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SUPPRESSION BY CYSTEAMINE OF RADIOSENSITIZATION IN 5-BROMODEOXYURIDINE SUBSTITUTED PHAGE T1.

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Radiosensitization of biological systems, by specific chemical alteration of molecular structure, has attracted great interest during recent years. The incorporation of analogues such as 5-bromouracil into the DNA, substituting naturally occurring bases, has been found to increase the radiosensitivity of phages, bacteria, and mammalian cells to ultraviolet light (UV) and X-rays (Stahl et al., 1960, Greer and Zamenhof, 1957, Djordjevic and Szybalski, 1960). Kaplan et al. (1962) found that substitution of certain DNA bases results in sensitization of Escherichia coli only if oxygen is present during ionizing irradiation. Erikson et al. (1961) demonstrated the same relative radioprotection by glycerol in normal and BUDR-substituted X-irradiated mammalian cells. But glycerol did not abolish the radiosensitization caused by the base analogue. In spite of all these studies no convincing hypothesis for the mechanism of sensitization against ionizing radiation by the analogues has been put forward. Recently, Müller, Köhnlein and Zimmer (1963) using the electron spin resonance method, showed that formation of a free radical by ionizing radiation in thymine requires 1200 eV but in 5-bromo-uracil 150 eV only. This finding may well be of importance for designing a model for the sensitizing action of halogen-substituted DNA-bases.

Sulfhydryl compounds of the cysteine-cysteamine group are well known to protect against some biological effects of ionizing radiation

and phages of the coli-T-group were demonstrated (Hotz 1962, 1963) to be a most convenient material for studying the action of sulfhydryls under a variety of conditions. It seemed promising to investigate the effect of a sulfhydryl compound on preventing or repairing particularly those additional radiolesions which have been observed in BUDR-labelled phage. In this communication preliminary results will be reported which are part of a current investigation on BUDR-substituted phage.

In order to replace thymidine by 5-bromodeoxyuridine, T1 wild-type phage was grown following a method described by Stahl et al. (1961). All strains of phages and their hosts were generously supplied by Dr.C.Bresch, Cologne. For irradiations a ^{60}Cobalt gamma-source and a 6-Watt Hanau low-pressure UV-lamp (radiation emitted predominantly at 2537 Å) were used. The irradiation techniques, dosimetry, and the methods of growing and assaying the plaque-forming ability of the phage have been described previously (Hotz and Müller, 1960).

Samples of BUDR-T1 were irradiated under various conditions with UV-light and gamma-rays. From the experimental results some of which are shown in figures 1-3 it is evident that:

(a) The sensitizing effect is observed in phage irradiated with UV- as well as with gamma-rays.

(b) BUDR sensitizes T1 by equal factors (about 2) independently of the conditions of ionizing irradiation, e.g. presence or absence of water and of the temperature in the range between 80° and 300° Kelvin.

(c) Cysteamine, if present during irradiation, is able to remove completely the radiosensitization in wet BUDR-phage, but not in dry systems.

To our knowledge, the observation reported under (c) is the first instance that a halogen-substituted DNA has the radiosensitivity of a normal structure in the presence of cysteamine, though showing pronounced sensitization to UV-light and ionizing radiation in the absence of cysteamine. Three explanations may be considered for this protective effect:

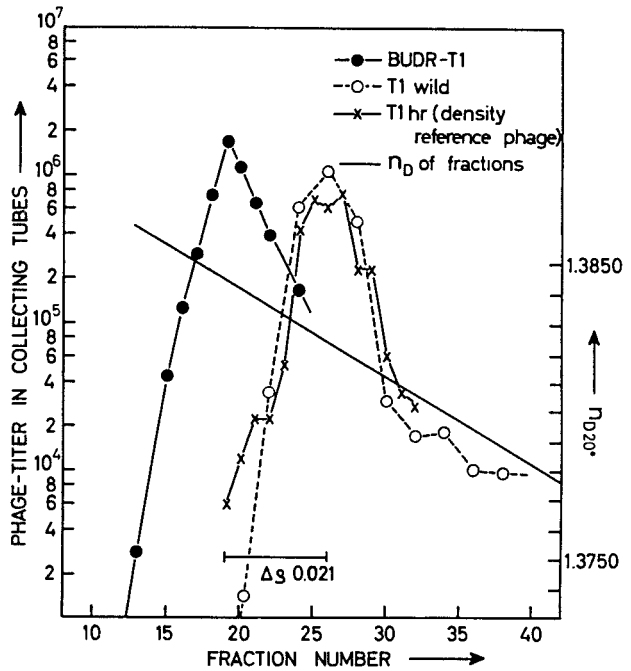

Figure 1. Density distribution in a "heavy" fraction of BUDR-T1 lysate. BUDR-T1 (filled circles), "light" wildtype phage (open circles), and density-reference phage T1hr (crosses). Phages were suspended in CsCl-nutrient broth solution containing 0.67 g CsCl/ml and centrifuged in the SW 39 Spinco rotor for 20 hours at 35 000 R.P.M. (Meselson et al., 1957). At the end of the run the bottom of the lusteroid tubes were pierced and the emerging drops collected in fractions of 5 drops each. The refractive index (n_{D20}) of the fractions corresponding to the density (ρ) of the CsCl-broth solution was measured by an Abbe-refractometer (Zeiss).

(i) The SH-compounds are "ordinary" radioprotectors - acting according to various hypotheses (Alexander et al., 1955, Eldjarn et al., 1956) suggested for the protective mechanism - with an extreme efficiency of preventing the radiation damage due to BUDR.

(ii) BUDR built into the DNA blocks an intrinsic system of the phage structure capable of protecting against or repairing lesions caused by irradiation. This "block" might be prevented by the addition of a substance like cysteamine.

(iii) The sulfhydryl-substances undergo a chemical reaction with the irradiated bromouracil. This reaction might result in preventing the

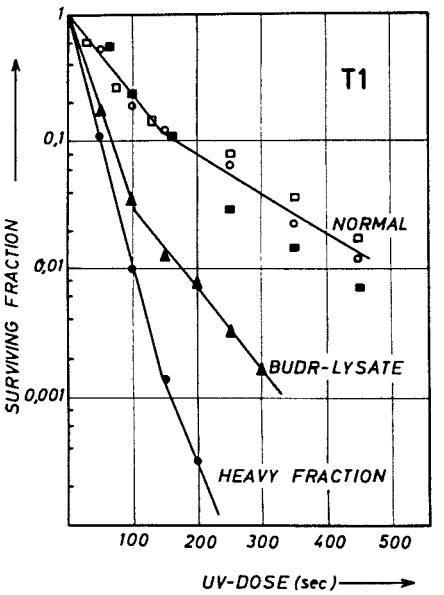

Figure 2. Surviving fraction of normal (O) and BUDR-substituted T1 (▲) after irradiation with ultraviolet light. Phage lysates were diluted one-hundredfold in 0.8 % NaCl-solution. Survival of a dense fraction (●) separated from a BUDR-lysate by gradient centrifugation. The effect of M/100 cysteamine on normal T1 (□) and on the heavy labelled fraction of BUDR-T1 (■).

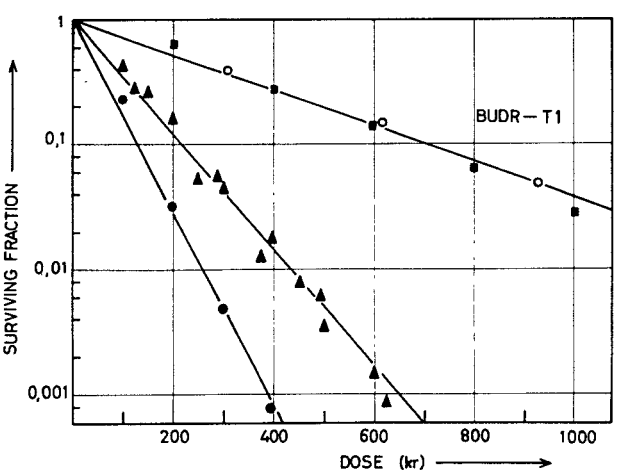

Figure 3. Surviving fraction of T1 suspended in 4 % Difco-nutrient broth after aerobic irradiation with a ^{60}Cobalt gamma-source. The curves represent the survival for normal T1 (▲), dense fraction of CsCl-gradient centrifugation of BUDR-T1 lysate (●), protective effect of M/100 cysteamine present during irradiation on normal T1 (O) and on the dense fraction of BUDR-T1 (■).

destruction of the analogue molecule and/or in scavenging phage-inactivating radiation products arising from the irradiated base analogue.

If explanation (i) were correct, the UV-irradiated normal phage should be protected by cysteamine as observed for many strains of X-irradiated normal phage (Hotz and Müller, 1960, 1961). Such protection, however, is not observed at all in our experiments, therefore making hypothesis (i) very unlikely.

The "intrinsic protective system" tentatively proposed in explanation (ii) can not be excluded by our experiments. It should be related neither to the enzymatic repair mechanism observed in phage T2v (Streisinger, 1956, Harm, 1958, Stahl et al., 1961), nor to photoreactivation, nor to the host-cell reactivation of phages like T1 (Sauerbier, 1961) all of which processes are efficient after UV-irradiation only.

Explanation (iii) is the most likely one, as an ordinary chemical reaction - possible in a wet system but not in the dry state - might account for our observations. It is of interest that dehalogenation of incorporated base analogues after UV-irradiation of "heavy" DNA was observed recently (Wacker et al., 1962). Such reactions and the influence of SH-compounds on these processes should occur after UV- and ionizing irradiation. This is the observation made in our experiments, and lead to the conclusion that the radiosensitization due to incorporation of halogen-substituted base analogues into phage-DNA, has the same origin for UV- and ionizing radiation. Sauerbier (1963) made the interesting observation that compared to free BUDR-phage, BUDR-T1 UV-irradiated after preadsorption to E.coli B_{s-1} does not show enhancement of radiosensitivity. It is tempting to speculate that in a phage-host cell complex, sulfhydryl compounds present in the cytoplasm of the bacterium eliminate the BUDR-effect.

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