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MICROWAVE SPECTROSCOPY OF BIOLOGICAL SUBSTANCES. I PARAMAGNETIC RESONANCE IN X-IRRADIATED AMINO ACIDS AND PROTEINS*

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The resonance of unpaired electrons in externally imposed magnetic fields of a few kilogauss gives rise to absorption frequencies in the microwave region analogous to nuclear magnetic resonance signals now widely observed in the lower-frequency radio region. Such microwave or radio signals can give much information about the immediate surroundings of the particle at resonance.[1] Unpaired electrons in biological materials exist normally in only very minute quantities on metallic ions or in organic free radicals. Nevertheless, Commoner, Townsend, and Pake[2] have been able to detect the resonance of organic free radicals in lyophilized biological substances, and we at Duke University[3] have been able to observe resonances of Mn^{++} and Cu^{+} ions and of natural free radicals in unlyophilized living plants. In the present work, X-irradiation is used to produce the unpaired electrons which are observed. This method appears to be applicable to almost any biological material. One can use it to study radiation damage to tissues as well as to obtain structural information. The results which are described here represent only the initial phases of the program of study of these and other biologically significant substances.

Several amino acids have been examined. Some typical resonance curves are shown in Figure 1. These represent essentially first derivatives of the absorption curves. They were obtained with the usual automatic recording spectrometer,[1] employing bolometer detection with low-frequency magnetic modulation of the resonance. Fifty-kilovolt X-rays were used for the irradiation. All measurements were made at room temperature.

In all the biological substances examined, a g factor very close to that for the free electron spin was obtained. This indicates almost complete quenching of the orbital momentum, as would be expected if the unpaired electron is in a molecular orbital. In practically all the samples a complex structure was observed. All samples were examined both at 9 kmc. and at 23 kmc. This allows one to ascertain whether the structure arises from nuclear interactions or from internal crystalline field splitting, since the spacings of the latter but not those of the former are sensitive to the strength of the externally imposed field. Also, the nuclear hyperfine structure can often be recognized by its symmetrical pattern.

In the strong fields employed in our experiments the nuclear-spin and electron-spin vectors precess separately about the applied field, and only the components of their magnetic moments which lie along this field experience an uncanceled interaction. Except for s orbitals, the splitting of the spin resonance by a nucleus of spin I and magnetic moment μ_I is given by

$$H = \frac{M_I \mu_I \beta_I}{I} \left\langle \frac{1}{r^3} \right\rangle_{\text{Av.}} (3 \cos^2 \theta - 1)_{\text{Av.}}, \quad (1)$$

in which M_I is the magnetic quantum number of the nucleus and β_I is the nuclear magneton. The co-ordinate r represents the distance of the electron from the nucleus, and θ is the angle between r and the direction of the applied field. For s electrons the above quantity averages to zero, but there is an additional orientation-independent term which arises from the nonvanishing of the s wave function at the nucleus. The magnitude of the coupling in the s orbital is directly proportional to the density $(\psi_s\psi_s^*)_0$ at the nucleus. If, therefore, the odd electron is in an s orbital when near the coupling nucleus or nuclei, the resulting structure of the resonance will be symmetrical and will not vary with orientation of the sample in the applied magnetic field. When the odd electron is in a p or a d orbital, the nuclear splitting will be orientation-dependent; and in a powder, a polycrystalline material, or a liquid the hyperfine structure will generally be smeared out.

All the nuclear interactions which we observed in the amino acids can be identified as arising from interactions with hydrogen nuclei. If the odd electron were to remain in $1s$ orbitals of hydrogen all the time, a total hyperfine spread of 500 gauss would be observed.[4] The number of lines of the structure increases with the number of coupling H nuclei. A single coupling H nucleus gives a doublet; two equally coupling ones give a triplet; n equally coupling H nuclei give a symmetrical pattern of $n + 1$ lines with a Gaussian distribution of intensities. Thus the observation of a large number of such components indicates a spreading of the wave function of the odd electron over a large number of H atoms.

The structural patterns shown in Figures 1–3 all appear to arise from interaction of the electron-spin moment with proton moments. That for glycine, predominantly a triplet, indicates two equally coupling protons. The outer satellites are too weak for the resonance to be a normal quintet from four protons. Because of its intensity distribution the resonance cannot result from N^{14} interaction. There is seemingly more than one way of getting two equivalent couplings here. The X-rays may ionize the glycine molecule and thereby cause it to dissociate into NH_3, CO_2, and $(CH_2)^+$. The $(CH_2)^+$ might then attach itself to something else—for example, to the O^- of the adjacent unhit zwitter ion to form the radical $(R—O—\dot{C}H_2)^+$. The shape of the resonance, particularly the two weak satellites or shoulders, suggests that the coupling is at least in part the direct dipole-dipole type. Evidently the tumbling motions are highly restricted even at room temperature. Possibly the ionization merely breaks off CO_2, leaving the radical $H_2\dot{C}—N^+H_3$. If such a radical were held firmly by hydrogen bridges, the odd electron localized on the C might interact through dipole-dipole coupling with the CH_2 protons to give the observed structure. The NH_3 nuclei would be too far away to give resolvable splitting.

For alanine a symmetrical, five-line hyperfine structure is observed. This indicates equal coupling of the electron to four protons. Our suggested explanation of such a symmetrical coupling is that the X-rays eject an electron, thereby causing the alanine to break up into NH_2, CO_2, and the radical $(H_2CCH_2)^+$. The CO_2 and NH_3 would escape, leaving the positively charged radical trapped in the hole made by the disintegration of the alanine molecule. At room temperature the rapid motions of this radical would probably nullify direct dipole-dipole interaction. The total spread of 95 ± 3 gauss therefore indicates that the odd electron spends about one-fifth of the time in $1s$ orbitals of the four hydrogens. The odd electron

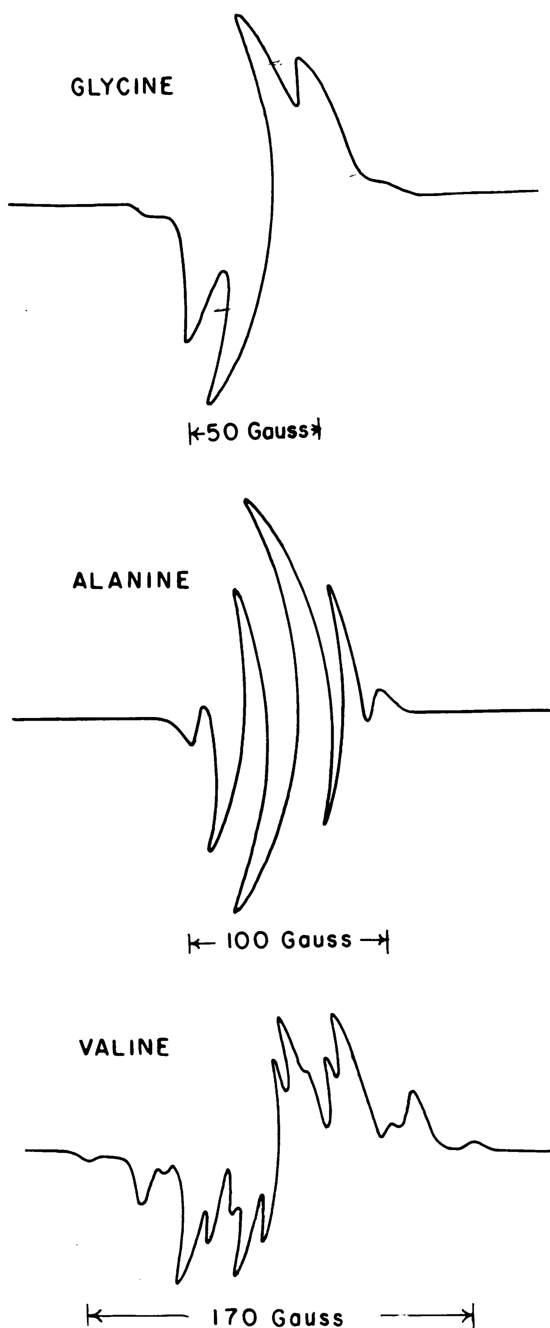
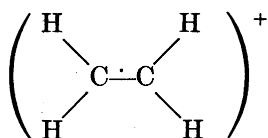
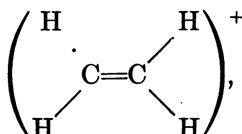


FIG. 1.—Tracings of electron-magnetic resonance spectra of X-irradiated glycine, alanine, and valine at room temperature. The tracing was made with an Esterline-Angus recorder on curved-co-ordinate recording paper. The curves represent first derivatives of the actual absorption lines. A phase-sensitive, lock-in detector was employed, with magnetic modulation of the absorption line at the lock-in frequency. The g factor for the center of the multiplets is essentially that for the free electron spin. The observation frequency is 9 kmc.

of the radical $(CH_2CH_2)^+$ could move into the $1s$ orbitals of the hydrogen through hyperconjugation. In terms of Pauling's concepts of the valence bond, the actual structure would be considered a resonant hybrid of

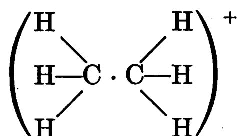


with the four equivalent forms

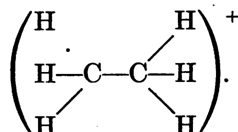


all of which contain a "one-electron bond." Because of the greater electronegativity of C over H, the electron of the C·H bond would be expected to divide its time between the C and the H atoms in the ratio of about 6 to 4. For 100 per cent C·H bonds, a multiplet spread of 40 per cent of 500 gauss (or 200 gauss) would be expected. The observed spread of 95 gauss indicates that the last four equivalent forms contribute a total of about 50 per cent to the actual structure.

For irradiated valine two superimposed hyperfine patterns were observed (Fig. 1), one of which definitely has seven components; the other is apparently a quintet like that of alanine. The seven symmetrical components must arise from six proton moments equally coupled to the moment of the electron. This pattern could be explained if the X-rays break up the valine molecule to leave, among other things, the symmetrical radical $(H_3CCH_3)^+$. The other things would probably be CO_2, NH_3, and H_2C_2. Again, the odd electron could migrate into the $1s$ orbitals of the six hydrogens through hyperconjugation or, in the valence-bond eoncepts, through resonance of the structure



with the six equivalent forms



Dipole-dipole interaction would again be nullified by tumbling motions of the radical. The spread of 160 ± 6 gauss indicates that the odd electron resides in a $1s$ orbital of an H about one-third of the time. This suggests a strong resonance or hyperconjugation of the one-electron CC bond with the CH bonds. From the coupling it is estimated that each of the seven structures contributes about equally.

The valine quintet has the same spacing, within the accuracy of the measurements, as has the alanine quintet. We therefore ascribe it to the same radical, $(H_2CCH_2)^+$, which the ionization could make, along with alanine, as an alternative to the dissociation which gives $(H_3CCH_3)^+$. From the relative intensities of the two multiplets, it appears that $(CH_3CH_3)^+$ is the more abundant.

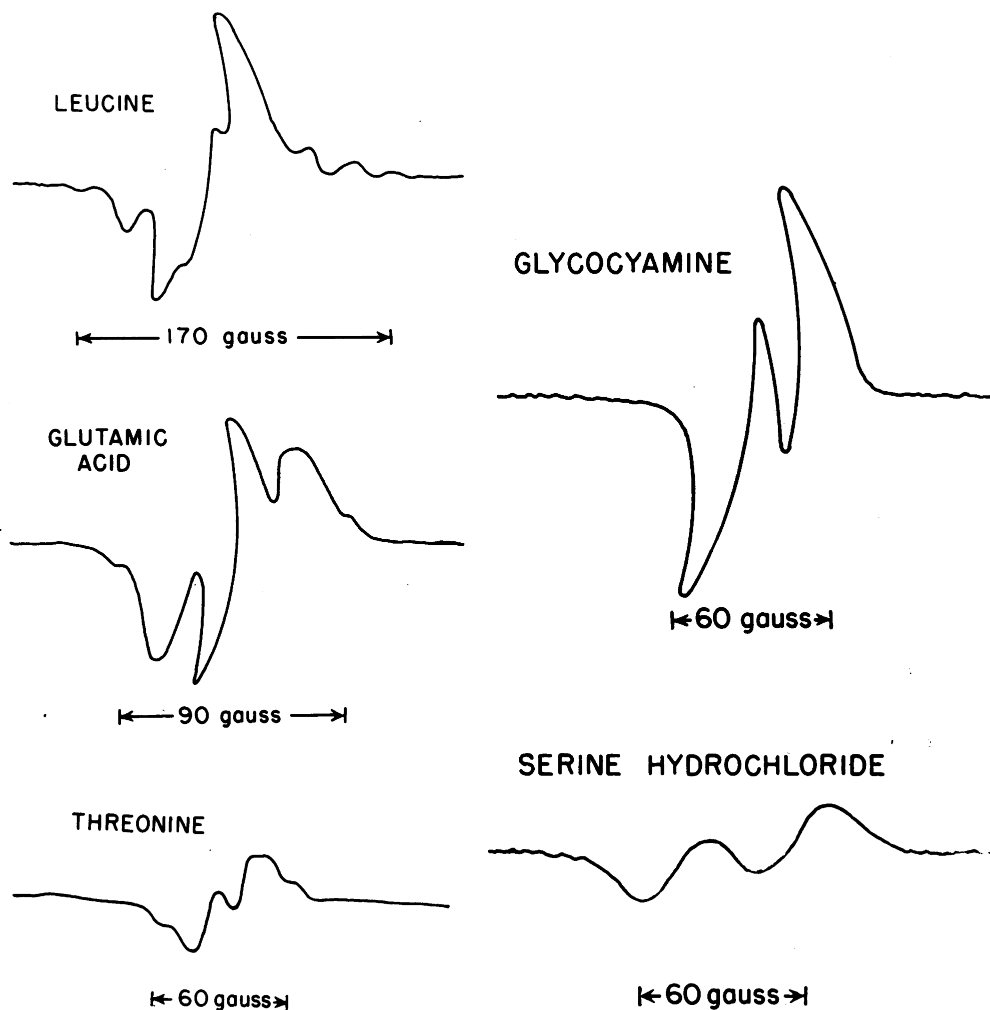


FIG. 2.—Tracings of electron-magnetic resonance spectra of leucine, glutamic acid, and threonine, with conditions as described for Fig. 1.

FIG. 3.—Tracings of electron-magnetic resonance spectra of glycocyamine and serine hydrochloride, with conditions as described for Fig. 1.

Leucine (see Fig. 2) gave a set of seven components with a total spread of about 160 gauss. This multiplet seems to be superimposed on other nonresolvable resonances. The seven components, like those of valine, probably arise from the $(CH_3CH_3)^+$ radical. The pattern obtained for isoleucine was essentially the same as that for leucine.

Glutamic acid,

```
H—O      H  H  H      O—H
   \     |  |  |     /
    C—C—C—C—C      ,
   //    |  |  |     \\
  O      H  H NH2      O
```

when X-rayed yields a complex paramagnetic resonance which appears to be a superposition, in about equal strength, of the glycine triplet and the alanine quintet. Interestingly, if the X-rays should remove an electron to form the radical $(CH_2)^+$, which is needed to give the glycine triplet, the remainder of the glutamic acid molecule would be simply alanine and CO_2. If the radiation should, by striking at another point, form the radical $(H_2CCH_2)^+$, which has been postulated to give the alanine quintet, the stable molecules H_2CO, CO, NH_3, and CO_2 could be formed from the remainder. We do not know, of course, that these two calamities actually occur when the quanta strike. They merely seem to be reasonable postulates which could account for the experimental observations.

The magnetic resonance of irradiated threonine, $CH_3CHOHCHNH_2COOH$ (see Fig. 2), appears to be a quartet resulting from three equally coupled protons. The multiplet spread is small, roughly 35 gauss. It may arise from the almost free $\cdot CH_3$ radical which the radiation could produce by breaking a single CC bond. If an electron is knocked away in the process, the relatively stable ionic group $HO^+{=}CH{-}CHNH_2{-}COOH$ would be left. Nevertheless, it seems surprising that the $\cdot CH_3$ radical could be trapped or that it would be stable so long at room temperature. The resonance of a sample of irradiated threonine which was kept in a sealed tube after irradiation showed no signs of weakening in a period of two months. It seems possible that the resonance is not a quartet but is a combination of different patterns which accidentally give the approximate form of an incompletely resolved quartet.

For serine hydrochloride (Fig. 3) a symmetrical doublet with separation of 35 gauss was observed. A possible origin is the radical of the type $R{-}O^+{-}H$. A second possibility is that the doublet arises from an OH radical stranded by neighboring groups through hydrogen bonds. A doublet splitting of this magnitude could be produced by direct dipole-dipole interaction of the proton and the odd electron on the O. With $\sqrt[3]{\langle r^3 \rangle_{Av.}} = 0.98$ A, a 30-gauss splitting is predicted by equation (2) below. A part of the splitting, however, is expected to arise from a nonvanishing of the odd-electron wave function at the hydrogen nuclei.

Doublets similar to that found for serine hydrochloride, but of smaller spacing, were found for glycocyamine, glycylglycine, and many other hydrogen-bridged complexes. They are believed to arise from an odd electron localized on an O and interacting with a bridging proton as explained later.

Cystine (Fig. 4) gives an asymmetric pattern with component separations depending upon the strength of the externally imposed field. This structure evidently result from crystalline field splitting. No structure from nuclear interaction is evident. The unpaired electron probably resides mainly on the sulfur atoms. When an electron is knocked out of the molecule at some other place, the vacancy probably shifts to the S atoms, where it has the lowest energy. Furthermore, a vacancy

on an S would allow it to form an additional three-electron bond with the S to which it is already linked by a normal electron-pair bond. This amounts to saying

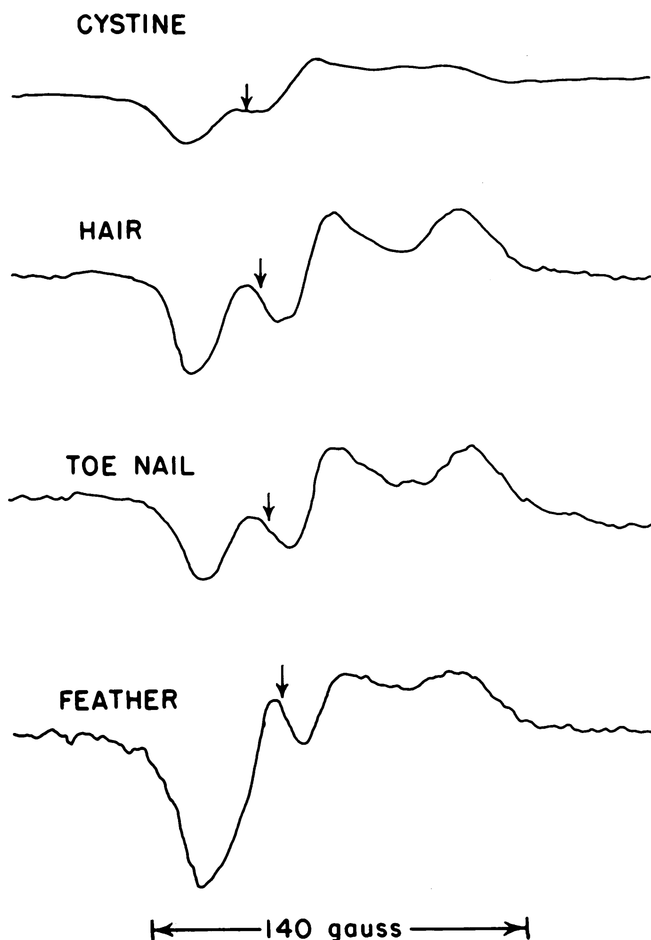
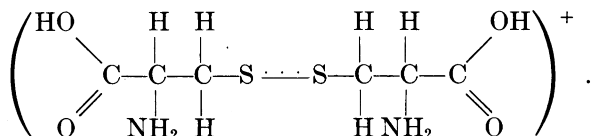


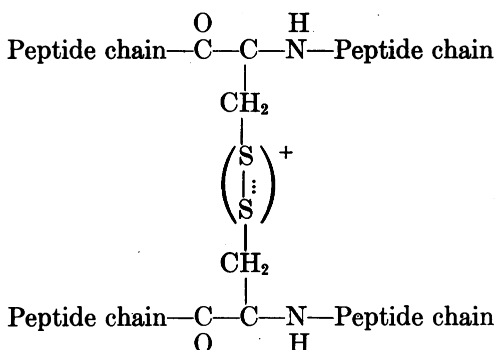
FIG. 4.—Tracings of electron-magnetic resonance spectra of cystine and some fibrous proteins, with conditions as described for Fig. 1. Arrows indicate position for g of the free electron spin.

that the hole would be shared by the two S atoms through exchange of their electrons. Thus the loss of an electron should strengthen (rather than weaken) the SS bonding, to form the radical



Patterns similar to that for cystine were observed also for the keratin proteins, hair, feather, and toenail (Fig. 4). We conclude that the resonance observed in these proteins arises predominantly from their cystine constituent. This was at

first a very surprising observation because cystine is only one of the many amino acids found in the side chains of these proteins and constitutes only about one-tenth of their total composition. No evidence for the patterns characteristic of other amino acids known to be in these proteins was detected even after several hours of irradiation with 50-kv. X-rays. On the other hand, it seems equally strange, considering the low concentration of cystine, that a detectable cystine resonance could be quickly produced by the irradiation. The evidence is strong that whenever radiation knocks out an electron to create a hole or vacancy at any given point in the protein, this hole or vacancy is quickly filled by an electron borrowed from a cystine group. This mechanism prevents the breaking of bonds and probably diminishes greatly the damage by the radiation. A polypeptide chain joined by the cystine link should not fall apart under ionizing radiations. On the contrary, the cystine link should be strengthened by a new three-electron bond,



to make the cross-bonding stronger.

It seems reasonable that the two sulfur atoms of the cystine molecule would act as an electron reservoir to supply electrons to fill vacancies at other points in protein molecules. The S atom has a low electronegativity, 2.5, as compared to 3.0 for N and 3.5 for O. Also, it has four spare or nonbonding electrons in its valence shell. Carbon, which has a comparably low electronegativity, uses all its valence electrons for bond formation, and so does hydrogen. A bond must be broken when C or H is ionized. In contrast, a cystine S which has lost an electron can form an additional one-half bond (three-electron bond) with the adjacent S, as already mentioned. From other results, partly described here, we conclude that ionization will leave a plus charge on an N or even on an O before it will sever a C—H or C—C bond, unless highly symmetrical radicals can be formed as a consequence—radicals like those already postulated which permit stabilization, through resonance, or the spreading of the wave function of the odd electron.

Raw silk, was found, when irradiated to have a symmetrical doublet. The component separation of about 12 gauss is the same at the two frequencies 9 and 23 kmc. This, with the equal intensities, suggests that the splitting results from interaction of the electron spin with that of a single proton. The doublet is within the accuracy of the measurement identical to that for glycylglycine (see Fig. 5). A similar doublet was observed for glycocyamine.

Silk is known from X-ray studies to have an elongated polypeptide-chain structure with the adjacent chains linked by hydrogen bonds between the C=O and

NH group. Glycylglycine is the shortest polypeptide chain. Its crystalline form also contains intermolecular C=O to NH hydrogen bridges. It is probably significant that these two substances were found to have the same resonance. Similar

GLYCYLGLYCINE
|← 60 gauss →|

CATTLE HIDE
|← 60 gauss →|

RAW SILK
|← 60 gauss →|

FISH SCALE
|← 60 gauss →|

FIG. 5.—Tracings of electron-magnetic resonance spectra of glycylglycine and some proteins, with conditions as described for Fig. 1.

doublets were observed for irradiated cattle hide, fish scale, and other structures containing similar hydrogen bridges. In them, the doublet resonance is ascribed to an odd electron localized on an O but experiencing direct dipole-dipole interaction with the bridging proton in the ionized structure,

```
      R       O
     /C\     /C\
    / H  \N/     \
          |
          H
          |
          |
          |
         ·O        ,
          |
   \ H   /C\\
    \C/      \\N+/
     R         H
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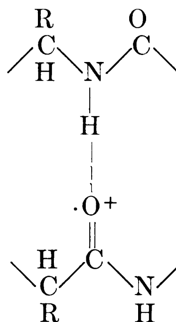
with perhaps some contribution from



This type of interaction would vary with orientation of the hydrogen-bridge axis in the external field, as indicated by equation (1); and for random orientations of the axes, as expected for our samples, it would give a doublet structure with peak separations of

$$\Delta H(\text{gauss}) = \frac{28}{\langle r^3 \rangle_{\text{Av.}}}, \quad (2)$$

where r is in angstroms. The theoretical line shape for the doublet is shown in Figure 6. Such a peaked shape, however, would not be realized experimentally, but

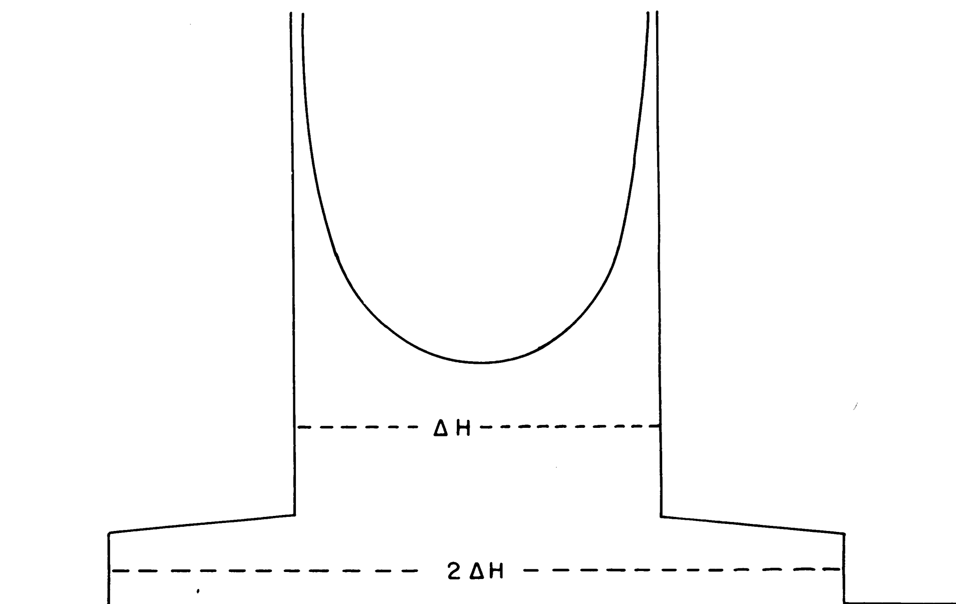


FIG. 6.—Theoretical absorption pattern for spin resonance of electron experiencing direct dipole-dipole interactions with a proton in a rigid polycrystalline sample, for the Paschen-Bach case.

in some curves the outer shoulders are evident. From the doublet splitting of about 12 gauss in glycylglycine or silk, etc., $\sqrt[3]{\langle r^3 \rangle_{\text{Av.}}}$ is found to be 1.35A. This value is somewhat less than the 1.6 A which is estimated for the O——H dis-

tance from the known length of 2.7 A for the usual O———H—N bridge. The difference is believed to result from the spatial distribution of the orbit of the old electron about the oxygen. Since the bonding orbital is probably hybridized, the nonbonding orbitals which contain the odd electron should be projected somewhat toward the proton. Even if the odd electrons were spherically distributed about the oxygen, $\sqrt[3]{\langle r^3 \rangle_{Av.}}$ would be less than the O———H internuclear distance.

Silk has no cystine content and probably no sulfur. Its spectrum is therefore understandably different from that of horn or hair, already described. Yet, if the mechanism which we have postulated actually occurs, again little immediate damage to the structure should result from the irradiation; in fact, the original structure might be restored when new electrons become available. This is true also for cattle hide and fish scale and probably would be true for all polypeptide chains connected by hydrogen bonds and cystine SS links or by either. Contrast this with the molecular catastrophe which seems to occur when the radiation strikes the individual amino acids. We suspect that the ready breakup of the basic amino acids arises from their zwitter-ion structure, which makes for easy formation of CO_2 and NH_3.

An interesting effect has been observed in irradiated skull bone. Immediately after irradiation, the resonance (Fig. 7) may be represented as a doublet, like that of silk, over which is superimposed a second resonance. At 9 kmc. the latter is a sharp singlet. After three days in air at room temperature, the doublet was gone, but the sharp singlet remained (see Fig. 7). Furthermore, three weeks later it had not noticeably diminished in intensity. At 23 kmc. it was split and appeared to have a shape like that of a resonance which was proved to result from bound O_2 in irradiated Teflon.[5] Additional tests are necessary for proof, but it seems possible that absorbed oxygen may tie to the free radical $R\cdot$ (made by the irradiation of the bone), to form the complex, R—O$\stackrel{...}{-}$O. Note that the odd electron of $R\cdot$ is transferred to the three-electron bond between the oxygens. This type of radical, found in irradiated Teflon after exposure to atmospheric oxygen, has been shown, by re-examination of the resonance, to remain stable for months. If it remains similarly stable in skull bone, as evidence to date indicates, radiation damage to the skull may be cumulative over longer periods. But for the protective coating of hair and scalp, our skulls might become noticeably paramagnetic!

The samples of glycine, alanine, leucine, valine, threonine, and glutamic acid were kept in sealed and evacuated glass tubes, and their signals were re-examined four months after irradiation. The resonance had not detectably changed. It seems remarkable that the charged radicals such as $(C_2H_6)^+$, which we have postulated, should enjoy such a long life. Nevertheless, it seems likewise remarkable that any radical in which the odd electron spends considerable time in $1s$ orbitals of H atoms should remain intact within a solid so long at room temperatures. Yet the experimental observations seem to demand that some form of complex but symmetrical radical remain for this period. If the positive radicals which we postulate actually exist, they are probably trapped in a cage of negative oxygens within the vacancy caused by the disappearance of their parent-molecule. Unfortunately, none of the proteins were kept in sealed tubes after irradiation. In general, their resonances disappeared within a few hours or days. More quantitative studies of the lifetime of the various radicals are planned.

Nothing has been said about possible resonance of the electrons knocked out by the ionizing radiation. These electrons evidently are trapped somewhere in the sample, presumably at sites of lattice imperfections or at impurity centers. If their resonances are of the order of 100 gauss in width, they would not have been detected with the small-amplitude-modulation technique which we used. In some of the curves, particularly those for leucine and glutamic acid, there seems to be a broad

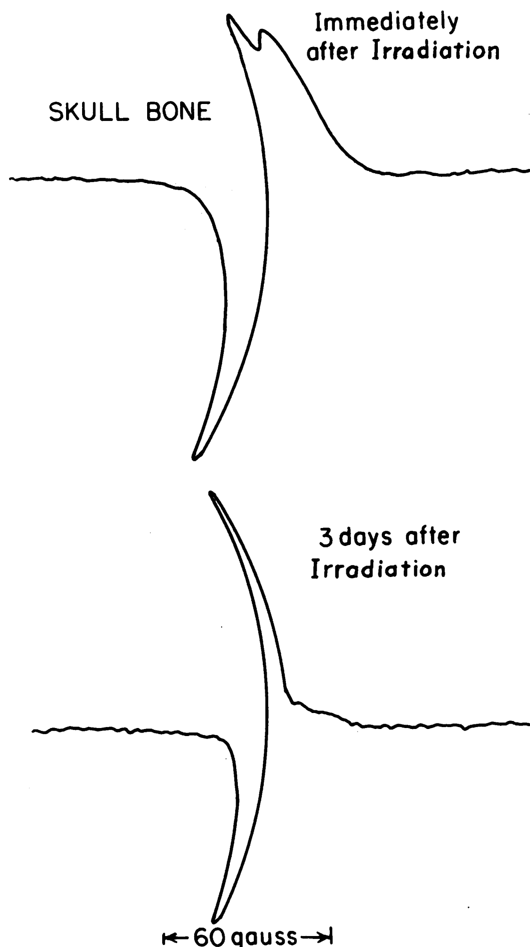


FIG. 7.—Tracings of electron-magnetic resonance spectra of skull bone immediately after and three days after irradiation with x-rays, with conditions as described in Fig. 1.

single resonance superimposed on the hyperfine pattern. This may result from trapped electrons.

If the mechanisms which we propose to account for the various electron resonances reported here are essentially correct, they may help to explain why fibrous proteins such as hair and hide so often form a protective coating for living things. It is rather evident that the simplest amino acids, like glycine, valine, and alanine,

could not alone survive the ionizing ultraviolet light from the sun, especially before the ozone layer was formed. They might do so by uniting in a polypeptide chain. We have here evidence for "survival of the fittest" on a molecular scale, evidence which may help to account for the protein buildup on the earth. A simple amino acid formed under such a protective coating as water or earth would be disintegrated by ultraviolet light when it drifted to the surface, whereas glycylglycine would only receive an ionizing wound from which it might later heal or because of which it might add another unit to grow bigger. Thus the sun would help to concentrate the fibrous proteins, which later would protect other molecular constituents of living matter from the sun. The same mechanism will not allow proteins to hold together or heal wounds inflicted by stronger radiations which knock out or break up entire atoms. Evolution has not yet prepared us for the atomic age!

At the Faraday Society Discussion on Microwave and Radio-Frequency Spectroscopy, in April, 1955, where our results were briefly described, we learned that J. Combrisson and J. Uebersfeld,[6] of Paris, had observed resonance in a number of amino acids irradiated with gamma rays from an atomic pile. The only similarity in our results and theirs is for glycine. The only structural pattern they reported was a triplet which they observed for several amino acids, including alanine and leucine. They failed to observe a resonance for several, including cystine and glutamic acid. Because they used very hard radiation which could remove hydrogen atoms, their results and conclusions are understandably different from ours.

We wish to thank Mr. W. M. D. Bryant, of the DuPont Laboratories, and Dr. B. Woodhall and Professor D. G. Sharp, of the Duke Hospital, for kindly supplying some of the samples.

NOTE ADDED IN PROOF: We have now kept a number of the proteins in sealed tubes away from oxygen during and after irradiation and have found that the resonance did not decay as when in air. Evidently the oxygen diffuses into these proteins and interacts with the radical to kill the resonance. In a few instances, as in the skull bone mentioned, the oxygen ties onto the radical in such a manner as to alter but not destroy the resonance. We think that the studies now in progress may lead to a better understanding of the adverse effects of oxygen in radiation damage to animals, just as the cystine results seem to account for protective effects of sulfur.

Two of us (W. G. and H. S.) are now using the method described here to study the effects of ionizing radiation on the nucleic acids and their constituents. Again, strong effects of oxygen have been noted. The resonances of DNA and RNA appear to remain indefinitely stable in an evacuated tube, whereas they both decay within a few hours in the normal atmosphere. In one constituent of nucleic acid, guanylic acid, a triplet structure was observed when the acid was irradiated and observed in an evaucuated tube. Upon exposure to air this was slowly converted to a stable singlet like that for skull bone which, as expected, has proved to result from absorbed O_2. We think that the singlet is due to bound, paramagnetic O_2 as in skull bone or Teflon.

* Supported by the Office of Ordnance Research and by the Office of Scientific Research of the Air Research and Development Command.

[1] For a description of the phenomena of magnetic resonance see W. Gordy, W. V. Smith, and R. F. Trambarulo, *Microwave Spectroscopy* (New York: John Wiley & Sons, Inc., 1953), chap. v. See also chap. ii. by W. Gordy, of *Chemical Applications of Spectroscopy*, soon to be published by Interscience Publishers, Inc., in the "Techniques of Organic Chemistry" series, ed. A. Weissberger.

[2] B. Commoner, J. Townsend, and G. W. Pake, *Nature*, **174,** 689, 1954.

[3] H. Shields, W. B. Ard, and W. Gordy (to be published).

[4] R. Livingston, H. Zeldes, and E. H. Taylor (*Phys. Rev.*, **94,** 724, 1954) have found that the free atom splitting of 500 gauss for H is also observed for atomic hydrogen trapped in the frozen acids H_2SO_4, H_3PO_4, and $HClO_4$.

[5] W. B. Ard, H. Shields, and W. Gordy, *J. Chem. Phys.*, **23,** 1727 (1955).

[6] J. Combrisson and J. Uebersfeld, *Compt. rend. Acad. sci.* (Paris), **258,** 1397, 1954. See also *Discussions Faraday Soc.*, No. 18, 1955.

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*MICROWAVE SPECTROSCOPY OF BIOLOGICAL SUBSTANCES. II. PARAMAGNETIC RESONANCE IN X-IRRADIATED CARBOXYLIC AND HYDROXY ACIDS**

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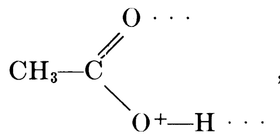
The microwave methods employed in the preceding paper on amino acids and proteins have been applied here to certain fatty and hydroxy acids.

The fatty acids contain a carboxylic head, COOH, and a hydrocarbon tail, *R*. They form dimers or higher polymers through two O—H · · · O hydrogen bridges per molecule. In this study we have included some with very short tails and some with relatively long tails but none yet with forked tails. The study has not proved monotonous. The saturated fatty acids do not all behave the same way when hit by X-ray quanta.

Figure 1 hints that different experimenters may not always get the same results in this field. The four curves obtained for acetic acid are qualitatively different. For the top curve the sample was irradiated and immediately observed at liquid-nitrogen temperature. The doublet of 25-gauss separation is believed to arise from the ionized hydrogen-bonded radical,



with the odd electron on the hydroxyl oxygen. At this temperature the radical is held fairly still by the hydrogen bridges, and the doublet splitting is produced mainly by dipole-dipole interaction between the electron and the hydroxyl proton. Equation (2) of the preceding paper with $H = 25$ gives the reasonable value of 1.05 A for $\sqrt[3]{\langle r^3 \rangle_{Av.}}$. If this interpretation is correct, the hydrogen bridge is obviously not a symmetrical one. In a symmetrical bridge the odd electron would have an equal probability of being on the other oxygens and of interacting with other bridging protons to give a more complex spectrum.