PARAMAGNETIC RESONANCE STUDY OF IRRADIATION DAMAGE IN CRYSTALLINE CARBOHYDRATES*

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Communicated September 10, 1958

INTRODUCTION

By subjecting organic molecules in solids to strongly ionizing radiation, such as X-rays or cathode rays, electrons can be removed from ground-state molecular orbitals with sufficient energy to free them from the molecule. When the organic molecule loses an electron in this manner, the ionized molecule, if it holds together, will have an unpaired electron in one of its orbitals. The electron which is removed from one molecule may become attached to a neighboring molecule and go into an excited orbital of this molecule, or it may be trapped at imperfections in the crystal lattice. It is possible for the ionizing radiation to produce either positively or negatively charged ions. These ions will be short-lived and will probably become stabilized as uncharged free radicals with unpaired electrons. In most cases the formation of the final radical will be a complicated process, in which the ionizing radiation will produce unstable entities, which in turn will decay to others until a stable radical is formed. If the barrier to the return passage of the electrons between the molecules is large, then sufficiently high concentrations of free radicals can be built up to give a detectable electron-spin resonance.

If the spin of the unpaired electron in the radical is entirely free from the perturbing influence of its environment, a single sharp line with \( g = 2.0023 \) might be expected. However, the electron is very sensitive to its environment, and information about the interaction of the electron with its surroundings can usually be obtained from the paramagnetic resonance spectrum. In the resonance spectrum
there are usually two sources of information: most importantly, the hyperfine structure, which arises from the interaction of the magnetic moment of the electron with the magnetic moments of the nuclei on which the electron is localized, and, second, the small residual spin-orbit coupling which in some cases causes the \( g \)-factor to be slightly anisotropic and different from the free-spin value.

**Hyperfine Structure.**—The hyperfine-structure splittings in polyatomic free radicals have been discussed in detail by Weissman. If the interaction of the electron with the external magnetic field is much larger than the interaction of the electron with nuclei in the radical, the magnetic fields at which the hyperfine lines occur for constant frequency are given by

\[
H = \left( \frac{\hbar \nu}{g \beta} \right) + \frac{1}{g \beta} \sum_{i} A_{i} m_{i}
\]

(1)

where \( A_{i} \) is the coupling constant of the electron with a particular nucleus \( i \) with spin \( I_{i} \) and the magnetic quantum numbers have the values \( m_{i} = I_{i}, I_{i} - 1, \ldots, -I_{i} \).

If all the coupling nuclei in a given free radical have the same coupling \( A_{i} \) to the electron spin, one can define

\[
T = \sum_{i} I_{i}
\]

(2)

and a total magnetic quantum number \( M = T, T - 1, \ldots, -T \). For this situation, equation (1) can be written in the simpler form

\[
H = \left( \frac{\hbar \nu}{g \beta} \right) + \left( \frac{A}{g \beta} \right) M.
\]

(3)

In this case the spectrum consists of \((2T + 1)\) components separated by \( \Delta H = (A/g\beta) \). The intensities of the components are proportional to the number of different combinations of \( m_{i} \)'s which give the same value of \( M \).

The interaction constant, \( A_{i} \), of the electron spin with the moment of a particular nucleus \( i \), in general, contains both an isotropic component and an anisotropic component. The isotropic component, which is the Fermi term, is independent of the orientation of the magnetic field and arises from the non-vanishing of the electronic wave function at the nucleus in question. Since only the \( s \) atomic orbitals are non-vanishing at the nucleus, the presence of an isotropic coupling term for a particular atom in a molecule generally indicates the \( s \) character in the bonding orbitals of the atom. The anisotropic contribution of \( A_{i} \) comes from the direct magnetic dipole-dipole coupling of the electron with the nucleus \( i \). In a single crystal, one can distinguish between the isotropic and anisotropic contributions to \( A_{i} \) by studying the hyperfine separations as the crystal is rotated with respect to the external magnetic field. For powder samples, one cannot, in general, distinguish between the isotropic and the anisotropic terms. Instead, an average \( A_{i} \) is obtained, because in a powder the chemical-bond axes are randomly oriented with respect to the magnetic field.

**\( g \)-Anisotropy.**—When the unpaired electron is localized on a non-\( s \) orbital of a single atom of a radical, the effects of spin-orbit interaction cannot be neglected. The orbital angular momentum is oriented by the strong electrical forces of the
chemical bond, and this gives rise to an anisotropy in the $g$-factor. If the electron-wave function is symmetric about a chemical bond, the $g$-factor reflects this symmetry. For an arbitrary orientation, $\theta$, of the bond axis with respect to the external magnetic field, the observed $g$-factor is given by

$$g^2 = g_{||}^2 \cos^2 \theta + g_\perp^2 \sin^2 \theta. \quad (4)$$

In the case of a powder sample, the resonance absorption is averaged over all possible orientations of the bond axis with respect to the magnetic field. Since the $g_\perp$ value has greater weight than $g_{||}$, the resonance has an asymmetric shape with two peaks. The peak with maximum intensity corresponds to the $g_\perp$ value, and the other peak corresponds to $g_{||}$.

In a powder sample one can distinguish between hyperfine-structure effects and effects due to $g$-anisotropy by studying the spectrum at several different frequencies. Once the strong-field or Paschen-Back case is realized, the hyperfine-structure separation is independent of the frequency of observation. The effect due to $g$-anisotropy depends on the frequency of observation.

The electron paramagnetic resonance study of radiation damage to organic molecules has been pursued by a large number of workers. Gordy and co-workers have carried out paramagnetic resonance studies of free radicals produced by X-irradiation of amino acids, peptides, fatty acids, nucleic acids, proteins, enzymes, hormones, and vitamins. Paramagnetic resonance studies on irradiated carbohydrates were reported by Combrisson and Ubersfeld and by O'Meara and Shaw. The results of similar studies on X-irradiated and cathode-ray-irradiated carbohydrates are reported in the present paper.

**EXPERIMENTAL RESULTS**

A total of sixteen irradiated carbohydrates have been studied by observing the paramagnetic resonance spectra of powder samples of these materials. $\alpha$-d-Glucopyranose monohydrate, $\beta$-glucitol (sorbitol), $\alpha$-d-galactopyranose, and $\alpha$-inositol have been studied after irradiation with both X-rays and cathode rays. In each of these samples the paramagnetic resonance spectrum of the X-irradiated sample was identical with the spectrum of the cathode-ray-irradiated sample. This result seems to indicate that the final radical products produced by X-irradiation are the same as those produced by high-energy cathode rays.

The spectrograph employed in the present study consisted of an X-band Klystron operating at constant frequency, controlled by a secondary frequency standard, which was monitored by WWV. Samples were mounted in a transmission-type resonant cavity having an unloaded $Q$ of approximately 5000. The cavity was situated between 6-inch diameter poles of an electro-magnet supplying a strong magnetic field, which could be varied slowly by means of a clock drive attached to a potentiometer in the magnet power supply. Magnetic field modulation at 800 cycles/sec was provided by means of two small coils mounted against the external walls of the sample cavity. A lock-in amplifier was employed, so that the trace displayed on the recorded chart was a first derivative of the absorption signal.

The high-energy electron beam (cathode ray) was supplied by a resonant transformer in conjunction with a permanently evacuated cathode-ray tube. The source utilized was a 1-Mev peak, 500 $\mu$a beam-out unit. The dose was measured.
Fig. 1.—Electron-spin resonance spectra of irradiated carbohydrates. The curves represent first derivatives of the actual absorption lines.

by a specially constructed, air ionization chamber. The unit was located at the General Electric Co., Milwaukee, Wisconsin. The soft X-rays were provided by a prototype unit located at the Battelle Memorial Institute, Columbus, Ohio. The X-ray tube was operated at 60 kvp. and 200 ma. and was fitted with a beryllium
window to allow the escape of long-wave-length radiations.\textsuperscript{12} All samples were irradiated with 5 megarads.

In most of the irradiated carbohydrates which have been studied, the spectra consist of a number of overlapping lines. To analyze the spectrum of an irradiated sugar which is composed of overlapping lines, it is necessary to be careful in identifying the positions of absorption lines. For precise determination of the position and intensity of a line in the spectrum, it is usually necessary to integrate the first derivative of the absorption spectra presented on the recorded chart. In the figures to be presented, the apparent position of lines are located, since maxima and points of inflection of the absorption curve are easily identified on the first derivative traces.

Irradiated $\alpha$-$d$-glucopyranose monohydrate, $\beta$-$d$-fructopyranose, $\alpha$-lactose monohydrate (4-O-$\beta$-$d$-galactopyranosyl-$\alpha$-$d$-glucopyranose monohydrate), L-sorbose, and $\alpha$-$d$-galactopyranose give very similar paramagnetic resonance spectra. The spectra of these irradiated sugars are shown in Figure 1. The spectrum of each of these samples shows a characteristic four-line hyperfine structure with an average spacing between lines $(A/\beta)$ of approximately 15 gauss. The $\beta$-factor, which is calculated from the center of the spectrum, is 2.003 ± 0.001. The hyperfine structure is best resolved for $\beta$-$d$-fructopyranose and $\alpha$-lactose monohydrate, and a detailed investigation of these spectra indicates that the hyperfine-structure components have an intensity ratio of 1:3:3:1. This type of hyperfine structure is to be expected if the electron is interacting with three protons $(I = 1/2)$, for each of which $(A/\beta) = 15$ gauss.

The hyperfine structure observed for L-sorbose (ring structure, if any, unknown) is not so well resolved as for $\beta$-$d$-fructopyranose and $\alpha$-lactose monohydrate, but the positions of the hyperfine lines coincide with those observed in $\beta$-$d$-fructopyranose and $\alpha$-lactose monohydrate. For $\alpha$-$d$-galactopyranose and $\alpha$-$d$-glucose monohydrate, the hyperfine structure is barely resolved. In $\alpha$-$d$-galactopyranose, only the two outermost lines can be located with reasonable accuracy; however, the position of these two outermost lines is almost the same as those observed for $\beta$-$d$-fructopyranose. For $\alpha$-$d$-glucopyranose monohydrate the four-line hyperfine structure is observed, but for this sugar two additional lines are observed near the center of the hyperfine pattern; this probably indicates that for this sugar more than one stable radical has been formed. It should be noted that this sugar was irradiated in the hydrated form.

From the paramagnetic resonance spectrum alone it is difficult to obtain a unique identification of the radical responsible for the hyperfine structure observed in $\alpha$-$d$-glucopyranose monohydrate, $\beta$-$d$-fructopyranose, $\alpha$-lactose monohydrate, L-sorbose, and $\alpha$-$d$-galactopyranose. However, the magnetic resonance spectra indicate that possible radicals are limited to types which contain three equally coupling protons.

The paramagnetic resonance spectra observed for irradiated erythritol and D-threitol are shown in Figure 1, and those of $\alpha$-$L$-rhamnopyranose monohydrate and myo-inositol are shown in Figure 2. For erythritol and D-threitol the spectrum consists of two lines of equal intensity. This type of spectrum would be expected if the electron were interacting with a single proton in the radical. The hyperfine spacing $(A/\beta)$ for erythritol and D-threitol is approximately 6 gauss. The hyper-
fine structure is centered on \( g = 2.003 \pm 0.001 \). Two lines of equal intensity are also observed in the \( \alpha-L \)-rhamnopyranose monohydrate spectrum; these two lines are assumed to be a hyperfine doublet with \( (A/gB) = 6 \) gauss and \( g = 2.002 \pm 0.001 \). In the \( \text{myo}-\text{inositol} \) spectrum (Fig. 2) four lines are observed. These four lines consist of a pair of intense lines and two weak lines. If the two strong lines in the spectrum, labeled \( A \) and \( C \) in Figure 2, are associated with a hyperfine doublet,
then \((A/gB)\) for this pair of lines is 25 gauss. The center of this assumed hyperfine-structure doublet is at \(g = 2.002 \pm 0.001\). If the weaker pair of lines is also considered a hyperfine-structure doublet, then \((A/gB)\) for this set of lines is 19 gauss and \(g = 1.991 \pm 0.001\).

The spectra observed for irradiated \(\beta\)-glucitol and \(\alpha\)-d-xylopyranose are shown in Figure 2. Each of these samples showed only a single absorption line with no hyperfine structure. The line observed in \(\beta\)-glucitol has \(g = 2.002 \pm 0.002\). The spectrum observed in \(\alpha\)-d-xylopyranose is asymmetrical and appears to be composed of a single sharp line with \(g = 2.003 \pm 0.001\), which is superimposed on a much broader line.

In Figure 2 the spectra observed for irradiated pentaerythritol and \(\beta\)-mannitol are shown. For each of these samples the spectrum is complex. In both spectra there is evidence that the quartet observed in \(\beta\)-d-fructopyranose is present; however, additional lines which are present prevent the quartet from being identified with certainty. In both samples the apparent positions of lines are indicated in the figures. It is known\(^1\) that the terminal primary hydroxyl groups are points of irradiation attack, and it may be of interest to note that \(\beta\)-mannitol possesses two stereoelectronically equivalent primary hydroxyl groups and that pentaerythritol possesses four.

Irradiated \(\alpha\)-d-mannopyranose and sucrose have also been studied, and the spectra of these sugars are shown in Figure 2. The \(\alpha\)-d-mannopyranose spectrum consists of three absorption peaks which can be associated in several ways. If peaks \(A\) and \(B\) are associated with a hyperfine doublet, then, for this pair of lines, \((A/gB) = 12\) gauss and \(g = 2.002 \pm 0.001\). Another possibility is that \(A\) is a single sharp line with \(g = 2.006 \pm 0.001\) and that \(B\) and \(C\) are lines in a hyperfine triplet with the third line unresolved because of its overlap with line \(A\). If \(B\) and \(C\) are lines in a hyperfine triplet with possible intensity ratio 1:2:1, then \((A/gB)\) is approximately 15 gauss and \(g = 1.999 \pm 0.001\). To distinguish between these various possibilities for \(\alpha\)-d-mannopyranose, it would be necessary to study the paramagnetic resonance spectrum of this sugar at a higher microwave frequency. The spectrum observed for sucrose is complex, and the possible positions of peaks are indicated in Figure 2. The center of the sucrose spectrum is at \(g = 2.002 \pm 0.001\). The complexity of the sucrose spectrum is not too surprising, since the crystal structure of this sugar is complicated. One of the carbon atoms (C4) of the furanose ring is displaced from the plane of the other four atoms. The configuration of both rings is such as to allow the larger attached groups to approach as nearly as possible to the plane of each ring.\(^1\)

Rhamnose is 6-deoxy-mannose, or the former carries a terminal methyl rather than a terminal hydroxymethyl group, with other parts being equal, save for enantiomorphism. The marked difference in their spectra perhaps reflects this structural dissimilarity.

In the carbohydrates thus far discussed, the only nuclei with non-zero nuclear spins were protons. In Figure 1 is shown the magnetic resonance spectrum of irradiated 2-amino-2-deoxy-\(\alpha\)-d-glucopyranose hydrochloride (\(\alpha\)-d-glucosamine hydrochloride). In addition to protons, \(\alpha\)-d-glucosamine hydrochloride contains one nitrogen atom. For N\(^1\) the nuclear spin \(I = 1\). The spectrum of \(\alpha\)-d-glucosamine hydrochloride shows five lines with an over-all separation between
outermost lines of 74 gauss. The center of this five-line structure is at $g = 2.001 \pm 0.001$. An analysis of this spectrum indicates that the spectrum consists of five equally spaced lines separated by 18.5 gauss. The spectrum can possibly be interpreted as hyperfine structure due to the electron coupling equally with four protons. Another possibility is that the spectrum is due to the electron coupling equally with two protons and a nitrogen. If the spectrum is actually due to the electron coupling equally with two protons and a nitrogen, the fact that the coupling constant for the proton is identical with the coupling constant for the nitrogen is an unexpected and surprising result. For four protons coupling equally to the electron, the expected intensity ratio of the lines would be 1:4:6:4:1, whereas, for the electron coupling equally to two protons and a nitrogen, the intensity ratio of the lines would be 1:3:4:3:1. One other possibility is that the spectrum is due to the electron coupling equally with two nitrogens, for which case the expected intensity ratio of the lines would be 1:2:3:2:1. To aid in making an estimate of the relative intensities of the five lines in the $\alpha\text{-d-glucosamine}$ hydrochloride spectrum, the first derivative record given in Figure 1 was integrated. An estimate of the intensities was made by trying to reproduce the observed spectrum from five lines of equal width but different peak heights. Only a very crude estimate could be made because of the large amount of overlap of the lines, but this crude analysis seemed to indicate that the ratio of the relative intensities was approximately 1:3:4:3:1.

**CONCLUSIONS**

In most of the irradiated carbohydrates studied, the existence of hyperfine structure in the paramagnetic resonance spectrum suggests the general nature of the radical produced by the radiation. From a consideration of the chemical structure of some of these sugars, some of the similarities observed in the spectra of different sugars are not surprising.

For $\alpha\text{-d-glucopyranose}$ monohydrate and $\alpha\text{-d-galactopyranose}$ the structures are given in Figure 1. The only difference in structure of these two sugars is the relative configuration about C4. Hence, since there is such a great similarity in the structure of these two sugars, it is not surprising that the radicals produced by irradiation of these molecules would give similar paramagnetic resonance spectra. The spectrum for $\alpha\text{-d-mannopyranose}$ is different from that of $\alpha\text{-d-glucopyranose}$ monohydrate and $\alpha\text{-d-galactopyranose}$. This would seem to indicate that the configuration of C2 in these sugars influences the type of radical formed.

The spectra of the two ketohexoses, $\beta\text{-d-fructopyranose}$ and L-sorbose (detailed structure unknown), are similar. The spectra of erythritol and D-threitol were found to be similar, and again the only difference in structure of these two is the relative position of a hydroxyl group.

Many questions about the effects of radiation damages to carbohydrates and the free radicals produced are still unanswered. However, it is hoped that the results of the paramagnetic resonance studies which have been carried out can be used in helping to decide what radicals are formed by the ionizing irradiation. On the basis of the present work, further studies seem justified. The study of irradiated single crystals of carbohydrates would probably yield additional information.
* This work was supported in part by the Office of Scientific Research, Air Research and Development Command, U. S. Air Force, and in part by the Quartermaster Institute for the Armed Forces QM Research and Engineering Command, U.S. Army, under Contract No. DA19-129-QM-932 with The Ohio State University Research Foundation (Project 765), and has been assigned No. 949 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the view or endorsement of the Department of Defense.


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**INCREASE IN FITNESS IN EXPERIMENTAL POPULATIONS RESULTING FROM HETEROSIS**

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*Communicated by Th. Dobzhansky, September 19, 1958*

**Introduction**—A local population of a species, or deme, consists of an array of individual genotypes. At any one time level, the deme represents the active evolutionary interface between the hereditary material and the environment. Genetic novelties due to new mutations or recombinations are automatically tested when the deme is under natural selection. If a genetic change improves the fitness of its carriers, natural selection may be expected to reproduce the change differentially. The result is adaptive evolution.

The experiments here described represent an attempt to study the process of adaptation directly. The equivalent of one gamete of foreign genetic material is introduced into an experimental population having a size which is held by natural selection in equilibrium with a rigidly controlled and limited environment. Although the environment is unchanged, this introduction is followed by an immediate threelfold increase in the population size. Such an increase, occurring as it does under continual strong natural selection, reflects a corresponding increase in the biological efficiency of the group under the specific environmental conditions embodied in the experiment. The evidence is strong that this increase is due to