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Observations on X-Ray-Induced Radicals in Whole Bacteriophage and Phage Nucleic Acid

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Observations on X-ray-induced radicals in whole bacteriophage and phage nucleic acid

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ESR measurements on x-rayed purified bacteriophage of strain *E. coli* T2 and on DNA extracted from this phage have yielded the following main results: (1) The ESR absorption of DNA irradiated *in vacuo* at various temperatures showed a pronounced hyperfine structure if measured *in vacuo* at room temperature. (2) When DNA is subjected to increasing doses of x-rays *in vacuo* at room temperature saturation of radical concentration occurs at a few hundred kr. (3) The initial energy expenditure measured at room temperature after irradiation at various temperatures ranges between 8 and 30 ev per radical induced. (4) The ESR absorption of whole phage irradiated *in vacuo* at various temperatures is at room temperature equal to the absorption of phage-DNA plus that of a similar quantity of protein. (5) The dependence of radical concentration on dose in whole phage irradiated *in vacuo* at room temperature is equal to the dose-dependence of DNA, plus that of a similar quantity of protein.

1. INTRODUCTION

Bacteriophages are organisms with unique properties for studies of radiation-induced radicals. Since phages are almost completely composed of protein and nucleic acid the correlation between radical production in the separated components and the whole organism is a rather direct procedure. Consequently electron-spin-resonance (ESR) studies on radiation-induced radicals in dry bacteriophage, strain *E. coli* T2, were initiated in our laboratory. This work was extended to deoxyribonucleic acid (DNA) extracted from the phage. T2 was used in our experiments since purification and extraction of DNA are well-known and practicable procedures for this strain. Unfortunately, T2 is easily inactivated by drying. Nevertheless, our results are probably valid for viable phage since the difference between dry viable and dry inactivated phage is not likely to affect the radicals measured appreciably. It is planned to test the validity of this assumption in the future.

2. MATERIALS AND METHODS

E. coli B was incubated at 37° in 20 litres of synthetic medium M9 (modified after Weidel and containing per litre 7·5 g $Na_2HPO_4 . 2H_2O$; 3 g KH_2PO_4; 1 g NH_4Cl; 0·5 g NaCl; 0·2 g $MgSO_4 . 7H_2O$; 4·4 g glucose; 0·27 mg $FeCl_3 . 6H_2O$). After counting a concentration of 5×10^8 bacteria ml and while the culture was still in the exponential phase of growth, one phage per bacterium was added and incubation continued at 37°C for another 18 hours. The lysates containing about 3×11^{11} T2/ml were, after addition of chloroform, cooled down to 4°C. T2 was precipitated and purified by HCl at pH 4 and concentrated by centrifuging for 45 min at 12 500 r.p.m. following the method of Herriott and

Barlow (1952). For further purification we added RNase and DNase to the preparations, precipitated the remaining coli-protein by antiserum and, finally, applied dialysis against aqua bidest. for three days. Purified phage preparations were frozen rapidly in an acetone–carbon-dioxide bath and freeze-dried *in vacuo*.

DNA was extracted from T2 following the method of Sevag, Lackmann and Smolens (1938) and lyophilized as described.

The phage preparations were used for ESR measurements in small quartz-tubes (3·5 mm i.d.). These were evacuated on a high-vacuum line in series with a liquid-nitrogen trap for at least 10 hours and subsequently sealed. For some measurements pellets (3·5 mm diameter and up to 10 mm length) were produced by pressing lyophilized phage at approximately 1000 kg/cm^2 (density of pellet approximately 1·2 g/cm^3) under a nitrogen atmosphere. The pellets were then transferred to quartz tubes, evacuated, and sealed as usual. Only the region of the tubes containing the samples of generally 50–100 mg weight (with the exception of unpressed whole phage which weighed only 5–10 mg per sample) were irradiated. The unirradiated part of the tubes was used for ESR measurements after shaking the specimen to the other end. Dose-effect curves were obtained by using soft X-rays (100 kv, 25 ma, filtered by 0·6 mm of quartz) at a dose-rate of 20 000 per minute. For irradiations at low temperatures (77°K) the sealed quartz tubes were flushed by a stream of cooled nitrogen gas. A commercial ESR-spectrometer (Varian) working at 3 cm wavelength was used in conjunction with variable temperature equipment (77°K to 600°K) and a double cavity previously described (Köhnlein and Müller 1960). Evaluations of the first derivatives of absorption spectra were performed with a momentum balance (Köhnlein and Müller 1962).

3. Results and discussion

Radical spectra of phage T2 DNA irradiated *in vacuo* are shown in figure 1. Both spectra obtained at room temperature (approximately 300°K) show a marked structure, which is much less prominent at liquid nitrogen temperature (approximately 77°K) after irradiation at 77°K. The spectrum obtained at 77°K irreversibly changes into the one observed at 300°K when the temperature is raised. Similar results were reported for salmon-sperm DNA previously (Alexander, Lett and Ormerod 1961).

In figure 2 an example for the variation of radical concentration with radiation dose is plotted. Experimental points were obtained with an evacuated sample of phage-DNA irradiated at room temperature. A conspicuous feature is the saturation at relatively low doses. The solid line in figure 2 represents the exponential function

$$C/C_\infty = 1 - \exp(-D/D_{37}), \tag{1}$$

where C is the radical concentration, D the radiation dose, $C_\infty = 44$ radicals per 10^{-16} g of DNA, and $D_{37} = 205$ kr. Under identical conditions similar curves were found with other samples. However, C_∞ and D_{37} were found to be up to about three times larger than the values derived from figure 2. The reason for these variations has not been determined. Experiments on commercial calf-thymus-DNA and yeast-RNA had yielded similar dose-effect curves. Maximum concentrations in these compounds were within the range found for phage-DNA, whereas values for D_{37} measured were about ten times as large.

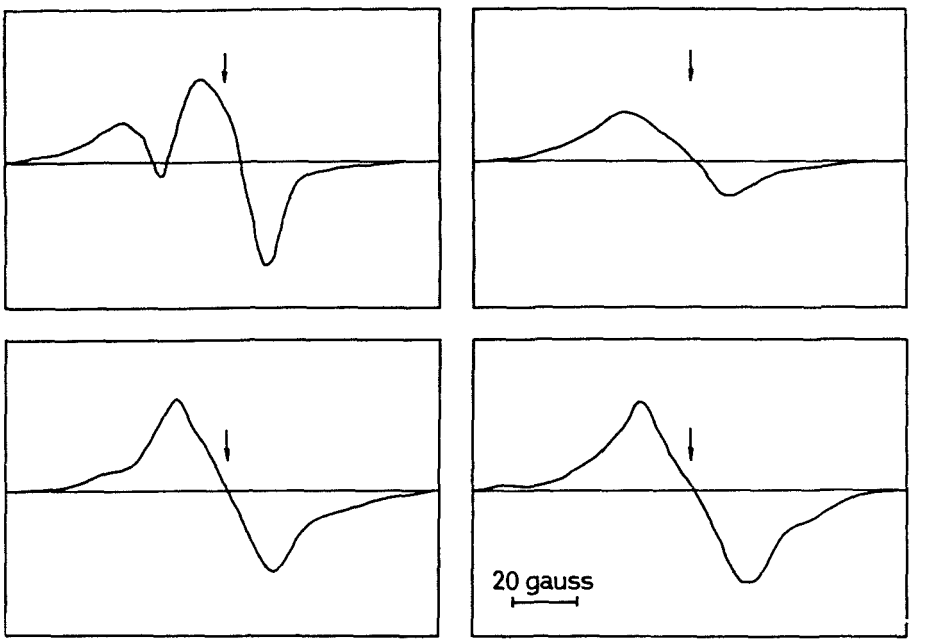

Figure 1. First derivatives of absorption spectra of dry phage T2-DNA *in vacuo* at 77°K irradiated at 77°K (top left), at 300°K irradiated at 77°K (bottom left), at 77°K irradiated at 77°K and warmed up to 300°K (top right), and at 300°K irradiated at 300°K (bottom right). Dose applied: 10^5 r. The arrow indicates a g-value of 2·0036.

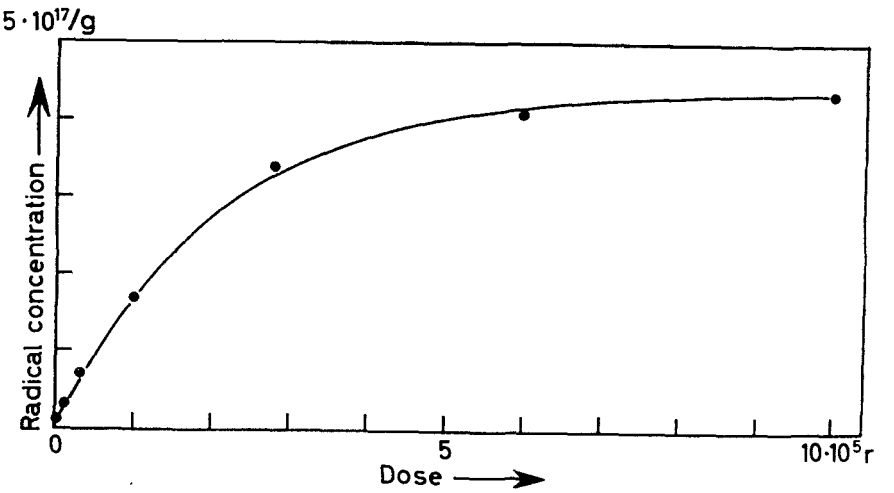

Figure 2. Radical concentration in dry phage T2-DNA irradiated *in vacuo* at 300°K. The solid is a best fit of equation (1) to the experimental points.

The exponential character of the curves obtained at room temperature suggests that a limited number of sites at which the radicals produced are stable and that the radicals are produced at random. Since the spectra show a pronounced hyperfine structure which does not depend on the dose, radicals are stabilized at

certain molecular groups only. C_∞ can be used to compute the number of radical sites per molecule, if the molecular weight is known. Since no special care was taken to avoid shearing of the DNA-molecules during preparation the value of 125×10^6 for the molecular weight of unbroken DNA extracted from T2 phage (Rubenstein, Thomas and Hershey 1961, Davison, Freifelder, Hede and Levinthal 1961) can serve as an upper limit, Using the data of figure 2 a value of about 100 for the maximum number of radicals per molecules is obtained.

However, instead of being caused by some quality of the irradiated compound itself, dose-saturation could be envisaged as being due to radiation-produced oxygen. This possibility was suggested by some recent findings on proteins (Usatyi and Lazurkin 1962). However, continuous evacuation during irradiation did not affect the dose-saturation. Since dose-saturation was not observed in proteins during evacuation, it must have a different cause in DNA. This finding is supported by the large difference in radiation doses at which saturation occurred in proteins and DNA.

When D is small in comparison to D_{37} equation (1) is approximated by

$$C/C_\infty = D/D_{37}. \tag{2}$$

From this initial linear part of the dose-effect curve the energy expenditure per radical

$$D/C = D_{37}/C_\infty \tag{3}$$

is derived. Experimental values are scattered between 8 and 30 ev per radical around a mean of 15 ev per radical. This result is similar to those obtained with proteins and nucleic acids and lower than those reported previously for other types of DNA (Alexander *et al.* 1961, Müller 1962).

Dose-effect curves for radical production in phage-DNA at 77°K deviated from linearity only above 3 Mr. The radical yield after warming up to 300°K was the same as after irradiation at 300°K. The results found for whole phage T2 can be understood on the basis of the results with DNA. In figure 3 spectra of phage T2 irradiated *in vacuo* are reproduced which correspond to those of DNA shown in figure 1. In the spectra obtained at room temperature the structure found with DNA appears to be still noticable, but strongly reduced by the absorption from other radicals which probably are those of the protein coat. This, however, does not hold for the spectrum obtained from whole phage at 77°K after irradiation at the same temperature. The strongly-asymmetric absorption which is found in this case was never observed in dry proteins or nucleic acids. Possible explanations have to be tested experimentally before discussing them.

The dependence of radical concentration on radiation dose in whole phage is not linear above 200 kr, but does not show a plateau as for phage-DNA (figure 4). This behaviour is quite similar to that found in higher organisms such as seeds (Zimmer, Ehrenberg and Ehrenberg 1957, Zimmer 1961). There also is a small amount of radicals present in unirradiated phage as in seeds, which is negligible in phage-DNA. This absorption is not due to those paramagnetic centres in unirradiated bacteriophage giving rise to extremely wide absorption lines reported previously (Müller, Hotz and Zimmer 1961). These lines are easily distinguished from radical-absorption spectra. The radical yields in whole phage scatter as much as do those for phage-DNA, but the mean value is higher by about a factor of two.

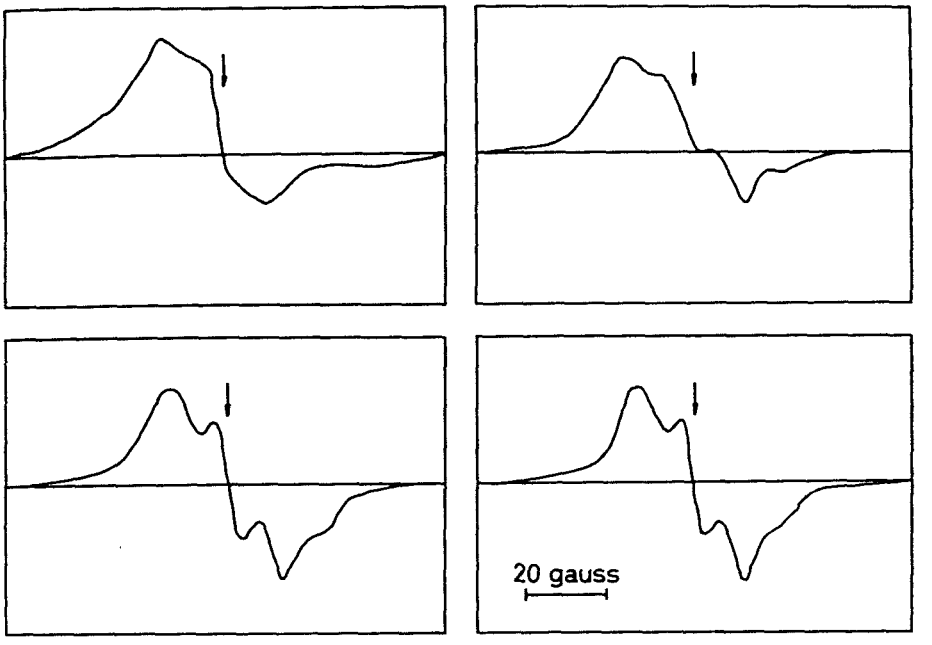

Figure 3. First derivatives of absorption spectra of dry whole phage T2 *in vacuo* at 77°K irradiated at 77°K (top left), at 300°K irradiated at 77°K (bottom left), at 77°K irradiated at 77°K and warmed up to 300°K (top right), and at 300°K irradiated at 300°K (bottom right). Dose applied 3×10^5 r. The arrow indicates a *g*-value of 2·0036

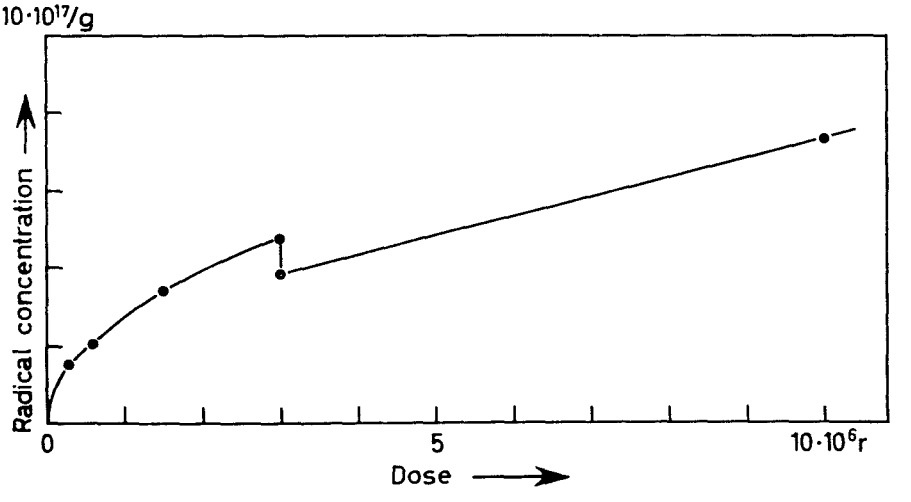

Figure 4. Radical concentration in dry whole phage T2 irradiated *in vacuo* at 300°K. The decrease at 3 Mr is due to storage at 300°K for 24 hours.

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Les mesures de résonance électronique paramagnétique (REP) sur des bactériophages purifiés irradiés aux rayons x de la variété *E. coli* T2 ainsi que sur la ADN extraite de ces phages, donnent les résultats suivants: (1) L'absorption de la ADN irradiée *in vacuo* et à différentes températures montre une structure hyperfine prononcée aux mesures *in vacuo* faites à des températures ordinaires. (2) La ADN étant exposée *in vacuo* et à des températures ordinaires à des doses croissantes de rayons x, on arrive à une saturation de la concentration radicalaire à une valeur de quelques centaines de kr. (3) La dépense d'énergie initiale mesurée à la température ordinaire, varie de 8 à 30 ev pour un radical produit. (4) Des phages complets irradiés *in vacuo* et à la température ordinaire, montrent une absorption de REP analogue à celle de la ADN extraite des phages plus une quantité comparable de protéines. (5) Des phages complets irradiés *in vacuo* et à des températures ordinaires, donnent lieu à une absorption de REP qui, d'une manière analogue à l'absorption de la ADN plus une quantité comparable de protéines, dépend de la dose d'irradiation reçue.

ESR-Messungen an röntgenbestrahlten gereinigten Bakteriophagen des Stammes *E. coli* T2 und an DNS, die aus diesen Phagen extrahiert wurde, ergaben folgende Hauptergebnisse: (1) Die ESR-Absorption von *in vacuo* bei verschiedenen Temperaturen bestrahlter DNS zeigt eine ausgeprägte Hyperfeinstruktur bei Messung *in vacuo* und Zimmertemperatur. (2) Wenn DNS *in vacuo* bei Zimmertemperatur steigenden Dosen von Röntgenstrahlung ausgesetzt wird, tritt Sättigung der Radikalkonzentration bei einigen hundert kr auf. (3) Der anfängliche Energieaufwand gemessen bei Zimmertemperatur liegt zwischen 8 und 30 ev pro erzeugtem Radikal. (4) *In vacuo* und bei Zimmertemperatur bestrahlte ganze Phagen zeigen die gleiche ESR-Absorption wie Phagen-DNS plus einer vergleichbaren Menge Protein. (5) *In vacuo* und bei Zimmertemperatur bestrahlte ganze Phagen verursachen ESR-Absorption, die in gleicher Weise von der Strahlendosis abhängt, wie die Absorption von DNS plus einer vergleichbaren Menge Protein.

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