CARBON RADICAL	GENERATION	ESR TEMP.	g-VALUES	SPLITTING PARAMETERS	REFERENCE
$CH_3(CH_2)_2CH_2\dot{C}H(COOH)$	x-irradiation of butyl malonic acid at 77° K; additionally warmed to 195° K	77° K		$a_{H\alpha}$ = 32.1, 21.4, 10.7 iso: 21.4 $a_{H\beta_1}$: 17.[illegible] $a_{H\beta_2}$: 6.4	Tamura, N. et al. Trans. Far. Soc. 62, 2[illegible] (196[illegible])
$CH_3(CH_2)_2CH_2\dot{C}(COOH)_2$	x-irradiation of butyl malonic acid at 77° K additionally warmed to 300° K	77° K		$a_{H\beta_1}$: 48.9 $a_{H\beta_2}$: 13.2	Tamura, N. et al. Trans. Far. Soc., 62 2[illegible]
$CH_3CH_2\dot{C}HCH_2CH(COOH)_2$	x-irradiation of butyl malonic acid at 77° K; additionally warmed to 195° K	77° K		$a_{H\alpha}$ = 32.1, 21.4, 10.7 iso: 21.4 $a_{H\beta_1}$ = $a_{H\beta_2}$ = $a_{H\beta_3}$ = $a_{H\beta_4}$: 35.7	
$CH_3\dot{C}H(CH_2)_2CH(COOH)_2$	x-irradiation of butyl malonic acid at 77° K	77° K		$a_{H\alpha}$: 32.1, 21.4, 10.7 iso: 21.4 $a_H^{CH_3}$: 24.2 $a_{H\beta_1}$ = $a_{H\beta_2}$ = 35.7	
$C_4H_9CH(COOH)_2^-$	x-irradiation of butyl malonic acid at 77° K	77° K		$a_{H\beta}$ 24.2	

ESR OF ORGANIC FREE RADICALS

AND

HUMAN BLOOD IN CANCER

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

In section A a review has been made of single crystal studies done from 1960-1973. This review has been put into tabular form (See Tables I, 2, 4-9). A critical analysis and INDO calculations of some of the radicals are reported.

Single crystal ESR studies of 2-imidazolidinone, 2-imidazolidine-thione, semicarbazide hydrochloride, succinimide and N-halo succinimide are reported.

$\dot{C}H_2NHCONH_2$ is observed for 2-imidazolidinone, $\dot{C}H_2NHCSNH_2$ is observed for 2-imidazolidinethione, $\dot{N}HNH_3^+$ is observed for semicarbazide hydrochloride, and $HC(O)\dot{N}HC(O)-CH_2$ is observed for succinimide and N-halo succinimide.

In section B, lyophilized whole blood from 183 persons with varying degrees of dysplasia and cancer of the cervix, uteri and with normal cervical epithelium were examined. A statistical regression analysis of the results shows that the nature of the radicals at q = 2 change as cancer progresses. The results show also that this technique is most probably not a suitable diagnostic tool.

La section A consiste en une analyse rétrospective, couvrant la période 1960-73, de la majorité des études par ESR portant sur les "cristaux uniques" (voir les tableaux 1, 2, 4-9). Une analyse critique, impliquant plusieurs calculs "INDO", nous a permis d'expliciter la présence de ces radicaux. Il nous a été possible d'identifier, par ESR, l'existence du radical $\dot{C}H_2NHCONH_2$ dans des "cristaux uniques" irradiés de 2-imidazolidone et de 2-imidazolidinethione, celle du radical $\dot{N}HNH_3^+$ pour l'hydrochlorure de semicarbazide et celle du radical $H\dot{C}CONHCOCH_2$ pour le succinimide et le N-halo-succinimide.

La section B consiste en une étude de 183 échantillons "lyophilés" de sang extraits de personnes souffrant, à des degrés différents, de "dysplasie, de cancer du cerveau ou de l'utérus. L'analyse statistique des résultats a montré que la nature des radicaux, à g = 2, change selon la progression de la maladie. Il ne semble pas cependant que cette technique soit applicable pour le diagnostic de ces cancers.

MOTIVATION

This research began as a study of the diagnostic value of electron spin resonance spectroscopy for carcinoma of the cervix uteri. One direct consequence of the chemical result was a desire for a more fundamental knowledge of free radical formation. An examination and review of the literature on single crystal ESR studies as well as an ESR study on small organic radicals in single crystals was therefore undertaken. Examination of the literature shows marked discrepancies even in the identification of the most simple radicals on crystalline media. An attempt is made to resolve some of the published errors and this led to the search for the elusive sigma radical. The results are summarized in the abstract and show that the thesis consists of two more or less separate research projects; studies of γ-irradiated crystals containing small organic molecules and studies of free radical formation in whole blood and its relationship to carcinoma of the cervix uteri.

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DEDICATED TO MY WOMAN AND CHILD AND TO ALL WHO HAVE HOPE.

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SECTION A.

ESR STUDIES OF SMALL ,ORGANIC ,,NITROGEN-CONTAINING FREE RADICALS.

INTRODUCTION

Since Zavoiski's fundamental experiments in 1946 the Electron Spin Resonance (ESR) experiment has developed to become a standard laboratory method of studying the configuration and dynamical behaviour of free radicals in low dielectric loss media. The advantage and sometimes disadvantage of the experiment lies in its insensitivity towards all that does not give an e.s.r. signal, thus giving a blissful sense of security to the researcher who attempts to postulate various reaction mechanisms.

The studies which follow immediately are limited to localized spin ½ radicals produced by γ-irradiation of small organic crystals. The review of the theory, and this includes the molecular orbital analysis of the radicals, is limited to the work done here. General references are given in appendix A of the thesis.

Electron Spin Resonance (ESR) Theory

The essential feature of the ESR technique is its ability to characterize molecules and free radicals by the resonance absorption due to the interaction of the electronic magnetic moment and its external magnetic field. The so-called spin Hamiltonian, $\hat{H}$, describing this interaction is written as

$$\hat{H} = \underset{\sim}{S} \cdot (\underset{\approx}{g} \cdot \beta \underset{\sim}{H} + \underset{\approx}{\hat{A}} \cdot I) - g_n \beta_n \underset{\sim}{H} \cdot \underset{\sim}{I} \qquad (1)$$

where $\underset{\sim}{S}$ and $\underset{\sim}{I}$ are the electronic and nuclear spin equivalent operators, $\underset{\sim}{H}$ is the magnetic field applied externally to the molecule, β_n is the nuclear magneton, g_n is the nuclear Zeeman splitting factor and β is the Bohr magneton. The electron Zeeman splitting factor $\underset{\approx}{g}$ and the hyperfine interaction $\underset{\approx}{\hat{A}}$ are tensorial quantities.

For the radicals observed in this thesis, the last term on the right-hand-side describing the nuclear Zeeman interaction is very small compared to the electronic interactions and thus is ignored in the following development.

The truncated Hamiltonian,

$$\hat{H} = \underset{\sim}{S} \cdot (\underset{\approx}{g} \cdot \beta \underset{\sim}{H} + \underset{\approx}{\hat{A}} \cdot \underset{\sim}{I}) \qquad (2)$$

appears to describe the interaction of the electron spin $\underset{\sim}{S}$ with two not necessarily parallel magnetic fields. The spin vector takes the direction described by the unit vector $\underset{\sim}{r}$ in the following equation which gives the magnetic field resonance conditions for

the three transitions involving an electron coupled to a nuclear spin, I = 1 (eg. Nitrogen).

$$H = [H_e - |\underset{\sim}{r} \cdot \underset{\approx}{A}|] / \underset{\sim}{r} \cdot \underset{\approx}{G} \cdot \underset{\sim}{r} \qquad (3)$$

$$H = H_e / \underset{\sim}{r} \cdot \underset{\approx}{G} \cdot \underset{\sim}{r} \qquad (4)$$

and $$H = [H_e + |\underset{\sim}{r} \cdot \underset{\approx}{A}|] / \underset{\sim}{r} \cdot \underset{\approx}{G} \cdot \underset{\sim}{r} \qquad (5)$$

In this derivation, $H_e = h\nu_e/2\beta$, $\underset{\approx}{G} = \underset{\approx}{g}/2$ and $\underset{\approx}{A} = \hat{\underset{\approx}{A}}/2\beta$. The three transitions are assumed to be equally intense and are observed by sweeping the applied magnetic field H while keeping the klystron resonance frequency ν_e constant.

In reality, the transition intensity depends on the r.f. field intensity and on the electronic relaxation time. The electron spin in a magnetic field can be described as occupying one of two energy levels, E_1 and E_2, and their relative population by the ensemble of electron spins found in the sample is described by the Boltzman equation,

$$\frac{N_1}{N_2} = e^{-(E_2 - E_1)/kT} \qquad (6)$$

where T is the temperature. When the system is perturbed by an r.f. field at resonance the ratio N_1/N_2 varies and in the absence of this field returns to its equilibrium value exponentially as described by a time constant T_{1e} , the spin-lattice relaxation time.

The relaxation mechanisms will not be discussed in detail here except to note that the line width of the individual esr transition is inversely proportional to a spin-spin relaxation time T_{2e}. T_{2e} requires that the electron spin have neighbours which are also paramagnetic. T_{2e} increases as the distance to its paramagnetic neighbours and is a function of the frequency of their motion. The presence of oxygen and metals can greatly reduce the T_{2e} and hence increase the linewidth of organic free radicals. The ESR spectrometer is essentially a Q meter where the resonant cavity Q is measured as a function of applied magnetic field. The magnetic field is modulated also at a high frequency, say 100 kHz to permit synchronous detection and thus greatly decrease the noise bandwidth of the spectrometer. A result of this is that the observed signals are derivatives of the absorption. Further noise reduction is obtained by stabilizing the frequency of the klystron to the resonance "Q drop" of the cavity. A block diagram of the spectrometer is shown in Fig. 1. The figure is self explanatory and is discussed in ref. 1.

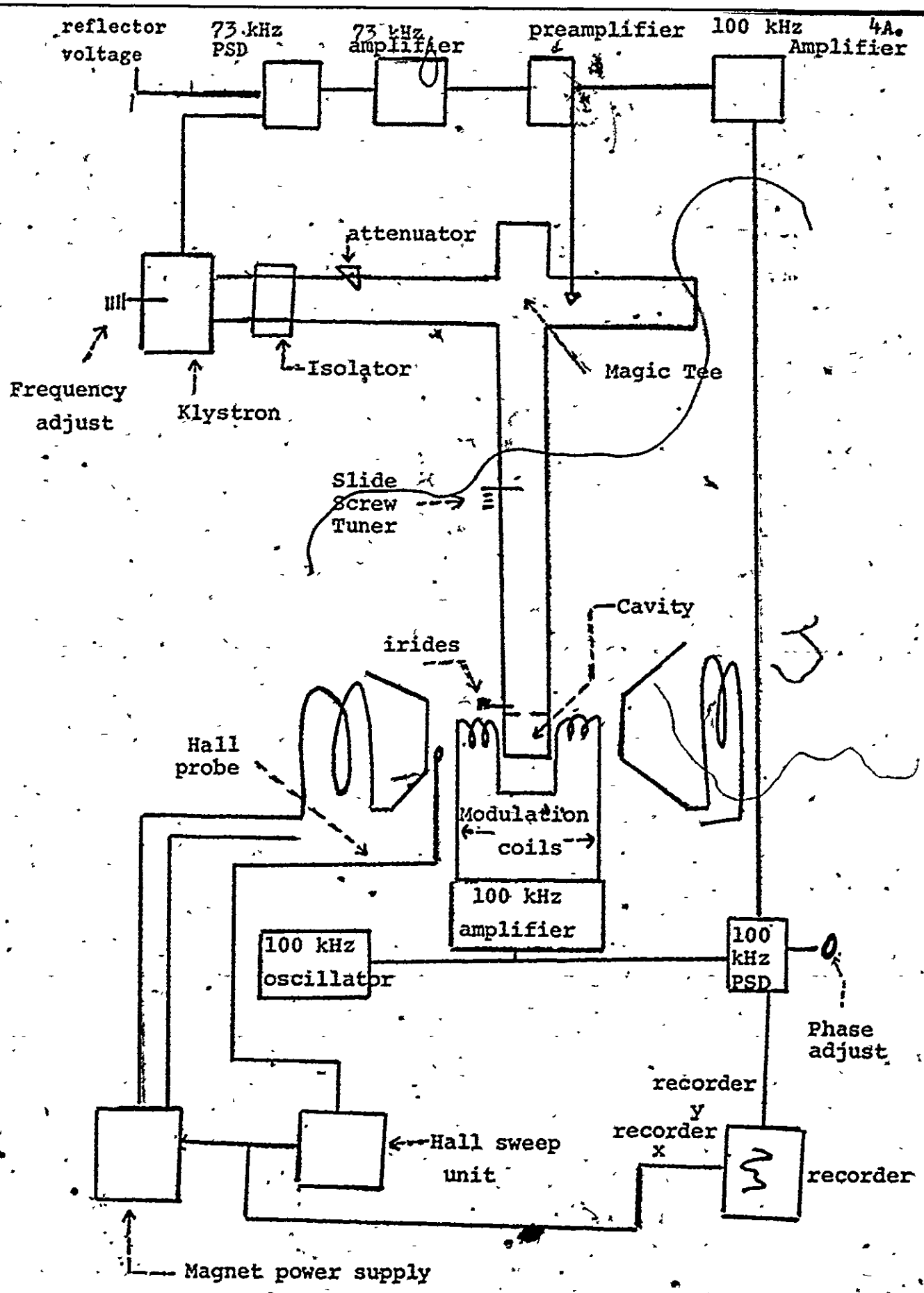


Fig.1 Typical spectrometer with essential components indicated

Free Radicals in Organic Single Crystals

The interaction of a high energy photon such as provening from X or γ-ray sources, with an organic molecule is a rather complicated process culminating often in the formation of free radicals. Free radicals are defined as molecules having unpaired electron spin. The ESR technique leaves the experimenter ignorant of all that does not give a signal. The mechanism of formation of the radical and its termination is not always obvious. The radicals are usually very dilute in the host crystal and therefore can result not from the destruction of the host crystal molecules but from the disintegration of an impurity. For example the radical H· results from the disintegration of H_2 introduced under pressure and at high temperature into fluorite (CaF_2) during single crystal formation (2). Analogous reactions occur in organic systems which are sometimes difficult to purify.

The first ESR studies of radiation damage in materials of biological interest were of powdered material (amino acids) (Gordy, et al., 1955 Ref. 3). This was quickly followed by reports of orientation effects in single crystals of alumine (van Roggen, et al., 1956 Ref. 4) and glycine (Uebersfield and Erb, 1956 Ref. 5). This last study was immediately pursued in great detail by Ghosh and Whiffen (1958, 1959 Ref. 6 and 7). Papers on dimethyl glyoxime (Miyagawa and Gordy, 1959 Ref. 8,9) and on malonic acid (McConnel et al., 1960 Ref. 10) soon followed. These papers confirmed the necessity of single crystal studies in obtaining information about the structure of the free radicals and the distribution of the odd

electron around the molecule. Free radicals in dilute solution give narrow line spectra since the rapid molecular tumbling effectively averages to zero the anisotropic component of the hyperfine coupling. The resultant spectra are less informative since they can only be interpreted in terms of their isotropic interactions. In amorphous or polycrystalline solids the summation over all orientations of the anisotropic term results in a broadening of the individual lines so that hyperfine structure may be obscured and be easily misinterpreted. In single crystals, the radicals are trapped at sites which usually have the same symmetry as the host lattice. Therefore, in addition to yielding information about the host lattice, the rotation pattern of the line spectra aid the experimenter in his analysis of the configuration and history of the radical.

Review and Analysis of Previous Single Crystal Studies

The first single crystal ESR study of an organic radical occurred, as we have pointed out before, in 1956. Since that time much literature has been published about such studies. A review of these studies published from 1960 to 1973 follows.

Although most of the radicals listed in tables 1 & 2 are organic, a few inorganic radicals have been included because of their relative importance to the biologist. The radicals have generally been produced by ionizing radiation. Nearly all the irradiations and observations have been done at room temperature.

TABLE 1--CARBON RADICALS (REVIEW OF SINGLE CRYSTAL STUDIES FROM 1950-1975)

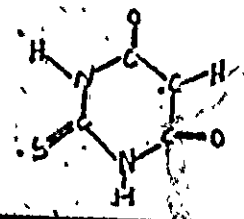
CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS IN GAUSS	REFERENCE
$CH_3\dot{C}HCH_2O_2C-CO_2CH_2-(CH_2)_2CH_3$	X-irradiation of di-N-butyloxalate-urea at 77°K	77°K	[illegible] 2.0023 2.0025 iso: 2.0025	aH_α: 22.7, 17.0, 17.0 iso: 22.2 aH_β: 26.6, 25.3, 25.3 iso: 25.7 aH_β: 23.0, 22.1, 22.1 iso: 22.4	Lai, [illegible] J. Chem. Phys., 53, 4399 (1970).
$CH_3\dot{C}H(CH_2)_2O_2C-CH_2-C(=O)-[illegible]-(CH_2)_3CH_3$	X-irradiation of di-N-butylmalonate urea at 77°K	77°K	2.0029 2.0026 2.0026 iso: 2.0027	aH_α: 32.6, 26.8, 26.8 iso: [illegible] aH_β: 26.[illegible], 25.5, 25.5 iso: 25.9 aH: 23.0, 22.1, 22.1 iso: 22.4	[illegible] J. Chem. Phys., 53, 4399 (1970).
$\dot{C}H(COOH)-CH_2(COOH)$	γ-irradiation of maleic acid at 300°K	300°K	2.0030 2.0039 2.0026 iso: 2.0032	aH_α: 31.0, 10.0, 19.6 iso: 20.3 aH_β: 1[illegible].8	Cook, [illegible] Phys. [illegible] 183 (1967).
$H-\dot{C}-COOH$ / $H-C-COOH$ / R*	γ-irradiation of maleic acid at 300°K	300°K	2.0035 2.0033 2.0033 iso: 2.0034	aH_α: 28.7, 11.7, 17.8 iso: 19.[illegible] aH_β: 12.8	Cook, J. [illegible] Phys., [illegible] 183 (1967)

*Nature of R unknown.

Carbon radical	Generation	ESR Temp.	g-factors	Splitting parameters	References
CH_2CONH_2	Y-irradiation of monofluoro-acetamide (CH_2FCONH_2) at 77°K	77°K		$a_{H_{\alpha_1}}$: 11.8, 19.9, 31.9 iso: 21.2 $a_{H_{\alpha_2}}$: 11.6, 19.8, 33.2 iso: 21.5	IWASAKI, [illegible] and TOKIYAMA, [illegible] J. Chem. Phys. 46, 4693 (1967).
CH_3, CH_3, CH_2-CH-COOH ⊕	X-irradiation of DL-valine at 77°K	225°K		a_{H_α}: 35.0, 26.1, 23.9 iso: 28.3 a_{H_β}: 32.8, 25.35, 3.2 iso: 22.1	BOX, H.C. et al. J. CHEM. PHYS. 46, 4470 (1967)
CH_3-CH_2CHCOOH	X-irradiation of α-amino isobutyric acid doped with cysteine hydrochloride (2%) at 77°K	220°K	2.002 2.026 2.061 iso: 2.0296	a_{H_β}: 30, 32, 31 iso: 30.6 a_{H_α}: 30, 16, 20 iso: 22.0	BOX, H.C. et al. J. CHEM. PHYS. 2324 (1966)
$NH_2COCH_{\beta_1}$-CH_{α}-$CONH_{\gamma_1}$ β_2 γ_2	X-irradiation of succinamide at 300°K	300°K	2.0032 2.0033 2.0040 iso 2.0032	a_{H_α}: 10, 19, 31.9 iso: 20.3 $a_{H_{\beta(1)}}$: 29.1, 30.7, 31.5 iso: 31.4 $a_{H_{\beta(2)}}$: 31.6, 32.1, 37.0 iso: 33.5 $a_{H_{\gamma_1}}$: 0.2, 0.4, 3.3 iso: 1.9 $a_{H_{\gamma_1}}$: 1, 3, 6 iso: 3.3 $a_{H_{\gamma_2}}$: 1, 3, 6 iso: 3.3	NAGAKURA, S. et al. J. CHEM. PHYS. 2823 (1966)

CARBON RADICAL	GENERATION	ESR temp.	g-VALUE	SPLITTING PARAMETERS	LITERATURE
$CH_3(CH_2)_2CH_2\dot{C}H(COOH)$	X-irradiation of butyl malonic acid at 77°K; additionally warmed to 195°K	77°K		a_{H_α} = 32.1, 21.4, 10.7 a_{iso}: 21.4 $a_{H_{\beta_1}}$ = 17.9 $a_{H_{\beta_2}}$ = 6.4	Tomura, M. et al., Trans. Far. Soc., 62: 2434 (1966)
$CH_3(CH_2)_2CH_2\dot{C}(COOH)_2$	X-irradiation of butyl malonic acid at 77°K additionally warmed to 300°K	77°K		$a_{H_{\beta_1}}$ = 48.9 $a_{H_{\beta_2}}$ = 13.2	Tomura, M. et al., Trans. Far. Soc., 62 2434
$CH_3CH_2\dot{C}HCH_2CH(COOH)_2$	X-irradiation of butyl malonic acid at 77°K; additionally warmed to 195°K	77°K		a_{H_α} = 32.1, 21.4, 10.7 a_{iso}: 21.4 $a_{H_{\beta_1}}$ = $a_{H_{\beta_2}}$ = $a_{H_{\beta_3}}$ = $a_{H_{\beta_4}}$ = 35.7	
$CH_3\dot{C}H(CH_2)_2CH(COOH)_2$	X-irradiation of butyl malonic acid at 77°K	77°K		a_{H_α}: 32.1, 21.4, 10.7 a_{iso}: 21.4 $a_{\beta}{}^{CH_3}$: 24.2 $a_{H_{\beta_1}}$ = $a_{H_{\beta_2}}$ = 35.7	
$C_4H_9\dot{C}H(COOH)_2^-$	X-irradiation of butyl malonic acid at 77°K	77°K		a_{H_β} 24.2	

CARBON RADICAL	GENERATION	ESR Temp.	g-FACTORS	SPLITTING PARAMETERS	REFERENCES
$CH_3\dot{C}H(CH_2)_2OC(=O)C(=O)CH_3$	X-irradiation of di-n-butyl oxalate urea at 77°K	77°K	2.0028 2.0023 2.0029 iso: 2.0026	a^{H}_{α}: 32.7, 17.0, 17.0 iso: 22.2 $a^{CH_3}_{H_\beta}$: 26.6, 25.3, 25.3 iso: 25.7 $a^{CH_2}_{H_\beta}$: 23.0, 22.1 22.1 iso: 22.4	LAI, A., J. CHEM. PHYS., 53, 4399 (1970)
$CH_3\dot{C}H(CH_2)_2O_2C-\underset{CH_2}{\underset{\vert}{C}}(=O)-C(=O)-O(CH_2)_3CH_3$	X-irradiation of di-n-butyl malonate urea at 77°K	77°K	2.0029 2.0026 2.0026 iso: 2.0027	a^{H}_{α}: 32.8, 16.8, 16.8 iso: $a^{CH_3}_{H_\beta}$: 26.7, 25.5, 25.5 iso: 25.9 $a^{CH_2}_{H_\beta}$: 23.0, 22.1, 22.1 iso: 22.4	LAI, A., J. CHEM. PHYS. 53, 4399
$CH_3(CH_2)_3CH_2-\dot{C}H-C(=O)-C\equiv C-CH_3$	X-irradiation of methyl-2-nonynoate-urea at 77°K	300°K	2.0037 2.0029 2.0024 iso: 2.0031	a^{H}_{α}: 24.2, 13.7, 13.7 iso: 17.2 $a^{CH_2}_{H_\beta(1)}$: 22.4, 22.4, 22.4 iso: 22.4 $a^{H}_{\beta(2)}$: 22.0, 21.8 21.8 iso: 21.9	BIENEL, G.B. et al, J. CHEM. PHYS., 54, 1630, (1971).
$HOOC\dot{C}H(CH_2)COOH$	X-irradiation of dipotassium malonate monohydrate at 77°K	300°K	2.0033 2.0036 2.0020 iso: 2.0030	a^{H}_{α}: 21.4, 19.0, 14.2 iso 21.66	BALES, B. et al, J. CHEM. PHYS. 51, 5178 (1969)
$HOOC\dot{C}H(CH_2)COOH$	X-irradiation of monopotassium succinate tetrahydrate at 300°K	300°K	2.0033 2.0050 2.0021 iso: 2.0029	a^{H}_{α}: 33.6, 20.7, 12.9 iso: 22.4 $a^{H}_{\beta(1)}$: 28.4, 25.1, 23.4 iso: 25.6 $a^{H}_{\beta(2)}$: 40.9, 38.3, 37.3 iso: 25.6	BALES, B. et al., J. CHEM. PHYS., 51, 5178 (1969).

CARBON RADICAL	GENERATION	ESR TEMP.	g-VALUE	SPLITTING PARAMETERS	REFERENCES
$^{\ominus}S\text{-}CH_2\text{-}\dot{C}H\text{-}NH_3Cl$	irradiation of cystine HCl at 77°C	77°K		a^{H}_{α}: 31.4, 29.6, 26.9 iso: 29.3 $a^{H}_{\beta_1} = a^{H}_{\beta_2} = a^{H}_{\beta_3}$: 31.4 29.6, 26.7 iso: 29.3	Box, H.C. et al. J. Chem. Phys., 45, 809 (1966)
$^{-}OOC\text{-}CH_2\text{-}\dot{C}H\text{-}C(=O)OH$	x-irradiation of β-succinic acid at 77°K	4.2°K	2.0020 2.0028 2.0038, iso: 2.0028	a^{13}_{C}: 134, 73, 61 iso: 89 a^{H}_{β}: 35, 22, 16 iso: 24.3 a^{H}_{β}: 84, 75, 75 iso: 78	Box, H.C. et al. J. Chem. Phys., 42, 1471 (1965)
	electron irradiation of 2-thiobarbituric acid @ 77°K	300°K	2.0114 2.0057 2.0025 iso: 2.0065	a^{H}: 28.1; 18.5; 10.1 iso: 18.9	Yelo, T.B. et al. Mol. Phys., 22 1055 (1971)
$^{-}OOCCF_2\dot{C}FCOO^{-}$	x-irradiation of sodium perfluorosuccinate at 77°K then warming to room temp	77°K		aF_{α}: 217, 7,2 iso: 75 aF_{β}: 122, 41, 41 iso: 68 aF_{β_2}: 9, 3 2 iso: 1.4 aF_{α}: 224, 5,4 iso: 77 aF_{β_1}: 125, 44, 41 iso: 70 aF_{β_2}: 14, 1, 2 iso: 5.6	Kispert, L.D and Rogers, M.T, J. Chem. Phys., 54, 3326 (1971)

*Splitting axes from 2 radicals having same chemical structure but different conformations.

CARBON RADICAL	Generation	ESR Temp	g values	Splitting Parameters	References
$^{\bullet}CH_2CO_2^-$	γ irradiation of sodium acetate at 77°K then warmed up to 160°K	77°K	2.0031 2.0021 2.0019 iso 2.0024	$a^{H}_{\alpha(1)}$: 20.6, 11.6, 33.3 iso: 21.8 $a^{H}_{\alpha(2)}$: 29.6, 6.9, 32.8 iso: 21.1 a^{13}_{C}: 13.4, 14.7, 35.5 iso: 34.1	FUJIMOTO, H. and J. JANIELKA, J. Chem. Phys. 52, 5 (1972)
		300°K		$a^{H}_{\alpha(1)}$ $a^{H}_{\alpha(2)}$: 23.3 15.9, 26.1 iso: 21.8 a^{13}_{C}: 13.4, 14.7, 14.3 iso: 14.1	
$^{13}CO_2^-$	γ-irradiation of strontium acetate 1-^{13}C	250°K	2.0011 1.9959 2.0030 iso 2.0000	a^{13}_{C}: 165.3, 140, 189.2 iso: 164.9	FUJIMOTO, H. and J. JANIELKA, J. Chem. Phys. 52, 5 (1972).
$^{\bullet}CH_3CO_2^-$	X-irradiation of strontium acetate hemihydrate at 77°K	150°K		$a^{H}_{CH_3}$: 14.1, 10.7, 10.7 iso: 11.8	ROLLES, W.M. et al. J. CHEM. PHYS. 54, 1532 (1970).
$CH_2CO_2^-$ +		233°K		a^{H}: 32.5, 20.9, 10. iso: 21.3 $a^{H}_{(2)}$: 32.7, 19.6, 11.2 iso: 21.2 a^{H}_{1}: 31.6, 20.6, 10.9 iso: 21.1 $a^{H}_{(2)}$: 32.1, 20.2, 21.2 iso: 21.3	

+ 2 magnetically distinct radicals having same chemical formula.

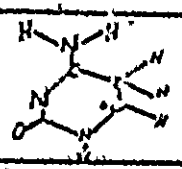
CARBON RADICALS	GENERATION	ESR Temp.	g-FACTORS	SPLITTING PARAMETERS	REFERENCE
$\dot{C}H_3$	X-irradiation of zinc acetate dihydrate at 77°K	77°K		aH: 22.1	TOLLES, W.M. et al., J. CHEM. PHYS., 19, 4765 (1965)
HCO *	γ-irradiation of formic acid at 77°K then annealation at 195°K for 30min	77°K	2.0037 2.0023 1.9948 iso: 2.0002	aH: 120.4, 123.5, 135.0 iso: 126.3 a^{13}_C: 121.8, 156.4 113.2 iso: 130.5	HOLSBERG, G.W., J. CHEM. PHYS. 51, 3456 (1969)
$H_3\dot{C}CHR$	γ-irradiation of d D-alanine and L-alanine at 300°K	77...210°K		$aH^{CH_2}_{\beta(2)}$: 24, 6.4, 5.1 iso: 5.3 $aH^{CH_3}_{\beta(2)}$: 28.1, 27.6, 27.6, iso: 27.8 $aH^{CH_3}_{\beta(3)}$: 41.6, 42.1, 46.1 iso: 43.3 aH^{CH}_{α}: 18.3, 21.2, 27.6 iso: 22.3	HIRAGUCHI, Y. and K. ITO, J. CHEM. PHYS. 36, 2197 (1962)
		293		$a_{H_\alpha}^{CH_3}$: 24.5, 25.4, 26.3 iso: 25.1 a_H^{CH}: 18.8, 19.9, 26.3 iso: 21.7	

* sigma radical

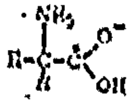
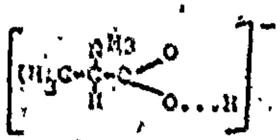
CARBON CENTERED RADICALS	GENERATION	ESR Temp.	g-FACTORS	SPLITTING PARAMETERS	REFERENCES
$CH_3-\overset{CH_3}{\underset{COOH}{\overset{|}{\underset{|}{C}}}}\cdot$	γ-irradiation of α-amino isobutyric acid at 300°K	300°K	2.0034 2.0030 2.0022 iso: 2.0024	$a_H^{CH_3}$: 24 ++	HORSFIELD, A. et al., TRANSFARADAY. Soc., 57, 1057 (1961)
$CH_3-\overset{CH_3}{\underset{HOOC-CH-NH_2}{\overset{|}{\underset{|}{C}}}}\cdot$	X-irradiation of D,L-valine at 300°K	300°K	2.0036 2.0031 2.0027 iso: 2.0031	$a_H^{CH_3}$: 25.6, 22.7 22.4 iso: 23.0 $a_N^{NH_2}$: 8.4, 8.0, 6.7 iso: 7.7 a_H^{CH}: ≈ 0	McDONELL, C.A. and H.C. DEN, INT. S SYMP ON MOLECUL. STRUCTURE AND SPECTROSCOPY, D201, Tokyo, 1962
$HOOC-CH_2-\overset{CH_3}{\underset{CH_3}{\overset{|}{\underset{|}{C}}}}\cdot$	X-irradiation of 2-methyl propane-1, 2-dicarboxylic acid at 300°K	300°K		$a_H^{CH_3}$: 23.2 ++ $a_{H_\beta(1)}^{CH_2}$: 9.6, 6.1 5.0 iso: 6.8 $a_{H_\beta(2)}^{CH_2}$: 25.0, 23.6 16.8 iso: 21.3	CVETKOV, Y., et al., J. CHEM. SOC., 1964, (310)
$HOOC-\dot{C}H-CH_2-COOH$	X-irradiation of aspartic acid hydrochloride $HOOC-\overset{NH_3Cl}{\overset{|}{CH}}-CH_2-COOH$ at 300°K	300°K		a_H^{CH}: 32, 21.4, 13.9 iso: 22.5 $a_{H_\beta(1)}^{CH_2}$: 24.3, (iso) $a_{H_\beta(2)}^{CH_2}$: 41.5 (iso	ROWLANDS J., J. CHEM. SOC., 4263, (1961)

++ nearly isotropic

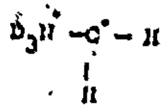
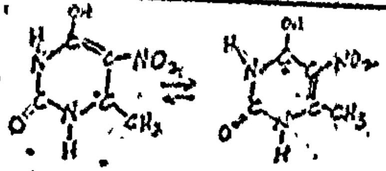
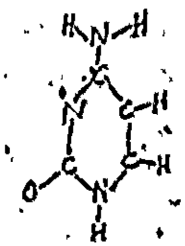
CARBON RADICAL	GENERATION	ESR Temp.	g-factors	SPLITTING PARAMETERS	REFERENCES
CH_2COO^-	X-irradiation of glycine at 300°K	300°K	2.0020 2.0034 2.0042 iso: 2.0032	$a_H^{CH_2}$: 10.7, 19.7, 32.5. iso: 22.2 $a_H^{CH_2}$: 33.6, 25.6, 3.6 iso: 21.0	HEINER, R.F. and W.S. KOSKI, J. AM. CHEM. SOC., 85, 873 (1963)
NH_4^+	X-irradiation of glycine			a_H: 52.0, 2.3, 41.1 iso: 31.8 H_1-H_2-H_3: 20.7, 13.3 15.1 iso: 16.4 a_N: 2.7, 4.3, 1.8 iso: 2.9	HEINER, R.F. and W.S. KOSKI, J. AM. CHEM. SOC., 85, 873 (1963).
CO_2...H or CO_2^-... ...H-O.	γ-irradiation of potassium bicarbonate and urea nitrate at 300°K	300°K	1.9971, 2.0012 2.0031 iso: 2.0040	$a^{13}C$: 133, 142, 179 iso: 15. all: < 5	CHANTRY, G.W. et al., M.P. 5, 589, (1962)
$^{13}CO_2^-$	γ-irradiation of potassium bicarbonate and urea nitrate at 300°K	300°K	2.006 2.0006 2.00184 iso: 2.0112	a: 13.5, 10.0	CHANTRY, G.W. et al., MP 5, 589 (1962)
$CH_3OCHCOO^-K^+$	γ-irradiation of K-methoxyacetate at 300°K	300°K		a_H^{CH}: 29.3, 18.6 0.1 iso: 17.8 $a_H^{CH_3}$: 4.3, 1.4, 0.7 iso: 2.1	BOCKFIELD M and J.R. MORTON TRANS FARADAY SOC. 59: 470 (1963)

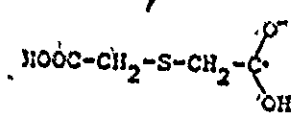
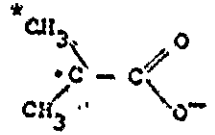
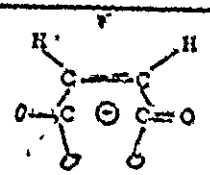
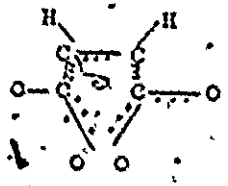
Carbon radical	Generation	ESR Temp.	G-factors	Splitting parameters	References
	γ-irradiation of cytosine monohydrate at 300°K	300°K		a: 30.3, 5.3, 17.5 iso: 17.9	HERAK, J. N. et al. Int. J. Radiat. Phys. Chem., 1, 585 (1969)
$HOOC-\dot{C}H_2$	X-irradiation of sarcosine·HCl ($HOOC-CH_2-NH_2-CH_3^+Cl^-$)	300°K	2.0056 2.0029 2.0040 iso: 2.004	$a^{H}_{\alpha_1}$: 21.5, 21.0, 13.7 iso: 22.0 $a^{H}_{\alpha(2)}$: 9.7, 21.0, 32.6 iso: 21.0	LASSMAN, G. and W. Dargrau, Mol. Phys. 21, 969
$HOOC-\dot{C}HNH_2-CH_3$	X-irradiation of sarcosine·HCl at 300°K	300°K	2.0033 2.0019 2.0057 iso: 2.003	$a^{CH}_{H_\alpha}$: 35.9, 23.0, 11.0 iso: 23.3 $a^{NH_2}_{H_\beta}$: 26.7, 22.7, 30.6 iso: 23.3 a^{N}: 3.4, 1.1, 3.9 iso: 2.8	LASSMAN, G. and W. Dargrau Mol. Phys. 21, 551 (1971)
$CH_3\dot{C}(COOH)-NHCO-C_6H_5$	X-irradiation of N-benzoyl alanine ($CH_3-CH(COOH)-NHCOC_6H_5$) at 300°K	300°K	2.0038 2.0014 2.0020 iso: 2.0034	a^{N}_{α}: 1.6, 2.0, 3.2 iso: 2.2 $a^{CH_3}_{H_\beta}$: 18.0, 17.0, 17.0 iso: 18.0	LASSMAN, G. and W. Dargrau Mol. Phys. 21, 551, (1971)
$CH_3\dot{C}H-NHCOC_6H_5$	X-irradiation of N-benzoyl alanine ($CH_3-CH(COOH)-NHCOC_6H_5$)		2.0020 2.0026 2.0025 iso: 2.0023	a^{N}_{α}: 3.2, 3.2, 2.7 iso: 3.0 $a^{CH_3}_{H_\beta}$: 20.3, 21.5, 21.0 iso: 20.9 $a^{CH}_{H_\alpha}$: 16.4, 15.0, 28.3	LASSMAN, G. and W. Dargrau Mol. Phys. 21, 551 (1971)

CARBON RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
$\dot{C}HFCONH_2$	X-irradiation of monofluoroacetamide at 300°K	300°K		a^{CH}_{α}: 34.3, 22.5, 11.1 iso: 22.5 a^{F}: 189, 16.1, 3.9 iso: 56.4 $a^{13}C$: 85	COOK, R.J. et al Mol. Phys. 7, 31 (1963)
$CF_3\dot{C}FCONH_2$	γ-irradiation of pentafluoropropionamide at 300°K	300°K	~2.0023	a^{CF}_{α}: 201, 12, ≤10 iso: 73.6 $a^{CF_3}_{\beta}$: 30,17,14 iso: 22.7	LOMIZ, R. J. Chem. Phys. 45, 1339 (1966)
$NaCO_2-CF_2\dot{C}FCO_2-Na^+$	X-irradiation of sodium perfluoro succinate at 300°K	300°K	2.0060 2.0039 2.0036 2.0045	a^{CF}_{α}: 151, 59, 4 iso: 71 $a^{CF_2}_{\beta}$(1): 62.8, 22.8, 18.6 iso: 34.7 $a^{CF_2}_{\beta}$(2): 71.4, 26.8, 22.8 iso: 40.4	FOCHER, H. and D. H. Whiffen J. Chem. Phys., 40, 2662 (1964)
$\dot{C}F_2CONH_2$	γ-irradiation of trifluoroacetamide ($CF_3CF_2CONH_2$) at 300°K	300°K	2.0025 2.0040 2.0049 iso: 2.003	$a^{19}F$: 178, 24.3, 24.3 iso: 75	LOMIZ, R. W. Gordy J. Chem. Phys., 37 (1962)
$HOOC-CH_2\dot{C}H-COOH$	γ-irradiation of fumaric acid-urea at 223°K	77°K 300°K		$aH_{\beta}(2)$: 33.2 $aH_{\beta}(2)$: 28.9 aH_{β}: 30.7	CORVAJA, C. J. CHEM. PHYS. 44, 1958 (1966)

CARBON RADICAL	GENERATION	ESR Temp.	g-FACTORS	SPLITTING PARAMETERS	REFERENCES
	X-irradiation of glycine at 77°K then warming to 110°K	77°K		a_{H_β}: 26	Sinclair, Y. J. Chem. Phys. 55, 245 (1971).
$H_3\overset{+}{N}\dot{C}HCOO^-$	γ-irradiation of glycine at 300°K	300°K		a_H: 3.46, 3.38, 2.50 iso: 3.1 a_H^{CH}: 34.26, 21.8, 11.32 iso: 22.6 $a_H^{NH_3}$: 19.12, 16.42, 15. iso: 17.17	Fedberg, A. and A. Ehrenburg, J. Chem. Phys. 48, 4822 (1968)
	X-irradiation of α-alanine - 1 ^{13}C at 4.2°K 150°K			135.5, 71.1, 62.1 iso: 89.7	Sinclair, J. and M.R. Hanna J. Chem. Phys. 50, 2125 (1969)
$CH_3\dot{C}HCOOH$	X-irradiation of alanine -1 ^{13}C at 4.2-150°K	300°K		$a\ ^{13}C_\beta$: 128 (i.e.) ⓐ	Sinclair, J. and M. R. Hanna J. Chem. Phys. 50, 2125 (1969)

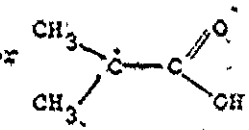
ⓐ Along 001 crystal axes

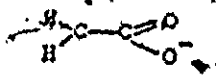
Carbon radical	Generation	ESR Temp.	g-factor	Splitting parameter	Reference
	X-irradiation of (deuterated) glycine at 77°K	77°K		a_H^{N}: 7 a_H: 26 (iso)	SINCLAIR, J. J. Chem. Phys. 55, 21[illegible] (1971)
	γ-irradiation of 5-NITRO-6 METHYL URACIL		2.002 2.005 2.003. iso.2.005	a_H^{OH}: 9.5, 0.5, 0.5 iso : 3.5 $a_H^{CH_3}$: 10.7, 8.8, 8.8 iso : 9.4	SNIPES, W. and DAWSON, [illegible] J. Chem. Phys 48, 4066 (19[illegible])
	γ-irradiation of cytosine monohydrate at 77°K	77°K	≈2.0023	a_H: 0.57, 2.26, 1.04 iso: $a_{N(3)}$: 0.22, 0.18, 0.93 iso a_N 0.77 (iso)	MORAN, J. and W. SNIPES[?] J. Chem. Phys. 50, 3101 (1969), values in millitesla
$(CH_3)_2\dot{C}CO_2^-$	irradiation of α-amino isobutyric acid at 77°K			$a_H^{CH_3}$: 24.2 (iso) $a_H^{CH_3}$: 43.5, 18.5, 10.7 iso: 24.3	Morton, J.R., J. Chem. Phys., 41, 2956 (19[illegible])

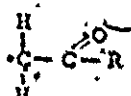
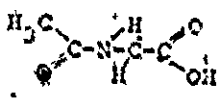
CARBON RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS	REFERENCES
 π	irradiation of thiodiglycolic acid [HOOC-CH_2-S-CH_2-COOH] at 4.2°K	4.2°K	2.0034 2.0030 2.0017 iso: 2.0027	CH_2: 9.8 ++ a_{H_β} CH_2: 22.1 a_{H_α}	Box, A.C. et al J. Chem. Phys., 3974 (1963).
	x-irradiation of α-amino isobutyric acid at 77°K	220°K	2.0024 2.0029 2.0033 iso:2.0029	** $a_H^{CH_3}$: 134.3 139.4 145.7 iso: 139.5	Box, H.C. & H.C. Freund, J. Chem. Phys., 44, 2345 (1966)
 †	x-irradiation or γ-irradiation of potassium hydrogen maleate at 77°K	77°K	2.0043 2.0039 2.0079 iso:2.0053	aH: 5.0 ††	Toriyama, K & Iwasaki, M., J. Chem. Phys., 55, 2181 (1971).
	x-irradiation or γ-irradiation of potassium maleate at 77°K or 300°K	300°K	2.0024 2.0045 2.0040 iso:2.0036	aH: 2.1, 7.0, 10.1 iso: 6.4	Toriyama, K & Iwasaki, M., J. Chem. Phys., 55, 2181 (1971); Iwasaki, M. and K. Itoh, Bull. Chem. Soc Japan 37, 44.

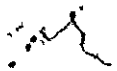
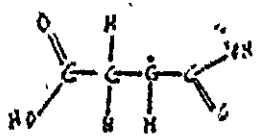
† sigma radical †† nearly isotropic ††ENDOR also used

** splitting arises from 2 sets of 3 equivalent protons.

* or 

CARBON RADICAL	GENERATION	ESR Temp	g-factors	SPLITTING PARAMETERS	REFERENCES
	X-irradiation of glycine-d_2 and glycine-d_5	145°K		a^{CH_2} aH: 31.0, 10.5, 19.2 iso: 20.2	SINCLAIR J., J. CHEM. PHYS., 55, 245 (1971).
$COO\text{-}\dot{C}HCH_2OH$	X-irradiation of dL-serine at 300°K	300°K		a^{H}_{α}: 34.2, 20.3, 11.6 iso: 22.0 $a^{H}_{\beta(1)}$: 39.9, 39.2, 35.2 iso: 37.8 $a^{H}_{\beta(2)}$: 16.0, 11.1, 9.7 iso: 12.3	CASTLEMAN, B.N., and G.C. MOULTON J. CHEM. PHYS., 55, 2598.
$(^{-}COO)\dot{C}H(^{+}NH_3)CH_2OH$	X-irradiation of dL-serine at 77°K	77°K	2.0031 2.0038 2.0023 iso: 2.0030	a^{H}_{β}: 19.4, 20.6, 22.9 iso: 21.0	CASTLEMAN, B.N., and G.C. MOULTON, J. CHEM. PHYS., 55, 2598 (1971)
$\cdot CH_2NH_3^+$	Y-irradiation of trithyl ammonium sulfate dodecahydrate at 300°K	300°K		$a^{NH_3}_{\beta}$: 19.2 (iso) $a^{CH_2}_{\alpha}$: 25 (iso) a^{N}: 4.1 (iso)	Kohn, R. and P.C. Nadeau J. Chem. Phys. 44, 691 (1966)

CARBON RADICALS	GENERATION	ESP-Temp	g-FACTORS	SPLITTING PARAMETERS	REFERENCES
	electron irradiation of glycylglycine at 77°K	77°K		$a^{H}_{\alpha(1)}$: 16.4 $a^{H}_{\alpha(2)}$: 27.2	MELO, T.B. INT. J. RADIAT. BIOL. 23, 247 (1973)
$H_3\overset{+}{N}CHCONHCH_2COO^-$	electron irradiation of glycyl glycine at 77°K	140°K			MELO, T.B., INT. J. RADIAT. BIOL. 23, 247, (1973).
$CNCHCONHCONH_2$	X-irradiation of cyanoacetyl urea at 300°K	300°K	2.0036 2.0034 2.0020 iso: 2.0030	a^{H}_{α}: 30.2, 19.7, 8.8 iso: 19.6 a^{N}: 11.1, 2.6, 0.45 iso: 4.7	LAU, P.H. and L.G. LIN, J. CHEM. PHYS. 51, 5139 (1969)
$H_2\dot{C}-\dot{C}H-R$	γ-irradiation of dL alanine at 300°K	300°K		all: 27, 27, 7 iso: 20.3 $a^{CH_3}_{H}$: 26 (iso)	HIYAGAWA, T. and GORDY, W. J. CHEM. PHYS., 32, 255 (1960).
	γ-irradiation of N-acetyl glycine at 300°K	300°K	2.0027 2.0032 2.0042 iso: 2.0034	$a^{CH}_{H_\alpha}$: 27, 17, 10 iso: 18	HIYAGAWA, T. et al, J. CHEM. PHYS., 33, 1599 (1960).
$H_3N^+CH_2CONHCHCO_2^-$	x-irradiation of α-glycyl glycine at 300°K	300°K		a^{H}_{α}: 16.8, 29.5 8.6 iso: 18.3	LIN, W.C. and C.A. McDOWELL, MP 4, 333 (1961)

CARBON RADICAL	GENERATION	ESR Temp.	g-FACTORS	SPLITTING PARAMETERS	REFERENCES
$CH_3(CH_2)_3\dot{C}H_2CHSCH_2CH_2)_4CH_3$ β α γ	X-irradiation of di-N-hexyl sulphide urea at 77°K	253... 293°K	2.0037 2.0037 2.0059 iso:2.0044	a^{H}_{α}: 25.4, 11.5, 11.5 iso: 16.1 a^{H}_{β}: 20.3, 18.9, 18.9 iso: 19.4 a^{H}_{γ}: 3.7	Griffith, O.H. H.M. Mallon J. Chem. Phys. 47, 837 (1967)
$CH_3\dot{C}HSCH_2CH_3$ β α γ 	X-irradiation of diethyl sulfido urea at 77°K	253... 293°K	2.0036 2.0059 2.0059 iso:2.0043		
$CH_3-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{\|}{N}}-\overset{\dot{C}}{\underset{H-C-H}{\overset{\|}{C}}}-\overset{O}{\overset{\|}{C}}-OH$ $H-C-H$ S CH_3	γ-irradiation of N-acetyl methione at 300°K	300°K	2.002 2.003 2.005 iso:2.0033	$a^{CH_2}_{H\beta}$: 10....12	Cipollini, E. and H. Gorpy J. Chem. Phys. 37, 13 (1962)
	X-irradiation of β-succinic acid at 300°K	300°K		a^{H}_{β}: 34.3, 35.2 39.2 iso: 35.9 a^{H}_{β}: 27.9, 29.2 29.9 iso: 29.0 a^{H}_{α}: 22.4, 29.3 18.1 iso: 23.3	Horsfield, A. and J.R. Morton Trans Faraday Soc. 58, 470 (1962)

CARBON RADICAL	Generation	ESR Temp	g factors	Splitting parameters in gauss	References
$CO_2^- \dot{C}F_2\ CO_2^-$	X-irradiation of sodium perfluoro-succinate at 200K	300°K	2.0060 2.0039 2.0036 iso: 2.004	a^F_α: 150, 58.9, 3.9 iso: 71.6 $a^F_{\beta_1}$: 62.8, 22.8, 16.5 iso: 34.04 $a^F_{\beta_2}$: 71.4 26.7, 22.8 iso: 40.35	Rodger, M.T. and D.H. Whiffen J. Chem. Phys. 40, 2662 (1964) Iwasaki, M. MP 20, 503 (1971)
$\dot{C}H_2\text{-}CO_2^-$	X-irradiation of zinc acetate dihydrate ($Zn(CH_2HCO_2)\cdot 2H_2O$)	120-140°K	2.00420 2.00242 2.00353 iso: 2.00338	$a^H_{\alpha 1}$: 9.5, 21.3, 32.9 iso: 21.2 $a^H_{\alpha 2}$: 9.4, 21.1, 33.1 iso: 21.2	Tollos, W.M. et al., J. Chem. Phys, 49, 4745 (1968)
$Cl^-(NH_3)^+\text{—}CH_2\text{—}C(=O)(OH)$	X-irradiation of Glycine Hcl at 4.2°K	4.2-175°K		$a^H_{\beta 1}$: 29 $a^H_{\beta 2}$: 11.0	Box, H.C. et al. J. Chem. Phys. 50(7), 2880 (1969)
$CH_3\dot{C}HCOO^-$	X-irradiation of Disodium succinate hexahydrate at 300°K	300°K	2.0044 2.0037 2.0025 iso: 2.0035	a^H_α: 32.7, 19.3, 12.6 iso: 21.5 $a^{CH_3}_{H\beta}$: 26.6, 24.5, 24.3 iso: 25.1	Bale., & et al. J. Chem. Phys. 50; 2880 (1969)

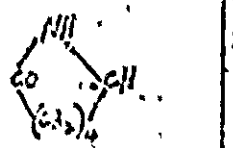
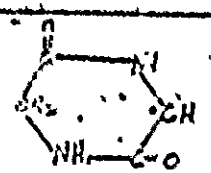
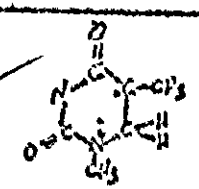
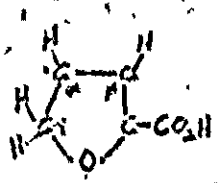
CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS in GAUSS	REFERENCES
$HOOCCH-\overset{\cdot}{C}(CH_3)-CH_2-COOH$	X-irradiation of 2-2'-methyl propane -1,3 dicarboxylic acid at 300°K	300°K		a_H^{CH}: 32.5,21.8,8.6 iso: 21.1	CVETKOV, Y.D. et al J. Chem. Soc. 810, (1964)
$HOOC-CH-\overset{\cdot}{C}(CH_3)-COOH$	X-irradiation of 2-methyl propane-1,2-dicarboxylic acid at 300°K	300°K		a_H^{CH}: 32.5,21.8,8.6 iso: 21.1	CVETKOV, YID. et al J. Chem. Soc, 810, (1964)
$\overset{\cdot}{C}H_2Cl-CONH-\overset{\cdot}{C}(CH_3)-COOH^*$	X-irradiation of Chloro Acetyl DL-alanine at 77°K	4.2... 77°K		$a_{H_\beta}^{CH_3}$: 22.3, 20.1, 17.2 * iso: 20.7	HELLS, J.N., and H.C. DON J. Chem. Phys. 48, 2542
$HOOC-\overset{\cdot}{C}H-CH_3$	Electron beam irradiation of acrylic acid at 77°K	77° 200°K		a_H^{CH}: 31.8,24.2,8.5 iso: 21.5 a_H^{CH3}: 22.3...28.1	SHIOJI, Y.S. et al J. Polymer Sci. Pt AI 3373 (1963)
$CH_3\overset{\cdot}{C}(COOH)-CH_2-COOH$	X-irradiation of Itagonic acid at 300°K	300°K	2.0025... 2.0038	$a_H^{CH_3}$: 24.6,23.8,23.0 iso: 23.8 $a_{H_\beta}^{CH_2}$: 14.9,18.8,16.0 iso: 16.8 $a_{H_\beta(1)}^{CH_2}$: 24 $a_{H_\beta(1)}$	FUJIMOTO H. J. Chem. Phys. 39(3) 846 (1963)

*ENDOR also used

CARBON RADICALS	GENERATION	ESR-Temp	g-FACTORS	SPLITTING PARAMETERS	REFERENCES
$CH_3\dot{C}HCO_2^-$	X-irradiation of strontium acetate hemihydrate at 77°K	300°K		$a_H^{CH_3}$: 30.9, 19.3, 11.1 iso: 20.5	Tollop, W.H. et al. J. Chem. Phys. 54, 1532 (197
$^{\bullet}CH_3$	X-irradiation of strontium acetate hemihydrate at 77°K	300°K		$a_H^{CH_3}$: 26.7, 24.1 24.1 iso: 25.0.	Tolles, W.M. et al. J.Chem. 54, 1532 (197
$\dot{C}H_3$	X-irradiation of zinc acetate dihydrate at 300°K	300°K		$a_H^{CH_3}$: 27.2, 24.9, 24.7 iso : 25.6	Tolles, W.M. et al J. CHEM. PHYS. 54, 1532 (1970)
	X-irradiation of deuterated DL-Tartaric acid at 77°K	77°K	2.0037 2.0032 2.0025 iso: 2.0031	a_H^{CH}: 26.2, 27.9 21.6 iso: 23.5	Moulton, G.C. and Cornachy, M.P. J. Chem. Phys. 51,2283 (1969)
$CH\dot{C}HCOO^-$	X-irradiation of disodium succinate hexahydrate	300°K	2.0044 2.0037 2.0025 iso: 2.0035	a_H^{CH}: 32.7, 19.3, 12.6 iso : 21.5 $a_{H_\beta}^{CH_2}$: 26.6, 24.5, 24.5 iso : 25.1	Bales, B. J. Chem Phys. 51, 1974 (1969)

*Radical formed at 2 magnetically inequivalent sites

CARBON RADICAL	GENERATION	ESR TEMP.	G-FACTORS	SPLITTING PARAMETERS	REFERENCES
$CH_3\dot{C}H_2O(CH_2)_4OCHCH_3$	X-irradiation of 1,4-diethoxybutane at 77°K	273°K	2.0040 2.0030 2.0030 iso: 2.0033	a^{CH}_{α}: 22.5, 8.6, 8.6 iso: 13.2 $a^{CH_2}_{\beta}$: 22.1, 20.2 20.2 iso: 20.8 $a^{CH_2}_{\gamma}$: 3.03	GRIFFITH, O.H. J. CHEM. PHYS. 42, 2651 (1965)
$CH_3\dot{C}^{=O}CH(CH_2)_5CH_3$	X-irradiation of 2-nonanone at 300°K	298°K	2.0040 2.0044 2.0044 iso2.0043	a_{α}: 27.1, 13.57, 13.57 iso: 18.1 a_{β}: 25.35, 16.07, 16.07 iso: 19.2 a_{β}: 16.7, 16.1, 16.1 iso: 16.3 a_{γ}: 1.9	GRIFFITH, O.H. J. CHEM. PHYS. 42, 2644 (1965)
$CH_3(CH_2)_4\dot{C}^{=O}CH(CH_2)_5CH_3$	X-irradiation of 6-undecanone at 300°K	298°K	2.0040 2.0044 2.0044 iso2.0043	a_{α}: 27.1, 13.6, 13.6 iso: 18.1 a_{β}: 25.4, 22.5, 22.5 iso: 23.5 a_{β}: 17.1, 16.1, 16.1 iso: 16.4 a_{γ}: 3.2	
$CH_3(CH_2)_{10}\dot{C}^{=O}CHCH_3$	X-irradiation of 3-tetradecanone	310°K	2.0040 2.0044 2.0044 iso2.0043	a_{α}: 26.7, 13.9, 13.9 iso: 18.2 a_{β}: 22.8, 20.7, 20.7 iso: 21.4 a_{β}: 22.8, 20.7, 20.7 iso: 21.4 a_{γ}: 3.2	

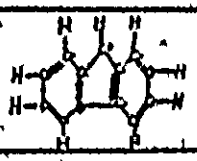
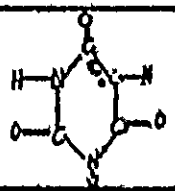
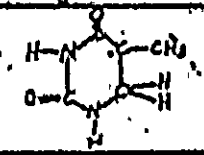
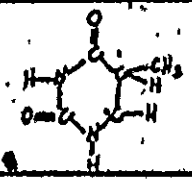
CARBON RADICAL	GENERATION	ESR Temp.	g-FACTORS	SPLITTING PARAMETERS	REFERENCES
	X-irradiation of ε-caprolactam at 300°K	300°K	2.0025 2.0032 2.0039 2.0032	a_{NH}: 0.1, 2.1, 3.1 a_{iso}: 1.7 a_{H_4}: 10.3, 18.3, 32.5 a_{iso}: 20.4 $a_{H_3^{CH}}$: 5.0, 7.0, 11.0 a_{iso}: 7.6 $a_{H_3^{CH}2}$: 34.3, 36.7, 43.2 a_{iso}: 38.1	KASHIWAGI, M. Y. KURITA, J. CHEM. PHYS., 40, 1780, (1964)
	X-irradiation of 1,4, diketopprporazine at 300°K	300°K	2.0045 2.0040 2.0025 a_{iso}: 2.0037	a_{CH}: 20.3, 15.9, 7.4 a_{iso}: 16.6 a_{CH_2}: 9(1?0) a_{NH}: 4, 1.5, 1 a_{iso}: 2	LIN, W.C. and C.A. MCDOWELL, CAN. J. CHEM. 41, 9, (1963) HIYAGAWA, I. Tech. Rept. ISSP A 27, (1961).
	γ-irradiation of 1-methyl thymine	85°K ↓ 133°K		a_{CH_3}: 19.9 a_{CH_2} (1): 36.8 a_{CH_3} (2): 42.0	SNIPES, W. and J. SCHMIDT, J. CHEM. PHYS., 40, 1445 (1964)
	γ-irradiation of furane-2-carboxylic acid at 300°K	300°K		a_{CH_α}: 13.9, 8.9, 4.3 a_{iso}: 9.0 a_{CH_β}: 3.6, 2.1, 1.1 a_{iso}: 2.2 a_{CH_γ}: 31.5	COOK, R.J. et al., M.P. 7, 57, 1964.

CARBON RADICAL	GENERATION	ESR Temp.	g-FACTORS	SPLITTING PARAMETERS	REFERENCES
$ROCH\,COOH$	γ-irradiation at 300°K of glycollic acid.	300°K	2.0053 2.0036 2.0017 iso:2.0036	a_H^{CH}: 30.7, 19.7, 10.7 iso: 20.4 a_H^{OH}: 10.0, 2.5, 1.4 iso: 2.3	Atherton, N.M. and D.H. Moffat Mol. Phys., 3, 1, (1960).
$CH(CO_2H)_2$	γ-irradiation of malonic acid at 300°K	300°K		a_{H_2}: 10.0, 20.7, 32.5 iso: 21.1	Horsfield, A. et al, Mol. Phys., 1, 327, (1961).
$CH_2(CO_2H)$	γ-irradiation of malonic acid at 300°K	300°K	2.0051 2.0034 2.0042 iso:2.0042	$a_H^{CH_2}(1)$: 10.7, 19.7, 32.5 iso: 22.1 $a_H^{CH_2}(2)$: 13.2, 21.0, 32.8 iso: 22.5	Horsfield, A. et al Mol. Phys., 1, 327, (1961). Horsfield, A. et al Nature 199, 169 (1961).
$HO\text{-}\overset{H}{C}\text{-}CCO^-Li^+ \cdot H_2O$	γ-irradiation of lithium glycolate monohydrate at 300°K	300°K	2.0051 2.0039 2.0031 iso:2.0037	a_H^{CH}: 28.1, 16.8, 8.2 iso: 18.4 a_H^{OH}: 6.3, 4.4, 2.9 iso: 4.6	Pooley, D and D.H. Whiffen, Trans. Faraday Soc., 57, 1445, (1961).
$HO\text{-}\overset{H}{C}\text{-}CCO^-Li^+$	γ-irradiation of lithium glycolate (anhydrous) at 300°K	300°K	2.0043 2.0042 2.0021 iso:2.0035	a_H^{CH}: 30.4, 18.2, 9.6 iso: 19.3 a_H^{OH}: 21.4, 12.5, 8.2 iso: 13.9	Pooley, D and D.H. Whiffen, Trans. Faraday Soc., 57, 1445 (1961).
$OOC - \overset{H}{C}H\text{-}CH_2\text{-}(CH_2)_2\text{-}COO^-.2\overset{+}{N}(CH_3)_3$	γ-irradiation of hexamethylenediammonium adipate at 300°K	300°K		a_H^{CH}: 31.7, 18.3, 8.9 iso: 19.6 $a_H^{CH_2}$: 45 $a_H^{CH_2}$: 20	Kashiwagi, H and Y. Kurita, J. Chem. Phys., 39, 3165 (1963).

CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS in GAUSS	REFERENCES
$^{\bullet}CF_3$	electron irradiation of trifluoroacetamide at 77°K	77°K	2.0046 2.0038 2.0024 iso: 2.003	aF: 92, 88, 25.3 iso: 144.6 $a^{13}C$: 236, 257, 318 iso: 271	Rodgers, M. and Kispert, L.D. J. Chem. Phys. 46, 3193 (1967)
$\dot{C}F_2CONH_2$	electron beam irradiation of trifluoroacetamide at 77°K	77°K	2.0026 2.005 2.004 2.0038	a F: 16,14,202 iso: 77.3 $a^{13}C$: 147.5 aF: 23.5, 14.0, 18.0 iso: 72.5 $a^{13}C$: 67.6, 66.1, 130 iso: 88.2	
$CF_3\dot{C}FCONH_2$	X-irradiation of			aF: 36, 17, 14 iso: 22.3	Iwasaki, M. JMP 20, 503 (1971)
$HOOC-\dot{C}H-CH_3$	γ-irradiation of l-α-alanine ($HOOC-\underset{NH_2}{CH}-CH_3$) at 300°K	300°K		a_{II}^{CH}: 31.9, 17.8, 8.9 iso: 19.5 $a_{II}^{CH_3}$: 23.9, 24.0, 27.1 iso: 25.0 ⊕	Morton, J.R. & A. Horsfield, J. Chem. Phys. 35, 1142 (1961)

⊕ nearly isotropic and temperature dependent

Carbon Radical	Generation	ESR Temp.	G-factors	Splitting parameters in gauss	References
$CH_3\dot{C}OCH(CH_2)_7CH_3$	X-irradiation of 2-undecanone at 300°K	313	2.0040 2.0044 2.0044 iso:2	all_δ: 1.8 all_α: 27.5, 13.92 13.9 iso: 18.4 all_β: 25.3, 24.3, 24.3 iso: 24.6 all_γ: 18.9, 17.8, 17.0 iso: 18.1	Griffith, O.H. J. Chem. Phys. 42, 2444 (1965)
$CH_3C\dot{C}H(CH_2)_8CH_3$ O	X-irradiation of 2-dodecanone at 300°K	322	2.0040 2.0044 2.0044 iso:	all_α: 20.7, 13.6, 13.6 iso: 17.9 all_β: 25.0, 23.6, 23.6 iso: 24.0 all_γ: 18.6, 17.5 iso: 17.8 all_γ: 1.9	
$CH_3(CH_2)_7\dot{C}OCH_3$ O	X-irradiation of 3-undecanone at 300°K	298	2.0040 2.0044 2.0044	all_α: 20.7, 13.6, 13.6 iso: 17.9 all_β: 23.2, 20.3, 20.3 iso: 21.2 all_β: 23.3, 20.3, 20.3 iso: 21.3 all_γ: 3.3	

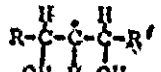
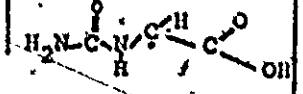
CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS in GAUSS	REFERENCE
	X-irradiation of dimethyl (9-fluorenyl) sulphonium bromide at 300°K	300°K	2.0038 2.0042 2.0045 iso2.0041	all: 20.3, 4.9, 3.9 iso: 9.7	LUCKEN, E.A.C. and C. HAZELINE J. CHEM. PHYS.48, 1942, (1968).
	γ-irradiation of Barbituric acid dihydrate at 300°K	300°K	2.0044 2.0056 2.0022 iso2.0040	all: 11.2, 30.0, 20.4 iso: 20.5	BERNHARD W. and W. SNIPES, J. CHEM. PHYS., 44, 2817, (1966).
	electron beam irradiation at 295 and 77°K of dihydrothymine	295°K 77°K			HENRIKSEN T. W. SNIPES, J. CHEM. PHYS., 52, 997 (1970).
	electron beam irradiation at 295°K and 77°K of dihydrothymine	295°K and 77°K	2.0039 2.0031 2.0022 iso2.0030	$all^{CH_3}_{\alpha}$: 7.8, 26.9, 17.5 iso: 17.4 all_{β}: 44.0	

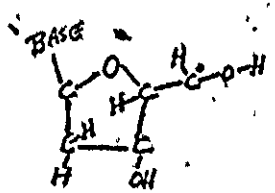
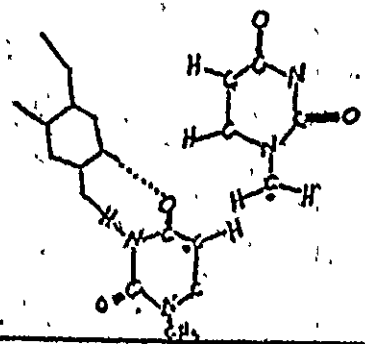
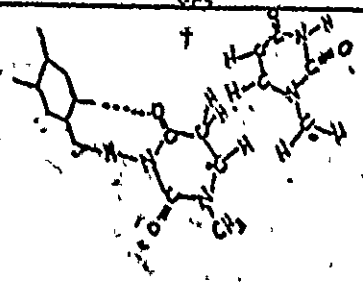
CARBON RADICAL	GENERATION	ESR TEMP.	G-FACTORS	SPLITTING PARAMETERS	REFERENCES
$CH_3CH_2O(CH_2)_4\dot{C}HCH_3$	X-irradiation of 2,6-diethoxybutane at 77°K	273°K	2.0030 2.0030 2.0030 iso: 2.0033	a_α^H: 32.5, 8.6, 8.6 iso: 13.2 $a_\beta^{CH_2}$: 22.1, 20.2 20.2 iso: 20.8 $a_\gamma^{CH_2}$: 3.03	GRIFFITH, O.H. J. CHEM. PHYS. 42, 2651 (2965)
$CH_3\dot{C}(=O)CH(CH_2)_5CH_3$	X-irradiation of 2-nonanone at 300°K	298°K	2.0040 2.0044 2.0044 iso2.0043	a_α^H: 27.1, 13.57, 13.57 iso: 18.1 a_β^H: 25.35, 16.07, 16.07 iso: 19.2 a_γ^H: 16.7, 16.1,16.1 iso: 16.3 a_δ^H: 1.9	GRIFFITH, O.H. J. CHEM. PHYS. 42, 3644 (1965)
$CH_3(CH_2)_4C(=O)\dot{C}H(CH_2)_5CH_3$	X-irradiation of 6-undecanone at 300°K.	298°K	2.0040 2.0044 2.0044 iso2.0043	a_α^H: 27.1, 13.6,13.6 iso: 18.1 a_β^H: 25.4, 22.5,22.5 iso: 23.5 a_β^H: 17.1,16.1, 16.1 iso: 16.4 a_γ^H: 3.2	
$CH_3(CH_2)_{10}C(=O)\dot{C}HCH_3$	X-irradiation of 2-tetradecanone	310°K	2.0040 2.0044 2.0043 iso 2.0043	a_α^H: 26.7, 13.9, 13.9 iso: 18.2 a_β^H: 22.8, 20.7, 20.7 iso: 21.4 a_β^H: 22.8, 20.7, 20.7 iso: 21.4 a_γ^H: 3.2	

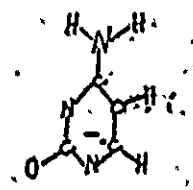
CARBON RADICAL	GENERATION	ESR Temp.	g-factors	SPLITTING PARAMETERS	REFERENCES
$NH_4OOCCH_2\dot{C}OHCOOH$	γ-irradiation of monoammonium L-malate at 300°K	300°K	2.0045 2.0028 2.0018 iso: 2.0034	$a_{\beta(1)}^{CH_2}$: 34.0, 34.0 36.0 iso: 34.3 $a_{\beta(2)}^{CH_2}$: 7.8, 11.0, 7.3 iso: 8.9	RAO, D. and R.S. ANDERSON, J. CHEM. PHYS. 43, 2899 (1965).
$NH_4OOCCH_2\dot{C}OHCOOH$	γ-irradiation of monoamonium L-malate monohydrate at 300°K	300°K	2.0038 2.0024 2.0032 2.0031	$a_{\beta(1)}^{CH_2}$: 32.5, 33.4 36.3 iso: 34.1 $a_{\beta(2)}^{CH_2}$: 13.2, 13.8 17.2 iso: 14.7 a_H^{OH}: 0, 1.0, 6.0 iso: 2.3	RAO, D. and R.S. ANDERSON, J. CHEM. PHYS. 43, 2899 (1965)
$NH_4OOCCHOHCOH\text{-}COONH_4$	γ-irradiation of ammonium tartrate at 300°K	300°K	2.0025 2.0025 2.0032 iso: 2.0027	a_β^{CH}: 0.7, 1.0, 4.6 iso: 2.1	
$KOOCCHOHCOHCOOK$	γ-irradiation of potassium tartrate at 300°K	300°K	2.0021 2.0027 2.0030 iso: 2.0026	a_β^{CH}: 0.2, 3.5, 2.8 iso: 2.2	
$CH_3(CH_2)_3\dot{O}CH(CH_2)_2CH_3$	X-irradiation at 77°K of dibutyl ether used	273°K	2.0040 2.0030 2.0030 iso: 2.0033	a_α^{CH}: 22.8, 28.4, 8.4 iso: 13.2 $a_{\beta 2}^{CH_2}$: 22.5, 21.5, 21.5 iso: 21.8 $a_{\gamma 2}^{CH_2}$: 8.2	GRIFFITH, O.H. J. CHEM. PHYS. 42, 2651, (1965)
$CH_3(CH_2)\dot{C}HOCH_3$	X-irradiation of methyl octyl ether urea at 77°K	273°K	2.0040 2.0030 2.0030 iso: 2.0033	a_α^{CH}: 22.5, 8.6, 8.6 iso: 13.2 $a_\beta^{CH_2}$: 22.5, 21.5 21.5 iso: 21.8 $a_\gamma^{CH_2}$: 3.03	

CARBON RADICAL	GENERATION	ESR	g-VALUES	SPLITTING PARAMETERS	REFERENCES
$CH_3\dot{C}HCOOCH_2(CH_2)_6CH_3$ β α γ	x-irradiation of n-octyl acrylate at 77°K	300°K		$a_{H_\alpha}^{CH}$: 30.5, 15.1, 15.1 iso: 20.2 $a_{H_\beta}^{CH_3}$: 26.1, 23.9, 23.9 iso: 24.6 $a_{H_\gamma}^{CH_2}$: 3.4	WEDUM, E. et al. Trans. Far. Soc. 63, 821 (1967)
$CH_3\dot{C}H_2CHCOOCH_2(CH_2)CH_3$ β α γ	x-irradiation of dodecyl crotonate at 77°K	300°K		$a_{H_\alpha}^{CH}$: 30.5, 14.8, 14.8 iso: 20.0 $a_{H_\beta}^{CH_2}$: 21.5, 19.7, 19.7 iso: 20.3	WEDUM, E. et al. Trans. Far. Soc. 63, 821 (1967)
$CH_3-\dot{C}-COOCH_2(CH_2)_{10}CH_3$ β CH_3 γ	x-irradiation of lauryl methacrylate at 77°K	300°K		$a_{H_\beta}^{CH_3}$: 21.6, 21.4, 21.4 iso: 21.4 $a_{H_\gamma}^{CH_2}$: 1.1	WEDUM, E. et al. Trans. Far. Soc. 63, 821 (1967)
$CH_3(CH_2)_5CH_2-\dot{C}H-COOCH_3$ β α γ	x-irradiation of methyl nonenoate at 77°K	300°K		$a_{H_\alpha}^{CH}$: 30.6, 14.9, 14.9 iso: 20.1 $a_{H_{\beta_1}}^{CH_2}$: 23.1, 22.6, 22.6 iso: 22.7 $a_{H_{\beta_2}}^{CH_2}$: 30.6, 30.6, 30.6 iso: 30.6 $a_{H_\gamma}^{CH_3}$: 1.1	WEDUM, E. et al. Trans. Far. Soc. 63, 821 (1967)
$CH(\dot{C}ONH_2)_2$	γ-irradiation of malonamide at 300°K	300°K	2.0016 2.0040 2.0040 iso: 2.0032	aH: 10.2, 21.1, 31.8 iso: 20.8	Rexroad, H.N. et al. J. Chem. Phys., 42, 324 (1965)

CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS in GAUSS	REFERENCES
$HOOCCH{=}\dot{C}H\,COOH$	X-irradiation of glutaconic acid at 300°K	300°K	2.0021 2.0036 2.0036 iso: 2.0031	$a^{CH}_{H_\alpha}$: 15.3, 3.5, 4.2 iso: 17.7 $a^{CH}_{H_\beta}$: 5.4, 0.96, 0.28 iso: 2.33	Holler C. and T. Cole J. Chem. Phys. 37, 382 (1962)
$CH_3-\dot{C}-(COOH)_2$	X-irradiation of methyl malonic acid at 300°K	4.2 °K 77 °K 300°K	2.0026 2.0034 2.0044 iso: 2.0035	$a^{CH_3}_{H_\beta}$: 26.9, 24.6, 24.5 iso: 25.3	Holler, C. J. Chem. Phys. 36, 175 (1962)
$HOOC-\dot{C}H-CH(NH_2)-COOH$	X-irradiation of dl-aspartic acid at 300°K	300°K		$a^{CH}_{H_\alpha}$: 27.5, 22.8, 17.1 iso: 22.5 $a^{CH_2}_{H_\beta}$: 41.1, 44.6, 40.7 iso: 42.1	Jascja, T. and Anderson R. J. Chem. Phys. 36, 2727 (196
$CH_3\dot{C}HR$	X-irradiation of l-alanine at 300°K	300...370°K		aD: 4.0	Itoh, K and Miyagawa, I. J. Chem. Phys. 40, 3328 (1964)

Carbon Radical	Generation	ESR Temp	g-factors	Splitting parameters in gauss	References
$CH_2\dot{C}HR$ or $CH_3\dot{C}DR$	X-irradiation of L-alanine at 300°K	300°K		$a^{H}_{H_\alpha} = a^{H}_{H_\beta}{}^{CH_3}$ 26.3	I Toh, K. and Miyagawa, I. J. Chem. Phys. 40, 3328 (1964)
$Cl^- \; NH_3^+-CH_2-CONH$ $HOOC-\dot{C}H$	irradiation of glycyl glycine-HCl at 300°K.	300°K	2.0026	$a^{CH}_{H\alpha}$: 25.4, 20.9, 10.8 iso: 19.1 a^{NH}_{H} 7.0	Box, H.C. et al, J. Chem. Phys, 37 2100 (1963)
	X-irradiation of sucrose at 300°K	300°K	2.0036	a^{CH}_{H}: ≈19 a^{CH}_{H}, a^{CH}_{H}: ≈5.0	Shields, H. and P. Hawrock J. Chem. Phys. 37 202 (1962)
	γ-irradiation of N-Carbamyl glycine	300°K		a^{CH}_{H}: 26.0, 22.0, 10 iso: 19.3 a_N: 3.0, 4.0, 2.0 iso: 3.0	Rao, D.V.G.L.N. and M. Katayama J. Chem. Phys. 37 382 (1962)

CARBON RADICALS	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS (in gauss)	REFERENCES
	x-irradiation of deoxyadonosine monohydrate at 300°K	300°K	2.0029 2.0052 2.0075 iso:2.0052	$a^{CH}_{H\alpha}$: 16.4,22.6,5.0 iso: 14.6 $a^{CH}_{H\beta}$: 7.0	Alexander, C and C.E. Franklin, J. Chem. Phys., 54, 909 (1971).
† 	γ-irradiation of 1-methyl uracil at 77°K	77°K.... 173°K		$a^{CH_2+CH}_{H_\alpha}$: 13.5 $a^{CH_2}_{H_\beta}$: 27.0	Dulcio, A., and J.N. Herak, Mol. Phys., 26, 605
† 	γ-irradiation of 1 methyl uracil at 77°K	77°K		$a^{CH_2+CH}_{H_\alpha}$: 13.5 $a^{CH_2}_{H_\beta}$: 18.0	Dulcio, A., J.N. Herak, Mol. Phys., 26, 605
† radical pairs formed					

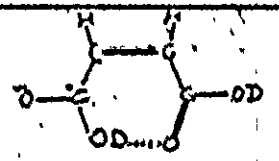
CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS in GAUSS	REFERENCES
	irradiations of cytosine monohydrate at 77° K	77° K		all: 11.0, 21.0, 11.0 iso: 14.0	Herak, J.N. and V. Galogazga, J. Chem. Phys. 50, 3101 (1969)
$\dot{C}H(CONH_2)_2$	x-irradiation of malonamide at 300 K	300° K	2.0028 2.0023 2.0034 iso: 2.0028	a_{IIa}^{CH}: 7.9, 21.9, 32.9 iso: 20.9	Cyr, N and N.C. Lin, J. Chem. Phys., 50 3701
NH_2COCH_2CONH *	x-irradiation of malonamide at 300 K	300° K	2.0019 2.0043 2.0032 iso: 2.0031	a_{α}^{III}: 84.3, 79.6, 80.0 iso: 81.3	Cyr, N and W.C. Lin, J. Chem. Phys, 50, 3701 (1969)
$CHCH_2CONHCONH$ *	x-irradiation of malonamide at 300 K	300° K	2.0032 2.0026 2.0018 iso: 2.0025	alla: 87.0, 85.4, 84.8 iso: 85.7 aN: 21.8, 7.4, 5.7 iso: 11.6	Lay, P.N. and W.C. Lin, J. Chem. Phys., 51, 5139 (1969)

* sigma radical

CARBON RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
$^{\cdot}CH_3$	Electron irradiation of sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) at N°K	N°K		$a^{13}C$: 82.7,15.5,15.0 iso: 37.7 all: 22.5, 22.5, 21.8 iso: 22.3	Rodgers, M.T. and L.D. Kispert, J. Chem. Phys., 45, 221 (1967).
$CH_3CH_2\dot{C}(COOH)_2$	γ-irradiation of ethyl malonic acid at 300°K	300°K		CH_2: a^{H}_{β}(1): 26.8,25.4,22.9 iso: 25.0 CH_2: a^{H}_{β}(2): 22.1,20.0,18.9 iso: 20.4	Rowlands, J.R. and D.H. Whiffen, Mol. Phys., 4, 349 (1961).
$^{\cdot}CH_3$	γ-irradiation of sodium acetate trihydrate at 77°K	77°K	2.0023	$a^{13}C$: 21.5,13.8,82.7 iso: 39.3 all: 22.4,22.5,21.3 iso: 22.1	Janecka, J., et al J. Chem. Phys., 54, 3229 (1971).
$H_3\dot{C}CHR$	γ-irradiation of l-alanine d-l-alanine at 300°K	77°K		CH_3 ⊕: all: 27.7,25.4,26.2 iso: 25.4 CH: a^{H}: 18.3,21.2,27.6 iso: 22.4	Miyagawa, I and K. Itoh, J. Chem. Phys., 36, 2157 (1962
		293°K		CH_3 ⊕: a^{H}: 24.3,23.4,26.3 iso: 25.4 CH: a^{H}: 18.8,19.9,26.3 iso: 21.7	
		300°K		CH_3 ⊕⊕: a^{H}: 27.5,25.0,25.0 iso: 25.8 CH: a^{H}: 8.4,16.6,32.0 iso: 19.6	⊕values are for non-deuterated compounds. ⊕values are for deuterated compounds.

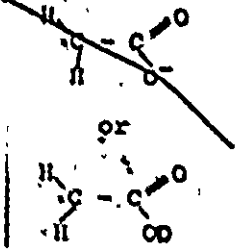
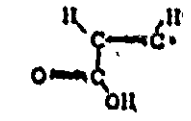
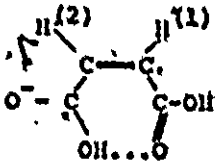
CARBON SKELETAL	GENERATION	USE TEMP.	g-FACTORS	HYPERFINE PARAMETERS (in Gauss)	REFERENCES
$(CO_2H)\dot{C}H(CH_2)_2(CO_2H)$	γ-irradiation of glutaric acid at 300°K and 77°K	300°K		$a_{H_\alpha}^{CH}$: 31.4;18.9; 9.3 iso: 20.0 $a_{H_{\beta(1)}}^{CH_2}$: 46.8;43.9;39.6 iso: 43.6 $a_{H_{\beta(2)}}^{CH}$:16.4;14.2; 19.2 iso: 16.8 a_{H}^{CH}: 28.2;18.6;8.2 iso: 18.2 $a_{H_{\beta(1)}}^{CH_2}$: 39.3;37.1;38.2 iso: 38.2 $a_{H_{\beta(2)}}^{CH_2}$: 13.6;11.1, 10.7 iso: 11.8	Horsfield A. et al Mol. Phys., 4, 169 (1961).
$(CO_2H)\dot{C}H(CH_2)_3(CO_2H)$	γ-irradiation of adipic acid at 300°K	300°K		a_{H}^{CH}: 33.5;17.7; 9.9 iso: 21.8 $a_{H_{\beta(1)}}^{CH_2}$: 26.4 $a_{H}^{CH_2}{}_{\beta(2)}$: 40.0	Morton, J.R. and A. Horsfield, Mol. Phys., 4, 219, (1961).

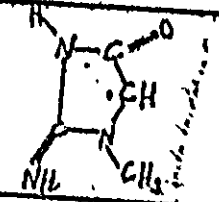
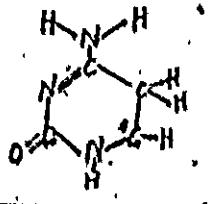
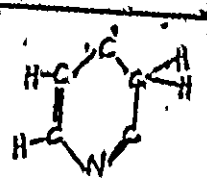
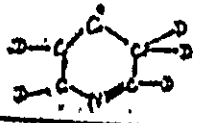
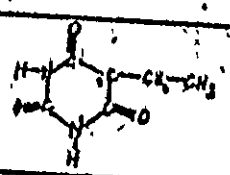
*Radical has 2 different sites giving 2 sets of splitting parameters.

Carbon radical	Generation	EPR Temp	g values	Splitting parameters	Reference
CO_2^-	X-irradiation of ^{17}O enriched sodium formate (HCOONa) at 300°K	300°K		$a^{13}C$: 155.0, 150.0, 195 iso: 167 $a^{17}O$: 22.1, 23.5, 50.0 iso: 32.2 $a^{17}O$: 21.8, 22.9, 51.2 iso: 32.0	SCHLICK, S. et al J. CHEM. PHYS. 55 867 (1971)
CO_2^-	γ-irradiation of calcite	77°K	2.0032 1.9980 2.0035 iso 2.0011	$a^{19}F$: 43.9, 93.2, 43.9 iso: 60.3 ⊕	MARSHALL, S.A. and McMillan, J.A. J. CHEM. PHYS. 49, 4887 (1968)
	X or γ-irradiation Maleic acid-d_2 at 77°K	77°K	2.0040 2.0043 2.0024 iso: 2.0035	all_β: 13.0, 3.2, 8.5 iso: 8.2 all_γ: 6.4, 1.9, 5.2 iso: 4.5	Eda, B. and H. Iwasaki Mol. Phys., 24, 589 (1972)
$HOCHCOO^-$	γ irradiation of Potassium glycollate at 300°K	300°K	2.0054 2.0039 2.0021 2.0038	all_α: 28.6, 18.2, 8.2 iso: 18.2 OH all_γ: 6.4, 4.3, 2.9 iso: 4.5	Atherton, N.M. and D.H. Whiffen, Mol. Phys., 3, 103 (1960).
$^-OCOCHO^-$	γ irradiation of sodium formate at 300°K	300°K	2.0022 2.006 2.006 2.0004	all_α: 22.2, 6.9, 14.1 iso: 14.4	Collins, R.F., S. Clough, Mol. Phys. 10, 33 (1965).

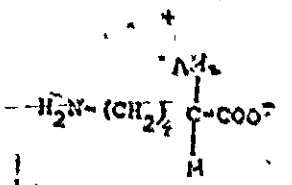
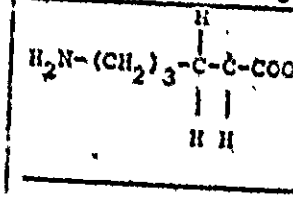
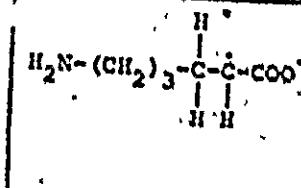
* ENDOR also used

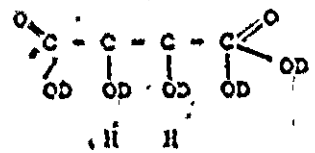
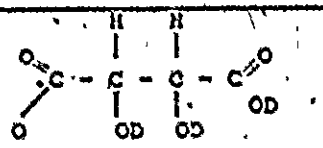
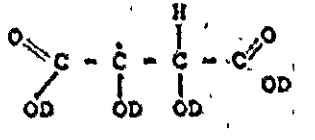
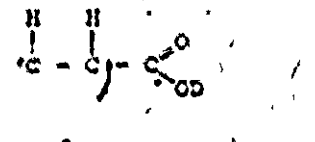
⊕ unpaired electron spends 3.3% of the time on CO_2^- molecule thereby creating CO_2^{2-} ion

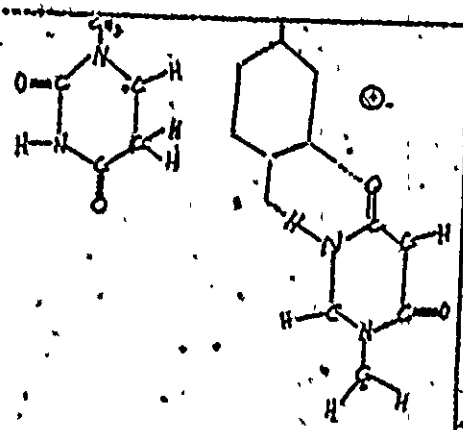
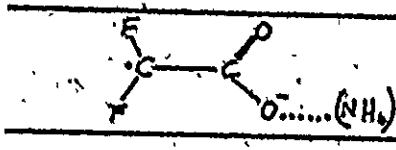
CARBON RADICAL	GENERATION	ESR TEMP.	g-VALUES	SPLITTING PARAMETERS in GAUSS	REFERENCES
	x-irradiation of deuterated glycine at 77°K, then warmed to 165°K	4.2°K	2.0025 2.0038 2.0044 iso:2.0035		Box, H., Freund, H., and Budzinski, E. J. Am. Chem. Soc. (1966) 659
	irradiation of deuterated malaic acid at 77°K	77°K	2.0032 2.0026 2.0018 iso:2.0025	$a_{H\alpha}$: 27, 9.6, 3.9 iso: 13.5 $a_{H\beta}$: 58.	Iwasaki, M. et al J. Am. Chem. Soc., (1970) 3211
	irradiation of deuterated malaic acid at 77°K	77°K	2.0043 2.0040 2.0024 iso:2.0036	$a^{(1)}_{H}$: 8.5, 3.2, 13.0 iso: 8.2 $a^{(2)}_{H}$: 6.4, 5.2, 1.9 iso: 4.5	Iwasaki, M. et al J. Am. Chem. Soc. (1970) 3211
CO_2^-	γ or x-irradiation of potassium hydrogen Malcate at 77°K in the dark and then continued exposure to visible light (for almost 20 min.)	77°K	2.0047 2.0021 1.9957 iso:		Tonyama, K. et. al J. Am. Chem. Soc. (1971) 6415
HOOC-CH-CH-COOH R	γ-irradiation of fumaric acid	300	2.0043 2.0033 2.0029 iso:2.0035	$a^{CH}_{H\alpha}$: 32.1, 20.3, 11.3 iso: 21.4 $a^{CH}_{H\beta}$: 22.8, 19.6, 18.2 iso: 20.0	Cook, R.J. et al. J. Chem. Soc. (1963) 3520

CARBON RADICAL	GENERATION	ESR TEMP.	g-VALUE	SPLITTING PARAMETERS	REFERENCES
	x-irradiation of creatinine at 300°K	300°K		a_H^{CH}: 24.3, 11.7, 18.0 iso: 18.0 (ϵ_{CN}(max)=4.1) +	Ueda, H., J. Chem. Phys., 40, 901 (1964)
	γ-irradiation of cytosine monohydrate at 300°K	300°K	2.0023	allα: 17.8, 12.8, 28.2 iso: 19.6 allβ: 37.1	Cook, J.S et al, Mol. Phys., 13 49 ( )
	electron irradiation at 77°K of pyridine	173°K		$a_2=a_4=a_6$: 12 Hα a_{3H_β}: 24	Tauji, K et al J. Chem. Phys., 45 2894 (1966)
	γ-irradiation of pyridine -d5 at 77°K	173°K		a_{3D_β}: 7	Tauji, K et al J. Chem. Phys., 45, 2894 (1966)
 **	x-irradiation of 5,5-diethyl barbituric acid	300°K		all_β 11.6	Haak, R. J. Chem. Phys., 55, 3693 (1971)

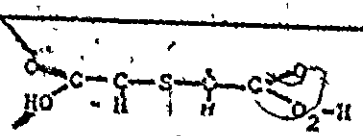
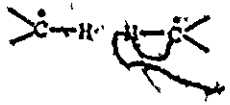
*Maximum value of anisotropic a_N; **unpaired electron stabilized via resonance structures

CARBON RADICAL	GENERATION	ESR TEMP.	g-FACTORS	COUPLING CONSTANTS IN GAUSS	REFERENCES
	γ-irradiation of L-Lysine·HCl·$2H_2O$ at 77°K and also α-amino isobutyric acid at 77°K	77°K	2.001... 2.003	a_{II_β}: 4,3,11 iso: 6	Fujimoto M., et al J. Chem. Phys., 48, 3345 (1968).
	γ-irradiation of L-Lysine·HCl·$2H_2O$ at 77°K, then stored overnight at 90°K or stored at 77°K for more than 1 month.	77°K... 130°K	2.001... 2.003	a_{II_α}: 31.8, 18.7, 9.6 iso: 20.0 $a_{II_\beta}^{CH_2}$: 37.5...38.5	Fujimoto, M., et al, J. Chem. Phys., 48, 3345 (1968).
	γ-irradiation of L-Lysine·HCl·$2H_2O$ at 77°K then warmed to 300°K	300°K 77°K	2.001... 2.003	a_{II_α}: 19.1, 29.4, 13.8 iso: 20.8 $a_{II_\beta(1)}$ 27.5...31.0 $a_{II_\beta(2)}$ 35.0...38.5 $a_{II_\beta(1)}$ and (2) 34.0 37.0 a_{II_α}: 21.6, 31.6, 10.9 iso: 21.3	Fujimoto M. et al, J. Chem. Phys., 48, 3345 (1968).
$CH_3\dot{C}HCOOH$	γ-irradiation at 195°K followed by UV irradiation			$a_{II_\beta}^{CH_3}$ 24.6	Klanck, R.S. J. Chem. Phys., 49, 1725 (1968).
$\cdot CHODCHOSCOOD \cdot D_2O$	X-irradiation of deuterated d-l-tartaric acid at 195°K			a_{II_α}: 23.0, 10.7 iso: 22.4 a_{II_β}: 37.2, 35.3, 30.5 iso: 34.5	Moulton, R.C., and B. Cornandy, J. Chem. Phys., 53, 3022 (1970).

CARBON RADICAL	GENERATION	ESR temp	g-values	Splitting (hfs) values	Reference
	γ-irradiation of deuterated D-tartaric acid at 77°K	77°K	2.0026 2.0020 iso:2.003	1) a_{H_β}: 20.0 iso: 28.2	Gulchinock, J. and F.R. Hallett Can. J. Chem., 50 3706 (1972)
			2.0035 2.0033 2.0017 iso:2.0028	2) a_{H_β}: 16.3, 11.1, 9.8 iso: 12.4	
	γ-irradiation of deuterated D-tartaric acid at 77°K	77°K	2.0041 2.0033 2.0029 iso:2.003	a_{H_β}: 19.4, 16.2, 5.8 g iso: 13.8	Gulchinock, J. and F.R. Hallett Can. J. Chem., 50 3706 (1972)
	γ-irradiation of deuterated D-tartaric acid at 77°K	295°K	2.0048 2.0034 2.0023 iso:2.003	a_{H_β}: 5.7, 4.4, 3.5, iso: 4.5	Gulchinock, J. and F.R. Hallett Can. J. Chem. 50 3706 (1972)
			2.0038 2.0028 2.0019 iso:2.0039	a_{H_β}: 31.0, 24.2, 23.2 iso: 26.1	
	γ-irradiation of deuterated D-tartaric acid at 77°K	253°K	2.0029 2.0025 2.0021 iso:2.002		Gulchinock, J. and F.R. Hallett Can. J. Chem. 50 3706 (1972)
			2.0046 2.0035 2.0033 iso:2.003	a_{H_α}: 33.5, 21.8, 12.1 iso: 22.4	
CO_2^-	γ-irradiation of deuterated D-tartaric acid at 77°K		2.000		Gulchinock, J. and F.R. Hallett Can. J. Chem. 50 3706 (1972)
					*Radical has 2 different sites.

CARBON RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
	γ-irradiation of 1 methyl uracil at 77°K, then heated to 173°K	77°K		$a^{CH}_{H_\alpha}$: 4.5 $a^{CH}_{H_\alpha 2}$: 13.5 $a^{CH}_{H_\beta 2}$: 18.0	Dulcio, A., and J.N. Herak, Mol. Phys., 26, 605 (1973).
$^{\bullet}CCl_2FCOND_2$ 	x-irradiation of N, N-dideuterodichlorofluoroacetamide at 77°K; then warmed to 300°K	170°K	2.0021 2.0069 2.0076 iso:2.0055	$a^{19}F$: 6.9,10.7,16,8 iso: 50.1 ^{35}Cl: 3.2,5.8,18.0 iso: , 9. $a^{D}C$: 90.6 $a^{14}N$: 3.8,5.09,5.09	Kispert, L., F. Myers, J. Chem. Phys., 56, 2623 (1972).
$^{\bullet}CF(CONH_2)_2$	γ-irradiation of difluoromalonamide at 300°K	300°K	2.0029 2.0047 2.0043 iso:2.0040	aF_α: 200,1,10 iso: 63	Iwasaki, M. et al Mol. Phys., 18 201 (1970).
	γ-irradiation of ammonium trifluoroacetate at 300°K	300°K	2.0030 2.0054 2.0054 iso:2.0045	aF : 188, 14, 14 iso: 72	Srygley, F. and W. Gordy, J. Chem. Phys., 46, 2245 (1967).

⊕ radical pair formed

CARBON RADICAL	GENERATION	ESR TEMP	g-VALUE	SPLITTING PARAMETERS	REFERENCES
HOOC $\dot{C}$H COOH	X-irradiation of malonic acid at 300°K	300°K		a : 75.9, 8.1, 15.1 iso: 33.1 a^{CH}_{β}: 32.5, 20.7, 10.4 iso: 21.1	Colo, T. and C. Heller, J.Chem. Phys. 34, 1085, 1961. McConnell, H.M. et al. J. Am. Chem. Soc. 82 766 (1960).
	γ-irradiation of THIOGLYCOLIC Acid at 300°K	300°K	2.002 2.005 2.011 iso: 2.006	a^{CH}_{α}: 7, 16, 23 † iso: 15.0 a^{CH2}_{β}: 5.5	Kurita, Y. et H. Gordy, J. Chem. Phys. 34, 1285 (1961)
$\dot{C}H_2$ COOH ††	γ-irradiation of BROMOACETIC ACID (CH_2 Br COOH) at 77°K	77°K	2.009 2.0025 2.0025	a^{CH2}_{H}: 27	Suttle, J.R. and J. Lontz J. Chem. Phys. 46, 1539 (1967)
 †††	electron beam irradiation of glycylglycine at 77°K	77°K		a^{H}_{α}: 29.5, 16.4, 12.1 iso: 19.4 a^{H}_{α}: 29.9, 17.3, 13.1 iso: 20.1 a^{H}_{β}: 10.0, 8.0, 5.7 iso: 7.9 aN: 3.0, 3.0, iso: 2.0	Nolo, T.B., Int. J. Radiat. Biol. 23, 247, (1973)

† Almost isotropic

†† or possibly ionized species CH_2 Br C(=O)OH

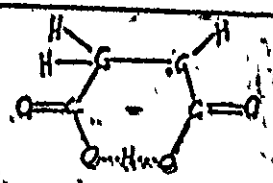
††† Endor also used

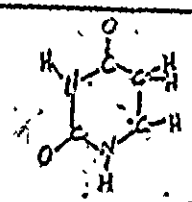
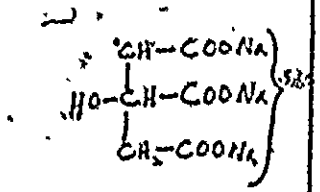
CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS	REFERENCES
CH_2COOH	x-irradiation of malonic acid at 77°K additionally warmed up to 195°K; additionally warmed up to 300°K	77°K		aH_α = 32.1, 21.4, 10.7 iso: 21.4	TAMURA, N. et al. Trans. Far. Soc. 62, 2434 (1966)
$CH(COOH)_2$	x-irradiation of malonic acid at 77°K, then warmed to 195°K additionally warmed to 300°K	77°K		aH_α = 32.1, 21.4, 10.7 iso: =21.4	
$CH_3CH_2CH(COOH)$	x-irradiation of ethyl malonic acid at 77°K; additionally warmed to 195°K; additionally warmed to 300°K	77°K		aH_α: 31.1, 19.6, 8.2 iso: 19.6 aH_{β_1}: 22. aH_{β_2}: 6.4	TAMURA, N. et al. Trans. Far. Soc. 62, 2434 (1966)
$CH_3CH_2C(COOH)_2$	x-irradiation of ethyl malonic acid at 77°K then warmed to 300°K	77°K		aH_α: 31.1, 19.6, 8.2 iso: 19.6 aH_{β_1} = 43.2 aH_{β_2} = 4.6	
HOOC CH CH_2CH_2COOH α β **	x-irradiation of glutaric acid	300°K	2.0021 2.0030 2.0040 iso: 2.0030	a_{H_α}: 8.2, 19.6, 31.7 iso: 20.0 $a_{H_{\beta(1)}}$: 13.6, 15, 16.4 iso: 15 $a_{H_{\beta(2)}}$: 42.8, 44.3, 48.9 iso: 45.3	Kwirami A., J. Chem. Phys., 55, 2484 (1971)

** ENDOR also used.

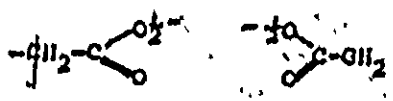
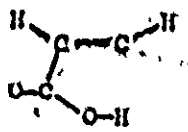
CARBON RADICAL	GENERATION	ESR	g-VALUES	FITTING PARAMETERS	REFERENCES
$-O_2CCH_2CH_2CO_2^{2-}$	γ-irradiation of disodium succinate $\cdot 6D_2O$ (triclinic) at 77°K	77°K		aH_{B_1}: 10 (iso) aH_{B_2}: 19, 25.6, 15.7 iso: 20.1	Fujimoto, M and W. Seddon, Can. J. Chem. 48, 2810 (1970)
$-O_2C(\dot{C}HCH_2)CO_2^-$	γ-irradiation of disodium succinate $\cdot 6D_2O$ (triclinic) at 77°K	77°K		aH_α: 24.9, 61.3, 42.1 iso: 42.8 aH_{β_1}: 8.3, 13.0, 7.9 iso: 9.7 aH_{β_2}: 19.3, 14.1, 15.8 iso: 16.4	Fujimoto, M. and N. Seddon, Can. J. Chem., 48 2810 (1970)
$-O_2C\dot{C}HCH_2CO_2^-$	γ-irradiation of disodium succinate $\cdot 6D_2O$ (triclinic) at 77°K.	300°K		aH_α: 30.6, 20.7, 11.5 iso: 42.8 aH_β: 39.4, 35.3, 33.0 iso: 35.9 aH_{β_2}: 24.5	Fujimoto, M and W. Seddon, Can. J. Chem., 48 2810 (1970)
$-O_2C\dot{C}HCH_3$	γ-irradiation of disodium succinate $\cdot 6D_2O$ (triclinic) at 77°K	300°K		aHα: 21.4	Fujimoto, M and W. Seddon, Can. J. Chem., 48 2810 (1970)
CO_2^-	γ-irradiation of disodium succinate $\cdot 6H_2O$ at 77°K	300°K	2.0145 1.9960 1.9923 iso:2.0009		Fujimoto, M and W. Seddon, Can. J. Chem., 43 2810 (1970)
$-COC\dot{H}_2$	X-irradiation of glycine at 300°K	300°K		$\dot{C}H_2$ H_1: 3.6, 25.6, 33.6 iso: 21.0	WEINER, B.B. and W.S. KOSKI, J. Am. CHEM. SOC. 85, 873, (1963).

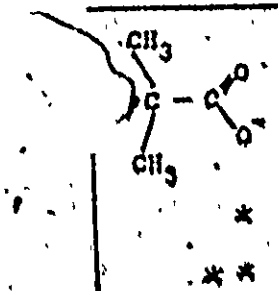
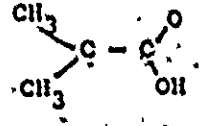
CARBON RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
$CH_3(CH_2)_3\overset{\gamma}{C}H_2\overset{\beta}{C}H\overset{\alpha}{C}O\overset{\zeta}{C}OCH_2CH_3$	x-irradiation of ethyl heptanoate at 300°K	300°K		a^H_α: 30.3; 14.5, 14.5 iso: 19.8 $a^H_{\beta(1)}$: 24.4, 23.3, 23.3 iso: 23.6 $a^H_{\beta(2)}$: 24.4, 23.4, 23.4 iso: 23.7	Griffith, O.H., J. Chem. Phys., 41, 1093 (1964).
$CH_3(CH_2)_4CH_2\dot{C}HCOCH_2CH_3$	x-irradiation of ethyl octanoate at 300°K	300°K		a^H_α: 29.3, 14.5, 14.5 iso: 19.4 $a^H_{\beta(1)}$: 27.8, 26.7, 26.7 iso: 27.1 $a^H_{\beta(2)}$: 24.6, 24.0, 24.0 iso: 24.2	Griffith, O.H., J. Chem. Phys., 41 1093, (1964).
$CH_3(CH_2)_5CH_2\dot{C}HCCH_2CH_3$	x-irradiation of ethyl nonanoate at 300°K	300°K		a^H_α: 14.5, 14.5 $a^H_{\beta(1)}$: ½ (48.6) ~½ (48.6 $a^H_{\beta(2)}$: ~½ (48.6), ½ (48.6)	Griffith, O.H., J. Chem. Phys., 41, 1093 (1964).
$CH_3(CH_2)_6CH_2\dot{C}HC\ CH_2CH_3$	x-irradiation of ethyl decanoate at 300°K	300°K		a^H_α...14.6, 14.6 a^H_β...~½ (52.1), ~½ (52.1).	Griffith, O.H., J. Chem. Phys., 41, 1093 (1964).
$CH_3(CH_2)_7CH_2\dot{C}HC\ CH_2CH_3$	x-irradiation of ethyl undecanoate at 300°K	300°K		a^H_α: 29.6, 14.7, 14.7 iso: 19.7 $a^H_{\beta(1)}$: 28.2, 27.8, 27.8 iso: 27.9 $a^H_{\beta(2)}$: 23.5, 23.3, 23.3 iso: 23.4	Griffith, O.H., J. Chem. Phys., 41, 1093 (1964).

CARBON RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
HOOC–C(•)H–COOK	X-irradiation of potassium hydrogen malonate at 300°K	300°K	2.00*	$a^{CH}_{H\alpha}$: 19.8, 13.3, 10.0 iso: 20.4 a^{OH}_{H}: 10.1, 10.3, 10.7 iso: 10.4	Micklin, R. et al. J. Chem. Phys. 56, 1279 (1972)
HOOC–C(•)H–COOK and HOOC–C(•)H–COOK or HOOC–C(•)H–COOK	X-irradiation of potassium hydrogen malonate at 300°K	300°K	2.00*	a^{13}_{C}: 30, 55, 45 iso: 43.3 $a^{13}_{C^1}$: 70, 3, 11 iso: 28.0	Micklin, R. et al. J. Chem. Phys. 1279 (1972)
$^{\ominus}OOC-\dot{C}H-CH_2-(CH_2)_3-\overset{\oplus}{N}H_3Cl^{\ominus}$ $NH_3 \cdot 2H_2O$	X-irradiation of lysine monohydrochloride-dihydrate		2.0042 2.0033 2.0025 iso: 2.0033	a^{CH}_{H}: 30.0, 18.6, 12.9 iso: 20.5 $a^{CH_2}_{B(1)}$: 29.6 iso: 28.7 $a^{CH_2}_{H\beta(2)}$: 40.0, 36.5, 36.5 iso: 37.3	Cvetkov, Y. D. et al., Trans Faraday Soc. 59, 2213 (1963)
	γ-irradiation of sodium hydrogen maleate trihydrate at 77°K in the dark	300°K	2.0023 2.0037 2.0042 iso: 2.0034	$a^{H}_{H\alpha}$: 11.9, 19.6, 31.1 iso: 20.9 $a^{H}_{H\beta1}$: 32, 33, 36 iso: 34 $a^{H}_{H\beta2}$: 26, 26, 30 iso: 27	TORIYAMA, K. et al., J. CHEM. PHYS. 55, 1885 (1971)

CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS in GAUSS	REFERENCES
	γ-irradiation of dihydrouracil at 300°K	300°K	2.0023 (iso)	aH: 21.5, 6.0, 31.0 iso: 19.5 $a_H^{CH_2}$: 24 $a_H^{CH_2}$: 4.2	Horak, J.H. J. Chem. Phys. 53 576 (1970)
(OOC-CH-CH-COOH) (OOC-CH-CH-COOH)	irradiation of hydrogen fumarate at 77°K	77°K	2.0023 2.0035 2.0049 iso: 2.0035	aH: 5.4, 11.1, 15.4 iso: 10.6	Iwasaki, M. et al J. Chem. Phys. 55 1472 (1971).
	γ-irradiation of sodium citrate pentahydrate at 300°K or 77°K	300°K		aHα: 32.5, 19.3, 13. iso: 21.6	Russell, D.B. J. Chem. Phys. 43 1996 (1965)
$CH(SO_3)_2^-$	γ-irradiation of potassium methane disulphonate at 300°K	300°K	2.0033 2.0023 2.0019 iso: 2.0025	aH: 20.3, 33.9, 10.0 iso: 21.4 $a^{13}C$: 92.8, 19.8, 22.1 iso: 44.8	HORSFIELD, A. et al M.P. 5, 241 (1962)
CO_2^-	γ-irradiation of sodium formate at 300°K	300°K	2.0014 2.0032 1.9975 iso: 2.0007	$a^{13}C$: 156, 195, 151 iso: 167 $a^{23}Na$: 7.5, 7.5, 9.3 iso: 8.2	OVENALL, G.W. and D.H. WHIFFEN, M.P. 4, 135, (1961)
$^-OOCCHCOO^-$	γ-irradiation of potassium hydrogen malonate at 300°K	300°K	2.0031 2.0054 2.0041 iso: 2.0042	aH: 19.1, 30.7, 10.0 iso: 19.9	LIN, W.C. and C.A. McDOWELL, M.P. 4, 343 (1961)

CARBON RADICAL	GENERATION	TEMP.	g-FACTORS	HYPERFINE COUPLING CONSTANTS (in gauss)	REFERENCES
$CH_3(CH_2)_8CH_2\dot{C}HCCH_2CH_3$	x-irradiation of ethyl dodecanoate at 300°K	300°K		a^H_α: ...14.6, 14.6 a^H_β: ...~½(52.8), ~½(32.8) a^H_β: ...~½(52.8), ~½(52.8).	Griffith, O.H., J. Chem. Phys., 41, 1093 (1964).
$CH_3(CH_2)_4CH_2\dot{C}HCOCH_3$	x-irradiation of methyl octanoate at 300°K	300°K		a^H_α: 29.6, 14.7, 14.7 iso: 19.6 $a^H_{\beta(1)}$: 31.8, 31.2, 31.2 iso: 31.4 $a^H_{\beta(2)}$: 23.7, 23.0, 23.0 iso: 23.2	
$CH_3(CH_2)_4CH_2\dot{C}HCO(CH_2)_5CH_3$	x-irradiation of hexyl octanoate at 300°K	300°K		a^H_α: 29.3, 14.6, 14.6 iso: 19.5 $a^H_{\beta(1)}$: 27.1, 25.8, 25.8 iso: 26.2 $a^H_{\beta(2)}$: 21.1, 20.4, 20.4 iso: 20.6	
$CH_3\dot{C}HCOCH_2(CH_2)_6CH_3$	x-irradiation of methyl propionate at 300°K	300°K		a^H_α: 30.1, 14.9, 14.9 iso: 19.9 a^H_β: 25.6, 23.2, 23.2 iso: 24.0 a^H_β: 25.6, 23.2, 23.2 iso: 24.0	

Carbon Radical	Generation	ESR Temp	g Value	Splitting Parameter	References
	x-irradiation of potassium hydrogen malonate at 4.2°K	4.2°K	2.0074 2.0072 2.0026 iso: 2.0057		Box, H.C. et al: J. Chem. Phys. 55, 315, 1971
	x-irradiation of potassium deuterium maleate at 77°K	77°K	2.0033 2.0026 2.0017	a^{H}_{α}: 23.6, 8.7, 0.1 iso: 10.8 a^{H}_{β}: 58.26	Toriyama, K. & M. Iwasaki J. Chem. Phys. 55, 2181 (1971)
$CH_2\ CO_2$	γ-irradiation of sodium acetate 2-^{13}C at 77°K	77°K 300°K		$a^{B}C$: 80.7, 25, 19.3 iso: 34.2 $a^{13}C$: 75.7, 30.2, 5.4 iso: 37.1	Fujimoto, H. and J. Janecka J. Chem. Phys. 55 5 (1971)
$CH_3CH\text{-}OSO_3^-$	γ-irradiation of potassium ethyl sulphate at 77°K	114°K		a^{CH}_{α}: 48, 20.9, 28 iso: 18.9	AYSCOUGH, P. and Roy, A., Trans. Far. Soc., 63: 1107 (1967)
$HOOC\text{-}CH_2\text{-}C(OH)COOH$	x-irradiation of ammonium malate monohydrate at 77°K	300°K 77°K		$a^{13}C$: 82.9 a_H^{OH}: 0.1, 1.1, 0 iso: 2.4 $a^{CH_2}_{\beta_1}$: 38.9 $a^{CH_2}_{\beta_2}$: 12.9	CORVAJA, C., Trans Far. Soc., 63: 2098 (1967)

CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS	REFERENCES
$HOOC-CH_2-O-\dot{C}H-COOH$	γ-irradiation of Di glycollic acid mono-hydrate 300°K	300°K	2.0022 2.0044 2.0061 iso: 2.0042	$a^{CH}_{H\alpha}$: 16.5, 27.0, 8.9 iso: 17.5 $a^{CH_2}_{H\beta\gamma}$: 3...5	Kurita, Y. J. Chem. Phys. 36, 560 (1962)
$H_3N-CH_2-CO-NH-\dot{C}H-COO$	γ-irradiation of α-glycyl glycine at 300°K	300°K	2.0028 2.0038 2.0033 iso: 2.0032	a^{CH}_{A}: 19, 28.9 iso: 18.6 aN: 4, 3, 2 iso: 3	Katayama, M. and N. Goroy, J. Chem. Phys 35, 117 (196)
$HOOC-\dot{C}HOH$	γ-irradiation of urea oscalate at 300°K	300°K	2.0024 2.0048 2.0047 iso: 2.0039	a^{CH}_{HB}: 6.2, 0.3, 8.3 iso: 4.7 a^{CH}_{H}: 15.7, 26.5, 8.1 iso: 16.8	Rao, D.J.G.N. and W. Gordy, J. Chem. Phys. 35, 362 (1961)
$HOOCCH(NH_3Cl)CH_2\dot{C}HCOOH$	X-irradiation of glutaric acid HCl at 300°K	300°K		a^{CH}_{H}: 20, 30, 10 iso: 20 $a^{H}_{\beta(1)}$: 25 $a^{H}_{\beta(2)}$: 35	Lin, W.C. et al. J. Chem. Phys. 35, 757 (1961) McConell et al. J. Chem. Soc. 82, 766, 1960
	x-irradiation of α-amino isobutyric acid at 77°K	165°K	2.002 a_H 2.0031 2.0042 iso: 2.0032	$a^{CH_3}_{H}$: 125.7, 130, 139.6 iso: 130.2	Box, H.C. and H.C. Freund J. Chem. Phys., 44, 2345 (1966). *splitting due to 2 sets of 3 equivalent protons **or 
		190	2.0024 2.0029 2.0033 iso: 2.0029	$a^{CH_3}_{H}$: 124.3, 129.6, 136.8 iso: 130.2	

Carbon Radical	Generation	ESR	g-factors	Splitting parameter in gauss	References
$CH_3\dot{C}HCOOH$	X-irradiation of (-α-alanine) at 300°K	300°K 77°K		a^{H}_{α}: 18.2, 20.3, 26. iso: 21.5 $a^{CH_3}_{H\beta}$: 23.9, 25.3, 26.0 iso: 25.0 a^{H}: 19.2, 21.0, 27.5 iso: 22.3 $a^{H}_{\beta(1)}$: 41.4, 42.1 45.7 iso: 42.8 $a^{H}_{\beta(2)}$: 27.5, 27.1 27.5 iso: 27.1 $a^{H}_{\beta(3)}$: 3.0, 5.7, 5.0 iso: 5.0	Morsfield, A., et al M.P. 4, 425 (1961)
$(CO_2H)\dot{C}H-CH_2(CO_2H)$	γ-irradiation of succinic acid at 300°K	300°K	2.0045 2.0026 2.0019 iso: 2.0030	a^{CH}_{H}: 32.9, 23.1, 10.7 iso: 21.4 $a^{CH_2}_{H\beta(1)}$: 30.6, 35.0 33.0 iso: 32.7 $a^{CH_2}_{H\beta(2)}$: 31.8, 28.2, 25.7 iso: 28.6	Pooley, D. and D. H. Whiffen M.P. 4, 81 (1961)
$CH_3(CH_2)_2\dot{C}H_2CHCOOCH_2CH_3$	X-irradiation of ethyl hexanoate at 300°K	300°K		a^{H}: 29.2, 14.8, 14.3 iso: 19.4 $a^{CH_2}_{H\beta}$: 26.8, 23.3, 23.3 iso: 24.4 $a^{CH_2}_{H\beta}$: 22.5, 21.96	Griffith, O.H. J. Chem. Phys. 41 1093 (1964)

CARBON RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS	REFERENCES
$H_3\overset{+}{N}-\dot{C}H_2-CO_2^{2-}$	γ-irradiation of glycine at 77°K	77°K		a_H^{CH} : 36.3 a_N^{1+} : 30.0	Sevdobov, M. et al. J. Chem. Soc. Perkin II 1808 (1973)
$H_2\dot{C}CO_2^{2-}$	γ-irradiation of glycine at 77°K	135°K		aH: 28, 17.5, 17.5 iso: 21	
$H_3\overset{+}{N}\dot{C}H_2$	γ-irradiation of glycine at 77°K	77°K		a_H^{NH}: 20 a_H^{CH}: 13 a_N^{1+}: 5	
$H_2N\dot{C}H_2$	γ-irradiation of glycine at 77°K	100°K		a_H^{NH} : 5 a_H^{CH} : 13 a_N^{1+} : 5	
$CH_3\dot{S}CH\cdot CH_2\cdot CH(\overset{+}{N}H_3)CO_2^-$	x-irradiation of DL-methionine at 77°K	77°K	2.0052 2.0017 2.0080 iso:2.0050	$a_{H\alpha}^{CH}$: 23.2, 12.8, 6.7 iso: 14.2 $a_{H\beta_1}^{CH_2}$: 18.2 (iso) $a_{H\beta_2}^{CH_2}$: 31.1 (iso) $a_H^{CH_3}$ 3.6 (maximum)	Cadona, D. & Rowlands, R., J. Chem. Soc. (B) 488 (1968)

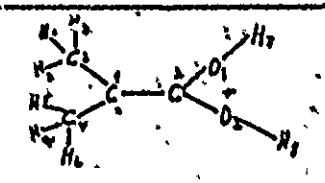
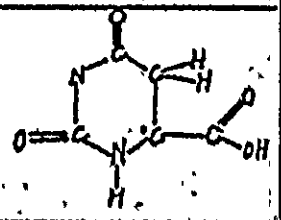
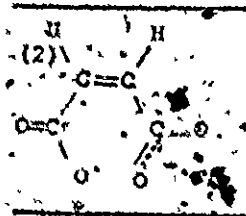
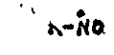
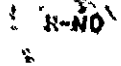
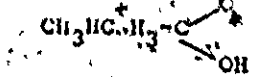
CARBON RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
	irradiation of α-aminoisobutyric acid at 77°K	77°K		$a_{H_1}=a_{H_4}$: 22.5 $a_{H_5}=a_{H_6}=a_{H_7}=a_{H_8}$: 4.6	Cadena, D. et al. Can. J. Chem. 47, 3429 (1973)
$^{\cdot}O_2C(\dot{C}HCH_2)CO_2^-$	γ-irradiation of disodium succinate $6D_2O$ (Monoclinic) at 77°K;	77°K		a_{H_α}: 52.2, 20.8, 35.1 iso: 36 a_{H_β}: 16.6 (iso) $a_{H_{\beta(2)}}$: 7.8, 7.2, 14.4 iso: 9.8	Vyas, H. et al. Can. J. Chem. 48, 2805 (1970)
$^{-}O_2C\dot{C}HCH_2CO_2^-$	γ-irradiation of disodium succinate $\cdot 6D_2O$ (monoclinic) at 77°K	300°K		a_{H_α}: 19.7, 12.4, 31.1 iso: 21.1 $a_{H_\beta}=a_{H_\beta}$: 32.0, 30.9, 34.5 iso: 32.4	Vyas, H. et al. Can. J. Chem. 48, 2805 (1970)
$CH_3\dot{C}HCO_2^-$	X-irradiation of zinc acetate dihydrate (stored after irradiation for 1 month at 300°K)	300°K		a_H^{CH}: 10.6, 18.8, 30.7 iso: 20.1	Tolles, W. M et al J. Chem. Phys. 54, 1532 (1970)
	γ-irradiation of orotic acid at 77°K	170°K, or 300°K	2.0023 2.0066 2.0031 iso: 2.004	$a_{H_{\beta_1}}^{CH_2}$: 22.8 (iso.) $a_{H_{\beta_2}}^{CH_2}$: 34.5 (iso)	HUTT & KMINN, J et al. J. Phys. Chem. 74, 4022 (1970)

TABLE 2---"NON-CARBON" RADICALS (REVIEW OF SINGLE CRYSTALS STUDIES 1960-1973)

RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS	REFERENCES
$^{32}SO_3^-$	γ-irradiation of taurine, $H_3\overset{+}{N}CH_2CH_2SO_3^-$	300°K	2.0035(iso)	$a^{33}S$: 135.2,99.2, 97.9 iso:	LIND, G. and R. Kowly Can. J. Chem. 50, 43 (1972)
$H_3\overset{+}{N}CH_2CH_2{}^{32}SO_2$	γ-irradiation of taurine at 300°K		2.0024 2.0056 2.0097 iso:2.0059		LIND, G. and R. Kowly Can. J. Chem. 50, 43 (1972)
$H_3\overset{+}{N}CH_2CH_2{}^{33}SO_2$	γ-irradiation of taurine, $H_3\overset{+}{N}CH_2CH_2SO_3^-$ at 300°K	300°K		$a^{33}S$: 49.6,9.1,1.2	LIND, G. and R. Kowly Can. J. Chem. 50, 43 (1972)
NO_3	γ-irradiation of KNO_3 at 77°K	77°K	2.005 2.0025 2.0025 iso:2.018	aN: 4.5	Cunningham, J. et al. Phys. Chem. Solids 23, 167 (1962).
$NO_3^{(2-)}$	irrad. of RNO_3	4.... 77°K	2.004 2.008 2.010 iso:2.007	aN: 3.2, 3.5, 3.5 iso: 13	Cunningham, J. et al. Int. Symp. Free Radicals, Uppsala 1961 (N013)
$NO_3^{(2-)}$	irrad. of KNO_3	4°K	2.002 2.006 2.006 iso:2.005	aN: 32, 32, 61 iso: 42	Cunningham, J. et al. Int. Symp. Free Radicals Uppsala 1961 (N013

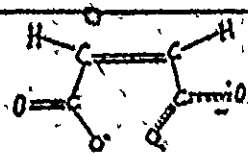
Radicals	Generation	ESR temp	g-values	Splitting parameters in gauss	References
CO_4^-	X-irradiation of K_2SO_4 at 77°K	77°K	2.0486 2.0082 2.0037 iso: 2.0201		Grozov, V. and Morton, J.R. Can. J. Chem. 44, 527 (1966)
SO_3^-	X-irradiation of K_2SO_4 at 77°K	300°K	2.0033 2.0033 2.0023 iso: 2.0029		
$CH_3\overset{+}{S}\cdot CH_2\cdot CH_2\cdot CH(NH_3^+)CO_2^-$	X-irradiation of DL-methionine at 77°K	77°K	2.06934 2.00552 2.01948 iso: 2.02811		Cadena, D. and Rowlands, R., J. Chem. Soc. (B) 488 (1963)
$[CH_3O\cdot C(NH_2)_2]^{++}Cl^-$	X-irradiation of O-methylisouronium chloride at 77°K	77°K	2.0279 2.0365 2.0267 iso: 2.0304	$a_H^{NH_2}$: 13.9, 16.0, 3.9 iso: 11.4 $a_H^{CH_3}$: 58.2, 33.9, 37.8 iso: 43.2	Cadena, D. and Rowlands, R., J. Chem. Soc. (B) 485: (1968)
$\dot{N}H_2CO$	electron irradiation of trifluoroacetamide at 77°K	77°K	2.003 2.004 2.007 2.003(iso)	a_N: 33.4, 7.5, 10.0 iso: 16.6 a_{H_1}: 20.1, 37.5, 4.8 iso: 21 $a_{H_{(2)}}$: 5.0, 2.0, 5.0 iso: 19.0	RODGERS M. and [illegible] J. Chim. phys., No. 3 (1967)

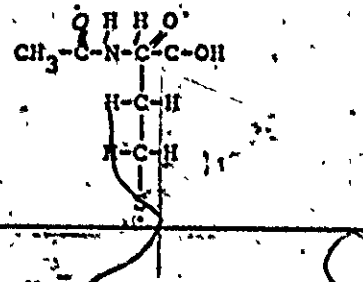
RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
$\overset{+}{N}H_2\,CH_2\,COO^-$	X-irradiation of glycine at 77°K	110°K 120°K		aN: 27.3, 16.8, 10.5 iso: 16.8 a_{α}^{H}: 23 (iso) a_{α}^{H}: 30 (iso) a_{β}^{H}: 6 (iso) a_{β}^{H}: 52 (iso)	Sinclair, J. J. Chem. Phys. 55, 245 (1971)
$(HCO_3)_2^-$	X-irradiation of potassium bicarbonate at 77°K	77°K	2.0261 2.0079 2.0063 iso: 2.01343	a: 2.8, 4.0, 4.6 iso:	Holmberg, R.W. J. Chem. Phys. 55, 1730 (1971)
$HCO_3^{\bullet}$	X-irradiation of potassium bicarbonate at 77°K	150 293	2.0189 2.0076 2.0060 iso: 2.0108 2.0184 2.0087 2.0590 iso: 2.0110	a: <2	Holmberg, R.W. J. Chem. Phys. 55 1730 (1971)
$CH_3(CH_2)_8\dot{C}H_2\text{-}NO\text{-}CH_3$	X-irradiation of decanal oxime O-methyl ether urea $CH_3(CH_2)_8CH_2=NOCH_3$ at 77°K	300°K	2.0074 2.0036 2.0036 iso: 2.0049	aN: 4.0, 20.0, 20.0 iso: 14.6 aH: 26.2, 26.0, 26.0 iso: 26.1 a_2H: 4.0, 1.6, 1.6 iso: 2.4	Ciecierska, Tworek, Z. et al., J. Chem. Phys. 56, 1001 (1972)
$N\equiv C\text{-}N=C(NH_2)\,\overset{\oplus}{N}H$	X-irradiation of decyandiamide $(N\equiv C\text{-}N=C(NH_2)_2$ at 300°K	77°K		aN: 31.7, 7.2, 2.4 iso: 13.7 aN: 8.7, 6.1, 4.9 iso: 6.5 a_{α}^{NH}: 75 ⊖	Lau, P.W. and W. C. Lin J. Chem. Phys. 54, 823 (1971) ⊖ sigma radical

RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
	γ or x-irradiation of potassium hydrogen maleate at 77°K in the dark and exposure to visible light	77°K	2.0233 2.0066 2.0037	$a_{H(2)}$: 5.2, 3.4, 3.0 iso: 3.9	Tonyama, K. et. al J. Am. Chem. Soc. (1971) 6415
$^{15}NH_3$	γ-irradiation of Ammonium perchlorate at 300°K	300°K		a_H: 26.1, 26.1, 24.8 iso: 25.6 ^{15}N: 30.6, 26.1, 24.8 iso: 27.1	Fujimoto, M., and J.R. Morton Can. J. Chem. 43 1012 (1965)
$^{15}NH_3$	γ-irradiation of Ammonium perchlorate at 300°K	77°K		a_H: 28.1, 25.1, 24.6 iso: 25.9 ^{15}N: 50.3, 17.1, 14.0	Fujimoto, M. and J.R. Morton Can. J. Chem. 43 1012 (1965)
$^{32}S_2O_2$	x-irradiation at room temperature of $Na_2S_2O_3 \cdot 5H_2O$	300°K	2.0083 2.0050 2.0030		Morton, J.R. Can. J. Chem. 43 1948 (1965)
	Mercury arc (UV) irradiation of pyrrolidine N-oxide			a_N^{14}: 5.1, 32.2, 5.3 iso: 14.2 a_H: 19.0, 18.4, 21.1 iso: 19.5	Bodnar, J. and C. Rowell J. Chem. Phys. 56, 707 (1972)
	Mercury arc (UV) irradiation of morpholine N-oxide			a_N^{14}: 8.6, 9.4, 31.3 iso: 16.4 a_H: 27.1, 32.7, 32.8 iso: 31.5	Bodnar, I. and Rowell, J. Chem. Phys. 56, 707 (1972)
	X-irradiation of L-alanine at 80°K	80°K	2.0021 2.0026 2.0040 iso: 2.0029	a_H: 10.6, 14.9, 21.0 iso: 15.3 a_H: 14.9, 18.2, 21.5 iso: 18.2	Miyagawa, I. et al. J. Chem. Phys. 51, 3520

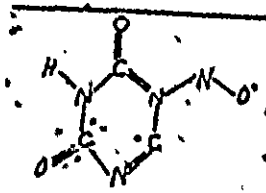
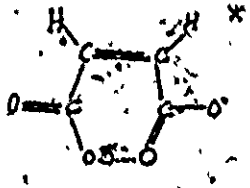
RADICALS	GENERATION	ESR TEMP.	g-VALUE	SPLITTING PARAMETERS	REFERENCES
$CF_3CFCONH_2-O-\dot{O}$	γ-irradiation of pentafluoro-propionamide at 300°K and then exposure to oxygen (air)	300°K	2.03 2.01 2.01 iso: 2.02		Lontz, R.J. J. Chem. Phys., 48, 1239 (1968)
$\dot{O}H$	electron irradiation of at 77°K	77°K	2.004 2.008 2.013 iso:2.008	aH 7.5, 43, 43 iso: 26.3	Gunter, T.E., J. Chem. Phys., 46, 3818 (1967)
$\dot{O}-N-CCH_3-CCH_3-N-OH$	x-irradiation of dimethyl glyoxime at 77°K	77°K	2.004 2.008 2.013 iso:2.008	$aN_{(1)}$: 38, 22, 22 iso: 27.3 $aN_{(1)}$: 52, 26, 26 iso: 34.6	Kurita, Y. J. Chem. Phys., 41, 3926 (1964)
SO_3^- *	γ-irradiation of potassium ethyl sulphate at 300°K (also at 77°K)	300°K	2.0038 2.0032 2.0029 iso: 2.0033	$a^{33}S$:137,102.0,101.1 iso: 113 $a^{33}S$:138.4,101.9,101.0 iso: 113.7	SYSCOUGH, P. and Roy, A., Trans. Far. Soc., 63: 2107 (1967)
OH	γ-irradiation of ice at 77°K	77°K	2.005 2.009 2.060 iso:	aH:26.0,44.0,0 iso: 23.3	BRIVATI, J. et. Val Trans. Far. Soc. 63:2112 (1967)
OH	γ-irradiation of ice at 77°K	77°K	2.005 2.009 2.0585	aH: 26.0,43.5 iso: 24.6	DIXON, G., Trans. Far. Soc. 63: 2098 (1967)
CO_3^-	γ-irradiation of potassium bicarbonate at 77°K	77°K	2.0013 2.0032 2.0032		Holroyd, R.W. J. Chem. Phys. 55, 1730 (1971)

* Radical has 2 different.

RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
$PhCH_2S\dot{O}_2$	x-irradiation of dibenzyl sulfone at 300°K	300°K	2.0027 2.0056 2.0094 iso:2.0059	$a^{33}S$: 96.3,56.2, 63.4. iso: 71.9	Geoffroy, M., and EAC Luckon, J. Chem. Phys., 55, 2719 (1971).
$PhS\dot{O}_2$	x-irradiation of phenylthioacetic acid-S, phenyl methylsulfone diphenyl sulfone at 300°K	300°K	2.0023 2.0051 2.0072 iso:2.0048	$a^{33}S$: 107.1, 71.3, 71.3 iso: 83.2	
$(R-\dot{S}-)^+$	x-irradiation of thiourea at 4.2°K	4.2°K	2.0067 2.000 2.003 iso:2.026		Box, H.C. et al J. Chem. Phys., 48, 1748 (1968).
$(R-S)^+$	x-irradiation of allylthiourea 4.2°K		2.058 2.007 1.997 iso:2.020		Box, H.C. et al, J. Chem. Phys., 48, 1748, (1968).
$(R-S)^-$	x-irradiation of allylthiourea at 4.2°K	4.2°K	*2.0023		Box, H.C. et al J. Chem. Phys., 48,1748 (1968).
$(R-S)^+$	x-irradiation of dimethylthiourea at 4.2°K	4.2°K	*2.025 2.017 2.001 iso:2.0143		Box, H.C. et al J. Chem. Phys., 48, 1748 (1968).
	γ-irradiation of sodium hydrogen maleate trihydrate at 77°K	77°K	2.0047 2.0071 2.0303 iso:2.0140		TORIYAMA, K. et al, J. CHEM. PHYS. 55, 1885 (1971)

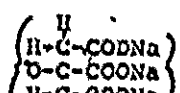
RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETES	REFERENCES
HOCC-CH-CH_2 – S· NH_3Cl	Electron irradiation at 77°K of L-cysteine hydrochloride.	77°K	1.99 1.99 2.29 iso:2.09	$a_H^{CH_2}$: 37... 39 β(1) $a_H^{CH_2}$: 12... 16 β(2)	Alasaka, K. J. Chem. Phys., 43, 1182, (1965).
HOOC-CH_2-HCS·COOH	γ irradiation of mercaptosuccinic acid (HCOC-HCSH-CH_2-COOH) at 300°K	300°K	2.054 2.026 2.003 iso:2.028		Hahn, Y. and H.N. Roxroad, J. Chem. Phys., 38, 1599, (1963).
	γ irradiation of N-acetyl-methionc at 300°K	300°K	2.004 2.029 2.064 iso:2.004	$a_H^{CH_2}$: 9.5 β H	Cipollini, E and W. Gordy, J. Chem. Phys. 37, 13 (1962)
N_2^-	UV irradiation of barium azide (anhydrous) at 300°K	77°K*	1.997 1.997 1.979 iso:1.991	a_N: 3.6; 20.0, 4.1 iso: 9.23	MARINKAS P. and BARTRAM, R. J. CHEM. PHYS. 48, 927, (1968)
N_2^-	X-irradiation of sodium azide (NaN_3) at 2°-4°K	2-4°K		a_N: 3.9, 23.5, 11.2 iso: 12.86	SILSBEE, R.H. J. CHEM. PHYS. 45, 1703 (1966)
NO_3^-	γ-irradiation of potassium bicarbonate and urea nitrate at 300°K	300°K	2.0066 2.0114 2.0203 iso: 2.0127		CHANTRY, G.W., et al., M.P. 5, 589 (1962)

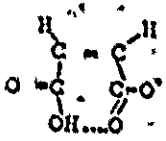
Radical	Generation	ESR Temp	g values	Splitting parameters in gauss	References
SO_2^+	Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) x-irradiated at 300°K	300°K	2.0070 2.0105 2.0305 iso: 2.016		Dolisle, J.M. J. Chem. Phys. 43 3298 (1965)
$(CH_3)_3-N^+$	γ-irradiation of Betaine Hydrochloride ($(CH_3)_3NCH_2COO$) at 300°K	300°K	2.0048 2.0059 2.0036 iso: 2.004	aN: 7.8, 14.3, 60.3 iso: 27.5 aCH_3: 27.8 iso	Schoffa, G. J. Chem. Phys. 40 593 (1963)
$(CH_3)_3-NH_3^+$	γ-irradiation of tetramethyl ammonium chloride ($N(CH_3)_4Cl$) at 300°K	300...403°K	2.0042 2.0048 2.0036 iso: 2.004	aN: 20.4, 20.4, 13.2 iso: 18.0 aCH_3: 26.7 iso	Tench, A.J. J. Chem. Phys, 38. 593 (1963)
	X-irradiation of histidine Hcl.	4.2°K		aH^{CH_2}: 53.2, 50.7, 49.5 iso: 51.1 $a'H^{CH_2}$: 50.2, 47.1, 46.6 iso: 48.0 iso: 3.2 aN) = aN(3): 0.10, 1.6	Box, H.C. et al. J. Chem. Phys. 46, 2130

RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
	γ-irradiation of ^{15}N labelled 5-Nitrouracil at 300°K	300°K	2.0116 2.0062 2.0023 iso: 2.0067	aN: 32.7, 37.4, 58.8 iso: 43.0	Benson, B.W. Mol. Phys., 24, 1175 (1972)
 *	X-irradiation of maleic acid-d$_2$ (DOOC-CH=CH-COOD) at 77°K in the dark	77°K	2.0261 2.0061 2.0035 iso: 2.0119	aH: 6.7, 2.7, 2.3, iso: 3.9	Eda, B. and M. Iwasaki J. Chem. Phys. 35, 563 (1971)
NO_2^-	γ-irradiation of sodium Nitrite ($NaND_2$) at 77°K	77°K	2.0057 2.0015 1.9910 iso: 1.99895	aN: 49.4, 67.9, 46.7 iso: 54.7	Zeldes, H. and R. Livingston J. Chem. Phys. 35, 563 (1961)
$NH-NH_3^+$	γ-irradiation of semicarbazide hydrochloride at 300°K	300°K	2.0023 2.0041 2.0044 iso: 2.0036	aN: 34.2, 11.8, 4.8 iso: 16.9 aNB: 5...3 (iso: 4) aHα: 36.7, 27.6, 10.5 iso: 24.9 aHB 16...14 (iso 15.)	Shrivastava, K., and Anderson, R. J. Chem. Phys. 48, 4599 (1968)

* sigma radical

Radicals	Generation	ESR Temp	g-factors	Splitting parameters in gauss	References
$HO_2^{\cdot}$	γ-irradiation of hydrogen peroxide urea: $(H_2O_2 \cdot CO(NH_2)_2)$	77°K	2.049 2.008 2.001 iso: 2.0193	aH: ~11.0	Ichikawa, T. and N. Iwasaki J. Chem. Phys. 44, 2979
OH(I)	γ-irradiation of sodium hydrogen maleate trihydrate at 77°K	77°K	2.0034 2.0081 2.0618 iso: 2.0244	aH: 27.0, 44.7, 3.5 iso: 32.7	Toriyama, K. and M. Iwasaki, J. Chem. Phys. 55, 1890 (1971)
OH(II)	γ-irradiation of sodium hydrogen maleate trihydrate at 77°K	77°K	1.9947 2.0065 2.1198 iso: 2.0040	aH: 29.4, 44.8, a.8 iso: 23.1	
OH	γ-irradiation of ice at 77°K	77°K	2.0077 2.0077 2.0127 iso: 2.009	aH: 35.3, 35.3, 53.3 iso: 41.3	McMillan, J.A. et al. J. Chem. Phys. 33, 609 (1960)
$H_2O^{\cdot}$	γ-irradiation of ice at 77°K	77°K	2.00 2.04 2.04 iso: 2.02		McMillan, J.A.. et al. J. Chem. Phys. 33, 609 (1960)

RADICAL	GENERATION	ESR TEMP.	G-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
NO_2	electron irradiation of $Pb(NO_3)_2$ lead nitrate at 300°K	77°K	2.005 1.995 2.004 iso: 1.998	aN: 56.9, 56.9, 50.0 iso: 54.6	Golding, R. and Honchman J. Chem. Phys. 40, 1554 (1964)
NO_3	electron beam irradiation of $Pb(NO_3)_2$ lead nitrate at 300°K	77°K	2.029 2.029 1.998 iso: 2.0186	a^{207}Pb: ~2, ~1	Golding, R. and Honchman J. Chem. Phys. 40, 1554 (1964)
 $\cdot 5H_2O$	γ-irradiation of sodium citrate pentahydrate at 300°K	300°K		a^{Cl}: 5.7, 7.6, 10.0 5.35	Russell, D.B. J. Chem. Phys. 43, 7996 (1965)
$H_2^+NCH_2CO_2^-$	γ-irradiation of glycine at 77°K	77°K		a^{NH} 22, 15, 15 iso: 17.3 a^{α} N: 41, 10, 10 iso: 20.3	Serdobov, M. et al. J. Chem. Soc. Perkin II, 1601 (1975)
SO_3^-	potassium sulphamate γ-irradiated at 300°K	300°K	2.004		Rowlands, J.R. Mol. Phys., 565 (1962).

RADICAL	GENERATION	ESR TEMP	g-VALUES	SPLITTING PARAMETERS	REFERENCES
$^{32}S\ ^{33}SO_2^-$ $^{33}S\ ^{32}SO_2^-$	x-irradiation of $Na_2S_2O_3 \cdot 5H_2O$ at Room Temp.	300°K.		$a^{33}S$: 183,145,145 iso : 157 $a^{33}S$: 38.9,21.1,11.4 iso: 23.8	NORTON, J.R. Can. J. Chem. 43 1948(1969)
$^{+}N(CH_3)_3$	γ-irradiation of $[(CH_3)_3\overset{+}{N}CH_2CO_2^3H]\ OH^-$ at Room Temp.	300°K		$a^{14}N$: 38.9,5.7,11.7 iso : 18.9 aH : 26.1	DREWS, R. et al Can. J. Chem. 43 2439(1965)
$\cdot NH_2$	γ-irradiation of $K^{15}NH_2SO_3$ at 77°K	77°K		$a^{1}H$: 31.4,25.3,25.0 iso: 27.2 $a^{15}N$:53.5,1.9,0.3 iso: 18.5	NORTON, J.R. and D.R. Smith Can J. Chem. 44: 1951 (1966)
$\cdot NH_2SO_3^-$	γ-irradiation of $K^{15}NH_2SO_3$ at 77°K	77°K		aH: 26.4,18.1,9.8 iso:18.1 $a^{15}N$: 44.9,6.8,2.9 iso: 18.2	NORTON, J.R. and D.R. Norton Can. J. Chem. 44 1951 (1966)
$\cdot NHSO_3^-$	γ-irradiation of $K^{15}NH_2SO_3$ at 77°K	77°K		$a^{1}H$: 37.5,21.4,8.3 iso: 22.4 $a^{15}N$: 51.1,3.3,0.7 iso: 18.3	NORTON, J.R. and D.R. Norton Can. J. Chem. 44 1951 (1966)
	irradiation of deuterated maleic acid (DOOCCH-CHCOOD) at 77°K	77°K	2.0261 2.0061 2.0035 iso:2.011	aH: 6.4, 3.6, 1.7 iso: 3.9	Iwasaki, M. et al J. Am. Chem. Soc., (1970) 3211

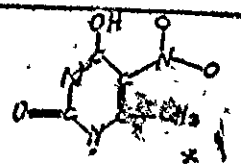
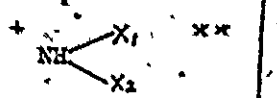
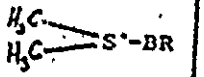
*Sigma radical

FREE RADICALS	GENERATION	RES. TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCE
O_2^-	γ-irradiation of hydrogen peroxide urea [$H_2O_2 \cdot CO(NH)_2$] at 77°K	77°K	2.088 2.088 2.000 iso: 2.0586		Ichikawa, T. and M. Iwasaki; J. Chem. Phys., 44, 2972
O_3^-	X-irradiation of sodium sulfate at 77°K	77°K 300°K	2.006120 (iso).		Hariharan, N. and Sobhanadri, J., Mol. Phys., 18, 713, 1970.
SO_3^-	X-irradiation of sodium sulphate at 77°K & 300°K	2.004 (iso.)			Hariharan, N. and J. Sobhanadri, Mol. Phys., 18 713, 1970.
$N_2O_4^-$	Y irradiation of sodium nitrite at 300°K	300°K	2.0055 2.0047 2.0142 iso: 2.0142	a_N: 8.0, 2.0, 2.0 iso: 4.0	Tatono, J. and K. Gesi, J. Chem. Phys., 40 317, (1964).
$(CS^+)_2(TCNQ)_3^-$	7,7,8,8 Tetracyanoquino dimethansalt	175°K	2.0025 2.0015 2.0003 iso: 2.0014		Chesnut, D.B. and P. Arthur Jr., J. Chem. Phys., 36 3969, (1962).
$TCNQ^-$	Quinolium $(TCNQ)_2$		2.002 2.0026 2.0004 iso: 2.0028		J. Chem. Phys., 35 4370 (1961).
SO_3^-	Irradiation of potassium methane disulphonate $(K_2CH_2(SO_3)_2)$	300°K	2.0036 (iso).	aS: 112,113,153 iso a: 129	Chantry, G. et al Mol. Phys., 5, 233 (1962).

*unpaired electron is delocalized over several TCNQ molecules

RADICAL	GENERATION	ESR TEMP.	g-VALUES	SPLITTING PARAMETERS IN GAUSS	REFERENCES
SO_2^-	X-irradiation of Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) at 300°K	300°K	2.0070 2.0047 2.0095 iso: 2.00706		Dolisio, J.M. J. Chem. Phys., 43, 3298 (1965).
OH	γ irradiation of Ammonium Oxalato monohydrate $(NH_4)_2 C_2O_4 \cdot H_2O$ at 300°K	300°K	2.002 2.002 2.026 iso: 2.01	aH: 6.6, 2.6, 9.3 iso: 15.6	Krishnamurthy, M. Mol. Phys. 24, 1353, (1972).
SO_2^-	X-irradiation of sodium sulphate at 77°K & 300°K	77°K & 300°K	2.0075 2.0013 2.0102 iso: 2.0063		Hariharan, N. and J. Sobhanadri, Mol. Phys. 18 713 (1970).
$N(SO_3)_2^=$	γ irradiation of potassium amino disulphonate at 300°K	300°K	2.0042 2.0082 2.0025 iso: 2.0049	aN: 37.9, 0, 2.1. iso: 13.3	Horsfield, A. et al, Mol. Phys., 5, 241 (1962).
N_4^-	γ-irradiation of (Rb N_3) Rubidium azide at 77°K	92°K	2.0047 1.9953 2.0013 iso: 2.0043	a_N 2.5, 1.6, 8.1 iso: 4.1	Guha, C. et al, Physical Rev. B 7, 4047, (1973)
N_4^-	UV irradiation of potassium azide (KN_3) at 300°K	77°K	2.005 1.997 2.002 iso: 2.0104		Shuskus, A. et al, J. Chem. Phys., 33, 622 (1960).
O_3^-	irradiation of $KClO_3$ at 300°K	77°K	2.0025 2.0113 2.0174 iso: 2.0104		Atkins, P.W. et al J. Chem. Soc., (1962) 4785

RADICAL	GENERATION	ESR TEMP.	g-FACTORS	SPLITTING PARAMETERS IN GAUSS	REFERENCES
$\dot{S}\text{-}CH_2\text{-}CH(NH_3Cl)COOH$	UV irradiation of cysteine HCl at 77°K and momentary warming.	77°K	1.985 2.004 2.251 iso:2.079	$aH_{\beta(1)}$: 32.5,35.7, 38.9 iso: 35.7 $aH_{\beta(2)}^{CH_2}$: 10.3,12.0, 13.7 iso: 33.7	Box, H.C. et al J. Chem. Phys., 45, 809 (1966).
SO_3^-	x-irradiation of sodium sulphate at 300°K	300°K	2.0045		Hariharan, H., and J. Sobhanadri, Mol. Phys., 17, 507 (1969).
O_3^-	x-irradiation of sodium sulphate at 300°K	300°K	2.0100		Hariharan, H., and J. Sobhanadri, Mol. Phys., 17 507 (1969).
SO_2^-	x-irradiation of sodium sulphate at 300°K	300°K	2.0218 2.0069 2.0076 iso:2.0121		Hariharan, H., and J. Sobhanadri, Mol. Phys., 17, 507 (1969).
SO_4^-	x-irradiation of sodium sulphate at 300°K	300°K	2.0337 2.0271 2.0028 iso:2.0212		Hariharan, H., and J. Sobhanadri, Mol. Phys., 17 507 (1969).
CO_2^-	neutron irradiation of calcite at 77°K	77°K	2.00161 2.0030 1.99227 iso:2.0006	^{13}aC: 177.28, 134.62, 131.69 iso: 147.8	Marshall, S.A., Mol. Phys., 8, 225, (1964).
$\{R\text{-}CH_2\text{-}S\cdot S\text{-}CH_2\text{-}R\}^-$	γ-irradiation or electron irradiation of L-cystine dihydrochlorine 77°K	77°K	2.018 2.018 2.002 iso: 2.0126	$aH_{\beta1}$: 8.5... 10.5 $aH_{\beta2}$: 6.0... 8.0	Alaska, K et al J. Chem. Phys., 40 3110 (1964).

Radicals	Generation	ESR Temp	G- factors	Splitting Parametes	References
$R_1-CR_2NO^{\cdot}$... +	X-irradiation of dimethyl glyoxime $-O_1\ O_1-d_2$ at 77°K	77°K			Kurita, Y. and N. Kashiwagi J. Chem. Phys. 44, 1727 (1966)
$R_1-CR_2NO^{\cdot}$.... ++	X-irradiation of Glyoxime at 77°K	77°K			
$R_1-CR_2NO^{\cdot}$.... +++	X-irradiation of methyl glyoxime at 77°K	77°K			
 *	electron irradiation of 5-Nitro 6 Methyl uracil at 77°K then warmed up to 110°—250°K	77°K	2.0066 2.0055 2.0021 iso2.0047	$a^{14}N$: 43.8, 17.8, 16.9 iso: 26.2	SAGSTUEN, E. Rad. Res., 55, 225 (1971).
 **	γ-irradiation of $(NH_4)_2HPO_4$ at 300°K	300°K	2.0026 2.0048 2.0089 iso2.0054	aN: 3.7, 8.1, 36.2 iso: 16.0 aH: 11.3, 22.7, 36.2 iso: 23.4	MORTON, J.R. Phys. Chem. Solids, 24, 209 (1963)
	X-irradiation of dimethyl (9 fluorenyl) sulfonium bromide at 300°K	300°K	1.9900 2.0753 2.0700 iso: 2.0481	$a^{81}Br$: 366.2, 85.8, 85.8 iso: 179.3 $a^{79}Br$: 339.7, 79.1 79.1 iso: 165.9	Lucken, E. and Mazolino, C. J. Chem. Phys., 48, 1942 (1968)
$CH_3\ S^{\cdot}$	X-irradiation of dimethyl (9 fluorenyl) sulphonium Bromide at 300°K	300°K	2.054 2.003 2.012 iso: 2.012		

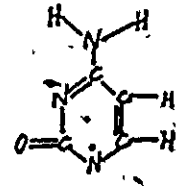
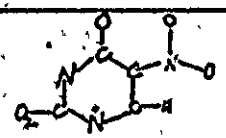
++ R_1= CHNOH and R_2= H

+ R_1= C(H$_3$)NOD and R_2= CH_3

* sigma radical

+++ R_1= CHNOH and R_2= CH_3

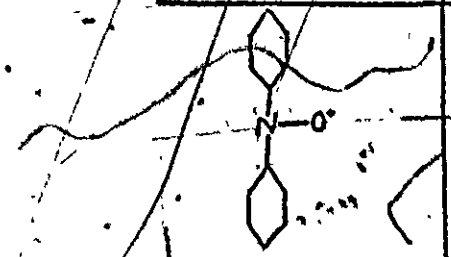
** structure of X unknown

radicals	Generation	ESR Temp.	g factors	SPLITTING PARAMETERS IN GAUSS	REFERENCES
$H_2N\text{-}CONH$	X-irradiation of hydroxy urea at 300°K	300°K	2.0108 2.0027 2.0062 iso2.0065	aH_α: 21.2, 13.5 1.5 iso: 12.07 aN: 22.2, 1.5, 1.2 iso: 8.3	SHIELDS, H.W. et al J. CHEM. PHYS. 46, 2510 (1967).
$^{\bullet}NH_2C(NH)NCN$	γ-irradiation of dicyandiamide at 300°K	300°K			LIND, G. and KENLY, R. Can. Journal of Chem. 49, 2514 (1971)
$\dot{N}H_2^+\text{-}SO_3^-$	γ-irradiation of $NH_3^+\text{-}SO_3^-$ at 300°K	77.. 300°K		aH: 29.6, 23.2 15.7 iso: 22.8 ++ aH: 36.8, 11.8, 6.1 iso: 18.2	ROWLANDS, J.R. and D.H. WHIFFEN, Nature 193, 61 (1962)
	cytosine monohydrate γ-irradiated at 300°K	300°K	2.003 †	$aN_{(a)}$: 0.35, 0, 15.3 iso: 5.0 $aN_{(b)}$: 07, 0, 7.8 iso: 2.5 aH_β: 21.8, 14.2, 13.6 iso: 16.4	COOK, J.B. et al MP 13, 49 (1967)
$\dot{N}H(SO_3^-)$	γ-irradiation of potassium sulphamate at 300°K	300°K	2.0078 2.0038 2.0037 iso2.0057	a_N^{14}: 34.8, 3.6, 1.96 iso: 13.4 aH: 38.2, 21.1, 8.7 iso: 22.7	ROWLANDS, J.R. MP 5, 565 (1962)
	γ-irradiation of 5-nitrouracil at 300°K	300°K	2.0117 2.0064 2.0027 iso2.0069	aN: 22.5, 25.2 40.0 iso: 29.2	BENSON, B. and SNIPES, W MP. 20 357 1970, SYMONS, M.C.R: MP. 22, 551 (1971)
+ nearly isotropic g factor * a sigma radical as well as π radical with same chemical formula is formed			++ at 300°K		

Radical	generation	ESR temp	g-factors	Splitting parameters in gauss	References
$[HOOC-CH_2-\overset{\oplus}{S}-CH_2-COOH]$ ⊕	irradiation of thiodiglycollic acid ($HOOC-CH_2-S-CH_2-COOH$) at 4°2°K	4°2°K	2.0022 2.011 2.004 iso: 2.0123		Box, H.C. et al. J. Chem. Phys. 41 3974 (1968)
$(S-CH_2-CH_2-CH-COOH)_2$ NH_3C ††	X-irradiation of L-cystine dihydro chloride at 4°2 K	77°K	2.033 2.028 2.005 iso: 2.022		Box, H.C. and H. Freund, J. Chem. Phys. 41, 2571 (1964)
$(S-CH_2-CH_2-CH-COOH)_2^{\oplus}$ (NH_3Cl) ⊕	X-irradiation of L-cystine dihydrochloride at 77°K	4°K	2.0024 2.0174 2.0178 iso: 2.0125	$a^{CH_2}_{H_\beta}$: 7.5, 9.6, 10.0 iso: 9.0 $a^{CH_2}_{H_\beta}$: 6.4, 8.6, 9.6 iso: 8.2	Box, H.C. and H. Freund, J.Chem. Phys. 40, 817 (1963)
$S-CH_2-CH(NH_3Cl)COOH$	UV-irradiation of cystring Hcl at 77°K and momentary warming	77°K	1.085 2.004 2.251 iso: 2.08	$a^{CH_2}_{H_\beta}$: 32.5, 35.7, 38.9 iso: 35.7 $a^{CH_2}_{H_\beta(2)}$: 10.3, 12.0 13.7 iso: 12.0.	Box, H. et al. J. Chem. Phys. 45, 809

⊕ Endor also used †† electron localized on disulphide group

Radical	Generation	ESR Temp	g factors	Splitting parameters in gauss	References
NH_3^+	X-irradiation of NH_4ClO_4 at 300°K	300°K	2.0039 2.0034 2.0032 iso:2.0035	aH: 25.8, 25.6, 26.2 iso: 25.9 aN: 22.1, 19.0, 16.8 iso: 19.5	Cold, J. Journal of Chemical Physics 35, 1169. (1961)
ND_3^+	X-irradiation of (deuterated) ND_4ClO_4 at 300°K	300°K	2.0039 2.0034 2.0032 iso:2.0035	aN: 21.2, 18.4, 16.7 iso: 18.8 aD: 4.0 (iso)	Cole, I., J. Chem Phys. 35, 1169
$N_2O_2^+$	electron irradiation of sodium nitroprusside ($Na_2Fe(CN)_5NO.2H_2O$) at 77°K	300°K	1.899 2.108 2.028 iso:2.011	a_N: 8, 2, 2 iso: 4	Muniz, R. and J. Danon, Molecular Physics 9, 999 (1965)
$N_2H_4^+$	γ-irradiation of hydrazinium hydrogen oxalate ($N_2H_5HC_2O_4$) at 300°K	300°K	2.0047 2.0022 2.0039 iso: 2.0036	aN: 27.6, 2.5, 2.5 iso: 10.9 aH: 2, 20.6, 13 iso: 11.9	Edlund, O. et al J. Chem. Phys. 49, 749 (1968)

RADICAL	GENERATION	ESR, TEMP	g-VALUES	SPLITTING PARAMETERS	REFERENCES
	diphenylnitric oxide diluted in benzophenone (single crystal)	300	2.0063	aN: 37.3, 4.4, 4.4 iso: 9.5 $a_2 = a_2^1 = a_4 = a_4^1 = a_6$: 1.17, 3.52, 3.52 iso: 1.96 $a_3 = a_3^1 = a_5 = a_5^1$: 1.76, 0.20, 0.20 iso: 0.98	Daguchi, Y. Bull. Chem. Soc. Jap. 34, 910 (1961)

In some cases (Box and Freund 1964 page 6-7 of table 2 ; Box, et al., 1965 page 6-5 of table 1 ; Arasaka, et al., 1964 page 6-60 of table 2) different radicals have been observed after irradiation and examination at liquid nitrogen temperature and subsequent warm up has not always yielded the same radicals as are produced by room temperature irradiation.

The most commonly observed radical results from the breaking of a C-H bond to give a π electron radical centered on a carbon. As a result bond hybridization is changed from tetahedral sp^3 to planar sp^2 and the unpaired electron occupies what is predominantly a carbon 2p orbital directed perpendicular to the plane of the radical. Other radicals formed by the rupture C-N, C-C, N-H, C-F and S-S bonds have also been observed.

It should be noted that many radicals are formed but only those which are stable are observed. In fact it is the stability of the product rather than the bond strengths of the precursor molecule which determines how many and what kind of radicals can be seen.

Interaction of Unpaired Electron and Neighbouring Atoms

In equation 1 the term $\underset{\sim}{S}\cdot\underset{\sim}{A}\cdot\underset{\sim}{I}$ describes the hyperfine interaction between an electron's magnetic moment and that of the nucleus. There are two fundamentally distinct components of hyperfine interactions. They are called the Fermi contact term which is isotropic

and independent of direction and the anisotropic or dipolar term which depends on the magnetic field direction but is symmetric to inversion. For the carbon centered radical, the isotropic interaction term is proportional to the s character of the orbital occupied by the electron and the anisotropic term is proportional to the p character of this orbital.

To simplify the following discussion, all except s- and p- orbital contributions to hyperfine interaction are neglected. An electron in an s orbital has a non zero probability of being found at the atomic nucleus while an electron described as being in a p orbital has zero probability of being found at the nucleus. In addition, of these two configurations only the latter has non zero angular momentum. Just as the wave function for a single electron in an atom is called an atomic orbital so by analogy a wave function describing an electron in a molecule is called a molecular orbital. A good model of a molecular orbital is a linear combination of atomic orbitals. Combinations of s- orbitals have zero angular momentum about their axis and are called σ (sigma) orbitals. These orbitals can also be formed from $2p_z$ orbitals. Linear combinations of p_x and p_y orbitals have an angular momentum of 1 unit, and are called π orbitals. Fig. 2 illustrates the formation of the σ and π molecular orbitals.

Since as pointed out before anisotropic interaction is proportional to the p- character of the orbital of the unpaired electron, it is possible to estimate this p character by comparing

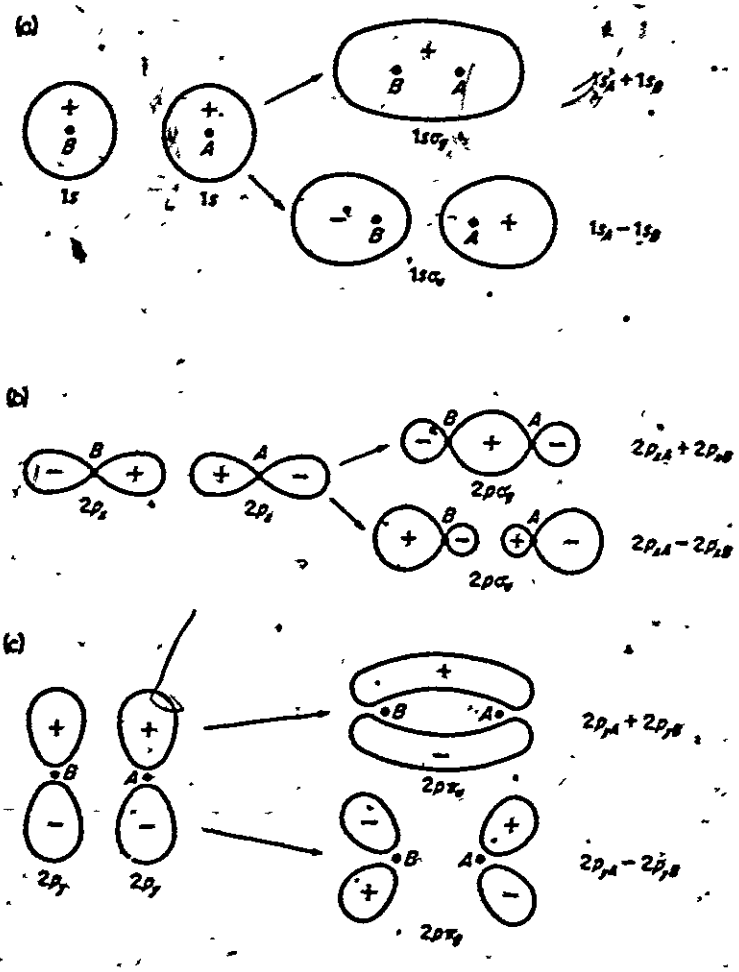


FIG. 2 Formation of molecular orbitals by linear combinations of atomic orbitals.

the observed anisotropic interaction, B with a parameter B_o calculated as if the unpaired electron were wholly in a p orbital. The S- character of the unpaired electron may similarly be estimated by comparing the observed isotropic interaction, A with a value A_o which has been calculated as if the electron was entirely in an S orbital. Table 3 gives a few hyperfine constants that have been calculated from available wave functions. A review article published by Morton in Chem. Rev. 64, 453 (1964) discusses this in greater depth with appropriate references.

Hyperfine Interaction with α Hydrogen

An α hydrogen is one which is directly attached to the free radical atom in a π electron system. According to McConnell and Chestnut (10) the isotropic proton hyperfine coupling A_H, is proportional to the spin density ρ, on the central atom ie:

$$A_H = Q\rho \qquad (7)$$

where Q is a constant approximately equal to -64 MHz. A large number of radicals containing α hydrogen interactions have been identified and some of them are listed in Table 4.

This table illustrates a number of points namely that for carbon centered radicals isotropic coupling for the α hydrogens is fairly constant and varies between 50 and 63 MHz. For the relatively few nitrogen centered radicals that have been identified, α hydrogen isotropic coupling is usually over 64 MHz. More examples are needed however before it can be definitively stated that α hydrogen iso-

Table 3

Atomic Parameters $\psi^2_{ns}(0)$ and $\langle r^{-3}\rangle_{np}$ (A.U.) and One-Electron Hyperfine Constants (M.c.p.s.)

Nucleus	n	$\psi^2_{ns}(0)$	A_s	$\langle r^{-3}\rangle_{np}$	B_p
H^1	1		1420		
B^{11}	2	1.408	2020	0.775	53
C^{13}		2.767	3110	1.692	91
N^{14}		4.770	1540	3.101	48
O^{17}		7.628	4628	4.974	144
F^{19}		11.906	47910	7.546	1515
Si^{29}	3	3.807	3381	2.041	87
P^{31}		5.625	10178	3.319	287
S^{33}		7.919	2715	4.314	78
Cl^{35}		10.643	4634	6.710	137
As^{75}	4	12.460	9582	7 111	255
Xe^{129}	5	26.71	33030	17.825	1052

This table has been taken from a review article published by Morton, Chem. Rev. 64, 453, 1964.

Table 4 - Hyperfine interaction (in MHz) with α-hydrogen atoms

Host crystal	Radical	Hyperfine interaction				References
		Isotropic	Anisotropic			
Glutaric acid	$HO_2C-(CH_2)_2-\dot{C}H-CO_2H$	-56	+30	+3	-32	Horsfield, A., Morton, J., Whiffen, D., Mol. Phys. 4. 169 (1961).
		-51	+28	-1	-28	
Succinic acid	$HO_2C-CH_2-\dot{C}H-CO_2H$	-60	+30	+1	-32	Heller, H., and McConnell, H., J. Chem. Phys. 32. 1535 (1960). Pooley, D., Whiffen, D., Mol. Phys. 4. 81 (1961).
Adipic acid	$HO_2C-(CH_2)_3-\dot{C}H-CO_2H$	-57	+29	+7	-37	Morton, J., Horsfield, A., Mol. Phys., 4. 219 (1961).
Hexamethylenediammonium adipate	$^-OOC-(CH_2)_4-\dot{C}H-COO^-$	-55	+30	+4	-34	Kawashita, M., and Kurita, Y., J. Chem. Phys., 39. 3165 (1963).
Diglycollic acid	$HO_2C-CH_2-O-\dot{C}H-CO_2H$	-49	+24	+3	-27	Kurita, Y., J. Chem. Phys., 36. 560 (1962).
Thiodiglycollic acid	$HO_2C-CH_2-S-\dot{C}H-CO_2H$	-43	+21	-2	-23	Kurita, Y., and Cordy, W., J. Chem. Phys., 34. 1085 (1961).
Glycylglycine	$H_3N^+-CH_2-CO-NH-\dot{C}H-CO_2^-$	-52	+27	-1	-26	Katayama, M. and Cordy, W., J. Chem. Phys., 35. 117 (1961).
		-51	+27	+4	-32	
Glycylglycine hydrochloride	$H_3N(Cl)-CH_2-CO-NH-\dot{C}H-CO_2H$	-53	+23	-5	-18	Box, H., et al., J. Chem. Phys. 39. 2100 (1963).
N-Carbamyl glycine	$H_2N-CO-CO-NH-\dot{C}H-CO_2H$	-54	+26	-8	-18	Rao, D., Katayama, M., J. Chem. Phys., 37. 382 (1962).

tropic couplings in nitrogen centered radicals have larger values than those for carbon centered radicals. Table 4 also shows that α hydrogen atoms trapped in π electron systems exhibit characteristic anisotropic interaction.

Anisotropic hyperfine energy is described by the following expression

$$g\beta\gamma M_I M_S \left\langle \frac{1-3\cos^2\alpha}{r^3} \right\rangle_{av} (1-3\cos^2\theta) \qquad (8)$$

where g is the gyromagnetic ratio for the electron, γ the gyromagnetic ratio for the nucleus, β is the Bohr magneton, M_I the nuclear spin quantum number, M_S the electron spin quantum number, r the distance from the nucleus to the unpaired electron, α the angle between this line and a principal axis of the tenson and θ the angle between the magnetic field and this same principal axis. To estimate the sign of the anisotropic hyperfine interaction we need only to determine the sign of the quantity $(1-3\cdot\cos^2\theta)$.

Hyperfine Interaction with β Hydrogens

A β hydrogen is one which is two bonds away from the free radical atom. The most common form is $\dot{C}-C-H_\beta$ with carbon as both the central and intermediate form. A β hydrogen is therefore not as near to the odd electron's orbital as the α hydrogen. Since anisotropic interaction is proportional to r^{-3} (See eqn. 8) little anisotropy is expected (13). Experimentally, it has been observed that the principal values for anisotropic β hydrogen couplings

rarely exceed 10% of the isotropic coupling. They have been found to vary from less than 10 MHz (14,15) to over 120 MHz (16). The size of this β coupling depends on the radical's geometry - being small if the β proton is in the nodal plane of the unpaired electron's 2p orbital and large if is far from that plane. Stone and Maki (17) have shown that the isotropic β coupling is given by the expression

$$\beta_1 + \beta_2 \cos^2\theta \qquad (9)$$

where β_1 and β_2 are constants such that $\beta_1 < \beta_2$, θ is the angle between the C_β-H_β bond and the axis of the odd electron's 2p orbital projection perpendicular to the plane of the $\dot{C}_\alpha$-C_β bond. From α-alanine studies at liquid nitrogen temperature, Horsfield et al. (18) have calculated a value of less than 10 MHz for β_1. β_2 has a value of 130 ± 10 MHz (19). Other theoreticians such as McLachlan (20) and Lykos (21) have shown that isotropic coupling is positive. In Table 5 β coupling values for several radicals are listed.

In some cases (Table 6) β hydrogen coupling involve methyl groups. The most common examples involving methyl groups are found in radicals of the type $\dot{C}$-CH_3 and $\dot{N}$-CH_3.

At room temperature, the methyl group rotates freely and so the protons become geometrically equivalent. This equivalence results in a spectrum of four equally spaced lines with intensity ratio 1:3:3:1. The weak anisotropy means that even polycrystalline samples give well resolved spectra. At lower temperatures, for

Table 5 - Hyperfine interaction (in MHz) with β-hydrogen atoms

Host crystal	Radical	Hyperfine interaction				References
		Isotropic	Anisotropic			
Deuterated DL-serine	$(DO)_2\dot{C}$-HCND$_2$-CH$_2$OD	+118	+5	-3	-3	Rao, D., and Gordy, W., J. Chem. Phys., 35. 764 (1961).
Succinic acid	HO_2C-CH_2-$\dot{C}H$-CO_2H	+100	+8	-1	-7	Keller, H., and McConnell, H., J. Chem. Phys. 32. 1535 (1960).
		+80	+9	-1	-8	Pooley, D., and Whiffen, D., Mol. Phys. 4. 81 (1961).
Valine	$(H_3C)_2$-$\dot{C}$-HC(NH_3^+)-CO_2^-	+8				Whiffen, D., Pure appl. Chem., 4, 185 (1962)
N-Acetyl methionine	H_3C-CO-NH-$\dot{C}$H(CO$_2$H)-$(CH_2)_2\dot{S}$	+27				Cipollini, E., and Gordy, W., J. Chem. Phys. 37 13 (1962).
L-Cystine dihydrochlorine	HO_2C-H_2NCH-CH_2-$\dot{S}$	+25				Kurita, Y., and Gordy, W., J. Chem. Phys., 34. 282 (1961).
Deuterated DL-tartaric acid	DO_2C-DO$\dot{C}$-DOCH-CO_2D	+6	+10	-3	-7	Rao, D., and Gordy, W., J. Chem. Phys., 36. 1143 (1962).
Fumaric acid	HO_2C-R$\dot{C}$H-CH-CO_2H	+56	+8	-4	-5	Cook, R., Rowlands, J., and Whiffen, D., J. Chem. Soc. 3520 (1963).
Maleic acid	HO_2C-$\dot{C}H_2$-CH-CO_2H	+81	+6	-2	-3	Cook, R., Elliot, J., and Wyard, S., Mol. Phys., 12. 185 (1967).
Itdconic acid	H_3C-$\dot{C}$(CO_2H)-CH_2-CO_2H	+47	+6	-2	-5	Fujimoto, M., J. Chem. Phys., 39. 840 (1963)
Thymidine	OC-NH-CH_2-$\dot{C}$(CH_3)-CO-NH	+114				Pruden, B., et al., Proc. Natn. Acad. Sci. U.S.A., 53. 917 (1965).

Table 6 - Hyperfine interaction (in MHz) with methyl groups

Host crystal	Radical	Hyperfine interaction				References
		Isotropic	Anisotropic			
Glycine	$H_3N^+-\dot{C}H-CO_2^-$	+53	+11	-3	-8	Morton, J., J. Am. Chem. Soc. 86. 2325 (1964).
Methyl malonic acid	$H_3C-\dot{C}-(CO_2H)_2$	+71	+5	-2	-2	Heller, H., J. Chem. Phys., 36. 175 (1962).
α-Alanine	$H_3C-\dot{C}H-CO_2H$	+70	+6	-3	-3	Miyagawa, I., and Gordy, W., J. Chem. Phys. 32. 255 (1960).
Acethyl DL-alanine	$H_3C-CO-NH-\dot{C}(CH_3)-CO_2H$	+53				Katayama, M., J. Mol. Spectrosc., 9. 429 (1962).
α-Amino isobutyric acid	$(H_3C)_2-\dot{C}-CO_2H$	+66	+4	-1	-3	Horsfield, A., Morton, J. and Whiffen, D., Trans. Faraday Soc., 57. 1657 (1961).
Ethylurea	$H_3C-\dot{C}H-NH-OCNH_2$	+64				Jaseja, T. and Anderson, R., J. Chem. Phys. 35. 2192 (1961).
Itaconic acid	$H_3C-C(CO_2H)-CH_2-CO_2H$	+67	+2	0	-2	Fujimoto, M., J. Chem. Phys. 39. 840 (1963).

example 77°K, the spectra is more complex, for the methyl protons are no longer equivalent. This means that the methyl group is either completely locked or rotates rather slowly.

One study illustrating the latter statements is that of Horsfield et al. (18). These investigators examined a crystal of α-alanine that had been γ-irradiated at 77°K. They observed that at 77°K β-hydrogen coupling values from the radical $H_3C\text{-}\dot{C}H\text{-}CO_2H^-$ formed in irradiated α-alanine single crystals are very different from those observed at 300°K. At 77°K the methyl group was rotating at less than 10 MHz and its three protons were inequivalent. The isotropic coupling was still 70 MHz but the individual couplings instead of having the room temperature values 67, 71 and 73 MHz were now 120, 76 and 14 MHz. Horsfield et al. calculated the degree of deviation from the crystal axes by the radical and showed that the methyl group had taken up a skew position in relation to the $-\dot{C}\text{-}\dot{C}\text{-}H_\alpha$ plane of the radical. Between 100 and 200°K there is a gradual change in the spectrum of the $H_3C\text{-}\dot{C}H\text{-}CO_2H$ radical (22, 23) and free rotation of the methyl group occurs only above 190°K (24). The temperature at which free rotation of the methyl group begins, differs from radical to radical depending on the steric factors involved. In the radical $H_3C\text{-}\dot{C}\text{-}(CO_2H)_2$ formed by irradiating methyl malonic acid (25) the methyl group still rotates freely at 4°K. The radical $(H_3C)_2\text{-}\dot{C}\text{-}CO_2H$ which can be formed by γ-irradiating α-amino isobutyric acid has 2 methyl groups. Morton (26) has shown that at 40°K the protons of one methyl group rotate freely while those from the other group do not. Table 6 gives some examples of

β hydrogen coupling from radicals with methyl groups at room temperature.

β hydrogens are also found in radicals of the type $>\dot{C}-OH$. Here oxygen is the intermediate atom. In these radicals hydrogen is usually in or near the nodal plane of the unpaired electron's orbital and so only small isotropic interaction is observed. The sign of coupling is as a rule negative (27, 28). However in the case of anhydrous lithium glycollate, the hydroxyl group is not in the plane of the radical (29). Spin polarization produces a positive spin density at the proton thereby giving rise to a relatively large positive coupling.

The hydroxyl hydrogen is usually nearer the free radical carbon than the β-hydrogen in a $>\dot{C}-C-H_\beta$ fragment. Dipole-dipole interaction is thus greater and the resultant anisotropics larger.

β-hydrogens are also found in radicals of the type $>\dot{C}-N-H_\beta$ where nitrogen is the intermediate atom. The β proton interacts with the unpaired electron in a similar manner to a β proton with carbon as the intermediate atom. The splitting from the nitrogen is often very close in magnitude to that from the β proton thereby making interpretation of the spectra difficult at times.

Hyperfine Interaction with γ hydrogen atoms

A γ hydrogen is one which is three bonds away from the radical

atom. The radical $\dot{C}-C-C-H_\gamma$ shows such an hydrogen. Hyperfine coupling between the unpaired electron's magnetic moment and that of the γ proton is quite weak and hence is often not detected (16,30). In cases where one of the intermediate atoms is oxygen or sulfur some of the unpaired electron spin density is delocalized from the 2p orbital of the free radical carbon and up to 25% may appear on the adjacent atom. As a result γ proton couplings have been detected in such radicals as $H_3C-O-\dot{C}H-CO_2$ in potassium methoxyacetate (31) and $(HO_2C)-CH_2-S-\dot{C}H-(CO_2H)$ in irradiated thiodiglycollic acid (32).

The isotropic splittings were 6 and 15 MHz respectively. Table 7 shows some γ-proton splittings obtained at room temperature.

Hyperfine Interaction with Nitrogen Nucleus

Unpaired electrons centered on nitrogen nuclei are as a rule localized mainly in the 2p orbital of this nucleus. The N^{14} nucleus has a spin quantum number of 1. Interaction between its magnetic moment and that of the unpaired electron results therefore in an ESR spectrum of 3 evenly spaced lines. Theoretical calculations (33,34) indicate that the isotropic interaction is positive.

Anisotropic interaction is described by the expression (11,12)

$$\beta_0(3\cos^2\theta - 1)\rho \qquad (10)$$

where β_0 is a constant that is 43 MHz for nitrogen, θ the angle

Table 7 - Hyperfine interaction (in MHz) with γ-hydrogen atoms

Host crystal	Radical	Hyperfine interaction		References
		Isotropic	Anisotropic	
1,4-diethoxybutane	$CH_3CH_2O(CH_2)_4\dot{O}CHCH_3$	8.5		Griffith, O.H., J. Chem. Phys. 42. 2651 (1965).
6-undecanone	$CH_3(CH_2)_4\overset{O}{\overset{\|}{C}}\dot{C}H(CH_2)_3CH_3$	8.9		Griffith, O.H., J. Chem. Phys. 42. 2651 (1965).
3-tetra decanone	$CH_3(CH_2)_{10}\overset{O}{\overset{\|}{C}}\dot{C}HCH_3$	8.9		Griffith, O.H., J. Chem. Phys. 42. 2651 (1965).
2-dodecanone	$CH_3\overset{O}{\overset{\|}{C}}\dot{C}H(CH_2)_8CH_3$	5.3		Griffith, O.H., J. Chem. Phys. 42. 2644 (1965).
n-octyl acrylate	$CH_3\dot{C}HCOOCH_2(CH_2)_6CH_3$	6.7		Wedum, E., et al., Trans. Far. Soc. 63. 821 (1967).
Methyl nonenoate	$CH_3(CH_2)_5\underset{\gamma}{\dot{C}H}\text{-}\underset{\alpha}{CH}\text{-}\underset{\beta}{COOCH_3}$	3.1		Wedum, E., et al., Trans. Far. Soc. 63. 821 (1967).

between the applied magnetic field and the 2p orbital axis and p the spin density. The diagonalized coupling tensor describing the hyperfine interaction between the nitrogen's nuclear magnetic moment and that of the unpaired electron in the 2p orbital is thus cylyndrically symmetric — being $+2\beta_0$ when the applied field is parallel to the axis of the unpaired electron's orbit and $-\beta_0$ when it is perpendicular to this axis. The values in Table 8 are in reasonable agreement with this model.

Interaction between the magnetic moments of an unpaired electron on a carbon atom and that of an adjacent nitrogen atom has also been observed. Anisotropic coupling are in these instances very weak.

Isotropic coupling is also generally weak and probably results from a small negative spin density on the nitrogen as a result of spin polarization of >C-N bond. The splitting parameters for nitrogen is rarely more than 12 MHz in these circumstances and so is usually only a contributory factor to linewidth in most ESR spectra.

The N^{15} nucleus has a spin quantum number of $\frac{1}{2}$ and hence in interaction with an odd electron gives an ESR spectrum of 2 lines. This doublet spacing is different from the triplet observed when the N^{14} nucleus interacts with the unpaired electron. Isotropic substitution can thus be used to determine whether hyperfine interaction observed results from the presence of nitrogen (35).

Table 8 – Hyperfine interaction (in MHz) with the N^{14} nucleus

Host crystal	Radical	Hyperfine interaction				References
		Isotropic	Anisotropic			
Potassium sulfamate	$\dot{N}H(SO_3)^{-}$	+38	+60	-28	-32	Rowlands, J., Mol. Phys. 5, 565 (1962).
Potassium amine disulfonate	$\dot{N}(SO_3)_2^{2-}$	+37	+69	-31	-37	Horsfield, A., Morton, J., Rowlands, J., and Whiffen, D., Mol. Phys. 5, 241 (1962).
Betaine hydrochloride	$(CH_3)_3-\dot{N}^{+}$	+77	+92	-37	-55	Schoffa, G., J. Chem. Phys., 40, 908 (1964).
Sulfamic acid	$H_2\dot{N}^{+}-SO_3^{-}$	+51	+52	-18	-34	Rowlands, J. and Whiffen, D. Nature, Lond., 193, 61 (1962).
Diammonium hydrogen phosphate	$H\dot{N}^{+}<$	+45	+56	-21	-35	Morton, J., J. Phys. Chem. Solids 24, 209 (1963).
Deuterated cytosine	$OC-\dot{N}-HC=CH-C(HD)_2-N$	+14	+29	-14	-15	Cook, J.B., Elliot, J. and Wyard, S., Mol. Phys. 13, 49 (1967).
Deuterated cytosine	$OC-\dot{N}=CH-CH=C(HD_2)-\dot{N}$	+7	+15	-7	-9	Cook, J., Elliot, J. and Wyard, S., Mol. Phys. 13, 49 (1967).

Hyperfine Interaction with the Carbon Nucleus

The C^{13} nucleus has spin $I = \frac{1}{2}$ and so when it couples with an unpaired electron a two-line spectrum results. C^{13} however has a natural abundance of only 1.1%. This spectrum is thus rarely seen in irradiated organic single crystals. Isotropic interaction is positive (33,34,36). As with the nucleus N^{14}, anisotropic hyperfine interaction is described by the expression 10 with the constant B_0 being 91 MHz. A few examples of hyperfine interaction with the C^{13} nucleus in single crystals are listed in Table 9. The interactions are in reasonable agreement with the expected pattern. The C^{12} nucleus has zero spin and so is not involved in hyperfine interaction.

Examples of the Misidentification of Free Radicals

It should be emphasized that the esr experimenter can be ignorant of much that does not give an esr signal and mistakes can be made. Radicals which can be identified uniquely by their powder spectra are rare indeed, and in spite of the many publications to the contrary, it is more speculation or good guessing when spectra are attributed to certain unique radicals. It is only with a complete analysis of the orientation pattern of the esr signal from a radical in a single crystal environment that one can be fairly confident that a proposed model is reasonable. Mistakes are still made however if complementary information from theoretical calculations is not available. The necessity of these theoretical

Table 9 - Hyperfine interaction (in MHz) with the C^{13} nucleus

Host crystal	Radical	Hyperfine interaction				References
		Isotropic	Anisotropic			
Malonic acid	$H\dot{C}(CO_2H)_2$	+93	+120	-50	-70	Cole, T. and Heller, H., J. Chem. Phys., 34, 1085 (1961)
Glycine	$H_3N^+-\dot{C}H-CO_2^-$	+127	+127	-60	-67	Morton, J., J. Am. Chem. Soc. 86. 2325 (1964)
Glycine	$H_2\dot{C}-CO_2^-$	+124	+97	+9	-106	Morton, J., J. Am. Chem. Soc. 86. 2325 (1964)
Potassium methane disulfonate	$H\dot{C}(SO_3)_2^{2-}$	+126	+134	-64	-70	Horsfield, A., Morton, J., Fowlands, J., and Whiffen, D., Mol. Phys. 5. 241 (1962)
Succinic acid	$HO_2C-(CH_2)_2-\dot{C}(OH)O^-$	+93	+134	-61	-73	Box, H., et al. J. Chem. Phys. 38. 2100 (1965).

or quantum chemical calculations is made evident by the following two examples.

Publications by Neta and Fessenden (37), Syrons (38) and Lin, Cyr and Toriyama (39) have suggested that X- or gamma irradiation of crystals of compounds containing amide or nitrile groups does not indeed form the sigma radical (Fig. 3) as previously reported (40).

Cyr and Lin (40) in a very careful study of an X-irradiated malonamide single crystal observed a radical spectrum with coupling constants for one nitrogen A_N = 19.3 gauss, B_N = 9.3 gauss and for one proton, A_H = 77 gauss, B_H = 2 gauss. They concluded that the radical has sigma structure (Fig. 3) and this is analogous to the sigma radicals HCO and HCN which also have unusually large large proton hyperfine coupling constants. Neta and Fessenber (37) and then later Syrons (38) suggested that the observed A_N was large enough for the radical to have the structure in Fig. 3 but that the iminyl radical (Fig. 4) was a more likely alternative structure.

Lin, Cyr and Toniyama (39) repeated their experiments using N^{15} substituted cyanoacetamide, $N \equiv C\text{-}CH_2CONH_2$ and observed the same spectra. Their experiment showed that the stable radical was most likely the radical depicted in Fig. 4 and that the production of the same radical from malonamide, $H_2NCOCH_2CONH_2$, requires a rather complicated reaction mechanism.

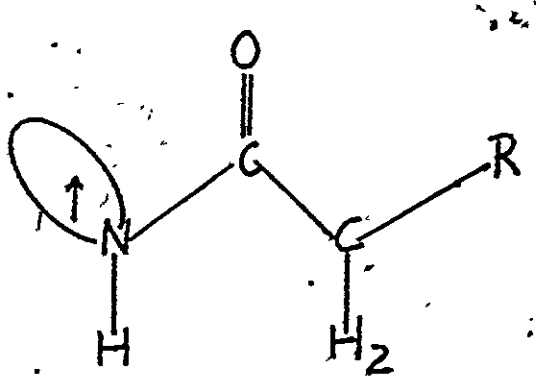


FIG.3. Sigma radical suggested by Cyr and Lin (40) as being formed in malonamide single crystals after X-irradiation.

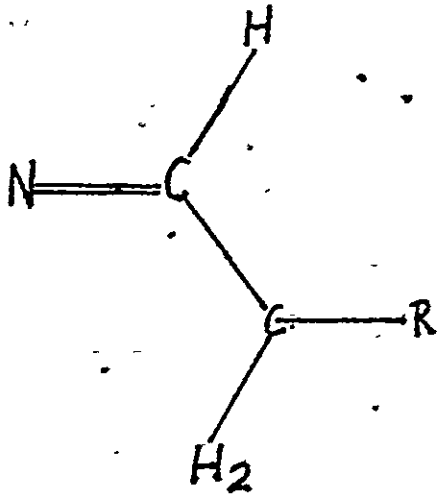


FIG.4. Iminyl radical suggested by Neta and Fessenden (37), Symons (38) and later experimentally confirmed by Lin, Cyr and Toriyama(39) as being formed in malonamide single crystals after X-irradiation.

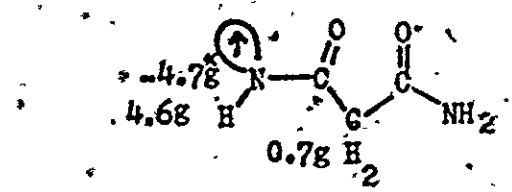
It must be pointed out here that identification might have been made more quickly and easily if aided by spin density calculations using either the extended Hückel or INDO methods (41).

The results of an INDO calculation using Pople's program modified* to be acceptable to the small memory capacity of the CDC 6600 computer at Montreal indicate clearly, (and at a cost of less than $30.00) which of the radicals proposed is the most likely.

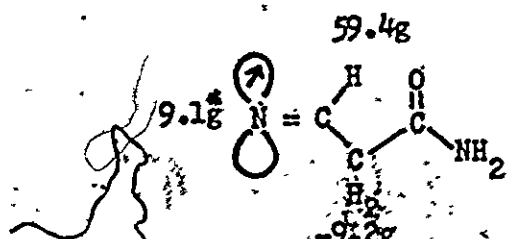
Figure 5 shows the hyperfine coupling constants calculated for each of the radicals. Standard geometries (41) were used throughout. Both the proton and the nitrogen coupling constants calculated for the sigma radical were an order of magnitude smaller than the experimentally observed values. The iminyl radical is shown in Fig. 5b with the calculated coupling constants. The splitting due to the two equivalent beta protons, with a calculated nearly isotropic coupling of 9 gauss, could well have been lost in the poorly resolved spectrum observed by Cyr and Lin. The rather smaller calculated than experimental values are due in part to the inadequacy of the chosen models which ignore intermolecular effects. However, the iminyl radical is definitely the best model!

In a separate study, Lind and Kewly (42) very carefully examined the esr signals obtained from gamma irradiated dicyandiamide, $(H_2N)_2C = N - C \equiv N$ and reported a spectrum which they attributed to the sigma radical $(NH^{\cdot})(NH_2)C = N - C \equiv N$. The hyperfine couplings attributed to this sigma radical are 73-80 gauss for a

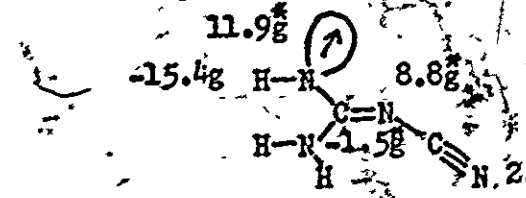
* The author has altered the program by storing all matrices in Hessendin or triangular form.



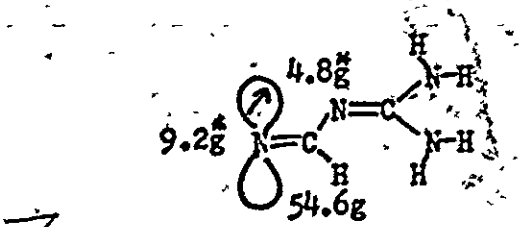
(5a)



(5b)



(5c)



(5d)

FIG. 5. Possible molecular structures of the "dicyandiamide" radical and their calculated (INDO) coupling constants.

proton, 0-25 gauss for a nitrogen and a coupling of less than 6.5 gauss for a third nitrogen. Presumably the radical was formed by the simple abstraction of a hydrogen from an amino group. The calculated coupling constants for the dicyandiamide radical proposed by Lind and Kewly Fig. 5c and that proposed here Fig. 5d, are shown. Again, it is most reasonable to conclude that the sigma radical is unlikely and that the iminyl radical is the best model.

It is unlikely that sigma radicals are stable at elevated temperatures (greater than 77° K). In the presence of hydrogen bonded systems as is the case for malonamide and dicyandiamide, the highly localized charge distribution required by a sigma radical may easily be destroyed by the highly mobile protons in such systems.

Electron Spin Resonance of γ-irradiated single crystals of ethyleneurea (2-imidazolidinone) and ethylenethiourea (2-imidazolidinethione).

Ethyleneurea and ethylenethiourea have been shown to be weakly radioprotective (43,44). Ethylenethiourea has been found also to have antiepileptic, anticonvulsant activity (45). The mechanism of drug action for both drugs possibly involves free radical formation.

The detailed crystal structure of ethylenethiourea has been determined by P.J. Wheatley (46) using X-ray methods. The crystal structure of ethyleneurea has not been published. However, its

molecular structure and the esr spectra of the γ-irradiated powder are similar to those of ethylenethiourea and this suggests they could have the same crystal structure. Moreover, the crystals grown from solution have the same form. The ethylenethiourea crystal belongs to the monoclinic system and the dimensions of the unit cell are a = 5.774 ±0.003 b = 4.540 ±0.005, c = 5.801 with β = 101.18. There are four molecules in each unit cell and the space group is P_{2_1}/a C_{2h}^5. The molecules are shown schematically in Fig. 6 and their arrangement about the unit cell is shown in Figure 7.

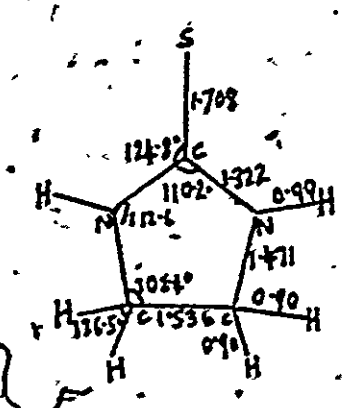
EXPERIMENTAL

Spectroscopic grade ethylenethiourea and ethyleneurea were purchased from the Aldrich Chemical Company and were used to grow single crystals.

At room temperature both ethylenethiourea and ethyleneurea are extremely soluble in amyl alcohol and so single crystals of both these compounds were obtained without too much difficulty by very slow evaporation from the saturated solution. It is noteworthy that other commonly used techniques were less successful. The external appearances of the two crystals were nearly identical and the angle between the crystal faces were examined.

A set of reference axes consisting of the crystallographic a and b axes together with c the third member of the orthogonal set were chosen for the ESR measurements. These axes are shown in Fig. 8.

Ethylenethiourea



Ethyleneurea

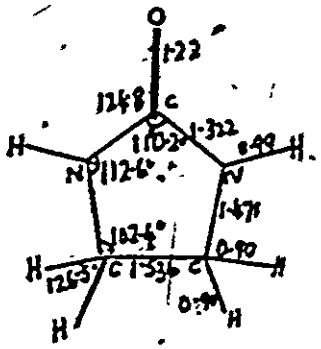


FIG. 6. Dimensions of the ethylenethiourea and the ethyleneurea molecules.

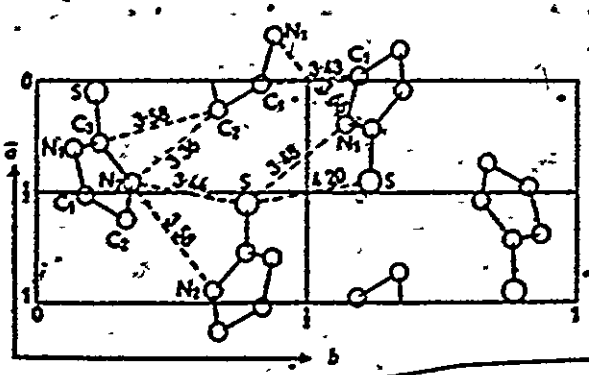


FIG. 7. Ethylenethiourea: projection on (001).

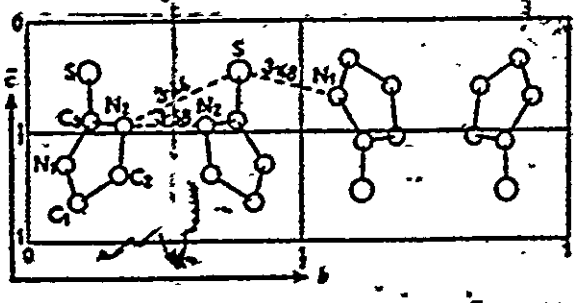
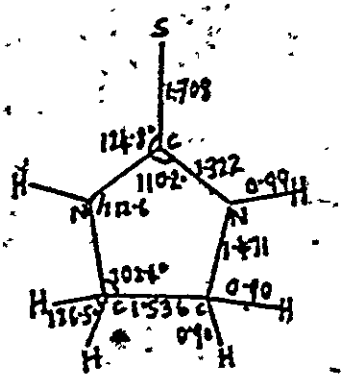


FIG. 7a. Ethylenethiourea: projection on(100).

Ethylenethiourea



Ethyleneurea

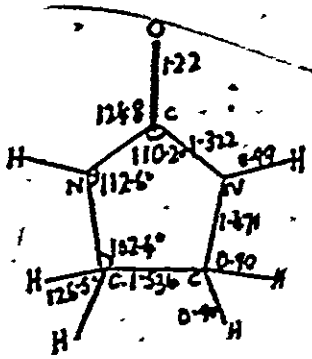


FIG. 6. Dimensions of the ethylenethiourea and the ethyleneurea molecules.

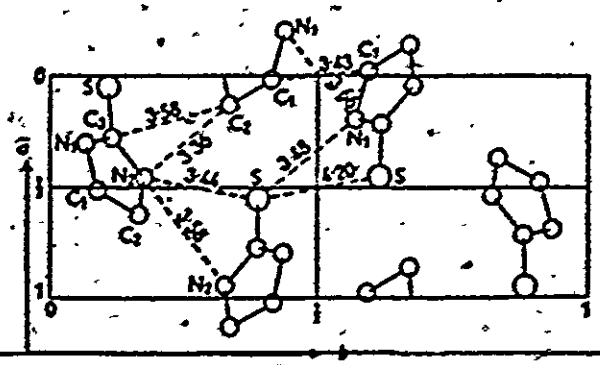


FIG. 7. Ethylenethiourea: projection on (001).

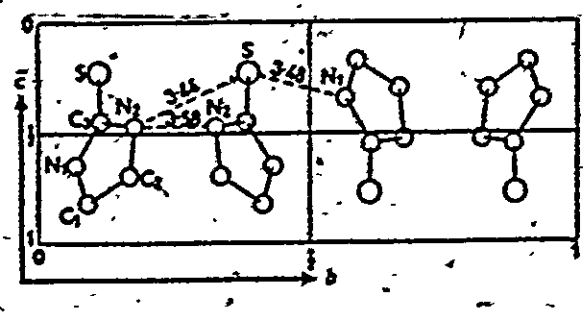


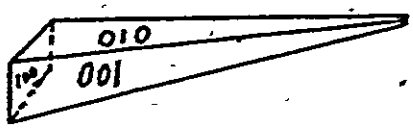
FIG. 7a. Ethylenethiourea: projection on(100).

The atomic coordinates given in unit cell fractions for the crystal monoclinic system were transformed (19) to an orthogonal system in terms of actual coordinates.

A monoclinic crystal having space group symmetry P_{2_1}/a possesses a center of symmetry and one twofold axis, the b axis (shown in Fig. 8). Radicals formed in crystals usually assume the symmetry of the host lattice and in this case spectra coming from radicals at different sites related by the P_{2_1}/a symmetry operations superimpose identically when the magnetic field lies along or is perpendicular to the b axis. Otherwise two magnetically distinct but symmetry related sites are observed.

The crystals were irradiated and examined by ESR at room temperature. Also these single crystals were* irradiated and examined at liquid nitrogen temperature. In the latter experiment, the crystals were warmed to room temperature during which time ESR spectra was taken every five minutes. Ethylenethiourea and ethyleneurea crystals and powders as well as their deuterated analogous were also examined similarly. To assure that surface effects were unimportant in spite of the strong concentrations of ozone present in γ-cell, a γ-irradiated single crystal of ethyleneurea was quickly swirled in methanol, dried and then re-examined by ESR at the same (room) temperature. Also single crystals of both compounds were γ-irradiated in the presence of trans-4, 4-dimethyl-2-pentene — an olefin which is known to react very readily with ozone (47). Additionaly γ-irradiation of evacuated samples, samples in air or

* The authors are indebted to Dr. Joseph Steinberg of the University of Montreal, Department of Physiology for the use of his ^{60}Co γ-cell.



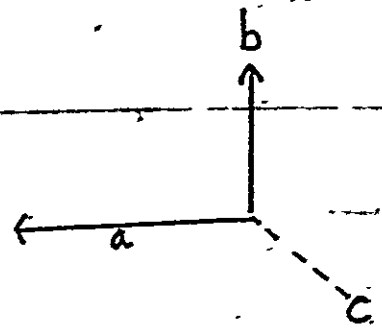


FIG. 8. Single crystal of ethyleneurea showing choice of reference axes.

nitrogen were carried out in order to see if changes could be caused in the ESR spectra obtained from the samples. It should be noted that γ-irradiation changed the appearance of the crystals from colourless to grey.

A Bruker X-band e.p.r. spectrometer having a standard TE 102 mode rectangular cavity and operating at nearly 9.6 GHz was used throughout these experiments.

Room temperature spectra was taken at 10° intervals as the crystal was rotated in the magnetic field. A similar procedure was adopted to record the spectra as the crystal was rotated about the other 2 axes of the orthogonal set. Spin density was determined by the INDO calculations (41) using Pople's program modified to be acceptable to the small memory capacity of the CDC 6600 computer at the University of Montreal.

DESCRIPTION OF SPECTRA

The single crystals of ethyleneurea, ethylenethiourea, their powder and deuterated analogues gave identical spectra at 300°K and 77°K. Rotation of the single crystals produced virtually no change in the spectra; nor did γ-irradiation in nitrogen, air, under evacuated conditions or in the presence of trans-4, 4-dimethyl-2-pentene — an ozone scavenger. When a γ-irradiated single crystal of ethylene urea was dipped in methanol, dried and re-examined, no spectral changes were seen. The spectra consisted of 3 lines whose relative intensities were near 1:2:1 with a splitting of 35.6 gauss,

linewidth was approximately 22.3 gauss. Fig. 9 shows a typical spectrum. Variation of microwave power in the resonant cavity showed that the lines saturated equally. Graphs of signal intensity against the square root of microwave power are shown in Fig. 10.

DISCUSSION:

(a) Radical Structure

Ethyleneurea and ethylenethiourea single crystals when grown from heavy water, have their labile protons, that is, those attached to the nitrogen, replaced by deuterons.

Deuteration of these compounds did not produce a change in the ESR spectra and so one may conclude that the hydrogens on the nitrogens of these compounds did not contribute significantly to the ESR spectra. The number and relative intensities of the lines indicate that they are due to the hyperfine interaction of the unpaired electron with 2 geometrically similar hydrogens. Since the hydrogens on the nitrogens of ethylene urea and ethylene thiourea are not responsible for the three lines observed, this suggests that the structure of the radical we have observed involves C_1 and C_2.

A radical that could be assigned to the ESR spectrum observed is:

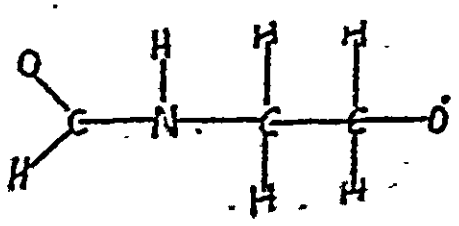


FIG. 11.

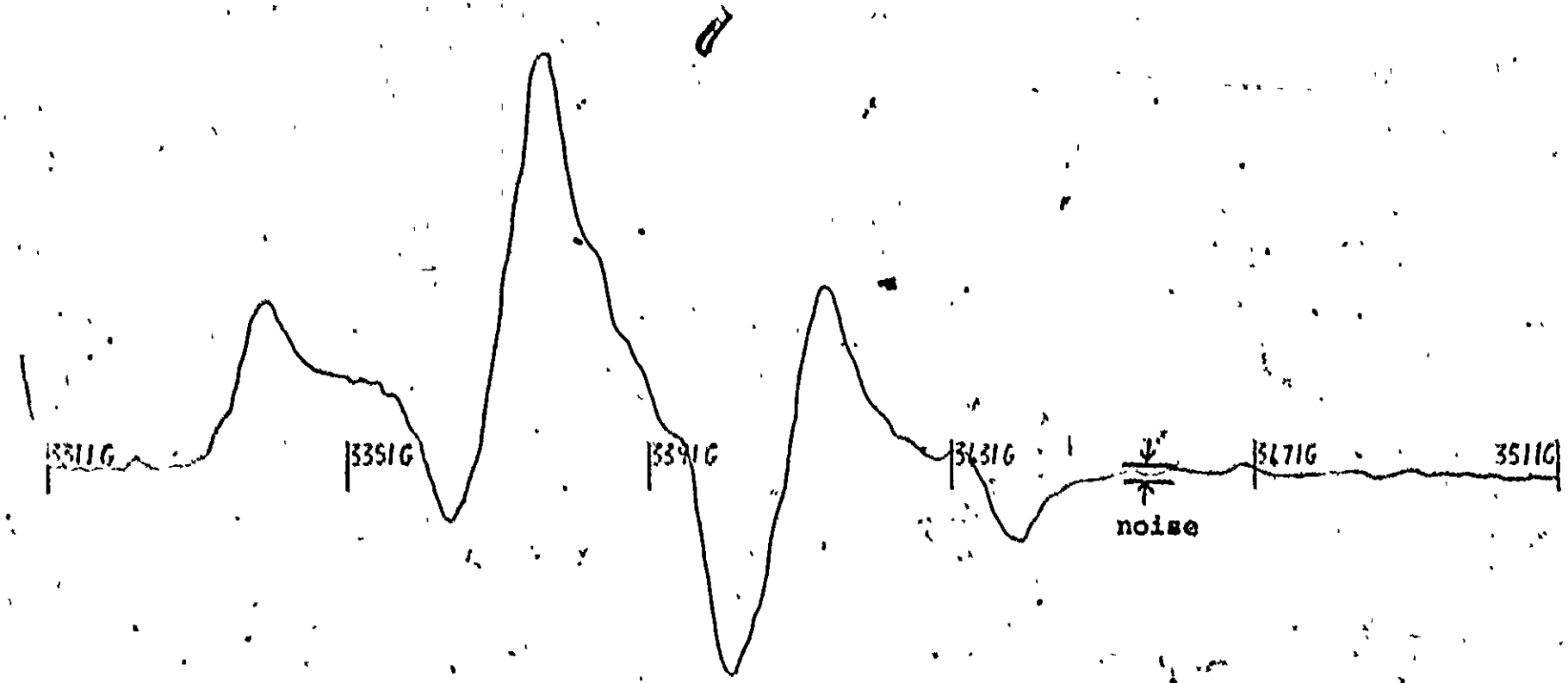


FIG. 9. STRUCTURE OF ETHYLENEUREA

	SAMPLE: ETHYLENEUREA	MODULATION AMPL.	2 GAUSS
	TEMP.: 300°K	TIME CONSTANT	.1 sec.
MICRON.	FREQ.: 9.35 GHz	RANGE	200 GAUSS
MICRON.	POWER: 20 mW.	SCAN TIME	200 secs.

Note that the noise level is rather excessive and is indicated at the right of the trace.

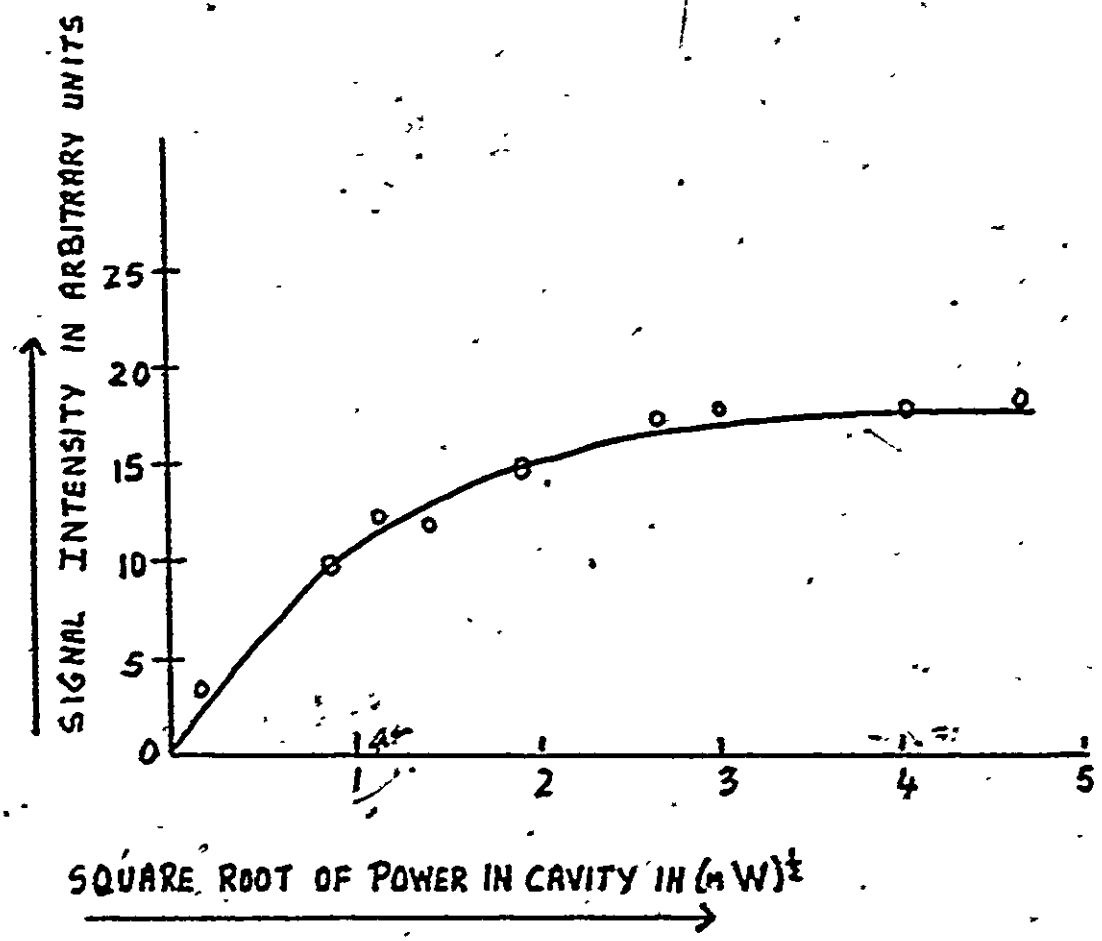
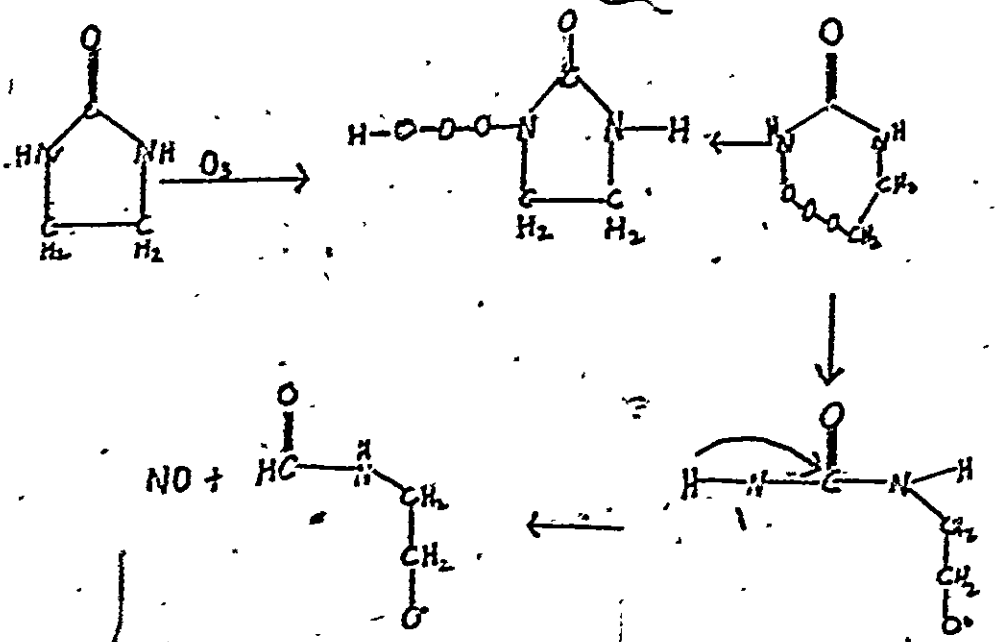


FIG. 10. Plot of signal intensity against the square root of the power in the microwave cavity for the radical species observed in the spectrum of ethyleneurea.

It is conjectured that such a radical could result from the reaction of ozone with ethyleneurea or ethylenethiourea by the following pathway:



One would however hesitate to propose a reaction mechanism without some chemical proof.

The size and geometry of the ozone molecule (See Fig. 12) and the molecular parameters of the ethyleneurea and ethylenethiourea suggest that such a reaction, if it occurs, would be primarily a surface reaction. However, when the ethyleneurea single crystal was dipped in methanol, dried and re-examined by ESR, the only spectral change observed was a very small decrease in signal size. Since irradiation of the sample in the presence of trans-4, 4-dimethyl-2-pentene a known ozone "scavenger" (47) did not cause any change in the spectra it can be concluded that ozone did not participate in the generation of the radical from ethyleneurea and its analogues.

The radical species responsible for the spectra observed was probably produced by the rupture of the C_1-C_2 bond as shown in Fig. 13.

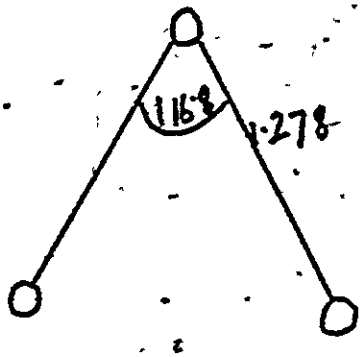


FIG. 12. Ozone molecule.

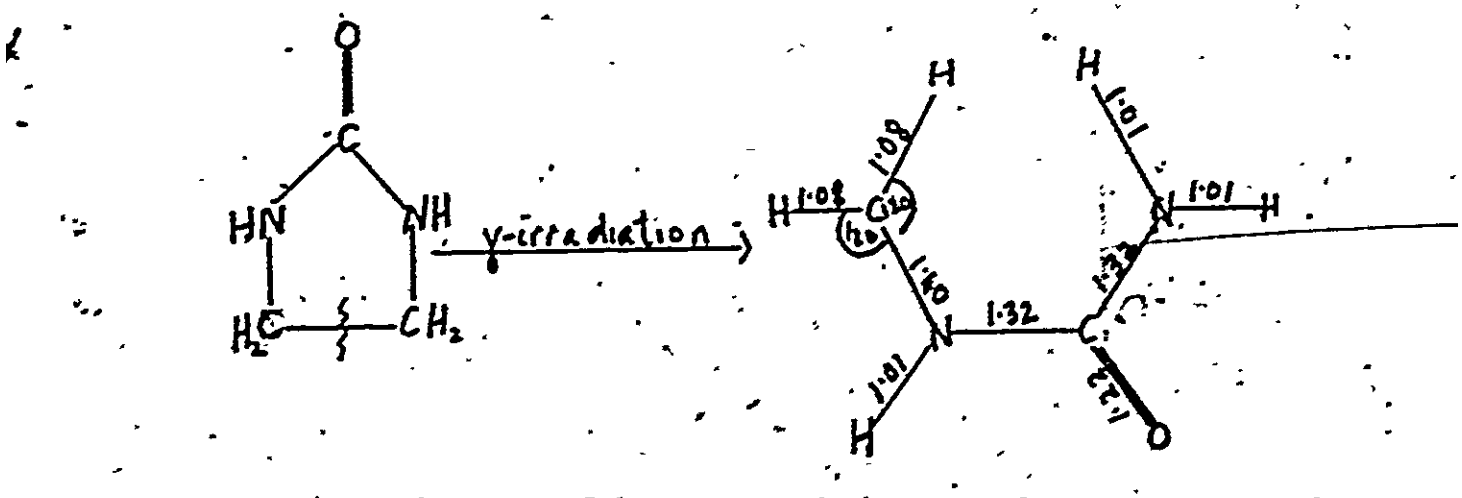


FIG. 13. Formation of radical species in γ-irradiated ethyleneurea(ethylenethiourea).

INDO calculations on several models show that the above radical is the one most likely responsible for the spectra observed. The INDO calculations show that the signs of the hyperfine values for the 2α hydrogens should be negative. This is exactly what McConnell concluded for similar radicals. The spin density on the central carbon was calculated to be 0.85 which in view of the large hydrogen coupling is smaller than that expected experimentally. The smaller calculated than experimental values are due in part to the inadequacy of the chosen models which neglect intermolecular effects which must be significant in this highly hydrogen bonded system. The radical proposed (Fig. 14) appears to be the best model however. INDO calculations are shown in Table 10.

Since ethyleneurea and ethylenethiourea single crystals are monoclinic as pointed out before, this means that resonance from two magnetically distinct radical sites should be observed when the magnetic field does not lie along or is not perpendicular to the twofold axis — the b axis. This was not observed in the experiments conducted and can only mean that the angle θ for the two sites are identical, such as would occur if the methylene group was rotating quite rapidly. The small anisotropy observed (less than 1 gauss) at 300°K and 77°K supports this conclusion. This small anisotropy occurs because rotation is not completely free and the methylene group is somewhat more likely to be found in certain preferred orientations. Splittings from the β-nitrogen or proton were not identifiable. However the linewidth of the α-hydrogen "triplet" signal (22.3 gauss) is large enough to mask these. INDO calcula-

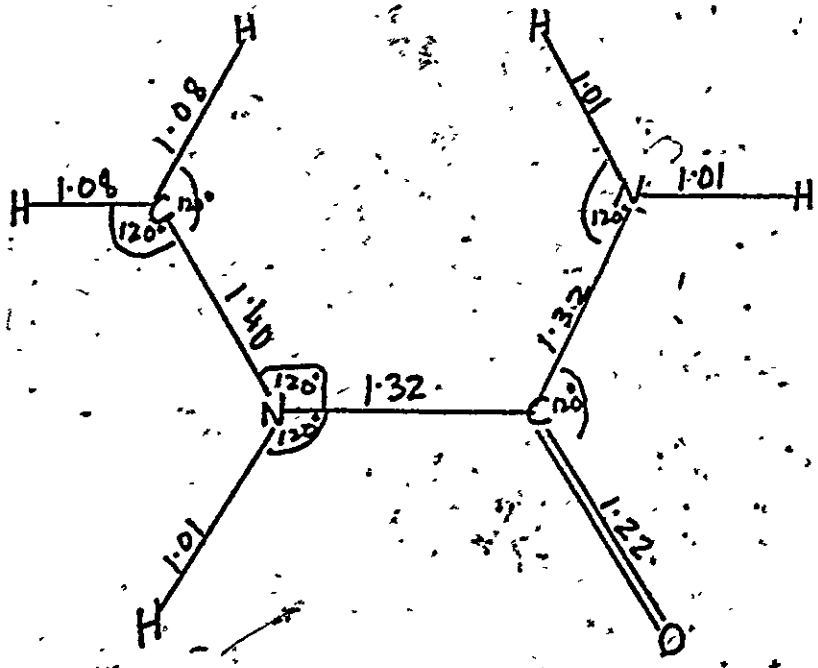


FIG. 14. Radical species formed in irradiated ethyleneurea(ethylenethiourea).

TABLE 10

INDO CALCULATIONS OF SPIN DENSITIES AND ISOTROPIC COUPLING CONSTANTS IN THE RADICAL $CH_2NHCONH_2$

Orbital	Atom	Spin Density	Coupling Constant
2s	Cα	0.0478	39.19
$2p_x$	Cα	0.0281	
$2p_y$	Cα	0.0236	
$2p_z$	Cα	0.8502	
1s	H1α	-0.0369	-19.91
1s	H1α	-0.0384	-20.73
2s	N_β	0.0024	0.90
$2p_x$	N_β	-0.0030	
$2p_y$	N_β	-0.0111	
$2p_z$	N_β	0.1214	
1s	H_β	-0.0055	2.9

tions (Table 11) show that the β nitrogen would have an isotropic splitting of less than 1 gauss with an unpaired electron spin density of 0.0024 in the S-orbital. If the unpaired electron was entirely in a p-orbital of the nitrogen, then its anisotropic coupling would be 34 gauss. The odd electron's spin density in the p-orbital according to the INDO calculations is (0.0030 + 0.0111 + 0.1214 =) 0.1355. Anisotropy should thus be (34 x 0.1355 =) 4.607 gauss and could easily be obscured by the largeness of the linewidth of the α hydrogen lines.

(b) Ozone Formation from the γ-Irradiation of Air

It should be stated finally that the ozone formed in the sample tube by gamma irradiation of air was identified by its small dark ink blue colour and by the fact that as the sample tube was warmed up from 77°K to room temperature the ink blue stain disappeared. (Ozone has a boiling point of approximately 162.0°K (48)). The formation of this compound from the γ-irradiation of air may have important biomedical implications. For example lung cancers are routinely treated by γ-irradiation in most hospitals. The long term prognosis for patients undergoing this type of treatment for lung cancer is however much poorer than for patients with other types of cancer who are undergoing similar treatment.

Current opinion is that this is largely due to the tendency of the primary lung lesion to metastazize quite rapidly. Since ozone is an extremely reactive substance its presence in the lung may have some effect on the "recovery rate" of these patients.

TABLE 11

INDO CALCULATIONS ON β NITROGEN OF THE RADICAL

$\dot{C}H_2NHCONH_2$

Orbital	Atom	Spin Density	Hyperfine coupling constant
2s	Nβ	0.0024	0.9039
$2p_x$	Nβ	-0.0030	
$2p_y$	Nβ	-0.0111	
$2p_z$	Nβ	-0.0000	

Electron Spin Resonance of γ-irradiated Single Crystals of Semicarbazide Hydrochloride.

Semicarbazide hydrochloride has a wide variety of pharmacological effects (49,50). Among these are (a) its ability to reduce hypertension (51). This appears to be associated with its action on the central nervous system; (b) its ability to increase the uptake of histamine by platelets in blood (52) and (c) its activating effect on cerebral acetylcholinesterase followed by alternate inhibition (53). These actions are related to the molecular structure of the compound. Whether or not free radical mechanisms are involved however has not been determined. Since semicarbazide is a nitrogen containing compound and the vast majority of compound in living organisms contain nitrogen, the effects of ionizing radiation on such a compound will be of interest to the biologist.

Previous ESR studies on these compounds indicate that there is no definite pattern to the location of bond rupture caused by ionizing radiation. For example in L-alanine (54) and urea oxalate (55) the terminal C-N bond is broken and the NH_2 group is lost, while in methyl urea (56) it is an N-H bond that is broken. ESR studies on ethyleneurea and ethylenethiourea (57) show that a C-C adjacent to an N-H bond is broken. The apparent lack of pattern with respect to the rupture of bonds in these molecules points up the necessity for further investigation of irradiated nitrogen containing compounds.

EXPERIMENTAL

Semicarbazide powder from Anachemia Chemicals Ltd., of Montreal was used to grow single crystals of semicarbazide hydrochloride by slow evaporation of aqueous solutions at room temperature. Deuterated semicarbazide hydrochloride crystals were obtained by recrystallization of normal material from D_2O.

The crystals were irradiated with a ^{60}Co γ-source for 45 minutes at room temperature. Crystal axis were determined by means of an X-ray precession camera and the faces identified under a phase microscope.

For the ESR measurements, a set of reference axes (an orthogonal set) consisting of the crystallographic axes a, b and c, were chosen. The crystals were aligned in the ESR cavity with the aid of an optical goniometer so that one of the selected axes was perpendicular to the main magnetic field. Spectra was recorded at room temperature with an X-band (9 GHz) spectrometer at 10° intervals as the crystal was rotated in the main magnetic field. A similar procedure was adopted to record the spectra as the crystal was rotated about the other two axes of the orthogonal set and to record the spectra from the deuterated crystal as it was rotated about each of the three selected axis. Coupling constants were evaluated using the method of Schonland (58).

CRYSTAL STRUCTURE

Semicarbazide hydrochloride crystallizes from aqueous solutions in colourless prisms elongated along the [001] axis; the deuterated analog forms crystals elongated along the [010] axis (See Fig. 15).

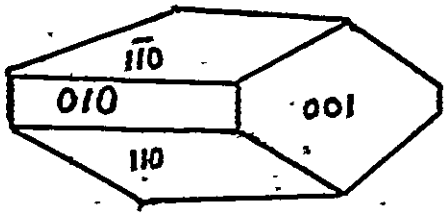
The detailed crystal structure of semicarbazide hydrochloride has been determined by Nardelli et al. (59) using precession and Weisenberg X-ray techniques.

The semicarbazide crystal belongs to the orthorhombic system. Each unit cell has four molecules. The space group is $P2_12_12_1$ (D_2^4) and dimensions of the unit cell are a = 7.51 ≠ 0.01, b = 13.13 ≠ 0.01 and c = 4.64 ≠ 0.01 Å. When the magnetic field lies along the a, b or c axes all four molecules are magnetically indistinguishable. With the magnetic field in the ab, bc, or ca planes two magnetically different set of molecules can be distinguished. With other orientations of the magnetic field towards the crystal axis, all four molecules become magnetically distinct.

DISCUSSION

The undeuterated γ-irradiated single crystal of semicarbazide hydrochloride gives a ten line ESR spectrum of relative intensities

Deuterated semicarbazide



Semicarbazidehydrochloride

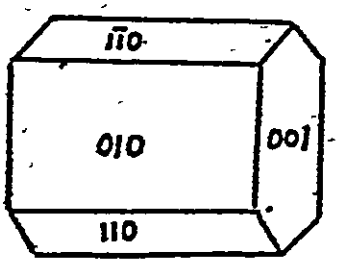


FIG. 15. Growth habits of Semicarbazide hydrochloride single crystals.

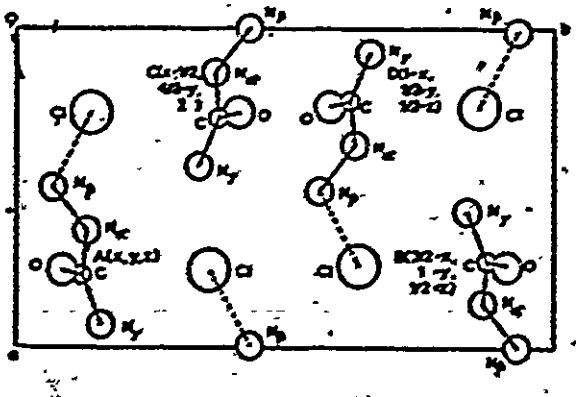


FIG. 15a. Projection of the crystal structure of semicarbazide hydrochloride on the (001) face.

1:3:5:7:8:8:7:5:3:1 with a linewidth of 9.9 gauss when its a, b, or c axis is aligned parallel to the main magnetic field. The crystal structure as we have pointed out before suggests that all the crystal sites are magnetically equivalent for these orientations. The best molecular model that would give such a spectrum consists of an α nitrogen and hydrogen and a β nitrogen with three hydrogens attached (See Fig. 16).

This analysis is made more evident when we examine the deuterated semicarbazide. In D_2O the exchange of D for hydrogen occurs most rapidly at the NH_3^+ group to produce $NH_2CONHND_3^+ Cl^-$. This is most easily seen by the rapid decrease of the NH_3^+ proton NMR signal when semicarbazide hydrochloride is placed in D_2O. The magnetic moment of deuterium is one sixth of that of hydrogen and so the hyperfine coupling to deuterium which replaces a proton should be reduced proportionally. The smaller deuterium coupling, note that we believe that the α hydrogen coupling is about 21 gauss, should therefore give a coupling of about 3½gauss. A three gauss splitting is sufficiently small to be lost in the residual line breadth. The lines are quite broad as a result probably of a rather short electron spin lattice relaxation time. The short relaxation time is a result of the spin projection angle of the anisotropy being quite large. At some angle where the nitrogen coupling reduces to zero, we expect very narrow lines. This subject will be treated in further detail in the following chapter of the succinimidyl radical.

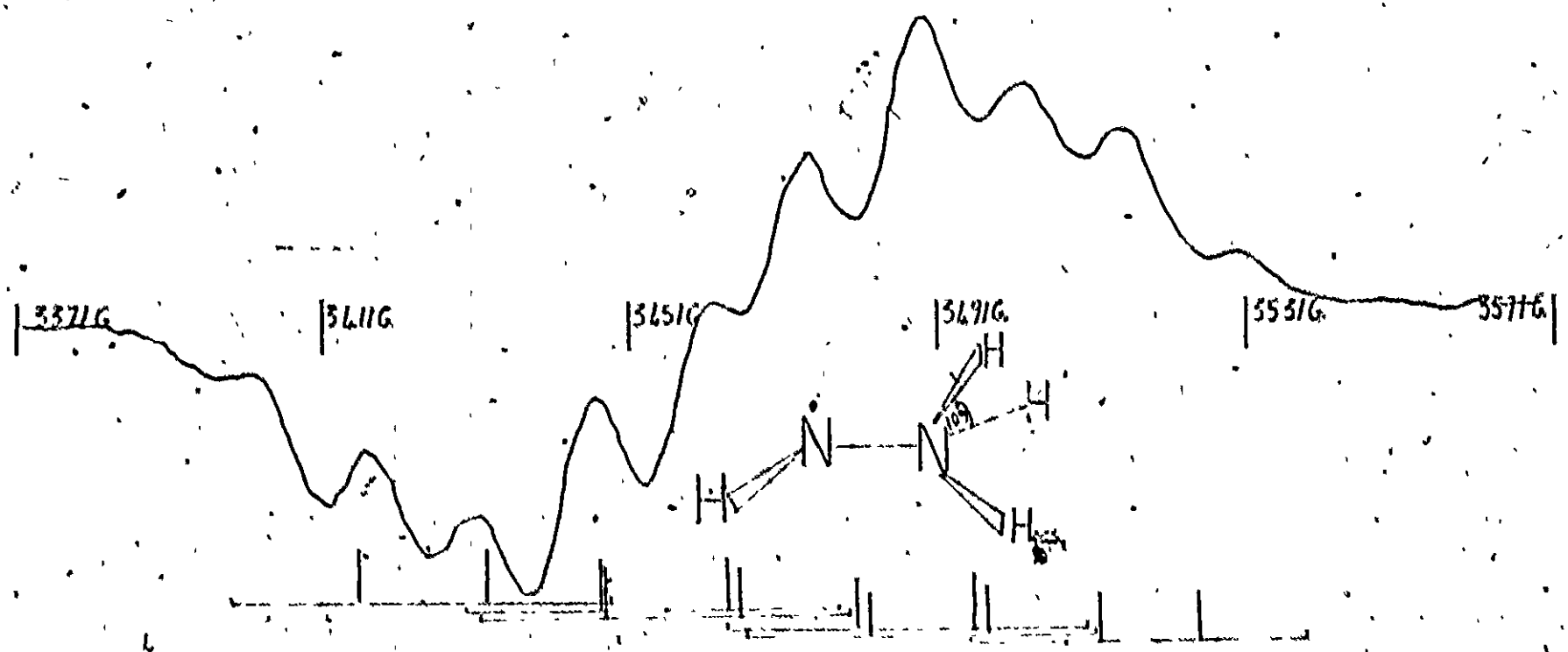


FIG. 16. ESR SPECTRUM OF NORMAL SEMICARBAZIDE HYDROCHLORIDE WITH MAGNETIC FIELD PARALLEL TO B AXIS. RADICAL ASSIGNED TO THIS SPECTRUM IS ALSO SHOWN.

SAMPLE: SEMICARBAZIDE HYDROCHLORIDE. SCAN RANGE: 200 GAUSS. SCAN TIME: 200 SEC. TEMPERATURE: 300K. TIME CONSTANT: 0.5 SEC. MICROWAVE FREQUENCY: 9.55 GHz. MICROWAVE POWER 1mW. MODULATION AMPLITUDE:1 GAUSS.

The γ-irradiated deuterated single crystal of semicarbazide (See Fig. 17) yields a four line ESR spectrum of relative intensities 1:2:2:1 when its a, b or c axis is parallel to the main magnetic field. These lines arise from the alpha nitrogen triplet being split by interaction with the α proton. The lines due to the coupling of the deuterium with the unpaired electron are lost in the linewidth and so these lines from the deuterated semicarbazide have a linewidth of 11.9 gauss as compared to a linewidth of 9.9 gauss for the lines from the undeuterated semicarbazide single crystal. β nitrogen splitting was not evident in spectra from both the deuterated and undeuterated crystal but was indicated by the anisotropic variation of the linewidth.

With the magnetic field parallel to the crystal's a, b or c axis only one radical's resonance should be observed. If the magnetic field however is in the ac, ab or ca planes then spectra from two magnetically distinguishable sets of radicals should be observed. The angular dependence of the spectral lines as the magnetic field is rotated in the crystallographic bc plane for example (See Fig. 18) is in agreement with these deductions.

INDO calculations show that the $\dot{N}H\text{-}NH_3$ radical is definitely the best model that can be assigned to the spectra observed. These calculations are given in Table 12. The actual coupling constants that have been evaluated from the spectra are given in Table 13. These coupling constants are in fair agreement with those obtained from a number of like radical moieties as shown in Table 14 and al-

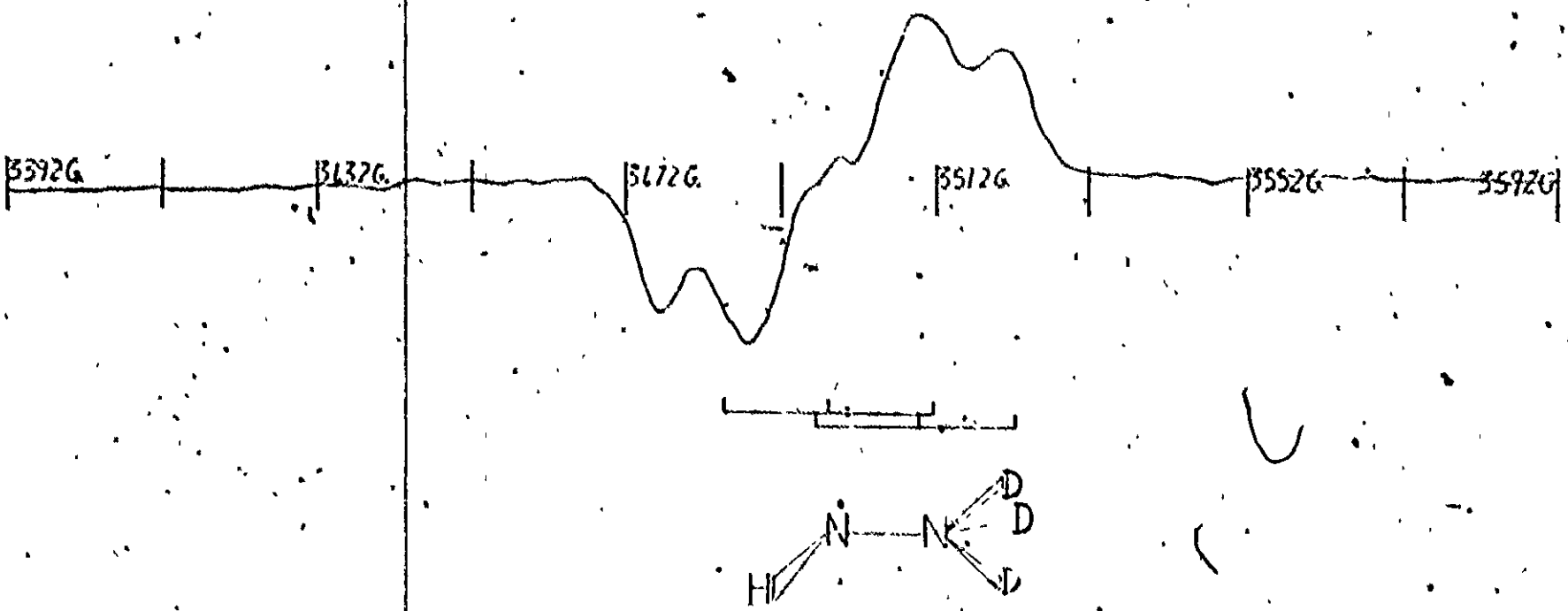


FIG.17. ESR SPECTRUM OF DEUTERATED SEMICARBAZIDE HYDROCHLORIDE WITH MAGNETIC FIELD PARALLEL TO B.AXIS. RADICAL ASSIGNED TO THIS SPECTRUM IS ALSO SHOWN.
SAMPLE: DEUTERATED SEMICARBAZIDE HYDROCHLORIDE.
TEMPERATURE: 300K. TIME CONSTANT: 0.5 SEC. MICROWAVE POWER: 1mW.
MICROWAVE FREQUENCY: 9.58 GHz. SCAN RANGE: 200 GAUSS. SCAN TIME: 200 SEC.

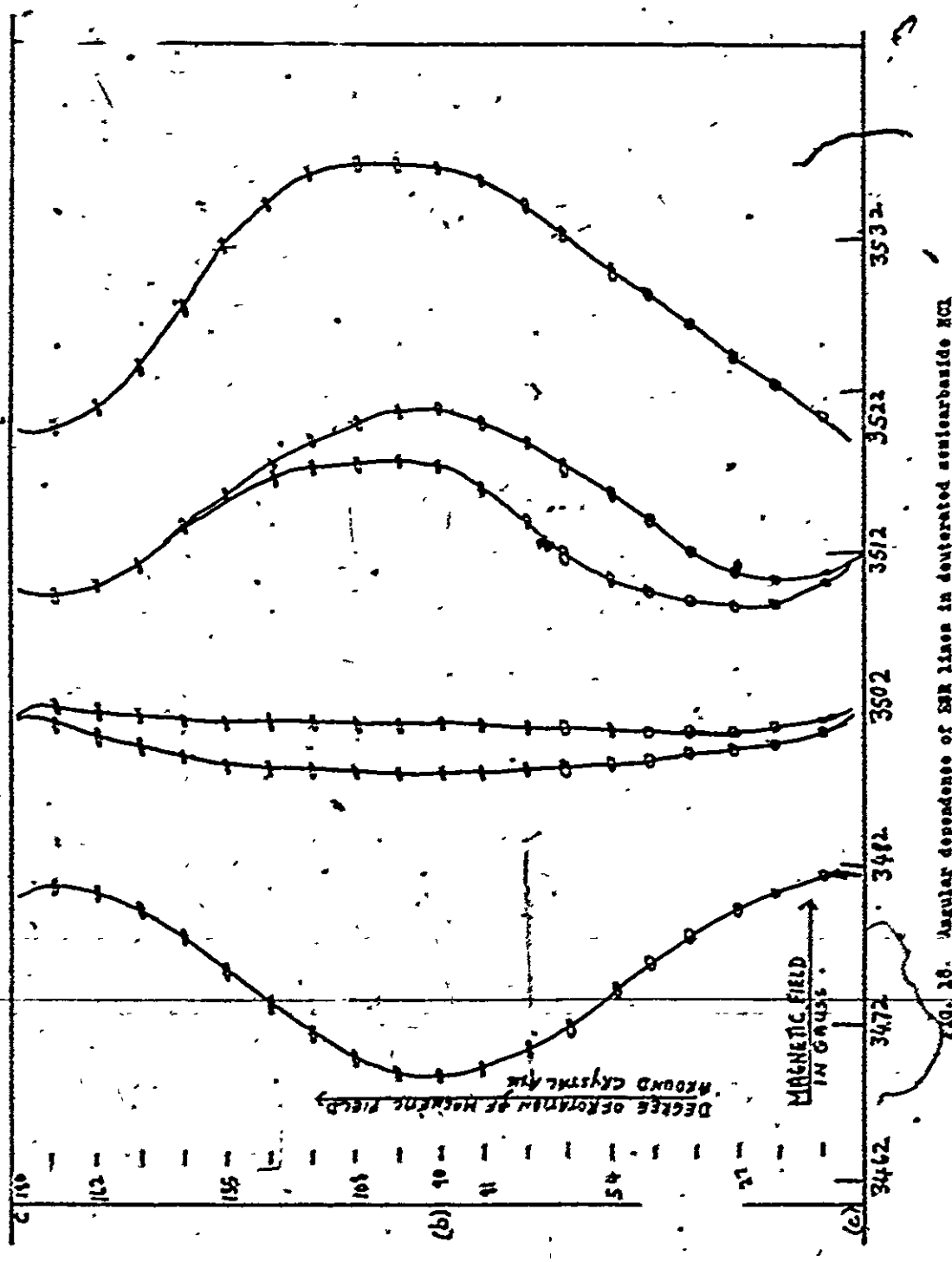


FIG. 18. Angular dependence of ESR lines in deuterated semicarbazide HCl

TABLE 12

ISOTROPIC COUPLING CONSTANTS CALCULATED BY THE INDO METHOD FOR THE RADICAL $\dot{N}HNH_3$

Atom	$H\beta_1$	$H\beta_2$	$H\beta_3$	$N\beta$	$N\alpha$	$H\alpha$
Coupling Constant	0.3941	19.9630	19.9630	-3.3755	13.4136	-18.2480

TABLE 13

COUPLING CONSTANTS EVALUATED FROM ESR SPECTRUM OF $NHNH_3$

Atom	Splitting Parameters (in gauss)	Isotropic Splitting (in gauss)	Anisotropic Splitting (in gauss)
Nα	30.6		15.9
	10.5	14.7	- 4.2
	3.1		-11.6
Hα	34.2		13.1
	20.3	21.2	- 0.9
	10.1		-10.1
Nβ		≃ 3	
Hβ		≃12	

TABLE 14

COMPARISON OF HYPERFINE COUPLINGS OF N AND H IN SIMILAR RADICALS AT ROOM TEMPERATURE

	Hyperfine Coupling (gauss)				
	Nα		Hα		
Radical	Isotropic	Anisotropic	Isotropic	Anisotropic	References
$N(SO_3)_2^{2-}$	13.2	24.6			Horsfield, A. et al. Mol. Phys. 5, 241 (1962)
		-11.0			
		-13.2			
$N^+H_2SO_3^-$	18.2	18.6	-22.9	-0.4	Rowlands, J. and Whiffen, D. Nature 193, 61 (1962)
		-6.4		-6.8	
		-12.1		+7.1	
$NH(SO_3^-)$	13.5	21.3	22.7	1.5	Rowlands, J.R. Mol. Phys. 5, 565 (1962)
		- 9.8		14.0	
		-11.5		15.5	
$HN-NH_3$	14.7	15.9	21.2	13.0	This work
		- 4.2		- 0.9	
		-11.6		-10.1	

31E.

so with those evaluated from the radical $NH\text{-}NH_3^+$ by Shrivastava and Anderson (60).

Spin density data obtained by INDO calculations on the $NH\text{-}NH_3$ radical are compared with spin density data calculated from the radical by using SCP wave functions. These latter values are also compared with spin density data calculated on related radicals by using the SCP wave functions (See Table 16).

There is reasonable agreement between the INDO calculations and the SCP calculations for $NH\text{-}\overset{+}{N}H_3$ and related radicals.

We conclude therefore that the resonance observed after γ-irradiation of semicarbazide single crystals at room temperature is that of $NH\text{-}\overset{+}{N}H_3$. It should be noted that this ESR examination of single crystals of semicarbazide hydrochloride differs from that done by Shrivastava and Anderson (60) in that (a) γ-rays instead of X-rays were used to create crystal damage and (b) INDO calculations of coupling constants of the radical $NH\text{-}NH_3$ were done. Shrivastava and Anderson did not do these calculations.

TABLE 15 a

INDO CALCULATIONS OF SPIN DENSITIES IN THE RADICAL $^{14}N'H-^{14}N'H_3^+$

Orbital	Atom	Spin Density	Orbital	Atom	Spin Density
2s	Nα	-0.0354	2s	Nβ	-0.0089
$2p_x$	Nα	0.0171	$2p_x$	Nβ	-0.0214
$2p_y$	Nα	0.0071	$2p_y$	Nβ	-0.0063
$2p_z$	Nα	0.9600	$2p_z$	Nβ	-0.0238
1s	Hα	-0.0338	1s	$H_1\beta$	0.0007
			1s	$H_2\beta$	0.0370
			1s	$H_3\beta$	0.0370

TABLE 15 b

SPIN DENSITIES IN $^{14}N'H-^{14}N'H_3$ RADICAL

CALCULATIONS BASED ON SCF COMPUTATIONS OF HYPERFINE CONSTANTS

Orbital	Atom	Spin Density	Orbital	Atom	Spin Density
2s	Nα	0.031	2s	Nβ	-0.009
2p	Nα	0.51	2p	Nβ	
1s	Hα	0.049	1s	Hβ	0.029

TABLE 16

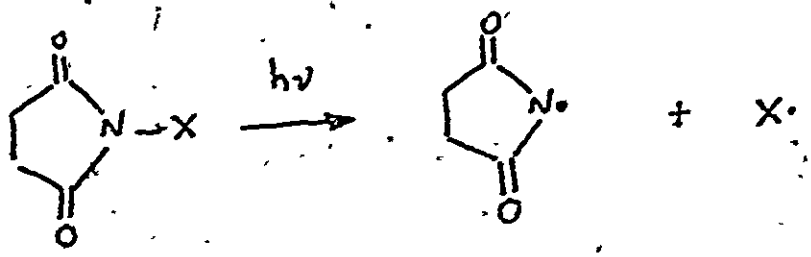
COMPARISON OF SPIN DENSITIES IN ^{14}N AND ^{1}H ORBITALS FOR $NH-NH_3$ AND OTHER RADICALS

Radical (α-atoms)	Spin Density ρH1s	ρH1s	ρN2p	Reference
$\dot{N}HSO_3^-$	0.045	0.024	0.63	Rowland, J. Mol. Phys. 5, 565 (1962)
$\dot{N}HNHCONH_2$	0.039	0.023	0.74	Hamrick, Jr., P. and al. Rad. Res. 48, 234 (1971)
$\dot{N}(SO_3^-)_2$	-	0.024	0.74	Horsfield, A. and al. Mol. Phys. 5, 241 (1962)
$\dot{N}HNH_3^+$	0.049	0.031	0.51	This work. See also Ref. 60

Radical (β-atoms)	Spin Density ρH1s	ρN2s	ρN2p	Reference
$CH_2CO_2-\dot{N}H_3^+$	0.032	-0.005	0.038	Ghosh, D. and Whiffen, D. Mol. Phys., 2, 285 (1959)

γ-Radiation damage of succinimide and N-halo succinimide as observed by electron paramagnetic resonance.

The decomposition of N-halosuccinimide such as N-bromoccinimide during irradiation by uv light to produce halogen radicals has received the profound attention of many organic chemists during the past twenty years (61). The proposed reaction,



suggests that a succinimidyl radical is formed. This succinimidyl radical has however been mysteriously ignored by investigators. Not wishing to permit such oversights we chose to examine what happens to the succinimide.

The succinimidyl radical should be easily observed by electron paramagnetic resonance (e.p.r.) if it has a sufficiently long lifetime. In a crystalline host matrix the mobility of a reactive molecule such as the succinimidyl radical is severely restricted and so it should be possible to observe its epr signal and identify it before it is consumed by chemical reaction. In our laboratories studies of the proton nmr of succinimide have shown that the molecules at room temperature are quite rigidly fixed in the crystal lattice and so the radicals, if formed, should not be too mobile.

Succinimide crystallizes from ethanol into an orthorhombic unit cell structure (62) with space group P_{bca}, a = 7.50 Å, b = 9.62 Å, c = 9.62 Å and Z = 8. The molecules are nearly coplanar with the bc plane and the eight molecules per unit cell are related by two-fold axes lying along the a, b and c axes. Usually, radicals formed in crystalline host matrices assume the symmetry of the host lattice. Such is the case with succinimide where the two fold symmetry serves to greatly simplify the esr spectra. Sites related by inversion symmetry are indistinguishable by epr. Similarly, in this case all the molecular sites are magnetically indistinguishable, unless however, the radical can sit in several orientations in its site.

EXPERIMENTAL

The succinimide crystals with dimensions ½ cm by ½ cm by 1 cm were formed by very slow evaporation of a 10% ethanol aqueous solution containing succinimide*. N-deuterosuccinimide was formed by repeated recrystallization from deuterated solvents. The best formed crystals were subjected to γ-irradiation dosage of 5 krad**. Subsequently the crystals were aligned on a goniometer head using a precession camera mounted on a Picker X-ray spectrometer. The orientation of the crystals was determined to better than 1°; this latter limit being determined by the imprecision of mounting the goniometer on the e.p.r. spectrometer cavity.

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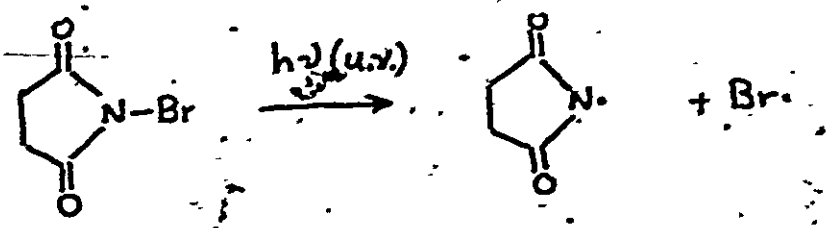
* White label succinimide

** The authors are indebted to Dr. Joseph Sternberg of the University of Montreal, Department of Physiology, who made available freely his ^{60}Co γ-cell.

A Bruker 414s X-band e.p.r. spectrometer having a standard TE102 mode rectangular cavity and operating at nearly 9.6 GHz was used throughout these experiments. All single crystal measurements were performed at room temperature (25° C).

RESULTS AND DISCUSSION

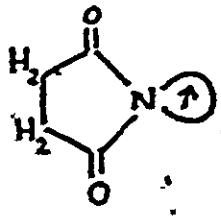
μv irradiated powders and $CHCl_3$ and hexane solutions of N-chloro and N-bromosuccinimide showed no e.p.r. spectra. A reasonable explanation for this is that the radicals produced, and there is adequate chemical evidence that they are produced prodigiously, are not long lived. The well known reaction (61)



has as products a highly reactive succinimidyl radical. Molecular orbital calculations show that the unpaired electron density is probably localized near the nitrogen but in a π orbital extending over the neighbouring carbonyl groups. The μ.v. irradiation experiment was repeated with the sample solution mounted in a high resolution n.m.r. spectrometer probe. There was no evidence of dynamic polarization of the nuclear spins, although it must be admitted that the experimental method was probably inadequate for the task presented.

γ-irradiation of polycrystalline succinimide, N-chlorosuccinimide and N-bromosuccinimide at 30°C and to a dose of 5kd showed no visible decomposition or discoloration of the powders. Epr spectra recorded at 23°C and ½ hour after the γ-irradiation are shown in Figures 19, 20 and 21. The spectra of succinimide and N-chlorosuccinimide are very similar whereas that of N-bromosuccinimide has a much larger splitting. These spectra are shown in Figure 22. It is evident that the bromine atom is near the radical site; the diffuse nature of the valence electronic orbitals of Br make its presence felt at many angstrom units distance. The spectra however do resemble those expected from a radical with two nearly equivalent protons, that is, a triplet spectrum with intensity ratios 1:2:1.

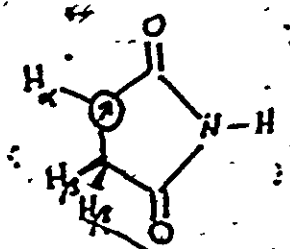
Molecular orbital calculations using the INDO approximation of two possible radicals are shown below. The calculated coupling constants for the succinimido radical,



$A_N = 7.1$ gauss $\quad B_N = 9.0$ gauss

$A_H = 3.0$ gauss

are much too small to explain the spectra. However, those calculated for the succinimidyl radical,



$A_{H_\alpha} = -17.5$ gauss $\quad A_N = 0.1$ gauss $\quad B_N = 1.2$ gauss

$A_{H_\beta} = +36.7$ gauss $\quad A_H \leq 0.1$ gauss

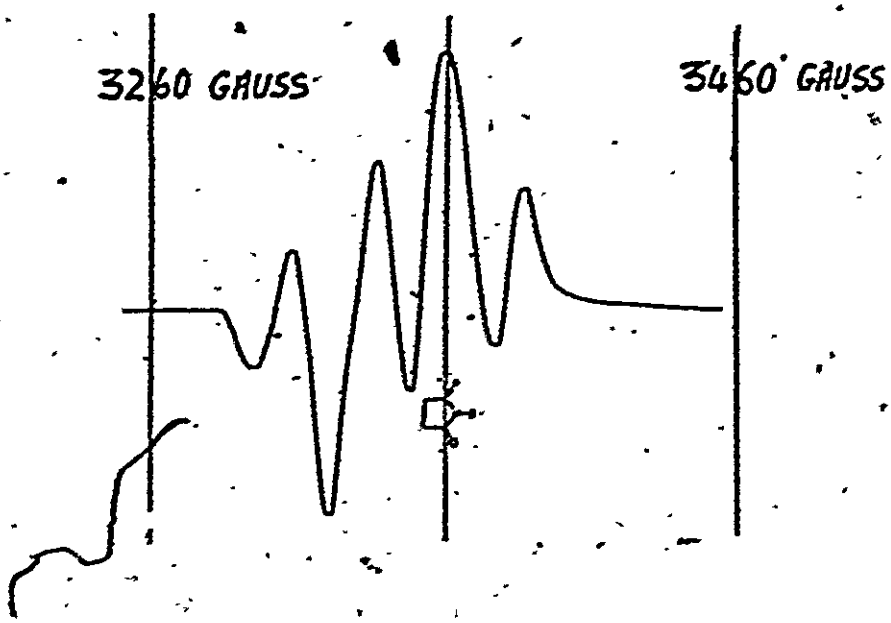


FIG. 19. X-band ESR spectrum of γ-irradiated pollycrystalline succinimide at 30°C. The conditions of ESR spectroscopy were microwave power 5mW; modulation amplitude 4 gauss; scan time 500 sec; time constant 1 sec; scan range 500 gauss and microwave frequency 9.55 GHz. Spectrum was taken 1/2 hr. after γ-irradiation.

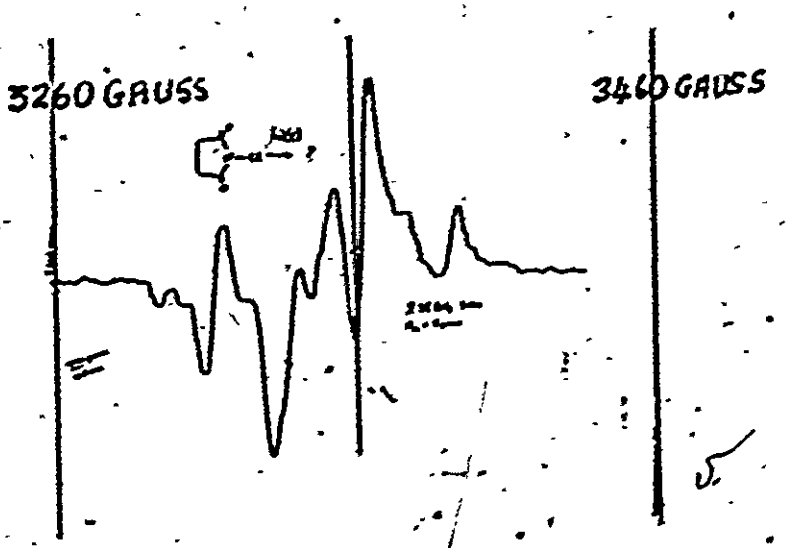


FIG. 20. X-band ESR spectrum of γ-irradiated pollycrystalline N-chlorosuccinimide at 30°C. The conditions of ESR spectroscopy were microwave power 5mW; modulation amplitude 4 gauss; scan time 500 sec; time constant 1 sec; scan range 500 gauss and microwave frequency 9.38 GHz. Spectrum was taken 1/2 hr. after γ-irradiation.

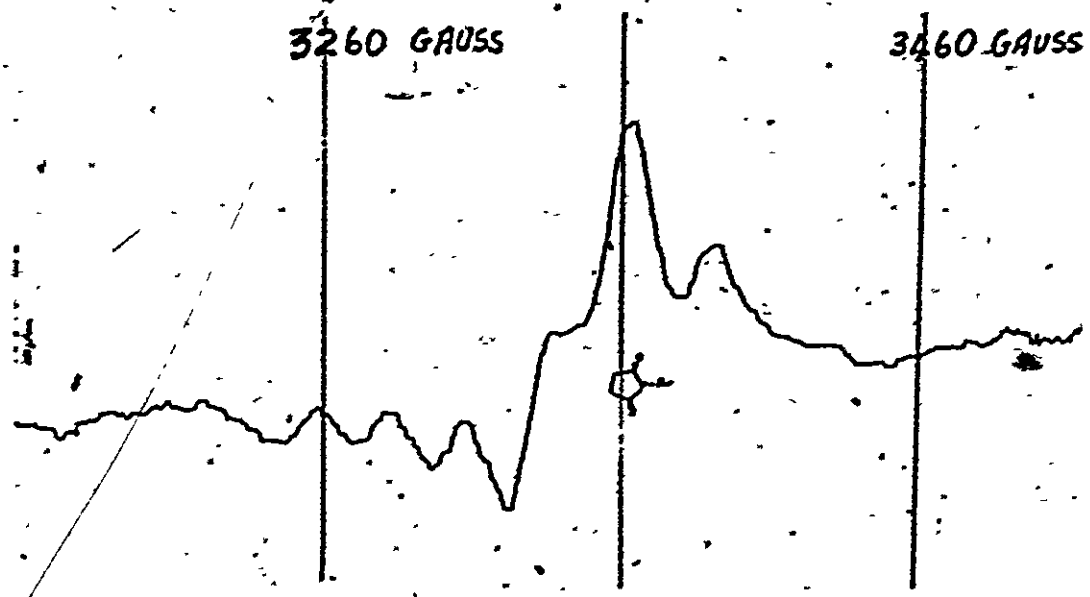


FIG. 21. X-band spectrum of γ-irradiated polycrystalline N-bromosuccinimide at 90°C. The conditions of ESR spectroscopy were microwave power 5mW; modulation amplitude 4 gauss; scan time 500 sec; time constant 1 sec; scan range 500 gauss and microwave frequency 9.58 GHz. Spectrum was taken 1/2 hr. after γ-irradiation.

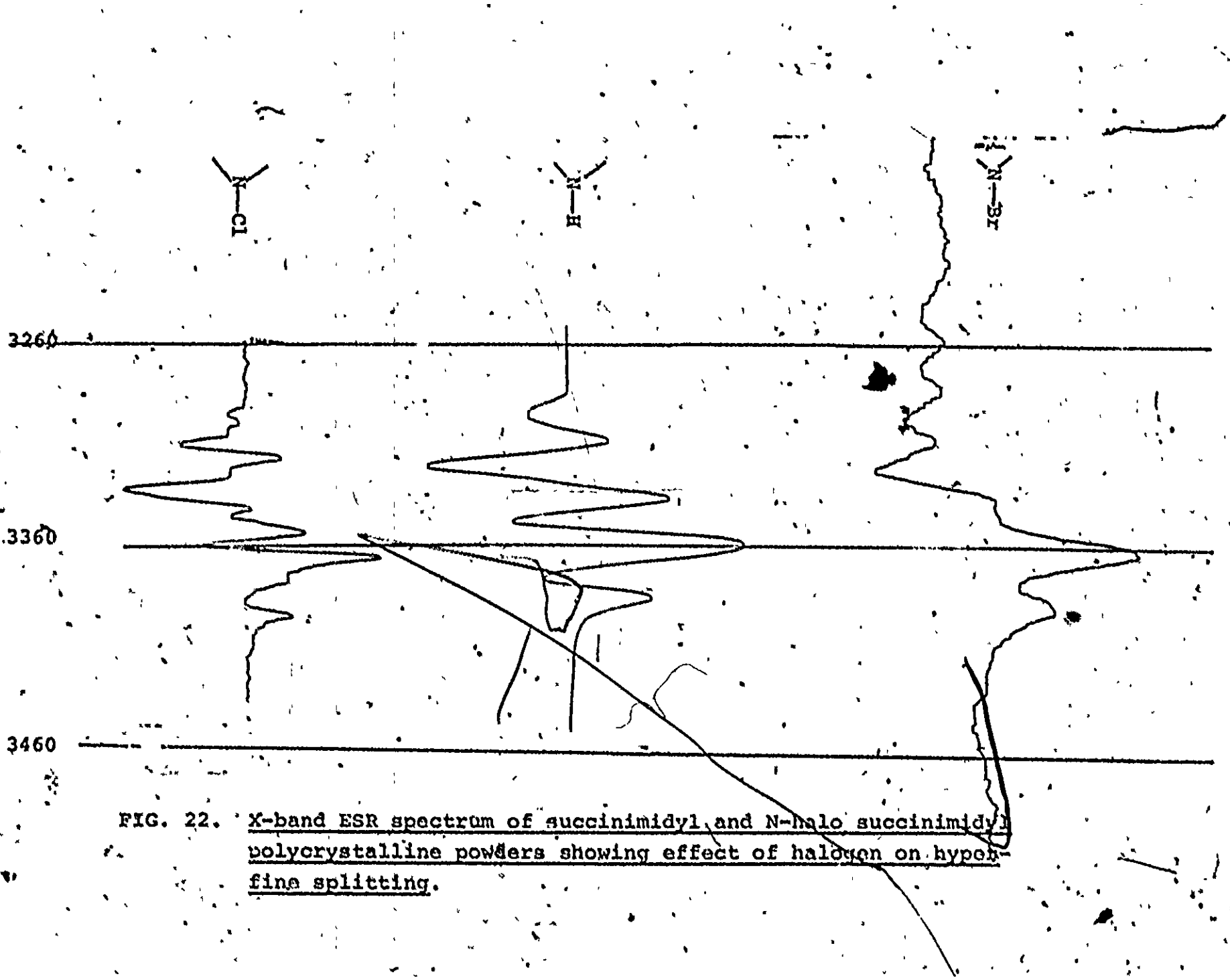


FIG. 22. X-band ESR spectrum of succinimidyl and N-halo succinimidyl polycrystalline powders showing effect of halogen on hyperfine splitting.

are much more reasonable. In the case of succinimide, it is easy to rationalize that the succinimido radical is a highly reactive radical combining readily with the neighbouring molecules or labile hydrogens in the hydrogen bonds.

An experiment with polycrystalline N-deuterosuccinimide did not show a spectrum different from that obtained from succinimide. It should be noted that the hyperfine coupling to a deuterium atom is approximately one-sixth that expected for a proton. Advantage of this fact was taken during the following single crystal experiments deemed necessary to properly identify the radical. Note that the succinimido radical, if present and visible to the e.p.r. would have been extremely interesting theoretically.

Single crystals of N-deuterosuccinimide were γ-irradiated and mounted in the epr spectrometer cavity with the goniometer rotation axis parallel to the a axis. The crystal orientation on the goniometer head was determined to better than 1° using a X-ray precession camera. Single crystal spectra were recorded at 5° intervals and some typical spectra are shown in Figure 23. The positions where the spectrum crosses the baseline are plotted as a function of orientation in Figure 24. One set of lines, with intensity ratios in accord with an electron seeing one alpha and two beta hydrogens, is shown darkly in Figure 25. The two sets of lines observed in Figure 24 indicate that the radical has two different sites in the crystal lattice. This is to be expected since an analysis of the succinimide crystal structure

FIG. 23

X-band ESR spectra of N-deutero succinimide with external magnetic field perpendicular to the c axis and lying in the plane of the radical where the nitrogen coupling is least.

FIG. 23a

DPPH

FIG. 23b

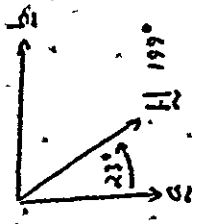
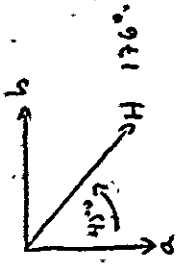
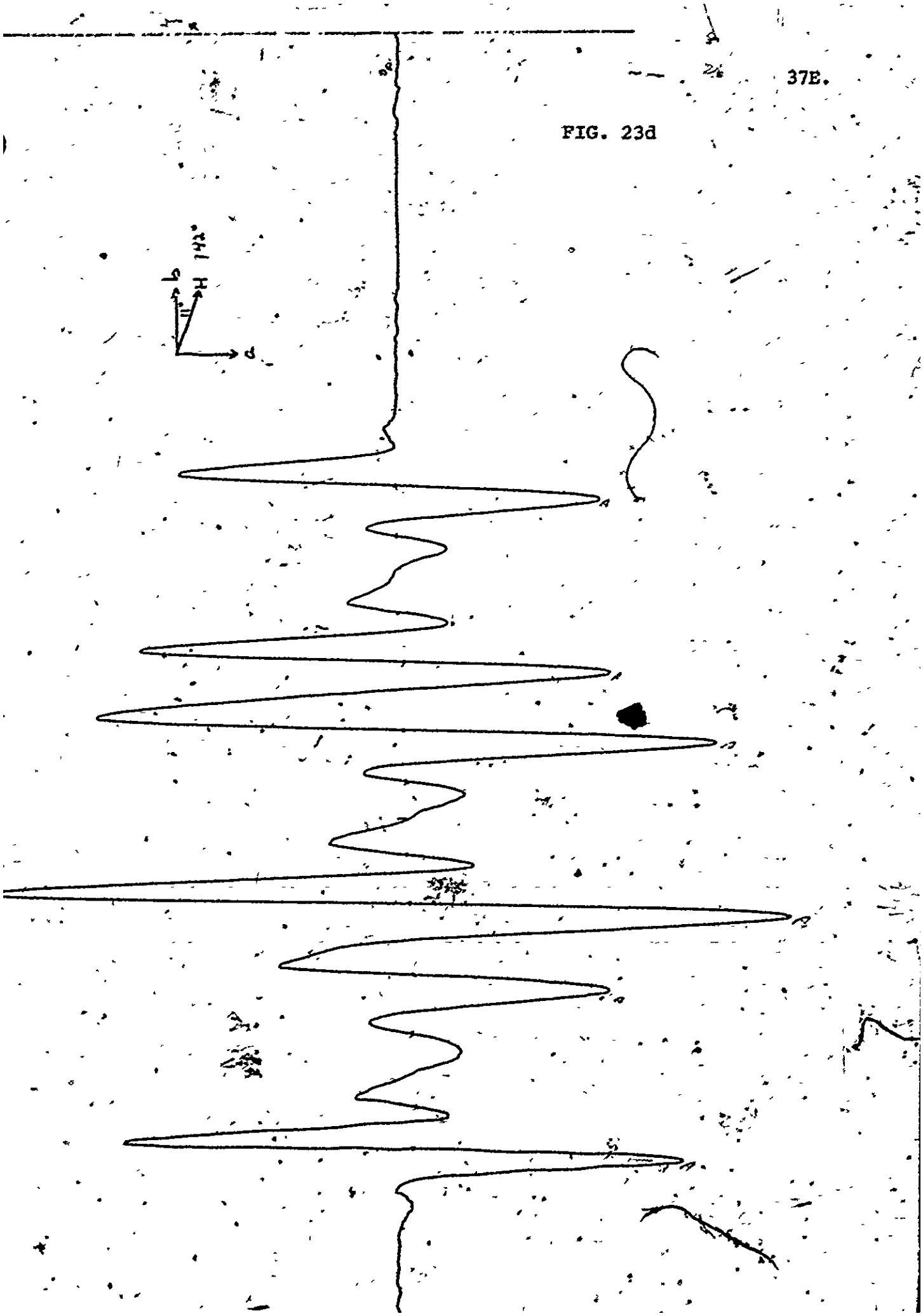


FIG. 23c



DPPH

FIG. 23d



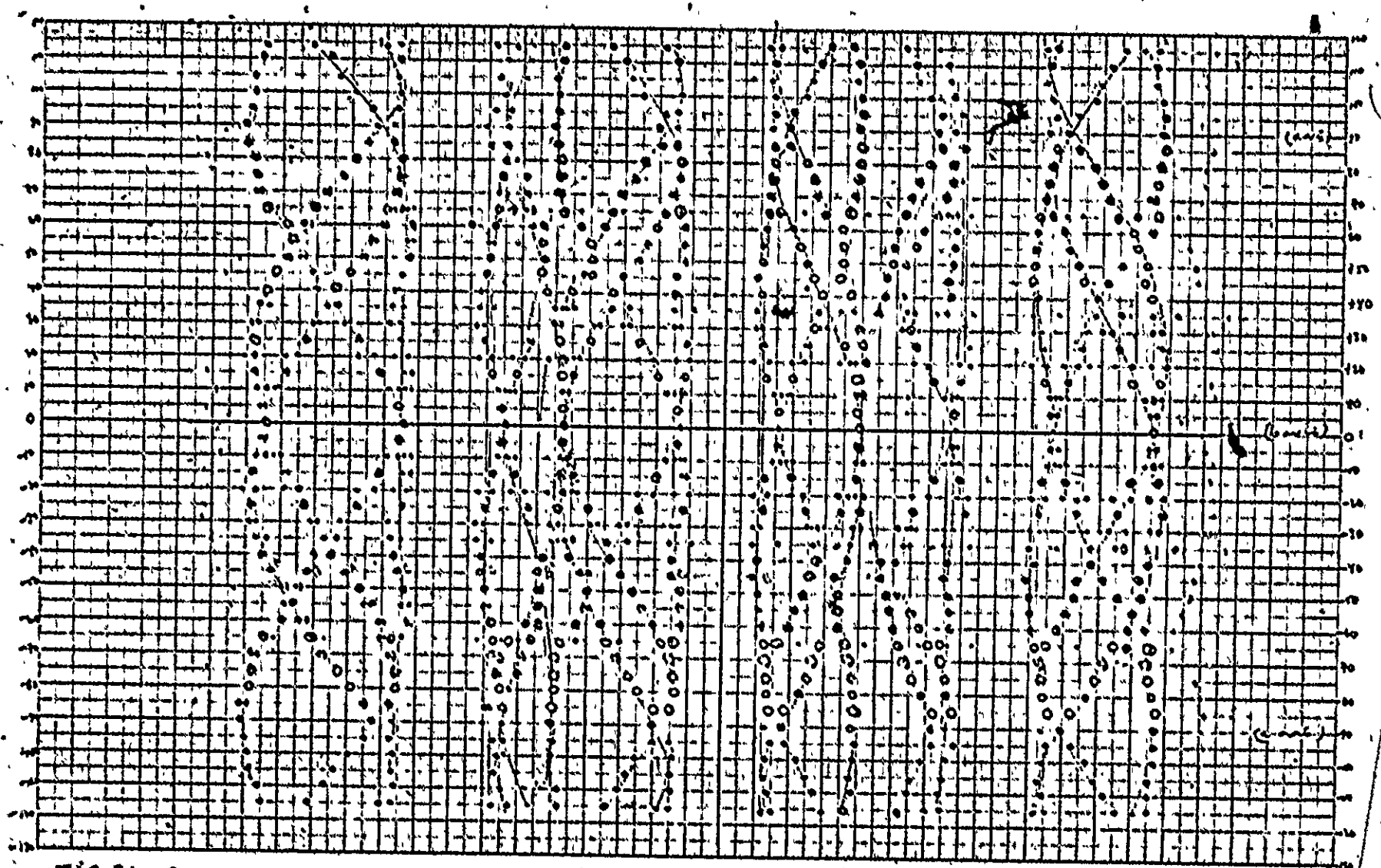


FIG. 24 Orientation pattern of X-band ESR spectrum of N-deutero succinimide with external field in radical plane where linewidths due to Nitrogen anisotropic coupling are at a minimum.

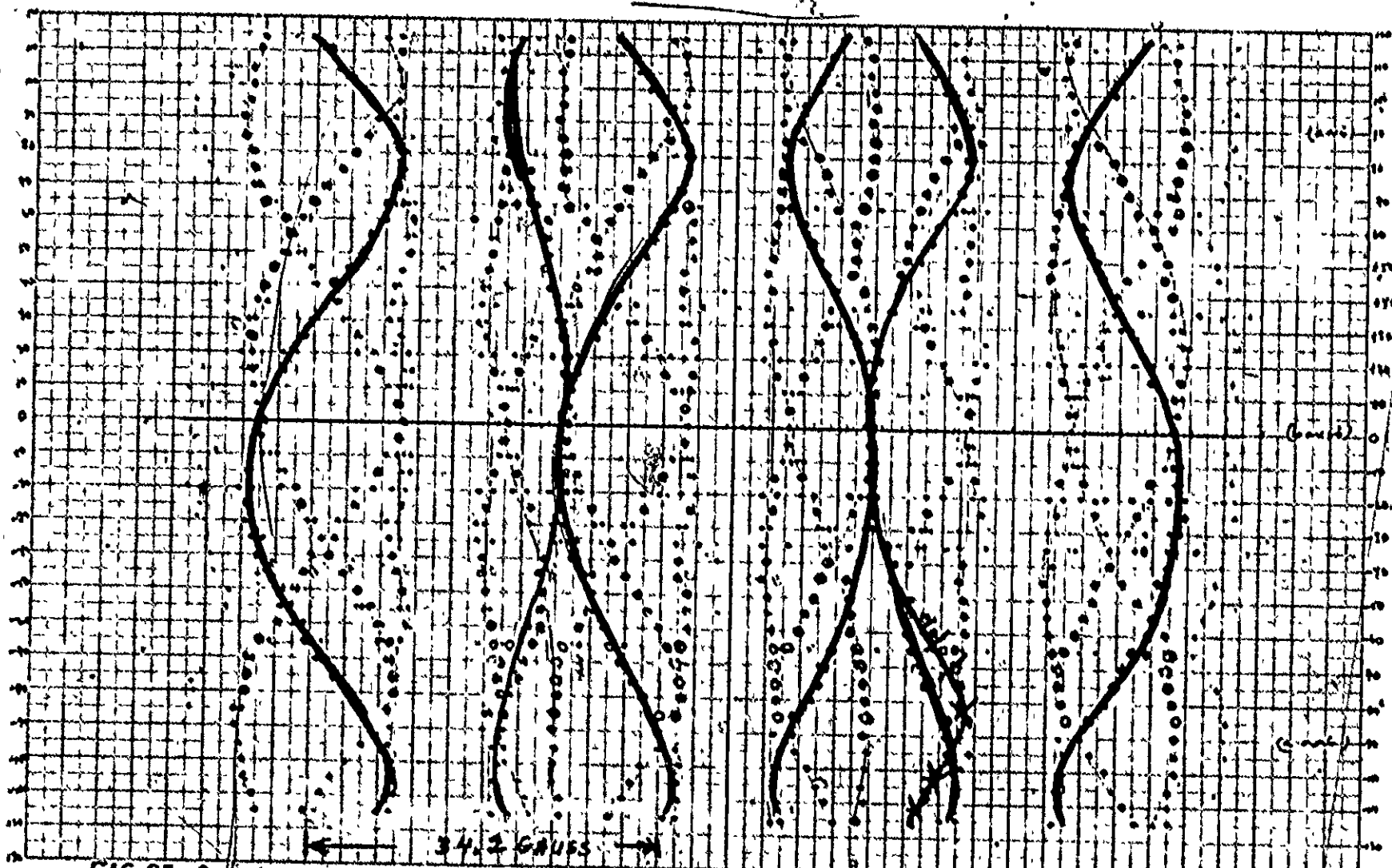
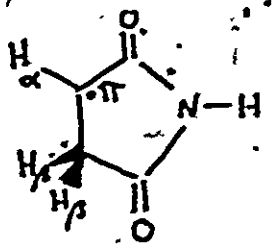


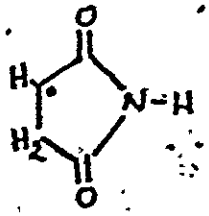
FIG. 25. Orientation pattern of X-band, ESR spectrum of N-deuterosuccinimide with external field in radical plane. Orientation pattern consistent with electron "seeing" one alpha and two beta hydrogens is shown as solid line.

shows that with the external magnetic field in the ac, ab, or bc crystallographic plane there should be 2 magnetically distinguishable groups of molecules.

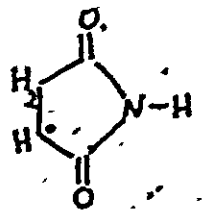
The radical species that has been assigned to the spectra observed is:



The coupling constants and g values evaluated from the spectra are given in Table 17. The coupling values are in gauss. Let us consider the radicals formed at one site. If the radical formed is the following:



then also at the same site can be formed the radical



The same lattice site can therefore give rise quite simply to two spectra. We say that there are two radical sites, i.e., one for each orientation. We see therefore at least two sets of lines. Indeed, four sets of lines are visible, but they superimpose at certain orientations. The spectrum in Figure 26 shows this superimposition.

TABLE 17

EXPERIMENTALLY OBSERVED HYPERFINE COUPLING CONSTANTS AND g VALUES OF γ-IRRADIATED SUCCINIMIDE

Direction	g value	Hyperfine Couplings (gauss) Hα	Hβ	N	Hγ
x axis	2.019_1	7.9	28.0	~0	~0
y axis	2.011_3	30.7	30.1	~0	~0
z axis ≈ c axis	2.01_I	~20	~30	~5	~0

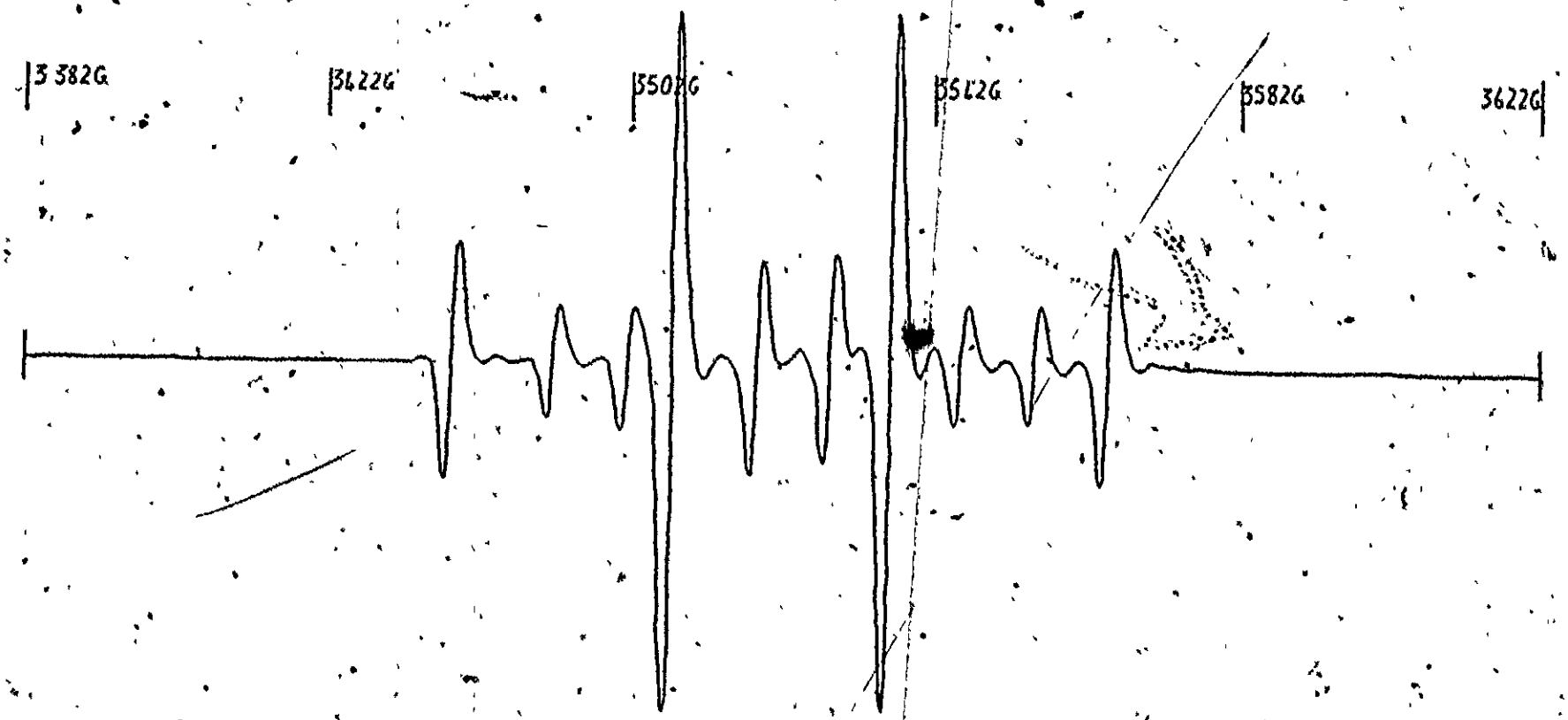


FIG. 26. N-deutero succinimide, X-band ESR spectrum of single crystal. Field lies along b axis and in plane of radical.

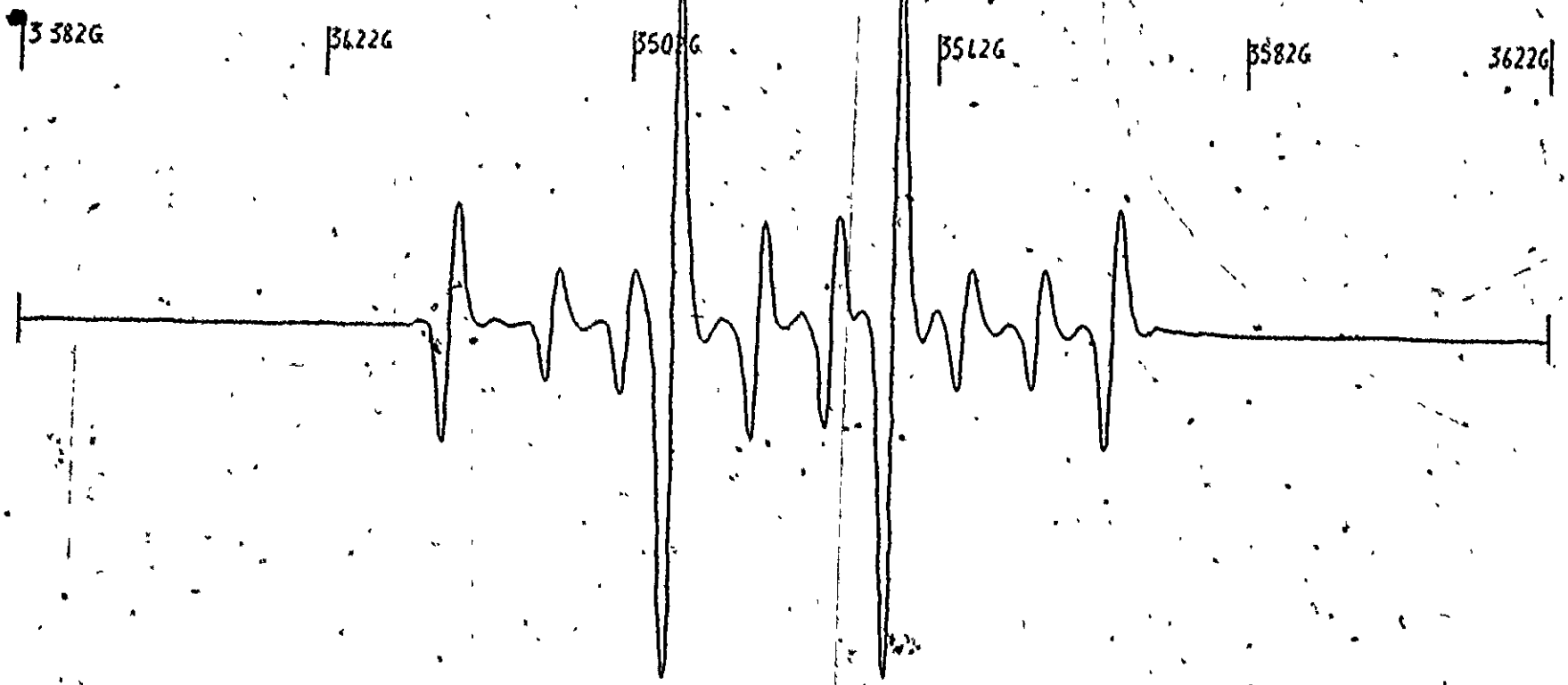
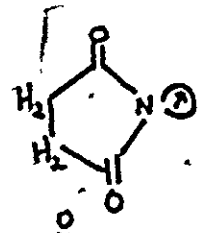


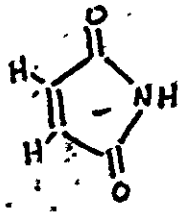
FIG. 26. N-deutero succinimide, X-band ESR spectrum of single crystal. Field lies along b axis and in plane of radical.

Some spectra at other oritentations not in the plane are also shown in Figure 27. The nitrogen hyperfine coupling is not visible for spectra with the field in the ab plane. However, when the field lies along the c axis, all the radical sites are equivalent and the triplet splitting ratios, 1:1:1, characteristic of nitrogen is ~5 gauss.

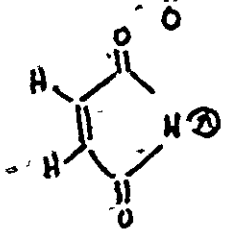
The molecular orbital calculations using the INDO open shell method give the values -1 gauss and 2.5 gauss for the field in and out of the plane, respectively. This is in good agreement with that observed for nitrogen.

The other hyperfine splittings are also in fairly good agreement with the calculated values and are shown in Table 18. Other radicals such as





and



were also considered. Their calculated hyperfine splitting values do not accord with the observed ones. It is most unlikely that the radical is other than that which has been assigned here.

FIG. 27

X-band ESR spectra of succinimide crystal with field oriented perpendicular to the a axis and lying in the b c plane. Note that linewidths increase rapidly as the nitrogen anisotropic coupling becomes significant.

FIG. 27a


231°
H
b axis is at 226° on goniometer.
a axis is perpendicular to field

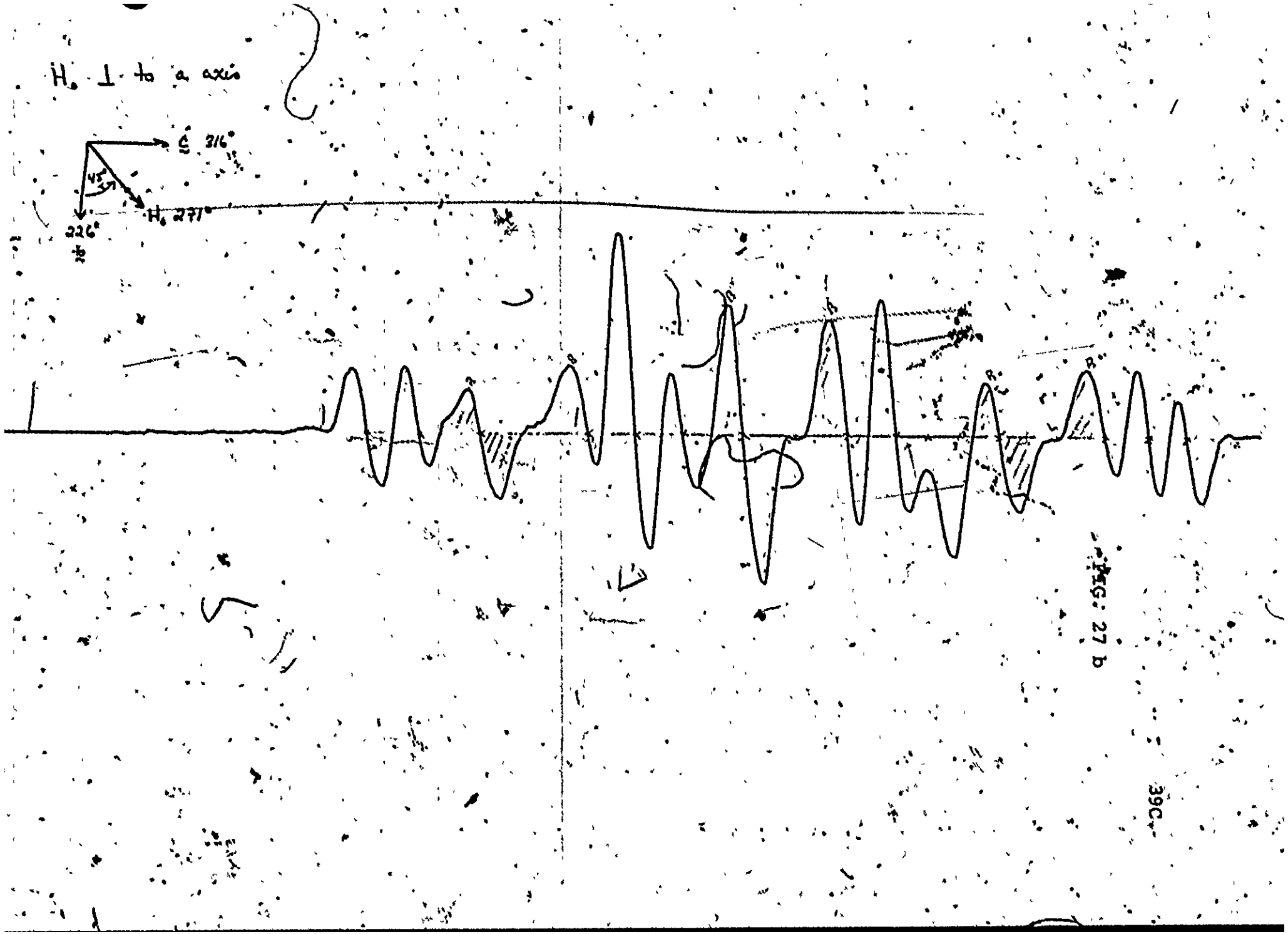


FIG. 27 b

39C-

FIG. 27d

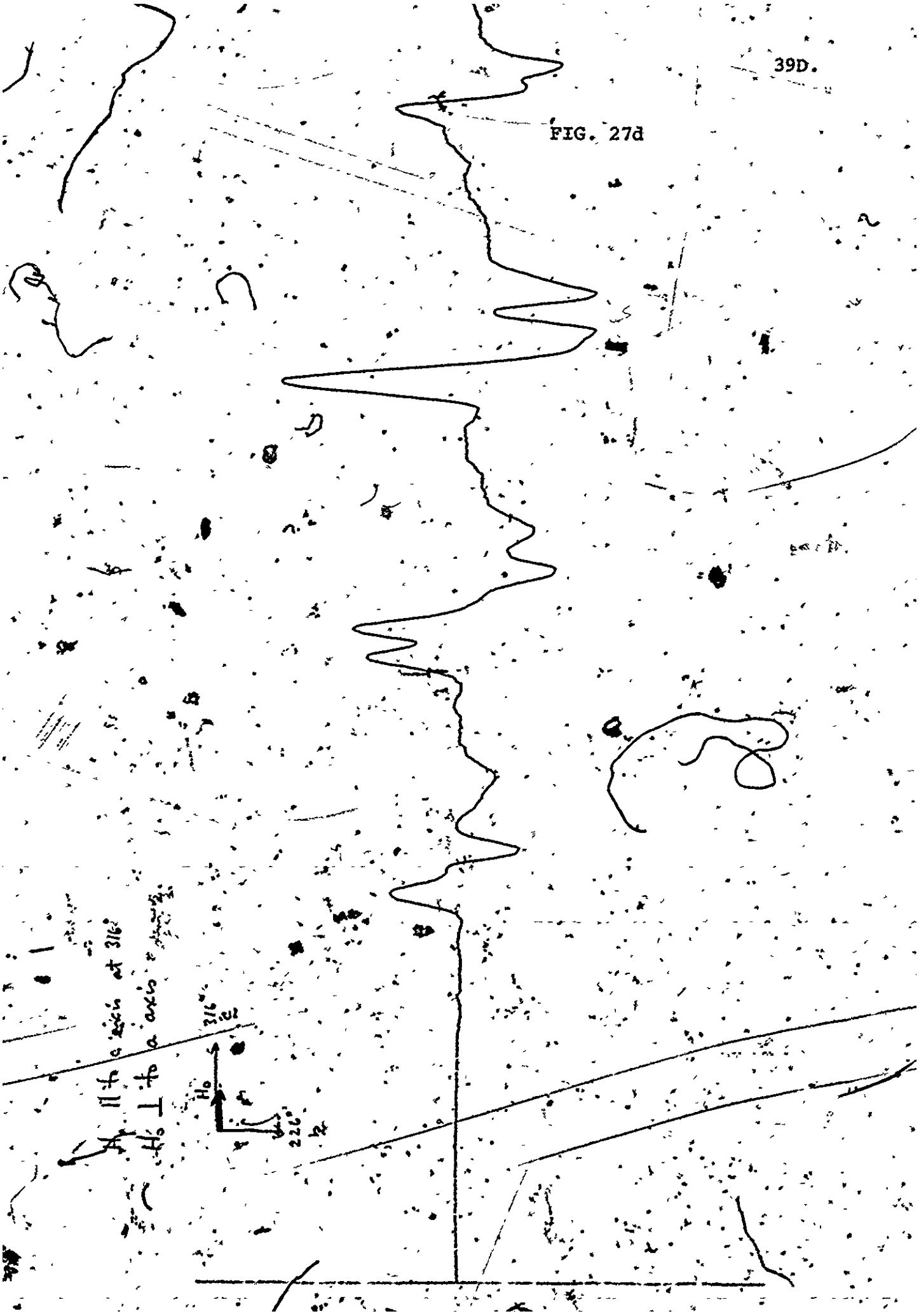


TABLE 18

EXPERIMENTALLY OBSERVED AND THEORETICAL
(CALCULATED USING POPLE'S INDO METHOD) COUPLING CONSTANTS

Hyperfine coupling constants (gauss)

Direction	H_α		H_β		N		H_γ	
	obs.	theor.	obs.	theor.	obs.	theor.	obs.	theor.
x axis	7.9	- 8.8	28.0	37.8	~0	-1.1	~0	0.1
y axis	30.7	-25.9	30.1	36.7	~0	-1.1	~0	0.1
z axis	~20	-17.8	~30	35.6	~5	2.5	~0	0.1

APPENDIX A

General references applying to ESR studies of single crystal studies

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SECTION B.

ESR STUDIES OF BLOOD AND ITS COMPONENTS FROM WOMEN HAVING CARCINOMA OF THE CERVIX UTERI.

INTRODUCTION

A. Cancer

The term cancer refers to a very large variety of conditions in which there is an abnormal mass of cells, the growth of which exceeds and is uncoordinated with that of normal cells and persists in the same excessive manner even after cessation of the stimuli which evoked the change" (1). In pathology cancer is described by the term malignant neoplasm and a distinction is made between carcinomas which are derived from the ectoderm and endoderm and sarcomas which are mesodermal in in origin. The word neoplasm signifies new growth and the adjective malignant implies that this growth is resistant to treatment. Neoplasms or tumours can be benign or malignant. However, benign tumours resemble in structure their cells of origin more closely than those of malignant tumours. Moreover they do not spread widely throughout the body, grow so rapidly or kill so certainly as malignant tumours.

Cancer consists of malignant cells which are generally supported by a nonmalignant stroma made up of connective tissue, blood vessels, nerves and lymphatics. (Thus in chemical analysis of entire cancers corrections have to be made for variations in the volumetric ratio of malignant cells to stroma and for chemical composition of the stroma). On a cellular level several distinctions can be made between cancer cells and normal cells:

Cancer cells usually do not function in the same manner as their

normal counterparts. They increase in number in unruly fashion, partly or wholly released from the usual restraints such as contact inhibition, basement membrane limitation and formation of immune mechanisms destructive to foreign cells. Cells of malignant tumours can spread directly to nearby tissues and metastazize via lymphatics and blood vessels to other more or less distant locations. Cancer cells act as amino acid traps, thus depriving their better behaved bretheren of needed nutrition. Histologically malignant cells from different primary lesions show a greater similarity in size and shape than their normal counterparts. But in a given cancer there is often greater diversity of size and shape of malignant cells than is exhibited by the normal cells of the same origin.

Intranuclear viscosity is often less in malignant cells than in normal ones.

Mineral constituents e.g. calcium and magnesium, are usually higher in neoplastic cells as compared to normal ones. The polarity of many cells are lost when they become malignant. This is sometimes evidenced by lack of uniformity in the position of the centrioles. Nuclear division is frequently more irregular in cancer cells than in normal ones. Graphic illustrations of some of these differences are shown in Figs 1 and 1A.

Carcinoma of the Cervix Uteri

Carcinoma of the cervix is the second most frequent cancer affecting women. 2 of each 100 women in North America has the disease and one of them will die of it. It occurs chiefly in parous women towards the end of their child bearing years. The disease is thus rarely seen under the

FIG.1 Continued.

1. Group of cancer cells, squamous cell carcinoma of the cervix, Grade2.

2. Group of cancer cells, squamouscell carcinoma of the cervix, Grade 2 (another case).

3. Group of cancer cells(same as 2)

4-8 Group of cancer cells, squamous cell carcinoma of the cervix, Grade 2-3.

9-11 Groups of cancer cells ,recurrent postradiational squamous cell carcinoma of the cervix.

12-27 Groups of cancer cells, adenocarcinoma of the endocervix.

28. Group of cancer cells from a scraping of the tumour surface immediately after its removal. Note striking inequality in size of nuclei, some reaching gigantic proportions. (Same as 12-27.)

29. Group of crowded cancer cells, adenocarcinoma of the cervix.

30. Group of cancer cells, squamous postradiational cell carcinoma of the cervix,Grade 2.

31-32. Degenerating cancer cells,squamous cell carcinoma of the cervix, Grade 2 to 3, after arecent treatment with x-ray.

33. Degenerating cancer cell, squamous cell carcinoma of the cervix,Grade 2, after irradiation

34-35. Degenerating cancer cells(same as 31-32)

FIG.1. Cancer cells found in the vaginal smear of women(carcinoma and adénocarcinoma of the cervix uteri)

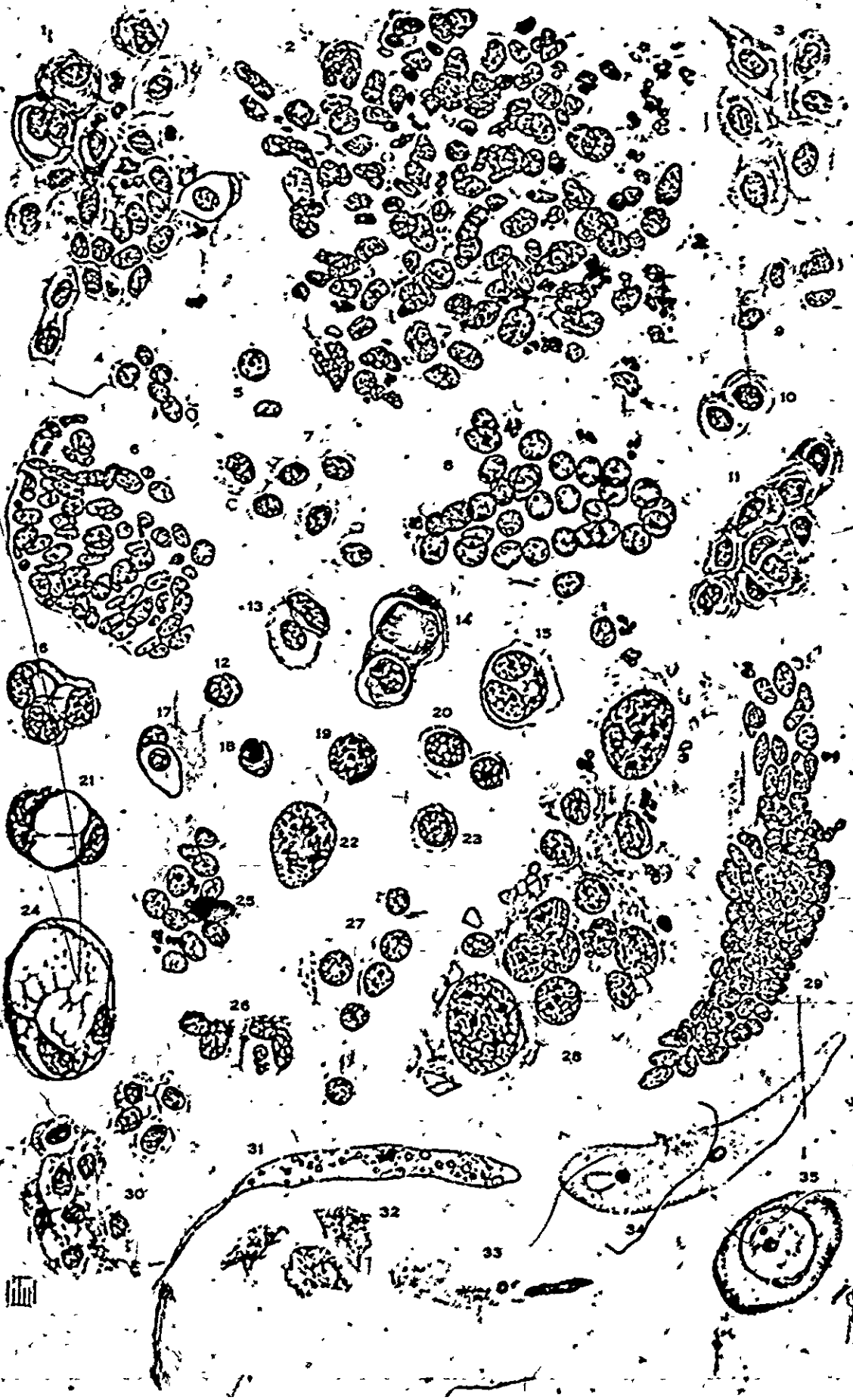


FIG.1A continued.

1-2. Superficial squamous cells of the normal follicular(preovulatory) phase.

3-9. Superficial squamous cells of the normal regressive(postovulatory) phase.

10-16. Navicular cells, intermediate type, of normal pregnancy.

17-19. Normal navicular cells, intermediate type, non-pregnant

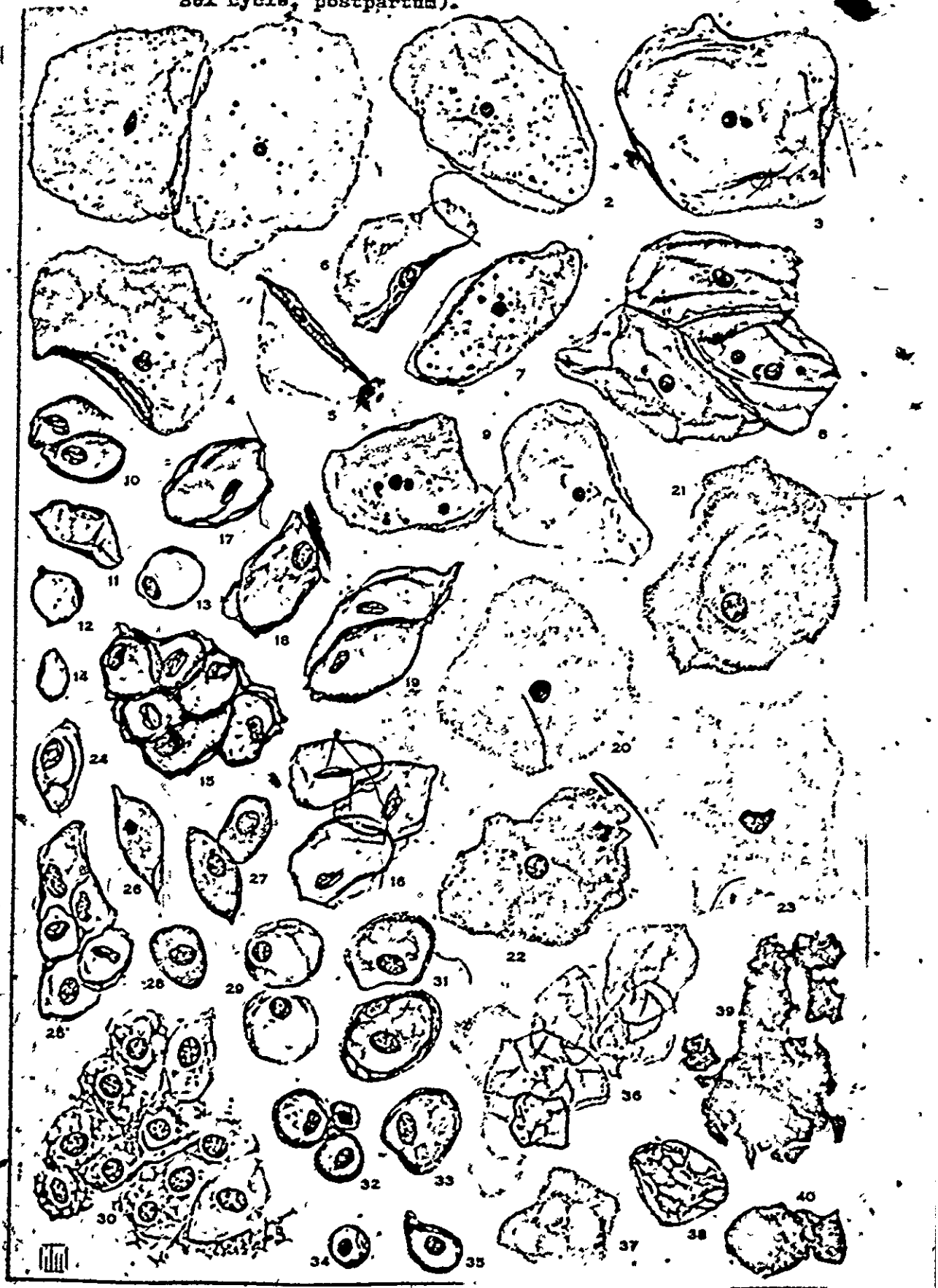
20-23. Superficial squamous cells of the normal premenstrual phase

24-30. Cells of the normal postpartum phase, outerbasal and intermediate types.

31-35. Normal cells of the cervical outer basal type.

36-40. Superficial squamous cells totally cornified.

FIG.1A. Epithelial cells found in the vaginal smear of women(normal sex cycle, postpartum).



age of 25 or over the age of 65. The mean age is approximately 46 years (3,4). The incidence is less common among jewish women, and other women whose spouses have had circumcision and among virginal women. Carcinogenic properties have been attributed to smegma (5) which may explain the importance of circumcision. Incidence is thus higher in ethnic groups where circumcision is uncommon in women who have married early and who have had multiple pregnancies (6). Sexual promiscuity, coupled with failure to use contraceptive devices appears to be a factor contributing to cervical carcinogenesis (7).

The disease is classified according to its cellular origin. When it originates in squamous cells (usually at the squamous-columnar junction of cervical epithelia) it is called squamous carcinoma of the cervix uteri. On the other hand when it originates in the columnar cells of the endocervix it is called adenocarcinoma. Squamous carcarcinoma of the cervix uteri accounts for 90.2% of all instances of cervical cancer, while adenocarcinoma of the cervix uteri occurs 7.1% of the times.

In a few instances (1.6% of all reported cases) lesions have characteristics of both types of cervical cancer. A few cases (0.8%) where primary lesions are found in both squamous epithelia as well as the endocervis have also been reported. In about 0.3% the cytologist is unable to determine the cellular origin of the primary lesion (8).

The extent of a carcinoma of the cervix is determined by physical examination, according to what the physician may see and feel in the vagina and cervix and feel in the uterus, parametrium and pelvis.

The rules that apply in determining the stage of progression of cancer of the cervix are accepted internationally. They were adopted by the International Federation of Gynecology and Obstretrics in 1961 (9). They apply to both epidermoid carcinoma and adenocarcinoma. (See Figs 2-1 to 2-7).

Stage 0 Carcinoma in situ, intraepithelial carcinoma

Stage 1 Carcinoma strictly confined to the cervix

Stage 1A cases of early stromal invasion

Stage 1B all other cases of Stage 1

Stage 11 The carcinoma extends beyond the cervix but has not extended on to the pelvic wall. The carcinoma involves the vagina, but not the lower third (subgrouping of Stage 11 cases into 11a (no parametrial involvement) and 11b (parametrial involvement) is recommended.

Stage 111 The carcinoma has extended on to the pelvic wall. On rectal examination there is no cancer free space between the tumour and the pelvic wall. The tumour involves the lower third of the vagina.

Stage IV The carcinoma has extended beyond the pelvic wall and has penetrated the mucosa of the bladder or rectum.

Diagnosis

The symptons of carcinoma of the cervix large enough to ulcerate,

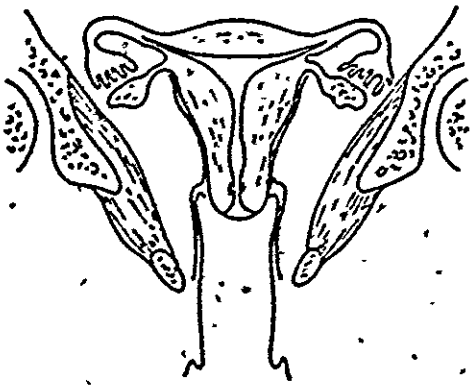


FIG. 2-1. Stage 1A carcinoma of the cervix. Early stromal invasion is present.

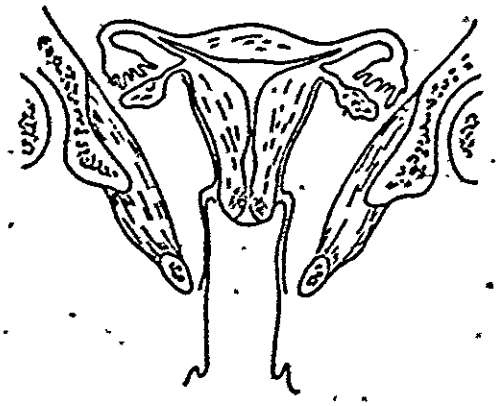


FIG. 2-2. Stage 1B carcinoma of the cervix. The tumour is small and confined to the cervix.

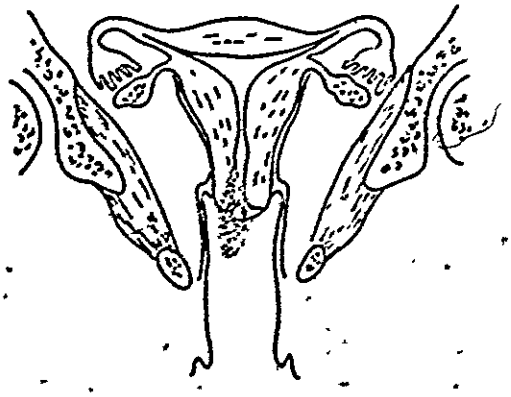


FIG. 2-3. Stage 1B carcinoma of the cervix. The tumour is large but does not extend into tissues beyond the cervix.

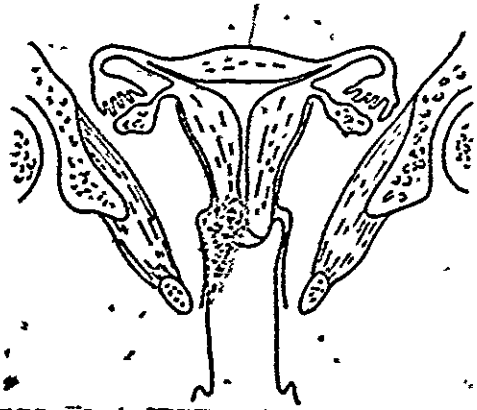


FIG. 2-4. Stage 2A carcinoma of the cervix. The tumour extends beyond the cervix(here into the upper third of the vagina). The parametrium is not involved. The lower third of the vagina is not involved.

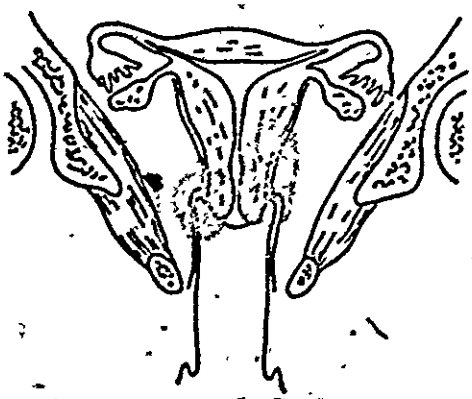


FIG. 2-5. Stage2B carcinoma of the cervix. The tumour extends into the parametrial tissues but not into the wall of the pelvis.

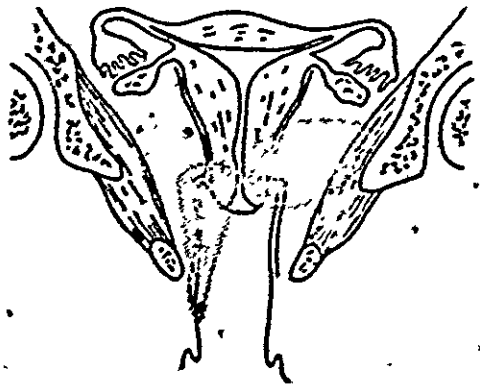


FIG. 2-6. Stage 3 carcinoma of the cervix. The tumour involves the lateral pelvic wall or lower third of the vagina(or both).

FIG. 2 continued.

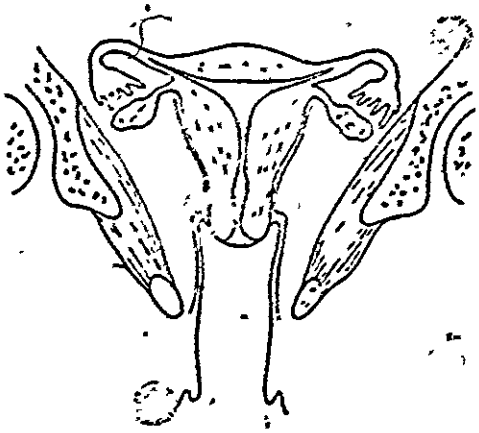


FIG. 2-7. Stage4 carcinoma of the cervix. The tumour has extended beyond the true pelvis(for instance, to soft tissue of the vulva or to a lymph node above the pelvic brim).

(See ref. 8 for above figures)

may be intermittent bloody discharge or spotting; large lesions with central necrosis may be the source of a foul yellowish discharge. Invasion or compression of the pelvic nerves late in the course of the disease causes pain in the lumbar regions, legs and hips (1,4).

In recent years a considerable amount of effort has been directed towards finding carcinoma of the cervix at an early stage before any symptoms have been produced and before the lesion is visible to the examiner. This has been accomplished by the use of stained smears (The papanicolaou smear) of exfoliated cervical cells (10,11) and by microscopic examination of tissue removed by punch or cone biopsies. (In very small lesions the sample may miss the abnormal tissue). Considerable controversy has been generated however, as to whether the early dysplasia of cervical tissue seen under the laboratory microscope necessarily progresses, if untreated, to carcinoma of cervix (12-18). Current belief is that it does and that instances in which it does not happen are due to the fact that the abnormal tissue has been removed by cone or punch biopsies etc (19-22).

B. ESR AND CANCER

1. Tissue Work

The first experimental applications of ESR to cancer were stimulated by theoretical considerations concerning the role of free radicals in living matter (23-29). These radicals originate either from the outside of the cell or from the inside (30-35); they are highly reactive (36-39) and so are capable of altering cellular biochemistry.

Investigations of normal and neoplastic tissues (40-77) show differences in ESR signal intensities. Usually the former give larger signals than the latter. The exceptions (65-69) to this rule may be a result of the dynamics of tumour growth (78-80) (See Fig. 3).

Paramagnetic transition elements are present in different amounts in some tumours than their normal counterparts (57, 58, 81-83).

For example Nebert and Mason (58,81) observed that at high microwave powers ESR signals from mouse hepatoma were less intense than the "normal" ones.

Mulay and Mulay (83) found that mouse melanoma (S91) had resonances at g = 2.05 and g = 4 while amelanoic derivatives (S91A) from the same animal had neither of these peaks. The line shapes of ESR signals from malignant growths may qualitatively not be similar to those observed for homologous normal tissue (82, 84). "Triplet" signals for example have been observed in the ESR spectra of neoplastic tissues (43, 59, 85-90). Such signals do not appear to be intrinsically related to tumour processes since they have been found for tissues in other pathological states (91-95). They may be due to NO-hemoprotein complexes with sulphur and iron involved (92-94).

Another difference between normal and neoplastic tissues is a shift in g-factors in the g = 2 region (71, 78, 78, 96). These shifts are generally quite small although Driscoll et al (78) has reported a ten-fold change between normal and cancerous tissue. Lyophilized samples were used so the origin and meaning of the shifts are not clear. Most studies do not show g-factor changes. This is not unexpected since

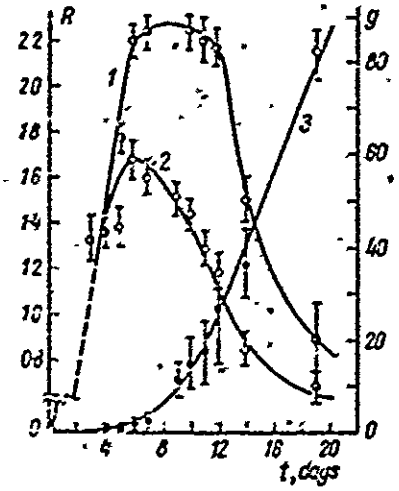


FIG. 3. Kinetics of concentration of free radicals in peripheral(1) and central(2) part of tumour(walker carcinosarcoma) and change in weight of tumour(3). Ordinate (left--R) relative amplitude of EPR signal. (See ref. 80)

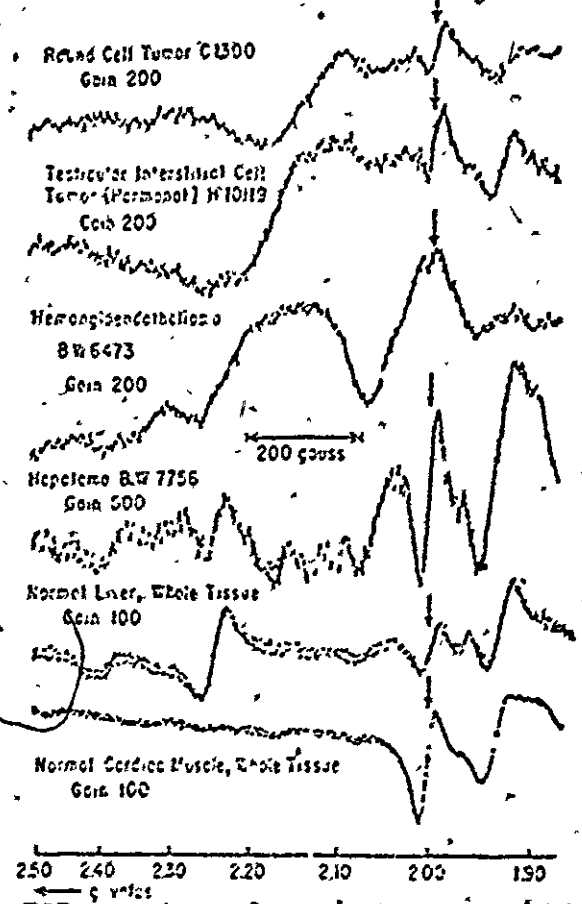
broad lines are seen in most tissues and organic radicals as a rule have only slightly differing g-values. Away from the g = 2 region larger g factor changes have been observed (58, 81, 83). In Figure 4 several examples of these signals are shown.

2. Blood Work

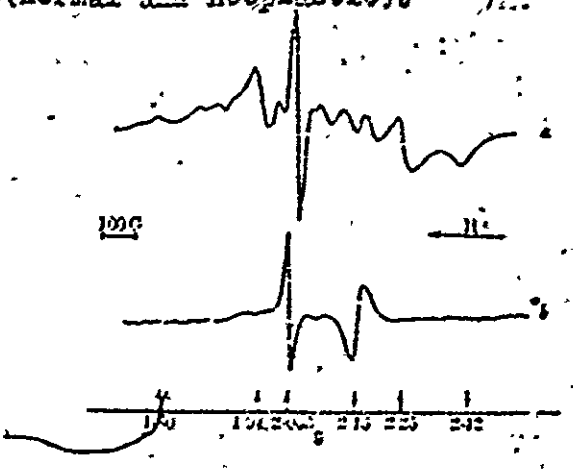
Most ESR investigations of cancer have been done on tumour tissues and only relatively few on blood or its components (97-103). During Cancer, several non specific disease changes occur in blood. Examples of these changes are (a) a rise in glycoprotein levels (104-114), (b) a decrease in haemoglobin content and sedimentation rate of red blood cells (115-117), (c) a rise in ceruloplasmin (100) and (d) a reappearance of "fetal" proteins (118). Changes that are specific for the type of cancer may also occur in blood as the disease progresses (119-124). Nevertheless if these changes are not associated with free radical formation they are not detectable by ESR.

Saprin's group when examining the response of Walker, carcinosarcoma to ThioTEPA also studied peripheral blood (80). This study was probably suggested by the earlier work of Pablova and Livenson (103) who investigated the concentration of free radicals in peripheral blood of patients with chronic lymphogenous leukemia. A 30 to 40 fold increase of free radical concentration was found in the leukocyte fraction of peripheral blood of the diseased patients as compared to the blood of the normal patients. In similar fashion to their work on tissues, Saprin et al examined the dynamic variation of free radical concentration

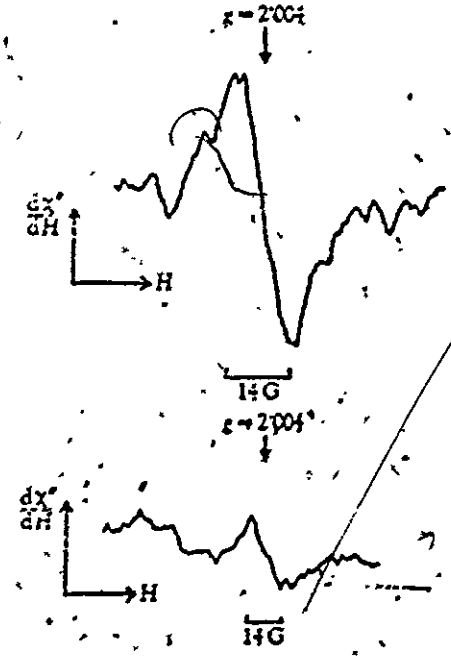
FIG. 4. ESR spectra of various tissues (normal and neoplastic).



ESR spectra of various mouse tumours and of normal-mouse liver and cardiac tissues. Rapid freeze technique used. (See ref. 58).



ESR spectra of normal tissues from mice: (a) liver; (b) muscle. Rapid freeze technique used. (See ref. 87).



ESR spectra of tissues from rat: (a) normal tissue from kidney; (b) neoplastic tissue from kidney. Surviving tissue technique used. (See ref. 65).

in whole peripheral blood taken from rats who were growing Walker carcinosarcomas. Their findings are summarized in Fig. 5. Wallace et al (99) using their own work as well as Saprin's work (30) have attempted to establish a generalized blood curve showing the variation of free radical concentration in peripheral blood as a function of tumour size.

Their findings are summarized in Fig. 6. Wallace's curve while quite intriguing must nevertheless be considered suspect since it is an extrapolation of rat data to human data. Such an extrapolation may not be valid.

3. Free Radicals as Carcinogens

Earlier work (37, 84, 125, 126) has shown that free radicals are probably involved in all cellular oxidation-reduction reactions and Commoner (45) has noted that there appears to be a relationship between concentration of free radicals in a tissue and its metabolic activity. The experiments of Truby and Goldzieher (50) show however that this relationship, if it exists is not a simple one since neither the stimulation of the guinea-pig adrenals by adreno corticotropin nor its suppression by hydrocortisone sulphate had much influence on the intensity of the ESR spectrum of the adrenal tissue. A closer correlation, as Commoner and Ternburg (44) note, is observed for free radical and mitochondrial concentration in animal tissues. These investigators found that resonance could not be detected in the liver, kidney or heart of rats at birth, were smaller a day after birth, but reached a constant intensity after 8 days.

Similar observations have also been noted by Brennan et al (42).

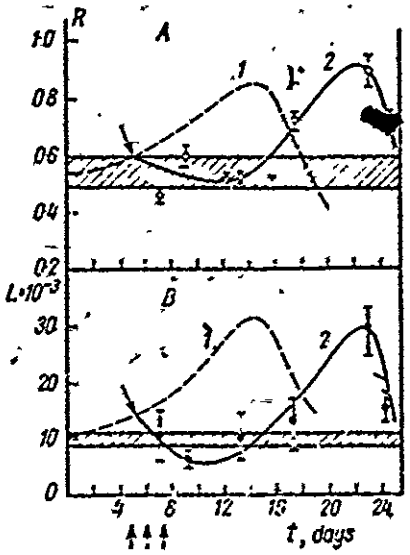


FIG. 5. A--Kinetic curves of change in concentration of free radicals in blood of controls (1) and animals given ThioTEPA (2). Ordinate, relative amplitude of ESR signal. B--Kinetic curves of change in number of leucocytes in blood of controls(1) and animals(rats) given ThioTEPA. (See ref. 80)

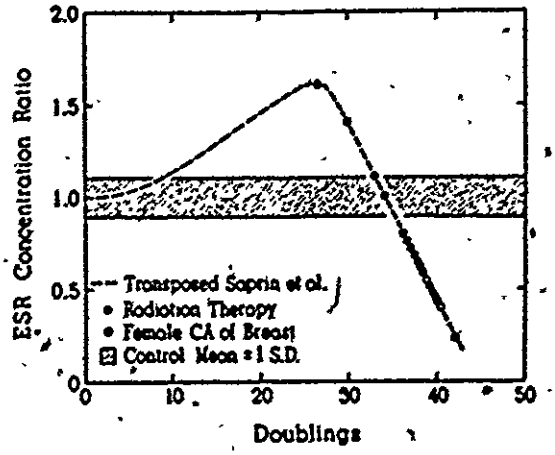


FIG. 6. Peripheral blood: free radical vs. tumour doublings in human cancer. (See ref. 99).

Dawkins (127) observed a parallel increase in rat liver succinoxidase activity with this mitochondrial concentration. These facts, along with the observed similarity of tissue spectra with those from isolated mitochondria and from redox systems found in mitochondria indicate that the origin of "tissue" signal is in the enzymatic redox activity of mitochondrial particles.

ESR of free radicals in normal animal tissue may then be only another way of measuring mitochondrial populations.

In malignant tissues free radical type and/or quantity appear to change during the progress of the disease. To date no consistent explanation has been offered. Oppenheimer et al (128) reported carcinogenesis after implantation of plastics in rodents. However, when a series of plastics of differing free radical concentration was used tumour incidence did not appear to be altered by this change in concentration (129). Boyland (130) and Peacock (131) observed similar results but with different carcinogens.

One problem in the above experiments is that only relatively stable free radicals can be observed under these conditions. Such free radicals are stable because they are not very reactive. Experiments of the type outlined above are therefore not likely to shed much light on the importance of highly reactive free radicals in carcinogenesis and the high reactivity of radicals continues to be of interest to the author and other ESR investigators in this field.

The administration of free radical inhibitors in both animals and humans reportedly causes a decrease in the rate of tumour growth (75, 80, 132) which is paralleled by a decrease in the intensity of the ESR signals originating from the malignant tissues (133, 134). These conclusions are rendered somewhat doubtful by contradictory reports. For example Kalmanson (135) has reported that the administration of tumour inhibitors such as propyl gallate cause an increase in the ESR signals seen in several types of tumours. This absence of clarity of experimental findings has led to the proposal of several different theories concerning the involvement of free radicals in carcinogenesis. Burlakova (136-138) for example has suggested that high intracellular levels of free radicals leads to an inhibition of the rate of cell division. She cites as evidence the fact that an increase in antioxidant ability is often associated with carcinogenic ability of drugs.

Harman (32) has theorized that cellular mutations such as those produced by cancer and aging are related and due to the action of free radicals on DNA and its precursors thereby affecting cellular metabolism.

4. Carcinogens as Free Radicals

Several studies (23, 139-145) have indicated that carcinogens in most instances readily form free radicals and that the case of free radical formation can often be correlated with the capacity to induce tumour formation.

Some carcinogens such as cigarette smoke, thioacetamide, p-dimethyl amino azobenzene etc give rise to distinctive ESR spectra in tissue (26,

146). Interestingly Vithayatil et al (26) found that after the administration of amino azobenzene to rats there was a change in liver tissue ESR spectra. This change appeared well before any histological or biochemical manifestigation of the developing cancer in the liver could be detected.

Another interesting finding is that certain polynuclear hydrocarbons when heated to temperatures above their melting points gave well resolved ESR spectra (147-149). Forbes and Robinson (147) showed that 3,4 benzpyrene, a well known carcinogen found in tobacco smoke, when heated in a vacuum gives rise to a spectrum which on exposure to air changes.

Neither spectrum is identical with the spectra of benzpyrene anion or cation radicals at ordinary temperatures (300°K). The authors have suggested that the carcinogenicity of tobacco smoke may be due to these reactive radicals produced by heating.

Ingram (1[redacted]) and Wyard (148) have investigated the production of free radicals by charring organic materials and found that free radicals are present in high concentration in such foods as toast. The medical significance of this has not been ascertained however.

Various authors have attempted to explain the mechanism of cancer induction by carcinogen free radicals. Among the hypothesis published are the following:

(a) Certain free radicals can form charge-transfer complexes which lead to the induction of cancer (151-153).

(b) The initiation of cancer by congugated hydrocarbons is a

consequence of the transfer of excitation energy by resonance from tryptophan to the carcinogen resulting in the formation of a specific protein carcinogen complex (154, 155).

(c) Some carcinogens react with sulfhydryl groups of proteins by free radical mechanisms (23, 156).

(d) In order that aromatic radicals may be carcinogenic they must possess a reactive band in the K region but must be devoid of reactive para positions (active L region) (156, 158).

(e) Carcinogenic activity is associated with electron transfer from the highest filled energy level of the protein to one of the empty levels of the "carcinogenic" hydrocarbon, thereby resulting in an unpaired electron in the molecular orbital of the protein (151, 152, 159).

The above hypothesis have certain features in common: they all suggest that free radicals are involved in carcinogenesis. Recent work (160-162) does not entirely support this view. Free radicals are probably involved in some types of chemical carcinogenesis especially in view of the demonstrated interaction between free radicals and DNA (163) and in view of the fact that many carcinogens are strong electrophiles (160). It is evident that chemical carcinogenesis is of such a diverse nature that free radical mechanisms must be considered within the overall physiochemical properties of each class of compounds (96).

5. Irradiation and Cancer

That ionizing irradiation can cause the induction of tumours is

well documented (164-174). The nature of the induction mechanism is not known although radiobiological considerations point to alterations in DNA with subsequent disruption of cellular metabolism (175, 176). Free radical formation is probably involved and a considerable amount of ESR studies on radiation damage of DNA has been done (177-181).

Recent work (182, 183) has shown that irradiation of radiosensitive tissues (i.e. tissues which are easily damaged by ionizing irradiation) leads to the formation of an ascorbate radical. The intensity of this irradiation induced ESR signal varies with the concentration of added flavin mononucleotide and it has been concluded that radiation damage may be a function of tissue flavin content (184).

6. General Problems involved in the study of ESR in relation to Carcinogenesis

(i) Techniques of Measurements.

The method of sample preparation can greatly affect the ESR spectrum. Ideally, ESR spectra of tissue should tell the experimenter something about the behaviour and environment of free radicals as they are found in living matter. Each of the methods now available to the investigator compromises this ideal. In the main, three preparatory techniques are used:

(a) the rapid freeze technique

(b) the surviving tissue technique

(c) the freeze dry technique

The Rapid Freeze Technique

Spectrometer sensitivity is adequate with this technique being of

the same order as for dry samples. If measurements are made at 77° K (as compared to 300°K) there is a fourfold gain in sensitivity due to the Boltzman factor. The problem of non resonant absorption by water in the tissues is also minimized since water's dielectric constant drops from 80 to approximately 2 when it is frozen. The rapid reduction of sample temperature to 77° K stops all cellular processes, thereby allowing the investigator to take many more samples and re-examine them by ESR at his convenience.

The possibility that this technique can produce artifactual changes in the ESR spectra should be borne in mind. In aqueous state samples at 273° K or higher, transition metal ions are not as a rule observed although Mallard and Kent (65) reported lines at g = 2.01 and g = 4 which they attributed to paramagnetic metal ions. These ions have not been observed in freeze dried material either, apart from whole blood (52, 185). At 77° however, most transition metal ions are readily detected and their resonances may obscure those of free radicals that are of more direct interest in this field and complicate the interpretation of these spectra. Swartz and Molenda (55) have shown that the two types of ESR signal can be differentiated by means of power saturation studies since at 77° K the relaxation times of the transition metal ions are much shorter than those of the usual tissue free radicals. Except for the factors pointed out above (paramagnetic ions and relaxation time effects) freezing has little if any affect on pre-existing free radicals (186, 187). If oxygen which is a free radical scavenger is present, the

number of free radicals can be markedly reduced by the time of measurement (188, 189). In spite of the great sensitivity of the rapid freeze method very few measurements of free radical concentration have been reported.

The Surviving Tissue Technique

In this technique introduced by Commoner et al (160) in 1956, the organs to be studied are removed from the animals as rapidly as possible, cut into thin slices about 0.5 mm thick and then suspended in either a 5% glucose or saline solution contained in a flat ESR cell. The tissue may be stored on dry ice for 3-4 weeks without any apparent change in radical concentration. Kerkut et al (53) observed that at room temperature free radical concentration can decay by as much as 50% during the first hour.

One of the difficulties with this technique is that the signal to noise ratio is usually very small and there has been considerable debate on the optimum design of the ESR cavity and associated cell (44, 46, 190-194). Another difficulty is that tissues become rapidly anoxic and the spectrum is liable to change during prolonged observation periods.

ESR spectra from surviving tissue are not distinguishable from those obtained from free radicals in redox enzyme systems such as cytochrome reductase, and B-hydroxy butyrate dehydrogenase. There is little doubt that these spectra are due to the semiquinone free radicals associated with these systems (44).

The ESR signals seen in lyophilized tissue are probably similar in origin to those seen in surviving tissue and furthermore have the advantage of being greater intensity. The additional signal intensity may be caused by the freeze drying process itself. Wyard (84) has suggested that certain enzyme cofactors are transformed into free radicals during the freeze drying process.

The Freeze Drying Technique

The dielectric constant of the samples are so reduced by freeze drying as to render practical the spectrometer operation and to yield a very good sensitivity. The signals observed are broad and without any exceptional characteristics; their origin is obscure but their intensities, and line widths vary markedly from sample to sample. In this thesis, we attempt to correlate the signal intensities, linewidths and g values of the "crossing points" to the type and incidence of cancer. A more detailed treatment of lyophilization is given in the section entitled discussion (See Page 24).

Comparison of the Three Techniques

In summary, the conditions in the surviving tissue technique are closest to normal though the ESR measurements are essentially under anaerobic conditions. Commoner and Ternburg (44) however did not notice any difference when the sample was saturated with O_2 or N_2 immediately before recording.

The Rapid Freeze Technique is the most sensitive of the three techniques but under certain circumstances gives results that are

different from those obtained using the other two techniques. For example with the surviving tissue technique malignant tumour tissues usually show a low and often barely detectable ESR signal. On the other hand a small but clearly defined signal is usually present when the freeze dried technique is used.

With the Freeze Dried Technique signal intensity differences between neoplastic and normal tissue are usually less than with the other two techniques.

6 (ii) Interpretation of Spectra.

Free Radicals and transition metal ions may be present in the samples. The Chief differences between their resonances is that free radicals have g values very close to the "free spin" value (2.00232) while individual transition metal ions have definite g values which fall between g - 1.93 and g = 6. Some of these metal ions thus have spectra quite close to g = 2 and can be mistaken for free radicals.

In such instances power saturation studies may enable a differentiation to be made between the two types of signal.

The identification of free radicals in biological samples is often not possible since the ESR spectra usually consists of a single featureless line, but by very precise measurements of g values, line widths and line shapes it may be possible to distinguish between spectra of this type and correlate them with biological phenomena.

Hyperfine splitting is sometimes observed. If there is doubt as to whether lines in a spectrum are due to hyperfine splitting or come from different radicals this can be resolved by comparing ESR

measurements at different frequencies. The absence of variation of hyperfine splitting implies that the spectrum is either isotropic or that the radical is tumbling so rapidly that the anisotropic part of the hyperfine interaction and the g value variation is averaged out. In solutions the tumbling, Tc, is approximated by the following expression.

$$Tc = 4 \pi n a^3/3k T$$

where n is the viscosity of the fluid and a is the radius of the radical. This equation is true for small molecules such as simple semiquinones but does not hold for large molecules such as proteins. In biological tissues of course semiquinones are likely to be tightly bound to proteins so that tumbling is restricted. Looser forms of attachment are sometimes possible (195).

Paramagnetic metal ions in biological media often give rise to very complex spectra. Beinert and Palmer (196) have made a compilation of these spectra. By comparing unknown ESR lines with these known spectra, it may be possible to identify the paramagnetic ions present.

The possibility that artifactual signals may be present in the spectra must also not be overlooked. A frequent source of such signals is the paramagnetic contamination of the sample cavity and/or holders and "background spectra" must be run frequently. The sample itself may influence cavity dielectric loss etc. in such a way as to make the reproducibility of instrumental conditions quite difficult

and so spectra may vary from one run to another. External paramagnetic impurities may contaminate the sample during its preparation. In some instances however, sample contamination may arise from paramagnetic substances that are indigenous to the cell but not an essential part of the molecular system being studied (197-206). Mechanical damage to biopolymers can also cause free radicals to be produced (207,208).

The introduction of oxygen into a sample (to avoid anoxia for example) can as has been pointed out before, affect radical formation (188,189). Relaxation times of radicals in frozen solutions can also be affected by the presence of oxygen (209).

C. REASONS FOR CHOOSING CARCINOMA OF THE CERVIX (UTERI)

As mentioned on page 7 Saprin et al (80) showed that free radical concentration in lyophilized peripheral blood of rats with Walker carcinosarcoma rises and falls as the tumour progresses reaching its maximum at halfway between the time of implantation of the tumour and death. Since Saprin et al blood measurements were made at the same time as the tumour tissue measurements in the rats with Walker carcinosarcoma and since both the tissue and blood findings were presented with a common abscissa, i.e. time since tumour implantation, Wallace was able to transpose the blood, curve to the generalized form shown in Fig. 6. In his studies on breast cancer in females, Wallace (99) found that free radical concentration in lyophilized peripheral blood decreased as the tumour progressed. He was thus able to observe the descending limb of the generalized blood curve (See Fig.7). However, Wallace used only 15 patients. The work presented in this section of the thesis is an attempt to find the ascending limb of the generalized blood curve and to have a cytological criterion of early cancer which would be available without undue inconvenience to the patient.

Cervical cancer was chosen because the early stages of the malignant process may be readily monitored by the use of Papanicolau smears, cone biopsies etc, and without much inconvenience to the patient. Possible blood findings can then be readily correlated with tumour growth. The women's pavilion, at the Royal Victoria Hospital, Montreal, Quebec, has a rather large and well organized cytology clinic for this disease.

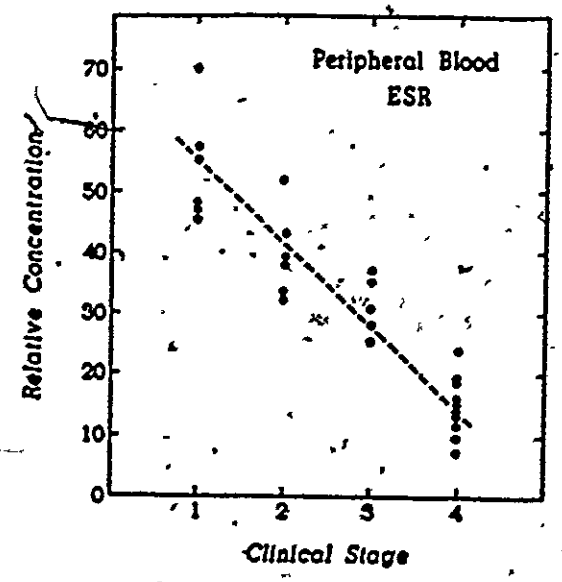


FIG. 7. Plot of relative concentration of free radicals in the peripheral blood of patients with clinically established cancers --- as a function of clinical stage. (See ref. 99).

This greatly facilitated our studies. Since blood is routinely drawn from patients at this clinic, no ethical difficulty was encountered in obtaining our samples.

METHODS AND RESULTS

183 samples of whole blood from humans have been examined by electron spin resonance and statistically analysed.

The pathological classification of these samples are given below

(a)	Normal epithelial cells	56
(b)	Mild dyplasia of the cervix uteri	35
(c)	Moderate dyplasia of the cervix uteri	16
(d)	Severe dyplasia in the cervix uteri	11
(e)	Carcinoma in sita of the cervix uteri	20
(f)	Invasive carcinoma in the cervix uteri	15
(g)	"Apparently" normal females	30

ESR characteristics determined from the examples are given in Tables 1-7. The statistical analysis of the results are given on Table 8. Sample preparation was done by Dr. C.G. Kalomeries. 28 measurements in Tables 1-7 are taken from Dr. E.C.B. Pederson's results. On the Clinical side help was received from Dr. J.P.A. Latour and Dr. K.G. Marshall of the Royal Victoria Hospital.

In some cases fractionation of whole blood was carried out by centrifugation. In these instances EDTA was used as the anticoagulant. This substance does not give any ESR signal under experimental conditions

The blood fractions obtained were plasma platelets and red blood cells (which included leukocytes). These fractions were then lyophilized for 72 hours. In the cases where fractionation was not done the whole blood samples as soon as they were obtained were dipped in liquid nitrogen and then lyophilized for 72 hours. The samples were then gently tapped to make them powdery and after this transferred to standard varian ESR spectroscopic grade quantity tubes having an external diemeter of 4mm, an internal diameter of 3mm, and length of 240mm. Being mindfull that mechanical damage (207, 208) can cause free radical formation in biopolymers, caution was taken to see that this did not occur. Each sample after lyophilization was packed to a height of approximately 50mm. Rubber gloves were changed between the handling of samples in order to avoid cross contamination and a surgical mask was also worn. To minimize exposure of the samples to moisture, a desicator containing anhydrous magnesium perchlorate was used for storage. The sample containing tubes were then removed evacuated for ten minutes and sealed. A total of 18 samples of non lyophilized fresh whole blood were examined by ESR at liquid nitrogen temperature and room temperature as soon as they were obtained. Plasma was also examined at liquid nitrogen temperatures and at room temperatures.

Additionally, plasma that was pooled from normal persons and plasma that was pooled from persons with invasive carcinoma of the cervix uteri were separated by ultrafiltration into 3 fractions; (1) a fraction containing molecules over 100,000 in molecular weight, (2) a fraction

containing molecules from 100,000 - 30,000 and (3) a fraction containing molecules from 30,000 - 0. These fractions were then examined by ESR at liquid nitrogen temperatures. A varian 4502 X - band ESR spectrometer and Varian TE 102 standard cavity were used to make all ESR measurements of samples and diphenyl picryl hydrazyl was used for field frequency calibrations. Varian strong pitch was used as an external standard. Tests with chromium in magnesium oxide as a secondary internal standard showed that the measurements were reliable. Varian precision quartz tubes with an external diameter of 4mm and an internal diameter of 3mm were used in the measurement of all samples:

Linewidth, g - value, peak to peak signal height and signal intensity were measured for each sample. Since the signal shape was essentially invariant the signal intensity was set equal to peak to peak height times the linewidth squared. A stepwise regression analysis (210, 211) was done on the data.

TABLE 1

Electron Spin Resonance Signal Characteristics obtained from blood samples from females who were apparently "normal".

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age in Years	Intensity of signal (App x H_{pp}^2)
2	3.9	9.6	0.0015	30	359.42
3	5.0	8.8	0.0017	39	387. 2
22	3.7	8.9	0.0015	54	293. 1
45	2.1	9.6	0.0019	27	193. 5
47	5.4	8.8	0.0017	52	418.77
49	7.7	9.0	0.0020	40	623.
65	3.5	8.4	0.0022	27	246. 9.
68	4.2	8.9	0.0021	18	332. 7
69	4.1	9.0	0.0021	27	332. 1
78	7.1	8.9	0.0022	28	562. 4
81	5.5	8.7	0.0023	25	416. 2
82	3.6	8.6	0.0023	20	266. 2
83	6.9	8.8	0.0023	21	534. 3
84	5.9	9.1	0.0021	34	488. 6
86	5.3	9.4	0.0022	41	468. 3
88	2.7	9.5	0.0022	55	243. 7
91	5.5	8.6	0.0023	47	406. 7
92	5.5	8.9	0.0023	25	435. 6
95	5.7	8.8	0.0023	30	441. 4
99	5.5	8.9	0.0023	21	435. 6

Table 1 - Continued,...

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}^2) in gauss	g	Age in Years	Intensity of signal (App x H_{pp})
100	7.2	8.9	0.0021	32	570. 3
110	6.0	8.6	0.0022	27	443. 7
163	5.3	9.2	0.0018	20	448. 6
165	7.2	9.6	0.0017	37	663. 5
168	4.6	9.5	0.0017	21	415. 1
6	5.0	9.5	0.0019	21	721. 1
196	9.2	9.4	0.0019	28	812. 9
197	8.3	9.7	0.0019	56	780. 9

TABLE-2

Electron Spin Resonance Signal Characteristics obtained from blood samples from females who were diagnosed as having Normal Cervical Epithelial Cells.

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age in Years	Intensity of signal (App x H_{pp}^2)
34	4.70	7. 9	0.0022	25	293. 3
36	5. 9	9. 7	0.0018	42	555. 1
37	5. 9	9. 2	0.0018	66	499. 3
38	5.30	9. 2	0.0018	26	448. 6
43	4.80	8.70	0.0020	22	363. 3
44	4.60	9.20	0.0020	45	389. 3
53	3.60	9.00	0.0018	32	291. 6
55	5.10	9.50	0.0021	28	460. 2
57	7.20	8.90	0.0019	33	570. 3
58	3. 3	9.10	0.0019	37	273. 2
63	7.80	8.70	0.0021	38	590. 3
72	3.75	8. 9	0.0021	22	293. 0
74	3. 8	9. 3	0.0022	65	328. 6
85	8. 1	9. 0	0.0022	28	656. 1
105	3.55	9.15	0.0020	26	289. 8
126	4. 4	9. 1	0.0015	28	364. 3
127	4. 9	9. 0	0.0022	21	396. 9
128	2.55	9. 4	0.0020	40	229. 7

Table 2 - Continued,...

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age in Years	Intensity of signal (App x H^2_{pp})
131	3. 2	9. 5	0.0022	34	288.8
135	2. 9	9. 4	0.0021	29	256.2
137	6. 1	8. 6	0.0023	27	451.1
138	4. 7	9.46	0.0019	42	424.2
142	2. 2	9.15	0.0018	50	182.2
143	6. 3	9.55	0.0015	17	568.5
146	3. 7	9. 4	0.0021	41	326.9
147	8. 4	9.55	0.0018	30	774.1
150	8. 3	8. 9	0.0019	33	657.4
154	4. 1	9. 3	0.0016	33	354.6
155	5. 2	9. 8	0.0016	28	499.4
156	6. 9	9.266	0.0019	25	596.8
157	6.16	9. 4	0.0018	28	547.8
59	6. 1	10.0	0.0019	21	610.0
160	5. 4	9. 3	0.0016	23	467.0
162	4. 7	9. 2	0.0018	36	397.8
164	5. 0	9. 7	0.0017	29	470.4
166	4.26	9. 2	0.0017	20	363.9
170	6. 9	9.36	0.0015	47	609.7
176	-	10.0	0.0021	23	-

Table 2 - Continued,...

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age in Years	Intensity of signal (App x H_{pp}^2)
180	8.9	10.0	0.0017	24	890.0
182	6.0	10.0	0.0017	27	600.0
186	4.2	10.0	0.0018	33	420.0
187	4.6	9. 7	0.0018	22	432.8
188	8.6	9. 7	0.0020	34	809.2
189	4.6	10.0	0.0018	36	460.0
191	6.5	9. 8	0.0017	20	624.2
193	5.8	9. 7	0.0021	30	545.7
194	-	9.35	0.0020	27	
199	9.05	10.1	0.0015	27	928.3
203	5.5	9. 9	0.0016	18	539.0
[illegible]	6.5	9. 2	0.0015	20	550.2
213	4.55	9. 2	0.0019	35	389.3
217	4.3	10.0	0.0015	57	430.0
218	5.2	9. 7	0.0018	34	489.2
219	6.2	9. 4	0.0017	30	547.8
222	6.3	9. 3	0.0019	49	544.8

TABLE 3

Electron spin resonance signal characteristics obtained from blood samples from females diagnosed as having mild dysplasia of the cervix uteri.

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age in Years	Intensity of signal (App x H^2_{pp})
35	3.4	9.7	0.0018	30	319.9
59	5.1	8.9	0.0019	24	403.9
62	4.6	8.3	0.0020	25	316.9
64	5.4	8.3	0.0021	25	372.0
67	3.8	8.8	0.0023	51	294.3
70	3.7	9.1	0.0021	21	306.4
80	3.1	9.3	0.0022	31	268.1
90	2.8	9.3	0.0022	19	242.1
94	4.1	9.0	0.0022	23	332.1
101	4.4	9.4	0.0021	29	388.7
103	4.4	9.1	0.0020	21	364.3
104	3.7	9.2	0.0021	41	313.2
108	7.6	8.5	0.0022	24	549.1
109	3.9	9.1	0.0020	33	356.7
113	7.4	8.8	0.0021	45	573.0
114	7.4	9.1	0.0021	24	612.7
122	5.2	9.4	0.0016	31	459.5
123	6.2	9.1	0.0016	23	513.4

Table 3.1 Continued,...

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age in Years	Intensity of signal (App x H_{pp}^2)
129	3.9	9.5	0.0023	31	351.9
130	2.7	9.7	0.0020	33	254.0
133	2.7	9.3	0.0022	20	233.5
144	6.5	9.1	0.0020	34	538.2
148	5.5	8.8	0.0017	21	425.9
149	9.9	8.9	0.0018	20	784.2
153	8.4	9.4	0.0016	32	742.2
171	4.7	9.7	0.0017	30	442.2
175	13.0	8.5	0.0020	44	939.2
181	11.8	9.4	0.0018	21	1042.6
192		8.9	0.0018	25	-
198	7.6	9.7	0.0016	50	715.1
201	6.0	9.3	0.0017	25	518.9
206	7.9	8.9	0.0018	23	625.7
209	7.9	9.4	0.0017	23	698.0
223	4.6	10.0	0.0018	29	460.0

TABLE 4

Electron spin resonance signal characteristic obtained from blood samples from females diagnosed as having moderate dysplasia of the cervix uteri.

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age in Years	Intensity of signal (App x H^2_{pp})
41	3.0	8.4	0.0018	24	211.6
79	3.8	8.6	0.0022	21	281.0
111	5.9	9.3	0.0021	22	510.3
112	3.5	9.6	0.0019	32	322.5
115	5.1	8.7	0.0021	20	386.0
145	6.4	8.8	0.0018	42	495.6
173	9.6	9.7	0.0021	32	903.26
185	5.1	9.4	0.0018	23	450.6
190	5.1	9.7	0.0018	31	479.8
200	9.7	9.1	0.0018	28	803.2
207	8.2	8.9	0.0017	29	649.5
208	4.8	9.2	0.0018	35	406.3
211	4.3	9.3	0.0022	25	371.9
215	8.7	8.6	0.0020	32	643.4
220	6.5	9.8	0.0016	25	624.2

TABLE 5

Electron spin resonance signal characteristics obtained from blood samples from females diagnosed as having severe dysplasia of the cervix uteri.

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age in Years	Intensity of signal (App x H^2_{pp})
54	5.4	8.8	0.0023	29	418.2
56	3.7	9.3	0.0019	39	320.0
61	4.8	9.2	0.0020	40	406.3
73	2.7	9.4	0.0021	24	238.5
102	5.7	8.7	0.0021	39	431.4
140	3.4	9.6	0.0019	28	313.3
167	5.6	9.5	0.0018	51	505.4
169	4.5	9.4	0.0017	28	397.6
178	5.9	9.8	0.0018	38	566.6
205	4.8	9.5	0.0018	36	433.2
216	8.4	9.4	0.0018	19	742.2

TABLE 6

Electron spin resonance signal characteristics obtained from blood samples from females diagnosed as having carcinoma in situ of the cervix uteri.

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g.	Age in Years	Intensity of signal (App x H^2_{pp})
39	4.0	8.6	0.0018	33	295.8
40	5.8	8.7	0.0018	37	439.0
42	6.7	9.2	0.0017	24	567.1
60	5.4	9.0	0.0021	21	437.4
116	7.7	8.8	0.0021	19	596.3
124	5.0	9.6	0.0017	23	460.1
125	6.6	10.1	0.0017	19	673.2
134	3.9	9.4	0.0022	33	344.6
136	3.3	9.4	0.0023	29	291.5
141	3.5	9.6	0.0019	41	322.5
151	5.0	9.4	0.0021	43	441.8
152	4.7	9.2	0.0020	49	397.8
158	6.7	9.6	0.0018	50	617.4
161	3.8	9.6	0.0017	38	350.2
172	4.6	9.8	0.0017	49	655.5
174	6.3	10.2	0.0017	31	655.5
179		9.4	0.0019	32	
183	-	-	-	-	-

Table 6 - Continued,....

Case No.	Peak to Peak height of Signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age in Years	Intensity of signal (App x H_{pp}^2)
202	5.8	8.4	0.0016	29	409.2
212	4.4	9.3	0.0023	39	380.6

TABLE 7

Electron spin resonance signal characteristics obtained from blood samples from females diagnosed as having invasive carcinoma of the cervix uteri.

Case No.	Peak to Peak height of signal (App) in (gauss)	Linewidth of signal (H_{pp}) in gauss	g	Age - in Years	Intensity of signal (App x H_{pp}^2)	Stage
75	5.4	8.7	0.0020	54	408.7	4
96	5.6	8.5	0.0022	32	404.6	4
97	5.4	8.3	0.0022	51	372.0	4
98	6.9	8.5	0.0022	58	498.5	4
106	6.5	9.0	0.0018	74	380.2	4
107	5.1	8.5	0.0020	67	368.5	4
117	8.1	8.6	0.0020	51	599.0	4
119	9.9	8.2	0.0019	47	692.6	4
120	6.9	8.7	0.0021	63	522.3	4
121	6.6	8.6	0.0018	61	488.1	4
132	5.7	9.0	0.0024	30	461.7	1
139	6.2	9.8	0.0017	55	211.2	3
177	9.9	9.3	0.0019	20	1193.5	5
224	5.6	8.2	0.0018	59	376.5	4
225	8.2	7.8	0.0017	59	498.9	4

TABLE 8

Stepwise regression analysis of spectral parameters for the different groups of donors.

	Group means (standard deviations) and F tests (=0.05)								
Number of Cases variable	Normal 55	Mild 35	Moderate 15	Severe 11	Insitu 19	Invasive 15	F to enter incl. age as variable	F to enter not incl. age nor invasive group	F to enter with line incl.
amplitude	5.45(1.63)	5.77(2.14)	5.98(2.17)	4.99(1.52)	5.24(1.26)	6.69(1.37)	1.8	0.8	0.75
line-width	9.38(0.42)	9.14(0.39)	9.14(0.45)	9.32(0.33)	9.33(0.48)	8.55(0.49)	9.6	2.2	-
SG from DPPH x 10^3	1.86(0.22)	1.95(0.22)	1.91(0.19)	1.93(0.18)	1.90(0.22)	1.97(0.22)	1.1	1.0	0.3
Age	32.4(10.7)	28.7(8.5)	28.1(6.0)	33.7(9.0)	33.6(9.9)	57.0(15.5)	18.2	-	-
Signal Intensity	481 (159)	477 (204)	502 (192)	433 (136)	461 (127)	425 (144)	0.5	0.3	0.5

DISCUSSION

In interpreting the above results, certain problems that are germane to the experimental method used (i.e. lyophilization) must be considered.

A. The Problem of Lyophilization

Since 1954 (45) a considerable body of evidence has grown up which indicates that the signals seen in lyophilized preparations do not accurately reflect free radical levels present in hydrated functioning cells. For example Berlin and Penskaia (1956) (212) showed that rapid freezing of high molecular polymers breaks some of the bonds, thereby giving ESR spectra. Varian associates (1957) (52) as well as our group (213) have found that freeze drying of whole human blood produces free radicals that are not present in quick frozen blood as observed in the ESR spectrum. Truby and Goldzhier (1958) (49) noted a difference in linewidth and signal intensity between lyophilized and frozen liver preparations and concluded that the free radicals seen in the freeze dried tissues were artifacts created during drying. Bliumenfeld et al (214) have made a summary of published ESR data showing that in general linewidth of the ESR spectra of lyophilized animal tissue is about 8G compared to 14G before drying. They noted also a large shift in g factor in many of the dried samples.

Malinovski and Kafalieva (215) in ESR studies of plant leaves reported that lyophilization destroyed the radicals observed in the fresh tissue and that a different radical appeared after continued

drying. The size of the ESR signal was also proportional to the length of drying at 0.01 torr. Thus they concluded that lyophilization produced free radicals; loss of water causes chemical bonds to break resulting sometimes in the formation of stable free radicals. Unfortunately, they did not consider the effect of oxygen.

The effect of oxygen on free radicals in lyophilized tissue was first reported by Miyagawa et al (216). They found that animal tissues, lyophilized and observed in vacuum, gave little or no ESR signal, but that after admission of air or oxygen a sizeable signal appeared. Removing the air or oxygen caused the spectra intensity to decrease considerably, to reappear again on the readmission of the gas. The spectra had a small asymmetry due to g - value anisotropy of the type expected for a unpaired electron on an oxygen molecule. Miyagawa et al thus concluded that the spectra appeared to be due to molecular oxygen weakly bonded to some site on the lyophilized sample. This effect of oxygen on lyophilized tissue has also been demonstrated by several other workers (217-222). Even traces of oxygen, such as are found in virtually all commercial nitrogen gases, can generate free radicals in lyophilized tissue and it is only when special oxygen scrubbed gases are used that such free radical formation can be prevented (223). Additionally, Morozova and Bliumenfeld in their ESR studies on rat liver and spleen have noted that if the tissues are lyophilized without preliminary freezing the oxygen effect was almost or completely absent (224). Moisture has also a demonstrable effect on free radical production

associated with lyophilization (225). Kalmanson et al (48) have shown that if evacuated lyophilized tissue is exposed to moist oxygen, the ESR signal increases by about five times and then subsequently decreases. Replacing oxygen by argon in the slightly moistened samples reduces free radical concentration by a factor of 2-3. In many instances (220,226) moisture affects only the rate of free radical production since with continued drying free radical production approaches the same concentration found in material at 15 and 30 percent relative humidity.

Other effects have also been observed. In lyophilized yeast, free radicals are present at g = 2.03 and g = 2.024 (227-229). In moist yeast, the absorption at g = 2.03 is considerably diminished but on drying this absorption is increased many fold. Studies with the cell free extracts (229) show that the g = 2.03 absorption can be obtained only if the enzyme preparation is incubated with glycogen. In the presence of glucose and ATP, resonance at g = 2.03 was detected. As a rule ESR signals from lyophilized tissue do not show hyperfine structure and so the free radicals involved are difficult to identify. Nevertheless several workers (45, 214, 219, 230-234) have concluded that the free radicals detected in dried biological material involve enzyme cofactors and or semiquinones in an oxidized state, because of the fact that redox enzyme systems (e.g. flavones) that have been isolated from mitochondrial fractions give ESR signals virtually identical to those derived from

lyophilized tissue. Kalmanson et al (48) while agreeing with the probable relation of ESR signals to enzyme cofactors and semiquinones found puzzlingly enough that boiling tissues for several hours before lyophilization did not remove the ESR signal. Chetverikov et al (225) found on the other hand that free radicals were not produced in lyophilized tissue in the absence of water (defined as less than 10 percent moisture). They concluded therefore that free radicals found in lyophilized tissue are not only unrepresentative of those present in living tissue but may not even be associated with "normal" oxidation-reduction systems. Chetverikov et al however, did not extend their observations for more than a few hours. Their conclusions may not be valid for longer periods of continued drying (220,226). There is abundant evidence (93, 221, 222, 234) that the free radicals detected in lyophilized tissue have some relationship to precursors in the fresh tissues observed under physiological conditions. Azhipa et al (93) for example have reported that when rats were asphyxiated the free radical content of lyophilized brain was reduced. Karitonenkov et al (232) have found that no ESR signal could be detected from lyophilized preparations of oxidized ethyl gallate or from mixtures of oxidized ethyl gallate and amino acids. However from a mixture of oxidized ethyl gallate and protein a large signal was detected. They concluded therefore that proteins in lyophilized mixtures act to stabilize free radicals. With respect to wet tissue, the free radicals originally present are not necessarily destroyed by the drying procedure although their concentration is usually so low that their contribution to the

ESR signal (from the lyophilized tissue) is negligible. Nevertheless if exposure to oxygen and moisture is rigidly controlled, the freeze drying - ESR technique can be used to determine true differences in the capacity of sample to interact with oxygen to produce free radicals. Such a capacity may be related to significant changes in the tissues.

B. The Cavity

The resonant cavity used in the x - band experiments is a rectangular box measuring 12 mm x 2.5 cm x 2.5 cm. The sides of the cavity are formed of gold plated brass and silver plated ceramic, the latter to permit transmission of the high frequency field modulation (100 kH_2). The high quality factors obtained, Q - 4000 for this type of empty cavity are indicative of a good sensitivity. The cavity resonates in the TE_{102} mode and the circulating r.f. fields are shown in the figure 8. The sample is inserted in the cavity at the position of maximum magnetic component of the r.f. field as shown in figure 9. As evident in figure 9, the sample location is rather important. For example, an aqueous or wet sample projecting into the electric field component will absorb much r.f. power independent of the externally applied magnetic field. The quality factor of the resonant cavity containing the sample is much reduced, so much so that in certain cases the fairly weak e.s.r. absorption is obscurred. This power loss observed for wet samples is due to the rapid but hindered reorientation of the electric dipoles of the water. The r.f. field intensity in the cavity is much reduced if the loss is great. Similarly, the magnetic component of the r.f.

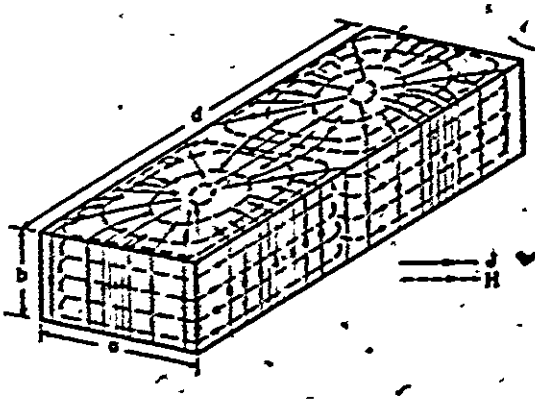


FIG.8. Current distribution J in a TE_{102} mode rectangular resonant cavity with dimensions a, b, and d.

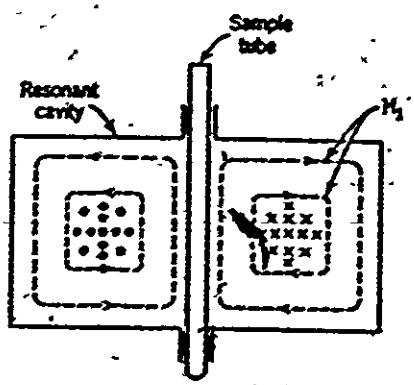


FIG. 9. ESR sample tube in rectangular TE_{102} resonant cavity.

field, H_1, which causes the e.s.r. transitions is reduced and a much smaller absorption is produced. The two factors reduced Q and reduced H_1, can severely alter the intensity of absorption of like samples placed in or out of the electric field in the resonant cavity.

Experimentally, precautions were taken to place all samples identically in the cavity. Moreso, the sample geometry and packing were maintained to close tolerances by using repeatedly the same procedures. For example, Table 9 , the variation of sample weight and machine factors are visible as variations in the e.s.r. spectra. The standard deviation in each of the e.s.r. parameters, peak to peak height, g value, line width about the mean for each sample, several aliquots being measured for more than one hundred samples, are shown in Table 10. Without analyzing in detail each of the experimental factors involved, the overall deviations are mainly due to sample packing since all other terms were experimentally observed to be relatively insignificant. Indeed, in spite of a very elaborated packing procedure, requiring freeze drying of all samples to the same degree, careful weighing techniques and tamping procedures, the signal variations about their mean could not be reduced further than those shown in the tables.

C. Nature and Origin of ESR spectra obtained from human blood

A typical spectra is shown in fig. 10. Relatively sharp lines are seen at g = 2 and g = 4 while a number of broad lines are seen over the entire spectrum. At liquid nitrogen temperature, the lines in the g = 4 region showed no change when the microwave power incident

Table 9

Typical variations of sample weight and peak to peak amplitude of sample - signal.

Sample (Case) No.	Sample Weight	Peak to Peak height of signal (in gauss)	Differences between peak to peak heights of same aliquots	Square of difference from mean	
138A 138B	173.9 167.7	4.0 3.6	0.7	.01	
139A 139B	175.6 171.2	5.3 4.8	0.5	.04	Standard Deviation of signal height is approximately 0.19
140A 140B	155.7 163.2	2.3 3.0	0.7	.01	
141A 141A	190.3 189.3	3.6 3.1	0.5	.09	
142A 142B	60.8 51.0	1.9 0.8	1.1	.09	
144A 144B	281 275	9.5 8.6	0.9	.01	
147A 147B	296.7 278.2	8.8 8.0	0.8	0	
148A 148B	173.1 188.2	4.9 6.1	1.2	.16	

Table 10

Typical variations of line width and g value of ESR spectra of samples

Sample (Case) No.	Δ g	Difference in Δ g of aliquots	Line width	Difference in line width of aliquots	
138A 138B	0.0019 0.0018	0.0001	9.5 9.4	0.1	Standard deviation of Δ g is approximately 0.0001
139A 139B	0.0017 0.0016	0.0001	9.8 9.7	0.1	Standard deviation of line width is approximately 0.1
140A 140B	0.0018 0.0020	0.0002	9.5 9.6	0.1	
141A 141B	0.0020 0.0017	0.0003	9.6 9.5	0.1	
142A 142B	0.0019 0.0017	0.0002	9.0 9.2	0.2	
143A 143B	0.0016 0.0017	0.0001	9.6 9.7	0.1	
144A 144B	0.0021 0.0019	0.0002	9.0 9.1	0.1	
145A 145B	0.0019 0.0017	0.0002	8.7 8.8	0.1	

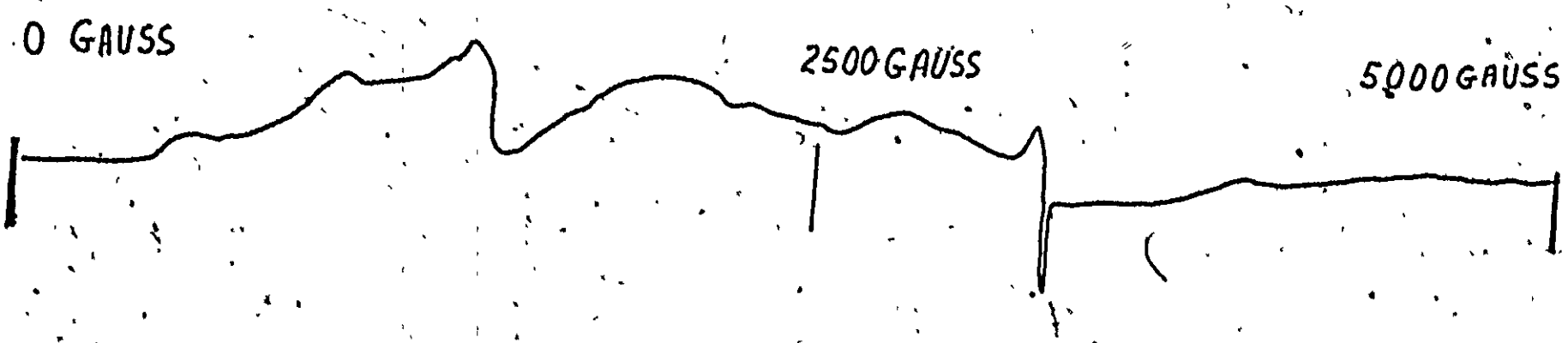


FIG. 10. TYPICAL ESR SPECTRUM OF LYOPHILIZED WHOLE HUMAN BLOOD.

RANGE: 5000 GAUSS
SCAN TIME: 50 MINUTES.
TIME CONSTANT: 1 SEC
MICROWAVE POWER 20 MW.
MODULATION AMPLITUDE: 2 GAUSS
TEMPERATURE: 300°K.

on the sample was changed from approximately 0.1 mW to 60 mW. The g = 2 lines however showed an increase in "structure". Fig 11 shows this effect. Free radicals have longer relaxation times than transition metal ions and so are more easily saturated. It was concluded therefore that the g = 4 ESR lines were probably from transition metal ions and the g = 2 lines from free radicals. Separation of whole blood into plasma platelet and red blood cell fractions show that the g = 4 lines arise primarily from the red blood cell and platelet fractions. All three fractions however, contribute to the g = 2 signal.

As noted in the section entitled METHODS plasma was also separated by ultracentrifugation into 3 fractions (1) a filtered fraction containing molecules over 100,000 in molecular weight (2) a fraction containing molecules from 100,000 to 30,000 and (3) a filtered fraction containing molecules from 30,000 to 0.ESR results from the lyophilized preparations of these fractions showed virtually no qualitative difference. However, the g = 2 signal relative intensities were roughly 10:4:1 respectively. Since only 2 sets of samples were examined in this way, it may be dangerous to place too much emphasis on the accuracy of the difference of the intensities. However the relative order is probably correct. The variation in packing could certainly not account entirely for the intensity differences. The g = 4 lines from each of the fractions were quite weak and no differences in signal intensities

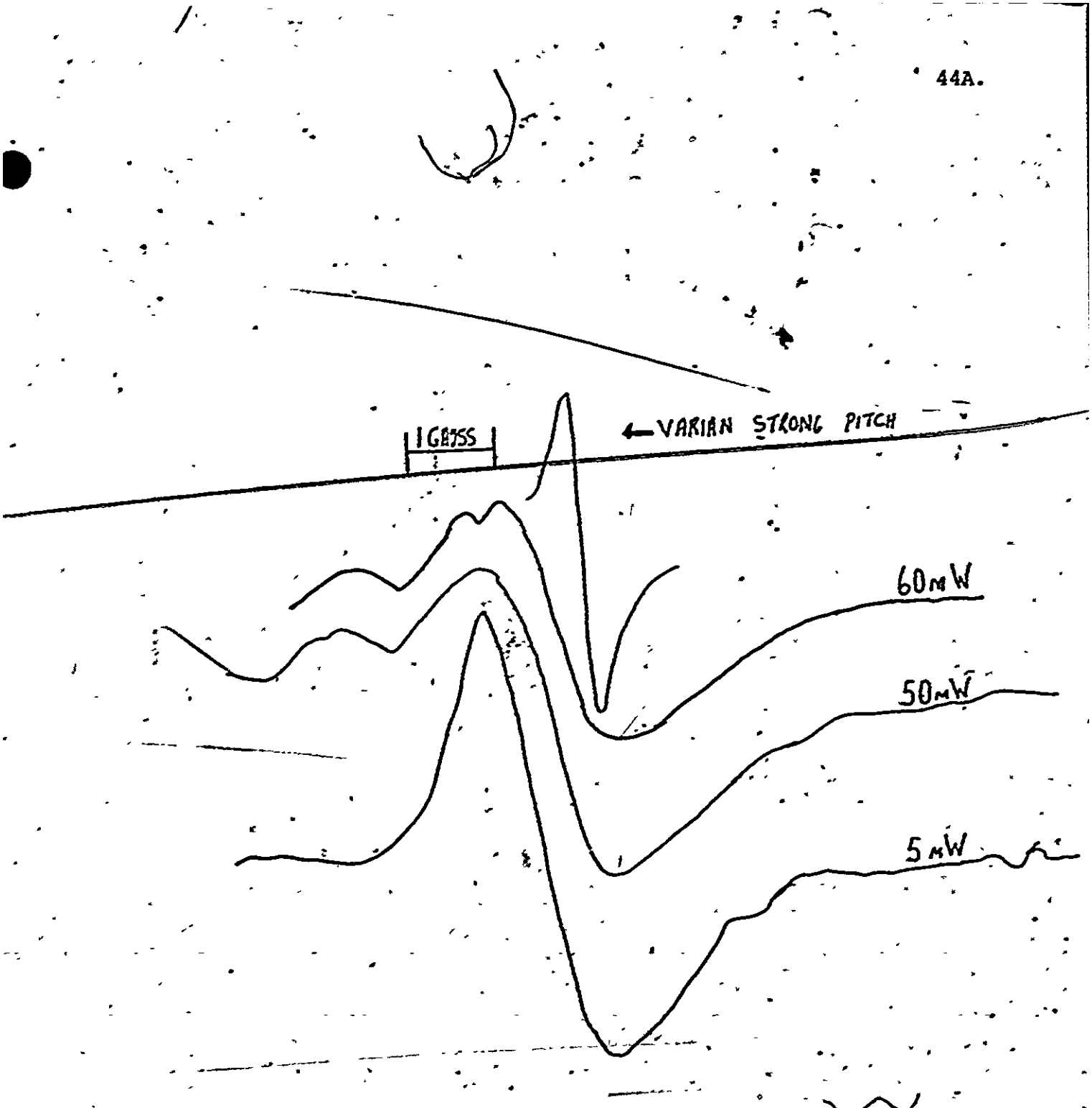


FIG. 11. ESR spectrum of the g=2 signal in lyophilized whole human blood. The effect of increasing microwave power incident on these "blood" samples is seen.

were evident. In quick frozen blood examined at liquid nitrogen temperature the g = 4 lines were very broad and only a weak resonance at g = 2 was observed (see fig 12). Quick frozen plasma also gives a weak resonance at g=2 and a typical spectrum is seen in fig. 13.

In the present study, the observations on lyophilized whole blood and fractions thereof are consistent with the hypothesis that the ESR lines in the g = 4 region are due primarily to iron in different spin states. In a variety of biological compounds (235-238) iron (III) in the high spin state gives resonances at g = 4.2 - 4.3. During the lyophilization of whole blood there occurs breaking of the porphyrin ring structure in hemoglobin of erythrocytes and heme proteins (239) and the subsequent oxidation of the associated iron which could rise to such iron (III) compounds. Resonances at g = 6 have been associated with iron (II) (240) and may contribute to the spectra of lyophilized whole blood.

The signal at g = 2 arises from a multitude of different free radicals. Transition metal ions are also probably involved. The present studies show that lyophilized plasma, platelet and red blood cell fractions each give very strong signals at g = 2.

In lyophilized plasma as in lyophilized whole blood the signals in the region of g = 2 probably arise mostly from semiquinone radicals formed by oxidation. It is believed that lyophilization facilitates this oxidation. These semiquinones can form complexes with oxidized (and reduced) forms of certain proteins (241-247). Unattached semiquinone radicals give multiline spectra in solutions but when they are

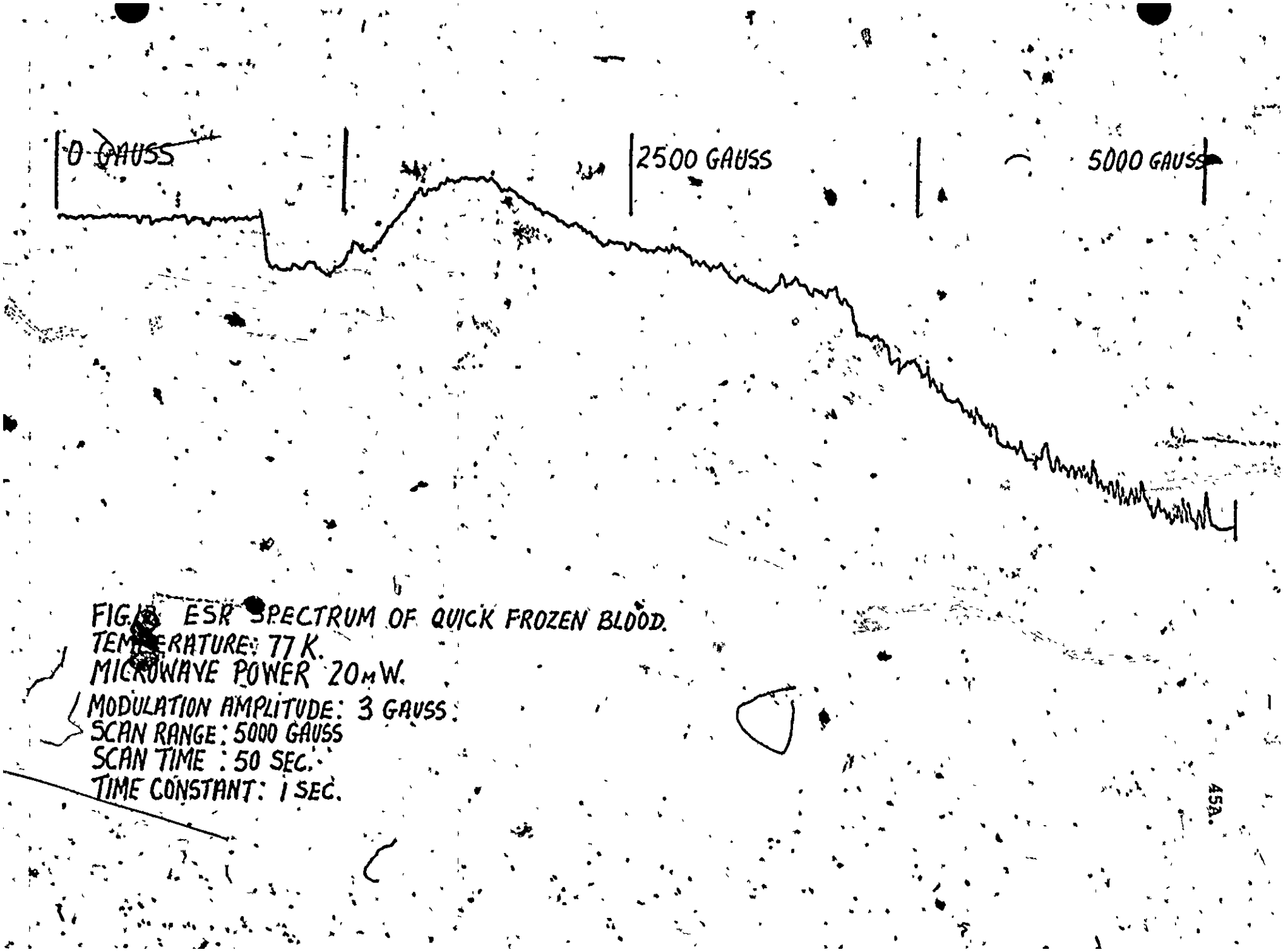


FIG. 8 ESR SPECTRUM OF QUICK FROZEN BLOOD.
TEMPERATURE: 77 K.
MICROWAVE POWER 20 MW.
MODULATION AMPLITUDE: 3 GAUSS.
SCAN RANGE: 5000 GAUSS
SCAN TIME : 50 SEC.
TIME CONSTANT: 1 SEC.

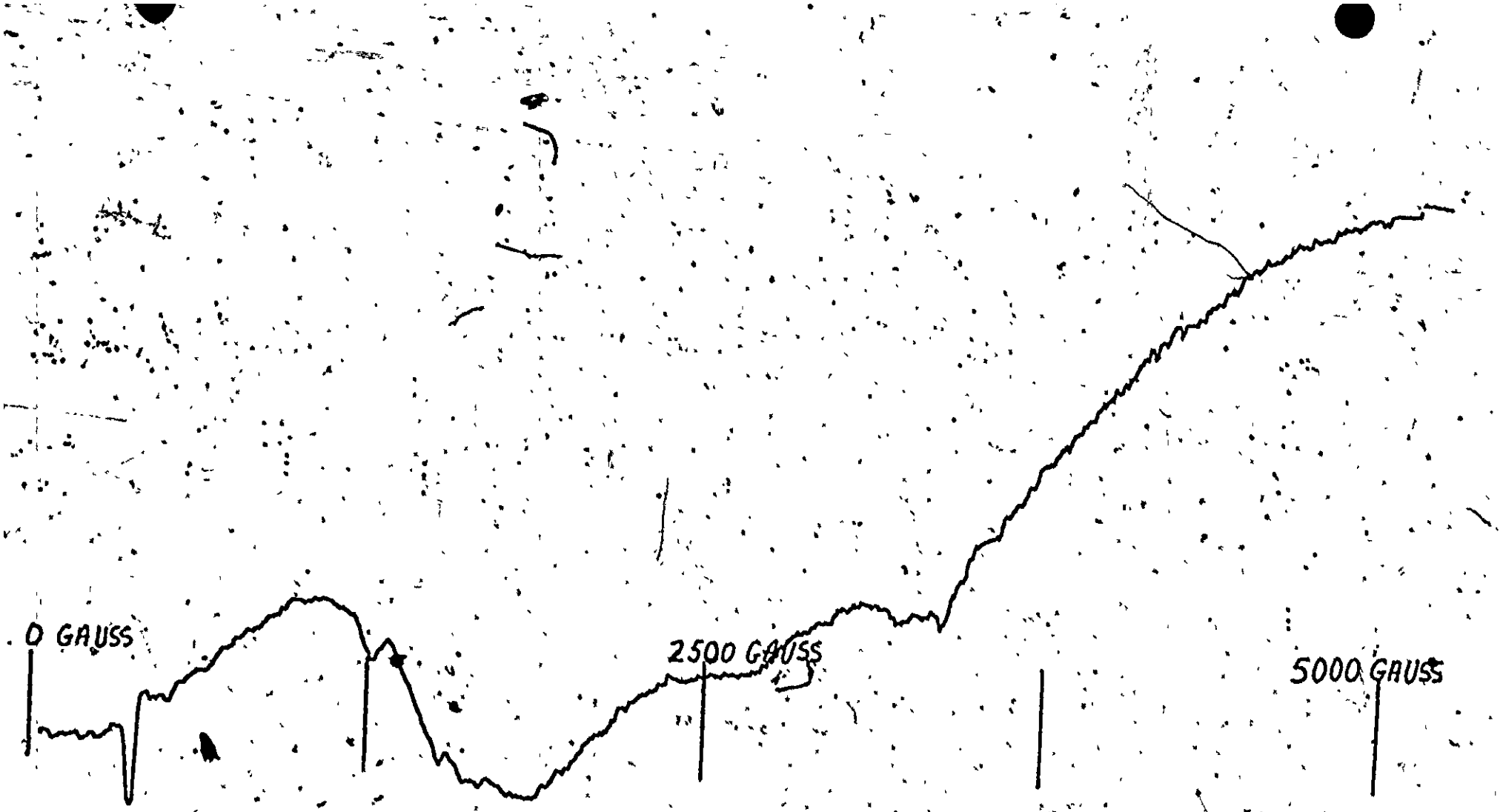
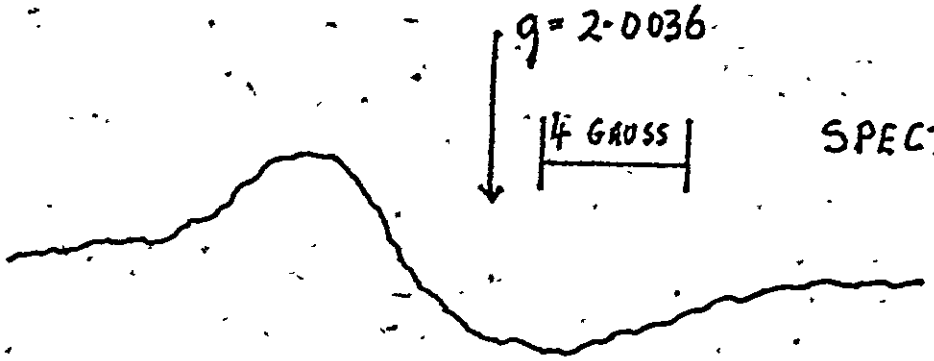


FIG. 13. ESR SPECTRUM OF QUICK FROZEN PLASMA.
TEMP: 77 K
MICROWAVE POWER: 20 m W.
MODULATION AMPLITUDE: 3 GAUSS.
SCAN RANGE: 5000 GAUSS
SCAN TIME : 500 SEC.
TIME CONSTANT: 1 SEC.

bound to proteins or are in non single crystal solid state a "broad" powder spectrum results. This powder spectrum is similar in g value, line width and line shape to the signals obtained from lyophilized plasma at g = 2 (See Fig 14). Rudge and Blumfeld (248) found that ESR spectra from a lyophilized mixture of L-ascorbic acid (Vitamin C) and albumin, on exposure to oxygen are qualitatively similar to that from lyophilized rat spleen. Nor were ESR signals obtained when ascorbic acid or albumin were lyophilized separately and exposed to oxygen. Heckley (249) has confirmed the above findings. He observed that the ESR spectra from any of a number of different proteins or DNA combined with ascorbic acid and lyophilized yielded spectra that are identical in line shape, linewidth and g factor. These spectra are similar to those obtained by us at g = 2 in lyophilized plasma. Heckley, concluded that ascorbic acid or semiquinone substances like it may be involved in spontaneously producing free radicals (probably oxidation) in animal tissue.

Ascorbic acid is present in human plasma and is easily oxidized to its semiquinone free radical form. Other semiquinones that are normally present in human plasma and so could contribute to the g = 2 signal seen in the lyophilized plasma are vitamins, B, E, K and Q. These vitamins are involved in many biological reactions some of which occur in sera (250). They function as cofactors of enzymes (to which they are often attached) and participate in reactions via their free radical forms (196, 239, 240). The chief contribution of the g = 2 signal in lyophilized plasma probably arises from these oxidized semiquinones.



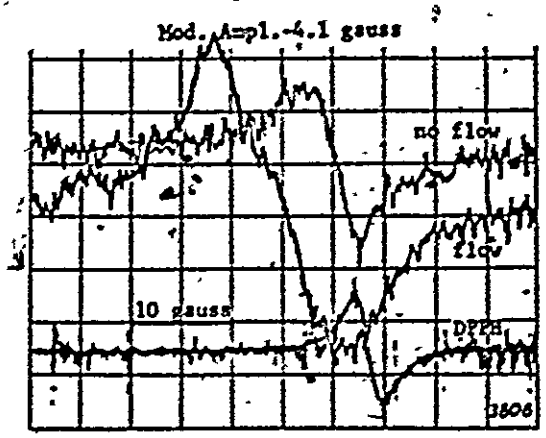


FIG. 14. Spectrum A is from lyophilized plasma. THe signals from lyophilized whole blood and from lyophilized red blood cells are similar in linewidth, lineshape and g-value.
Spectra B are of free radicals from triiodothyronine oxidized by $KMnO_4$ in akaline solution. These spectra were obtained with continuos flow apparatus in conjuction with a dual cavity containing a reference DPPH sample in one half. Magnetic field increases left to right, so singlet spectrum recorded from the transient radical present during rapid flow has a higher g-factor (g=2.0186) than the metastable radical persisting after flow has stopped(g=2.0059). (See ref.239).

Recently (251, 252) the superoxide onion radical O_2^- has been identified as a species occuring in semiquinone - substrate - oxygen systems. This radical has resonances in the g = 2.08 to 1.95 region and may make small contributions to the g = 2 absorbtion seen in quick frozen plasma and lyophilized plasma. ESR spectra of the superoxide onion radical is shown in Fig 15.

A multitude of other substances that could give rise to free radicals are present in human plasma and may make minimal contributions to g = 2 signal observed in this study. Cathecholamines such as epinephrine, norepinephrine, and dopamine are normally present in human plasma and do undergo oxidation to form semiquinone free radicals (253-258). They therefore, may contribute to g = 2 ESR signal in lyophilized plasma. Other hormones that are in human plasma and do give rise to ESR signals after oxidation are the thyroid hormones thyroxine and triodothronine (259-262), the estrogens (263-266), serotonin (259) and insulin (267-268). These ESR signals are also at g = 2. Other examples of substances that may contribute to g = 2 ESR signal observed in lyophilized human plasma are:

(a) ceruloplasmin (269-274). This is a protein containing between 6-8 copperatoms per molecule (275-278). It constitutes the main copper component of human serum (279). Each molecule contains two types of Cu^{2+} (280-281). Ceruloplasmin functions as an oxido-reduction enzyme linking ferrous iron with oxygen (282,283). In some types of disease its concentration in

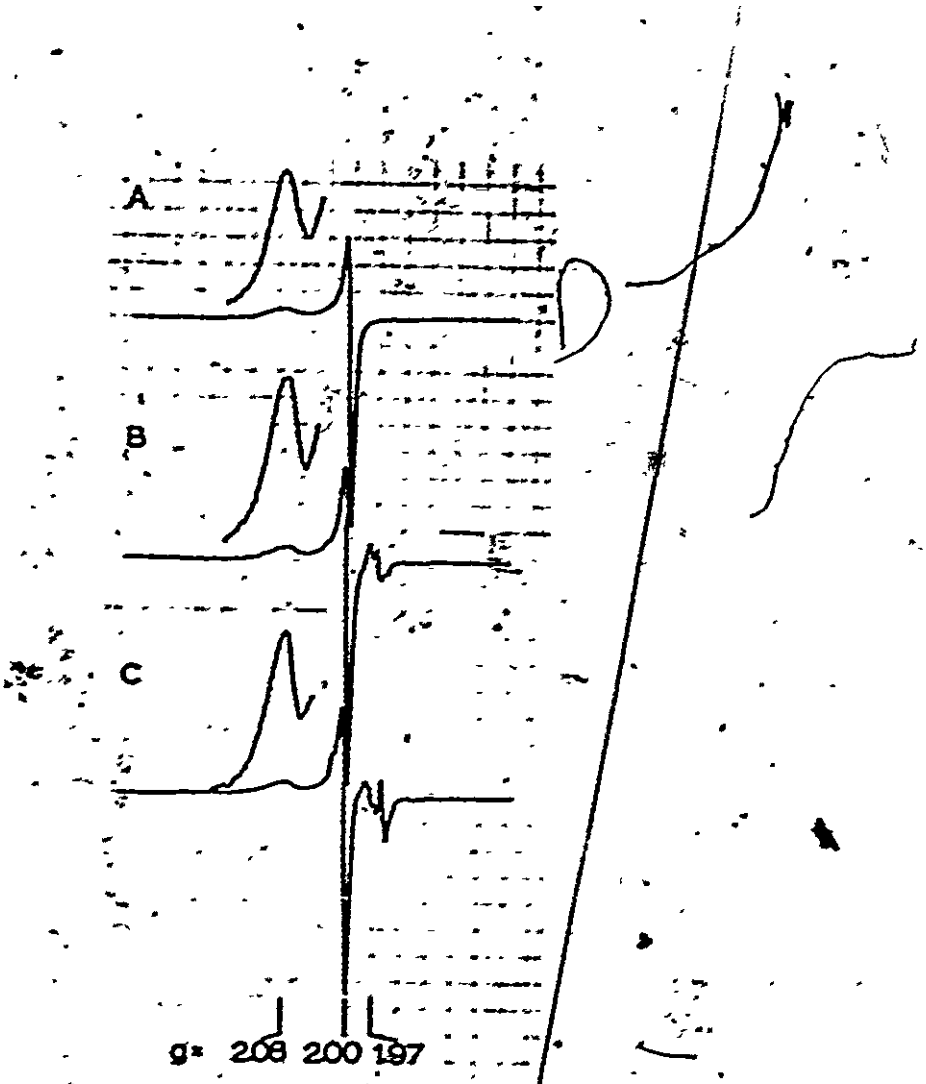


FIG. 15. ESR Spectra of superoxide anion radical, $O_2^{\cdot-}$ produced in different ways; according to Orme-Johnson and Beinert(252). A--C, ESR spectra observed 150 msec after mixing various reducing mixtures with an equal volume of oxygenated buffer. A--0.31 mM sodium dithionite in 0.01 M potassium phosphate of pH 7.4 was mixed with 1 M glycine buffer of pH 10.5. B--0.053 mM xanthine oxidase in 0.01 M pyrophosphate of pH 8.5 was reduced anaerobically with sodium dithionite, corresponding to 14 eq per mole of enzyme, and mixed and frozen as in A. C--0.025mM xanthine oxidase, dissolved as in B, was mixed with oxygenated glycine buffer as in A, which contained 2 mM xanthine. The conditions of ESR spectroscopy were microwave power, 45 mwatt; modulation amplitude 2 G, and temperature 102 K. The enlarged low-field portion at g=2.08 was recorded at an amplification fourfold higher than that used for the complete spectra and at 6 G modulation amplitude. The relative amplifications used for recording spectra A to C were 1,2,1.25. At g=1.97 signals of Molybdenum(5) are seen with xanthine oxidase. Under the experimental conditions used the flavin and molybdenum signals are partly saturated. The signal at g=2.00 in B and C may have a small contribution from the flavin semiquinone. All ESR spectra shown in this figure represent the first derivative of the absorption line versus magnetic field, with the field increasing from left to right.

human serum is markedly reduced (284). In cancer and a variety of infectious diseases however, there is evidence that the ceruloplasmin level in blood rises (100). Resonance at g = 2 has been observed with ceruloplasmin (285, 286).

(b) Transferrin. This serum protein is in vivo an iron containing protein which requires the presence of equimolar amounts of bicarbonate ions for the strong binding of iron (287,288). The apoprotein however can bind several other metal ions (289). At physiological pHs it has resonances in the g = 2.04 - 2.05 range (287).

(c) Antibody to carcinoembryonic antigen. As was indicated before recent work has shown that in humans a group of glycoprotein substances loosely referred to as carcinoembryonic antigen (CEA) increases in plasma when cancer occurs (106). Our studies show that this "antigen" when lyophilized or quick frozen does not give an ESR signal. However the lyophilized antibody to the carcinoembryonic antigen gives an ESR signal in the g = 2-3 region (See Fig 16). This antibody was derived from the rabbit and its presence in humans has not been specifically demonstrated, although an antibody that similarly binds CEA has also been found in humans (290).

(d) Drugs. ESR studies on such commonly used drugs as phenothiazine (291-299), chlorpromazine (300-304) and the salicylates

FIG. 16. ESR signal of lyophilized antibody to carcinoembryonic antigen.

(305, 306) have led to the hypothesis that free radical formation may be relevant to drug action (302, 307). The resonances resulting from these compounds can thus contribute to the lines seen in lyophilized whole blood. On the other hand studies on nitrates and nitrites (which may be consumed in drinking water for example) indicate that such drugs are not likely to give signals in whole blood (308) although signals may result in such tissues as liver (133).

Red blood cells give signals also. This signal may result from iron in an oxidized state. Hemoglobin in red cells contain iron in its porphyrin moiety and an ESR signal at g = 1.94 has been attributed to iron in a whole range of biological material (309-313).

Platelets contain large amounts of proteins (314) and semiquinones such as epinephrine and norepinephrine (315). These substances as explained before can give rise to the ESR signal observed at g = 2.

The signals from lyophilized red blood cell platelet and plasma fractions as well as those from lyophilized whole blood itself were observed to diminish with time. Periodic measurements of the samples however, showed that no changes in line shape occurred. In summary then, one may say that whole blood contains a vast array of substances which are capable of giving rise to the ESR signals observed. The amount of many of these substances in the blood stream may be related to dietary intake.

D. STATISTICAL ANALYSIS OF RESULTS

It was immediately evident in these studies that the scatter in experimental results was large. Spectra obtained from different samples from the same donor were nearly identical, but when obtained from different patients, the spectral widths and intensities showed little evident correlation with the disease stages. In all cases the line-shape as shown in Figure 14(A) was essentially invariant. A statistical analysis was deemed necessary. The results of a stepwise regression analysis is shown in Table 8. Among the different classifications of disease progress, normal, mild, moderate and severe dysplasia, in situ and invasine carcinoma, the observed parameters in general show little significance. The linewidth is the statistically most important variable and it readily separates the group having invasine carcinoma from the others. Unfortunately this is also the group for which there was no control, the mean age of this group being 57 years whereas the other groups and their control ranged in age from 33 to 35 years. The partial F-test to remove or enter the linewidth as a variable was always more than 2.2 even when the group having invasine carcinoma was eliminated from the analysis.

Figure 17 shows histograms of the groups as a function of the signal linewidth. It is easily seen that the signals obtained for the invasive carcinoma group have a much narrower linewidth and that this distinction is significant. Figure 18 shows the mean linewidth for each of the groups. The general trend towards a decreasing linewidth is notable. It is important to note again that the other spectral parameters, including

FIG. 17A

Histogram of linewidths from 'invasive' samples and that from 'normal' samples (heavy solid line).

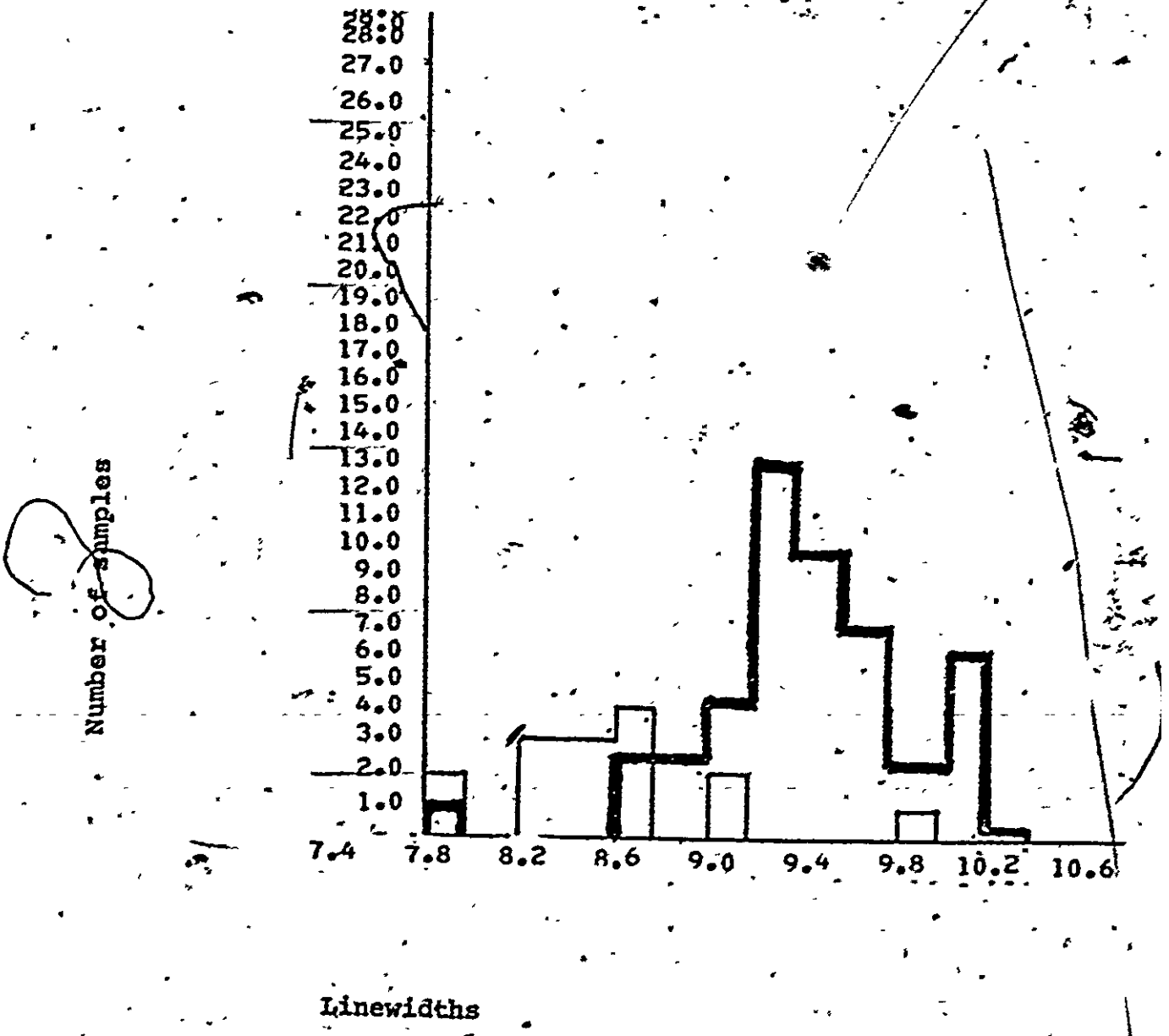


FIG. 17B

Histogram of linewidths from 'in situ' samples and that from 'normal' samples (heavy solid line).

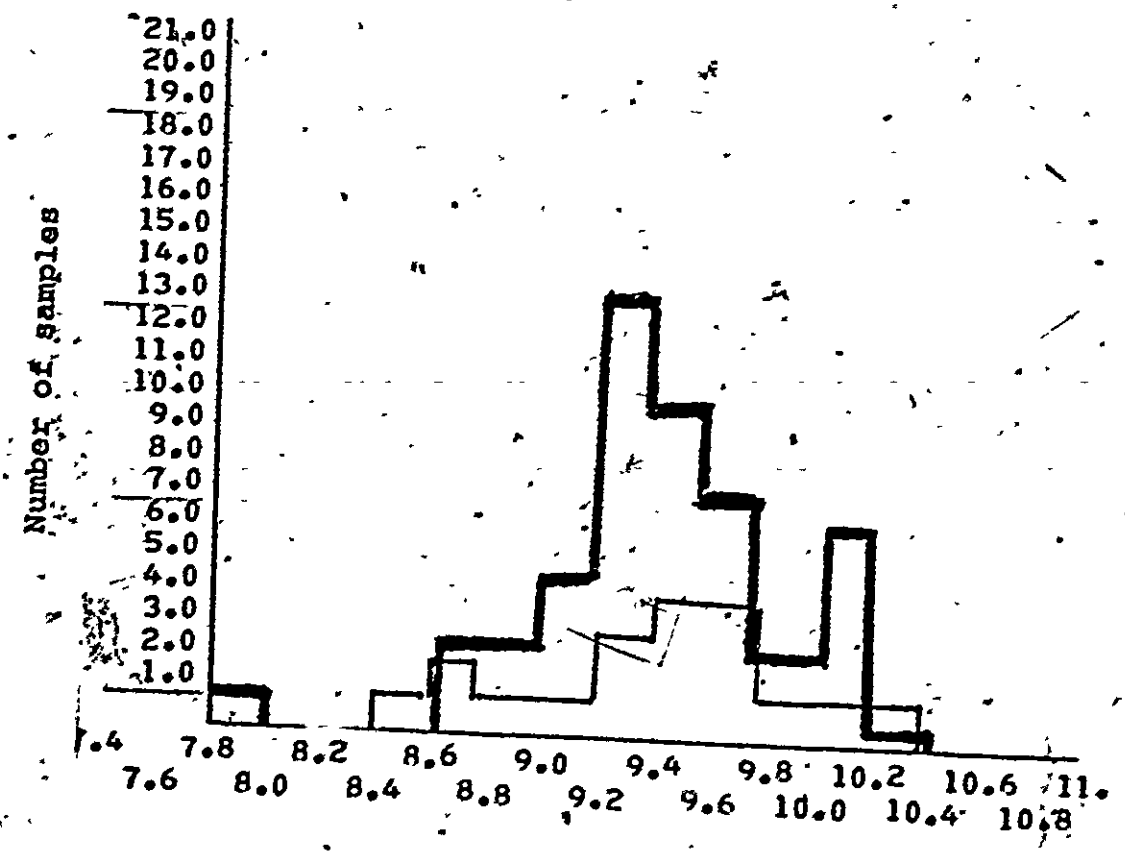


FIG 17C

Histogram of 'severe dysplasia' sample linewidths and that of normal samples (heavy solid line).

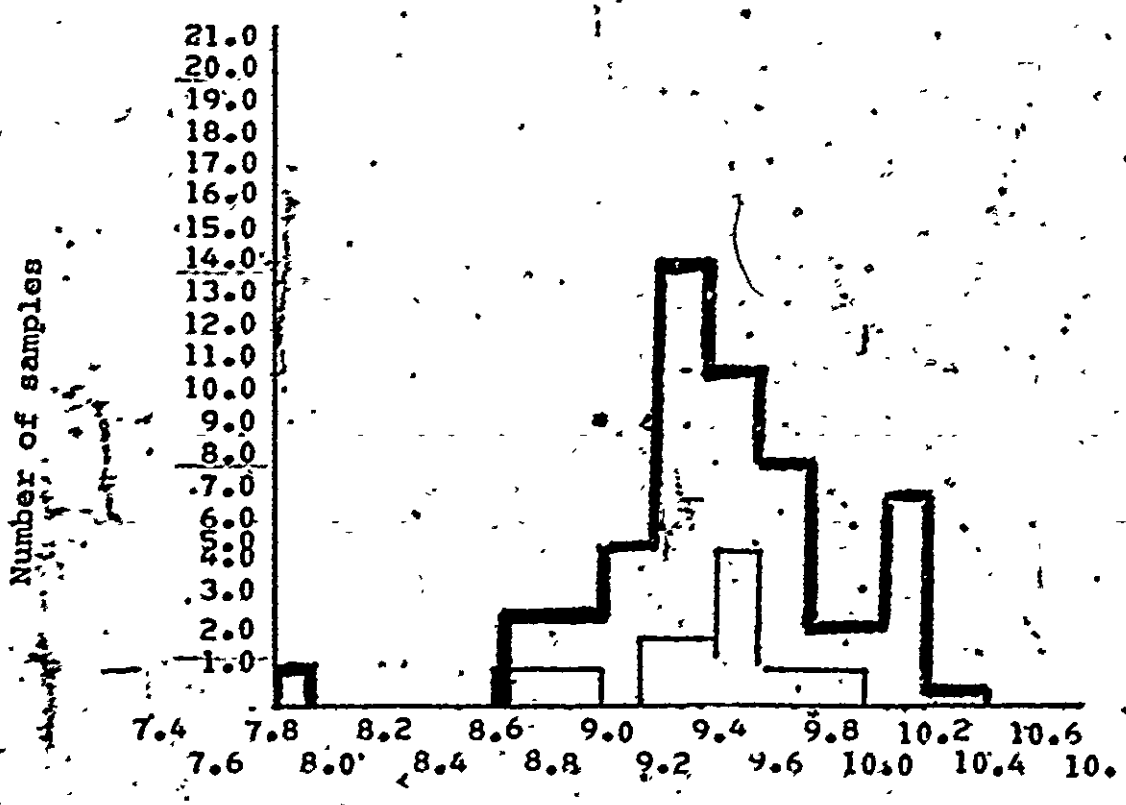


FIG. 17d

Histogram of linewidths from 'moderate dysplasia' and that from normal samples (heavy solid line).

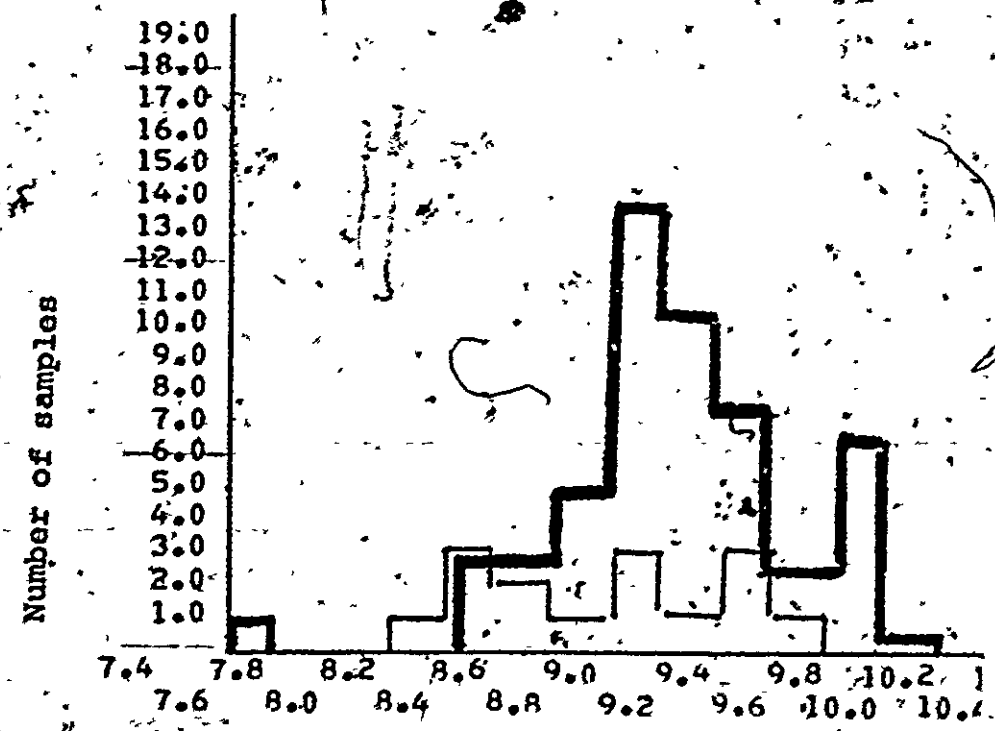


FIG. 17e

Histogram of linewidths from 'mild dysplasia' and that from normal samples (heavy solid line).

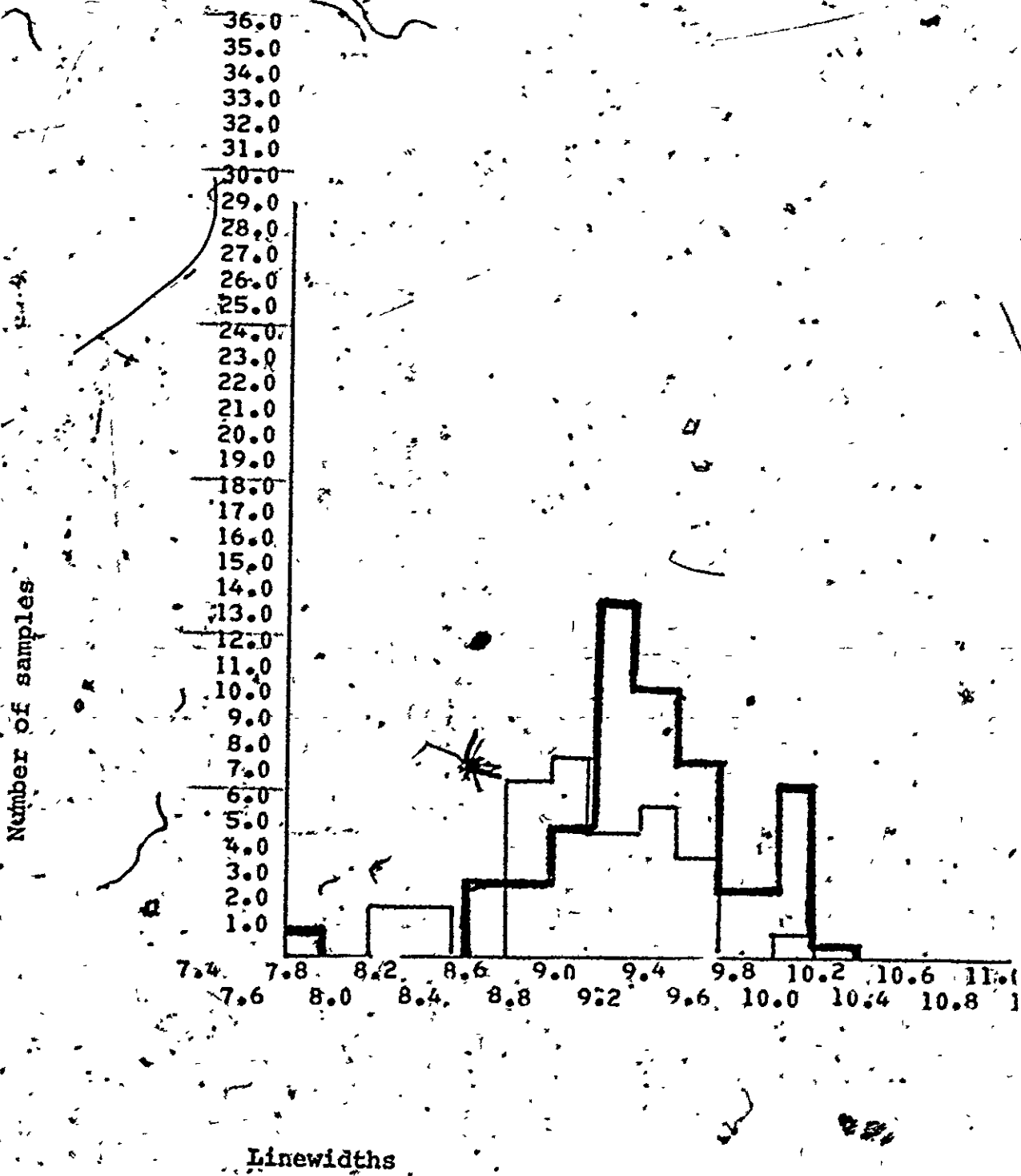


FIG. 17f

Histogram of linewidths from all samples less invasive samples and that from normal samples (heavy solid line).

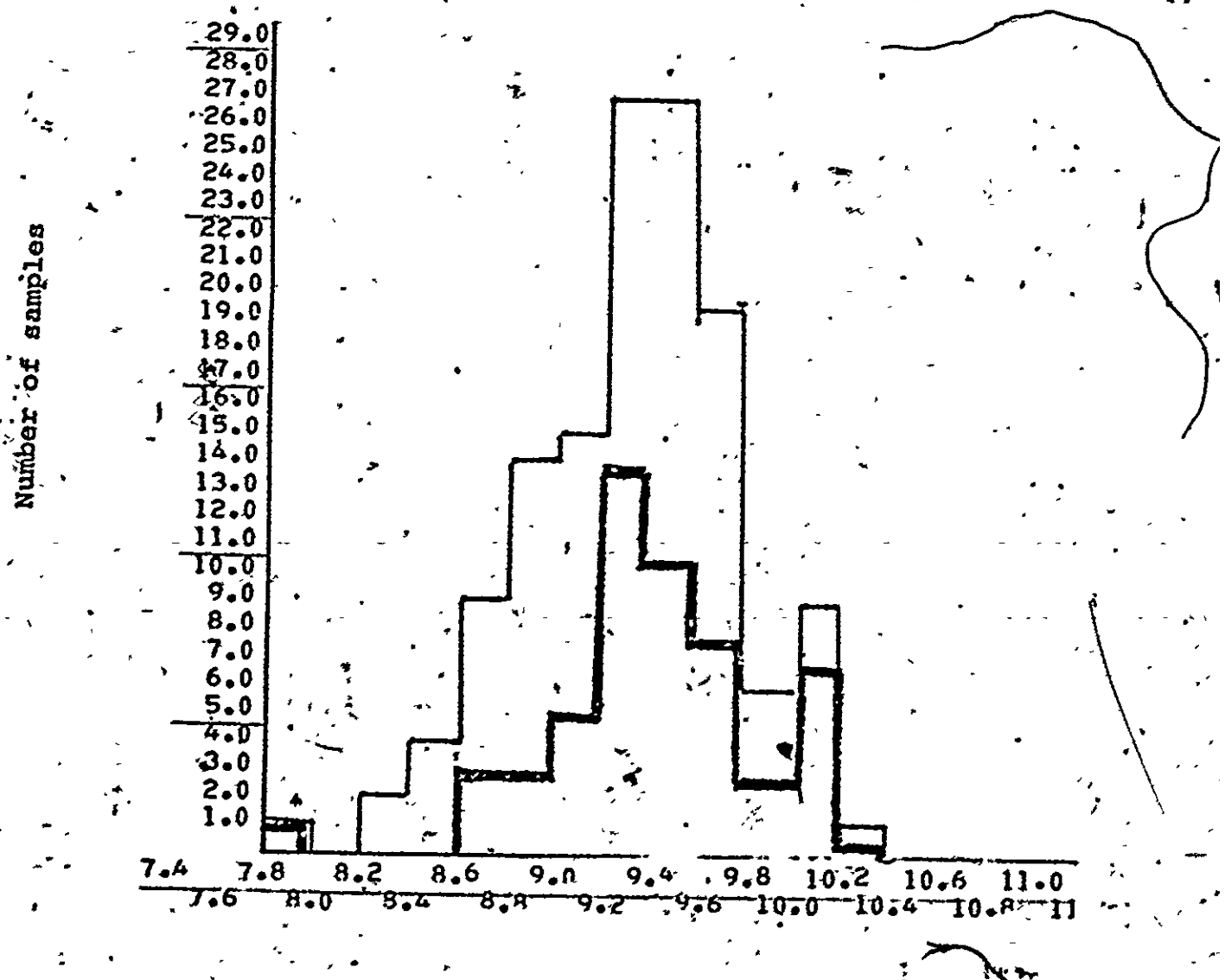
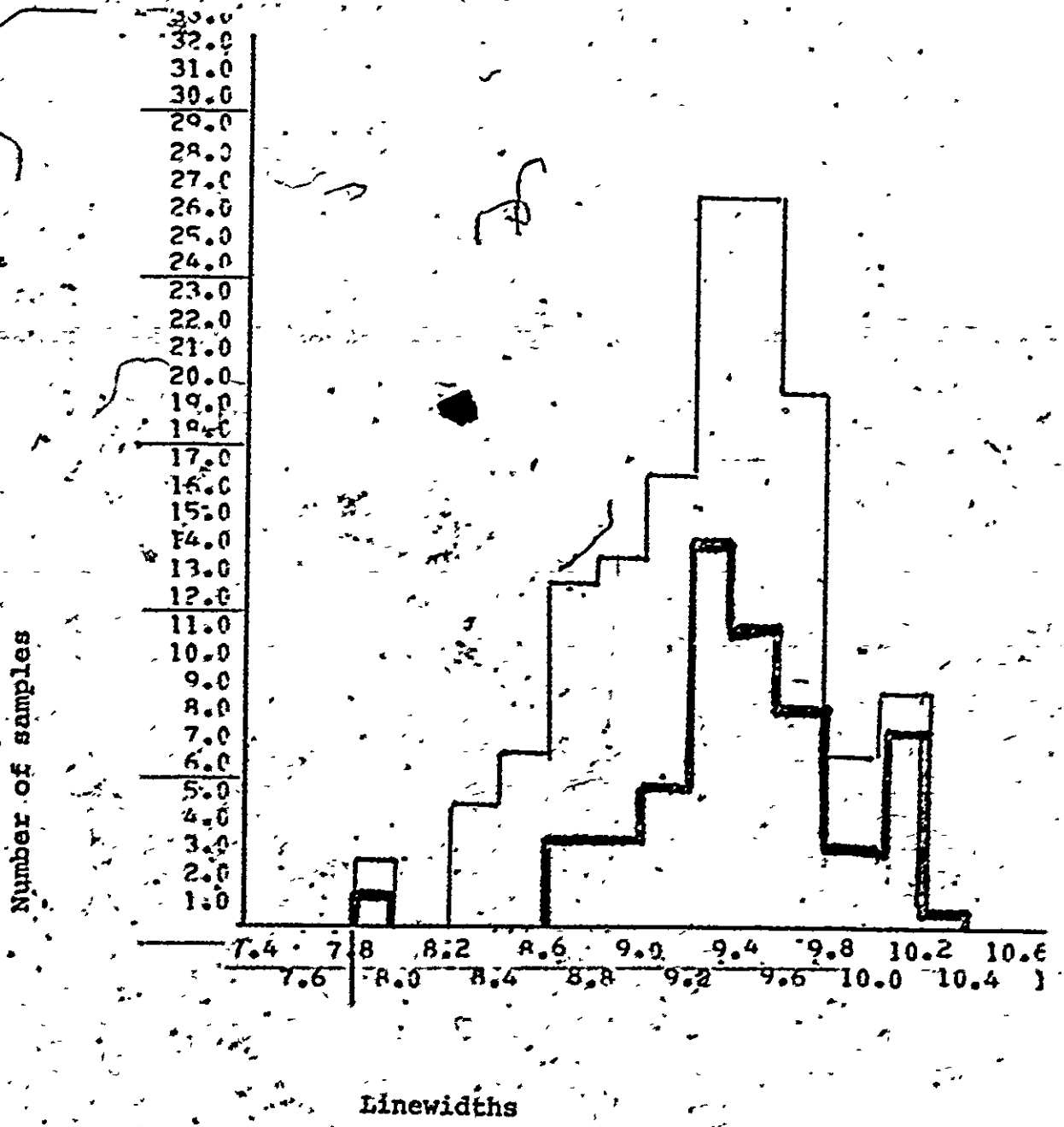


FIG. 17g

Histogram of linewidths from all samples and that from normal samples only (heavy solid line).



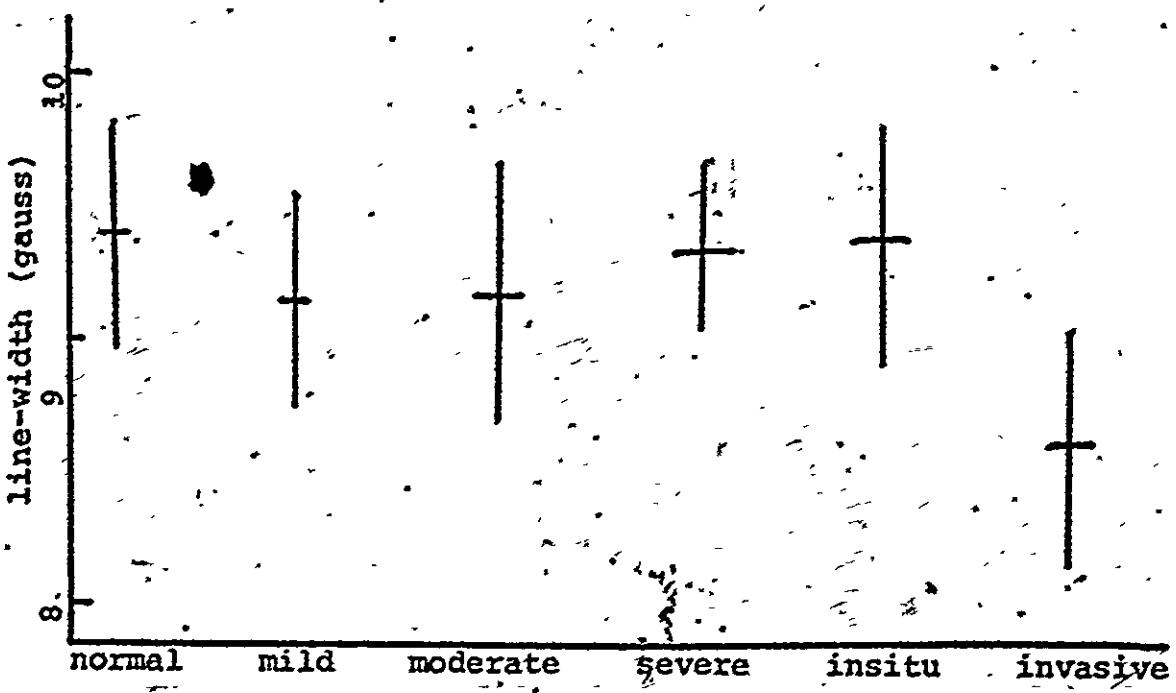


FIG. 18. Plot of mean value of linewidth versus group. Vertical lines show extent of standard deviation.

the concentration of free radicals, did not appear to be statistically important.

Although the radicals giving rise to the e.s.r. signals have not been identified, it may be conjectured that they are semiquinone radicals As pointed out before Heckley and others have observed spectra of ascorbic acid and protein and they noted that the spectra obtained were not unlike those of the semiquinone radical. The spectra obtained are not unlike those of the semiquinone radical. Since semiquinone containing substances such as drugs, vitamins, etc., are a routine part of many diets, it is expected that their concentration in the blood should be a function of ingestion and general physical health of the patient. It is thus not surprising that the signal intensity was the least significant variable. On the other hand, a variation in signal linewidth implies that the dynamic behaviour or more likely the essential character of the radicals is a variable. Finally, it is interesting to note that examination of neoplastic breast tissue shows that the mean linewidth of malignant tissue is narrower than that of benign tissue which is in turn narrower than that of normal breast tissue (7/). The question then of the changing nature of the radicals in blood, whether they be semiquinone or otherwise is fundamental to further study.

In conclusion it should be emphasized that the results indicate that the ESR technique applied to the analysis of the g = 2 signal of peripheral blood is not suitable.

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CLAIMS TO ORIGINAL RESEARCH

The material presented in this thesis is original with respect to the following points.

(1) A critical review of the literature from 1960 onwards of ESR studies of organic single crystals is presented and published spectra are assigned to new previously unidentified radicals.

(2) ESR studies of γ-irradiated nitrogen containing organic single crystal have shown spectra. The orientation pattern of these spectra permit the radicals to be identified and characterized. They are: $\dot{C}H_2NCONH_2$ observed in 2-imidazolidinone, $\dot{C}H_2NCONH_2$ observed in 2-imidazolidinethione, $H\dot{C}CONHCOCH_2$ observed in succinimide, and N-halosuccinimide.

(3) ESR studies of lyophilized whole blood obtained from 183 patients showing invasive carcinoma, carcinoma insitu, and varying degrees of cervical dysplasia of the cervix, uteri and from normal persons, have shown that the character of the radicals in blood apparently changes as the disease progresses. However the number of radicals do not appear to vary in a statistically significant fashion. This is the first significant statical analysis applied to such a study and it has shown that the diagnostic utility is much more limited than has been previously supposed.

An additional study of some of the components of blood, show that carcinoembryonic antigen, an antigen associated with several types of cancer including cervical cancer, and found in the blood, does not give a signal. However, the antibody to this antigen gives a signal at $q = 2.3$.