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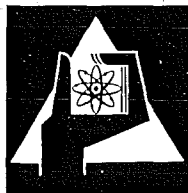
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A Comparative E.P.R. Study on Free Radical Formation in
DNA Constituents after Exposure to Discharge-excited Argon,
Hydrogen and Deuterium

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A comparative E.P.R. study on free radical formation in DNA constituents after exposure to discharge-excited argon, hydrogen and deuterium

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Since the observation of the thymine E.P.R.-signal in γ-irradiated DNA (Ehrenberg, Ehrenberg and Löfroth 1963), radical formation in irradiated DNA constituents has been studied in numerous investigations. The results of these studies revealed that many of the radicals observed are H-atom adducts. Herak and Gordy (1965) produced radicals of this type by treating powdered samples of nucleic acid bases with atomic hydrogen generated in a glow discharge. Recent investigations in our laboratory, on thymine solutions bubbled with discharge-excited hydrogen, have shown that an ' indirect effect ' exerted mainly by OH radicals contributes significantly to the destruction of thymine (Carpy and Dertinger 1970). It was concluded that these species result from water decomposition caused by energy uptake from excited discharge products of hydrogen. The aim of the present study was to discover whether discharge-excited gaseous species are also involved in free-radical formation in powdered samples.

For this purpose a conventional gas-flow apparatus was used that enabled exposure and subsequent transfer of the powdered material into an E.P.R.-sample tube. Technical details of the radiofrequency discharge and the flow system have been reported elsewhere (Carpy and Dertinger 1970). The exposure time was 40 min in every experiment at 30 Torr gas pressure and approximately 10 Watts radiofrequency power. Special precautions were taken to keep u.v.-radiation away from the sample. This was achieved by an L-shaped discharge tube being interrupted in downstream direction by a ' u.v.-labyrinth '. In this interface, made of non-reflecting material (Teflon), the gas-flow was deviated by four-fold reflection on the walls before re-entering the tube. The effect of these precautions on the yield of excited discharge products is small. Moreover it is known that Teflon stabilizes atomic hydrogen very effectively (Berg and Kleppner 1962). Fine powders of DNA bases and deoxyribose (Fluka and Calbiochem products) were exposed at room temperature to pure argon, hydrogen, and deuterium. Free-radical detection was performed by means of an E.P.R. spectrometer operating at 9·5 GHz (AEG, Berlin).

Three typical results are presented here. Figure 1 shows the E.P.R.-spectrum of thymine after exposure to hydrogen or argon. In either case identical spectra are observed which result from addition of a H atom to the C_6-position of the thymine molecule (Herak and Gordy 1965). On the other hand, the exposure to discharge-excited deuterium exclusively yields the corresponding D-adduct (Herak and Gordy 1965), which could also be confirmed in the present investigation. This indicates that, in the case of the hydrogen discharge, the thymine radical is produced by the direct H-attack of the 5,6 double bond. However, to explain the formation of the same radical by excited

argon, it is necessary to assume energy transfer from the excited atoms to a thymine molecule with subsequent decay and release of atomic hydrogen, which itself attacks another molecule to produce the H-adduct. (Thymine is very susceptible to U.V.-action, which manifests itself in the production of an additional signal in the centre of the spectrum. This is, however, completely prevented by the use of the U.V.-labyrinth.) Another interesting observation was made with the purine bases. Figure 2 *a* shows the E.P.R.-signal of adenine exposed to discharge-excited hydrogen. It consists of a 1 : 2 : 1 triplet with
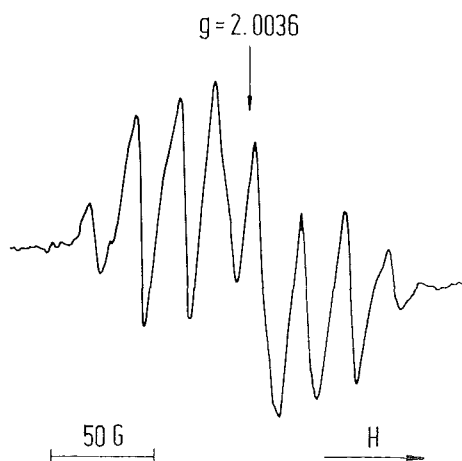

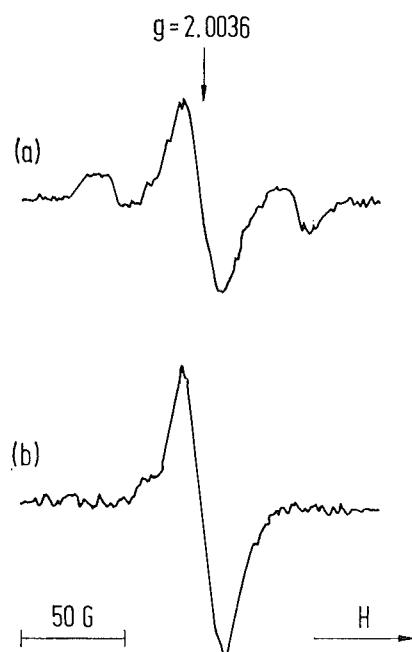Figure 1. E.P.R.-spectrum of thymine exposed to discharge-excited argon or hydrogen.

Figure 2. E.P.R.-spectra of adenine. (*a*) Exposure to discharge-excited hydrogen. (*b*) Exposure to discharge-excited argon.

approximately 40 Gauss hyperfine coupling and has been attributed to the H-adduct in the position C_8 of the adenine (Herak and Gordy 1965). However, an additional signal is superimposed on the central triplet line, thus masking the correct 1 : 2 : 1 intensity ratio. Figure 2 *b* shows the adenine signal after exposure to argon, which consists only of the central line without appearance of the outer lines of the triplet. It is concluded that the strong central line is a stable paramagnetic primary product caused by transfer of energy from the excited gases to the adenine molecule. In contrast to thymine, this product does not seem to undergo fragmentation at room temperature. Therefore unlike the thymine case no ' conversion ' into the H-adduct is observed.

Finally, the radical formation in $\beta2$-deoxy-D-ribose will be considered. Exposure to argon, hydrogen, and deuterium yields identical spectra (figure 3); the 3 gases are also approximately equally effective in the production of the underlying radicals. Therefore we conclude that the deoxyribose signal is indicative of radicals formed mainly by energy uptake and subsequent destruction of the molecule. On the other hand the spectrum in figure 3 resembles the signal obtained by ionizing irradiation of deoxyribose. Single crystal studies on irradiated deoxyribose revealed that the spectrum is most probably due to a radical formed by dissociation of a CH bond and rupture of the C_1-O-C_5 bond (Hüttermann and Müller 1969). Thus atomic hydrogen is not required for the formation of the deoxyribose radical from either ' outside ' or from fragmentation processes.
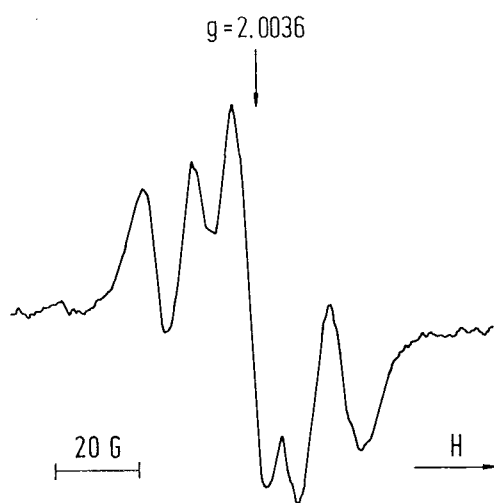

Figure 3. E.P.R.-spectrum of $\beta2$-deoxy-D-ribose exposed to discharge-excited argon, hydrogen, or deuterium.

The results presented here show excited molecules and atoms, generated in a glow discharge, to play a dominant role in the process of radical formation. This possibility has been completely neglected in earlier experiments. As energy transfer and superexcitation are involved in the action of the discharge-excited species common features with the ionizing radiation are obvious. This is reflected also in the similarities between the E.P.R.-spectra presented here and

the spectra produced by ionizing irradiation (Köhnlein and Müller 1964). It is evident that experiments with discharge-excited noble gases differing in their excitation levels are very useful for studying primary processes in radiation biology and radiation chemistry, e.g. energy transfer and fragmentation pathways. Further investigations are in preparation to study selectively the possible pathways of radiation damage, including physico-chemical and biological tests.

REFERENCES

BERG, H. C., and KLEPPNER, D., 1962, *Rev. scient. Instrum.*, **33**, 248.
CARPY, S., and DERTINGER, H., 1970, *Int. J. Radiat. Biol.*, **18**, 359.
EHRENBERG, A., EHRENBERG, L., and LÖFROTH, G., 1963, *Nature, Lond.*, **200**, 376.
HERAK, J. N., and GORDY, W., 1965, *Proc. natn. Acad. Sci. U.S.A.*, **54**, 1287.
HÜTTERMANN, J., and MÜLLER, A., 1969, *Radiat. Res.*, **38**, 248.
KÖHNLEIN, W., and MÜLLER, A., 1964, *Int. J. Radiat. Biol.*, **8**, 141.