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ON THE ROLE OF HYDROGEN ATOMS IN FREE RADICAL SITE MIGRATION

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September 1969

Free radical site migration in irradiated proteins and other biologically important compounds has been a topic of interest in a number of publications. In one way or another, site migration is involved in such processes as (a) selective formation of radicals on cysteine residues in proteins; (b) transfer of radicals from proteins to sulfur-containing radiation protectors; (c) thermally-induced free radical decay; and (d) radiation-induced free radical destruction. All these processes require the migration of unpaired electrons for distances of about 10 Å or greater in an irradiated sample.

In their early work on proteins, Gordy and Miyagawa (1960) suggested that the migration is intramolecular in nature and mediated by electron-hole transfer. Sulfur-containing radiation protectors were thought to "scavenge" the unpaired electron by becoming bonded to the protein during sample preparation. However, Henriksen and coworkers (1968) presented good evidence that the transfer of unpaired electrons can be intermolecular in the protein-scavenger system. These workers and others (Braams 1963, Sommermeyer, Stegle, and Schnepel 1967, Copeland, Sanner, and Pihl 1968) proposed that site migration is

mediated by the diffusion of small radicals, in particular, hydrogen atoms. Braams (1963) presented a rather detailed theory to explain a number of radiation processes, including thermal decay, by the migration of small radical fragments. Müller (1966) suggested that hydrogen atoms are responsible for the radiation-induced free radical destruction that leads to dose-saturation, and Copeland et al. (1968) suggested that hydrogen atoms are intermediates in the intermolecular formation of secondary protein radicals. In the case of thermal decay and free radical destruction by radiation, Horan and coworkers (Horan, Taylor, Strother, and Snipes 1968, Horan and Snipes 1969) have recently presented evidence that favors an inter- and intramolecular site migration via electron-hole transfer rather than diffusion of hydrogen atoms.

Because hydrogen atoms have been so strongly implicated in free radical site migration, it seemed appropriate to look for this process in a compound that does not contain hydrogen. In this communication we wish to report experiments that were performed on hexachloroethane (C_2Cl_6). The sample, obtained commerically, was checked using infrared spectroscopy to ascertain that it was free of water. The sample was sealed in a quartz tube under one atmosphere of nitrogen. Irradiation was carried out with 6.5 MeV electrons from a linear accelerator at a dose rate of 1 MR/min. E.P.R. spectroscopy was completed using a Varian 4500 spectrometer with a TE_{104} dual cavity. The standard used was pitch in KCl.

Figure 1 shows the E.P.R. spectrum of irradiated hexachloroethane. In Figure 2a the dose response curve is plotted; R/N gives the number of radicals per molecule. At saturation, there is approximately one radical per 750 molecules. The data fit the empirical equation

$$R = R_{\infty}(1 - e^{-kD}), \tag{1}$$

where R is the number of radicals, R_{∞} is the number of radicals at saturation, k is a constant, and D is the dose. A straight line plot is obtained (Figure 2b) if the equation is written in the form

$$\log(1 - R/R_{\infty}) = -kD, \tag{2}$$

and the value of k is found to be 0.035 MR^{-1}.

If equation (1) is differentiated with respect to dose, the relation

$$\frac{dR}{dD} = kR_{\infty} - kR \tag{3}$$

is obtained. Müller (1966) has interpreted this equation as representing a constant production and a first-order destruction of free radicals. Recently, the existence of the first-order destruction of free radicals by gamma-irradiation has been experimentally verified for alanine (Snipes and Horan 1967) and several other compounds (Horan and Snipes 1969).

It has been pointed out previously (Horan and Snipes 1969) that the destruction process is extremely efficient, so that energy absorbed in other parts of the crystal must be capable of effecting radical destruction. Because radicals can only be destroyed by recombination

with other unpaired electrons, this implies some form of radical site migration. In the case of hexachloroethane, this cannot be mediated by hydrogen atoms, as none are present in this compound. It seems likely that electron-hole conduction mediates the intermolecular site migration by one or more of the mechanisms suggested previously (Horan et al. 1968) for thermal decay.

These experiments show that site migration can occur by a process that does not involve hydrogen atoms. In other compounds, hydrogen atoms may indeed be the mediators for site migration. The point to be made is that all previous evidence, as well as that presented here, has been only suggestive; more conclusive experiments are needed to discover the nature of free radical site migration.

ACKNOWLEDGMENTS

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Figure Captions

Figure 1. First-derivative E. P. R. spectrum of hexachloroethane irradiated at room temperature. The arrow marks the field position of DPPH, for which g = 2.0036.

Figure 2. (a) Dose-response curve for irradiated hexachloroethane.

(b) Same data, plotted according to equation (2).

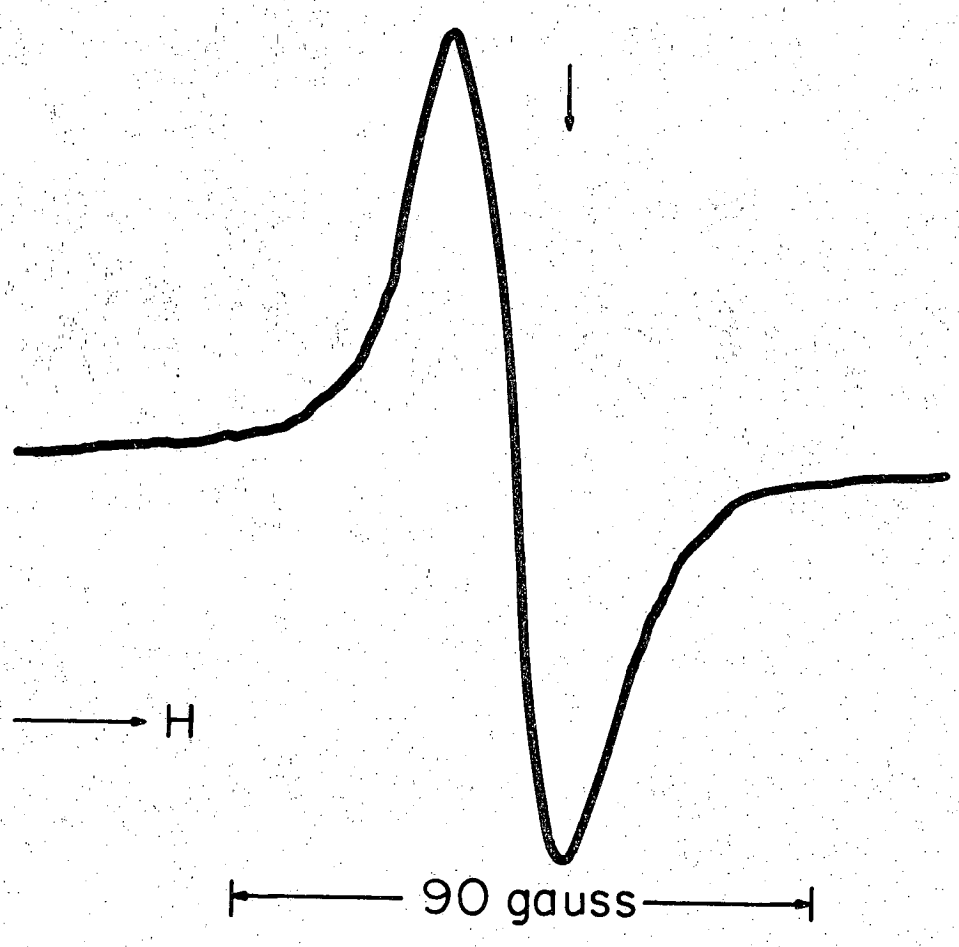

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Figure 1.

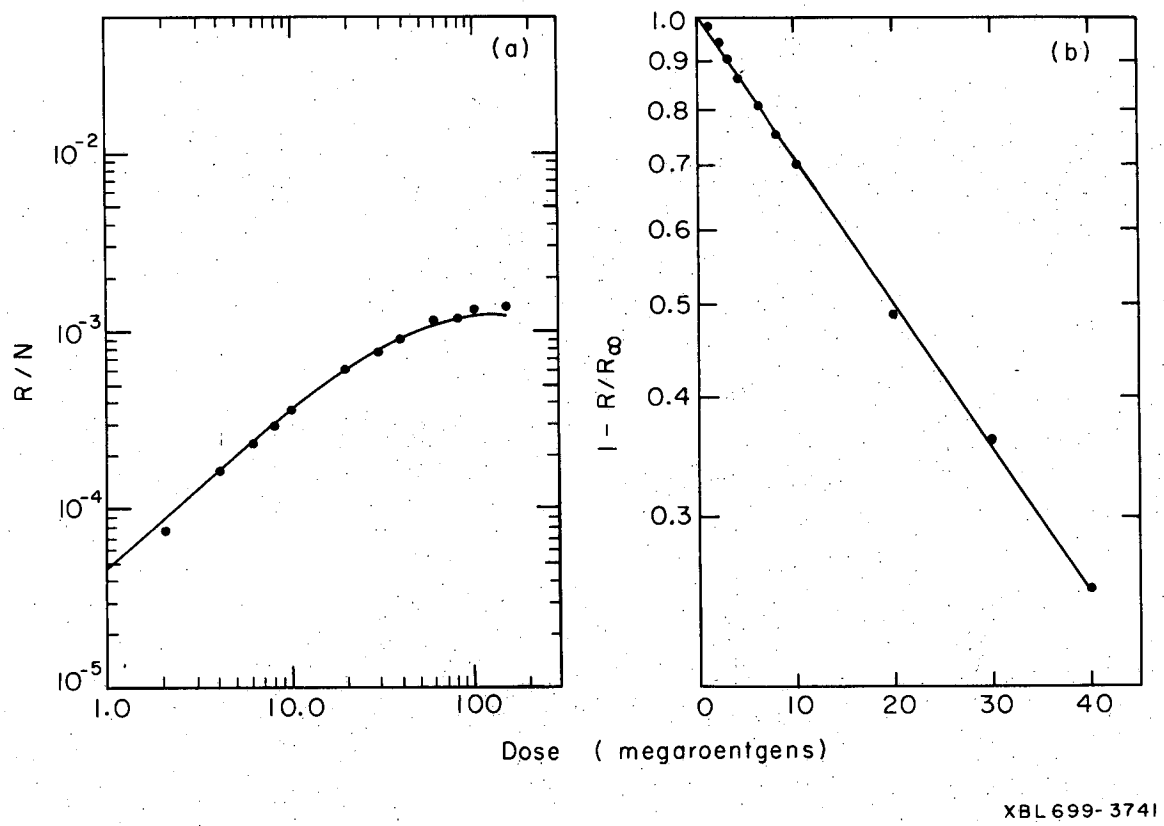
(a)
(b)
R / N
10^{-2}
10^{-3}
10^{-4}
10^{-5}
1.0
10.0
100
$1 - R/R_\infty$
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0
10
20
30
40
Dose (megaroentgens)
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Figure 2.

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