KERNFORSCHUNGSZENTRUM

KARLSRUHE

Juli 1963

KFK 206

Institut für Strahlenbiologie

Experiments in Radiation Chemistry of T1-Phage

G. Hotz, K. G. Zimmer

GESELLSCHAFT FÜR KERNFORSCHUNG M. B. H.

KARLSRUHE

Reprinted from
INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, Vol. 7, No. 1, p. 75, July 1963

Experiments in radiation chemistry of T1-phage

G. HOTZ and K. G. ZIMMER

Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, Germany

(*Received* 13 *June* 1963)

Addition of sulphhydril-compounds, freeze-drying and low temperature reduces inactivation by ionizing radiation of plaque-forming ability in T1-phage by various and nearly additive amounts : if combined these changes of circumambient conditions during irradiation lead to ' factors of protection ' of $p=16$ (as compared to irradiation in highly concentrated broth). The results fit a model of radiation effects as proposed by Alexander, Howard-Flanders *et al.* after its extension to provide for the effects of low temperature. Existing physico-chemical evidence in support of the model is discussed briefly. Replacing some of the thymine in the DNA of T1 by 5-bromouracil leads to a sensitization of inactivation by ionizing radiation which neither depends on nor changes the efficiency of the ' protective measures ' mentioned above with one exception : when irradiation is done in sulphhydril-containing broth *sensitization* by 5-bromouracil vanishes completely.

1. INTRODUCTION

The inactivation by ionizing radiation (IoR) of various abilities of viruses was the subject of innumerable studies. Nevertheless, we know little about the physico-chemical reactions started by the absorption of IoR in phage, i.e. about the radiation chemistry leading to inactivation of some ability or other. Previous studies can be divided into groups concerned mainly with (i) the application of the target hypothesis (e.g. Lea 1946, Pollard 1954, Bonét-Maury 1955), or (ii) with the radiation chemistry of acqueous solutions and its bearing on radiobiology (e.g. Alper 1955), or (iii) with the use of radiations as a tool for investigating problems of virus-genetics (e.g. Stahl 1959). The mechanisms of inactivation *per se* attracted less interest (for an excellent summary and bibliography of earlier work cf. Gard and Maaløe 1959).

Inactivation of plaque-forming ability (PFA) is the ability of phage lending itself most easily to experimental work and was, consequently, the reaction studied by most investigators, though parallel investigations of other reactions furnish valuable additional information (e.g. Watson 1950, 1952).

2. INACTIVATION OF PFA : DAMAGE TO PROTEIN OR TO DNA ?

Inactivation of PFA may be caused by damage to protein as well as to DNA. In work with ultra-violet (U.V.)-radiation, damage to protein can, because of specific absorption of radiations of different wavelengths, be separated fairly easily from damage to DNA (Winkler, Johns and Kellenberger 1962). With IoR specific absorption by organic molecules is absent, and equivalent experiments

are much more difficult to design. If we exclude the actions by diffusible agents generated by IoR in aqueous suspensions (for which reactions with the proteins of phage were shown to occur), inactivation of PFA by IoR-damage to the protein of phage has, to our knowledge, never been demonstrated directly. In fact, we know of but few related experiments: (i) Irradiation of a thin layer of dry T1 by electrons in the energy-range of 0–1500 ev showed very little or no inactivation of PFA until the energy of impinging electrons was raised to about 700 ev (Davis 1954). (ii) X-ray-inactivated T2 phage is as effective a coating agent for heat-killed bacteria as active T2, thus demonstrating the adsorption ability of x-ray-inactivated phage (Watson 1950). (iii) The ability of T1 and T2 to attach to bacteria can be inactivated by high doses of IoR (Pollard and Setlow 1956). (iv) Serological affinity of T1 is changed by very large doses of IoR (Pollard and Setlow 1954).

The experiment with slow electrons was interpreted to indicate that no inactivation of PFA occurred until the range of the electrons surpassed the thickness of the head membrane which, from the known range of electrons, was estimated to be about 120 Å. This interpretation implies that damage by electrons absorbed in the protein of the head membrane does not lead to inactivation of PFA. Such an assumption, though obviously correct for the doses of electrons used, does not necessarily hold for much larger doses, nor would we expect sufficiently heavy doses of electrons impinging on the phage-tails to be without effect on PFA. Consequently, both the experiments (i) using slow electrons and (ii) measuring adsorption ability of x-ray-inactivated phage, seem to indicate a preponderance of damage to DNA over that to protein at low doses of IoR†, whereas at high doses damage to adsorption ability and other forms of protein damage occur. Experiment (iii) permits a rough estimate to be made: Inactivation of PFA in dry T1 by IoR requires a D_{37} of about 2×10^3 ev absorbed per phage and inactivation of the ability to adsorb a D_{37} of about 4×10^4 ev absorbed per phage, or 20 PFA-hits.

As mentioned before, it is not easy to design experiments yielding information on the radiation chemistry of phage-DNA. In the present paper we describe attempts to get such information by experiments on inactivation of PFA by IoR: (*a*) under various circumambient conditions (temperature, presence or absence of water and of oxygen), (*b*) in the presence of so-called ' radioprotective ' substances (glycerol, cysteine, cysteamine), (*c*) after incorporation of the ' radiosensitizing ' base analogue 5-bromouracil. In the discussion we shall also make use of the results of experiments on the production of free electron spins by IoR in relevant materials conducted in parallel with the inactivation experiments and reported in detail elsewhere (Köhnlein 1963, Müller 1963 a, Müller, Köhnlein and Zimmer 1963, Zimmer, Köhnlein, Hotz and Müller 1963, Zimmer 1961).

3. Materials and methods

(i) *Bacteriophage* T1, T1hr and the hosts *Escherichia coli* B and B/1g were kindly supplied by Dr. C. Bresch, Cologne, in 1958. The methods of growing and

† If one wants to take into consideration arguments based on the target hypothesis, the correlation of target-weight and DNA-content found in a variety of viruses might be mentioned here (Epstein 1953, Ginoza and Norman 1957). On the other hand, observations like ' early-step damage ' (Weigle and Bertani 1956, Harm 1958) might be interpreted as being due to some form of protein-damage by IoR.

assaying the plaque-forming ability of the phage have already been described (Hotz and Müller 1960). Purified and concentrated suspensions of T1 were diluted tenfold into 4 per cent Difco-nutrient broth.. After this dilution the actual phage titer was usually around 10^{10} particles per millilitre. For irradiation in the presence of the SH-compounds, cysteine or cysteamine was dissolved in the broth before adding the phage.

(ii) *X-irradiations* of T1 in suspensions equilibrated with various gases were performed according to a standard technique previously described (Hotz and Müller 1960, 1962, Hotz 1961, 1963 a).

(iii) *Gamma-irradiation* was carried out using a ^{60}Co gamma-source (Gamma-cell 220, Atomic Energy of Canada Ltd.). The source was loaded with 6×10^3 Curie delivering during the time of this investigation about 7 kr/min at the point the sample was mounted. Irradiation of phage-suspensions was performed in a closed plexiglass cup of 30 mm diameter (thickness of the walls 4 mm, for electron equilibrium) containing 4 ml of the suspension.

(iv) *Desiccation of phage*: For experiments with phage in the dry state, 0·2 ml samples of appropriate phage-suspensions were pipetted into glass ampoules and desiccated by evaporation or freeze-drying, both methods giving the same results concerning the effectivity of dehydration. Samples containing SH were always freeze-dried to avoid oxidation and local concentration of SH, as the latter led to irregular toxic effects on phage. After the samples looked fairly dry, evacuation was continued for another 48 hours. Thereafter the ampoules were sealed in vacuum better than 10^{-2} torr. After irradiation the ampoules were opened and the dry substance resuspended in 5 ml of 0·8 per cent Difco-nutrient broth.

(v) *Low temperature*: To irradiate phage at low temperature, the ampoules with dry phage were placed in a small Dewar bottle with liquid nitrogen fitting into the irradiation chamber of the gamma-source.

(vi) *Dosimetry* of the ionizing radiation absorbed in the irradiated biological material was done as described previously (Hotz and Müller 1960, Hotz 1963 a).

(vii) *Ultra-violet irradiation* was carried out with a 6 w Hanau low-pressure ultra-violet lamp, model 6/20, emitting radiation of 2537 Å mainly. Phage stocks were diluted hundred-fold in buffer and 4 ml of the suspension were irradiated in a 10 cm Petri dish at room temperature while stirring magnetically. After a given ultra-violet dose 0·1 ml samples were withdrawn and diluted for phage assay.

(viii) *Incorporation of 5-bromodeoxyuridine (BUDR)*: Stocks of BUDR-T1 were prepared by the 'method I' described by Stahl, Crasemann, Okun, Fox and Laird (1961). Quantitative analysis for base substitution and distribution of BUDR was performed by CsCl density-gradient centrifugation (Meselson, Stahl and Vinograd 1957). Phages were suspended in nutrient broth solution containing 0·67 g CsCl/ml and centrifuged in the SW 39 rotor of a Spinco model L for 20 hours at 35 000 r.p.m. At the end of the run the lusteroid tubes were pierced, and the emerging drops collected in fractions of five drops each. The refractive index (n_{D20}) of the fraction corresponding to the density (ρ) of the CsCl-broth solution was measured by an Abbe-refractometer (Zeiss). The difference in density ($\Delta\rho$) between a heavy fraction of BUDR–T1 and the density-reference phage T1hr was calculated to be 0·021.

4. Results

4.1. *Irradiation of phages in suspension*

There is no point in giving here a detailed description of our rather extended experiments published previously (Hotz 1961, 1962, 1963 a, Hotz and Müller 1960, 1961, 1962). In these the addition of glycerol, cysteine and cysteamine during irradiation of various phages (T-even, T-uneven and ØX174) suspended in broth was tested as to its influence on PFA in the presence and absence of air. Many of the results, though interesting in themselves, have but a limited bearing on our present problems. The relevant points can be summarized briefly as follows.

(i) The relation between dose of IoR and inactivation of PFA was found to follow a simple exponential function (first-order or one-hit law) in all experiments, except for cases of trivial complications. Hence we can describe the changes due to varying physical or chemical parameters by ratios of slopes of inactivation curves which, in a purely formal way we denote, according to the directions of change in slope, as protection-factors p or as sensitization-factors s.

(ii) The presence, in appropriate concentrations, of sulphhydril-compounds (SH) in broth such as H_2S, cysteine, cysteamine during irradiation with IoR reduces the inactivation of PFA in all phages tested by a factor of about $p=2$ to $p=3$.

(iii) In the absence of added SH, inactivation of PFA by IoR is in all phages tested largely independent of the presence or absence of oxygen, in striking contrast to the results found in the majority of other biological entities.

(iv) In the presence of added SH, oxygen increases the inactivation of PFA by IoR in all phages tested by a factor of about $s=2$.

(v) Phages that are resistant to osmotic shock (T1, T5, T7, T4Bo^r, P22) require the presence of SH in the suspension during irradiation, whereas non-resistant phages (T2, T4, T6) can form, during previous immersion in SH-solution, some kind of a fairly stable 'complex' with SH (they can bind, adsorb or encage SH), thus rendering the phage less sensitive to IoR in SH-free suspension. This 'complex' and the protection afforded decay with time at a rate depending on temperature (Hotz 1962, 1963 a, Marcovich 1962).

The results (i)–(iv) are compatible with a general model of the damage by IoR (Alexander and Charlesby 1955, Howard-Flanders 1960, Howard-Flanders and Jockey 1960, Alexander and Ormerod 1962). Result (v) neither contradicts the model, nor does it permit in the present state of our knowledge conclusions concerning our main problem.

4.2. *Irradiation of T1* in vacuo *and at low temperature*

Another series of experiments was, therefore, undertaken in which we limited ourselves to using T1, but extended the range of additional parameters. Again, all the dose–effect curves were of one-hit type. Consequently, the experiments and the results can be described and discussed very conveniently in terms of 37 per cent doses and of factors p and s as shown in table 1. Some of the effects observed are known from earlier work (e.g. Doerman as cited in Watson 1952, Bachofer, Ehret, Mayer and Powers 1953, Epstein and Schardl 1957), but we are not aware of any set of similar experiments using the same phage, the same source of radiation, and the same technique throughout. All the results we report are

based on complete curves of dose versus inactivation of PFA as obtained by several repetitions of the measurements for all the conditions studied (figure 1). The mean inactivation doses of PFA (D_{37}) are given in kiloroentgen and also in units of energy (ev) transferred to a volume of 10^{-16} cm^3 approximately representing the volume of a T1 phage. It is quite noteworthy what considerable amounts of energy are needed per phage to inactivate PFA: energies range from 500 ev for T1 at room temperature and suspended in broth to 8200 ev, if irradiation takes place at the temperature of liquid nitrogen in vacuum and in the presence of SH. This range corresponds to an overall protection-factor of $p_5 = 16{\cdot}4$ (cf. table 1) and illustrates clearly that the calculation of target-volumes does not necessarily lead to geometrically-meaningful results. The so-called indirect effects, i.e. actions produced by diffusible agents generated in water by IoR, are often assumed to be excluded if irradiation is done in concentrated broth, but careful freeze-drying brings about an additional protection of $p_1 = 4{\cdot}3$. The protection afforded by desiccation is obviously in part due to reducing the possibility of producing active species by IoR in the water inside the phage. But desiccation may also reduce damage to DNA by changing the course of reaction of 'DNA-radicals' formed by IoR. Addition of SH to the suspension in broth leads to a protection of $p_3 = 3{\cdot}3$ and combined with desiccation to $p_4 = 7{\cdot}9$. The finding that $p_1 > p_7$ (desiccation in the absence of SH gives a greater relative protection than desiccation with SH added) seems to support the view that part of the protection by desiccation in the absence of SH is due to avoiding damage by active species produced in water, which in the presence of SH would have been caught by this substance. On the other hand, $p_7 > 1$ shows other effects of desiccation such as inhibition of reactions of 'DNA radicals' to be of importance. The protection afforded by lowering the temperature during irradiation to 80°K ($p_6 = 2{\cdot}3$) is usually ascribed to making impossible reactions of 'DNA radicals' that otherwise might have led to damage. Such an assumption is not contradicted by $p_8 \approx p_6 > 1$ (about equal protection by low temperature in the presence and absence of SH), but needs, in our opinion, careful consideration for other reasons. The problem of protection afforded by low temperature and the finding that $p_5 > p_2$ will be discussed below.

4.3. *Irradiation of T1 containing 5-bromouracil*

To gain additional data on the radiation chemistry of T1-phage, a third series of experiments was run completely analogous to the second one, but using T1 containing the DNA base analogue 5-bromouracil (BU). From many previous studies in a variety of biological materials, we know that the effects of ultra-violet as well as of IoR are increased if the DNA contains certain analogues instead of the naturally-occurring bases (e.g. Stahl *et al.* 1961, Kaplan, Zavarine and Earle 1962), though no convincing model has been put forward for the mechanism of this sensitization towards IoR.

The results of the third series of our experiments are shown, together with the results of the second series, in table 2. It is at once evident that introduction of BU sensitizes the inactivation of PFA in T1 by equal amounts under all three conditions of irradiation studied ($s_{11} = s_{12} = s_{13} = 2$). When irradiating dry powder *in vacuo* and irrespective of temperature, the protection of phage by SH is not influenced by the introduction of BU ($p_{12} = p_{22} = p_{13} = p_{23} = 2$). Because of numerical equality $p_{22} = s_{12}$ and $p_{23} = s_{13}$, these cancel leading to $p_{32} = p_{33} = 1$.

Irradiation by Co-60 source: Material (amounts as in Table 1)	Conditions	D_{37} kr	Factors p of protection or s of sensitization
T1, H_2O, NB	air 300°K	95	
T1, NB	vac 300°K	410	
T1, NB	vac 80°K	950	
			$p_{11}=3$ $p_{12}=2$ $p_{13}=2$
T1, H_2O, NB, CY	air 300°K	310	
T1, NB, CY	vac 300°K	750	
T1, NB, CY	vac 80°K	1560	
			$s_{11}=2$ $s_{12}=2$ $s_{13}=2$
BU-T1, H_2O, NB	air 300°K	55	
BU-T1, NB	vac 300°K	180	$p_{31}=3$ $p_{32}=1$ $p_{33}=1$
BU-T1, NB	vac 80°K	510	
			$p_{21}=6$ $p_{22}=2$ $p_{23}=2$
BU-T1, H_2O, NB, CY	air 300°K	300	
BU-T1, NB, CY	vac 300°K	360	
BU-T1, NB, CY	vac 80°K	900	

Table 2. Second and third series of experiments on inactivation of PFA in T1 by IoR. D_{37} stands for mean inactivation dose, NB for nutrient broth, CY for cysteamine and BU–T1 for phage in the DNA of which about 80 per cent of the thymine is replaced by 5-bromouracil.

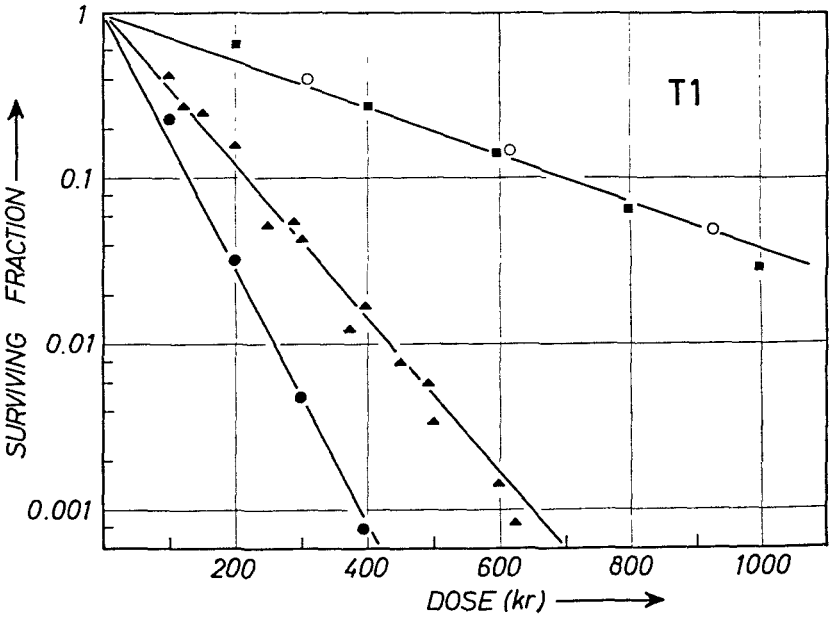

Figure 2. Inactivation of PFA in T1 and in BU–T1 by various doses of ^{60}Co gamma-rays. All irradiations done in air on phage suspended in 4 per cent Difco-nutrient broth. Normal T1 (▲), dense fraction of CsCl-gradient centrifugation of BU–T1 lysate (●), M/10 cysteamine in the suspension of normal T1 (○) and of BU–T1 (■).

Though this numerical equality is quite accidental, the results indicate clearly that in the dry systems sensitization by BU is not changed by the presence of SH. In the wet system, however, we find $p_{11}=3$ and $p_{21}=6$, and the relation $p_{21}/s_{11}=p_{11}=p_{31}$ indicates a complete protection by SH against the sensitization by BU in addition to the 'ordinary' protection afforded by SH (cf. figure 2). It is difficult to avoid the conclusion that protection by SH against sensitization by BU is due to an ordinary chemical reaction, which is possible in the wet system but not in the dry state.

5. Discussion

At this stage we should briefly consider what experimental evidence we have for and against the applicability to inactivation of PFA in phage of the various models of protection by SH suggested in the literature. In our experiments action of SH by competing for active species formed in water by IoR may be involved to a certain extent (as noted above), but not when carefully-desiccated phage is irradiated *in vacuo* at 80°K. The same experiments leave no room for explaining the protective action of SH by deoxygenation, though this mode of action may be of importance in other biological material. Vigorous reducing action on 'biomolecules' by SH has been suggested as cause for its protective ability, particularly for cases in which protection of some duration was obtained after immersion of T-even phages in SH-solutions before irradiation (Marcovich 1962). There is no need to consider this model here, as T1 does not show such an effect. Another model has attracted much interest: the formation of disulphide linkages between SH and proteins (Eldjarn and Pihl 1956). According to this model protective action of SH is caused by migration of a hole produced by IoR in protein to the S–S linkage, which is reopened. Earlier in this paper we quoted evidence that inactivation of PFA in the range from zero to several PFA-hits is due preponderantly to DNA-damage. It is difficult to imagine an extension from proteins to DNA of the disulphide-linkage model which, therefore, does not seem to apply in our case.

After quoting evidence against a number of models for explaining the protective action of SH against damage by IoR in phage-DNA, we should mention that such an action has indeed been demonstrated as occurring in suspension of transforming principle (Hutchinson 1961) and in freeze-dried nucleic acid of tobacco-mosaic virus (Ginoza and Norman 1957), i.e. for DNA and for RNA. We do not, therefore, loose touch with reality if we look for positive evidence in favour of the only model we are left with for explaining our experiments: the hydrogen-donation model developed by Alexander, Howard-Flanders *et al.* (Alexander and Charlesby 1955, Alexander and Ormerod 1962, Howard-Flanders 1960, Howard-Flanders and Jockey 1960, Howard-Flanders, Levin and Theriot 1963, Ormerod and Alexander 1963). Briefly, the model proposes five main reactions:

(1) irradiation by IoR: $RH \rightarrow R^{\cdot} + H$;
(2) in the absence of O_2: $R^{\cdot} + R^{\cdot} \rightarrow R\text{–}R$;
(3) in the presence of O_2: $R^{\cdot} + O_2 \rightarrow RO^{\cdot}_2$;
(4) SH present, O_2 absent: $R^{\cdot} + SH \rightarrow RH + S^{\cdot}$;
(5) SH present, O_2 present: Competition between (3) and (4).

For a discussion of the applicability of this model to inactivation by IoR of PFA in T1 we assume RH to stand for phage-DNA (in agreement with the findings set

out in §2) and consider the experimental evidence for reactions (1) to (5) to occur in DNA.

Evidence for reaction (1) rests so far mainly on electron-spin-resonance (E.S.R.) measurements. The formation by IoR of free radicals in purified preparations of phage-DNA was demonstrated clearly (Müller 1962, 1963 a), and this process was found to need a remarkably small amount of energy: less than 100 ev per free spin produced.

The evidence for the occurrence of reactions $R^{\cdot} + R^{\cdot} \rightarrow R\text{–}R$ and $R^{\cdot} + O_2 \rightarrow RO^{\cdot}_2$ describing the mechanisms of damage in the model we are discussing is quite good, at least *in vitro* (Lett, Stacey and Alexander 1961, Alexander, Lett and Itzhaki 1961), though not much is known about the same reactions *in vivo*, nor about their biological consequences. Nevertheless, one can easily imagine the formation of R–R (cross-linking of DNA), or of peroxyradicals $RO^{\cdot}_2$, to lead to severe damage in biological systems.

The process of protection by hydrogen-transfer from SH to other materials forms another basic assumption of the model. Some evidence for this was obtained, again by E.S.R., first in salmon sperm heads, which partly consist of protein, but recently in pure DNA too (Müller 1963 b). It was found that a freeze-dried suspension of sperm heads and cysteamine showed a certain type of E.S.R. spectrum when measured at 80°K immediately after irradiation by IoR done at the same temperature. On warming up to room temperature, the original spectrum gradually disappeared, and new lines developed which were attributed to the radical -S$^{\cdot}$ assumed to originate from reaction of SH with the 'DNA-radicals' (Alexander and Ormerod 1962, Ormerod and Alexander 1963). Although earlier measurements on nucleoproteins indicate that the results obtained with sperm heads are probably not blurred by the presence of protein, similar measurements with pure DNA are more decisive. They show clearly that the reaction $R^{\cdot} + SH \rightarrow RH + S^{\cdot}$ occurs and lend considerable support to the model.

We may, therefore, state that there is at least some experimental evidence for every reaction required in the model. Moreover, the model and the well-established fact that SH scavenges reactive species formed in water by IoR describe the data given in table 1 quite satisfactorily with one exception: the protection afforded by low temperature, irrespective of the presence or absence of SH ($p_6 \approx p_8 \approx 2$). Similar observations have frequently been made before, and in various biological materials. It is quite usual to 'explain' such a finding by saying that 'limitation of diffusion prevents the effects of active species that might otherwise have led to damage'. However plausible such a statement may sound, we should consider more carefully what it really means. The reasoning would be quite simple if the biological reactions could be measured at the same low temperature as applied during irradiation. Unfortunately, this cannot be done: any biological test needs warming-up first, which means re-establishing diffusion. The usual explanation seems, therefore, to assume tacitly that the active species, while frozen in during irradiation at low temperature, decay in some way not leading to damage and *before* diffusion, and thereby damage becomes possible during the unavoidable warming-up. One might, of course, postulate that a reaction leading to harmless removal of the dangerous reactive species becomes possible at lower temperatures than does the reaction leading to damage. But there is, to our knowledge, no evidence for such an assumption. Nevertheless, one might extend the model in the following way: assume another path of reaction

leading to splitting or reorganization of RH by IoR which, at low temperature, would be hindered by the Franck–Rabinovich effect. Alternatively, one might invoke here, as in many observations in radiation biology, hypothetical processes of 'energy-transfer' combined with the assumption that the particular process acting shows the required dependence on temperature. Even for explaining $p_5 > p_2$ (additional protection afforded by SH at low temperature, cf. table 1), we see no immediate necessity for invoking intermolecular energy transfer, as has occasionally been done. Migration of protons is not seriously restricted at 80°K and, consequently, repair by donation of hydrogen might be about equally effective at 80°K as at 300°K. Repetition of the experiments with more densely-ionizing radiation is under way, as the formation of 'thermal spikes' by such radiation might lead to different results and enable us to decide between various possibilities of explaining protection by low temperature.

It is more difficult to design a model explaining the various effects observed in experiments with BU-containing phage. In the dry state at room temperature as well as at 80°K, sensitization by BU and protection by SH are obviously quite independent of each other (cf. table 2 and §4.3). This permits the H-donation model to be applied to SH-protection as before and gives no clue as to the mechanism of sensitization by BU. In the wet state, however, SH protects as usual and,

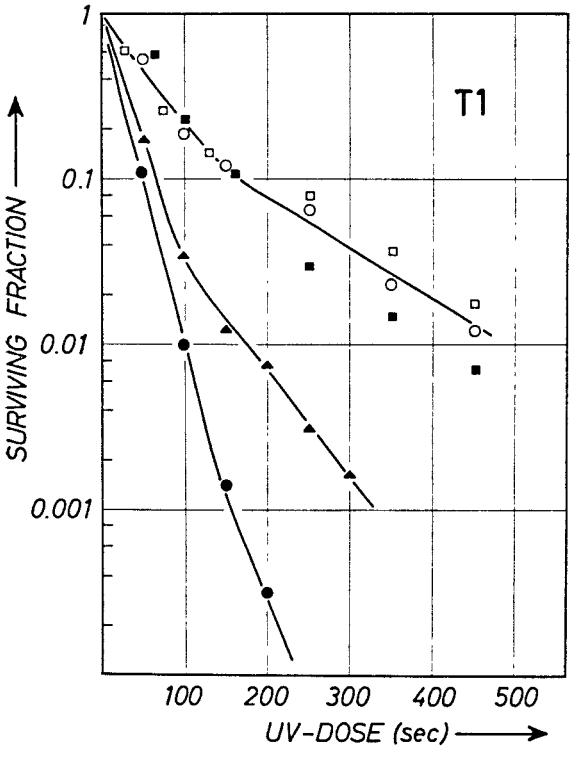

Figure 3. Inactivation of PFA in T1 and in BU–T1 by various doses of ultra-violet radiation. Phage lysates were diluted one-hundredfold in 0·8 per cent NaCl solution. Normal T1 (○), BU–T1 lysate (▲), dense fraction (●) obtained from BU–T1 lysate by gradient centrifugation, M/100 cysteamine in the suspension of normal T1 (□) and of BU–T1 (■).

in addition, completely eliminates sensitization by BU. This result seems to indicate the formation by IoR of some product of BU that either brings about damage to DNA (observed as sensitization), or may be neutralized by chemical reaction with SH in the wet state (unlimited diffusion). As to the nature of this reaction nothing much can be said at this moment. Measurements using the E.S.R.-method showed that formation by IoR of a free radical in thymine requires 1400 ev and in BU 160 ev only (Müller *et al.* 1963). This finding may well be of importance for designing a model for the sensitizing action of BU and for the protection against this sensitization by SH in wet systems. Moreover, the pronounced hyperfine structure found in the E.S.R. spectrum of irradiated BU at g-values higher than that of the free electron is likely to yield additional information (Köhnlein 1963).

In support of the view that protection by SH against sensitization by BU is quite different from 'ordinary' protection by SH another experiment should be quoted (in addition to the difference between the two kinds of protection already deduced from table 2): Inactivation of PFA in T1 by ultra-violet (2537 Å) in aqueous suspension is not protected against by SH (figure 3), it is sensitized by BU and this additional inactivation (due to BU) is fully protected against by the simultaneous presence of SH (Hotz 1963 b).

L'addition des composés sulphhydril, un séchage à froid et une température basse réduisent l'inactivation par les radiations ionisantes du pouvoir de former des plaques en phage-T1 aux degrés divers et presque toujours additifs: si combinés, ces changements des conditions circumambiantes pendant l'irradiation mènent aux 'facteurs protecteurs' de $p=16$ (par comparaison à l'irradiation d'un bouillon concentré). Les résultats sont conformes à une modèle des effets d'irradiation comme celui qui a été proposé par Alexander, Howard-Flanders *et al.*, compte tenue des effets de la température basse. L'évidence de nature physico-chimique qui donne appuy au modèle est discutée de façon courte. La remplacement d'une part de la thymine dans la DNA de T1 par 5-bromouracil mène à une sensibilisation de l'inactivation par les radiations ionisantes, inactivation qui semble ni dépendre de ni changer l'efficacité des 'mesures protecteurs' susdites, à l'exception suivante: lorsque l'irradiation est mise en oeuvre dans un bouillon qui contient de sulphhydril, la sensibilisation par moyen de 5-bromouracil s'évanouit complètement.

Zusatz von Sulfhydril-Verbindungen, Gefriertrocknung und tiefe Temperatur vermindern die Inaktivierung der Plaque-Bildungs-Fähigkeit bei T1-Phagen durch ionisierende Strahlung in verschiedenem nahezu additivem Ausmass. Eine Kombination dieser Änderungen der Umgebungsbedingungen während der Bestrahlung führt zu 'Schutzfaktoren' von $p=16$ (verglichen mit Bestrahlung in hochkonzentrierter Bouillon). Die Resultate fügen sich in ein von Alexander, Howard-Flanders *et al.* vorgeschlagenes Modell ein, wenn man dieses für den Einfluss von Tieftemperatur erweitert. Physikochemische Befunde, die das Modell stützen, werden kurz diskutiert. Ersetzt man einen Teil des Thymins in der DNS von T1 durch 5-Bromouracil, so erhält man eine Sensibilisierung der Inaktivierung durch ionisierende Strahlung, die weder von den erwähnten 'Schutzmassnahmen' abhängt noch diese beeinflusst, jedoch mit einer Ausnahme: Bei Bestrahlung in Sulfhydril-haltiger Bouillon verschwindet die Sensibilisierung durch 5-Bromouracil völlig.

References

Alexander, P., and Charlesby, A., 1955, *Radiobiology Symposium*, Ed. Z. M. Bacq and P. Alexander (London : Butterworths), p. 49.

Alexander, P., Lett, J. T., and Itzhaki, R., 1961, *Radiat. Res.*, **14**, 363.

Alexander, P., and Ormerod, M. G., 1962, *Biological Effects of Ionizing Radiation at the Molecular Level* (Wien : International Atomic Energy Agency), p. 399.

ALPER, T., 1955, *Radiat. Res.*, **2**, 119.
BACHOFER, S. C., EHRET, C. F., MAYER, S., and POWERS, E. L., 1953, *Proc. nat. Acad. Sci., Wash.*, **39**, 744.
BONÉT-MAURY, P., 1955, *Radiobiology Symposium*, Ed. Z. M. Bacq and P. Alexander (London : Butterworths), p. 75.
DAVIS, M., 1954, *Arch. Biochem. Biophys.*, **49**, 417.
ELDJARN, L., and PIHL, A., 1956, *Progress in Radiobiology*, Ed. J. S. Mitchell, B. E. Holmes and C. L. Smith (Edinburgh and London : Oliver & Boyd), p. 249.
EPSTEIN, H. T., 1953, *Nature, Lond.*, **171**, 394.
EPSTEIN, H. T., and SCHARDL, D., 1957, *Nature, Lond.*, **179**, 100.
GARD, S., and MAALØE, O., 1959, *The Viruses*, Vol. 1, Ed. F. M. Burnet and W. M. Stanley (London and New York : Academic Press), p. 359.
GINOZA, W., and NORMAN, A., 1957, *Nature, Lond.*, **179**, 520.
HARM, W., 1958, *Virology*, **5**, 337.
HOTZ, G., 1961, *Experientia*, **17**, 498 ; 1962, *Z. Naturf.* B, **17**, 37 ; 1963 a, *Int. J. Rad. Biol.*, **6**, 143 ; 1963 b, *Biochem. Biophys. Res. Commun.*, **11**, 393.
HOTZ, G., and MÜLLER, A., 1960, *Z. Naturf. B*, **15**, 450 ; 1961, *Ibid.*, **16**, 282 ; 1962, *Ibid.*, **17**, 34.
HOWARD-FLANDERS, P., 1960, *Nature, Lond.*, **186**, 485.
HOWARD-FLANDERS, P., and JOCKEY, P., 1960, *Int. J. Rad. Biol.*, **2**, 361.
HOWARD-FLANDERS, P., LEVIN, J., and THERIOT, L., 1963, *Radiat. Res.*, **18**, 593.
HUTCHINSON, F., 1961, *Radiat. Res.*, **14**, 721.
KAPLAN, H. S., ZAVARINE, R., and EARLE, J., 1962, *Nature, Lond.*, **194**, 662.
KÖHNLEIN, W., 1963, *Strahlentherapie* (in the press).
LEA, D. E., 1946, *Actions of Radiations on Living Cells* (Cambridge University Press).
LETT, J. T., STACEY, K. A., and ALEXANDER, P., 1961, *Radiat. Res.*, **14**, 349.
MARCOVICH, H., 1962, *Z. Naturf.* B, **17**, 23.
MESELSON, M., STAHL, F. W., and VINOGRAD, J., 1957, *Proc. nat. Acad. Sci., Wash.*, **43**, 581.
MÜLLER, A., 1962, *Int. J. Rad. Biol.*, **5**, 199 ; 1963 a, *Ibid.*, **6**, 137 ; 1963 b, personal communication.
MÜLLER, A., KÖHNLEIN, W., and ZIMMER, K. G., 1963, *J. mol. Biol.*, **7**, 92.
ORMEROD, M. G., and ALEXANDER, P., 1963, *Radiat. Res.*, **18**, 495.
POLLARD, E., 1954, *Advanc. Virus Res.*, **2**, 109.
POLLARD, E., and SETLOW, J., 1954, *Arch. Biochem. Biophys.*, **50**, 376 ; 1956, *Radiat. Res.*, **4**, 87.
STAHL, F. W., 1959, *The Viruses*, Vol. 2, Ed. F. M. Burnet and W. M. Stanley (London and New York : Academic Press), p. 353.
STAHL, F. W., CRASEMANN, J. M., OKUN, J. M., FOX, E., and LAIRD, C., 1961, *Virology*, **13**, 98.
WATSON, J. D., 1950, *J. Bact.*, **60**, 697 ; 1952, *Ibid.*, **63**, 473.
WEIGLE, J. J., and BERTANI, G., 1956, *Virology*, **2**, 344.
WINKLER, U., JOHNS, H. E., and KELLENBERGER, E., 1962, *Virology*, **18**, 343.
ZIMMER, K. G., 1961, *Studies on Quantitative Radiation Biology* (Edinburgh and London : Oliver & Boyd).
ZIMMER, K. G., KÖHNLEIN, W., HOTZ, G., and MÜLLER, A., 1963, *Strahlentherapie*, **120**, 161.