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ELEKTRON SPIN RESONANCES IN BACTERIOPHAGE: ALIVE, DEAD, AND IRRADIATED.

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ELECTRON SPIN RESONANCES IN BACTERIOPHAGE: ALIVE, DEAD, AND IRRADIATED

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In the course of our investigations into the actions of ionizing radiations on T-phages (Hotz and Müller 1960, 1961, Zimmer 1961) e.s.r. spectra of phage were taken under various conditions and after various treatments of phages E.coli T1 and T2. The extensive measurements of which a few examples only are given here led to the following conclusions.

(i) Both kinds of phage show a very broad absorption starting at zero external magnetic field and extending over several thousands of gauss (Fig.1, obtained with unirradiated vacuum dried phage preparations of very high purity). A rough analysis of the spectra indicates that the

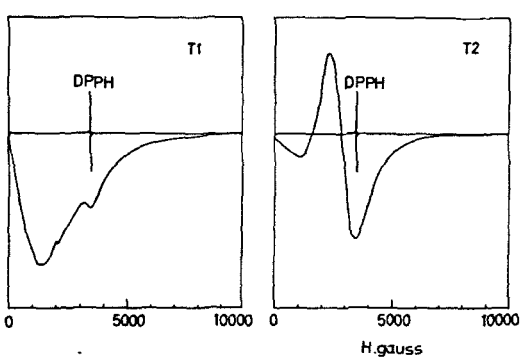

Fig.1. Typical examples of derivatives of e.s.r. absorption at 9500 Mc in highly purified phage T1 and T2 compared with the absorption signal of the free radical diphenylpicrylhydrazyl (DPPH).

absorption is due to the presence of 10^{18}-10^{19} unpaired electrons per gram of phage.

(ii) The spectra are rather similar for T1 and T2. T1 will, to a high percentage, survive the process of purification, the drying in vacuo

and the pressing (at 1000 kgms./cm^2) of purified dry phage into a handy pellet containing 10^{13}-10^{14} particles. Hardly any T2 will survive this procedure. Hence the spectra appear to be a consequence of nucleoproteid structure rather than of being alive or dead.

(iii) The spectra disappear completely and irreversibly on dry heating of phage, done inside the cavity of the e.s.r.-spectrometer, to more than 200° C (Fig.2). Hence they are definitely not due to any impurities: para-, ferro-, or antiferromagnetic.

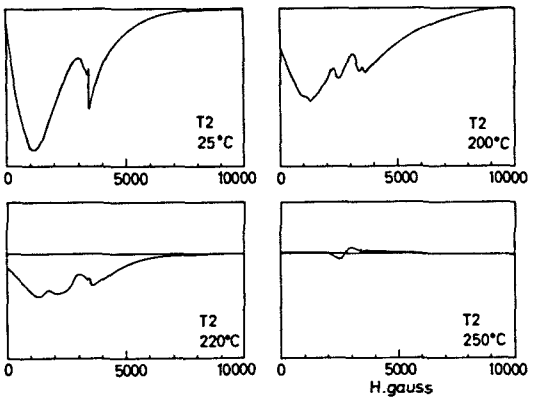

Fig.2. Derivatives of absorption of highly purified phage T2 before and after heat treatment.

(iv) The high concentration of unpaired electrons observed is another important piece of evidence against ascribing the e.s.r. spectra to the presence of impurities.

(v) After irradiation by X-rays the spectra of the unirradiated phage remain essentially unchanged (Fig.3) but a narrow additional line appears due to the production by radiation of free organic radicals (Zimmer, Ehrenberg and Ehrenberg 1957, Zimmer 1958).

(vi) If phage is dried in vacuo together with the 'radioprotective' agent cysteamine and irradiated by X-rays the narrow additional line shown by irradiated phage is greatly reduced (Fig.3) due to a strong molecular interaction (Norman and Ginoza 1958). Several activities of

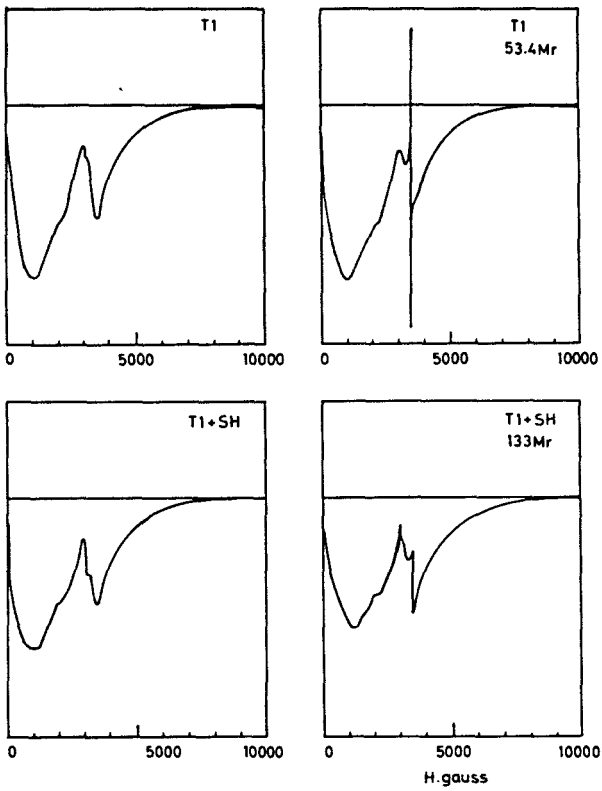

Fig.3. Derivatives of absorption of phage T1 dried without cysteamine before and after irradiation with 53.4 x 10^6 r of X-rays or dried with cysteamine (SH) before and after irradiation with 133 x 10^6 r of X-rays.

virus usually attributed to nucleic acids can be protected against radiation damage by cysteamine, and it is known that cysteamine protects proteins by forming mixed disulphides with the latter (Eldjarn and Pihl 1958). Consequently some form of unidirectional energy transfer may be postulated from nucleic acid to protein. Irradiations by slow electrons depositing all their energy in the protein coat of T1 did not influence the normal activities of phage (Davis 1954) showing energy transfer from protein to nucleic acid not to occur. Our detailed experiments with phage and protective substances will be described elsewhere.

(vii) Our results obtained in phage and the findings of earlier studies on e.s.r. spectra of nucleic acids and artificial complexes thereof

with proteins (Blumenfeld 1959) seem to support each other. The more so as our observations resulted quite independently and as a by-product from investigations in a very different field. On the other hand our experiments have the advantage of using a very well defined nucleic acid protein complex: the phage. Moreover, they show, for the first time, the existence of a high concentration of unpaired electrons in something that may be considered as living.

(viii) At variance with the work done on biochemicals we did not observe a reversible disappearance of the e.s.r. spectrum at liquid nitrogen temperature (Fig.4). Occasionally, the spectra would disappear

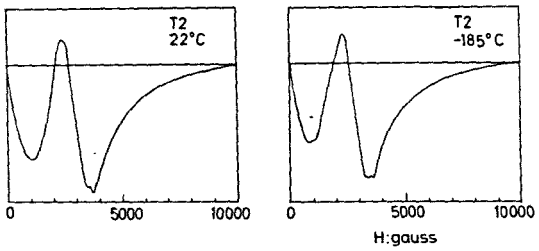

Fig.4. Derivatives of e.s.r. absorption in highly purified phage T2 at low temperature.

irreversibly at low temperature but these observations might be due to secondary effects such as condensation of water or oxygen from the air. We cannot help feeling that the failure to observe an antiferromagnetic curie-point in phage strengthens rather than weakens the evidence - and facilitates the theoretical interpretation of the origin of the huge cloud of unpaired electrons in nucleoproteid complexes.

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