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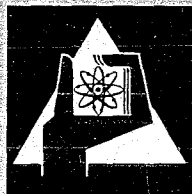
Mai 1969

KFK 1004

Institut für Strahlenbiologie

Free Radicals in Gamma-Irradiated 2-Deoxy-D-ribose:  
Electron Spin Resonance of Single Crystals and Polycrystalline Material

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## Free Radicals in Gamma-Irradiated 2-Deoxy-D-ribose: Electron Spin Resonance of Single Crystals and Polycrystalline Material

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HÜTTERMANN, J., AND MÜLLER, A. Free Radicals in Gamma-Irradiated 2-Deoxy-D-ribose: Electron Spin Resonance of Single Crystals and Polycrystalline Material. *Radiation Res.* **38**, 248-259 (1969).

Single crystals of 2-deoxy-D-ribose have been studied by electron spin resonance (ESR) spectroscopy at 8-mm wavelength following  $\gamma$ -irradiation. The main feature of the spectra recorded was a quintet pattern of about 10 gauss hyperfine splitting. The splitting as well as the mean  $g$  factor of 2.005 was found to be almost independent of crystal orientation. Minor spectral variations were attributed to the presence of other radicals. This conclusion was proved by observation of ESR spectra from irradiated polycrystalline 2-deoxy-D-ribose at 8-mm and 3-cm wavelengths. Heat-treatment of these samples revealed the presence of at least two other radicals. The quintet pattern is attributed to interaction of a radical electron at a carbon atom with four  $\beta$ -protons bound to neighboring carbon atoms in radicals  $\text{COOHCH}_2\text{CHOH}\dot{\text{C}}\text{OHCH}_3$ , which are assumed to be produced by rupture of a CO bond following initial dissociation of atomic hydrogen. The proposed reaction scheme is discussed as a model for the initial processes of chain ruptures in DNA.

### INTRODUCTION

Production of free radicals in nucleic acids and their constituents by ionizing radiations has been studied extensively by means of electron spin resonance (ESR) spectroscopy. Polycrystalline material was used in most of this work, which supplied valuable information on radical reactions induced by irradiation. Generally, radical yields were found to increase in the order base, nucleoside, nucleotide, nucleic acid, pentose, whereas qualitative results showed that radical stabilization at room temperature occurs preferentially in base groups. Hence, radical transfer from pentoses to bases was assumed to prevail (for a review see Müller, 1).

In recent years interest has been focused on studies of radiation damage in single crystals of nucleic acid constituents, since such investigations provide detailed knowledge about the chemical structure and conformation of radicals. Positive identification of stable radicals has been achieved by now for a number of nucleic acid bases (2-4), nucleosides (5-8), and one nucleotide (9). Addition of atomic hydrogen to the unsaturated bonds of base rings was shown to be a major pathway of radical formation. ESR studies of polycrystalline mixtures containing thymine and deoxyribose proved that the radiation-produced hydrogen originated mainly from deoxyribose (10). The fate of the latter, however, remained obscure. Radical stabilization outside base groups has been established for single crystals of 3'-cytidylic acid (9) and cytidine (8) only. No information on deoxyribose radicals has come from single-crystal work so far. In view of the important function of this compound in the structure of DNA and its unknown fate during radical formation, we extended our ESR investigations to single crystals of pure 2-deoxy-D-ribose.

#### EXPERIMENTAL PROCEDURE

Polycrystalline samples of 2-deoxy-D-ribose were obtained from Nutritional Biochemicals Corporation. The compound was dissolved in freshly distilled acetone. From the saturated solution, which was seeded with a small amount of polycrystalline material and placed in a desiccator containing  $P_2O_5$ , single crystals were grown by slow evaporation at room temperature. Special care had to be taken to work in the absence of humidity, as the compound is highly soluble in water. The crystals obtained were small, colorless needles, approximately  $6 \times 0.8 \times 0.8$  mm, elongated along the  $c$  axis. The external habit of the crystals with axes  $a$ ,  $b$ , and  $c$  is shown in Fig. 1 together with the chosen right-handed orthogonal ESR reference axes  $a'$ ,  $b'$ , and  $c$ .

Furberg (11) has determined the crystal and molecular structure of the compound using x-ray diffraction methods. He reported the crystals to be orthorhombic with space group  $P2_12_12_1$ , containing four molecules in the unit cell of dimensions 11.37 Å, 10.64 Å, and 4.86 Å. Within experimental error, x-ray diffraction data taken on some of our crystals gave the same space group and unit cell dimensions, and we

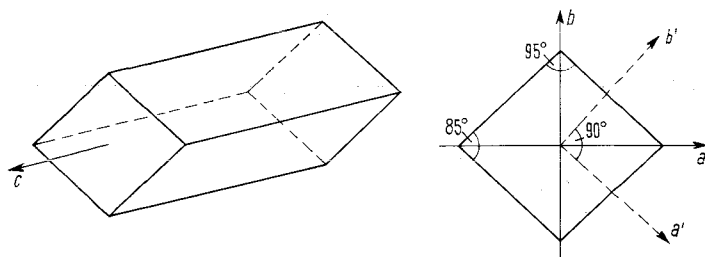


Fig. 1. External configuration of orthorhombic single crystals of 2-deoxy-D-ribose showing crystallographic axes  $a$ ,  $b$ ,  $c$  and ESR reference axes  $a'$ ,  $b'$ ,  $c$ .

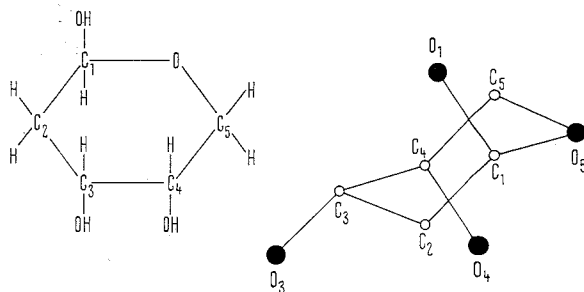


Fig. 2. Chemical and spatial structure of 2-deoxy-D-ribose molecules within single crystals after Furberg (11).

shall assume our crystals to be identical to those of Furberg. Thus we can confirm that the sugar is in the pyranose form. The schematic representation and the molecular structure of 2-deoxy-D-ribose as reported by Furberg are shown in Fig. 2.

The crystals were irradiated in air at room temperature with a  $^{60}\text{Co}$  source up to a  $\gamma$ -ray dosage of 0.2 to 0.4 Mrad. After irradiation they showed a pronounced fragility. At higher irradiation doses the crystals became opaque, broke into small fragments, and turned into a viscous liquid on standing for several hours in air.

ESR measurements were carried out at room temperature with a conventional reflection spectrometer (AEG), operating at a microwave frequency of 35 GHz (Q-band). For single-crystal measurements the spectrometer was equipped with a goniometer allowing rotation of the crystals about two mutually perpendicular axes within the cylindrical  $\text{TE}_{011}$ -type resonator. Spectra from irradiated polycrystalline samples of 2-deoxy-D-ribose were recorded both at 9.3-GHz (X-band) and at 35-GHz microwave frequency. The  $g$  factor of all spectra was calibrated, using polycrystalline DPPH as a standard. The magnetic field was measured by proton resonance in conventional manner.

## RESULTS

In Fig. 3, an ESR spectrum obtained from an irradiated single crystal of 2-deoxy-D-ribose is shown. The magnetic field,  $H$ , is parallel to the  $ab$  plane of the crystal. In this case the spectrum consists essentially of five lines with equal spacing of about 10.5 gauss and an approximate intensity ratio 1:4:6:4:1. The bars indicate the theoretical pattern for the corresponding free radical. On rotation of the crystal about the  $c$  axes, the hyperfine coupling of the quintet is nearly isotropic, varying from 10 to 11 gauss. In some orientations additional hyperfine structure of the quintet is seen. By following the spectral lines at sufficiently close intervals of crystal orientations, this feature can be interpreted in terms of two magnetically distinct molecules with slightly different  $g$  factors. The maximum splitting of each of the five lines is observed when  $H$  is along the  $a$  or  $b$  axis of the crystal. Its value of

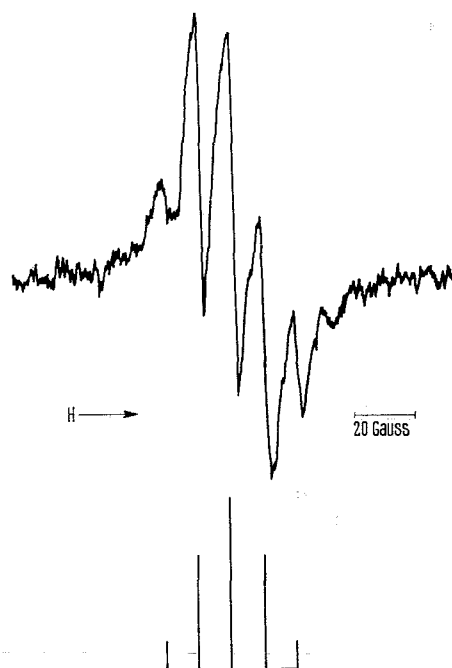


FIG. 3. First-derivative ESR spectrum at 35 GHz of single crystal irradiated with 0.4 Mrad. The magnetic field is oriented parallel to the  $ab$  plane. Apparent positions and amplitudes of hyperfine lines are indicated below.

about 4 gauss represents a difference in  $g$  factor of  $6.5 \times 10^{-4}$ . Within these limits, the  $g$  factor is isotropic in the  $ab$  plane of the crystal, having a mean value of 2.0054.

Rotation of the crystal about the two other axes,  $a'$ , and  $b'$ , produces similar results. Both the hyperfine coupling and the  $g$  factor of the prominent quintet are nearly isotropic, the latter varying from 2.0054 to 2.0045. Position splitting arising from magnetically distinct molecules could be observed neither in the  $cb'$  nor in the  $ca'$  plane of the crystal.

Besides the quintet we have found additional, less prominent lines not fitting this scheme, one of which can be seen at the high-field end of the spectrum in Fig. 3. This observation suggests that lines of one or two more radicals must be present in our spectra. From the records of all spectral lines in the three crystal planes it is assumed that one of the additional radicals possesses an anisotropic  $g$  factor. Selective formation or annealing of the prominent quintet or of the lines of one of the other radicals in the single crystals was attempted with little success. A few heat-annealing experiments which were carried out successfully will be discussed later. Most experiments failed, owing to the small size of the crystals and the high fra-

gility observed after irradiation. Thus, only the free radical giving rise to the predominant quintet is open to a satisfactory interpretation.

To obtain more information about the varieties and the reactions of radiation-produced free radicals in 2-deoxy-D-ribose, our investigations were extended to polycrystalline samples. This procedure appeared to be especially promising in view of the nearly isotropic  $g$  factor of the quintet, which means that conclusions drawn from such spectra remain valid for the single crystal.

The spectra of polycrystalline samples irradiated at 77°K and at 300°K are shown in Figs. 4*a* and 4*b*, respectively. Both spectra were recorded at room temperature and a microwave frequency of 9.3 GHz. The spacing of the prominent five lines in both spectra is of the same magnitude as it is in the spectrum of Fig. 3, which means that it is due to hyperfine interaction only. The variation of intensity ratios of the quintets in Figs. 4*a* and 4*b*, indicated by the numbers beneath the spectra and the marked superposition of additional lines at the high- and low-field ends of both spectra, strongly suggests at least two different types of radicals to be present. Heat-annealing at 60°C exhibits an additional feature which is the same in both samples and is shown in Fig. 5. After this treatment the central line of the quintet has decreased more than any other line, giving an intensity ratio of about 1:4:5:3:1. In

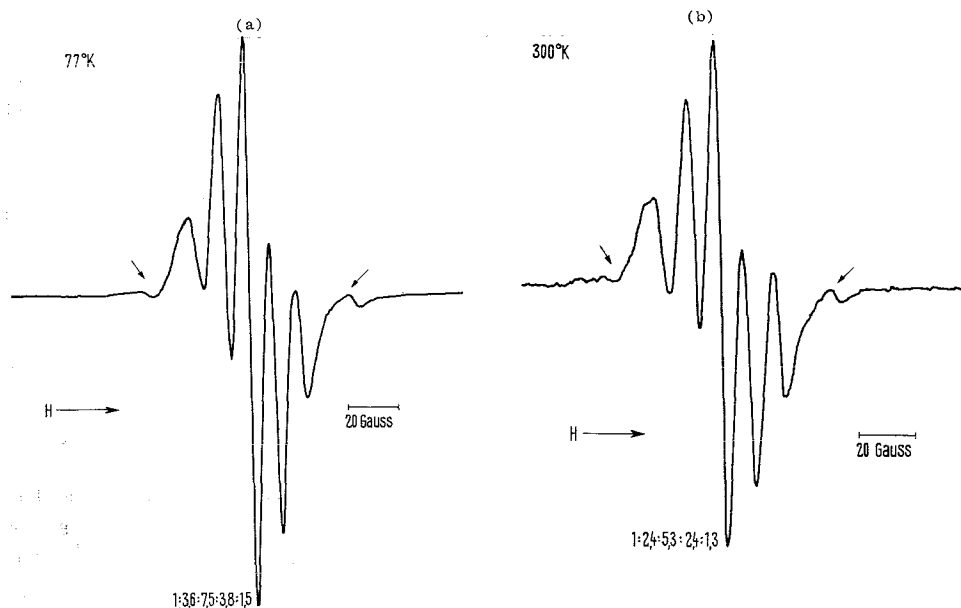


FIG. 4. First-derivative ESR spectra of polycrystalline 2-deoxy-D-ribose recorded at 9.3 GHz and at room temperature after irradiation with 1.2 Mrad at 77°K (*a*) and at 300°K (*b*). Intensity ratios of main lines are given. Satellite lines are marked by arrows.

addition, the outermost small lines have disappeared. Prolonged annealing up to the point where the sample becomes a viscous liquid and all radicals disappear leads to a pattern shown in Fig. 6. The inner three lines of the quintet are of nearly equal amplitude, whereas the intensity ratio of the two outer lines to the central line is of

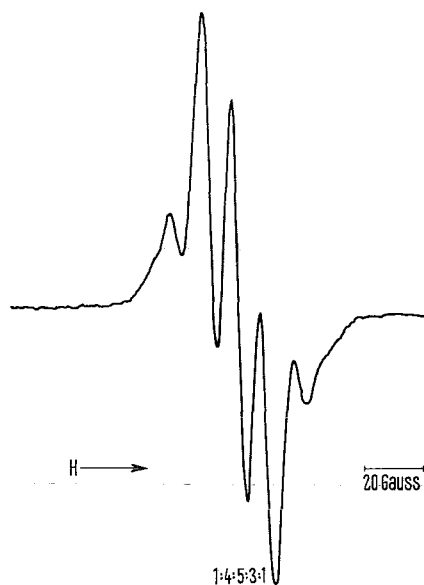


FIG. 5. Modified spectrum of Fig. 4a or 4b after heat-treatment at 60°C for 40 minutes.

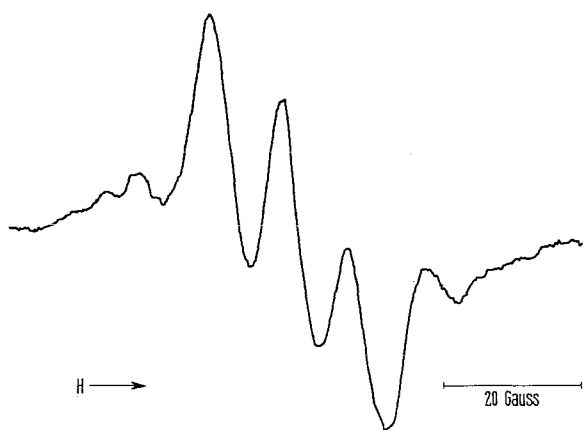


FIG. 6. Derivative spectrum obtained on prolonged heat-treatment of samples of Fig. 4a or 4b at 60°C recorded at 9.3 GHz and at room temperature.



the theoretically correct magnitude 1:6:1. From these observations we assume that radiation produces at least three different types of radicals in 2-deoxy-D-ribose, one of which is more heat-resistant than the one accounting for the quintet.

Spectra from the same samples as described in the last section were also recorded at 35-GHz frequency. In general, the spectra obtained are the same, though the five lines are not as clearly separated even in the spectra of the heated samples. This is easily explained by the assumption that the radical, which is known from single-crystal spectra to possess an anisotropic  $g$  factor, is the most heat-resistant one. Clearly, deformations of the isotropic quintet in polycrystalline samples due to a  $g$  factor anisotropy of a second radical are much more effective in Q-band than in X-band spectra. The results of the few successful heat-annealing experiments carried out with irradiated single crystals agree with this assumption.

#### DISCUSSION

Our investigations have proved that the prominent feature obtained from irradiation of 2-deoxy-D-ribose at room temperature is a five-line spectrum with intensities 1:4:6:4:1. Because of its  $g$  factor, this must arise from interaction of an unpaired electron in a carbon  $2p$ -orbital with four equivalent protons. The hyperfine coupling is isotropic, indicating that the protons are in  $\beta$ -positions with respect to the carbon atom. Therefore, the free radical responsible for the quintet pattern must be described by either scheme I or scheme II of Fig. 7, where  $R$  represents groups lacking substantial hyperfine interaction with the unpaired electron. Comparison of Figs. 2 and 7 shows that no configuration resembling scheme I or II can be formed from the original molecule without rearrangements of the molecular structure. A radical of type I may be built up with a minimum number of atomic displacements by an initial loss of a hydrogen atom from C-3 or C-4 followed by an interchange of a hydroxyl group in the  $\beta$ -position with a  $\gamma$ -hydrogen atom. However, exchange reactions between OH and H appear to be rather improbable under the conditions of the experiment. Thus we are left with scheme II, which fortunately may be obtained by a more likely sequence of reactions. An initial hydrogen loss from C-1 is followed by a proton jump from C-4 to C-3 and a simultaneous reshuffling of valence electrons resulting in a rupture of the pyranose ring to give the radical shown in Fig. 8a. The same end product is obtained if the hydrogen atom is not dissociated

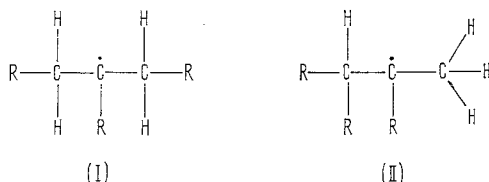


Fig. 7. Possible structures of radical giving the observed quintet pattern

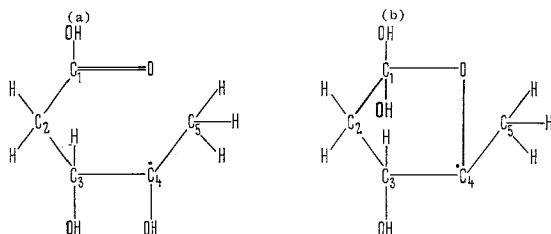


Fig. 8. Proposed structure of predominant radical produced by  $\gamma$ -irradiation of 2-deoxy-D-ribose (a) and alternative structure (b).

from C-1 initially but from any other carbon atom. Since all carbon atoms of the pentose molecule are bonded to hydrogen, this case is reduced to the former by further shifting of protons along the carbon chain. The occurrence of intramolecular migration of hydrogen in organic radicals is a well-known fact (12, 13).

In radicals of type II the equivalent methyl protons on C-5 may be assumed to rotate freely about the C-4 to C-5 bond at room temperature. Under this provision an approximate calculation of the spin density,  $\rho_{C-4}$ , of the unpaired  $\pi$ -electron on C-4 may be performed by using the relation introduced by Heller and McConnell (14):

$$A_i^\beta = B \cdot \rho_{C-\alpha} \cos^2 \Phi_i$$

In this equation  $A_i^\beta$  is the hyperfine splitting of the proton on carbon atom  $i$  and  $\Phi_i$  is the dihedral angle due to bond C- $i$  to H. The constant  $B$  is usually taken to be about 50 gauss. For a freely rotating methyl group,  $\cos^2 \Phi_i$  must be averaged over all angles. Thus, with  $A^\beta = 10.5$  gauss, we obtain

$$\rho_{C-4} = 0.42$$

Since the coupling of the C-3 proton is equivalent to that of the methyl group, the value of  $\cos^2 \Phi_3$  must also be 0.5, which means that  $\Phi_3$  is  $45^\circ$ .

It remains to discuss the absence of observable hyperfine coupling due to the hydroxyl group bound to C-4. The interaction of an OH group with the radical electron is composed of an isotropic component depending on the dihedral angle  $\theta$  between the CO and OH bonds and an anisotropic part which is proportional to the inverse cube of the distance  $r$  between the hydroxyl proton and C-4 (15). A relatively small increase of  $r$  which may originate from hydrogen bonding could result in a large reduction of the latter. Similarly, the isotropic component becomes very small if  $\theta$  approaches  $90^\circ$ . Under such conditions the OH interaction would not be resolved experimentally. Strong dependence of OH coupling on the environment has been reported by Pooley and Whiffen (15). It hardly appears possible to derive bond lengths and angles of the radical proposed from the original nonplanar "easy-chair" structure (Fig. 2) of the deoxyribose molecule (11). Therefore, the structure

shown in Fig. 8a is not regarded as incompatible with the absence of anisotropic hyperfine splitting in the spectra recorded.

An alternative radical structure is shown in Fig. 8b. Comparison with Fig. 8a reveals that radical (a) above is transformed into radical (b) by shifting an OH group from C-4 to C-1 and ring closure between the carbonyl oxygen and C-4. The radical thus formed obviates the necessity to explain the absence of an OH interaction with the unpaired electron and, in addition, facilitates understanding the small value of  $\rho_{C-4} = 0.42$  by a possible spreading of electron density over the oxygen bridge. These attractive features, however, are outweighed by the necessity to assume the unlikely transfer of an OH group and, decisively, by the results of infrared measurements discussed below. The further discussion is therefore based on the structure shown in Fig. 8a.

We shall now consider in more detail several questions arising from the model proposed. For this purpose we turn to some chemical investigations on irradiated carbohydrates in the solid state. Though only one publication (Jacobs, 16) is concerned with 2-deoxy-D-ribose, the main features used for our argument appear to be well established for all carbohydrates. The initial loss of a hydrogen atom has been shown by numerous authors to be one of the most frequent consequences of irradiating saturated organic compounds. This also holds true for 2-deoxy-D-ribose, as shown by Jacobs, who identified the volatile products originating from irradiation of the sugar constituent of DNA. Besides water vapor and CO<sub>2</sub> he obtained large quantities of molecular hydrogen. From his investigations he concludes that the primary process of irradiation is the dissociation of a hydrogen atom which subsequently abstracts another hydrogen atom either from the same or from a neighboring molecule to form H<sub>2</sub> or, alternatively, a hydroxyl group to form water. These assumptions are in complete agreement with our results and offer a straightforward explanation of the instable behavior of single crystals after irradiation.

In view of the very high radiation doses of about 50 to 100 Mrad applied by Jacobs, we shall make use of his arguments only as far as the abstraction of atomic hydrogen from the pyranose ring is considered to be the initial event of radiation damage in 2-deoxy-D-ribose. It has been mentioned already that dissociation of a hydrogen atom from any carbon of the pyranose ring is equivalent to hydrogen loss from C-1. In this case an unpaired electron is primarily localized at C-1. As a consequence, the C-5 to O bond will break under formation of a C-1=O double bond, while the unpaired electron is transferred to C-5. The free radical proposed in Fig. 8 is produced by an additional reaction involving an intramolecular hydrogen migration from C-4 to C-5, forming a methyl group on the latter and shifting the unpaired electron to C-4. The mechanism of radical formation just outlined is strongly supported by the following observations. First, to confirm the C-5 to O bond breakage and the formation of the C-1=O carbonyl function, we have recorded some infrared spectra from irradiated polycrystalline 2-deoxy-D-ribose. A strong band was ob-

tained in the region of about  $1730\text{ cm}^{-1}$ , which is not present in the spectra of unirradiated samples, and is certainly due to the carbonyl stretching vibrations. Further indications, confirming the above mechanism, come from two recent ESR investigations on irradiated 3'-cytidylic acid (9) and cytidine (8). In the latter compound, a free radical on the sugar moiety is observed, arising from abstraction of a hydrogen atom from C-1. For 3'-cytidylic acid, too, a free radical residing on the sugar is postulated, and the mechanism of radical formation proposed by Bernhard and Snipes (9) is of strong resemblance to that presented here, lacking only the intramolecular hydrogen migration. In view of the differences in chemical structure and conformation between ribose and deoxyribose, the absence of hydrogen migration in the former does not appear to be surprising. The fact that 2-deoxy-D-ribose is able to change its conformation from the pyranose form to the furanose form simply by solvation shows the high probability of hydrogen transfer in this compound, since such a process is accompanying the conformational change. From the arguments listed above, the chemical structure of the predominant radical shown in Fig. 8 as well as the mechanism leading to its formation appears to be well established.

A further question arising from this study is concerned with the importance of the sugar moiety for the effects of ionizing radiation on DNA. Without giving a complete discussion of all the problems involved, we want to outline some of the main questions, primarily from the viewpoint of ESR results. We shall deal first with events in which the sugar is believed to play an important, but more indirect role. Quantitative ESR measurements have shown (17) that the radical yield of dry nucleic acid constituents increases in the sequence base, nucleoside, nucleotide, and pentose. Though the radical yield of the latter is much higher than that of the bases, the observed spectra of nucleosides and nucleotides were not characteristic of their constituent pentoses and have been shown later by single-crystal studies to arise from free radicals stabilized on the base group of the compound investigated. Moreover, most of the base radicals proved to be H addition radicals. Therefore, the energy transfer from the sugar group to the base postulated earlier is now explained by the transfer of atomic hydrogen. These assumptions were supported by studies on molecular mixtures of thymine and deoxyribose (10). The results of the present investigations on single crystals of pure 2-deoxy-D-ribose are in complete accordance with the outlined scheme, since hydrogen loss from the pyranose ring has been shown to be indeed one of the major consequences of irradiation and might lead to the observed addition radicals in the base groups of nucleosides and nucleotides.

The question of whether and in what way the predominant radical in 2-deoxy-D-ribose is a direct precursor of biological radiation damage is, of course, much more difficult to answer. Nevertheless a complete scission of the pentose ring appears quite likely to occur on dissolving radical-containing dry material in water. This

pathway of reaction is supported by observations of Jacobs, who obtained production of volatile fragments on addition of water to irradiated dry sugars (16). Moreover, it was demonstrated by infrared spectroscopy of glucose and sucrose that these products were not just set free from the lattice by the process of solvation but resulted from chemical reactions yielding volatile compounds. Thus, the formation of the radical demonstrated here may well prove to lead by further reaction to the breaks of the polynucleotide chains of DNA which are currently being studied intensively (18).

## ACKNOWLEDGMENTS

We wish to thank Professor Dr. K. G. Zimmer for continued and encouraging interest in this work and Drs. W. Snipes and H. Dertinger for helpful discussions. Furthermore, we thank Dr. W. Schneider for x-ray diffraction measurements and Mr. W. Muhr for providing the infrared spectra.

RECEIVED: September 17, 1968

## REFERENCES

1. MÜLLER, The formation of radicals in nucleic acids, nucleoproteins, and their constituents by ionizing radiation. *Progr. Biophys. Mol. Biol.* **17**, 99-147 (1967).
2. H. DERTINGER, Der Ursprung des ESR-Dubletts in der Nucleinsäurebase Cytosin: eine Einkristallstudie bei 8 mm Wellenlänge. *Z. Naturforsch.* **22b**, 1266-1269 (1967).
3. J. B. COOK, J. P. ELLIOT, and S. J. WYARD, Electron spin resonance of an irradiated single crystal of cytosine. *Mol. Phys.* **13**, 49-64 (1967).
4. C. ALEXANDER, JR., and W. GORDY, Electron spin resonance of an irradiated single crystal of guanine hydrochloride dihydrate. *Proc. Natl. Acad. Sci. U.S.* **58**, 1279-1285 (1967).
5. H. DERTINGER, Freie Radikale in bestrahlten Einkristallen von Purin-Nucleosiden: Adenosin, Desoxyadenosin und Desoxyguanosin. *Z. Naturforsch.* **22b**, 1261-1266 (1967).
6. B. PRUDEN, W. SNIPES, and W. GORDY, Electron spin resonance of an irradiated single crystal of thymidine. *Proc. Natl. Acad. Sci. U.S.* **53**, 917-924 (1965).
7. J. J. LICHTER and W. GORDY, Electron spin resonance of an irradiated single crystal of deoxyadenosine monohydrate. *Proc. Natl. Acad. Sci. U.S.* **60**, 450-455 (1968).
8. W. GORDY, Free radicals from biological purines and pyrimidines, *Ann. N.Y. Acad. Sci.* in press.
9. W. BERNHARD and W. SNIPES, Electron spin resonance of a gamma-irradiated single crystal of 3'-cytidylic acid. *Proc. Natl. Acad. Sci. U.S.* **59**, 1038-1044 (1968).
10. A. MÜLLER, Zum Mechanismus der Strahlenschädigung von DNS. In *Molekulare Struktur und Strahlenwirkung* (H. Glubrecht et al., eds.), pp. 147-150, G. Thieme, Stuttgart, 1968.
11. S. FURBERG, The structure of 2-deoxyribose. *Acta Chem. Scand.* **14**, 1357-1363 (1960).
12. G. G. PHILLIPS, P. BAUGH, and M. YOUNG, Energy transfer in carbohydrate systems In *Energy Transfer in Radiation Processes* (G. O. Phillips, ed.), pp. 15-28, Elsevier Publishing Company, Amsterdam, 1966.
13. C. J. M. STIRLING, *Radicals in Organic Chemistry*. Oldbourne Press, London, 1965.
14. C. HELLER and H. MCCONNELL, Radiation damage in organic crystals. II. Electron spin resonance of  $(\text{CO}_2\text{H})\text{CH}_2\dot{\text{C}}\text{H}(\text{CO}_2\text{H})$  in  $\beta$ -succinic acid. *J. Chem. Phys.* **32**, 1535-1539 (1960).
15. D. POOLEY and D. H. WHIFFEN, Conformational isomerism in the  $\text{H}\dot{\text{C}}(\text{OH})\text{CO}_2^-$  radical ion. *Trans. Faraday Soc.* **57**, 1445-1448 (1961).

16. S. S. JACOBS, The solid state gamma irradiation of crystalline amino acids and sugars. Thesis, University of Colorado, 1962.
17. A. MÜLLER, Spektrographische Untersuchungen mittels paramagnetischer Elektronenresonanz über die Wirkung ionisierender Strahlen auf elementare biologische Objekte. *Akad. Wiss. Lit. Mainz (Math.-Nat. Kl.)* No. 5 (1964).
18. K. G. ZIMMER and colleagues, Some recent studies in molecular radiobiology. *Current Topics Radiation Res.* 5, 1-38 (1969).