ELECTRON NUCLEAR DOUBLE RESONANCE STUDIES
OF FREE RADICALS TRAPPED IN X-IRRADIATED
SINGLE CRYSTALS OF PHENAZINE, HIPPURIC ACID
AND N-ACETYLGLYCINE

by

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We accept this thesis as conforming to the
required standard.

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ABSTRACT

The technique of electron nuclear double resonance (ENDOR) spectroscopy has been used to determine the identity and structure of radicals trapped in room temperature X-irradiated single crystals of some biologically important compounds, namely, phenazine, hippuric acid and N-acetylglycine.

The ESR spectra of phenazine which had not previously been reported, were too complex to be analyzed. However, the high resolution of ENDOR has made it possible to study all the proton hyperfine interactions in detail. The radicals are formed as a result of the addition of atomic hydrogen to the nitrogen atom of phenazine molecule. The appearance of twice the number of certain ENDOR lines than expected from the crystal symmetry was interpreted by postulating that the radical was stabilized in two different conformations.

In hippuric acid (N-Benzoylglycine), the stable N-Benzoylaminomethyl radical formed by room temperature X-irradiation of single crystals has been identified and its structure established by detailed ENDOR studies at 77 K. Besides proton signals, ENDOR transitions arising from the hyperfine and quadrupole interactions of the $^{14}\text{N}$ nucleus were detected and a detailed analysis is presented. The assignment of exchangeable protons has been accomplished by use of partially deuterated crystals. The radical is most likely formed from oxidised hippuric acid by deprotonation and subsequent decarboxylation.

Electronic and molecular structure of the stable radical produced by room temperature X-irradiation of single crystals of N-acetylglycine has
been reinvestigated by detailed ESR and ENDOR studies. The results indicate that the radical structure and the nature of the wavefunction of the unpaired electron are significantly different from those reported in a previous 'negative' ENDOR study. In agreement with earlier ESR and ENDOR studies, it is found that the stable radical at room temperature is \( \text{CH}_3\text{CONHCHCOOH} \). ENDOR lines due to the \( \alpha \)-proton, three equivalent methyl protons and six exchangeable protons have been identified and the hyperfine tensors determined. According to the findings, the radical exists in four slightly different conformations. Isotopic substitution with \( ^{15}\text{N} \) and deuteration of the exchangeable protons were helpful in the analysis of the complex ESR and ENDOR spectra. It has been conclusively shown that the multiplet structure in the ESR spectrum of irradiated N-acetylglycine arises from the interaction of the unpaired electron with protons of the methyl group and the \( ^{14}\text{N} \) nucleus. It has been also demonstrated that the radical concentration in the crystal has to be very carefully controlled for the success of an ENDOR investigation.

In order to confirm the radical identification INDO-MO calculations have been performed on all the radicals reported here. Excellent agreement has been obtained between the observed spin densities and those computed by the INDO-MO calculations.
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DEDICATED TO
THE LOVING MEMORY
OF
MY FATHER
CHAPTER ONE

INTRODUCTION

Radiation chemistry is the study of the chemical effects of high energy ionizing radiation. High energy radiation includes electromagnetic radiation (x-rays and γ-rays), particles (α-particles, β-particles or electrons, protons and neutrons) and fission fragments. Radio chemistry, which is often confused with radiation chemistry, however, deals with the chemistry of radioactive elements and with the use of radioactive tracers and the measurement of their radioactivity. Nuclear chemistry, on the other hand, is concerned with nuclear transformations, particularly fission products and transuranium elements.

The principal characteristic of high energy radiation is that it causes ionization and subsequent chemical change during its passage through matter. There is sufficient energy available to break any bond, but in practice certain bonds may be broken preferentially. The ionizing photon or particle and the displaced electron are often both capable of producing further ionization. Thus one incident photon may affect many thousands of molecules.

Out of numerous uses of radiation chemistry, biological effects of radiation have always been of interest. Studies in this field range from physiological and biochemical effects in living animals, through those on tissues and other body components to the chemistry of proteins and amino acids. One aim of such work is the treatment of people inadvertently exposed to irradiation. Radiation biological studies are also helping to reveal the chemistry of many life processes and the formation mechanism of various radiation products.

Magnetic resonance spectroscopy has provided a new and powerful approach to the study of radiation effects. Most organic molecules contain an even
number of electrons and hence are diamagnetic. The net magnetic moment
of the nonvalence electrons in the inner filled shells of each atom or of
any unshared pair of valence electrons is zero. The net magnetic moment of
each pair of valence electrons involved in bond formation is also zero,
since it is axiomatic in quantum chemistry that covalent bonds are formed
by pairs of electrons of opposite spin. The effect of ionizing radiation
is to disrupt this pairing. Obviously unpairing occurs during ionization
or whenever a covalent bond is ruptured. A molecule having an unpaired
electron is called a free radical. Free radicals are necessarily para-
magnetic. The unpaired electrons in the damaged species interact magnetically
with any nucleus in the environment which possesses a magnetic moment. This
is especially true in the organic realm where the nearly ubiquitous proton
and other magnetic nuclei are often the origin of magnetic interaction with
the unpaired electron.

The field of magnetic resonance began with the demonstration of Electron
Spin Resonance (ESR) spectroscopy by E. Zavoisky\textsuperscript{1} in 1945 who observed the
resonance absorption of energy by unpaired electrons in the presence of a
magnetic field. Initially only paramagnetic transition metal complexes
were studied because the sensitivity of the instrumentation was very low.
By the mid 1950's, however, ESR spectra of organic free radicals\textsuperscript{2} trapped
in polycrystalline solids had been observed. In 1956, Uebersfeld and Erb\textsuperscript{3}
showed that it was possible, by high energy irradiation, to form radicals
inside a single crystal and for them to be trapped in specific orientations.
The ESR spectrum of such an oriented radical yields more information about
its structure than can be obtained from spectra of the radical in solution
or in a polycrystalline solid. The magnetic moment of the unpaired electron
is coupled to the magnetic moments of nuclei in its vicinity. This coupling
is responsible for the hyperfine structure of an ESR spectrum and is the source of most of the structural information obtainable from this spectrum. One component of this coupling is analogous to the classical dipole-dipole interaction and is direction dependent, or anisotropic. The rotational motion of a radical in solution averages out the anisotropic component so that only the isotropic coupling remains. In polycrystalline solids, the radicals are randomly oriented with respect to the magnetic field, so that the anisotropic contribution to this coupling leads to a blurring of the spectrum. ESR technique has been successful in identifying the major radicals formed in single crystals of large number of organic compounds on the basis of the largest hyperfine interactions. However, detailed information on the electronic structure of the radicals could not always be obtained, because important intramolecular hyperfine splittings were buried within the ESR line-width. Furthermore, more than one radical was often formed in these crystals and their spectra could not always be distinguished. In general, the radicals produced by ionizing radiation in aliphatic compounds yield well resolved ESR spectra and hence they have been studied in detail. In aromatic systems, however, the unpaired electron is extensively delocalized and hence coupled to a large number of nuclei, a situation which generally yields inhomogeneously broadened ESR spectra. For these compounds in the solid state, the ESR spectra appear very poorly resolved and thus yield little information.

In 1956 Feher demonstrated the technique of Electron Nuclear Double Resonance (ENDOR) in his famous work on defects in silicon. The much better resolution of ENDOR spectroscopy makes it possible to study the hyperfine interactions of nuclei which interact magnetically with unpaired electrons in both aliphatic and aromatic radicals. One of the main applications of
the ENDOR technique is resolution enhancement. A classic example of this is Holton, Blum and Slichter's study of the F-centres in lithium fluoride\(^5\). Although the ESR spectrum showed one broad structureless line, the ENDOR technique allowed them to resolve the hyperfine splittings out to the seventh coordination sphere. Furthermore, the ENDOR spectrum is inherently simpler than its parent ESR spectrum whose hyperfine structure has approximately \(2^n\) lines for \(n\) interacting protons. The ENDOR spectrum contains only two lines for each group of equivalent protons.

**SHORT REVIEW ON ENDOR OF STABLE ORGANIC RADICALS:**

The science of radiation chemistry attempts to establish the mechanism by which varieties of free radicals are formed. By mechanism, we mean the entire sequence of physical and chemical events initiated by the primary event in which energy is transferred to matter from a beam of radiation. As mechanisms become clear, the physical, chemical and biological consequences of radiation become correspondingly more comprehensible. The ENDOR technique is being extensively employed for the study of organic and biological radicals. The subject was first reviewed by Kwiram\(^6\) in 1971 and subsequently discussed in two excellent books\(^7,8\) and some reviews\(^9,10\).

Four types of radicals are encountered in solid organic matter as a result of ionizing radiation.

1. Removal of single electrons from electron pairs of nonradical molecules (oxidation).

\[ \text{M} \rightarrow [\text{M.}]^+ \quad \text{(Cation)} \]

2. Addition of single electrons to neutral nonradical molecules (Reduction).

\[ \text{M} \rightarrow [\text{M.}]^- \quad \text{(Anion)} \]
3. Dissociation of covalently bonded atomic hydrogen from nonradical molecules.

\[ \text{MH} \xrightarrow{-H} \text{M} \]

4. Association of atomic hydrogen on unsaturated positions of nonradical molecules.

\[ \text{M} \xrightarrow{+H} \text{MH} \]

Of these, the ionic radicals resulting from electron removal or attachment are usually unstable at room temperature and have been found to be stable only at low temperatures.

The present thesis deals with the detection and characterization of stable radicals trapped in organic compounds at room temperature. Hence we shall not discuss the ionic radicals resulting from low temperature irradiation. Rather, we shall briefly discuss here the application of ENDOR to stable radicals mainly from carboxylic acids, amino acids, nucleic acids and aromatics.

**Carboxylic Acids:**

The first observations of ENDOR signals from organic free radicals were made as early as 1961 by Cole, Heller and Lambe\textsuperscript{11} and Kwiram\textsuperscript{12} on a series of dicarboxylic acids. In succinic acid, Cole et al.\textsuperscript{11} have observed the ENDOR lines corresponding to the hyperfine splitting of the radical \( \text{HOOCCHCH}_2\text{COOH} \), whereas Kwiram and Hyde\textsuperscript{13} have successfully studied the weak interactions which caused inhomogeneously broadened ESR spectrum of the radical \( \text{HOOC(CH}_2)_3\text{CHCOOH} \) in adipic acid. Kwiram\textsuperscript{14} himself has made a detailed study of radicals formed on x-irradiation of glutaric acid. Out of two radicals, one was removed by u.v. irradiation, so that the remaining
HOOC\(\text{CH}_2\)COOH could be studied without interference.

Wells and Ko\(^{15}\) have reported temperature dependent sequence of molecular fragmentation processes occurring in x-irradiated single crystals of creatine monohydrate, a molecule with carboxylic and guanidine structures. The primary reduction product was found to undergo series of changes leading to deamination followed by hydrogen abstraction and the initial oxidized species was found to decarboxylate forming stable radicals at ambient temperature. Recently\(^{16}\) free radicals produced by irradiating single crystals of citric acid, a tricarboxylic acid, has been studied by ENDOR. At room temperature, two stable radicals were identified. One of these was the oxidized species produced by decarboxylation in a different conformation from that at low temperature and the other radical showed coupling to only one alpha-hydrogen and thus it was a hydrogen abstraction radical.

**Amino acids:**

There has been considerable effort to understand the mechanism involved in the production of radiation damage in amino acids because of their biological importance in the synthesis of proteins. Box and coworkers\(^{17}\) have studied the radical formation in glycine hydrochloride x-irradiated at 4.2K. They have shown that upon warming a neutral radical \(\cdot\text{CH}_2\text{COOH}\) is formed. Recently\(^{18}\) Welter et al. have reported an ENDOR investigation of the structure of glycine radical in x-irradiated triglycine sulphate single crystals at 77 and 300K. ENDOR spectra showed the lines of one \(\alpha\)-proton and three nonequivalent \(\beta\)-protons indicating strongly hindered rotation of the amino group. The structure of the radical was also confirmed by INDO calculations. On the other hand, three stable radicals have been identified in the study of radiation damage products formed in \(\beta\)-alanine\(^{19}\) at room
temperature. From controlled warming experiments, the authors have suggested that the primary oxidation and reduction products stable at low temperature decay into the more stable room temperature products.

Histidine is one of the more common of the biologically important amino acids characterized by an imidazole ring that often contributes importantly to biological function. ENDOR spectroscopy was used to characterize more completely the final hydrogen adduct radical obtained on warming the crystals irradiated at 4.2K to room temperature. The unpaired electron is delocalized over the imidazole ring and experiences a large interaction with β-protons via hyperconjugation. Detailed ENDOR investigation on x-irradiated L-asparagine hydrate was undertaken, since ESR data show a great deal of unresolved hyperfine structure. From the agreement of experimental results with INDO calculations, it was concluded that the dominant free radical at room temperature is \( \text{H}_2\text{NCOCHCH(NH}_3\text{)CO}_2^- \).

By x-irradiating α-amino isobutyric acid at 77K, Wells and Box were able to study three distinct radicals which appeared sequentially on warming. At room temperature, the ENDOR spectrum consists of four lines which are attributed to the protons of one rotating methyl group and three nonequivalent protons of a nonrotating methyl group. Similar behaviour was noted by Box et al. in the study of valine. They also obtained hyperfine coupling parameters for the nitrogen nucleus.

Whelan used ENDOR to confirm C-H bond scission product in glutamic acid and reported the hyperfine interactions for the three strongly coupled protons. Castleman and Moulton have examined Serine by x-irradiating at 77K and identified two stable radicals by subsequently warming to room temperature. A more detailed study on serine single crystal x-irradiated at 300K revealed the ENDOR transition of nitrogen in one of the two stable radicals.
One of the simplest substituted amino acids with a peptide bond is N-acetylglycine. Series of attempts through ENDOR have been made within the past decade to investigate the effect of ionizing radiation on this linkage that unites amino acids together in protein. Piazza and Patten measured hyperfine interaction for the methyl protons and proposed the radical \( \text{CH}_3\text{CONHCHCOOH} \), same as predicted from ESR studies. The negative ENDOR technique has been used to study x-irradiated crystal of N-acetylglycine at room temperature. Although signals from at least 13 different types of protons were detected, radical identification remains uncertain.

**Nucleic Acids:**

Knowledge of the radiation chemistry of deoxyribonucleic acid (DNA) and its constituents is very important to radiobiologist, who seeks to understand the effects of radiation on living organisms. The technique of ENDOR was not applied to single crystals of nucleic acid constituents until 1973, when Hampton and Alexander studied one of the radicals formed in x-irradiated single crystal of cytidine. This was a species with hyperfine coupling to three protons and is identified as a radical in the ribose group, with the spin localized on \( C_4^- \) and \( C_5^- \). Recently Close et al. have studied the dominant radical observed in room temperature x-irradiated deoxy cytidine 5'-phosphate monohydrate. With the help of detailed SCF-MO calculations at the INDO level, they have shown that 3aH radical has the allylic structure.

Perhaps the best known radical in radiation biology is thymine radical. In an attempt to characterize this free radical in irradiated thymidine, Box and coworkers have shown that the radical is formed by the addition...
of hydrogen to C₆ carbon atom. They have also deduced the methyl group rotational splitting constant from ENDOR measurements at 4.2K. Recently Hüttermann and coworkers³³,³⁴ have attempted 5-chloro and 5-bromo analogs of uracil and identified α-halo radicals formed by hydrogen addition to the pyrimidine 5,6-unsaturated bond.

There have been substantial contributions in the past from this laboratory on nucleic acid bases. Herak, Krilov and McDowell³⁵ have studied in detail the two types of radicals in x-irradiated deoxy cytidine-5'-phosphate. One of the radicals is formed by the decomposition of the furanose ring, thus giving rise to couplings of two protons at C₅ and one at C₄. In the second radical the unpaired electron is located mostly on C₄. In the study of x-irradiated single crystals of thymidine, Herak and McDowell³⁶ have reported a minority radical which is formed by the abstraction of a hydrogen atom from the methyl group. Other studies of stable radicals derived from nucleic acid constituents such as 1-methyl uracil³⁷, cytosine monohydrate³⁸ etc. have been mainly concerned with long range interactions.

Aromatics:

Irradiation of aromatic molecules frequently results in stable radical products corresponding to the addition of a hydrogen atom at the double bond. For example Bohme and Jesse³⁹ studied such a radical formed in anthracene crystal. ENDOR lines of all the C-H protons have been found. From the agreement of experimental results with McLachlan Theory, it is concluded that the radical is formed by hydrogen addition to C₉. On the other hand, x-irradiation of naphthalene⁴⁰ single crystals at room temperature produces two types of a-hydronaphthyl radicals which differ by the position of hydrogen addition. While these positions in the molecule are chemically
equivalent, its crystal environment is different. This leads to different concentrations of the two radicals depending on their different forming rates and hence unequal line intensities.

The complete ENDOR study of radicals from $\gamma$-irradiation at room temperature of single crystals of imidazole$^{41}$ and 1,2,4-triazole$^{42}$ enabled Lamotte and Cloux to deduce the spin density distribution in the radical from the determination of all the proton hyperfine tensors. For example, there are three protons in the parent imidazole molecule. ENDOR spectra show six proton lines for a single crystallographic site. Two tensors have nearly zero isotropic coupling and have been assigned to two hydrogen bonded protons. This leaves four tensors which successfully account for the hydrogen adduct radical.

**SCOPE OF THE PRESENT WORK:**

In the work presented here, the technique of ENDOR has been used to study the free radicals trapped in x-irradiated single crystals of phenazine, hippuric acid (N-benzoylglycine) and N-acetylglycine. A brief introduction to the theory and experimental techniques of ESR and ENDOR will be presented in order to show how the structure of a radical can be determined from spin Hamiltonian parameters. This theory will then be used to interpret the ENDOR spectra of radicals trapped in the above mentioned compounds of biological importance. The ENDOR study of phenazine and hippuric acid has not previously been attempted. Although a series of attempts through ENDOR have been made to study the radical in N-acetylglycine, the nature of radical has been a matter of controversy. However, from detailed ESR and ENDOR studies, we have been able to establish the structure of the radical.
CHAPTER TWO

THEORETICAL

2.1 Introduction

Several excellent articles\textsuperscript{6,43-47} and books\textsuperscript{7,8,48-52} provide comprehensive accounts of both ESR and ENDOR. Therefore this chapter contains a brief discussion on those aspects of the above phenomena which are relevant to the work described in this thesis.

Electron Spin Resonance (ESR) is a sensitive tool for the study of free radicals. We will restrict our discussion to systems having only one unpaired electron, as these are the only systems studied in the present work. ESR is sometimes confused with Electron Paramagnetic Resonance (EPR). EPR, strictly speaking, refers to the magnetic resonance of permanent magnetic dipole moments. ESR is more specific, but it is perhaps inaccurate in its implication because orbital angular momentum as well as spin angular momentum contributes in general to the electronic magnetic dipole moment. For all practical purposes, we shall use the term ESR in the remainder of the thesis.

For the general case of a crystal containing paramagnetic molecules, it is necessary to determine the energy levels by solving the Schrödinger equation for the electrons and nuclei in the entire crystal in the presence of a static magnetic field. However, it is not possible to arrive at a solution and one is forced to simplify this many body problem. A great simplification occurs if the individual free radicals are considered to be independent and non-interacting. Another useful approximation is known as
Born-Oppenheimer approximation which states that the total wavefunction of a molecule can be separated into an electronic and a nuclear part. Electrons move so fast (its mass being very small) that nuclei are effectively stationary. Thus the electronic wave function can be obtained with respect to fixed positions of the nuclei.

The total Hamiltonian for the molecule can be divided into two parts.

\[ H = H_E + H_M \]  \hspace{1cm} (2-1)

where \( H_E \) describes the electronic and kinetic terms and \( H_M \) describes the magnetic interactions.

In order to describe the effects of these magnetic interactions, we shall adopt the formalism of a spin Hamiltonian introduced first by Pryce and Abragam, whereby ESR and ENDOR spectra are analyzed in terms of transitions between energy levels which are the eigenfunctions of a Hamiltonian containing only spin operators. The eigenvalues of the spin Hamiltonian are the energies measured relative to the energy of the molecule in the absence of any magnetic interactions.

2.2 The Spin Hamiltonian:

2.2.1 The Electronic Zeeman Interaction:

An unpaired electron is characterized by a spin vector \( \mathbf{S} \), which gives the value of the spin angular momentum vector \( \hbar \mathbf{S} \). The magnetic moment of the electron possessing both spin and charge is proportional to \( \hbar \mathbf{S} \) and is given by:

\[ \mu_e = -\gamma \hbar \mathbf{S} = -g \beta \mathbf{S} \]  \hspace{1cm} (2-2)
where $\gamma$ is a constant called the magnetogyric ratio of the electron equal to $1.76 \times 10^7$ rad sec$^{-1}$ G$^{-1}$, $g$ is a dimensionless constant called the electron $g$-factor which equals 2.0023 for a free electron and is about 2.0 for most organic free radicals and $\beta$ is the Bohr magneton represented by

$$\beta = \frac{e\hbar}{2mc} = 0.927 \times 10^{-20} \text{ erg G}^{-1} \quad (2-3)$$

For a free electron, the interaction of its magnetic moment with the field determines the energy which is represented by

$$E = -\mu_e \cdot H \quad (2-4)$$

In order to obtain the quantum mechanical Hamiltonian, we replace $\mu_e$ by the appropriate operator, eq (2-2)

$$\mathcal{H} = gS \cdot \mathbf{H} \quad (2-5)$$

If the magnetic field is assumed to be in the $z$-direction, $H_x = H_y = 0$. Thus

$$\mathcal{H} = gS_z \cdot H_z \quad (2-6)$$

The energy of this system is then

$$E = gS_z M_s \quad (2-7)$$

where $M_s$ is the magnetic quantum number representing the value of $S_z$. The
spin magnetic moment for $S=\frac{1}{2}$ system can thus be aligned either parallel or antiparallel to the magnetic field. This gives rise to two states of differing energy with

$$E = \pm \frac{1}{2} g \beta H_z \quad (2-8)$$

The lowest state has the negative sign and corresponds to the magnetic moment aligned parallel to the field and hence the spin antiparallel to the field.

The difference in energy between the two states can be matched to a quantum of radiation through the Planck-Einstein formula i.e. $\Delta E = h\nu$, so that transitions can be induced between the levels by irradiating at the frequency given by the resonance condition:

$$\Delta E = h\nu = g\beta H_z \quad (2-9)$$

Our ESR spectrometer operates with $\nu = 9.5$ GHz and the field for resonance of a free electron is about 3,400 gauss.

In addition to the contribution of spin angular momentum, there is usually a contribution to the magnetic moment from electronic orbital motion. The spin orbit coupling can only arise for non-spherical orbitals and introduces an orientation dependence into the Hamiltonian. Because $g$ is a measure of the effective magnetic moment associated with an angular momentum $S$, this orientation dependence is included in the Hamiltonian as an anisotropy in the $g$-factor. A more general expression for the electronic Zeeman interaction in terms of some convenient cartesian axis system is:
\[ \mathbf{H} = \beta \mathbf{H} \cdot \mathbf{g} \cdot \mathbf{S} \]

\[ = \beta (H_x, H_y, H_z) \begin{pmatrix} g_{xx} & g_{xy} & g_{xz} \\ g_{yx} & g_{yy} & g_{yz} \\ g_{zx} & g_{zy} & g_{zz} \end{pmatrix} \begin{pmatrix} S_x \\ S_y \\ S_z \end{pmatrix} \tag{2-10} \]

where \( g \) is a symmetric tensor. A suitable reference frame can always be chosen to diagonalize the \( g \)-tensor which is then represented by its principal values: \( g_{xx}, g_{yy}, g_{zz} \).

In the organic free radicals we have studied, the orbital angular momentum is strongly quenched and so the \( g \)-tensor being relatively less anisotropic is close to the free electron value, i.e., 2.0023.

2.2.2 The Nuclear Zeeman Interaction:

If a nucleus in the free radical also possesses a spin, the magnetic moment of the nucleus can be expressed as:

\[ \mu_N = g_N \beta_N I \tag{2-11} \]

where \( g_N \) is the nuclear \( g \)-factor, \( \beta_N \) is the nuclear magneton and \( I \) is the nuclear spin angular momentum operator. Thus the Hamiltonian which represents the electronic and nuclear Zeeman contributions is given by

\[ \mathbf{H} = g \beta \mathbf{H} \cdot \mathbf{S} - \sum_{i=1}^{k} g_N \beta_N H_i I_i \tag{2-12} \]

where the summation is over all nuclei with \( I \neq 0 \).
2.2.3 The Hyperfine Interaction:

If we consider an organic free radical containing at least one magnetic nucleus with \( I \neq 0 \), the unpaired electron will experience a local magnetic field due to this nucleus. The magnitude of this local field is determined by the electronic structure of the radical, the magnetic moment of the nucleus and the orientation of the nuclear spin in the total field which it experiences. The electron resonance occurs when the total field which it experiences, made up of contributions from the applied field of the spectrometer and the local field, has the value given by the resonance condition. The value of the applied field required to cause a resonance in a particular radical will therefore depend on the spin states of the magnetic nucleus in the radical. Thus while sweeping the applied field to record the spectrum, there will be observed as many absorption lines as there are spin states of the magnetic nucleus and the spectrum will contain \((2I+1)\) lines.

2.2.3.1 The Dipolar Interaction:

The interaction energy between two magnetic moments \( \mu_e \) and \( \mu_N \) can be derived by considering the energy of one in the magnetic field of the other and is given by

\[
E = \frac{\mu_e \cdot \mu_N}{r^3} - \frac{3(\mu_e \cdot r)(\mu_N \cdot r)}{r^5}
\]  

(2-13)

where \( r \) is the radius vector from \( \mu_e \) to \( \mu_N \) and \( r \) is the distance between the two moments. The above formula is valid for point dipoles. It gives the energy of interaction of two bar magnets if their size is small compared to the distance between them.
The Hamiltonian for the magnetic interaction of a nuclear and electron spin can be obtained by replacing \( \mu_e \) and \( \mu_N \) by their operator equivalents. Thus the dipolar interaction Hamiltonian is represented by

\[
H_{\text{Dip}} = g \gamma_g \gamma_N \left\{ \frac{3(I \cdot r) \cdot (S \cdot r)}{r^5} - \frac{(I \cdot S)}{r^3} \right\} \quad (2-14)
\]

The expression (2-14) has to be averaged over the entire probability distribution \( |\psi(r)|^2 \) of the odd electron. If we now try to evaluate a spin Hamiltonian by integrating over the spatial coordinates, we meet a difficulty. In general, the integral will be of the form:

\[
\int \int \int \int \psi^*(r, \theta, \phi) H_{\text{Dip}} \psi(r, \theta, \phi) r^2 \sin \theta d\theta d\phi
\]

and unless the wavefunction goes to zero very rapidly at \( r=0 \), the integrand becomes infinite at this point. The difficulty is that the point dipole treatment breaks down at very short distances as for classical magnets. To simplify the treatment and obtain useful results, we will proceed by discussing more explicitly the case of an atom with one electron, neglecting the spin orbit coupling.

If the unpaired electron is in a p,d or any other orbital with \( \ell > 0 \), the wavefunction goes to zero exponentially as \( r \) goes to zero. Expanding the scalar products in Eq. (2-14) in a cartesian coordinate system with the nucleus at the origin, we obtain

\[
H_{\text{Dip}} = \frac{g \gamma_g \gamma_N}{r^5} \left\{ \begin{array}{l}
3(3x^2 - r^2)I_x S_x + (3y^2 - r^2)I_y S_y \\
+ (3z^2 - r^2)I_z S_z + 3xy(I_x S_y + I_y S_x) \\
+ 3yz(I_y S_z + I_z S_y) + 3xz(I_x S_z + I_z S_x) \end{array} \right\} \quad (2-16)
\]
where \( r = (x^2 + y^2 + z^2)^{\frac{1}{2}} \). The integration over the spatial part of the electron wavefunction can now be carried out term by term and it is clear that the spin Hamiltonian for the dipolar coupling can be written in the tensorial form:

\[
\mathbf{H}_{\text{Dip}} = \mathbf{S} \cdot \mathbf{A}^o \cdot \mathbf{I} \\
= A^o_{xx} S_x I_x + A^o_{yy} S_y I_y + A^o_{zz} S_z I_z
\]

(2-17)

The coupling tensor \( A^o \) is symmetric with its diagonal elements given by:

\[
A^o_{ii} = g_\beta g_\mathbf{N} \beta_N \left< \frac{3i^2 - r^2}{r^5} \right>, \quad i=x, y, z
\]

(2-18)

and with its off-diagonal elements given by

\[
A^o_{ij} = g_\beta g_\mathbf{N} \beta_N \left< \frac{3ij}{r^5} \right>
\]

(2-19)

The most important property of the anisotropic hyperfine tensor is that its trace, i.e., sum of the diagonal elements, is zero. An immediate consequence of this property is that for a radical undergoing rapid tumbling in highly fluid solution, there is no contribution to the hyperfine splitting from the dipolar coupling. A second consequence of the traceless property of the dipolar coupling is that the dipolar Hamiltonian averages out to zero whenever the electron cloud is spherical, as for an electron in an atomic s orbital.

2.2.3.2 The Isotropic Hyperfine Coupling:

The magnetic moments of the electron and nucleus are also coupled via
the Fermi contact interaction \(^{58}\) which represents the energy of the nuclear moment in the magnetic field produced at the nucleus by electric currents associated with the spinning electron. If we treat the nucleus as a spinning charged spherical shell, the energy of the electron spin magnetic moment interacting with the magnetic field (H) inside the sphere is given by

\[
E = -\frac{4\pi}{3} r^3 |\psi(0)|^2 \mu_e \cdot (2\mu_N / r^3)
\]  

(2-20)

where \(r\) is the radius of the sphere and \(|\psi(0)|^2\) is the probability of finding the electron at any point in the sphere. Substituting the magnetic moments by appropriate spin operators, we obtain the spin Hamiltonian:

\[
\mathcal{H}_{\text{iso}} = \frac{8\pi}{3} g \beta_N \beta_e |\psi(0)|^2 \mathbf{S} \cdot \mathbf{I}
\]

\[
= a S \cdot I = a (I_x S_x + I_y S_y + I_z S_z)
\]  

(2-21)

where \(a\) is the isotropic hyperfine coupling constant which is proportional to the squared amplitude of the electronic wavefunction at the nucleus.

\[
a = \frac{8\pi}{3} g \beta_N \beta_e |\psi(0)|^2
\]  

(2-22)

The contact interaction can only occur when the electron has a finite probability density at the nucleus. In other words, the electron must have some s orbital character.
The complete Hamiltonian describing the hyperfine interaction is:

\[ \mathcal{H}_{\text{Hyp}} = \mathcal{H}_{\text{iso}} + \mathcal{H}_{\text{Dip}} = a \mathbf{S} \cdot \mathbf{I} + \mathbf{A} \cdot \mathbf{I} \]

\[ = \mathbf{A} \cdot \mathbf{I} = A_{xx} I_x^2 + A_{yy} I_y^2 + A_{zz} I_z^2 \]  

(2-23)

where

\[ A_{xx} = A_{xx}^0 + a \]

\[ A_{yy} = A_{yy}^0 + a \]

\[ A_{zz} = A_{zz}^0 + a \]

Since the dipolar tensor has zero trace i.e. \( A_{xx}^0 + A_{yy}^0 + A_{zz}^0 = 0 \),

\[ a = \frac{1}{3} (A_{xx} + A_{yy} + A_{zz}) \]  

(2-24)

The evaluation of these parameters provides a valuable method for determining the electron distribution in a radical.

2.2.4 The Quadrupole Interaction:

For nuclei with \( I = \frac{1}{2} \), the energy levels are mainly determined by electron and nuclear Zeeman interactions and the hyperfine interaction. For nuclei with \( I > 1 \), there is another contribution to the energies of the hyperfine levels. This contribution is due to the nuclear quadrupole moment.

Nuclei with \( I > 1 \) have quadrupole moments because their charge distributions can deviate from spherical symmetry. The symmetry axis of the charge distributions is also the axis of the spin and magnetic moment. The nuclear quadrupole moment \( Q \), whose magnitude is a measure of the deviation of the charge distribution from spherical symmetry is defined as:
\( eQ = \int \rho_N(3z^2-r^2)\,d\tau \) \hspace{1cm} (2-25)

where \( \rho_N \) is the distribution function of the nuclear charge, \( z \) is the \( z \)-coordinate of the charge element at a distance \( r \) from the origin, \( e \) is the proton charge and the integral is evaluated over the volume of the nucleus.

A quadrupole moment has no interaction with a homogeneous electric field. However, it interacts with an inhomogeneous electric field, the energy of interaction depending on the magnitude of quadrupole moment and the gradient of the electric field, and is given by:

\[
E_Q = \frac{1}{6} \sum_{\alpha,\beta} V_{\alpha\beta} Q_{\alpha\beta} \quad (2-26)
\]

where \( V_{\alpha\beta} = \frac{\partial^2 V}{\partial \alpha \partial \beta} \), \( \alpha,\beta = x,y,z \)

and \( Q_{\alpha\beta} = \int (3\alpha\beta - \delta_{\alpha\beta}r^2)\rho_N\,d\tau \). The Hamiltonian in terms of nuclear spin is given by:

\[
\mathcal{H}_Q = \frac{eQ}{6I(2I-1)} \sum_{\alpha,\beta} V_{\alpha\beta} \left\{ \frac{3}{2}(I_\alpha I_\beta + I_\beta I_\alpha) - \delta_{\alpha\beta}I^2 \right\} \quad (2-27)
\]

This can be expressed more shortly as a tensor coupling of the nuclear spin with itself.

\[
\mathcal{H}_Q = I \cdot P \cdot I \quad (2-28)
\]

where \( P \) is called the quadrupole coupling tensor. The field gradient generally obeys Laplace's equation, hence the field gradient tensor is traceless i.e. \( \sum_{\alpha} V_{\alpha\alpha} = 0 \). As a result the quadrupole coupling tensor is also traceless.
There is no field gradient at the nucleus of a free nitrogen atom because of spherical symmetry, but in molecules, the non-spherical charge distribution of electrons, particularly the valence electrons, gives rise to field gradients. The quadrupole interaction influences the orientation of the nuclear spin, whose axis is colinear with that of the quadrupole moment. Therefore the energies of the hyperfine levels are determined by the electrostatic quadrupole interaction in addition to the magnetic Zeeman and hyperfine interactions. The total Hamiltonian required to describe a free radical in a solid is:

$$H = \beta H \cdot S + \sum_{i=1}^{n} (S \cdot A_i \cdot I_i + I_i \cdot B_i \cdot I_i - g_N H \cdot I_i)$$  \hspace{1cm} (2-29)$$

The interaction does not involve the electron spin explicitly and hence to first order there is no strong effect on the frequency of a $\Delta M_z=0$ ESR transition. However, when the energies of the hyperfine levels are calculated to higher order, the quadrupole interaction causes a shift in the transition frequencies and in extreme cases there may be appreciable intensity for certain forbidden transitions. The quadrupole coupling can be observed more directly in ENDOR spectra where there can actually be a splitting. Further aspects of the effect of the quadrupole interaction will be described under ENDOR principles.

2.3 Theory of ESR:

In the following section, it will be shown how ESR spectra can be described in terms of the spin Hamiltonian. The first task is to find the forms of the stationary state spin wavefunctions and their energies.
The final step is to determine which transitions may be induced by an oscillating magnetic field.

2.3.1 Energy levels for $S=\frac{1}{2}$, $I=\frac{1}{2}$ system:

We will discuss in detail the case of a free radical containing a single nucleus of spin $I=\frac{1}{2}$ and generalize the most important results for the case of interactions with several nuclei. For simplicity, we will consider radicals in solution with only isotropic interaction. The spin Hamiltonian is:

$$\mathcal{H} = g_B \mathbf{S} \cdot \mathbf{H} - g_N \mathbf{S} \cdot \mathbf{I} + a \mathbf{S} \cdot \mathbf{I}$$

(2-30)

The energy levels are determined by solving the equation:

$$\mathcal{H} \psi = E \psi$$

(2-31)

This equation is usually solved with the help of perturbation theory, in which the Hamiltonian is separated into two distinct parts, $\mathcal{H}_0$ and $\mathcal{H}_1$. $\mathcal{H}_0$ is the main component of the Hamiltonian and $\mathcal{H}_1$ is treated as a small perturbation. The spin wave functions are then expressed in terms of linear combinations of the basis functions $\phi_n$ which are chosen to be eigenfunctions of $\mathcal{H}_0$. The eigenvalues of $\mathcal{H}_0$ are the unperturbed energies $\varepsilon_n$. The perturbation $\mathcal{H}_1$ yields modified wave functions and energies of the form:

$$\psi_n = \phi_n - \sum_{m \neq n} \frac{\langle m | \mathcal{H}_1 | n \rangle}{\varepsilon_m - \varepsilon_n} \phi_m$$

(2-32)

$$E_n = \varepsilon_n + \langle n | \mathcal{H}_1 | n \rangle - \sum_{m \neq n} \frac{\langle m | \mathcal{H}_1 | n \rangle \langle n | \mathcal{H}_1 | m \rangle}{\varepsilon_m - \varepsilon_n}$$

(2-33)
The two terms on the right side of (2-33) are the first order and second order corrections respectively and \( \langle n|H_1|n\rangle \) and \( \langle m|H_1|m\rangle \) are the matrix elements of \( H_1 \).

The electron has a spin vector \( S \) equal to \( \frac{1}{2} \) and there are two allowed components of the spin along any chosen direction, i.e., z-axis. The two possible spin functions are denoted by symbols \( |\alpha_e\rangle \) and \( |\beta_e\rangle \) with spin quantum numbers \( M_s = +\frac{1}{2} \) and \( -\frac{1}{2} \) respectively. In terms of operator equations:

\[
S_z |\alpha_e\rangle = \frac{1}{2} |\alpha_e\rangle \\
S_z |\beta_e\rangle = -\frac{1}{2} |\beta_e\rangle
\]  

(2-34)

For nuclear spins, equations analogous to the above can be written

\[
I_z |\alpha_N\rangle = +\frac{1}{2} |\alpha_N\rangle \\
I_z |\beta_N\rangle = -\frac{1}{2} |\beta_N\rangle
\]  

(2-35)

The appropriate basis functions are written as the products of the electron and nuclear spin functions:

\[
\phi_1 = |\alpha_e \alpha_N\rangle, \quad \phi_2 = |\alpha_e \beta_N\rangle, \quad \phi_3 = |\beta_e \alpha_N\rangle, \quad \phi_4 = |\beta_e \beta_N\rangle
\]  

(2-36)

These functions are all eigenfunctions of \( H_0 \) and the matrix of \( H_0 \) is diagonal. For example:
The zero order energy values for the four states are:

\[ \varepsilon_1 = \frac{1}{2}g\beta H - \frac{1}{2}g_N^e\beta_N^e H \]
\[ \varepsilon_2 = \frac{1}{2}g\beta H + \frac{1}{2}g_N^e\beta_N^e H \]
\[ \varepsilon_3 = -\frac{1}{2}g\beta H - \frac{1}{2}g_N^e\beta_N^e H \]
\[ \varepsilon_4 = -\frac{1}{2}g\beta H + \frac{1}{2}g_N^e\beta_N^e H \]

(2-38)

The perturbation, the hyperfine interaction may be expanded as follows:

\[ \mathcal{H}_1 = aS\cdot I = a(S_z I_z + S_x I_x + S_y I_y) \]

(2-39)

The first term contributes to first order energies. The effect of the term \( a[S_x I_x + S_y I_y] \), however, is to produce a second order change in the energies which will be calculated later. The first order energy shifts are:
The operator \( S^I_z \) gives a diagonal matrix and can be included in zero order energies.

In order to consider the effects of \( a(S^I_x x + S^I_y y) \) on the spin states, it is convenient to define the ladder operators.

\[
S^+ = S_x + iS_y \\
S^- = S_x - iS_y \tag{2-41}
\]

\[
S^+ |a_e\rangle = 0 \\
S^+ |\beta_e\rangle = |\alpha_e\rangle \\
S^- |\beta_e\rangle = 0 \\
S^- |\alpha_e\rangle = |\beta_e\rangle \tag{2-45}
\]

Similar operators also exist for the nuclear spin.

The operator \( a(S^I_x x + S^I_y y) \) can be written in terms of the ladder operators

\[
a(S^I_x x + S^I_y y) = \frac{a}{2}(S^+ I^+ + S^- I^-) \tag{2-46}
\]
The only nonvanishing matrix elements are:

\[
\begin{align*}
\langle \alpha e_N \vert (S^+ I^- + S^- I^+) \vert \beta e_N \rangle &= 1 \\
\langle \beta e_N \vert (S^+ I^- + S^- I^+) \vert \alpha e_N \rangle &= 1
\end{align*}
\]  
\hspace{1cm} (2-47)

The complete matrix of \( \mathcal{H} \) is thus represented by

\[
\begin{array}{l|llll}
\langle \alpha e_N \rangle & \vert \alpha e_N \rangle & \vert \beta e_N \rangle & \vert \beta e_N \rangle \\
\hline
\langle \alpha e_N \rangle & \frac{1}{2}(Z_e - Z_N^+ + \frac{a}{2}) & 0 & 0 & 0 \\
\langle \beta e_N \rangle & 0 & \frac{1}{2}(Z_e + Z_N^+ - \frac{a}{2}) & \frac{1}{2}a & 0 \\
\langle \alpha e_N \rangle & 0 & \frac{1}{2}a & -\frac{1}{2}(Z_e + Z_N^+ + \frac{a}{2}) & 0 \\
\langle \beta e_N \rangle & 0 & 0 & 0 & -\frac{1}{2}(Z_e - Z_N^+ - \frac{a}{2})
\end{array}
\]

where \( Z_e = g\beta H \) and \( Z_N = g_N^\beta H_N \)  
\hspace{1cm} (2-48)

The eigenvalues and eigenfunctions of \( \mathcal{H} \) can be determined by diagonalization of the above matrix. The same result is also obtained by using the perturbation formulae (2-32) and (2-33), where the second order terms involve the off-diagonal elements of matrix. The eigenvalues are:

\[
\begin{align*}
E_1 &= \frac{1}{2}g\beta H - \frac{1}{2}g_N^\beta H_N + \frac{a}{4} \\
E_2 &= \frac{1}{2}g\beta H + \frac{1}{2}g_N^\beta H_N - \frac{a}{4} + \frac{a^2}{4(g\beta H + g_N^\beta H_N)} \\
E_3 &= -\frac{1}{2}g\beta H - \frac{1}{2}g_N^\beta H_N - \frac{a}{4} - \frac{a^2}{4(g\beta H + g_N^\beta H_N)} \\
E_4 &= -\frac{1}{2}g\beta H + \frac{1}{2}g_N^\beta H_N + \frac{a}{4}
\end{align*}
\]  
\hspace{1cm} (2-49)
For most free radicals, the hyperfine coupling energy is much less than the electron Zeeman energy. Hence the second-order corrections approach zero and can be neglected from the above equations. The energy levels are shown in fig. 1.

2.3.2 ESR selection rules:

If an oscillating magnetic field of strength $2H_1 \cos \omega t$ produced by microwave radiation is applied perpendicular to steady field, $H$ in $x$-direction such that $\omega$ is correct for resonance, several kinds of spin transitions can be induced. Transitions like $\alpha^e + \beta^e \rightarrow \alpha^e + \beta^e$ where only the electron spin changes are called ESR transitions, while those like $\alpha^e \rightarrow \alpha^n$ which involve the nucleus alone are NMR ones. The third kind of transition $\alpha^e \beta^e \rightarrow \alpha^e \beta^e$ where both spins change has a very low probability and is known as a "forbidden transition".

The resulting time dependent perturbation is:

$$V(t) = 2(g^e H^e_x - g^e N^e N^e H^I_x) \cos \omega t$$

$$= 2V \cos \omega t$$  \hspace{0.5cm} (2-50)

The transition probability for state $n$ to state $m$ is equal to

$$P_{nm} = \frac{2\pi}{\hbar} |<n|V|m>|^2 \delta(\omega_{mn} - \omega)$$  \hspace{0.5cm} (2-51)

where $\omega_{mn} = E_m - E_n$, expressed in frequency units. The deduction of selection rules is based on finding whether the matrix elements of $V$ between each pair of states vanishes or not. As the electron resonance transitions are caused by the effect of $H_1$ on the electron spins, we can omit the nuclear spin operator $I_x$ from (2-51). Thus
Fig. 1. Energy levels of an $S=\frac{3}{2}$, $I=\frac{1}{2}$ system, with first order ESR transitions.
The typical matrix element for ESR transition is calculated using the ladder operators.

\[
\langle \alpha_e \alpha_N | S_x | \beta_e \alpha_N \rangle = \langle \alpha_e | S^+ | \beta_e \rangle \langle \alpha \alpha_N | \alpha_N \rangle
\]

\[
= \frac{1}{2} \langle \alpha_e | S^+ | \beta_e \rangle = \frac{1}{2}
\]

Thus the spin quantum numbers in allowed ESR transitions obey the selection rules:

\[
\DeltaM_S = \pm 1 \quad ; \quad \DeltaM_I = 0
\]

These transitions marked by the single arrows in Fig. 1, have equal probabilities and hence equal intensities. The frequencies of the transitions are:

\[
h\nu_{1-3} = (\frac{1}{2}g_3^H - \frac{1}{2}g_N \beta_{H}^3 H^\frac{a}{4}) - (-\frac{1}{2}g_3^H - \frac{1}{2}g_N \beta_{H}^3 H^\frac{-a}{4}) = g_3^H \frac{a}{2}
\]

\[
h\nu_{2-4} = (\frac{1}{2}g_3^H + \frac{1}{2}g_N \beta_{H}^3 H^\frac{a}{4}) - (-\frac{1}{2}g_3^H + \frac{1}{2}g_N \beta_{H}^3 H^\frac{-a}{4}) = g_3^H \frac{a}{2}
\]

The first order ESR spectrum, therefore, consists of two equally intense lines separated by 'a', the hyperfine coupling constant. Since the spectrum is recorded by varying the magnetic field, the transition corresponding to the largest energy difference occurs at the lowest field.

2.3.3 Thermal equilibrium and spin relaxation:

Resonance absorption can be detected only if there is a population
difference between the upper and lower spin levels. When a macroscopic specimen containing \( N \) spins is placed in a steady magnetic field, the degeneracy is lifted and there are \( N_\beta \) spins in the lower state and \( N_\alpha \) in the upper. In thermal equilibrium, there is slight excess of spins in the \( \beta \)-state which gives rise to a small temperature dependent paramagnetism. The ratio of \( \beta \) to \( \alpha \) spins is determined by the Boltzmann factor:

\[
\frac{N_\beta}{N_\alpha} = e^{g_\beta H/kT}
\]  

(2-56)

At ordinary temperature \( g_\beta H \ll kT \) and the Boltzmann factor in (2-56) is approximately \([1+g_\beta H/kT]\). On the average \((N/2)[1+g_\beta H/kT]\) electrons have spin \( \beta \) and \((N/2)[1-g_\beta H/kT]\) have spin \( \alpha \).

The application of microwave energy in the proper orientation (microwave magnetic field perpendicular to the static magnetic field) causes transitions between the magnetic levels. The microwave induced transitions have equal probabilities in either direction. As a result, it can be shown that application of the resonant microwave field results in exponential decay of the population difference and eventually the levels will be equally populated. This is known as saturation. There would then be no net absorption of microwave energy and the resonance absorption line will ultimately disappear. For ESR spectrum to be observed there must then be some mechanism through which the spin system returns to thermal equilibrium. The establishment of thermal equilibrium between the \( \alpha \) and \( \beta \) states after application of steady magnetic field, \( H \) must inevitably require that there are interactions between the electrons and the thermal motion of lattice or surroundings which cause the spin orientations to change, while the excess energy is transferred to other degrees of freedom. This process of nonradiative transitions between the two states is called spin lattice
relaxation.

Because the lattice is at thermal equilibrium, the probabilities of spontaneous spin transitions up and down are not strictly equal. Consideration of these points leads to an expression for the rate of absorption of microwave energy

\[
\frac{dE}{dt} = n_0 \Delta E \frac{P}{(1+2PT_1)}
\]

(2-57)

where \(n_0\) is the population difference at thermal equilibrium, \(P\) is the transition probability and \(T_1\) is called spin lattice relaxation time (time taken for energy to be transferred to other degrees of freedom, i.e., for the spin system to approach thermal equilibrium). Equation (2-57) indicates that as long as \(2PT_1<<1\), it is relatively easy to avoid saturation. In an ESR experiment, one normally operates with low microwave power to avoid saturation.

In addition to spin-lattice relaxation, in which energy is transferred from the spin system to the lattice, there exist spin-spin relaxation mechanisms in which energy is redistributed within the spin system. The characteristic time for spin-spin relaxation or transverse relaxation is symbolized by \(T_2\). Spin relaxations play an important role in determining the width and shape of resonance absorption line.

2.3.4 Hyperfine Interaction With More Than One Nucleus:

The above discussions can be easily extended for the case where there is hyperfine interaction with several nuclei. To first order, the nuclear Zeeman interaction does not affect the appearance of ESR line positions. Hence the nuclear Zeeman term can be omitted. The spin Hamiltonian for an
electron coupled to more than one nucleus can be written as:

\[ \hat{H} = g\hat{B} \cdot \hat{S} + \sum_i a_i \hat{I}^{(i)} \cdot \hat{S} \]  

with eigenvalues:

\[ E = g\hat{B} \cdot \hat{M}_S + \sum_i a_i \hat{M}_I^{(i)} \cdot \hat{M}_S \]  

The resulting number of \( \Delta M_S = 1, \Delta M_I = 0 \) transitions is given by:

\[ n = \pi (2I^{(i)} + 1) \]  

Thus there will be \( 2^n \) transitions in the ESR spectrum of an unpaired electron interacting with \( x \) protons. It often happens that several protons have identical coupling constants. Such protons are termed equivalent and usually occupy symmetrically equivalent positions in the molecule. The number of lines in the spectrum is reduced but the intensities vary. In general, number of lines in the spectrum of \( n \) equivalent nuclei is \( 2nI+1 \). Thus the interaction of odd electron with \( n \) equivalent protons \( (I=\frac{1}{2}) \) results in \( (n+1) \) lines whose relative intensities are proportional to the coefficients of the binomial expansion of \( (a+b)^n \).

The ESR spectrum of a radical in which the unpaired electron is coupled to many nuclei often contains so many lines that many of them overlap and the resulting broadened spectrum cannot be analyzed. In solid state, there is further complication as a result of the anisotropic hyperfine interaction. The line positions will vary with the magnetic
field orientation and unless the lines are well separated, their angular variations cannot be followed. These are two of the major reasons why the ENDOR technique is so useful.

2.4 Theory of ENDOR:
2.4.1 The Isotropic Hyperfine Interaction:

In order to understand the basic principles of the ENDOR experiment, it will be useful to start with the simplest system, that of an unpaired electron interacting with a proton through an isotropic hyperfine interaction. The Hamiltonian is:

\[
\mathcal{H} = g_\beta H S - g_N^\beta_H N I + a^\prime S I
\]  

(2-61)

To first order, the energy levels for this system are determined by:

\[
E(M_S, M_I) = g_\beta H M_S - g_N^\beta H M_I + a^\prime M_S M_I
\]  

(2-62)

It is convenient to work in frequency units and define \( g_\beta H / h = \nu_e \), \( g_N^\beta _H / h = \nu_N \); \( a^\prime / h = a \)

\[
E(M_S, M_I) / h = \nu_e M_S - \nu_N M_I + a M_S M_I
\]  

(2-63)

Fig. 2(a)(b) shows the energy levels for two cases i.e. \( \nu_N > a/2 \) and \( a/2 > \nu_N \), respectively.

Because the nuclear Zeeman energy is much smaller than that of the electron, the population differences between the levels \( |e_a^N\rangle \) and \( |e_\beta^N\rangle \) can be ignored. The thermal equilibrium populations can be calculated on
Fig. 2. Energy levels for ENDOR experiment in an $S=\frac{1}{2}$, $I=\frac{1}{2}$ system.  
(a) $\nu_N > a/2$  (b) $a/2 > \nu_N$.  

the basis of Boltzmann distribution and can be shown to be $1+\epsilon$ for $\beta_e$ state and $1-\epsilon$ for $\alpha_e$ state where $\epsilon=g\beta H/kT$. If sufficient microwave power is applied to one of the ESR transitions, i.e., $|\beta_e, e_N\rangle \leftrightarrow |\alpha_e, e_N\rangle$, the populations of the two levels will be equalized.

In the ENDOR experiment, a radiofrequency field is applied to the spin system, while continuing to saturate the ESR transition. As the radiofrequency is varied, it will match the separation of $|\alpha_e, e_N\rangle$ and $|\alpha_e, \beta_N\rangle$ levels and induce transition between them when:

$$v_{rf} = v - \frac{3a}{2}$$

Removal of spins from $|\alpha_e, e_N\rangle$ by this absorption, restores a population difference between $|\beta_e, e_N\rangle$ and $|\alpha_e, e_N\rangle$ levels and there is an increase in the ESR absorption. Thus the ENDOR spectrum is a display of the enhancement of a partially saturated ESR transition as a function of the radiofrequency. The response has a linewidth more nearly that of the narrower nuclear resonance absorption than the electron resonance absorption.

As the radiofrequency is further varied, the separation between $|\beta_e, e_N\rangle$ and $|\beta_e, \beta_N\rangle$ levels will also be matched when:

$$v_{rf} = v + \frac{1}{2}a$$

For $v_N>a/2$, the two ENDOR transitions are given by $v_N+a/2$ separated by 'a' and centered at $v_N$. For $a/2>v_N$, the two ENDOR transitions are given by $a/2\pm v_N$, separated by $2v_N$ and centered at $a/2$. If $a/2=v_N$, then only one ENDOR line will be observed at $v_N+a/2$. 

It may be noted that the same two ENDOR transitions are observed when either ESR line is monitored. However, since \( v_N \) depends on \( H \), there will be a shift in the center of spectrum as different ESR lines are monitored by sweeping the magnetic field.

### 2.4.2 The Anisotropic Hyperfine Interaction:

An anisotropic or dipolar hyperfine interaction always exists between two magnetic dipoles, i.e., electron dipole-nuclear dipole. This interaction is orientation dependent. In the liquid phase where radical rotation is fast, the dipolar hyperfine interaction averages out on the ESR or ENDOR time scale, so it does not enter in the spectra. If the motion is arrested, except for vibration or some internal motion, as in solids, particularly, single crystals, we encounter anisotropic hyperfine interaction to analyze the ENDOR spectra.

We consider the typical case for organic radicals in single crystals where the hyperfine interaction is generally anisotropic. The spin Hamiltonian is:

\[
\mathcal{H} = g_S \mathbf{H}. \mathbf{S} + g_A \mathbf{I} - g_N \mathbf{N}. \mathbf{H} \cdot \mathbf{I}
\]  

(2-64)

In the preceding section we have seen that, to first order, the ENDOR transition frequencies are independent of the electron Zeeman energy. Thus the ENDOR frequencies can be written as:

\[
v^2 = M_S^2 h^2 A_h^2 h - 2M_S v_N h A_h + g_N^2 N^2 h^2 
\]  

(2-65)

where \( \mathbf{h} \) is a unit vector along the external field direction \((\mathbf{H} = H \mathbf{h})\).

The nuclear transitions are induced by an oscillating rf field \( H_2 \)
in the x-direction if the static field is along z. The first order ENDOR selection rules can be determined in a manner analogous to that shown for ESR in Sec. 2.3.2 using the time dependent perturbation:

\[ \mathbf{\mathcal{H}}(t) = (g_{\beta}B_{x}S_x - g_{\beta}B_{z}I_x) \cos \omega t \]  

(2-66)

The ENDOR selection rules can thus be found as:

\[ \Delta M_S = 0 \quad , \quad \Delta M_I = \pm 1 \]

Thus if both the ENDOR transitions (for \( I = \frac{1}{2} \)) are observed, the hyperfine tensor can be determined using the equation:

\[ v_+^2 - v_-^2 = 2v_p \mathbf{h} \cdot \mathbf{A} \cdot \mathbf{h} \]  

(2-67)

If for example, a crystal is mounted in the spectrometer such that the magnetic field is rotated in the yz plane, the equation (2-67) becomes:

\[ v_+^2 - v_-^2 = 2v_p \sin^2 \theta(A_{yy}) + 4v_p \sin \theta \cos \theta (A_{yz}) + 2v_p \cos^2 \theta (A_{zz}) \]  

(2-68)

where \( \theta \) is the angle between the z-axis and the field direction. These three elements of \( \mathbf{A} \) can be determined by measuring \( v_+^2 - v_-^2 \) for a minimum of three values of \( \theta \) in this plane. The remaining elements can be obtained by remounting the crystal and rotating the magnetic field in zx and xy planes.

When the hyperfine splitting is approximately 50 MHz or larger, the second order corrections to the energy levels become significant. The best way to analyze the ENDOR spectrum is to use a least squares procedure to
match the observed frequencies to those calculated by diagonalizing exactly by computer the Hamiltonian (2-64) for trial values of parameters.

2.4.3 The Quadrupole Interaction:

In general, information about nuclear quadrupole couplings is not readily derived from the normal ESR spectrum unless the couplings are fairly large. However, quadrupole couplings can be measured directly from an ENDOR spectrum. Let us consider a simple case of \(^1\)N nucleus (I=1) with an isotropic hyperfine coupling \(a/2\nu_N\) and axially symmetric quadrupole coupling. The spin Hamiltonian is:

\[
\mathbf{H} = g\beta_H \cdot S - g_N^e \nu_N \cdot I_z + aS_z \cdot I_z + \frac{P(I^2 - \frac{1}{3}I(I+1))}{3} \tag{2-69}
\]

The eigenvalues are:

\[
E(M^e_S, M^e_I) = \nu^e S - \nu^e N \cdot M^e_I + aM^e_S M^e_I + \frac{P(M^2}{3} - \frac{1}{3}I(I+1)) \tag{2-70}
\]

The frequencies of ESR transitions (\(\Delta M^e_S = 1, \Delta M^e_I = 0\)) are independent of \(P\), but those of the ENDOR transitions are not. Fig. 3 shows the effect of quadrupole interaction on the energy levels and the resulting four ENDOR frequencies for \(I=1\). In the absence of a quadrupole interaction, the hyperfine levels in each \(M^e_S\) manifold are equally spaced. Thus there is only one ENDOR transition frequency in each manifold and the complete spectrum consists of only two lines for each ESR line. However, with an observable quadrupole interaction, the four different ENDOR transition frequencies are:
Fig. 3. Energy levels for an $S=\frac{1}{2}$, $I=1$ system.
The appearance of ENDOR lines will depend on ESR transition being saturated. If $|{-\frac{1}{2},0}\rangle \leftrightarrow |{\frac{1}{2},0}\rangle$ ESR line is saturated, all the four ENDOR lines can be observed. Saturation of either of the other two ESR transitions results in only two lines in the ENDOR spectrum.

In addition to a direct measure of the quadrupole coupling, we can determine the relative signs of the hyperfine and quadrupole couplings from the ENDOR spectrum. If the signs of $A$ and $P$ are the same, saturation of the low field ($M^\pm =+1$) ESR line gives the highest and lowest frequency ENDOR lines. But if the signs of $A$ and $P$ are opposite, saturation of the low field ESR line gives the intermediate frequency ENDOR lines.

2.4.4 ENDOR spectrum for an Electron Coupled to More Than One Nucleus:

Very often radicals of interest contain groups of equivalent and non-equivalent nuclei and if there are several such groups, the ESR spectrum can contain an enormous number of lines. However, the ENDOR spectrum remains comparatively simple. If the internuclear interactions are neglected, the first order energies are:

$$E = M^S \nu_e - \nu_N (\sum_{i=1}^N \nu M^{i(S)}_I) + \sum_{i=1}^N a_i M^{i(S)}_I$$

(2-72)

Applying the ENDOR selection rules:

$$\Delta M^S = 0, \quad \Delta M^{(i)}_I = \pm 1$$
where the spin of only one nucleus changes, the ENDOR transitions are described by

\[ v_{\text{rf}}^{(i)} = v_N^{(i)} \pm \frac{a_i}{2} \]  

(2-73)

Thus, ENDOR spectrum consists of a pair of lines for each interacting, nonequivalent nucleus and can be analyzed in terms of an electron interacting with each nucleus separately. Equivalent nuclei only contribute a pair of lines to the ENDOR spectrum, although their intensity depends directly on the number of nuclei if the relaxation rates are equal. The ENDOR spectrum is, therefore, often much simpler than its parent ESR spectrum, which for \( x \) protons may contain up to \( 2^x \) lines while the ENDOR spectrum consists of no more than \( 2x \) lines.

When the radical contains several different couplings, it may be that even the ENDOR lines overlap and then for the highest precision, it may be necessary to study partially deuterated samples. Replacement of a proton by a deuteron completely removes its contribution to the spectrum because of the change in free nuclear frequency. As a result, one obtains an unequivocal assignment of the couplings to the various protons in the radical.

2.4.5 Determination of Spin Hamiltonian Parameters:

In practice, the spin Hamiltonian parameters are best obtained by comparing the observed frequencies to those calculated by exact, computer diagonalization of the matrix for the Hamiltonian:

\[ \mathcal{H} = g_S^E \cdot S - g_N^E \cdot N \cdot I + S \cdot A \cdot I + I \cdot P \cdot I. \]  

(2-74)
with appropriate spin functions and trial values of the parameters. The parameters are then adjusted by the method of least squares until the calculated frequencies match the observed ones.

In this work, we have used a computer programme written by Dickinson et al.\textsuperscript{59,60} and Hebden\textsuperscript{61} based on this approach. This program calculates the ESR and ENDOR transition frequencies for any specified spin Hamiltonian and performs the least squares refinement of the Hamiltonian parameters.

For this program, the values of $S$ and $I$ are read in, together with the nuclear magnetic moment and the other elements of the spin Hamiltonian \textsuperscript{(2-74)} where these are nonzero. For our study, the electronic $g$-tensor was taken to be isotropic. The experimental data are read in as a series of observed transition frequencies, with the corresponding field strengths and direction. For each value and orientation of $H$, the total spin Hamiltonian is diagonalized numerically; the transition frequencies are calculated and compared to the experimental values and the resulting set of residuals is used to make a first order correction to the parameters to be refined according to the least squares criterion. The whole process is cycled until the errors and parameters remain constant. In general, the initial guess at the required parameters need be accurate only to within an order of magnitude and convergence is usually achieved within 5 iterations.
CHAPTER THREE

INTERPRETATION OF SPIN HAMILTONIAN PARAMETERS

3.1 Introduction:

Hyperfine tensors are interpreted first in terms of the signs and magnitudes of their components; next they are interpreted in terms of the direction cosines for the principal axes of the hyperfine tensor relative to the crystal axes. In order to obtain the most detailed picture of the electronic structure of a radical in a single crystal, one needs to know the crystal structure. It is usually found that organic radicals produced in single crystals by ionizing radiation are oriented nearly the same as the undamaged molecules. In this chapter, we will discuss the methods used to deduce the radical's electronic structure from the observed hyperfine tensors. First of all we will consider proton hyperfine tensors which are roughly classified into four groups based on the magnitude of the anisotropy and on the ratio of dipolar hyperfine components to isotropic components. These groups correspond to $\alpha$-protons, $\beta$-protons, more distant protons, including matrix protons and protons bonded to atoms other than carbon. Finally we will discuss the hyperfine and quadrupole couplings for nitrogen nucleus.

3.2 Hyperfine Coupling Tensors for $\alpha$-Protons:

A proton adjacent to an atom possessing appreciable spin density in a $p$-type orbital and lying in the node of that orbital is conveniently called an $\alpha$-proton.

3.2.1 The Isotropic Hyperfine Coupling:

Because the $\alpha$-proton is in the node of the unpaired electron orbital,
it should have zero isotropic coupling. In fact, the proton is experimentally observed to have a nonzero coupling which is directly proportional to the 2p spin population on the adjacent carbon atom.

The problem was first discussed, independently and simultaneously by Jarrett, Weissman, Bersohn and McConnell in terms of correlation of the spins of the electrons in the C-H bond and the π electrons in aromatic compounds. The treatment refers particularly to the $\hat{C}$-H fragment and is therefore applicable with considerable success to both aromatic and aliphatic systems. This correlation which can be explained in terms of either valence bond or molecular orbital theory, results in a slight polarization of the electron spins in the C-H σ bond.

The extent to which the C-H σ electrons are polarized is directly proportional to the π-electron spin density, $\rho_\pi$, representing the fractional probability of finding the unpaired electron in the carbon 2p$_z$ orbital. The proportionality is usually expressed in terms of McConnell's relation:

$$a_H = \frac{Q_{CH}}{Q_{CH}^H} \rho_\pi$$  \hspace{1cm} (3-1)

where $Q_{CH}^H$ is the proportionality constant for a proton in a C-H fragment and has a value of about $-63$ MHz. The determination of the isotropic hyperfine coupling constant for an α-proton is thus a very useful method for deducing the electronic structure of a π-radical. McConnell's relation is valid for the N-H fragments as well, with an appropriate change in Q.

3.2.2 The Anisotropic Hyperfine Tensor:

Experimental determination of α-proton anisotropic hyperfine tensors in several radicals has shown that the principal values of the tensors
have approximately the form: \( t,0,-t \) with the principal value \( 't' \) directed along the X-H bond direction, principal value \( '0' \) directed parallel to the axis of the p-orbital and the principal value of \(-'t'\) directed perpendicular to the X-H bond in the molecular plane.

These observations were first qualitatively explained in terms of the electronic structure of a π-radical by Ghosh and Whiffen.\(^6\) They explained the case of an isolated C-H fragment, with the unpaired electron in a carbon 2p orbital. The local symmetry of the problem required the three principal axes to lie along the directions experimentally observed for an α-proton. Using the dipolar Hamiltonian, they determined the relative sizes and signs of the three principal values given by:

\[
-g^2 g_N \beta_N \langle (1-3\cos^2\theta) r^{-3} \rangle_{\text{ave}}
\]

where \( r \) is the distance from the electron to the nucleus, \( \theta \) is the angle between the radius vector and a principal axis of the coupling tensor and \( \langle \rangle_{\text{ave}} \) signifies an average over the spatial distribution of the unpaired electron. The angular function, \((1-3\cos^2\theta)\) has a node at \( \theta_0 = 53^\circ \) dividing the space into two parts: in one part \((1-3\cos^2\theta)\) is positive and in the other negative. When the magnetic field being in Z-direction lies along C-H bond, electron will most probably be found inside the cone of half angle, \( \theta_0 \) thus giving a positive hyperfine coupling. When the field is along Y-axis in the plane of the radical and is perpendicular to the C-H bond, the electron would be found outside this cone, hence the hyperfine coupling is negative. When this field is parallel to the axis of the p-orbital i.e. X-direction, both positive and negative contributions will be appreciable, hence we expect a value much smaller in magnitude than either of the others and perhaps not far from zero. Comparing these predictions
with experimental possibilities, we see that they are consistent with the choice of negative sign for the isotropic coupling.

α-proton coupling tensors differ very little from one saturated hydrocarbon radical to another. If, however, the odd electron is delocalized such as in conjugated and aromatic compounds, the principal values are reduced proportionately. Sometimes the total anisotropic hyperfine tensor of a proton does not bear a simple relationship to the crystal structure. In such cases, the assignment of the tensor to a particular proton is made by comparison of its directions and tensors with the theoretical ones calculated analytically by the method of McConnell and Strathdee.

McConnell and Strathdee's calculation is not restricted to the anisotropic tensor of an α-proton, but can be applied to the interaction of the unpaired electron with any proton. However, the form of the tensor depends on the relative orientations of the unpaired electron's orbital and the proton and the distance between them. At sufficiently large distances, this treatment reduces to the expected point-dipole formula and the principal values of the tensor have axial symmetry.

3.3 Hyperfine coupling Tensors for β-Protons:

The methyl protons in the ethyl radical (CH$_3$-CH$_2$.·) are two bonds distant from the unpaired electron and hence they are called β-protons. The β-proton dipolar tensors exhibit relatively small anisotropy amounting to <10 MHz for unit spin density on the α-carbon. The maximum anisotropy is often ~10% of the isotropic coupling for unit spin density on an aliphatic or aromatic α-carbon. This small anisotropy is due, of course, to the greatly reduced value of $\langle r^{-3} \rangle$ compared to an α-proton. The coupling
tensors often show near axial symmetry and consequently the orientations of the principal values tend to be relatively imprecisely determined. However, $\beta$-protons have a large contact hyperfine interaction, $\alpha$, which varies with the angle $\theta$ between the plane of the C-C and C-H bonds and the axis of $p_z$ orbital.

$$a = B_1 + B_2 \cos^2 \theta$$  

where $B_1$ and $B_2$ are constants. The $\cos^2 \theta$ dependence can be explained by hyperconjugation. This is an extreme approach to the electronic structure of the radical, but it provides a mechanism whereby the electron can penetrate into the hydrogen $1s$ orbitals. Such a process assumes that the unpaired electron in the carbon $2p_z$ orbital couples slightly with an electron in the C-H $\sigma$-bond, leaving the other with a small positive spin density (the sign of isotropic coupling constant for $\beta$-protons is in fact found to be positive).

There have been a number of attempts to interpret the whole $\beta$-proton interaction in terms of spin polarization. For example, calculations by McLachlan$^{70}$ and Derbyshire$^{71}$ based on this concept also yield an angular dependence such as that given in (3-2). In this mechanism, polarization occurs by the admixture of electronic configurations which leave unpaired spin in one of the atomic orbitals constituting the C-H bond. There is no actual transfer of spin as there is in hyperconjugation; the total spin density in the C-H bond remains the same and the change in spin density of one of the atomic orbitals is matched by an opposite change in the other. In some ways this kind of mechanism appears more acceptable, particularly when applied to aromatic anions. In these, hyperconjugation suggests that
there is transfer of charge from $\beta$-protons to the $2p_\pi$ antibonding orbitals of the ring carbons, but these have already accepted one electron in the formation of the anion from the hydrocarbon and are likely to resist further additions.

However, recent calculations\(^7\) are tending away from the spin polarization mechanism. Thus hyperconjugation remains a very plausible mechanism based on a widely accepted concept.

3.4 Interactions with Distant Protons:

Interactions with more distant protons are rarely observed in ESR. For instance, where interaction with $\gamma$-protons has been observed, the splitting is normally less than 1 gauss.\(^7\) There are even one or two cases of interaction with still more distant protons in aromatic ions.\(^7\)

For radicals studied in solid matrices, the linewidths are such that only $\alpha$- and $\beta$-proton splittings are observed.

It is through ENDOR that interactions for such weakly coupled protons have been measured. More distant protons, including protons on adjacent molecules to the radical (matrix protons) and hydrogen bonded protons generally exhibit a nearly pure dipolar coupling in which the maximum anisotropy is small (<10 MHz), but is greater than the isotropic coupling. A point dipole model for the dipolar interaction is axially symmetric along the direction between the two point dipoles, with the largest principal component of the dipolar tensor lying along the bond direction.

3.5 Interaction of Protons Bonded to Atoms Other Than Carbon:

In general, the hyperfine tensors of protons bonded to other atoms such as nitrogen and oxygen are somewhat analogous to protons bonded to
carbon. A large anisotropy for α-protons bonded to nitrogen is expected. This is observed in the 1,2,4-triazole H-adduct radical. Protons bonded to oxygen generally show small anisotropy because the protons are often β to the site of primary spin density.

3.6 Hyperfine Coupling for Nitrogen Atoms:

3.6.1 The Isotropic $^{14}$N Hyperfine Coupling:

The theory of isotropic $^{14}$N coupling is more complicated than for protons, although no new principles are involved. The spin polarization mechanism introduced earlier which produces spin density at an α-proton also produces spin density at the nucleus of the atom to which that proton is attached. The expression for this spin density produced at the nitrogen atom yields a McConnell relation completely analogous to equation (3-1).

This simple relation may not hold if there is appreciable spin density on adjacent atoms. The unpaired π-electron density on the adjacent atoms polarizes the adjoining σ-bonds and induces negative spin density in the nitrogen 2p orbital. However, the nuclear moment of $^{14}$N is relatively small and the polarization produced by spin density on adjacent atoms can often be neglected. Thus the $^{14}$N splitting is roughly proportional to the odd electron density in the nitrogen 2$p_z$ orbital with a Q value of about 75 MHz.

3.6.2 The Anisotropic $^{14}$N Hyperfine Tensor:

The anisotropic $^{14}$N-hyperfine tensor possesses axial symmetry. The diagonal hyperfine tensor has the form: ($-B_0$, $-B_0$, $2B_0$) where:

$$ B_0 = \frac{2\beta e g_{N} B_N}{r^{-3}} $$

(3-3)

The direction of the principal value $2B_0$ is parallel to the nitrogen 2$p_z$
orbital and perpendicular to the molecular plane. The theoretical value
of \( B_0 \) for a spin density of unity in the p-orbital is estimated to be
47.9 MHz.\textsuperscript{77} Using the value of \( B_0 \) from experiment one can estimate
the spin density of p\(_\pi\) orbital of the nitrogen atom. The agreement with
the spin density calculated from the isotropic coupling of proton attached
to nitrogen nucleus gives a clue to assign the tensor to nitrogen nucleus.

3.7 \(^{14}\)N Quadrupole Interactions:

Physically the quadrupole coupling is sensitive to the total electron
density in contrast to the hyperfine coupling which is sensitive only to
the unpaired spin density. Thus quadrupole couplings are more directly
related to chemical bond strengths.

Because of the prevalence of nitrogen among organic and biochemicals,
the \(^{14}\)N quadrupole coupling is potentially a valuable source of information.
The interpretation of this coupling is made somewhat uncertain because of
complicated bonding on N which makes difficult the estimation of the un­
balanced p-electrons and because of some uncertainties in the field gradient
at the nitrogen nucleus. However, successful results have been obtained
using approximate methods developed by Townes and Dailey\textsuperscript{78-80} and later
modified and extended by Gordy.\textsuperscript{81-83} In its simplest forms the theory
attributes the field gradient to an unequal filling of the p-orbitals in
the valence shell of the atom containing the coupling nucleus, all other
charges being ignored.

If we define an axis system (x,y,z) such that x is parallel to N-C
bond, z is perpendicular to the molecular plane and y is perpendicular to
both x and z, the principal elements of the theoretical coupling tensor
are given by:\textsuperscript{84}
\[ x_x = \left[n_x - \frac{1}{2}(n_y + n_z)\right]eQ_{210} \]

\[ x_y = \left[n_y - \frac{1}{2}(n_x + n_z)\right]eQ_{210} \]

\[ x_z = \left[n_z - \frac{1}{2}(n_x + n_y)\right]eQ_{210} \]  \hspace{1cm} (3-4)

where \( e \) is the proton charge, \( Q \) is the nuclear quadrupole moment, \( n_x, n_y, n_z \) are the numbers of unbalanced p-electrons and \( q_{210} \) represents the field gradient due to an electron in an atomic 2p-orbital. \( eQ_{210} \) is assumed to be \(-10 \text{ MHz}\) for \( ^{14}\text{N} \).

Comparison of theoretical tensors with those from experimental observations does not always yield good agreement. The disagreement between the tensors results from many simplifying assumptions made in the field gradient calculations.

3.8 Spin Density Calculations:

Recently there have been various sophisticated molecular orbital (MO) methods available for the calculation of unpaired electron spin density distribution in \( \pi \)-radicals. Such MO calculations have been advanced to the level that they can be used to support the assignment of hyperfine couplings to the various radicals.

The calculated spin densities should of course be compared with the experimental ones. The application of experimental techniques, i.e., ESR and ENDOR to radicals produces proton hyperfine coupling constants which can be converted into the experimental spin densities via McConnell relation.
In a series of papers, Pople and coworkers developed an approximate self-consistent-field molecular orbitals (SCF-MO) theory based on an intermediate neglect of differential overlap (INDO). Their aim was to design an approximate all valence electron MO method for estimating hyperfine coupling constants in various radicals. In an earlier approximate MO procedure known as complete neglect of differential overlap (CNDO) devised by Pople and coworkers the one-center exchange integrals were neglected and the restricted Hartree-Fock wavefunction was used, so that \( \sigma-\pi \) correlation observed in isotropic hyperfine couplings is not properly treated at this level of approximation.

The INDO approximation is less severe in that all one-center exchange integrals are retained and the unrestricted Hartree-Fock wavefunction is used. As a result, INDO method is a substantial improvement over CNDO in any problem where electron spin is important. The unpaired electron population is equal to the difference between \( \alpha \) and \( \beta \)-electron densities:

\[
\rho_{rs}^{\text{spin}} = \rho_{rs}^{\alpha} - \rho_{rs}^{\beta}
\]  

(3-5)

Form this expression it is clearly seen how a negative spin density can arise at a given point. The simple Hückel method, on the other hand, is applicable only to planar conjugated free radical and predicts only positive spin densities. The matrix of elements \( \rho_{rs}^{\text{spin}} \) is usually called the spin density matrix. Thus with this matrix, one can obtain the spin density at any point in the system.

Unlike the simple Hückel and McLachlan methods, which are applicable only to planar molecules, the INDO method is applicable to molecules of any geometry. It provides a good means of investigating the validity of the
McConnell relation because it implies the direct proportionality between the unpaired electron population ($\rho_\pi$) of the adjacent carbon ($2p_\pi$) orbital and the unpaired electron population ($\rho_s$) of the hydrogen 1s orbital. We have, therefore, used the INDO approximation to calculate the spin densities for various radicals in order to support our radical assignments, the computer program being provided by Pople and collaborators.
CHAPTER FOUR

EXPERIMENTAL METHODS:

4.1 Introduction:

This chapter outlines the operation of the ENDOR spectrometer as well as general treatment of ENDOR data. Details on crystal preparation and handling will be discussed in the chapters dealing with the individual samples.

4.2 The ENDOR Spectrometer:

The ENDOR spectrometer used in these experiments has been described in detail by a previous worker from this laboratory. The main features of the instrument together with a few modifications are given below and a modified block diagram of the spectrometer is given in Figure 4.

The spectrometer is built around an X-band ESR spectrometer and is capable of operating in either the homodyne or superheterodyne mode. Invariably the experiments at the usual temperatures of 77 or 4.2 K require the use of such low microwave powers that the resulting AFC instability and noise levels make homodyne operation difficult. Hence our experiments were performed exclusively in the superheterodyne mode, using an intermediate frequency of 30 MHz.

The source of microwave power was a klystron with a Hewlett-Packard 716B power supply. The microwave frequency was stabilized by phase-locking to a microwave systems model MOS 1 frequency stabilizer. Some of the microwave power was coupled out of the main waveguide with a 3dB directional coupler and fed into a balanced modulator for the generation of the side bands. The balanced modulator was tuned such that the out-put power at the carrier frequency, $v_0$ was minimum while the power in the two side bands...
Fig. 4. Block diagram of the ENDOR spectrometer.
was maximum. The main branch of microwave power at the carrier frequency was led through the three 20dB attenuators to the cavity through a magic Tee bridge.

A $\text{TE}_{101}$ rectangular cavity was modified and designed by Dr. V. P. Chacko for ENDOR work. Two vertical columns of five straight gold plated brass posts of 0.5 mm diameter traverse (in the x-direction) the cavity piercing the two end walls. The posts are insulated from the end walls by araldite. The posts are connected by thin copper wires outside the cavity to form a single loop. One end of the loop was connected to the output of an ENI3100L r.f. amplifier, the other end was soldered to the body of the cavity to complete the r.f. circuit. In this design, both the microwave magnetic field, $H_1$ and r.f. field, $H_2$ are vertical, allowing the magnet to be rotated in the horizontal plane for studies of the anisotropy of the ENDOR spectra. The posts are everywhere perpendicular to the microwave electric field, which is in the y-direction and have only a minor effect on the cavity Q. No net flux due to the microwave $H_1$ threads the loop; therefore, the loop does not harm the quality factor. The spacing between the two columns (5 mm in our case) is determined by the sample size. The width of the cavity can be increased to accommodate larger spacing between the two columns. The cavity frequency for the $\text{TE}_{101}$ mode is unaffected by the increased width. The space outside the rectangular cross section solenoid of posts, but inside the cavity provides the return path for r.f. field lines. A similar construction of an ENDOR cavity operating in the $\text{TE}_{201}$ mode has been reported by Castner and Doyle. The crystal is mounted on a Perspex wedge and fixed onto the side wall of the cavity using silicone grease, between the two columns of posts.
The reflected microwave power from the cavity is led through the third arm of the magic Tee to a balanced detector; it is detected at 30 MHz, amplified and passed to a PAR model 122 lock-in amplifier. The output of the lock-in amplifier is used to provide the Y-drive of an X-Y recorder. The X-drive is provided by either the field sweep from the Fieldial for ESR spectra or by a signal generated by the frequency sweep for ENDOR.

The magnetic field is provided by a Varian rotatable electromagnet with 9" pole faces controlled by a Fieldial Mark II power supply unit. For ESR work, variable audio-frequency magnetic field modulation was provided by modulation coils wound on the magnet pole-pieces. The coils were driven by the output of the lock-in amplifier.

For ENDOR work radio frequency was introduced at the sample site by connecting the r.f. coil to the output of an ENI3100L r.f. amplifier. The r.f. source was PRD7808 signal generator which can be frequency modulated to a depth of 5 to 50 kHz. The r.f. frequency was varied by sweeping the signal generator by means of a variable voltage ramp generator constructed by the electronic shop of this department. A parallel output from the ramp generator was used to drive the X-axis of the recorder. The r.f. frequency was monitored by a Hewlett-Packard 5246L counter which triggered a home-built digital event marker producing calibration pips in the spectrum at 1 MHz intervals.

The spectrometer was equipped with two concentric pyrex dewars designed to cool the cavity to 4.2 K with liquid helium in the inner dewar and liquid nitrogen in the outer dewar. Our experiments were performed with liquid nitrogen in both dewars. As long as the outer dewar was kept full, there was little evaporation from the inner dewar. The outer dewar was refilled
from time to time without disturbing the spectrometer. The cavity was enclosed by a copper tube and immersed in the liquid nitrogen. The copper tube cooled the cavity through conduction and prevented the liquid nitrogen from entering the cavity.

4.3 ENDOR Technique:

4.3.1 Crystal Alignment Through Site Splitting:

The commonly encountered crystals in which spectra show site splitting are of monoclinic or orthorhombic symmetry and we shall discuss these cases since the crystals under investigation were either monoclinic or orthorhombic.

In some cases of single crystal studies, the spectra are symmetrical and can be interpreted straightforwardly in terms of hyperfine splittings for a number of orientations but become more complicated along other orientations. This indicates that there are magnetically inequivalent radicals in the crystal. These are chemically identical species but having different orientations, so that in general they will have different spectra for a given orientation of the magnetic field. The difference between the line positions of the two radicals in the spectrum is termed site splitting.

A monoclinic crystal has \(a \neq b \neq c\), and \(\alpha = \gamma = 90^\circ \neq \beta\). The b-axis is a two-fold axis. In order to understand how the site splitting varies with the orientation of the magnetic field, it is useful to think of the orientations of a pair of radicals as represented by two vectors. The two radicals will have different spectra if the magnetic field makes unequal angles with the two vectors. If the b-axis is in the plane and the ac-plane is perpendicular to the plane of the paper, the vectors defining the orientations of the two radicals lie in the plane of the paper symmetrically with respect to b-axis. A magnetic field parallel to b or anywhere in the ac-plane makes
equal angles with the two vectors and so for those orientations, the spectra would show no site splitting. For other orientations, there would be site splitting.

In orthorhombic symmetry, there would be four magnetically distinct molecules. This sort of crystal has $a \neq b \neq c$, but $\alpha = \beta = \gamma = 90^\circ$. The $b$-axis remains fixed, but now we fix $a$ in the plane of the paper and $c$ perpendicular out of the plane of the paper. Out of four vectors, two are below the plane and other two above the plane related to the first two by reflection through the $ab$-plane. For a general orientation of the magnetic field, there will be site splitting into four spectra, but in the $ab$-, $ac$-, or $bc$-planes, the radicals become magnetically equivalent in pairs. The only orientations for which there is no site splitting are when the field is along one of the crystallographic axes.

Radicals trapped in single crystals are often very precisely oriented and if there is appreciable anisotropy, the spectra can be very sensitive to site splitting. The most serious uncertainty in the ENDOR data is the absolute orientation of the crystal in the magnetic field. However, the reproducibility of the data with different crystals and the double check provided by the site splitting present in different crystallographic systems indicate that $\pm 1^\circ$ is a reasonable estimate of this error. Because the crystal could not be re-aligned without disassembling the cavity, it was very difficult to reduce this error.

4.3.2 Operation of ENDOR Spectrometer:

The spectrometer was allowed to stabilize for at least an hour with the cavity maintained at 77 K. The ESR spectrum was obtained and a particular point on the ESR lineshape was selected for ENDOR study. The
microwave power level for maximum ESR signal was determined and the ESR transition was partially saturated by increasing the microwave power above this level. The magnetic field modulation was then switched off and the spectrometer was adjusted for higher sensitivity by increasing the lock-in amplifier and recorder gains over two hundred-fold. The radio frequency was scanned until an ENDOR signal was obtained. The frequency modulation used for detection was between one and four kHz with a depth depending on the ENDOR linewidth. Once the entire ENDOR spectrum was observed and its intensity optimized with the lock-in phase, modulation frequency, field strength and microwave power level, the magnet was rotated so that the angular variation of the spectrum could be studied. The optimum conditions were found to be quite reproducible from day to day.

4.3.3 Data Reduction:

The calibration of spectral line positions was achieved by fitting the positions of frequency markers to a quadratic expression and using this function to interpolate the line positions. A least squares fitting routine written by Dr. J. A. Hebden for a Monroe 1656 programmable desk calculator was used for this. At least 3 calibration points were fitted each time and the fit reproduced the frequencies of the calibration markers to within 5 kHz. In most cases, the line position could be taken as the center of the line, where it crossed the base line. For overlapping lines which were asymmetric or did not cross the base line, the line position was taken as the point of maximum slope.

The ENDOR data were taken in three orthogonal planes by rotating the magnet in intervals of 2 or 5° depending on the density of lines. The line
positions were then plotted as a function of magnetic field orientation for each of the three planes. The curves belonging to the same hyperfine coupling in each of the three planes were readily identified by comparing the curves in different planes at coincident axes. Experimentally observed points belonging to the same coupling were then fitted to the spin Hamiltonian with the least-squares-adjustment programme LSF, mentioned earlier, using an Amdahl 470 V/6 model II computer.
5.1 Introduction:

A thorough knowledge of the spin density distribution and the geometrical structure of the organic radicals can be achieved only through the complete determination of the hyperfine interaction tensors (i.e., of their principal values and directions) for the different nuclei in the radicals under study. This is possible only by working with the oriented systems such as those having the radical species trapped in a single crystal. In general, the radicals produced by ionizing radiation in aliphatic compounds yield well-resolved ESR spectra and hence they have been studied in detail. In aromatic systems, however, the unpaired electron is extensively delocalized and hence coupled to a relatively great number of nuclear spins. As a result, the ESR linewidth becomes essentially inhomogeneous and broad. For these compounds in solid state, the ESR spectra appear very poorly resolved or completely unresolved and thus generally yield little information.\(^{94-96}\)

The much better resolution of ENDOR spectroscopy makes it possible to study the hyperfine interactions of nuclei in such aromatic radicals in single crystals. Using this technique Bühme and Jesse\(^{39}\) have determined the structure of a stable dibenzo-cyclohexadienyl radical produced by room temperature X-irradiation of anthracene. While ENDOR lines of all the C-H protons have been detected, no ENDOR lines are found that could be assigned to methylene protons possibly due to unfavourable relaxation behaviour of \(\text{CH}_2\) protons. However, from a number of evidences, it was
unequivocally concluded that the radical was formed by hydrogen addition. Later, Böhme and Wolf \(^{40}\) carried out detailed ENDOR studies of X-irradiated naphthalene single crystal. From the determination of spin density distribution and hyperfine principal values of all CH and CH\(_2\) protons, they have concluded that two types of radicals are formed by addition of a hydrogen atom to the 1 and 4 positions of the naphthalene molecule. The different intensities for the two types have been ascribed to different concentrations of the radicals. Gloux and Lamotte \(^{41,42}\) have reported ENDOR investigations of radicals in aromatic heterocyclic compounds. In both imidazole and 1,2,4-triazole systems, their results correspond to a π-radical formed by the addition of one hydrogen atom to a carbon of the ring.

We decided to study phenazine for a number of reasons. The molecule is very similar to anthracene except that it has two nitrogen atoms. In all aromatic compounds, including heterocyclic, so far studied, the radicals were shown to be formed by the addition of hydrogen to carbon of the ring. Prior to this study, an ENDOR investigation of an X-irradiated single crystal of acridine (with one nitrogen) was taken up in this laboratory. \(^{92}\) It was found that the radical is formed as a result of hydrogen addition to the nitrogen atom of the acridine molecule. If the hydrogen addition radical detected in acridine was also produced in phenazine, it could only arise from hydrogen addition to either of the two nitrogen atoms of phenazine ring.

Moreover, phenazine resembles the flavin moiety of riboflavin which is a constituent of several enzyme systems called flavoproteins involved in intermediary metabolism. \(^{97}\) Riboflavin is relatively heat stable, but
sensitive to light and irradiation. Thus phenazine served as an excellent model system for determining the electronic structure of the radicals in riboflavin. There has not been any report of ESR or ENDOR on single crystals of phenazine. Our work is the first ENDOR analysis of radical formed by hydrogen addition to nitrogen atom of aromatic heterocyclic compounds. We have also observed two types of radicals produced by hydrogen addition to either of the nitrogen atoms of phenazine.

In this chapter, the results of an ENDOR study of the prominent radicals produced by room temperature X-irradiation of single crystal of phenazine are reported. No useful results could be obtained from the ESR spectrum because of very poor resolution as a result of the extensive delocalization of the unpaired electron. The hyperfine interaction tensors of all the protons in this radical could be determined by the ENDOR technique. No ENDOR lines for the $^{14}$N nuclei were observed. This did not, however, cause any difficulty in identifying the nature of the paramagnetic species.

5.2 Experimental:

Single crystals of phenazine were grown by slow evaporation of its saturated solution in propanone at room temperature. Well developed crystals of approximate dimensions 8x4x3 mm were easily obtained. Owing to differences in the relative development of different faces, there appeared to be two varieties of crystals, but they were found to be crystallographically identical. The crystal morphology is shown in Fig. 5, along with the crystallographic and reference axes.

The crystal structure of phenazine was reported by Herbstein and Schmidt and two years later, by Hirshfeld and Schmidt near 80 K. The atomic positions are essentially the same in both of these works.
Fig. 5. Crystal morphology of phenazine with reference axes.
Comparison of bond lengths shows that rms difference between the two sets of data is typically 0.010 Å.

The crystal is monoclinic with unit cell dimensions a=12.967, b=4.981, c=7.056 Å, β=109.0° and space group P2₁/a. There are two molecules per unit cell, both of which are magnetically equivalent along the b-axis and in the ac-plane; in all other orientations they are distinct. The crystallographic axes were located by external morphology and by identifying the crystal planes with the help of a two-circle optical goniometer. ENDOR measurements were carried out in the reference coordinate system a, b, c* (c*=axb). The molecular structure with the numbering of ring atoms is depicted in Fig. 6.

The crystals were X-irradiated at room temperature using a Machlett type OEG-60 X-ray tube operating with a voltage of 40 kV and a beam current of 20 mA. The optimum concentration of the radicals was obtained after the crystals were irradiated for about 80 hours. The irradiated crystals could be stored indefinitely in closed vials at room temperature without any significant decrease in the radical concentration.

ENDOR measurements were made at 77 K with the spectrometer described in chapter four. Since the ENDOR lines were found to be well separated, the spectra were recorded at intervals of 5°, as the magnet was rotated about each of the reference axes.

5.3 Experimental Results:

The ESR and ENDOR spectra of an X-irradiated phenazine single crystal for the magnetic field parallel to the b-axis are shown in Figure 7. Although the ESR spectrum in this orientation appears quite well-resolved, the resolution was very much poorer in most other orientations. No useful data could, therefore, be obtained from the analysis of the ESR spectra.
Fig. 6. Molecular structure and numbering of the ring atoms of phenazine.
Fig. 7. (a) First derivative X-band ESR spectrum at room temperature (b) ENDOR spectrum at 77 K of the N-hydrophenazinyl radical in an X-irradiated single crystal of phenazine for the magnetic field parallel to the b-axis. Assignment of the ENDOR lines is indicated.
As can be seen from Figure 7, the ENDOR spectra were, however, quite well-resolved. Not shown in the figure are the ENDOR lines in the immediate vicinity of $v_p$ which might belong to the weakly coupled intermolecular protons. No ENDOR lines due to the hyperfine coupling of the unpaired electron with nitrogen nuclei were observed in our study. All the observed lines arise from interactions with protons. Only those lines appearing at the higher frequency side of $v_p$ were used in the analysis. A maximum of 20 ENDOR lines could be observed in an arbitrary orientation, coalescing into ten when the magnetic field was oriented along the b-axis or in the ac-plane. By selectively saturating various positions throughout the ESR lineshape, we established that some of the weaker lines belong to a different radical. The nature and origin of these weaker lines will be discussed later.

For calculation and graphical presentation, $v_p$ was set equal to 13.820 MHz and the observed transition frequencies were corrected accordingly. The angular variation of the proton ENDOR frequencies is shown in Figures 8 and 9 in the three orthogonal planes. Figure 8 presents the high frequency branch of ENDOR lines belonging to radical(I), whereas Figure 9 presents those from radical(II). The curves belonging to the same proton coupling in each of the three planes were readily identified by comparing curves in different planes at coincident axes (the reference axes).

The ESR spectrum in most orientations consists of an ill-resolved broad line. Therefore the g-tensor could not be determined accurately. For convenience, we used the isotropic g-value of 2.0023 in place of the g-tensor. Approximately 100 data points were used to fit each tensor and
Fig. 8. Angular dependence of the ENDOR frequencies of radical (I) in an X-irradiated phenazine. The assignment of various ENDOR lines to specific protons is indicated on the right.
Fig. 9. Angular variation of the ENDOR frequencies of radical (II) in X-irradiated phenazine.
the rms error was typically less than 10 kHz. The diagonalized forms of the hyperfine tensors calculated by LSF, along with their direction cosines, are presented in Tables 1 and 2 for the radicals I and II, respectively.

5.4 Assignment of the couplings and Radical Identification:

5.4.1 Radical(I):

The assignment of the hyperfine coupling tensors to specific protons in radical(I) is indicated in Table 1. At the outset, we should note that these coupling tensors are typical of an aromatic π-radical. The directions of all the intermediate couplings are closely parallel to each other and perpendicular to the plane of the molecule as expected for a radical with the unpaired electron in a π-molecular orbital.

We attempted to relate the directions of the largest positive component of the anisotropic part of the coupling tensor to various C-H bond directions in the undamaged crystals. For protons attached to carbon atoms expected to have positive spin densities, the directions of the largest positive component of the dipolar tensor are very nearly parallel to the C-H bond directions. For other protons, deviations from the C-H bond directions were observed. All the hyperfine tensors could, however, be assigned to specific protons without much difficulty on the basis of the direction cosines, the sign of the isotropic part, symmetry considerations and comparison with the INDO results. It must be noted that because the unpaired electron is extensively delocalized over the whole molecule, the direct comparison of small coupling tensors to bond directions cannot yield unequivocal conclusions.

Four types of radicals are encountered in solid organic matter as
### TABLE 1

**PROTON HYPERFINE INTERACTION TENSORS FOR RADICAL(I) IN X-IRRADIATED SINGLE CRYSTAL OF PHENAZINE**

<table>
<thead>
<tr>
<th>Proton</th>
<th>Principal Value $^1$</th>
<th>Isotropic</th>
<th>Dipolar</th>
<th>Direction Cosines $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coupling (MHz)</td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>1,8</td>
<td>2.64</td>
<td>-10.50</td>
<td>13.14</td>
<td>+0.697</td>
</tr>
<tr>
<td></td>
<td>-14.22</td>
<td>3.72</td>
<td>-9.41</td>
<td>-0.336</td>
</tr>
<tr>
<td></td>
<td>-19.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.38</td>
<td>7.58</td>
<td>4.20</td>
<td>-0.552</td>
</tr>
<tr>
<td></td>
<td>+9.21</td>
<td>1.63</td>
<td>2.57</td>
<td>-0.641</td>
</tr>
<tr>
<td></td>
<td>+10.15</td>
<td></td>
<td></td>
<td>-0.533</td>
</tr>
<tr>
<td>3</td>
<td>-4.01</td>
<td>-9.46</td>
<td>5.45</td>
<td>-0.032</td>
</tr>
<tr>
<td></td>
<td>-9.43</td>
<td>0.03</td>
<td>5.47</td>
<td>-0.589</td>
</tr>
<tr>
<td></td>
<td>-14.93</td>
<td></td>
<td></td>
<td>-0.808</td>
</tr>
<tr>
<td>4,5</td>
<td>2.42</td>
<td>6.19</td>
<td>3.77</td>
<td>+0.574</td>
</tr>
<tr>
<td></td>
<td>+7.75</td>
<td>1.56</td>
<td></td>
<td>-0.799</td>
</tr>
<tr>
<td></td>
<td>+8.39</td>
<td>2.20</td>
<td></td>
<td>-0.179</td>
</tr>
<tr>
<td>6</td>
<td>-4.13</td>
<td>-9.62</td>
<td>5.49</td>
<td>+0.700</td>
</tr>
<tr>
<td></td>
<td>-9.58</td>
<td>0.04</td>
<td></td>
<td>-0.603</td>
</tr>
<tr>
<td></td>
<td>-15.15</td>
<td>5.53</td>
<td></td>
<td>-0.383</td>
</tr>
<tr>
<td>7</td>
<td>3.12</td>
<td>7.20</td>
<td>4.08</td>
<td>+0.782</td>
</tr>
<tr>
<td></td>
<td>+8.83</td>
<td>1.63</td>
<td></td>
<td>-0.583</td>
</tr>
<tr>
<td></td>
<td>+9.64</td>
<td>2.44</td>
<td></td>
<td>-0.220</td>
</tr>
<tr>
<td>10</td>
<td>-2.93</td>
<td>-8.50</td>
<td>5.57</td>
<td>+0.286</td>
</tr>
<tr>
<td></td>
<td>-9.79</td>
<td>1.29</td>
<td></td>
<td>-0.799</td>
</tr>
<tr>
<td></td>
<td>-12.79</td>
<td>4.29</td>
<td></td>
<td>+0.529</td>
</tr>
</tbody>
</table>

1. Signs of the principal elements are assumed on the basis of INDO results. Uncertainty in the principal values is ±0.01 MHz.

2. The two sign combinations chosen consistently relate one distinguishable crystal site to the other.
## TABLE 2

PROTON HYPERFINE INTERACTION TENSORS FOR RADICAL(II) IN X-IRRADIATED SINGLE CRYSTAL OF PHENAZINE

<table>
<thead>
<tr>
<th>Proton</th>
<th>Coupling (MHz)</th>
<th>Direction Cosines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Principal value</td>
<td>Isotropic</td>
</tr>
<tr>
<td>-3.97</td>
<td>-9.40</td>
<td>+0.697 ±0.712 ±0.091</td>
</tr>
<tr>
<td>-14.81</td>
<td>-5.41</td>
<td>-0.326 ±0.200 +0.924</td>
</tr>
<tr>
<td>+3.09</td>
<td>-4.08</td>
<td>+0.754 ±0.400 +0.520</td>
</tr>
<tr>
<td>+8.79</td>
<td>+7.17</td>
<td>+1.62 ±0.628 +0.394</td>
</tr>
<tr>
<td>+9.62</td>
<td>+2.45</td>
<td>-0.192 ±0.624 -0.758</td>
</tr>
<tr>
<td>+2.26</td>
<td>+12.55</td>
<td>+0.636 ±0.229 -0.737</td>
</tr>
<tr>
<td>-12.60</td>
<td>-10.29</td>
<td>-0.658 ±0.659 -0.364</td>
</tr>
<tr>
<td>-20.52</td>
<td>-10.23</td>
<td>-0.402 ±0.717 -0.570</td>
</tr>
<tr>
<td>+3.27</td>
<td>-4.12</td>
<td>-0.507 ±0.004 +0.862</td>
</tr>
<tr>
<td>+9.01</td>
<td>+7.39</td>
<td>+1.62 ±0.658 -0.379</td>
</tr>
<tr>
<td>+9.88</td>
<td>+2.49</td>
<td>-0.566 ±0.753 -0.337</td>
</tr>
</tbody>
</table>

Bond directions from crystal structure

$C_1-H_1, C_4-H_4, C_5-H_5, C_8-H_8$ : +0.678 ±0.210 -0.704

$C_3-H_3$ and $C_7-H_7$ : -0.005 ±0.489 +0.872

$C_2-H_2$ and $C_6-H_6$ : ±0.685 ±0.714 ±0.143

1. See footnotes to Table 1.
a result of ionizing radiation involving either removal or attachment of
an electron, dissociation of covalently bonded atomic hydrogen or
association of atomic hydrogen on unsaturated positions of the molecules. Of these, the ionic radicals resulting from an electron removal or attach-
ment have been found to be stable only at low temperatures and can,
therefore, be ruled out in the present study. Of the remaining two
possibilities, namely the radicals produced either by the addition or
removal of a hydrogen atom to the undamaged molecule, only the former
is relevant in the present case because we observe more intramolecular
proton couplings than could be accounted for without an extra hydrogen
added to the phenazine molecule. Therefore it can be concluded with
reasonable certainty that the radical is formed as a result of the
attachment of a hydrogen atom to one of the ring positions.

Now if the hydrogen atom is attached to any of the carbon atoms
(other than at positions 11-14), the resulting radical would have a
methylene group with a large spin density on the adjacent carbon atom. These two protons of the methylene group (β-protons) should then have
fairly large isotropic coupling. Our results do not indicate the
presence of such a large isotropic coupling.

The next possibility is, of course, the attachment of a hydrogen
atom to one of the nitrogen atoms, resulting in a large spin density on
the other (c.f. figure 10). For example, if a hydrogen atom is attached
at N(10), then the resulting radical would have a large spin density on
the π-orbital of N(9). In an earlier study of N-hydroacridinyl radical in
molecule A and B of acridine, the large spin density (≈0.5) on C9 was
verified by the determination of the hyperfine tensor of proton at position
9. This was the largest coupling having an isotropic value of ≈30.2 MHz
Fig. 10. Spin density distribution in (a) N(10)–H radicals in molecule A and B of acridine (b) N(10)–H and N(9)–H radicals in phenazine. The INDO spin densities are given in parentheses.
and assigned to C\textsubscript{9}-H proton. Based on structural and chemical similarities between acridine and phenazine, N-hydrophenazinyl radical should be the first choice to be considered. A large π-spin density on N\textsubscript{(9)} would give rise to large anisotropic hyperfine coupling with the \textsuperscript{14}N-nucleus. Although we have not observed any ENDOR lines due to nitrogen, the orientation dependence of the ESR linewidth and spectral spread indicate the highly anisotropic nature of the coupling. None of the proton hyperfine couplings observed in this radical was large enough to account for the ESR spectral spread. These observations point to a large π-spin density on the nitrogen atom. However, on account of the poor resolution of the ESR spectra, the \textsuperscript{14}N coupling tensor could not be determined.

5.4.2 Radical(II):

In figure 7(b), the weaker lines with primed numbers do not belong to the same radical as the other lines. This was verified by selectively saturating different portions of ESR spectrum. In all orientations, these lines were about five times weaker than the main lines. Although only three such weak lines are seen in Figure 7(b), all the main lines had such accompanying weaker lines but they were clearly resolved only in some orientations. Only four of these lines could be followed unambiguously in all the three planes. The results obtained from the analysis of these lines are presented in Table 2. Comparison of hyperfine tensors presented in Table 2 with those in Table 1 show that they belong to a radical which is almost identical in structure and orientation to the N-hydrophenazinyl radical.

Because of the center of inversion of the phenazine molecule, the two nitrogen atoms are completely equivalent in the undamaged crystal. Addition of hydrogen to either N\textsubscript{(9)} or N\textsubscript{(10)} should, therefore, yield
radicals identical in all respects. The atomic positions 1 and 8 of the phenazine molecule are equivalent to positions 4 and 5; so also 2 and 3 to 6 and 7, respectively. Thus the ENDOR lines from the symmetry-related protons should, therefore, be indistinguishable for the two radicals. But if there are some slight conformational differences between the two radicals because of constraints in the solid state, they would yield different set of ENDOR lines for the corresponding protons. We propose, therefore, that these weaker lines belong to the same type of radical, but with the hydrogen atom added to \(N_{(9)}\). The crystal surrounding of \(N_{(9)}-\text{H}\) differs from that of \(N_{(10)}-\text{H}\) resulting in a slight conformational change of \(N_{(9)}\)-hydrophenazinyl radical with respect to radical formed by the addition of hydrogen to \(N_{(10)}\).

It is difficult, however, to understand the intensity differences between these two sets of ENDOR lines. Since the structure of the two radicals are identical, the relaxation pathways in both the radicals must be similar leading to similar ENDOR enhancements. The different intensities, therefore, indicate different concentrations of the two radicals in the crystal. As the two nitrogen atoms are completely equivalent in the undamaged crystal, it is hard to understand why the addition of a hydrogen atom to one of them is more favourable than to the other. Similar intensity differences have been observed by Böhme and Wolf\(^{40}\) between two sets of ENDOR lines in the \(\alpha\)-hydronaphthyl radicals produced by X-irradiation of naphthalene single crystals. They ascribe the intensity difference to the different rates of formation of the two symmetry related radicals.

5.5 Spin Densities:

The spin densities on the ring atoms were deduced from the experimental
isotropic couplings by using the familiar McConnell relation. In order to confirm that our radical identification is correct INDO-MO calculations were performed on several possible radical structures. Of the many radicals considered, only the one with the hydrogen atom added to the nitrogen yielded good agreement for the spin densities. Moreover, this radical has significantly lower total energy (INDO) as compared to all other structures considered. Thus we are fairly certain of the identity of the radical. The experimental and INDO spin densities for N-hydrophenazinyl radicals are presented in table 3 along with those reported for N-hydroacridinyl radicals for comparison. Inspection of table 3 reveals that the spin density distribution in the N-hydrophenazinyl radical is quite similar to that found in the acridinyl radical. The large spin density on the second nitrogen atom could not be experimentally verified because there was no proton coupling at this position. On the whole the agreement can be deemed excellent considering the INDO approximations. The carbon atoms expected to have negative 2p spin densities have unusually large spin populations. Similar large negative spin densities were also found in N-hydroacridinyl and α-hydronaphthyl radicals.

5.6 Direction Cosines:

As mentioned earlier, for the radicals reported in this study, all tensors have an axis in common indicating that the unpaired electrons in these radicals occupy molecular orbitals with \( \pi \)-symmetry. However, in the course of discussion, mention must be made of a significant deviation from collinearity of the common principal direction. As can be verified from tables 1 and 2, all the couplings are approximately of \( \alpha \)-proton nature with fairly small spin densities on the respective ring atoms.
### TABLE 3

**SPIN DENSITIES ON THE RING ATOMS OF N-HYDROACRIDINYL AND N-HYDROPHENAZINYL RADICALS**

<table>
<thead>
<tr>
<th>Position</th>
<th>N-hydroacridinyl radical</th>
<th>N-hydrophenantrazinyl radical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\rho_{\pi}^{\text{(INDO)}}$</td>
<td>$\rho_{\pi}^{\text{(Observed)}}$</td>
</tr>
<tr>
<td>Molecule A</td>
<td>Molecule B</td>
<td>Ref.103</td>
</tr>
<tr>
<td>1,8</td>
<td>+0.144</td>
<td>+0.114</td>
</tr>
<tr>
<td>2</td>
<td>-0.087</td>
<td>-0.157</td>
</tr>
<tr>
<td>3</td>
<td>+0.142</td>
<td>+0.165</td>
</tr>
<tr>
<td>4,5</td>
<td>-0.089</td>
<td>-0.145</td>
</tr>
<tr>
<td>6</td>
<td>+0.161</td>
<td>+0.163</td>
</tr>
<tr>
<td>7</td>
<td>-0.101</td>
<td>-0.138</td>
</tr>
<tr>
<td>9</td>
<td>+0.473</td>
<td>+0.475</td>
</tr>
<tr>
<td>10</td>
<td>+0.208</td>
<td>+0.138</td>
</tr>
<tr>
<td>11,14</td>
<td>-0.127</td>
<td>-</td>
</tr>
<tr>
<td>12,13</td>
<td>+0.171</td>
<td>-</td>
</tr>
</tbody>
</table>
ENDOR line assigned to the N-H proton has anomalously low intensity and large linewidth as compared to all other lines. This may be due to an indirect effect of the fast spin lattice relaxation of $^{14}N$ nucleus. The dipolar component of smallest magnitude for the N-H protons (designated as proton 10 in table 1) shows a deviation of approximately $20^\circ$, indicating that the N-H bond may be bent with respect to the molecular plane. This is further confirmed by comparing the direction of the largest positive dipolar component expected to be along the N-H bond direction with those calculated from crystallographic data. Our results thus indicate that the hydrogen atom that is added on to nitrogen is out of the plane by approximately $20^\circ$.

The largest couplings are assigned to protons at positions 1,8 in radical(I) and 4′,5′ in radical(II). These two tensors are very similar in nature with a spin density of about 0.163 on the ring atom. The direction of the positive anisotropic component is within $3^\circ$ of the C$_1$-H$_1$ direction in the undamaged molecule.

Interactions 2, 6 and 7 of radical(I) correspond to interactions 6′,2′ and 3′ of radical(II), respectively. In the case of protons 3 and 6 of radical(I) which are bonded to carbons with positive spin densities, the directions of the positive dipolar components coincide with the bond directions within $2^\circ$. However, deviation up to about $15^\circ$ can be seen at protons 2 and 7. These deviations are caused by the fact that these protons are bonded to carbons with negative spin density. This negative spin density results from a polarization of the $\sigma$-electrons of the carbon skeleton whose influence on the local field must be accounted for. Although in all the cases we have assumed that the unpaired electron is only localized
in a carbon p-orbital, this is not the case with carbon with negative spin
density because of the polarization of σ-electrons. Similar notable
deviations have been reported by Böhme and Wolf\textsuperscript{40} in X-irradiated naphthalene
single crystals.

A significant deviation from collinearity is observed for the tensors
associated with protons 4 and 5. No relevant explanation for the deviation
can be given, except that the ENDOR data for these two protons are less
accurate because of very low signal intensity (cf. figure 7(b)).

5.7 Discussion:

As can be verified from tables 1, 2 and 3, there are small differences
in the corresponding values for the two types of radicals produced from
phenazine. The isotropic couplings of radical(I) are consistently higher
than those of radical(II). Hence the dipolar parts of these coupling
tensors are also slightly different. These differences have been attributed
to the fact that the radicals occur in slightly different conformations.
The dipolar part of the tensors for all the protons were calculated
analytically by the method of McConnell and Strathdee\textsuperscript{68} for the two
radicals separately. However, the accuracy of these calculations is not
good enough to compare such small differences. Therefore, no further
effort was made to explain them.

As expected, the spin density distribution in the N-hydrophenazinyl
radical is quite similar to that found in the acridinyl radical studied
earlier in this laboratory.\textsuperscript{92} Although the spin density on N(9) in the
phenazinyl radical was not experimentally determined, the INDO calculations
indicate that it is slightly larger than on C(9) in the acridinyl radical
(cf. table 3). This difference can probably be justified by the difference
in the electronegativities of the two atoms. Accordingly, the spin densities on the other atoms in the two radicals are also slightly different.

Subsequent to the completion of this work, Furrer et al. have reported the ESR studies of radical pair formation from excited states in the guest-host systems (i) acridine-fluorene (ii) phenazine-fluorene crystals. Their results indicate that a radical pair is formed by hydrogen abstraction from fluorene and hydrogen addition to acridine or phenazine. Table 3 lists the central spin densities for acridinyl and phenazinyl radicals as reported by these authors for comparison. While excellent agreement can be stated for acridinyl radical, the central spin densities in the phenazinyl case for these authors are quite different from those of ours and INDO, although the sum of the two central spin densities seems comparable. This difference has been attributed by them to the larger angle $\angle(Z^*,b)$ observed for phenazinyl and corresponding move of the charge density center towards the NH fragment of the phenazinyl radical. The work of these authors provides a substantial additional support to the identification of our radical.

Whereas there is little doubt about the identity and nature of the radicals discussed here, the mechanism of their formation cannot be ascertained from the present study. Most probably ionic species are produced as primary radicals as a result of the impact of X-rays. As these ionic radicals are unstable at room temperature, they undergo proton addition to form the radical identified in this study. However, this can be verified only by low temperature irradiation studies.

There is little we can say about the source of the hydrogen atoms. They are probably extracted from other molecules. However, no hydrogen
abstraction radicals are identified in the present work. In some orientations there were some weak ENDOR lines which did not belong to the hydrogen addition radicals. The experimental data were either incomplete or unreliable, so that their identification was not seriously attempted. These weak lines most certainly belong to another radical the nature of which we cannot ascertain in the present study.
CHAPTER SIX

$^{14}$N AND $^1$H ENDOR STUDIES OF RADIATION DAMAGE IN HIPPURIC ACID:

6.1 Introduction:

Hippuric acid is one of the few naturally occurring amino acids found in the urine of most mammals including humans. The compound was isolated from horse urine in 1829 and its composition determined in 1839 by Liebig. Metabolically produced benzoic acid is rendered harmless by reaction with glycine to give hippuric acid, which is then excreted. The reaction takes place in the liver and may be used as a diagnostic test of liver function.

Although ESR and ENDOR techniques have been extensively used in the study of radiation chemistry of many biologically important amino acids and their derivatives, very little attention has been focussed on the study of radiation damage in hippuric acid. Votinov has reported an ESR study of $\gamma$-irradiated polycrystalline hippuric acid. He has suggested that the radical is formed by the loss of the N-H hydrogen atom. Our results, however, indicate that the stable paramagnetic species formed by room temperature X-irradiation of hippuric acid is the N-Benzoyl-amino-methyl radical which is thought to be the end product of oxidation, caused by ionizing radiation in hippuric acid.

In addition to detecting ENDOR lines due to $\alpha$-protons and weakly coupled distant protons, we have observed $^{14}$N ENDOR lines in this radical. Although the $^{14}$N nucleus is present in many biologically important compounds, its ENDOR spectrum has been detected in only a few of these compounds. This is due in part to its small nuclear magnetic moment and rapid nuclear spin...
lattice relaxation. Box, Freund and Budzinski\textsuperscript{23} were the first to report \(^{14}\text{N}\) hyperfine constants obtained from an ENDOR study of \(x\)-irradiated valine. Rist and Hyde\textsuperscript{107} observed ligand \(^{14}\text{N}\) ENDOR signals from a study of Cu-8-hydroxyquinolinate doped in crystals of phthalimide and 8-hydroxyquinoline. Rustgi and Box\textsuperscript{108} have reported the \(^{14}\text{N}\) hyperfine and quadrupole coupling tensors of the inorganic species, \(\text{NO}_2^+\) and \(\text{NO}_3^-\), formed in \(x\)-irradiated glycylglycine-HNO\(_3\). An isotropic \(^{14}\text{N}\) ENDOR with a low hyperfine coupling has been obtained by Deigen and coworkers\textsuperscript{109} in \(\gamma\)-irradiated glycine single crystals. This unique observation is consistent with the finding that the maximum spin density is found not on the nitrogen, but on the adjacent carbon atom in the radical, \(\text{NH}_3\text{CHCOO}\) proposed by these workers. Nelson, Atwater and Gordy\textsuperscript{110} obtained the \(^{14}\text{N}\) hyperfine and quadrupole coupling tensors from an ENDOR analysis of free radicals trapped in \(\gamma\)-irradiated dimethylglyoxime single crystals. Nitrogen hyperfine and quadrupole data have also been presented by Schweiger and Günthard\textsuperscript{111} from the single crystal ESR and ENDOR of bis-(salicylaldoximato)-Cu(II) doped in bis-(salicylaldoximato)Ni(II). More recently \(^{14}\text{N}\) ENDOR lines have been detected and analyzed by a number of workers from this laboratory in \(x\)-irradiated caffeine hydrochloride dihydrate\textsuperscript{112} and Cu(II) impurity complexes in \(\alpha\)-glycine\textsuperscript{113} and alanine.\textsuperscript{114} A detailed analysis of hyperfine and quadrupole coupling of a ligand nitrogen in the system of Cu(II) in L-alanine has been presented by Calvo, Oseroff and Abache.\textsuperscript{115}

In this chapter we report for the first time \(^{14}\text{N}\) and \(^{1}\text{H}\) ENDOR studies of a stable radical formed by room temperature \(x\)-irradiation of single crystals of hippuric acid.

6.2 Experimental:

Single crystals of hippuric acid were grown by slow evaporation of its
saturated aqueous solution maintained at 30°C. Deuterated crystals were obtained similarly from heavy water solutions. Well developed colorless crystals of approximate dimensions 8x5x3 mm were easily obtained. The crystal morphology is shown in Fig. 11 along with the crystallographic axes. In this figure, the c-axis is perpendicular to the plane of the paper.

There have been three different crystal structure determinations of hippuric acid, including one by neutron diffraction. These reports are in substantial agreement regarding the structure, while differing in their choice of the crystallographic axes. We have used the neutron diffraction results. The crystal is orthorhombic with \( a = 10.586, b = 9.123, c = 8.880 \) Å, \( Z = 4 \) and space group \( P2_12_12_1 \). The four molecules in the unit cell are magnetically equivalent along the crystallographic axes; they are pairwise equivalent in the crystallographic planes; in all other orientations they are distinct. The molecular structure of hippuric acid is depicted in Fig. 12.

The crystallographic axes were located prior to irradiation by x-ray diffraction measurements. The crystals were x-irradiated at room temperature using a Machlett type OEG-60 x-ray tube operating at 40 kV, 20 mA. Optimum ENDOR signals were obtained after about 8 hours of irradiation. On increasing the radiation dose, noticeable line broadening occurred. The irradiated crystals can be stored indefinitely at ambient conditions in closed vials.

ENDOR measurements were made at 77 K with our X-band superheterodyne spectrometer described in chapter four. Spectra were recorded at intervals of 5° as the magnet was rotated about each of the crystallographic axes;
Fig. 11. Crystal morphology and axis system of a single crystal of hippuric acid.
Fig. 12. Molecular structure of hippuric acid.
however, in the vicinity of free proton NMR frequency, spectra were recorded at 2° intervals because of very high spectral density.

6.3 Experimental Results:

A preliminary ESR investigation revealed a considerably anisotropic 1:2:1 triplet along the crystallographic axes (the four molecules in the unit cell are equivalent along these directions), indicating the presence of two nearly equivalent protons strongly interacting with the unpaired electron. ESR spectra of an x-irradiated hippuric acid crystal along the three crystallographic axes are presented in Fig. 13. Each of these triplet lines showed further ill-resolved structures indicating the existence of other weaker hyperfine interactions, however, poor resolution in most orientations, combined with the presence of site splitting, prevented us from reaching any quantitative conclusions regarding the nature of the radical. Examination of the ESR spectra of crystals grown from heavy water indicated that these interactions were not due to exchangeable protons.

A typical ENDOR spectrum of the x-irradiated hippuric acid crystal can be divided into three sections: (i) a high frequency region of 25-55 MHz consisting of highly anisotropic ENDOR lines from two nearly equivalent protons (Fig. 14) (ii) a relatively small region of 10-17 MHz around $\nu_p$, consisting of many closely spaced proton ENDOR lines with considerably smaller anisotropy (Fig. 15) and (iii) a region of 1-7 MHz of $^{14}\text{N}$ ENDOR lines (Figure 16). Analysis of the spectra in regions (i) and (iii) was straightforward; however, the region around $\nu_p$ was crowded and only lines from five different proton couplings could be followed in all the three planes. For proton tensor calculations and graphical presentation $\nu_p$ was
Fig. 13. Typical ESR spectra of X-irradiated single crystal of hippuric acid.
Fig. 14. ENDOR spectra due to the α-protons in the N-benzoylaminomethyl radical in an X-irradiated single crystal of hippuric acid at 77 K.
Fig. 15. ENDOR spectra due to the weakly coupled protons in an X-irradiated single crystal of hippuric acid.
Fig. 16. ESR and ENDOR spectra of $^{14}\text{N}$ in X-irradiated single crystal of hippuric acid for the magnetic field parallel to $a$-axis. Three ENDOR spectra correspond to three different points of saturation of ESR line.
set equal to 13.906 MHz and the observed transition frequencies were corrected accordingly.

Fig. 16 shows the $^{14}$N ENDOR spectra for the magnetic field along the a-axis. The effect of saturating different ESR transitions is illustrated in the three spectra in this figure. The angular variations of proton and nitrogen ENDOR frequencies as measured in three orthogonal planes are depicted in Fig. 17, 18 and 19.

For strongly coupled protons, only the high frequency branch of the ENDOR spectra was used in the analysis, whereas for weakly coupled protons around $v_p$, both $v_+$ and $v_-$ were used. For nitrogen coupling, however, data from all four sets of lines were analyzed.

The experimentally observed frequencies belonging to the same interaction were fitted to the spin Hamiltonian with the help of the computer program discussed earlier. Approximately 120 data points were used to fit each tensor and the rms error was typically less than 30 kHz for the large couplings and 10 kHz for the others. The diagonalized forms of the hyperfine and quadrupole tensors are presented in Tables 4, 5 and 6.

6.4 Discussion:

6.4.1 α-proton Interactions:

Two large couplings observed in the x-irradiated single crystals of hippuric acid are both markedly anisotropic. These coupling tensors clearly indicate a radical structure ending at $-\text{CR}^-$ in which the two protons attached to the carbon atom having large spin density are responsible for the anisotropic couplings. The hyperfine coupling tensors in Table 4 represent these very nearly identical interactions. Small differences between the two tensors can be ascribed to the environmental differences
Fig. 17. Angular variation of the high frequency branch of the ENDOR frequencies of the α-protons in X-irradiated single crystal of hippuric acid.
Fig. 18. Angular variation of the ENDOR frequencies of the weakly coupled protons in X-irradiated hippuric acid. The experimental points are omitted from the figure for the sake of clarity. Data points were collected at 2° intervals and excellent agreement was obtained with the calculated values.
Fig. 19. Angular variation of $^{14}$N ENDOR frequencies in an X-irradiated single crystal of hippuric acid.
### Table 4

**Hyperfine Coupling Tensors for the α-Protons of N-Benzoylaminoethyl Radical**

<table>
<thead>
<tr>
<th>Proton</th>
<th>Coupling (MHz)</th>
<th>Direction Cosines(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Principal Value(^1)</td>
<td>Isotropic</td>
</tr>
<tr>
<td>(^2\text{H}_1)</td>
<td>-24.22</td>
<td>-51.37</td>
</tr>
<tr>
<td></td>
<td>-48.70</td>
<td>+ 2.67</td>
</tr>
<tr>
<td></td>
<td>-81.20</td>
<td>-29.83</td>
</tr>
<tr>
<td>(^2\text{H}_2)</td>
<td>-25.46</td>
<td>-51.17</td>
</tr>
<tr>
<td></td>
<td>-48.98</td>
<td>+ 2.19</td>
</tr>
<tr>
<td></td>
<td>-79.06</td>
<td>-27.89</td>
</tr>
</tbody>
</table>

\(^1\) Sign of the principal values is assumed on the basis of theory. Uncertainty in the principal value is ±0.03 MHz.

\(^2\) The four different sign combinations refer to the four inequivalent sites in the crystal.
of the two protons in the crystal. The neutron diffraction results indicate unequal C-H bond lengths for the two hydrogen atoms in the undamaged crystal. In x-irradiated N-acetylglycine Box et al. observed similar differences for the two nearly equivalent protons.

In a planar $-\text{CH}_2$ radical the hyperfine tensors of both the protons should have a common principal direction, which is perpendicular to the plane containing the three atoms. This was verified from the fact that the angle between the two intermediate couplings of $\alpha$-protons was found to be $\sim 2^\circ$. For the tensors reported in Table 4, the directions of the smallest principal values make an angle of $125.8^\circ$ with each other. From theory these directions are expected to be along the C-H bond directions. These observations lead us to the conclusion that the radical under investigation has a planar $-\text{CH}_2$ group, with a fairly large spin density on the terminal carbon $\pi$ orbital. The structure of the radical and numbering of atoms are shown in Fig. 20.

Now, such a radical structure is possible if the carboxyl group is homolytically cleaved from a molecule of hippuric acid. The most probable pathway leading to such a process could be deprotonation of the oxidized hippuric acid followed by the loss of a molecule of carbon dioxide. Supporting evidence for the radical structure comes from the following observations.

From chemical analysis of amino acids irradiated in the dry state, Gottschall and Tolbert concluded that oxidation of glycine and $\alpha$-alanine causes decomposition accompanied by the production of carbon dioxide. Recent studies on the oxidation products in x-irradiated carboxylic acids, amino acids and N-acetylglycine suggest that the overall process
Fig. 20. Structure of N-Benzoylaminomethyl radical.
initiated by oxidation is decarboxylation.

From the familiar McConnell relation \(^65\), we calculate a spin density of 0.809 on the \(\pi\)-orbital of the \(\alpha\)-carbon atom using a Q-value of -63.5 MHz. The experimental spin densities along with the spin densities obtained from INDO-MO calculations are given in Table 7. A spin density of 0.842 on the \(\alpha\)-carbon was obtained from INDO-MO calculations on the N-benzoylaminomethyl radical. The agreement can be deemed excellent considering INDO approximations. Thus we are almost certain about the identity of the radical.

If the radical was formed by the dissociation of the N-H bond as suggested by Votinov\(^6\), the radical would have a methylene group with a large spin density on the adjacent nitrogen atom. These two protons of the methylene group (\(\beta\)-protons) should then have fairly large isotropic hyperfine coupling. Moreover, large spin density on the \(\pi\)-orbital of the nitrogen atom would give rise to large anisotropic coupling with the \(^{14}\N\) nucleus. Our experimental results do not support these possibilities.

6.4.2 \(^{14}\N\) Hyperfine and Quadrupole Interactions:

Selective saturation study on the ESR line-shape showed that all the four lines in Fig. 16 are due to coupling of \(^{14}\N\). The results reported in Table 5 show that the \(^{14}\N\) hyperfine coupling tensor is fairly isotropic in character. This unique observation is consistent with the finding that maximum spin density is found not on nitrogen, but on the adjacent carbon atom. A similar observation has been noted by Deigen et al.\(^{109}\) in \(\gamma\)-irradiated glycine. The nearly isotropic coupling is probably caused by the small spin density induced in the \(s\)-orbital of nitrogen by the unpaired electron in the \(\pi\)-orbital of the carbon atom, through the spin


TABLE 5

NITROGEN HYPERFINE AND QUADRUPOLE COUPLING TENSORS IN N-BENZOYLAMINO-METHYL RADICAL

<table>
<thead>
<tr>
<th>Principal Values (MHz)</th>
<th>Direction Cosines $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Hyperfine Coupling $A_{11}$</td>
<td>$-7.38 \pm 0.01$</td>
</tr>
<tr>
<td>$A_{22}$</td>
<td>$-8.77 \pm 0.01$</td>
</tr>
<tr>
<td>$A_{33}$</td>
<td>$-9.44 \pm 0.01$</td>
</tr>
<tr>
<td>Quadrupole Coupling $\chi_{11}$</td>
<td>$-0.843 \pm 0.005$</td>
</tr>
<tr>
<td>$\chi_{22}$</td>
<td>$+0.582 \pm 0.005$</td>
</tr>
<tr>
<td>$\chi_{33}$</td>
<td>$+0.261 \pm 0.005$</td>
</tr>
</tbody>
</table>

$C_2-N$ bond direction: $\begin{pmatrix} 0.996 \\ 0.064 \\ -0.058 \end{pmatrix}$

$^1$The four different sign combinations refer to the four inequivalent sites in the crystal.
polarization mechanism.

The unpaired electron density in the π-orbital of nitrogen can be estimated from the anisotropy of the nitrogen hyperfine coupling. The $^{14}$N hyperfine tensor can be treated as approximately axially symmetric with the direction of the positive dipolar component parallel to the π-orbital. The deviation from axial symmetry is probably caused by the influence of the large spin density on the adjacent carbon atom. The dipolar part of the tensor has the form $-B_0$, $-2B_0$, $2B_0$, where $B_0$ is related to the unpaired electron density on the π-orbital of nitrogen. The value of $B_0$ in the present case can be taken as the mean of two smaller dipolar values i.e. 0.58 MHz. The theoretical value$^{125}$ of $B_0$ for unit spin density is 48 MHz. The spin density on the π-orbital of nitrogen is calculated to be 0.012. This is in excellent agreement with the value of 0.013 calculated from the isotropic part of the N-H proton coupling using McConnell relation.$^6$ (Table 7)

From symmetry considerations the principal axes of the quadrupole tensor are expected to coincide with those of the hyperfine coupling tensor. Inspection of table 5 shows that the principal element of hyperfine ($A_{11}$) and quadrupole ($\chi_{11}$) tensors are parallel to π- orbital of nitrogen within $\approx 7^\circ$. $A_{22}$ and $\chi_{22}$ are parallel to C$_2$-N bond direction in the undamaged crystal within $\approx 7^\circ$ and $A_{33}$ is parallel to $\chi_{33}$ within $\approx 2^\circ$.

A rigorous interpretation of the quadrupole coupling would be very difficult because of the complex dependence of the field gradient at the nitrogen nucleus on all the extra nuclear charges. However, reasonable results have been obtained using an approximate method developed by Townes and Dailey$^{78-80}$ and later extended by Gordy.$^{81-83}$ In its simplest form,
the principal values of the theoretical quadrupole coupling tensor are given by:

\[
\begin{align*}
\chi_{11} &= \left[ n_x - \frac{1}{2}(n_y + n_z) \right]eqQ \\
\chi_{22} &= \left[ n_y - \frac{1}{2}(n_z + n_x) \right]eqQ \\
\chi_{33} &= \left[ n_z - \frac{1}{2}(n_x + n_y) \right]eqQ
\end{align*}
\] (6-1)

where the symbols have their usual meanings and \( n_x, n_y, n_z \) are the occupation numbers of the 2p orbitals of the nitrogen atom. Employing the electron densities obtained from our INDO-MO calculations and using a value of \(-10 \text{ MHz}\) for \( eqQ \) as suggested by Gordy and Cook, we obtained 2.35, 3.16 and \(-5.51 \text{ MHz}\) for the principal values of the quadrupole coupling tensor. These values are much larger than the experimental values of 0.261, 0.582 and \(-0.843 \text{ MHz}\) (Table 5). Since the least squares fitting program for the assigned transitions converged with rms error of less than 5 kHz for 120 input data points, the disagreement must have resulted from the assumptions leading to equations (6-1). Similar disagreement has also been reported in the hydrogen addition radical trapped in an X-irradiated single crystal of caffeine hydrochloride dihydrate.112

The signs of the principal values of the quadrupole tensor relative to those of the hyperfine coupling tensor were determined by the method outlined by Cook.126 The energy level diagram shown in Fig. 3 is drawn for the case in which \( a \) and \( P \) were both positive. If the low field ESR line, corresponding to the \( |{-\frac{1}{2}, 0}> \text{ to } |{\frac{1}{2}, 0}> \) transition is saturated, then the two observed ENDOR transitions have frequencies given by:
\[ \Delta 1 = \frac{a}{2} + \nu_N - P \]  
\[ \Delta 4 = \frac{a}{2} - \nu_N + P \]  

If, however, the relative sign of \( a \) and \( P \) is reversed, saturation of the same ESR line would give rise to two ENDOR transitions with frequencies:

\[ \Delta 1 = \frac{a}{2} + \nu_N + P \]  
\[ \Delta 4 = \frac{a}{2} - \nu_N - P \]

As illustrated in fig. 16, saturating different portions of the ESR signal resulted in dramatic intensity differences of the various \(^{14}\text{N} \) ENDOR lines. Since by saturating the low field ESR line, the two outermost lines become prominent, the hyperfine and quadrupole couplings have opposite signs along \( a \)-axis. Similar observations along other directions led to the choice of signs indicated in Table 5.

6.4.3 Weakly coupled Protons:

From among the large number of closely spaced ENDOR signals in the vicinity of \( \nu_p \), only five could be followed unambiguously in all the three planes. The results obtained from the analysis of these lines are presented in Table 6. The assignment of these tensors to specific protons in the crystal was accomplished on the basis of direction cosines, symmetry considerations and comparison of INDO-MO results. In support of our assignment of ENDOR lines to exchangeable protons, we carried out a few ENDOR measurements on partially deuterated samples. Figure 21 shows a typical ENDOR spectrum of \( x \)-irradiated hippuric acid single crystals grown from heavy water for the magnetic field parallel to the \( a \)-axis. A consistent reduction in the intensity of lines \( A \) and \( B \) relative to other prominent lines
### TABLE 6

HYPERFINE COUPLING TENSORS FOR THE WEAKLY COUPLED PROTONS IN N-BENZOYLAMINO-METHYL RADICAL

<table>
<thead>
<tr>
<th>Proton</th>
<th>Coupling (MHz)</th>
<th>Direction Cosines&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Principal Value&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Isotropic</td>
</tr>
<tr>
<td>(B) N-H</td>
<td>σ: +0.45</td>
<td>-1.00</td>
</tr>
<tr>
<td></td>
<td>π: +3.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓: -6.47</td>
<td></td>
</tr>
<tr>
<td>N-H bond direction:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(D) H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>σ: 2.25</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td>π: 4.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓: 1.34</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;4&lt;/sub&gt;-H&lt;sub&gt;4&lt;/sub&gt; bond direction:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E) H&lt;sub&gt;6&lt;/sub&gt;</td>
<td>σ: 1.05</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td>π: 3.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓: 4.69</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;6&lt;/sub&gt;-H&lt;sub&gt;6&lt;/sub&gt; bond direction:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C) H&lt;sub&gt;8&lt;/sub&gt;</td>
<td>σ: 1.83</td>
<td>2.88</td>
</tr>
<tr>
<td></td>
<td>π: 3.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓: 2.94</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt;-H&lt;sub&gt;8&lt;/sub&gt; bond direction:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A) H.B.</td>
<td>σ: -1.97</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>π: -1.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓: +3.44</td>
<td></td>
</tr>
<tr>
<td>N-H...O&lt;sub&gt;3&lt;/sub&gt; bond direction:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Signs are assumed on the basis of INDO spin densities, except for the hydrogen bonded (H.B.) proton. Uncertainty in the principal value is ±0.01 MHz.

<sup>2</sup>The four different sign combinations refer to the four inequivalent sites.
Fig. 21. ENDOR spectrum in the vicinity of $\nu_p$ from an X-irradiated single crystal of hippuric acid grown from heavy water for the magnetic field parallel to a-axis.
in the spectrum of deuterated sample suggests that they belong to exchangeable protons.

The ENDOR line B is fairly anisotropic and is readily assigned to the coupling of the N-H proton. A relatively small spin density on the nitrogen atom induces a small negative isotropic component. The correspondingly large anisotropy of the tensor arises from the large spin density on the $\alpha$-carbon. From the isotropic part of the tensor, a spin density of 0.013 is calculated on the $\pi$-orbital of nitrogen, from McConnell relation using a Q-value of 78.5 MHz. This is in excellent agreement with the spin density calculated from the anisotropy of the nitrogen coupling. The direction of the intermediate component of the tensor is within 5° of the common principal direction of the $\alpha$-protons. However, the direction of $\sigma$-component of the tensor does not match very well with the N-H bond direction calculated from crystal structure. The disagreement goes up to the extent of ≈18° which might have resulted from the effect of the large spin density on $\alpha$-carbon. That the tensor is associated with an exchangeable proton was verified from the spectrum of the deuterated sample (figure 21).

Out of five phenyl protons, only three could be identified in our study. In the frequency range of 10-17 MHz around $\nu_p$, there were so many overlapping lines, that it was hard to pick up ENDOR lines of the weakly coupled metaprotons. Besides, an attempt to study all the lines does not shed any additional light on the electronic structure of the radical. Therefore, we did not make any further attempt to analyze these lines. Interactions C, D and E are assigned to the para-and two ortho- protons on the basis of their direction cosines and the larger spin densities expected at these positions as compared to the meta positions. Inspection
of tensors $H_4$, $H_6$ and $H_8$ in Table 6 reveals that they all have one principal axis in common which is perpendicular to the phenyl ring. The $\sigma$-components of the tensors C, D and E are parallel to $C_8$-$H_8$, $C_4$-$H_4$ and $C_6$-$H_6$ bonds in the undamaged crystal within $3^\circ$ and hence assigned to ortho-(H$_8$,H$_4$) and para-(H$_6$) protons respectively. Furthermore, the direction of the $\sigma$-component of para proton is found to be close to $\sim 120^\circ$ with respect to those of two ortho protons within experimental error. From the isotropic parts of these tensors, spin densities of -0.046 and -0.043 are calculated for the $\pi$-orbitals of the para- and the ortho-positions, respectively, from McConnell relation with a Q value of 63.5 MHz. These are in good agreement with the results of INDO calculations (cf. Table 7).

The remaining hyperfine coupling tensor (A) in Table 6 is assigned to an intermolecular hydrogen bonded proton. That the tensor belongs to an exchangeable proton was further verified by the inspection of spectrum of a partially deuterated sample (cf figure 21). Just as the ENDOR line B, the intensity of line A is also reduced relative to other strong lines. This tensor is nearly dipolar in nature with an isotropic component of -0.07 MHz. The axial symmetry of this tensor is also obvious. These characteristics are typical of hydrogen bonded protons. Of the two possible hydrogen bonded protons in hippuric acid, N-H proton of the neighbouring molecule hydrogen bonded to the amide oxygen is thought to be responsible for the interaction reported here, on the basis of the direction cosines. This is consistent with our observation that the carboxyl group is removed on radical formation, thus the second hydrogen bond which was present in the undamaged molecule is no longer present in
<table>
<thead>
<tr>
<th>ATOM</th>
<th>C₁</th>
<th>N</th>
<th>C₂</th>
<th>O</th>
<th>C₃</th>
<th>C₄₈</th>
<th>C₅₇</th>
<th>C₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>ρπ (INDO)</td>
<td>0.842</td>
<td>0.094</td>
<td>-0.054</td>
<td>0.124</td>
<td>0.007</td>
<td>-0.008</td>
<td>0.004</td>
<td>-0.007</td>
</tr>
<tr>
<td>ρπ (obs.)</td>
<td>0.809</td>
<td>0.013</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.043</td>
<td>-</td>
<td>-0.046</td>
</tr>
</tbody>
</table>
the radical. The calculated N-H ... O bond direction agrees with the
direction of σ-component within ±6°.

6.5 Conclusion:

The hyperfine tensors for two α-protons, five weakly coupled
protons and hyperfine as well as quadrupole tensors for $^{14}\text{N}$ have been
determined. Our results demonstrate that the superior resolution of
the ENDOR technique is very useful in the study of radicals trapped in
irradiated single crystals of organic compounds. In the present study
the ESR spectrum only exhibited a partially resolved triplet splitting in
an approximate ratio of 1:2:1 indicating the presence of two equivalent
protons attached to a carbon atom with large spin density. Detailed
structural information about the radical could not be obtained from the
ESR study, because important intramolecular hyperfine splittings were
buried within the ESR linewidths. Using the ENDOR technique, the electronic
structure of the radical along with its identification could be unambiguously
accomplished.

$^{14}\text{N}$ ENDOR lines belonging to such a low hyperfine coupling have been
reported only once before in the free radical trapped in the γ-irradiated
glycine single crystal. For the radical analogous to ours in N-acetyl-
glycine, Box and coworkers$^{119}$ did not observe $^{14}\text{N}$ ENDOR. This is not
particularly surprising because detection of $^{14}\text{N}$ ENDOR signals is often
hampered by the rapidity of its spin lattice relaxation and the small size
of its nuclear magnetic moment. Often the $^{14}\text{N}$ signals lie below the
observation range of most ENDOR spectrometers.

In a survey of radiation effects on organic compounds, it has been
established$^{119,120,123,124}$ that the carboxyl group is the site of the
primary oxidation with subsequent decarboxylation. However, it has been reported that the decarboxylated radical is usually unstable at room temperature, and decays with hydrogen abstraction processes. The present study establishes that in hippuric acid, the decarboxylated radical is stable at room temperature. A similar observation was made in the case of creatine monohydrate containing only one carboxyl group.\textsuperscript{130} In a structurally related compound, N-acetylglucose, the decarboxylated radical is stable only at low temperatures\textsuperscript{119,131}, indicating that phenyl group plays a critical role in the unusual stability of the decarboxylation radical in hippuric acid.
CHAPTER SEVEN

ENDOR STUDY OF X-IRRADIATED SINGLE CRYSTALS OF N-ACETYLGLYCINE - A REINVESTIGATION

7.1 Introduction:

Radiation induced radicals in proteins have been studied for a long time using the electron spin resonance (ESR) technique. In the first ESR papers by Gordy, Ard and Shields and Gordy and Shields, the protein spectra were divided into the sulfur pattern due to a cysteine sulfur radical and a doublet resonance. The nature of the doublet has been discussed thoroughly and there have been lot of controversies over the identity of the radical. The original suggestion that the unpaired electron was localized on an oxygen atom in a hydrogen bond was replaced by a model where the unpaired spin density is localized mainly in a \( \pi \)-orbital on an \( \alpha \)-carbon atom in the protein back bone.

![Diagram of amino acid structure]

A sensible approach to the study of radiation damage in exceedingly complex protein molecule begins with an investigation of simpler model compounds. The main objective of these studies is to examine the effects of ionising radiation on the peptide linkage (\(-\text{CO-NH}\)-) that joins amino acids together in proteins. We believe that these studies also will have
some bearing with regard to the processes taking place in irradiated proteins.

N-acetylglycine is one of the simplest compounds with a peptide bond. When irradiated in powders, the ESR absorption shows a doublet which is approximately of the same spacing as the doublets of proteins. In subsequent years, the irradiated single crystal of N-acetylglycine has been the subject of several ESR and ENDOR studies. In a recent ENDOR study, Box and coworkers identified three different radicals in X-irradiated single crystals of N-acetylglycine at 4.2K, two of which were reduction products and the other an oxidation product. The oxidized species decompose losing a proton and a molecule of carbon dioxide; the two reduced species are formed by electron addition to the peptide oxygen and the carboxyl group, the latter product occurring in two conformations.

In this chapter we deal with the stable radical produced by room temperature X-irradiation of N-acetylglycine. The room temperature radical has been identified by several groups of workers as

\[ \text{CH}_3\text{-CO-NH-CH-COOH} \]

Miyagawa and coworkers reported the \( \alpha \)-proton tensor for this radical in an ESR study. Mangiaracina observed a small nitrogen hyperfine splitting in the ESR spectra of deuterated acetylglycine. Later Piazza and Patten detected the ENDOR lines due to a rotating methyl group in the same radical. In a detailed ESR observations and MO calculations on the same radical, Saxebo et al. found that the unpaired spin density could migrate through the peptide group even upto \( \delta \)-proton. The Sextet pattern of ESR
spectra of N-acetylglycine single crystals grown from heavy water was explained to be due to the interaction of the unpaired electron with the methyl protons and the nitrogen in the peptide bond. Sinclair and Codella in a detailed variable temperature ESR study, established the pathways leading to the stable radical from the initial radicals produced at low temperatures.

In a more recent 'negative' ENDOR study Helms, Suzuki and Miyagawa (hereafter referred to as HSM) raised doubts about the structure of the radical produced by room temperature X-irradiation of N-acetylglycine. They proposed that the radical is essentially a cation:

\[
\text{CH}_3\text{-CO-NH-CH-COOH} \quad \text{III}
\]

They also asserted that the hyperfine coupling due to the methyl protons of the radical was identical to the methyl proton coupling from the neighboring molecule, which in the undamaged crystal is related by a centre of inversion. These proposals were based on intensity measurements of the ENDOR lines. These workers also detected ENDOR lines due to several weakly coupled protons which were not observed in the earlier ENDOR work of Piazza and Patten. The hyperfine tensors reported for these weakly coupled protons have considerable isotropic parts, indicating the presence of non-negligible spin densities at the respective atoms. Based on these observations, they concluded that the wave function of the unpaired electron extends significantly over the neighboring molecules.

We started ENDOR investigations on this radical in search of \(^{14}_N\) ENDOR lines, after completing a detailed \(^{14}_N\) and \(^1_H\) ENDOR study of the radiation damage in a related compound, hippuric acid (N-benzoyleglycine). Although
we were unsuccessful in our attempt to find $^{14}\text{N}$-ENDOR signals, we realized
that a reinvestigation of the $^1\text{H}$-ENDOR of this radical was in order. This
was due in part because unlike the ENDOR spectra reported by Piazza and
Patten, we obtained many well-resolved lines in the vicinity of the
free proton N.M.R. frequency. We also observed several lines due to the
$\alpha$-proton coupling, instead of the expected single line. These preliminary
findings prompted us to undertake a detailed investigation. In the course
of this study we have been able to gather sufficient evidence to propose
a definite picture of the radiation damage in N-acetylglycine, at variance
with the 'negative' ENDOR results of HSM.

7.2 Experimental:

Single crystals of N-acetylglycine were grown by slow evaporation of
its saturated aqueous solution at room temperature. Deuterated crystals
were grown in a similar fashion from heavy water. Well-developed crystals
of approximate dimensions 8x5x4 mm were easily obtained. $^{15}\text{N}$-acetylglycine
was prepared by condensing glycine ($^{15}\text{N}$-99\%) with acetic anhydride in the
presence of a small amount of water. The crystals were colorless and
exhibited a very regular external form which is shown in figure 22 together
with the orthogonal reference system used in these experiments.

The crystal structure of N-acetylglycine is known to be monoclinic
with a tetramolecular unit cell of dimensions $a = 4.86$, $b = 11.54$, $c = 14.63\text{Å}$
with $\beta = 138.2^\circ$ and space group $\text{P2}_1/c$. The four molecules in the unit cell
are pairwise equivalent, and therefore, only two sites can be distinguished
by ESR or ENDOR spectroscopy. When the magnetic field is either parallel
or perpendicular to the b-axis, only one site can be observed. All measure-
ments were carried out in the reference coordinate system $a^*$, $b$, $c$ ($a^* = bxc$).
Fig. 22. Crystalline form and crystallographic axes of N-acetylglycine.
The molecular structure of N-acetylglycine is shown in figure 23, along with the numbering system.

The crystals were X-irradiated at room temperature using the device described in the earlier chapters. The optimum concentration of radicals was obtained after about two hours of irradiation. ENDOR measurements were made at 77K with our X-band superheterodyne spectrometer described previously, using an f.m. frequency of \( \gamma \)1.1 kHz. Spectra were recorded at intervals of 2° in the vicinity of \( \nu_p \) and at 5° intervals for the \( \alpha \)-proton lines.

Preliminary ESR measurements were made on a Varian E-3 spectrometer. Second derivative ESR spectra were recorded on a home-built ESR instrument using 50 kHz field modulation and detecting the second harmonic.

No ENDOR lines due to the hyperfine coupling of the unpaired electron with the nitrogen atom were observed in our study. All the observed lines arise from interactions with protons. The experimentally observed frequencies belonging to the same proton coupling were fitted to the spin Hamiltonian with the least-squares-adjustment program LSF. Approximately 120 data points were used to fit each tensor and the rms error was typically less than 20 kHz. For the \( \alpha \)-proton tensor only the high frequency branch of the ENDOR spectra was used in the analysis, while both the branches were used for the weakly coupled protons.

7.3 Results and Discussion:

7.3.1 Experimental Results:

Although the hyperfine coupling tensor for the \( \alpha \)-proton interaction has been reported from an ESR study \(^{28}\), we decided to redetermine it from our ENDOR data in order to obtain more accurate results. When we recorded
Fig. 23. Molecular structure and numbering system of N-acetylglycine.
the ENDOR spectrum in the frequency region expected for the \( \alpha \)-hydrogen coupling, we discovered that, instead of the expected single line for each distinguishable crystal site, there were three to four closely spaced lines exhibiting similar angular variations. Figure 25 shows the high frequency branch of the \( \alpha \)-proton ENDOR lines for an orientation where the magnetic field makes five degrees with the \( b \)-axis in the \( bc \)-plane. The separation between the two groups of lines is due to site splitting. When the magnetic field is oriented along the \( b \)-axis, the two sets of lines merge together to yield a closely spaced ill-resolved four-line spectrum.

From the doublet pattern in the ESR spectrum (figure 24), it is evident that the unpaired electron is strongly coupled only to one proton. This apparent contradiction between the ESR and ENDOR observations prompted us to pursue the study further. Of these four ENDOR lines, we have been able to follow only three in all the three planes. For simplicity, the angular variation of the high frequency branch of the ENDOR frequencies belonging to one line only is shown in figure 26. The diagonalized forms of the \( \alpha \)-proton hyperfine tensors for these three ENDOR lines are presented in Table 8. It is evident from these data that these small differences could not be resolved in the ESR spectra.

Figure 27 shows the ENDOR spectrum in the vicinity of \( v_p \) for the magnetic field parallel to the \( \alpha^* \)-axis. On comparing this spectrum with Fig. 1 of HSM\(^{29}\), one notices several salient differences between the two, the foremost among them being the better resolution of our spectrum. The broad line referred to as \( \text{Hc}_2 \) and \( \text{Hc}'_2 \) by HSM\(^{29}\) and assigned to two equivalent methyl groups by them, is clearly resolved in our spectrum. These two lines along with the one designated by \( \text{Hc}_1 \) have very similar angular variations, analogous to the three lines due to the \( \alpha \)-proton
Fig. 24. First derivative ESR spectrum of X-irradiated single crystal of N-acetylglucine for the magnetic field parallel to $a^*$-axis.
Fig. 25. High frequency branch of the ENDOR spectrum due to the α-proton interaction in X-irradiated single crystal of N-acetylglycine for the magnetic field making 5° with b-axis in the bc-plane.
Fig. 26. Angular variation of the high frequency branch of the ENDOR frequencies for the α-proton in an X-irradiated single crystal of N-acetylglycine.
Fig. 27. ENDOR spectra in the vicinity of up from an X-irradiated single crystal of N-acetylglycine for the magnetic field parallel to a*-axis: (a) crystal grown from H₂O (b) crystal grown from D₂O.
<table>
<thead>
<tr>
<th>PROTON</th>
<th>Coupling (MHz)</th>
<th>Direction Cosines $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Principal Value $^1$</td>
<td>Isotropic</td>
</tr>
<tr>
<td>aH(A)</td>
<td>-23.65</td>
<td>+25.59</td>
</tr>
<tr>
<td></td>
<td>-48.64</td>
<td>-49.24</td>
</tr>
<tr>
<td></td>
<td>-75.42</td>
<td>-26.18</td>
</tr>
<tr>
<td>aH(B)</td>
<td>-23.31</td>
<td>+25.60</td>
</tr>
<tr>
<td></td>
<td>-48.47</td>
<td>-48.91</td>
</tr>
<tr>
<td></td>
<td>-74.96</td>
<td>-26.05</td>
</tr>
<tr>
<td>aH(C)</td>
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<td>+25.38</td>
</tr>
<tr>
<td></td>
<td>-48.02</td>
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</tr>
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<td></td>
<td>-74.44</td>
<td>-25.90</td>
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TABLE 8 (cont'd)

<table>
<thead>
<tr>
<th>PROTON</th>
<th>Coupling (MHz)</th>
<th>Direction Cosines</th>
</tr>
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<tr>
<td></td>
<td>Principal Value</td>
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</tr>
<tr>
<td></td>
<td>(MHz)</td>
<td></td>
</tr>
<tr>
<td></td>
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<tr>
<td>CH₃(A)</td>
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<td>- 8.69</td>
</tr>
<tr>
<td></td>
<td>-10.94</td>
<td>- 2.25</td>
</tr>
<tr>
<td>CH₃(B)</td>
<td>- 6.98</td>
<td>+ 1.33</td>
</tr>
<tr>
<td></td>
<td>- 7.37</td>
<td>- 8.31</td>
</tr>
<tr>
<td></td>
<td>-10.58</td>
<td>- 2.27</td>
</tr>
<tr>
<td>CH₃(C)</td>
<td>- 6.81</td>
<td>+ 1.31</td>
</tr>
<tr>
<td></td>
<td>- 7.15</td>
<td>- 8.12</td>
</tr>
<tr>
<td></td>
<td>-10.39</td>
<td>- 2.27</td>
</tr>
</tbody>
</table>

1 Sign of the principal values is assumed on the basis of theory. Uncertainty in the principal value is ±0.02 MHz.

2 The two sign combinations chosen consistently relate on distinguishable crystal site to the other.
interaction. In some orientations a fourth line is evident, but it could not be followed throughout. The hyperfine coupling tensors obtained from the analysis of these three lines are included in Table 8.

A careful examination of figure 27 reveals that most of the lines designated as $Hx_1-Hx_6$ show similar splittings. HSM$^{29}$ reported that the splittings observed for $Hx_1$ and $Hx_4$ by them was due to a slight deviation of the orientation from the $a^*$-axis. However, we find that the number of lines double as we rotate the magnetic field direction away from the axis, thus confirming that the splitting seen in the spectrum along $a^*$-axis is not due to misalignment of the crystal.

In our spectrum the lines due to $Hx_1$ in fact are resolved into three lines along $a^*$-axis, and in some other orientations even a fourth line appears. Besides the lines due to $Hx_1$ and $Hx_4$, all the other lines also show similar splittings in most orientations, although some of them appear as single lines in the spectrum shown in figure 27.

The angular variation of the ENDOR frequencies in the vicinity of $vp$ is shown in figure 28. Although we have collected data at two degree intervals in the three planes, figure 28 shows data points only at every 10°, this display being chosen for the sake of clarity. On comparing this diagram with figure 2 of HSM$^{29}$, it is apparent that at least in the ac-plane the variation of most of the ENDOR lines has been misassigned by them; this no doubt was due to the complex angular variation of these lines and the need for many data points. It is also to be noted that, except for the lines attributed to the methyl group, the ENDOR lines of all the weakly coupled protons cross $vp$ at least in one plane, thus indicating that not all the principal values of their hyperfine tensors would be of the same
Fig. 28. Angular variation of the ENDOR frequencies of the weakly coupled protons in an X-irradiated single crystal of N-acetylglycine.
sign. The hyperfine tensors obtained from these data are presented in Table 9. For the sake of brevity only one tensor per proton is included in this table although two or three were determined for each.

Although HSM$^{29}$ reported partial or complete tensors for several additional non-exchangeable protons, we have chosen not to analyze those closely spaced ENDOR lines in the immediate vicinity of $v_p$. We feel that such an analysis would not yield reliable data. Moreover, study of these extremely weak couplings, would not shed any additional light on the radical structure or the nature of the wavefunction of the unpaired electron.

7.3.2 Discussion:

In order to explain the appearance of more ENDOR lines than expected from the crystal symmetry, several possibilities were considered. Misalignment of the crystal could not give rise to the additional splittings because of the presence of the inversion centre in the monoclinic unit cell. Twinning of the crystal was ruled out by checking several crystals; all of them gave the same number of ENDOR lines with the same intensity ratio. The splitting could occur if the symmetry of the crystal was changed on cooling to 77 K, where our ENDOR measurements were made. However, when we measured the spectra at room temperature, essentially the same type of spectra was observed except for poorer signal to noise ratio. Therefore, any temperature effect can safely be ruled out as the cause of the observed splittings.

Another likely explanation is that the splittings arise because of very small differences in the precise geometrical shape or the orientation of the radical in the crystal lattice. Horsfield$^{140}$ et al. observed a
similar phenomenon in an ESR study of γ-irradiated single crystals of glutaric acid and postulated several reasons for the effect. They could not obtain sufficient experimental evidence to establish the mechanism producing the splitting. Later Kwiram re-investigated this system using the ENDOR technique. On the basis of the weakly coupled proton interactions, he postulated that the splitting resulted from two different conformations of the same radical species, differing in the orientation of the carbonyl group relative to the skeleton of the radical

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{C} & \quad \text{C} \\
\text{O} & \quad \text{H} \\
\end{align*}
\]

In our ENDOR study of X-irradiated single crystal of phenazine (chapter 5) we have observed two radicals which were chemically identical but differing in their precise geometrical shape. It is not uncommon to find radicals produced by ionizing radiation occurring in more than one conformation. Based on these examples it is reasonable to assume that the splittings we observe in N-acetylglycine are caused by very small conformational differences of the same radical.

Now, if the splitting of the ENDOR lines of the α-proton and the protons designated by \( H_x^1 - H_x^6 \) arises from small conformational differences of the same radical, it would seem unlikely that the lines due to the methyl protons are split for a different reason. Therefore, we have assigned the three lines at \( \sim 17.4 \text{ MHz} \) in figure 27 to the methyl protons.
The previous assignment of these lines by HSM\textsuperscript{29} to two methyl groups and a proton in the vicinity of the radical was based on intensity measurements of the ENDOR lines. Our contention is that the intensity measurement of these lines would be unreliable because the lines are not well separated. Our results show that the line they measured as a single line for Hc\textsubscript{2} and Hc\textsubscript{1} is in fact a composite of two closely spaced lines separated by \(\sim 100\) kHz.

In support of their assignment, HSM presented the second derivative ESR spectrum of a crystal grown from heavy water. They analyzed the multiplet structure in the ESR spectrum (figure 3 of HSM) as eight equally spaced lines with binomial intensity ratio. The eight lines, they argued, arise from seven equally coupled protons: six methyl protons and another in the vicinity of the radical. Fig. 29(a) shows the ESR spectrum under identical conditions with a stick diagram immediately beneath the spectrum. We strongly believe that the multiplet structure in the spectrum can be analyzed as six equally spaced lines with intensity ratio 1:4:7:7:4:1. Such a six line spectrum is possible if we assume that the hyperfine coupling of the three protons of a rotating methyl group is approximately equal to the coupling due to the \(^{14}\text{N}\) nucleus. HSM probably missed the important paper of Saxebol\textsuperscript{125} et al. who have satisfactorily analyzed the ESR spectra on the assumption that the sextet pattern arises from hyperfine interaction with the methyl protons and the \(^{14}\text{N}\) nucleus.

In order to confirm that the additional lines in the ESR spectrum are due to \(^{14}\text{N}\) interaction and not a second methyl group and an extra proton, we prepared single crystals of \(^{15}\text{N}\) labelled acetylglycine from heavy water. The ESR spectrum for such a crystal under identical
Fig. 29. Second derivative ESR spectra of deuterated N-acetylglycine for the magnetic field parallel to $a^*$-axis: (a) $^{14}$N-acetylglycine (b) $^{15}$N-acetylglycine.
conditions is shown in fig. 29(b). Now, if the additional lines were due to protons, no change should be noticed between the two spectra. However, one notices that the six line spectrum has changed to a poorly resolved five line spectrum. $^{15}\text{N}$ has a nuclear spin of $\frac{1}{2}$ and its magnetic moment is slightly higher than that of $^{14}\text{N}$ with $I=1$. The stick diagram beneath fig. 29(b) is calculated on the assumption that $a(CH_3)^{14}\text{N}$, and taking into account the difference in the magnetic moments of $^{14}\text{N}$ and $^{15}\text{N}$. It is to be noted that the ESR spectra of Fig. 29 are in very good agreement with the calculated ones. Based on these observations we can safely rule out a second methyl group and a proton in the vicinity of the radical as being responsible for the additional structure in the ESR spectrum. In an ESR study of irradiated acetylglucose, Mangiaracina observed well resolved $^{14}\text{N}$ hyperfine splitting in a crystal where the methyl group was deuterated. This observation supports our analysis of the ESR spectra.

In a detailed variable temperature ESR study of X-irradiated N-acetylglucose, Sinclair and Codella have established that the room temperature radical is produced from an undamaged molecule by a hydrogen atom transfer to radicals stable only at lower temperatures. The overall scheme, they have suggested for acetylglucose is:

$$\text{CH}_3\text{CONDCH}_2\text{COOD} \xrightarrow{\text{Ionizing radiation}} \text{CH}_3\text{CONDCH}_2\text{C}^{0} + e^- + D^+$$  \hspace{1cm} (1)

$$\text{CH}_3\text{CONDCH}_2\text{C}^{0} \xrightarrow{\text{below 77 K}} \text{CO}_2 + \text{CH}_3\text{COND-CH}_2$$  \hspace{1cm} (2)
\[ e^- + CH_3CONDCH_2COOD \rightarrow CH_3\overset{\ominus}{C}NDCH_2COOD \]  
\[ CH_3CONDCH_2\overset{\ominus}{C}OD \rightarrow CH_3\overset{\ominus}{C}NDCH_2COOD \]  
\[ CH_3CONDCH_2\overset{\ominus}{C}OD \rightarrow CH_3\overset{\ominus}{C}NDCH_2COOD \]  
\[ CH_3\overset{\ominus}{C}NDCH_2COOD \rightarrow CH_3\overset{\ominus}{C}ND + CH_2\overset{\ominus}{C}COOD \]  
\[ CH_3\overset{\ominus}{C}NDCH_2COOD \rightarrow CH_3\overset{\ominus}{C}ND + CH_2\overset{\ominus}{C}COOD \]  
\[ CH_3\overset{\ominus}{C}NDCH_2COOD > CH_3\overset{\ominus}{C}ND + CH_2\overset{\ominus}{C}COOD \]  

It is evident from the above steps that there would be no free proton available in the vicinity of the radical to form a weak bond with it as argued by HSM.\textsuperscript{29} Our results on irradiated \textsuperscript{15}N-acetylglycine have established beyond any reasonable doubt that the existence of such a weakly bonded proton in the vicinity of the radical is highly improbable.

From Table 9 it should be noted that the tensors associated with Hx\textsubscript{1}...
<table>
<thead>
<tr>
<th>PROTON</th>
<th>Coupling (MHz)</th>
<th>Principal value</th>
<th>Isotropic</th>
<th>Dipolar</th>
<th>Direction Cosines</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a*</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>b</td>
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<td></td>
<td></td>
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<td>c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>Hx₁(NH)</td>
<td>+2.87</td>
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<td>±0.923</td>
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<td>-0.854</td>
<td>±0.195</td>
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<td>(-0.021</td>
<td>+0.877</td>
<td>-0.482)</td>
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<tr>
<td>Hx₂(OH)</td>
<td>-1.81</td>
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<td>+6.61</td>
<td>-3.08</td>
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<td>+0.995)</td>
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<td>Hx₃(O₂---HN)</td>
<td>+1.61</td>
<td>+1.61</td>
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<td>+0.884</td>
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<td>+0.038</td>
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<td></td>
<td>+2.26</td>
<td>2.26</td>
<td>+0.046</td>
<td>±0.883</td>
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<td>(+0.021</td>
<td>-0.877</td>
<td>+0.482)</td>
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</table>
TABLE 9 (cont'd)

<table>
<thead>
<tr>
<th>PROTON</th>
<th>Coupling (MHz)</th>
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</thead>
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<td></td>
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<td>Isotropic</td>
<td>Dipolar a*</td>
<td>b</td>
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<td>Hx&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>+1.28</td>
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<td>±0.283</td>
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<td>+0.06</td>
<td>+0.920</td>
<td>±0.385</td>
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<td>±0.883</td>
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<tr>
<td>Hx&lt;sub&gt;5&lt;/sub&gt;</td>
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<td>-1.36</td>
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<td>+3.06</td>
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<td>±0.872</td>
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<td>Hx&lt;sub&gt;6&lt;/sub&gt;(O&lt;sub&gt;3&lt;/sub&gt;---HO&lt;sub&gt;1&lt;/sub&gt;)</td>
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<td>-1.71</td>
<td>-0.059</td>
<td>+0.998</td>
</tr>
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<td></td>
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<td>-0.03</td>
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<td>+0.059</td>
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<td>+3.24</td>
<td>-0.037</td>
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<td>(-0.002</td>
<td></td>
<td>+0.068</td>
<td>±0.998</td>
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</tbody>
</table>

1 See footnotes to Table 8.
2 Numbers in parentheses represent bond directions.
and Hx₂ have considerable isotropic parts. Based on this and their direction cosines these two tensors are assigned to the N-H and O-H protons in the radical. Both the protons have one principal axis in common which is also parallel to the intermediate coupling of the α-proton. On comparing the directions of the largest couplings with the bond directions, it is found that the agreement is within 6°. The remaining four exchangeable proton tensors are essentially dipolar in nature, indicating that they belong to intermolecular protons. The tensor Hx₃ has one component perpendicular to molecular plane and another component parallel to C₁-O₂ bond. Hence this was assigned to NH proton of the neighboring molecule hydrogen bonded to O₂ of parent molecule. Similarly the interaction Hx₆ has one component which is parallel to C₃-N bond direction which in turn parallel to O₃---H-O. Thus both the hydrogen bonded protons are accounted for. The remaining two exchangeable proton tensors belong to intermolecular exchangeable protons i.e. N-H or O-H protons of neighboring molecules not hydrogen bonded to the parent molecule.

From the isotropic part of the α-proton tensor we calculated an unpaired electron density of 0.75 on the pₓ orbital of the α-carbon, using the McConnell relation with a Q-value of -65 MHz. This is in very good agreement with the results of INDO calculations (pₓ INDΟ = 0.698). The calculated (INDO) and observed spin densities are given in Table 10. From the N-H proton isotropic coupling, a spin density of 0.065 on the pₓ orbital of nitrogen was calculated using a Q-value of 78 MHz (pₓ INDΟ = 0.111). Our INDO results in agreement with similar calculations by Saxebol et al. indicate that the remaining spin density is delocalized over the entire molecule.
**TABLE 10**

COMPARISON OF EXPERIMENTAL AND INDO UNPAIRED ELECTRON DENSITIES IN THE HYDROGEN ABSTRACTION RADICAL OF N-ACETYLGLYCINE

<table>
<thead>
<tr>
<th>ATOM</th>
<th>$C_1$</th>
<th>$O_1$</th>
<th>$O_2$</th>
<th>$C_2$</th>
<th>N</th>
<th>$C_3$</th>
<th>$O_3$</th>
<th>$C_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho\pi$(INDO)</td>
<td>-0.146</td>
<td>-0.024</td>
<td>0.299</td>
<td>0.698</td>
<td>0.111</td>
<td>-0.064</td>
<td>0.130</td>
<td>0.003</td>
</tr>
<tr>
<td>$\rho\pi$(obs.)</td>
<td></td>
<td></td>
<td></td>
<td>+0.752</td>
<td>0.065</td>
<td></td>
<td></td>
<td>+0.129</td>
</tr>
</tbody>
</table>
In an attempt to find out the reason for the failure of Piazza and Patten\textsuperscript{27} to find a well-resolved ENDOR spectrum in the vicinity of $\nu_p$, we carried out ENDOR measurements as a function of irradiation dose. The optimum signal to noise ratio was obtained with irradiation time of about two hours. Longer irradiation resulted in rapid decrease in the intensity and resolution of the main ENDOR spectrum while shifting intensity to the 'distant' ENDOR line. Finally after about 10 hours of irradiation a spectrum similar to that reported by Piazza and Patten\textsuperscript{27} was obtained. ESR spectra of the heavily irradiated crystals showed a significant increase in intensity, indicating that the radical concentration increased with irradiation dose. These observations suggest that cross relaxation\textsuperscript{142} is a dominant relaxation route in irradiated N-acetylglycine. Our observation that the irradiation dose and hence the radical concentration is rather critical for a well-resolved spectrum may well explain the frequent failures to detect ENDOR signals in molecular crystals.

7.4 Concluding Remarks:

In conclusion it should be noted that our results give no indication that the wavefunction of the unpaired electron significantly extends over the neighboring molecules. All the intermolecular hyperfine interactions we have observed are essentially dipolar in nature and hence do not indicate any appreciable spin density at the respective nuclei. The results of HSM\textsuperscript{29} showed considerable spin density on the neighboring molecules, but their results were obtained most likely from erroneous analyses of the ENDOR data.

There is also no evidence that an atom or a group which is broken from a molecule upon irradiation remains trapped in the immediate vicinity of the damaged molecule in the crystal. We have conclusively shown that the
multiplet structure in the ESR spectrum of irradiated N-acetylglycine arises from the interaction of the unpaired electron with protons of the methyl group and the $^{14}$N nucleus. Although we have not been able to obtain $^{14}$N ENDOR, it should be noted that the failure to find $^{14}$N ENDOR does not exclude the presence of nitrogen hyperfine interaction.

While it may be true that 'negative' ENDOR technique has some advantages over its conventional counterpart, however, the failure to detect conventional ENDOR may be due to too large a concentration of the radical in the crystal. We note that a carefully controlled ENDOR experiment should yield at least as good results as negative ENDOR, thus avoiding the need for an intense r.f. field, and hence, the resulting heat generation.
CHAPTER EIGHT

CONCLUSIONS:

In the foregoing chapters, the ENDOR investigations on three room temperature X-irradiated compounds, namely, phenazine, hippuric acid and N-acetylglycine have been described. Phenazine is exclusively aromatic, hippuric acid is aromatic with an aliphatic side chain and N-acetylglycine is purely aliphatic. Impressive consistencies have been observed while going from fully aromatic to fully aliphatic compounds. For example, phenazine requires about 80 hours, hippuric acid about 8 hours and N-acetylglycine about 2 hours of irradiation for an optimum ENDOR signal intensity.

The crystal structure of phenazine is monoclinic. The two molecules in the unit cell are magnetically equivalent along b-axis and in ac-plane; in all other orientations they have two distinct sites. All the ENDOR lines arise from interaction of unpaired electron with protons and no lines due to the hyperfine coupling of unpaired electron with $^{14}$N were observed in our study, although the ESR spectral spread indicates large $\pi$-spin on nitrogen. The ESR spectrum exhibited very much poorer resolution in most orientations, because the unpaired electron is extensively delocalized and coupled to large number of nuclei in aromatic systems. Our well resolved ENDOR spectra of phenazine have enabled us to characterize all the protons of an aromatic molecule which is otherwise impossible by ESR studies. The result indicates that the radical is formed by the addition of hydrogen atom to $N_{(10)}$ of phenazine. Analysis of some weaker accompanying lines suggests that they belong to the same type of radical but with the hydrogen atom added on $N_{(9)}$ with a slight conformational change with respect to the previous radical.
The crystal of hippuric acid is, on the other hand, orthorhombic. The four molecules in the unit cell are magnetically equivalent along the crystallographic axes and they have two distinct sites in all the crystallographic planes. A preliminary ESR spectrum revealed a highly anisotropic 1:2:1 triplet along the crystallographic axes indicating only two nearly equivalent protons strongly interacting with the unpaired electron. However, poor resolution combined with the presence of site splitting prevented us from reaching any quantitative conclusion regarding the nature of the radical. ENDOR investigations on this system fully clarified the identity and nature of the radical. In addition to strong α-proton coupling and some weak intra- and intermolecular proton interactions, we have detected $^{14}$N ENDOR lines belonging to a low hyperfine and quadrupole coupling which are rarely observed. The radical is believed to be formed by deprotonation of oxidized hippuric acid followed by the loss of a molecule of carbon dioxide. Among weakly coupled protons, N-H proton, three ring protons and one hydrogen bonded proton have been assigned. The exchangeable protons have been confirmed by partial deuteration. Although the decarboxylated radicals in carboxylic acids are usually unstable at room temperature, the present study establishes that in hippuric acid at least, it is stable at room temperature. In a structurally related compound N-acetylglycine the decarboxylated radical is stable only at low temperatures indicating that the phenyl group probably plays a critical role in the unusual stability of decarboxylated radical in hippuric acid.

The ENDOR investigation on X-irradiated N-acetylglycine is a reinvestigation of an earlier 'negative' ENDOR study by Helms, Suzuki and Miyagawa (HSM). Based on their results, they proposed that the room temperature radical was
essentially a cation, and that the wavefunction of the unpaired electron extends significantly over the neighboring molecules.

On reinvestigation we discovered several inconsistencies in the results of HSM. First of all, probably the radiation dose was not properly controlled for the detection of well-resolved spectra. Longer irradiation resulted in the production of a large concentration of the radicals most likely leading to cross relaxation, thus rapidly decreasing the intensity and resolution of the ENDOR spectrum while shifting intensity to the 'distant' ENDOR line. Because of poor resolution they were not able to follow unambiguously the closely spaced ENDOR lines in the vicinity of free proton NMR frequency. This led to misassignment of several lines in the angular variation plots, leading to erroneous values for the hyperfine tensors. Their results show appreciable isotropic parts for the tensors of several intermolecular protons.

On the other hand, we were able to obtain well-resolved ENDOR spectra by carefully controlling the irradiation dose. Upon careful analysis of the data we find that the hyperfine tensors of intermolecular protons are all nearly dipolar in nature with negligible isotropic parts.

Based on intensity measurements of ENDOR lines HSM concluded that a methyl group on the neighboring molecule had identical coupling to that on the radical itself. They also claimed that one of the ENDOR lines they observed belonged to a weakly bonded proton in the vicinity of the radical. These claims were 'substantiated' by presenting the second derivative ESR spectrum along the a*-axis. They analyzed the extra hyperfine structure in the spectrum as eight lines with binomial intensity ratio, arising from seven nearly equivalent protons - two methyl group protons and a weakly
bonded proton. We have unequivocally shown that the extra hyperfine structure in the ESR spectrum arises from interactions with a methyl group (that on the radical itself) and the $^{14}$N nucleus present in the radical. This was achieved by replacing $^{14}$N by $^{15}$N and demonstrating the accompanying change in the ESR spectrum. Our analysis of the ESR spectra is supported by the reports of Saxebol et al. and Mangiaracina.

Based on our results we have established that the stable radical in room temperature X-irradiated N-acetylglycine is CH$_3$CONHCHCOOH. We also find that the radical is present in the crystal in four slightly different conformations giving rise to the complex ENDOR spectra. There is no evidence for extensive delocalization of the unpaired electron beyond the bounds of the radical itself.

One of the fundamental aspects of these studies is the control of radical concentration in the crystals for the success of an ENDOR investigation. It has been demonstrated that the optimum signal to noise ratio is obtained with irradiation time of definite hours depending on the system. Longer irradiation results in rapid decrease in the intensity and resolution because of cross relaxation. This is probably the main reason why the previous workers on N-acetylglycine have failed to detect well-resolved ENDOR spectra of irradiated N-acetylglycine.

It is difficult to relate the radicals trapped in single crystals with radiation damage in living organisms as a result of the far greater complexity of the living system. However, the radicals which can be trapped in single crystals, can also be formed in the living organisms, although their life times are too short to allow their analyses by conventional ENDOR techniques. As a result of biological action of radiation, flavoprotein undergoes reduction
and it is thought that the site of the radical is the flavin mononucleotide moiety in the flavoprotein. The radical anion is formed by electron addition to a neutral flavin and the radical cation is formed by effective \( \text{H}^+ \) addition to a neutral flavin; so both anion and cation are the reduced forms of flavin molecules. Under some conditions, a neutral flavin radical is formed by hydrogen addition to nitrogen atom of flavin moiety; this is similar to cation radical and has a similar spin density distribution.

Since single crystals of flavoprotein are difficult to prepare and single crystal analysis gives the most complete information about a radical, it is easier to start with flavin moiety as a model compound to study the effect of radiation on flavoproteins. Our work on phenazine which resembles flavin moiety shows that the radical is essentially formed by hydrogen addition at one of the nitrogen atoms of the molecule.

Hippuric acid and N-acetylglycine are the simplest compounds with peptide bonds that unite individual amino acids into proteins. Since single crystals of proteins are difficult to prepare, it has been useful to start with the single crystals of these model compounds in order to interpret the radiation damage in proteins.

A variety of chemical agents are known that can protect organisms against the effects of radiation and sensitize tumour cells to radiation improving the effectiveness of radiation therapy in the treatment of cancer. It has long been known that ionizing radiation is considerably more lethal to cells. In view of compelling need for increasing radiation therapy in the treatment of cancer, the use of sensitizing agents has been receiving increased attention. Moreover the enhancement of radiation damage by certain compounds could lead to new methods for the treatment of cancers.
If these compounds could be selectively introduced into cancer tissue, radiation damage and subsequent cell death would be enhanced in this tissue. Thus the dosage required to destroy a cancer could be reduced, thereby reducing radiation damage to healthy tissues.

These compounds could also exhibit a remarkable capacity to protect living organisms from the effects of radiation. Mechanisms of radiation protection are of fundamental interest in radiation biology. The radiation protectors intervene in the radiation damage process by scavenging some of the primary reactive species produced in the radiolysis of body fluid. Thus the organism is protected, at least partially, against indirect effects of radiation. A bio-molecule having been oxidised by removal of hydrogen at some earlier stage of radiation damage process, is subsequently repaired by a donation of hydrogen from a protector molecule.
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