Comparing with our co-ordinates of Fig. 4, b,

\[ \begin{align*}
(Q_1)_w &= Q_1, \\
(Q_2)_w &= -(Q_1 + Q_2)/\sqrt{2}, \\
(Q_3)_w &= (Q_2 - Q_1)/\sqrt{2}, \\
(Q_4)_w &= Q_4.
\end{align*} \]

There are similar relations between the Wigner co-ordinates and our co-ordinates of Fig. 4, d.

Wigner states that if the number of particles is a power of 2 and the masses are equal, there is a co-ordinate system of high symmetry in which all particles are treated equally. Unfortunately the form of the potential energy in the collision of diatomic molecules would make these Wigner co-ordinates inconvenient.

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ELECTRON-SPIN RESONANCE STUDIES OF RADIATION DAMAGE TO CERTAIN LIPIDS, HORMONES, AND VITAMINS*

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In the present work we have applied the method of microwave electron-spin resonance to the study of radiation damage to certain lipids, hormones, and vitamins. In the accompanying paper on the nucleic acids and their constituents, references are given to descriptions of the theory and experimental methods which are employed. The samples, which were in a powdered form, were irradiated by a kilo-curie cobalt 60 γ-ray source. They were irradiated and observed under vacuum, were later exposed to air or oxygen and observed again at various intervals.

The steroid lipids and hormones are too complicated in structure to permit any detailed or complete interpretation of the paramagnetic resonance spectra induced by irradiation of them. Nevertheless, it has proved possible by comparison of the resonances produced in related steroids which differ only slightly in composition or structure to gain information about the relative effects of ionizing radiation upon various members of this biologically significant class of compounds.

Sitosterol, Cholesterol, and Cholic Acid. Sitosterol and cholesterol have the same steroid ring structure,

\[
\begin{align*}
&\text{CH}_3 \\
&\text{HO-} \\
&\text{CH}_3 \\
&\text{X} \\
&\text{CH}_2
\end{align*}
\]

and differ only in the group X, which is \(\text{CH}(\text{CH}_3)(\text{CH}_2)_2\text{CH}(\text{CH}_3)_2\) for cholesterol and \(\text{CH}(\text{CH}_3)((\text{CH}_2)\text{CH}_2)\text{CH}(\text{C}_2\text{H}_5)\text{CH}(\text{CH}_3)_2\) for sitosterol. Upon irradiation in a high vacuum with ionizing gamma rays, they give the same type of resonance pattern, a triplet with a doublet substructure, apparently arising from three coupling protons, two with equivalent coupling and the third with somewhat less cou-
CHOLESTEROL

IN VAC.

IN AIR 12 MIN

IN AIR 36 MIN.

SITOSTEROL

IN VAC.

IN AIR 4 MIN

IN AIR 16 MIN.

Fig. 1.—Electron-spin resonance curves for γ-irradiated cholesterol and sitosterol with samples under vacuum and exposed to air. Observations were made at room temperature on powdered samples. The curves represent second derivatives of the actual absorption curve. Bars represent the theoretical pattern caused by three hydrogen nuclei interacting with the electron spin, two of which have equal coupling. The irradiation dosage was of the order of 10⁷ r. Markers at base are spaced 68 gauss apart. Arrows pointing downward represent the position for DPPH resonance, g = 2.0036. The observation frequency was 9 kMc/sec. Recordings were made on curved co-ordinate paper with an Esterline-Angus automatic pen and ink recorder.

pling than the two equivalent ones. The observed resonances are shown in Figure 1. Such a combination should give a symmetric pattern, as indicated by the bars in Figure 1. The symmetry in the observed curves is slightly marred, possibly by the resonance of a secondary radical or by small anisotropies in the g factors and coupling constants.¹

It is not possible to be certain which of the three protons is coupling to the electron spin in these complicated systems. One seemingly reasonable possibility is that a C-H bond is broken in the vicinity of the C=C bond, leaving the free radical,
which should be stabilized through contributions of such hyperconjugated structures as

![Diagram of structures II and III](image)

These could account for the observed coupling with three protons.

The resonances for cholesterol and sitosterol produced by irradiation in a vacuum decay very rapidly upon exposure to air or oxygen. The effects of decay, although comparably rapid for both, appear to differ. For cholesterol, the resonance is first converted to a singlet, which then decays rapidly. For sitosterol, the original pattern decays with no apparent change of form. These effects are demonstrated in Figure 1.

A possible mechanism for the observed oxygen effect in cholesterol is that molecular oxygen combines with the proposed radical I to form the peroxide free radical IV:

![Diagram of structure IV](image)

which gives the singlet. Since the unpaired electron spin would be localized on the O₂ of this radical, no proton hyperfine structure would be expected. Furthermore, the flopping about of the O₂ axis at room temperature should reduce the spin-orbit coupling in the peroxide-free radical to make its resonance sharp. This possibility has been pointed out by V. V. Voevodsky.² The trapped O₂ of this group might then react with the adjacent group to open the ring, release atomic hydrogen, and form two carbonyl bonds:

![Diagram of structure V](image)
The atomic hydrogen released in this process would escape too rapidly to be detected in these experiments. Thus the decay of the resonances might be explained. Similar reaction mechanisms for \( \text{O}_2 \) have been postulated by others.

Because of their similar patterns when observed in a vacuum and because of their similar structure, cholesterol and sitosterol would be expected to have the same oxygen effect. A mechanism like that proposed for cholesterol could account for the oxygen effect observed for sitosterol if for it (but not for cholesterol) the decay of the final peroxide radical is more rapid than the diffusion rate of oxygen into the sample. Then the peroxide-free radical might never reach detectable concentrations in sitosterol, and one would observe only the decay of the original signal. If this interpretation is correct, the decay rate of the secondary resonance for cholesterol gives a measure of the reaction rate of the peroxide radical with its surrounding groups, whereas the decay rate of the signal in sitosterol gives a measure of the diffusion rate of \( \text{O}_2 \) into the sample.

Cholic acid,

\[
\begin{align*}
\text{CH}_3 & \quad \text{HO} \\
\text{CH}_3 & \quad \text{OH} \\
\text{X} = \text{CH(CH}_3\text{)}_3\text{CH}_2\text{CH}_2\text{COOH}
\end{align*}
\]

has a steroid ring structure similar to that for cholesterol or sitosterol but has a carboxylic acid group and no \( \text{C} = \text{C} \) bond. Its resonance is entirely different from that of cholesterol when it is subjected to ionizing irradiation. Compare Figures 2 and 1. Furthermore, there is no corresponding decay of the resonance in air for cholic acid, as was observed for cholesterol and sitosterol. The resonance produced by irradiation of cholic acid in a vacuum can be observed after days of exposure to air (see Fig. 2).

One can hardly attribute the absence of an oxygen effect for cholic acid to an inability of oxygen to diffuse into the powdered sample which we used. Evidently, oxygen does not readily attack the radical produced in the cholic acid. Possible reasons for this will be discussed after related effects in other steroids are described.

Steroid Hormones. Stigmasterol has the same steroid ring structure as that for sitosterol and cholesterol (see structure I above) but has a pure hydrocarbon \( \text{X} \) group, \( \text{CH(CH}_3\text{)}_3\text{CH} = \text{CHCH(C}_2\text{H}_5\text{)}_\text{CH(CH}_3\text{)}_2 \) containing a \( \text{C} = \text{C} \) bond. In fact, the complete structure of stigmasterol and sitosterol differ only in this double bond. When stigmasterol is irradiated in a vacuum, its resonance (see Fig. 3) is a triplet with doublet substructure, like that for sitosterol and for cholesterol. It is believed to arise from a free radical similar to that described for cholesterol, with the electron-spin density concentration in the steroid ring structure and interacting with three
protons. Furthermore, its oxygen effect is similar to that for cholesterol (compare Figs. 1 and 3).

The steroid ring for ergosterol,

\[
\begin{array}{c}
\text{HO} \\
\text{CH}_3 \\
\text{CH}_3
\end{array}
\]

has two double bonds but is otherwise similar to that of cholesterol. Its X group attached to the ring, \( X = \text{CH} (\text{CH}_3) \text{CH} = \text{CHCH} (\text{CH}_3) \text{CH} (\text{CH}_3)_2 \), is a pure hydrocarbon group similar to that of stigmasterol, with one \( \text{C} = \text{C} \) bond.

If the electron-spin density is concentrated in the steroid rings of radicals formed from all these substances, one would expect the second double bond of the ring to cause ergosterol to have a proton hyperfine structure different from that of stigmasterol. The patterns do appear to differ. Both decay rapidly in air, although the decay for stigmasterol is preceded by a conversion of the resonance pattern to a different one, whereas that for ergosterol is not. Our explanation for this difference is the same as that given for the similar difference between cholesterol and sitosterol.

The molecular structures of progesterone, testosterone, and testosterone propionate differ from those of the steroids already discussed mainly in that they have a carbonyl group in the place of the alcohol group in one of the four rings. Their structural formula is
where \( X = \text{COCH}_3 \) for progesterone, \( X = \text{OH} \) for testosterone, and \( X = \text{COOCH}_2\text{-CH}_3 \) for testosterone propionate. These steroids all give different resonance patterns when irradiated in a vacuum. The \( X \) group therefore has significant influence on the radical formation in them. In fact, the electron-spin density may be concentrated in the external \( X \) group of progesterone and testosterone propionate rather than in the steroid ring structure. For evidence of this, see the later section on oxygen effects. Unfortunately, the resonances are too complicated to be assigned to a specific free radical. Unequal interaction of the electron spin with three or more protons is indicated. The signals of none of these three hormones decay rapidly in oxygen or air. The stability of the patterns when the samples are exposed to air is illustrated for progesterone and testosterone propionate in Figure 4.

The ring structure for pregnenolone,
PHYSICS: REXROAD AND GORDY

PREGNENOLON

TESTOSTERONE

**Fig. 5.**—Electron-spin resonance curve for γ-irradiated pregnenolone and testosterone, with conditions as described for Figure 1.

is similar to that for cholesterol except that the C = C bond is switched to an adjacent position. Figure 5 shows its observed spin resonance, which is similar to, but not exactly like, that for cholesterol. Unlike that for cholesterol, its resonance does not decay rapidly when the irradiated sample is exposed to air. Its X group, COCH₃, is the same as that for progesterone. In fact, pregnenolone is formed from progesterone simply by the addition of an H to the carbonyl O of its ring, to form the OH group.

**Hexestrol.** The very potent hormone hexestrol does not have the steroid configuration but has two rings connected by saturated carbon groups to form the interesting symmetrical structure

![Hexestrol structure](image)

When we irradiated this hormone in a vacuum at room temperature, we obtained the electron-spin resonance shown in Figure 6. It has a proton hyperfine structure which appears superficially to be a triplet with a subtriplet structure. The subtriplet structure, however, does not have the symmetrical spacing or the relative subintensity distribution for two equally coupling protons. Also, one component is sharper than the other two. A possible interpretation is that there are two superimposed patterns, one a triplet of doublets, labeled (a), with a g factor of 2.00, and the other a triplet, labeled (b), with a slightly different g factor. These two patterns might arise from the same radical with parallel and perpendicular orientation in the applied field or with different relative orientations of the two rings. The latter possibility is an interesting one, for it seems that a variation in the relative orientations of the symmetrical groups in hexestrol might vary its hormone activity. As an indication of possible orientation effects, we were able to produce marked changes
in the resonance pattern by varying the temperature of the irradiated sample moderately, but we were not able to obtain any consistent or reproducible changes. These temperature effects are being investigated further.

The resonance pattern of irradiated hexesterol is converted in air to that characteristic of the peroxide radical and thereupon decays rapidly.

*Thyroid and Parathyroid.* Figure 7 shows the electron-spin resonance of $\gamma$-irradiated thyroid and parathyroid and the effects of air on them. The samples employed were commercial ones obtained from the Nutritional Biochemical Company.

The resonance for parathyroid is a doublet with component spacing of 18 gauss, similar to that for many irradiated proteins. Although the doublet components are not of equal intensity, it seems probable that their inequality arises from the presence of small amounts of oxygen which could not be pumped from the sample. Upon the admission of air to the irradiated sample, this asymmetry increases rapidly until, after 12 minutes, there is essentially only one component left (see the lower curve for parathyroid in Fig. 7). This effect suggests that oxygen interacts with the free radical to convert it to a peroxide one, which then proceeds to decay.

The resonance of irradiated thyroid is a reasonably symmetric quartet with approximate intensity ratios of 1, 3, 3, 1 and with a total spread of 56 gauss. This pattern suggests a free radical with three equally coupling hydrogens. There is evidence, however, for a substructure in the components which may arise from other interacting protons with weaker coupling.

In contrast to that of parathyroid, the resonance for thyroid shows no significant
change when the sample is exposed to air after irradiation. At least it shows no significant change after 7 days in air (see the lower curve for thyroid in Fig. 7).

**Vitamins.** Vitamin D$_2$ (calciferol) has a chemical structure

\[
X = \text{CH(CH$_3$)CH} = \text{CHCH(CH$_2$)CH(CH$_2$)CH$_3$}
\]

very similar to that of ergosterol. It is formed from ergosterol by the opening of a C-C bond in the second ring and the formation of an additional C=C bond. Figure 8 shows the electron resonance of γ-irradiated vitamin D in a vacuum and im-

![Diagram of electron resonance curves](image)

**Fig. 8.—**Electron-spin resonance curves for γ-irradiated vitamin D$_2$ (calciferol) and biotin, with conditions as described for Figure 1.

mediately after air is admitted to the irradiated sample. The resonance pattern for the evacuated sample is qualitatively different from that of ergosterol. It is not well resolved but shows hyperfine structure apparently caused by an even number of hydrogens, probably caused mainly by two, probably the two of the CH$_2$ group held by the double bond. As might be expected, signals of irradiated vitamin D$_2$ decay rapidly like those of ergosterol when the irradiated sample is exposed to air (see Fig. 8).

Biotin, or vitamin H, has the structure
and is seen to contain sulfur, nitrogen, and two carbonyl groups. The electron-spin resonance of \( \gamma \)-irradiated sample of biotin, shown in Figure 8, has a complex and incompletely resolved proton hyperfine structure which we shall not attempt to assign to a specific radical. The resonance does not decay rapidly when the irradiated sample is exposed to air.

Riboflavin, riboflavin 5-phosphate sodium, folic acid, and vitamin K\(_5\) when \( \gamma \)-irradiated gave moderately broad resonances with no resolvable structure (see Fig. 9). None of these resonances decayed rapidly when the samples were exposed to air. After several hours of exposure to air they were essentially unchanged in amplitude and form.

Fig. 9.—Electron-spin resonance curves for various \( \gamma \)-irradiated vitamins, with conditions as described for Figure 1.
Riboflavin has a three-membered ring system containing four nitrogens and two carbonyl groups. It seems reasonable that the unpaired electron in irradiated riboflavin is trapped in the ringed system and interacts with the nitrogens and C-H hydrogens of the rings. The nuclear interactions should broaden the resonance without producing a resolvable structure. The resonance for riboflavin 5-phosphate sodium has only about half the width of that for riboflavin. This smaller width may result from a lower concentration of the spin density on the atoms with interacting nuclei. Possibly the spin density is concentrated on the O's of the phosphate group. The rather broad width of the resonances for vitamin K₅ and folic acid may also result from unresolved structure caused by interacting nuclei. The ringed system of folic acid has four nitrogens, and that for vitamin K₅ has several C-H groups.

Ascorbic acid when subjected to ionizing radiations gives a doublet electron-spin resonance with a weaker central component (see Fig. 10). Because the intensity of the central component can be made to differ relative to that of the doublet, it is believed to arise from a radical other than that which gives rise to the doublet.

The doublet spacing, $\Delta H = 27$ gauss, for the ascorbic acid radical is the same at 2.7 kMc/sec as at 9 kMc/sec. Thus it most likely arises from coupling of a single proton with the electron spin. From our present observations we cannot be sure which of the several protons gives rise to the doubling. An interesting possibility is that the ionized ascorbic acid loses an H⁺ to form the free radical,
which could couple to the OH proton through 5 per cent contribution of II. Also forms III and IV

![Chemical Structures](image)

among others, should contribute heavily to the ground state. Here $X = \text{CHOHCH}_2\text{OH}$. The O-H group would probably form a hydrogen bridge with one of the adjacent carbonyl groups, as indicated in I.

Evidence for the above interpretation is that calcium ascorbate,

![Calcium Ascorbate](image)

gives no doublet but only a sharp singlet. When irradiated, it should yield a free radical represented as a hydrid of the two equivalent forms II and III.

![Additional Chemical Structures](image)

**Oxygen Effects.** A surprising correlation has been noted between the absence of an oxygen effect on the resonance and the presence of carbonyl groups in the molecule. The electron resonances for all unsaturated hydrocarbons such as ergosterol or hexesterol with no C-O groups were found to decay rapidly upon exposure to air or oxygen. A mechanism for this decay is discussed above for cholesterol. However, very similar substances having C=O groups exhibited no such decay in air. The evidence indicates that the carbonyl group acts as a protector against oxygen attack in certain irradiated substances. This simple correlation cannot, of course, be expected to apply in all cases, but, to our amazement, it seems to hold for all the
diverse biochemicals examined in the present work. The correlation is shown in Table 1.

It may be that the electronegative oxygen of the carbonyl bond in some way shields the electron spin from attack by O₂. If this is true, the odd electron density must be concentrated on, or near, the carbonyl group—as is indicated, for example, in the various structures proposed for the free radicals in irradiated ascorbic acid or calcium ascorbate. Thus the correlation found indicates that the carbonyl group has a strong influence on radical formation, at least in the compounds of the type examined here. Our results imply that in large, unsaturated hydrocarbon molecules the odd electron density produced by irradiation is likely to be concentrated in the vicinity of the C = C bond or bonds but that when both C = O and C = C bonds are present, the odd electron is more likely to be concentrated in the vicinity of the C = O group. The tendency of the odd spin to be formed in the vicinity of a double bond can be accounted for on the basis of resonance stabilization, as indicated in a few instances above.

It is interesting to speculate whether the absence or presence of an oxygen effect divides the hormones and vitamins into two classes with basically different functions, as, for example, thyroid and parathyroid, testosterone and estradiol.

### TABLE 1

**Correlation of Effects of Air or Oxygen on Resonance with Presence of C=O Group in Molecule**

<table>
<thead>
<tr>
<th>Substance</th>
<th>C=O Group</th>
<th>Oxygen Effect*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cholecalciferol</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Hexasterol</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Parathyroid</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Ergosterol</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Testosterone propionate</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Riboflavin 5-phosphate sodium</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Vitamin K₃</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Folic acid</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Calcium ascorbate</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Biotin</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

* Oxygen effect is here defined as a noticeable change or decay of the resonance signal within a few minutes or hours after exposure of the sample to air or oxygen.

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ELECTRON-SPIN RESONANCE STUDIES OF RADIATION DAMAGE TO THE NUCLEIC ACIDS AND THEIR CONSTITUENTS*

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Communicated by Charles R. Hauser, December 19, 1958

We have sought to apply the method of electron-spin resonance to gain information about the effects of ionizing radiations on desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) as well as on their constituents and the enzymes which attack them. Earlier we applied this method to the proteins and their constituents.¹

The nucleic acids are believed to be the constituents in the genes which carry the information for constructing all living matter. These acids² are long-chain polymers, the hydrolysis of which yields pyrimidine and purine bases, a pentose sugar, and phosphoric acid. Ribonucleic acid (RNA), found chiefly in the cytoplasm of the cell, upon hydrolysis yields phosphoric acid; the pentose sugar, d-ribose; the pyrimidine bases, cytosine and uracil; and the purine bases, adenine and guanine. Desoxyribonucleic acid (DNA), found in the nucleus of the cell, upon hydrolysis yields phosphoric acid; a desoxypentose sugar; the pyrimidine bases, cytosine and thymine (5-methyl-uracil); and the purine bases, adenine and guanine. In some samples of DNA the pyrimidine base 5-methyl-cytosine is found. By partial hydrolysis the nucleic acid polymers can be broken down into the nucleosides, segments containing one of the bases and a sugar unit, or into the nucleotides, the more complete segments which contain a base, a sugar, and a phosphate unit.

In the present study we have included the various constituent units obtained by partial as well as complete hydrolysis of the nucleic acids. However, our study is neither complete nor final. A knowledge of radiation effects upon these “patterns of life” is of sufficient importance to justify more extensive studies with the powerful method of electron-spin resonance.

The reader not familiar with the subjects of electron resonance can find descriptions of it in other, more lengthy works.³ It is sufficient to remark here that ionizing irradiations produce paramagnetic species within organic matter by separating electrons, normally paired in chemical bonds, in such a way that their electron-spin moments and orbital moments are canceled. If the paramagnetic species is placed in a microwave cavity which is in a magnetic field of the proper magnitude, the unpaired electrons “resonate with” and absorb microwave radiation which is