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GESELLSCHAFT FUR KERNFORSCHUNG M. B. H.

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Short Communication

A Characteristic Difference of Radiosensitivity between Phage Containing Single- and Double-Stranded Nucleic Acid

GERHART HOTZ
Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, Germany

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The protective effect of glycerol against biological damage caused by ionizing radiation has been established for a variety of biological systems. Modification of radiation damage in phage T1 is assumed to result from exchange of water by glycerol on surfaces usually occupied by water molecules (HOTZ, 1962).

Comparing the influence of drying on the radiosensitivity of single- and double-stranded DNA-phage a significant difference was observed (HOTZ, 1968). The inactivation dose (i.e. $D_{37\%}$) of gamma irradiated dried phage T1 was increased by a factor of 4.2 (± 0.06) compared to phage suspended in 4% Difco nutrient broth. When phage ΦX-174, however, was studied under the same experimental conditions this factor amounted to only 1.95 (± 0.08). From this observation the conclusion is drawn that the amount of biological damage due to radiation induced chemical reactions occurring in the water layer surrounding nucleic acids is dependent on the structure of these macromolecules, i.e. single- or double-strandedness. Consequently, a different radiobiological response is expected if phages with different types of nucleic acid structures are inactivated by ionizing radiation in the absence and presence of glycerol. The purpose of this short communication is to give some experimental evidence in favour of this hypothesis.

The phages used are from our collection of strains which originates from samples kindly supplied by the following laboratories. The T-phages and P22 from Dr. STARLINGER, Köln, 1958; ΦX-174 and MS2 from Dr. SINSHEIMER, Pasadena, 1960 and 1963, respectively; f2 from Dr. ZINDER, New York, 1963. The technique used for inactivation of the plaque-forming ability by ^{60}Co-gamma and X-irradiation as well as the radiation dosimetry have already been described in detail (HOTZ, 1962; HOTZ and ZIMMER, 1963).

Fig. 1 shows the effect of different concentrations of glycerol on the sensitivity of some DNA and RNA containing phages suspended in 4% Difco nutrient broth against ionizing radiation. Most of the so-called indirect effect of ionizing radiation in aqueous solutions should be avoided in this system. Since all dose-survival curves follow a simple exponential function we describe the changes due to varying the concentration of glycerol by the slopes of the inactivation curves. Factors of protection and sensitization are given by ratios of slopes in the absence and presence of glycerol. From the data shown it is evident that:

(i) Relatively high concentrations of glycerol are necessary to cause an appreciable radioprotection in phage.

(ii) After reaching a maximum of protection sensitization by high concentration of glycerol is observed.

(iii) Only phage strains containing double-stranded DNA are protected effectively by glycerol. Phage containing single-stranded DNA or RNA, however, do not show this effect.

Result (i) can best be explained by simple competition of glycerol with water for adsorption sites in the phage. This hypothesis is strongly supported by the ability of the LANGMUIR adsorption equation to describe radioprotection by

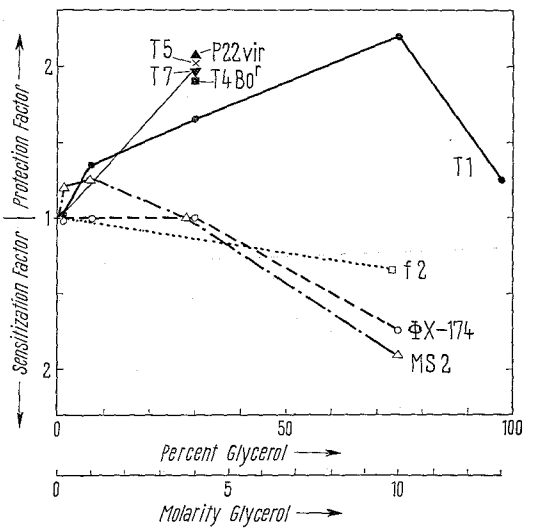

Fig. 1. Dependence of relative radiosensitivity (plaque-forming ability) of different phage under aerobic conditions on concentration of glycerol in 4% Difco nutrient broth. The effect of glycerol is expressed in terms of protection- and sensitization-factors against gamma radiation. Five strains of phage with double-stranded DNA (T1, T4 *Bo^r*, T5, T7, and P22*vir*), one containing single-stranded DNA (ΦX-174) and two strains of RNA-phages (MS2 and f2) have been tested

glycerol (WEBB, 1963). The observation that dehydration protects phage ΦX-174 against damage by ionizing radiation (HOTZ, 1968), while glycerol gives no protection at all, suggests that exchange of the molecules water-glycerol is an important part of the protective mechanism rather than simple removal of water.

It seems to be reasonable to explain result (ii) by a phage-inactivating effect of radiation induced reaction products of glycerol which are less abundant and, in addition, buffered effectively by the unspecific protein of the nutrient broth at low molarity.

Either the structural variations of the protein membranes or the configurational differences of the nucleic acids between the large phages containing double-stranded DNA and the small phages may be responsible for result (iii). As regards damage to the phage protein by diffusible species of irradiated aqueous solutions we assume our glycerol-nutrient broth system to be comparable (up to a glycerol concentration of 80%) to the widely used 0.8% Difco nutrient broth concerning the high capacity of this solution to react with phage-inactivating agents from irradiated water. Furthermore damage to protein by direct radiation effects is supposed to be rather negligible compared with damage to the nucleic acid of

phage. Therefore we propose that the radioprotective action of glycerol is on the nucleic acid of bacteriophage. It seems plausible to assume that water molecules bound to nucleotides mediate radiochemical reactions within the Watson-Crick helix from the radiation energy absorbing strand to the other sister-strand. This energy transfer is blocked when the water molecules are replaced by glycerol. If this hypothesis is valid we should expect a reduced radiation damage in all double-stranded phage but no effect of glycerol on the number of lethal hits per unit dose in a single-stranded nucleic acid structure. If this remarkable radiobiological behaviour of the phage found in our experiments would hold generally a glycerol test on the basis described might work as an easy assay to classify phages of unknown nucleic acid structure.

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Dr. G. Hotz
Institut für Strahlenbiologie
Kernforschungszentrum Karlsruhe
75 Karlsruhe 1, Postfach 3640