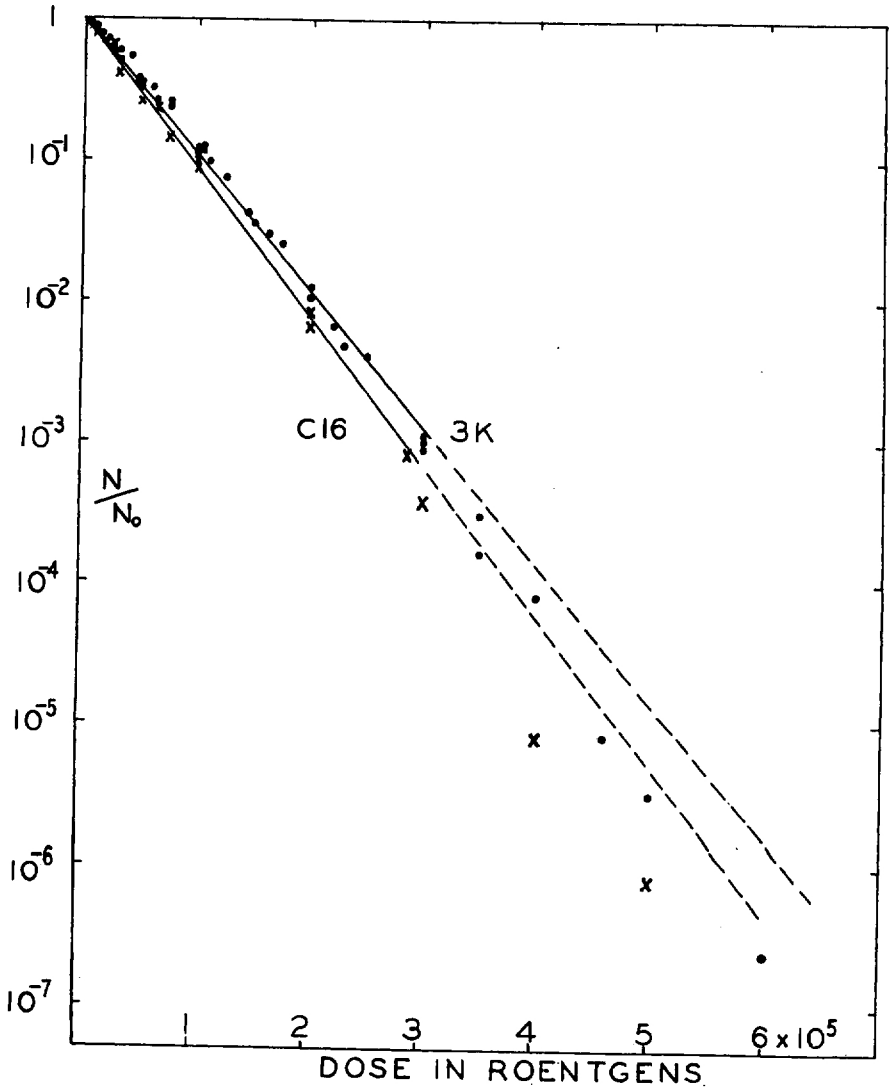

FIGURE 2

Extension of two of the inactivation curves shown in figure 1.

workers.[5] We have found such inactivation to be much more rapid in distilled water, saline, etc., than in those media (broth, gelatin, etc.) which protect phages against the action of x-rays. Concentrations of protective

substance necessary to give effective protection are the same in both cases. On the other hand concentrations of thioglycolate and cysteine low enough to have slight or no effect on the activity of phage gave no protection against x-ray inactivation.

(*g*) Broth, gelatin and albumins give complete protection against the slow spontaneous inactivation which phages undergo when highly diluted in distilled water or saline solution.

(*h*) With progressively lower dilution in distilled water, so that the concentration of original broth filtrate is progressively higher, the inactivation rate of phage by x-rays diminishes. At concentrations of broth above 1 per cent the sensitivity to x-rays becomes constant, and identical with that of suspensions in undiluted broth. The protection is not due to substance of bacterial origin present in the filtrate since the addition of 1 per cent of new broth or of as little as $1/10^4$ gm. per cm.^{3} gelatin to dilute suspensions of phage in water is enough to protect phage in the same measure. These quantities of broth and gelatin represent roughly comparable amounts of protein.

(*i*) No amount of gelatin or albumin added to the broth suspensions of phage gives any additional protection. *There exists a minimum sensitivity to x-rays for each phage*, which is the sensitivity measured in broth suspensions and analyzed in part 1 above.

(*j*) The action of x-rays on phage suspensions in water is not, as in broth, a function of the dose of radiation only. A large "time factor" is found, in the sense that a given dose is more effective the longer the time over which it is spread (within the range tested). We have not yet been able to establish whether inactivation continues after the end of the irradiation. However, water irradiated alone and inoculated immediately thereafter with phage failed to show higher inactivating power than un-irradiated water.

(*k*) The inactivation by x-rays of *different phages* suspended in water does not follow the same rules as the inactivation in broth. First it should be said that it is difficult to establish the shape of the inactivation curves owing to the presence of spontaneous inactivation and to the "time factor." However, the order of difference in sensitivity between small and large phages is clearly reversed: small phages suspended in water are inactivated somewhat more easily than large ones. In this connection it is of interest that these small phages are more sensitive also to the action of heat.[6]

Discussion.—The observations listed under parts 1 and 2 above appear as a whole to fit the following interpretation:

The inactivation of bacteriophages by x-rays is at least a twofold effect.

First there is an "indirect" effect predominant in water or saline suspensions, as described under part 2 above. This effect is probably exerted

through some inactivating agent produced in the medium as a consequence of the irradiation. This agent has a short life, as no inactivating effect is shown by previously irradiated water.

The mode of action of this agent on phages resembles that of oxidizing and reducing agents in that protection is afforded in both cases by the presence of small quantities of foreign proteins. This protection seems likely to be due to competition between the foreign protein and phage in reacting with the inactivating agent.

In the presence of foreign proteins bacteriophages are still inactivated by x-rays, but with reduced sensitivity and with other relationships as listed under part 1 above. This action we consider to be a "direct" effect on the phage particle itself, which can be analyzed according to the pattern of the "hit theory." This treatment will be developed more fully in a subsequent paper.

The indirect effect of x-rays on bacteriophages through the medium appears similar to the effects obtained by different authors[7] with enzymes and simple proteins.

A protective action of foreign proteins against x-ray inactivation of papilloma virus suspended in saline has recently been observed.[8] This is apparently another instance of the type of protection described above for phage.

It has been shown that with bacteriophages under suitable conditions both a direct and an indirect effect of radiation can be distinguished. This should serve to emphasize the importance of considering both types of action when analyzing the effect of radiation on a particular biological system.

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