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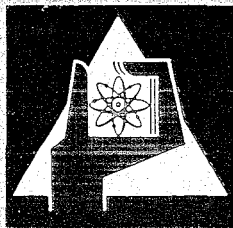
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Institut für Strahlenbiologie

Erzeugung von Ionenradikalen in bestrahlten Einkristallen von
Adenin-Hydrochlorid und Adenosin-5'-Monophosphat

Electron-Spin-Resonance Spectroscopy of Radiation-Induced Free
Radicals in Irradiated Single Crystals of Thymine Monohydrate

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Als Beitrag zur Klärung von Primärmechanismen der Wirkung ionisierender Strahlung auf Nucleinsäuren wurden mit Hilfe der Elektronenspin-Resonanz-(ESR)-Methode die in bestrahlten Einkristallen der Purin-Derivate Adenin·HCl und Adenosin-5'-Monophosphat erzeugten freien Radikale untersucht. Die Kristalle wurden aus gesättigten wäßrigen Lösungen gezogen und bei 300 °K in einer ^{60}Co - γ -Quelle mit Dosen von etwa 10 Mrad bestrahlt. Die Messung erfolgte bei Raumtemperatur mit einem konventionellen ESR-Spektrometer (Hilger & Watts). Die erhaltenen Spektren wurden als 1. Ableitung der Absorption registriert.

In den ESR-Spektren von Adenin·HCl für verschiedene Orientierungen des Kristalls im Magnetfeld (Abb. 1) überlagern sich die Linien dreier spektraler Komponenten, von denen eine aus einem schwach ausgebildeten Triplett mit einem Linienabstand von etwa 40 Gauß und einem Amplitudenverhältnis der Linien von annähernd 1:2:1 besteht, wie es durch die Balken unter einem Spektrum der Abbildung angedeutet wird. Ein Triplett dieser Struktur wird in allen bislang unter-

suchten Einkristallen von Purin-Derivaten beobachtet und gewöhnlich einem Radikal zugeschrieben, welches durch Anlagerung atomaren Wasserstoffs an einen Kohlenstoff einer N=C-Doppelbindung des Purin-Ringes unter Aufbrechen dieser Bindung entsteht. In Guanin-Abkömmlingen findet die Anlagerung an der Position C-8, in Adenin-Derivaten an C-2 statt¹⁻⁴. Eine ausführliche Analyse dieses Radikals soll hier nicht gegeben werden.

Die übrigen, in den Spektren von Adenin·HCl gegenüber dem Triplett vorherrschenden Komponenten werden von einem breiten Singulett (Halbwertsbreite ~ 20 Gauß) und einer überlagerten scharfen Einzelinie dargestellt. Eine genaue Bestimmung der g -Faktoren dieser Linien wird sehr erschwert durch die gegenseitige Überlappung aller drei Liniengruppen und die teilweise erhebliche Breite der Linien. Innerhalb der so begrenzten Genauigkeit ist der g -Faktor g_E der breiten Linie nahezu isotrop mit einem Wert von etwa $g_E = 2,003$. der g -Faktor des schmalen Singulett erscheint demgegenüber schwach anisotrop.

Die von Einkristallen von Adenosin-5'-Monophosphat nach Bestrahlung erhaltenen ESR-Spektren setzen sich aus denselben Liniengruppen zusammen, wobei jedoch nunmehr das Triplett gegenüber den anderen Komponenten dominiert (Abb. 2). Die breite Einzelinie drückt sich hier in einer Erhöhung der Amplitude der Mittellinie des Triplett gegenüber den beiden äußeren Linien aus; das scharfe Singulett ist wiederum der Mittellinie überlagert. Das starke Anwachsen der Tri-

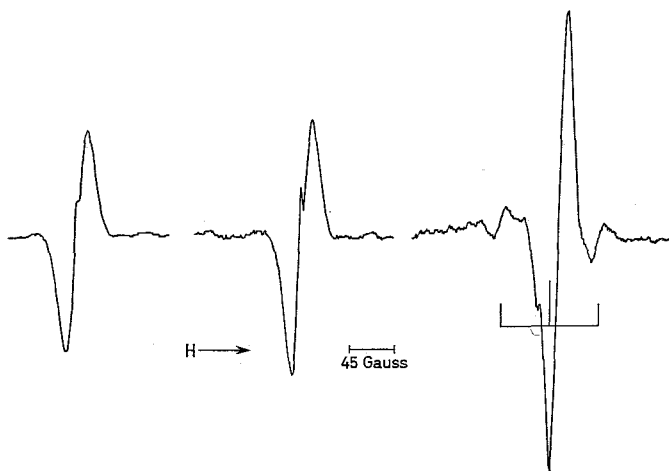


Abb. 1. ESR-Spektren eines bestrahlten Adenin·HCl Einkristalls in verschiedenen Orientierungen zum Magnetfeld.

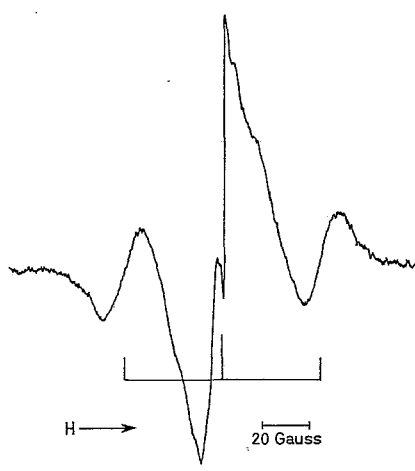


Abb. 2. ESR-Spektrum eines bestrahlten Einkristalls von Adenosin-5'-Monophosphat.

¹ H. DERTINGER, Z. Naturforschg. 22b, 1261 [1967].

² C. ALEXANDER, JR. u. W. GORDY, Proc. nat. Acad. Sci. USA 58, 1279 [1968].

³ J. J. LICHTER u. W. GORDY, Proc. nat. Acad. Sci. USA 60, 450 [1968].

⁴ J. SCHMIDT u. W. SNIPES, Radiat. Res. 38, 274 [1969].

plett-Gruppe gegenüber den anderen Linien beim Übergang von der Base zum Nucleotid wird darauf zurückgeführt, daß der für das Anlagerungsradikal benötigte Wasserstoff hauptsächlich aus der Radiolyse der Zuckerguppe herrührt. Diese Hypothese ist im Einklang mit den an β -2-Desoxy-D-ribose erhaltenen Ergebnissen⁵.

Eine eindeutige Zuordnung der neben dem Triplet in den untersuchten Purin-Derivaten beobachteten Linien zu bestimmten Radikalstrukturen kann auf Grund des Fehlens einer charakteristischen Hyperfeinstruktur nicht getroffen werden. Die qualitative Übereinstimmung der spektralen Komponenten der Base und des Nucleotids legt jedoch den Schluß nahe, daß die Radikalstabilisierung ausschließlich am Basenring stattfindet. Da an Hand einer Korrelation zwischen einer g -Faktor-Verschiebung der ESR-Spektren und der berechneten Elektronegativität die Bildung negativer Ionenradikale für die Purin-Basen gefordert wurde⁶, und da ferner ein breites Singulett das für diese Substanzen typische Spektrum nach Bestrahlung pulverförmiger Proben darstellt⁷, besteht die Möglichkeit, der breiten Linien ein Anion-Radikal des Adenins zuzuschreiben. Zwar sollte nach den Berechnungen der zugehörigen Spindichten⁸, wie sie in Abb. 3 gezeigt werden, das am Kohlenstoff C-8 des Purin-Ringes befindliche α -Proton nach MCCONNELLS Formel⁹ eine isotrope Aufspaltung von annähernd -14 Gauß ergeben, jedoch könnte diese durch die mit der auf weitere

Atome stark delokalisierte Spindichte verbundenen Wechselwirkungen unaufgelöst bleiben.

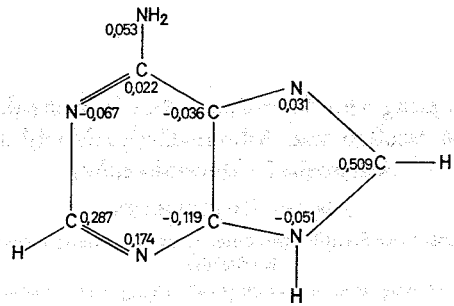


Abb. 3. Spindichten des Anion-Radikals des Adenins nach BAUDET et al.⁸.

Diese Hypothese bedarf weiterer Experimente, die durch eine bessere Trennung der drei spektralen Komponenten eine genaue Analyse des breiten Singulett ermöglichen. Versuche, dies durch eine Variation der Kristallmodifikation und -größe oder der Höhe der Bestrahlungsdosis zu erreichen, blieben bislang erfolglos. Hingegen erscheint es auf Grund einer neueren Arbeit an Ionenradikalen in Cytosin¹⁰ aussichtsreich, durch Benutzung deuterierter Einkristalle eine verbesserte Auflösung der Spektren zu erzielen. Diese Versuche sind in Vorbereitung.

⁵ J. HÜTTERMANN u. A. MÜLLER, Radiat. Res. **33**, 248 [1969].

⁶ M. LACROIX, J. DEPIREUX u. A. VAN DE VORST, Proc. nat. Acad. Sci. USA **53**, 399 [1967].

⁷ A. MÜLLER, Akad. Wiss. Lit. Mainz, Abh. math.-naturwiss. Klasse 1964.

⁸ J. BAUDET, G. BERTHIER u. B. PULLMAN, C. R. hebdom. Séances Acad. Sci. **254**, 762 [1962].

⁹ H. M. MCCONNELL u. D. B. CHESTNUT, J. chem. Physics **28**, 107 [1958].

¹⁰ J. N. HERAK u. V. GALOGAŽA, J. chem. Physics **50**, 3101 [1969].

Electron-spin-resonance spectroscopy of radiation-induced free radicals in irradiated single crystals of thymine monohydrate

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The molecular structures of free radicals formed in irradiated single crystals of thymine monohydrate were investigated by ESR spectroscopy. Two different types of radicals were observed, their relative contribution in the spectra depending on the experimental conditions. One is the previously known 5,6-dihydro-5-thymyl radical, the conformation of which is discussed briefly. It is proposed that the additional radical is formed by loss of a hydrogen atom from the methyl group, leaving an unpaired electron in a $2p_z$ orbital of the methyl carbon atom. The hyperfine spectrum of this radical originates from the interaction of the unpaired electron with the two remaining α -protons of the methyl group and, via resonance delocalization, with the α -proton attached to carbon atom C_6 of the thymine molecule. The hyperfine tensors of these couplings are given together with the g -tensor of this radical.

1. Introduction

The formation of free radicals in the irradiated DNA constituent thymine and its derivatives in the solid state has been extensively studied by electron spin resonance (ESR) spectroscopy. Most of these investigations involved polycrystalline samples (for a review see Müller 1967), but a single crystal study has been performed with thymidine (Pruden, Snipes and Gordy 1965). This work has established that one of the major pathways of radiation damage in these compounds is the addition of atomic hydrogen to the 5,6-double bond of the pyrimidine ring (radical (I) in this report). This type of radical is also observed in DNA (Eisinger and Shulman 1963). It was concluded that the adjacent hydrogen atoms originate mainly from the radiolysis of the deoxyribose group (Müller 1968). However, in the free base thymine, additional radicals are formed on irradiation (Müller 1964), the molecular structure of which cannot be inferred from the ESR spectra of polycrystalline samples. Furthermore, the source of the hydrogen atoms which form the 5,6-dihydro-5-thymyl radical in this compound was not known. As part of a programme to elucidate the primary events in the radiation damage of molecules of biological interest, we have therefore investigated the free radicals produced in irradiated single crystals of thymine monohydrate.

2. Experimental methods

Single crystals of thymine were grown from dilute aqueous solutions. Several crystal modifications are usually obtained by this procedure, and thymine samples from different manufacturers give different results. Only one type of crystal,

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however, the monohydrated form, was grown from samples of Nutritional Biochemical Corporation thymine (Lot No. 6404), which was used throughout this investigation. According to Gerdil (1961), these crystals are monoclinic with space group $P2_1/c$ and contain four molecules in the unit cell of dimensions $a=6.077 \text{ \AA}$, $b=27.862 \text{ \AA}$, and $c=3.816 \text{ \AA}$. The planes of the thymine molecules are nearly parallel to $(\bar{1}21)$. The four molecules form pairs of two magnetically equivalent molecules owing to crystal symmetry. On storage in air at room temperature, there is a rapid loss of the crystal transparency, followed by a pronounced cleavage parallel to (010) . This has been explained by loss of crystal water (Gerdil 1961). The crystals were therefore stored in mother liquor, where changes in transparency are slow.

Figure 1 shows the thymine molecule, the numbering system used, and the external form of the crystals. For ESR measurements, an orthogonal a^*bc -axis system was chosen in which b and c coincide with the crystallographic axes.

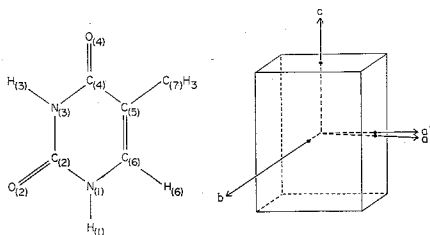


Figure 1. Schematic representation of the thymine molecule and thymine monohydrate crystal. ESR reference axes are a^*bc .

Irradiations were carried out in liquid nitrogen at 77°K or at room temperature, using either 100 kV x-rays or a ^{60}Co γ -source. No differences were observed in the spectra due to the different radiation sources. During irradiation at room temperature, the crystals were kept in a drop of mother liquor.

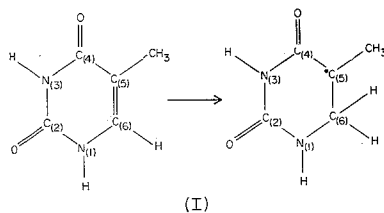
ESR spectra were recorded as the first derivative of the absorption, using commercial spectrometers (AEG, Hilger & Watts, Varian) operating either at 35 GHz (Q-band), or at about 9 GHz (X-band) microwave frequencies. Conventional accessories were used for low-temperature measurements and for the calibration of magnetic fields. The crystals were oriented by means of a goniometer capable of rotation about two orthogonal axes in the cavity. The accuracy of the initial crystal placement was about $\pm 10^\circ$, that of the goniometer rotation within $\pm 2^\circ$.

The g - and the hyperfine tensors were evaluated with computer programmes, using the methods of Schonland (1959) and Lund and Vänngård (1965).

3. Results and discussion

In figure 2, ESR spectra of irradiated single crystals of thymine monohydrate are shown. The upper spectrum was taken at X-band, the lower at Q-band frequencies. Here, the magnetic field H is parallel to the a^* -axis of the crystal. The overall hyperfine spacing of the lines is about 140 Gauss. This value is characteristic of the radical formed by addition of a hydrogen atom to the 5,6-double bond of the thymine molecule according to scheme (I). Since this radical

has been examined in detail in a single-crystal study of thymidine (Pruden *et al.* 1965), no thorough analysis is attempted here. The bars given under the upper spectrum refer to radical (I). Its conformation will be discussed briefly below.



Additional hyperfine lines are seen to overlap the lines of radical (I) in the centre of the spectra of figure 2, indicating the presence of other radicals. For this orientation, six lines are apparent. The relative contribution of the radicals in the spectra varies with the experimental conditions, such as the temperature of irradiation, the temperature of ESR measurements, irradiation dose, incident

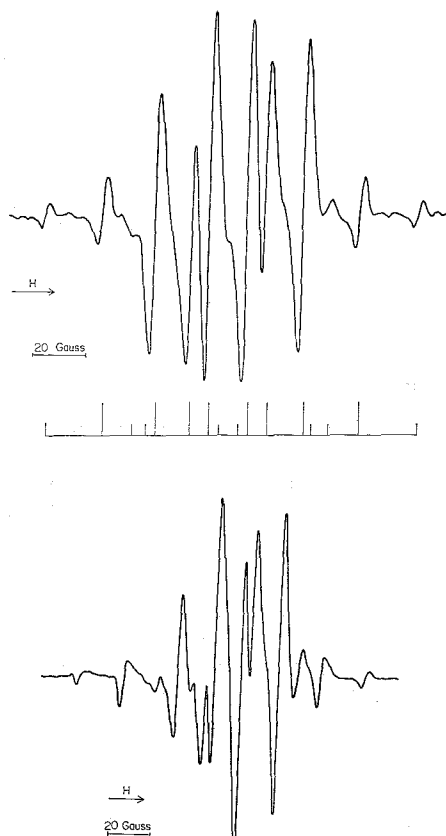


Figure 2. First derivative ESR spectra of thymine crystals irradiated at 300°K with 10 mrads. Magnetic field H is parallel to a^* -axis. Upper spectrum was taken at 9 GHz (X-band), lower at 35 GHz (Q-band) microwave frequencies. Temperature of observation is 300°K for both spectra. Bars under upper spectrum show theoretical hyperfine lines of radical (I).

microwave power, and treatment of the irradiated crystals with u.v.-light. This is illustrated by the series of spectra in figure 3 obtained from one crystal, irradiated at room temperature with about 50 mrads. Again, the magnetic field H is parallel to the a^* -axis of the crystal. The experimental parameters, are given in the figure legend. The notation A, A' and B in the spectra refers to lines of the different radicals, A and A' being lines of radical (I). The approximate intensity ratio of lines B and A is shown beneath each spectrum. Since this ratio changes markedly, depending on the experimental conditions used, the presence of distinct radicals is confirmed.

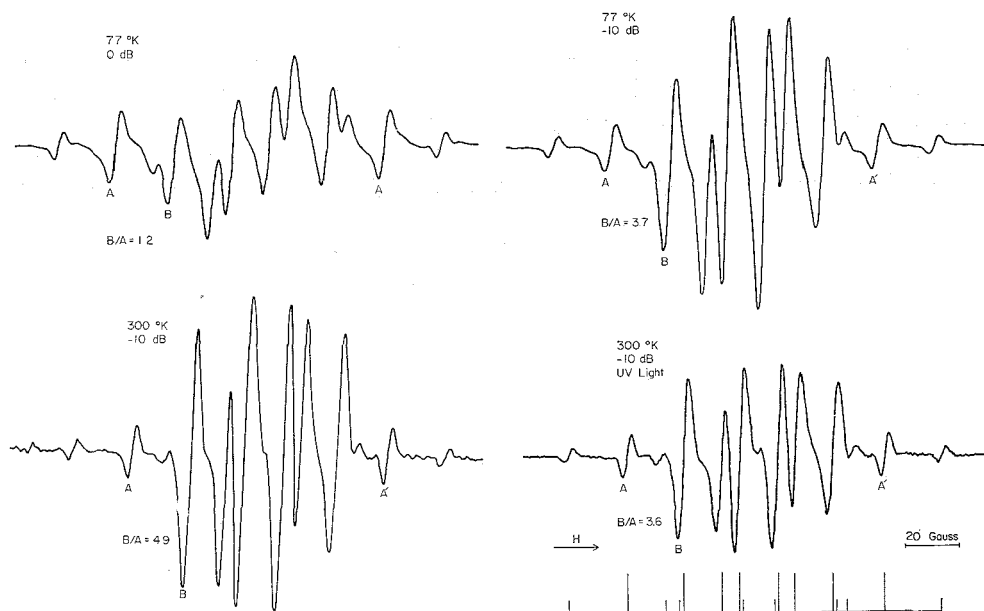


Figure 3. First derivative ESR spectra of thymine crystal irradiated at 300°K with 50 mrads. Spectra are taken at X-band frequencies at 77°K (top left and right) and 300°K (bottom left and right). Incident microwave powers shown in each spectrum are relative to spectrum top left. Bars beneath spectrum bottom right refer to radical (I) and are valid for all four spectra.

In contrast to the ratio of lines B and A, the relative intensities of the lines within the lines of radical (I), A and A', remain nearly constant. This strongly suggests that only one radical, represented by the six hyperfine lines in this orientation, is produced in addition to radical (I). Otherwise, changes similar to those observed for lines B and A should be expected to occur within this group of six lines. Further confirmation of this assignment stems from the fact that the spacing of the six lines is independent of the applied microwave frequency (figure 2). If the lines were due to more than one radical, the differences in their g -factors would result in changes of the line spacings at different microwave frequencies.

The above conclusion that two radicals are produced in irradiated thymine single crystals is in agreement with earlier findings in polycrystalline samples (Pietsch 1967).

The conformation of radical (I) in thymine monohydrate may be inferred from figure 3, on the assumption that its lines completely overlap those of the other radical in this crystal orientation. Its hyperfine spectrum then originates from the interaction of the unpaired electron on carbon atom C_5 with three equivalent protons of the methyl group and two unequivalent methylene protons attached to carbon C_6 . In table 1 the different couplings are listed. The conformation given here differs from that observed in thymidine (Pruden *et al.* 1965), where the methylene protons couple equivalently, but is consistent with the one reported for the analogous radical in crystals of 1-methyl-thymine (Snipes and Schmidt 1968).

Temperature of observation	Methyl	Methylene (a)	Methylene (b)
77°K	21.7	36.19	43.75
300°K	21.7	34.21	40.79

Table 1. Hyperfine splittings of radical (I) in gauss.

The identification of the second radical formed in irradiated single crystals of thymine is not straightforward because of the overlap with radical (I). Figure 4 shows the total spacing of the lines belonging to the second radical in the three crystal planes. The open circles represent the experimental data, the solid lines

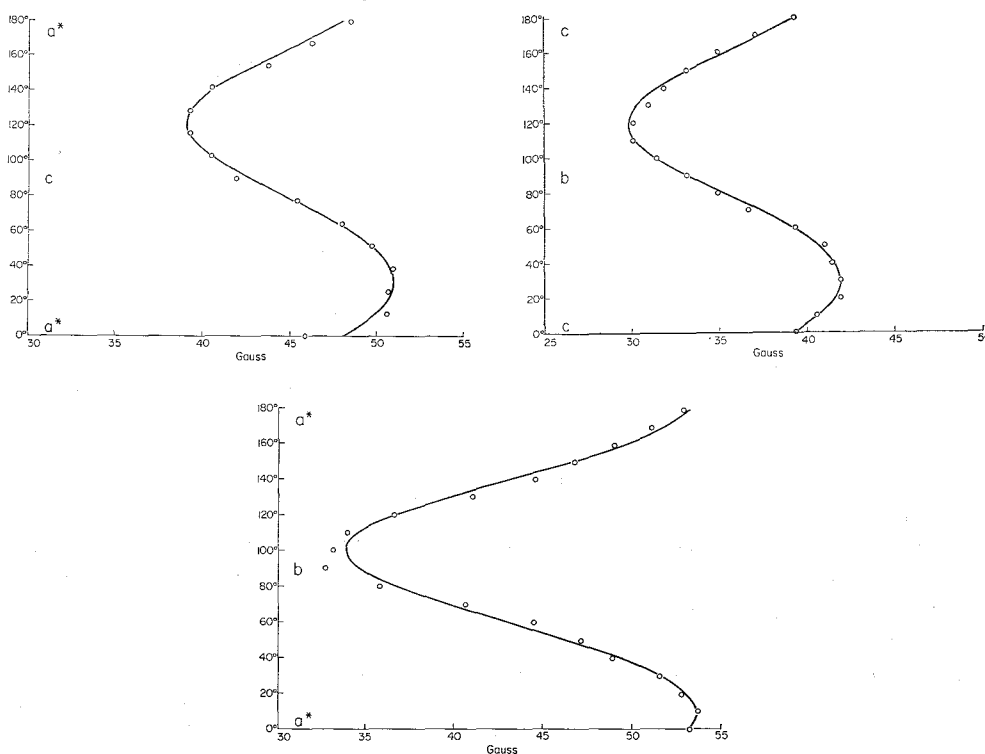


Figure 4. Total spacing of all hyperfine lines of radical II in the three crystal planes (shown at right of ordinate).

are the theoretical curves. It is seen, that the spacing is strongly anisotropic, in contrast to the total hyperfine splitting of radical (I). Furthermore, no marked amount of position splitting can be inferred from the figure. Since this also holds true for radical (I), it is concluded that the planes of the two magnetically unequivalent molecules in the crystal must nearly coincide.

Careful analysis of the spectra obtained from various crystals establish that the hyperfine spectrum of the second radical consists of four, five or six lines, depending on the orientation of the crystal. Because of the anisotropic behaviour of the line splittings, the extent to which individual lines overlap those of radical (I) varies with the orientation. Therefore, an assignment of the intensity ratio of the hyperfine lines of the second radical is complicated. However, in a number of orientations, especially in the *ac*- and *cb*- plane, the lines can be separated into two sets of three lines of roughly equal spacing. This is shown in figure 5 for two crystal orientations. For these and several other similar configurations, the intensity ratio of the triplet lines was verified by means of a curve resolver (Dupont 310) as being approximately 1 : 2 : 1. This suggests the interaction of the unpaired electron with three protons, two of which couple equivalently in these orientations. The above configuration is indicated by the bars beneath the spectra.

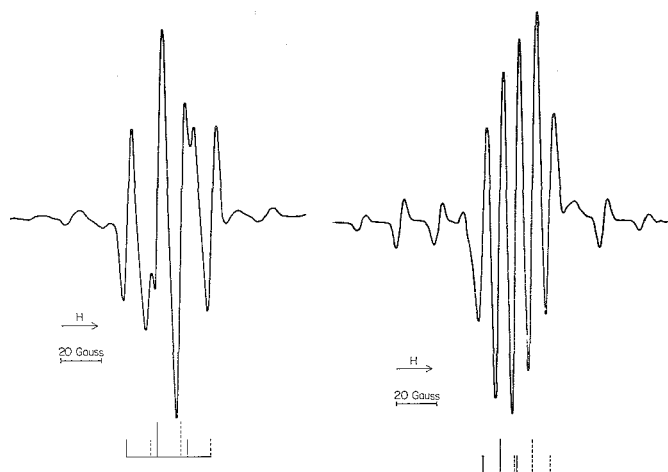


Figure 5. First derivative ESR spectra of irradiated thymine crystals with magnetic field H parallel to c (left) and with H in the ab -plane (right). Bars under spectra refer to radical (II). Spectra are taken at Q-band.

In figure 6 the experimental data for the splitting of the three lines (open circles) and the spacing of the two sets of triplets (crosses) are shown for the three crystal planes. The solid lines indicate the theoretical curves. Obviously, the simple pattern outlined above holds only for the *cb*- and the *ac*-plane. In these planes, both splittings run nearly parallel to each other and can be followed easily from one orientation to the next. Quite a different picture is obtained, however, in the *ab*-plane of the crystal. Here, the hyperfine pattern must be accounted for as consisting of four distinct splittings A, B, C and D, some of which nearly coincide at various orientations, thus complicating the assignment. This feature must be due to position splitting of the two pairs of unequivalent molecules in the crystal, though this could not positively be detected in the total spacing of

the hyperfine lines in figure 4. Since position splitting can be derived from the plot of the individual splittings in the *ab*-plane, the same might be expected to occur in the *cb*-plane, where it could not be observed. But, as will be discussed below, the molecular symmetry for the proposed radical reveals that this kind of splitting occurs only in the *ab*-plane.

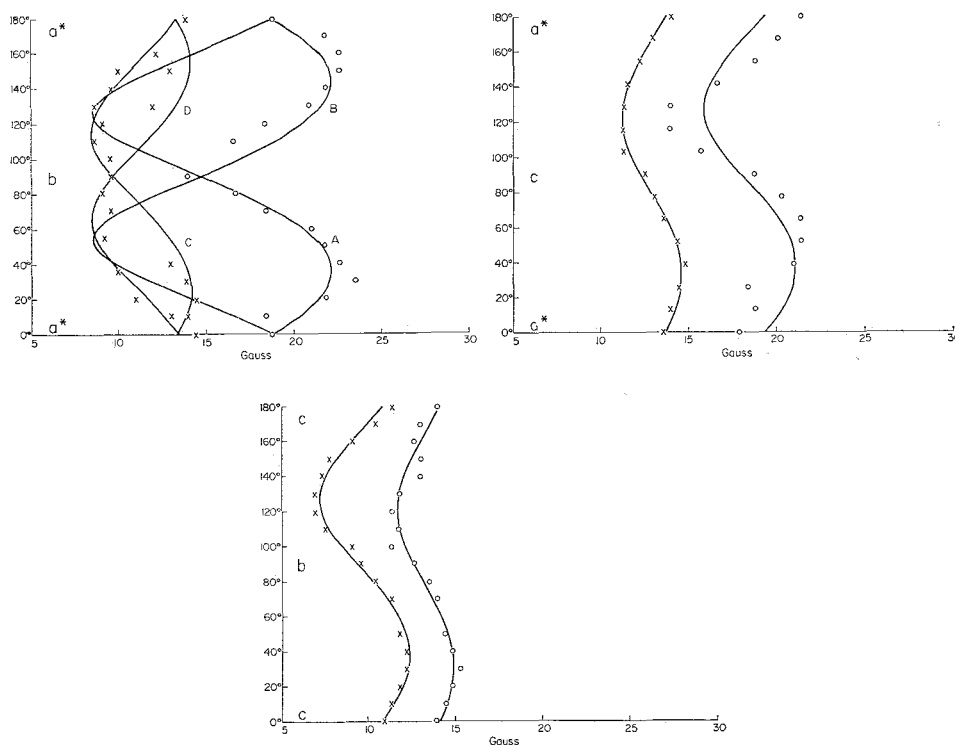


Figure 6. Hyperfine splitting within the triplet (open circles) and spacing of the two triplet sets (crosses) in three crystal planes.

In figure 7, the slopes of the *g*-factor for the second radical is shown in the three crystal planes, as measured at Q-band frequencies. Owing to a systematical error detected in the magnetic-field sweep unit of the spectrometer used (Hilger & Watts), the absolute values of the *g*-factor are an estimated 0.005 too high. The relative values, however, appear to be reliable. The observed *g*-factors are typical of carbon-centred free radicals.

The strong orientation dependence of the splittings in all crystal planes indicates the interaction of the unpaired electron with protons in α -positions, the number of which must be at least three. Without drastic molecular rearrangement of the thymine molecule, this can be achieved only by taking the methyl group into account. We propose that the radical under consideration is formed by removal of a hydrogen atom from the methyl group, leaving an unpaired electron on carbon atom C_7 . The hyperfine pattern then arises from the coupling of the electron with two methylene protons. The additional splitting observed stems from the proton attached to carbon C_6 according to the resonance structures given in Scheme (II).

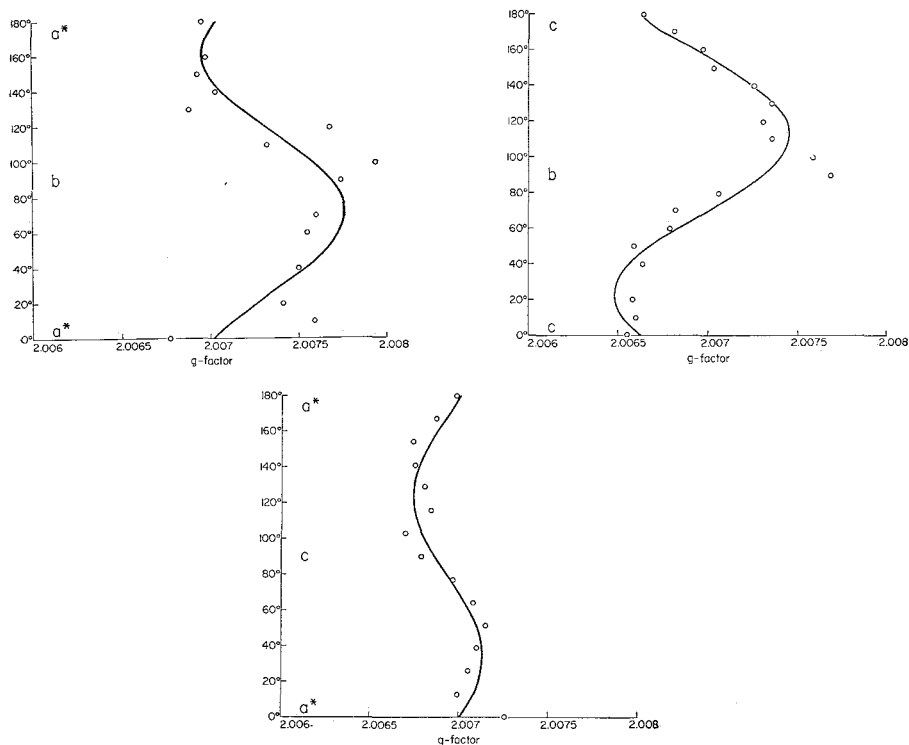
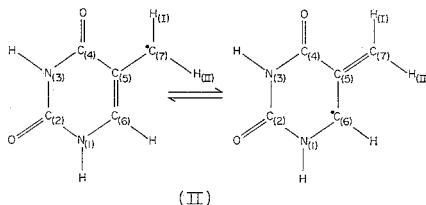


Figure 7. Variations of g -factors for radical (II) in three crystal planes.

For the bonds C_7-H_I , C_7-H_{II} and C_7-C_6 , sp^2 bonding is assumed with the plane of the sp^2 bonding identical to the pyrimidine ring plane and the direction of the C_6-C_7 bond unaltered.

The known crystal and molecular structure of the thymine crystals facilitate the confirmation of the proposed radical structure (II). In figure 8, a projection of the two magnetically nonequivalent molecules in the crystals are shown, using the data of Gerdil (1961). The solid line G represents the trace of the glide plane. According to Scheme (II), the directions of the methylene proton bonds are indicated as dashed lines, which are marked A and B. The approximate directions of the C_6-H_6 are shown as dotted lines, marked C and D. This notation corresponds to the one given for the splittings in figure 6.

Because of the symmetry of this molecular arrangement, it is evident that the molecules are magnetically equivalent with respect to the coupling species of radical (II) in two orthogonal planes perpendicular to the pyrimidine ring,

provided that one of the planes contains the glide plane. Furthermore, the methylene protons must couple equivalently in these planes, leading to a 1:2:1 hyperfine pattern. The extrema of the splittings of the methylene protons and the proton on C₆ should coincide approximately. These features hold for the experimental data in the *ac*- and *cb*-plane of the crystal, as may be inferred from figure 6.

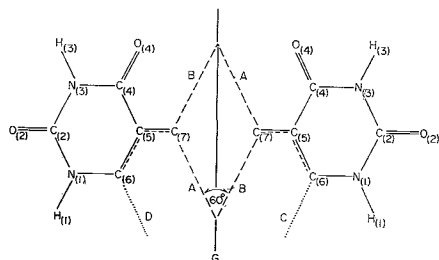


Figure 8. Projection of the two magnetically inequivalent thymine molecules in the crystal unit cell, using data of Gerdil (1961). Directions of methylene protons are shown as dashed lines A and B and protons on C₆ as dotted lines C and D.

For a plane containing the pyrimidine ring, both the positions of the methylene protons and the protons on C₆ are distinguishable, the phase angle between the minimum coupling of the positions being about 60°. Again, the extrema of the coupling of three of the six protons should coincide pair-wise. Comparison with the data for the *a***b*-plane given in figure 6 shows complete accordance with the outlined model, provided that the plane of the pyrimidine ring is closely parallel to the *a***b*-plane of the crystal with the C₆-C₇ bond perpendicular to the glide plane. Taking into account the uncertainty of ±10° of the initial crystal setting, there is agreement between the model derived from the ESR data and the molecular structure of the crystal.

In table 2, the *g*-tensor is given together with the hyperfine tensors of the coupling species for radical (II). The isotropic components a_{H} of the couplings allow an approximate calculation of the unpaired spin densities ρ_e on carbon atoms C₆ and C₇ using the relation (McConnell and Chesnut 1958):

$$a_{\text{H}} = Q \cdot \rho_e,$$

where Q is taken to be -28 Gauss. The spin densities obtained are 0.54 for C₇ and 0.4 for C₆.

4. Conclusion

Of the two different radicals formed in irradiated single crystals of thymine monohydrate, one has been shown to be the previously known hydrogen addition product, the other being due to a removal of a hydrogen atom from the methyl group. The assignment of the latter radical is in complete accordance with earlier findings of Heitkamp, Merwitz and Späth (1968), who reported a considerable amount of radiation-induced hydrogen atom loss from the methyl group of polycrystalline samples of thymine, tritium-labelled at that position.

In terms of a radical balance, it may be assumed that both radicals are formed in equal amounts. Though no attempt has been made in this report to evaluate quantitatively the amount of both radicals in the spectra, it can be stated that such

	Principal values	Isotropic component	Direction cosines		
			a^*	b	c
g	2.0063	—	0.953	0.271	0.132
	2.0078		-0.093	0.681	-0.726
	2.0069		0.287	0.679	0.674
A $^{\alpha}$	15.59	$a_{\text{H}} = (-)15.03$	0.959	-0.206	0.190
	8.12		0.137	0.937	0.320
	22.39		-0.244	-0.281	0.928
B $^{\alpha}$	11.74	$a_{\text{H}} = (-)11.26$	0.827	-0.409	0.384
	7.26		0.359	0.912	0.196
	14.78		-0.431	-0.025	0.902

Table 2. Principal values of g -tensor and hyperfine tensors (in gauss) for one of the methylene protons (A $^{\alpha}$) and the proton attached to carbon C $_6$ (B $^{\alpha}$) for radical (II) in irradiated thymine. Direction cosines relating the principal axes to the ESR-reference a^*bc are given.

a simple balance holds true only very roughly and under restricted experimental conditions regarding the dose and the temperature of irradiation. However, the results indicate that the methyl group is a possible source of hydrogen atoms in thymine nucleosides and nucleotides leading to the formation of radical (I). Since in the presence of the sugar group the contribution of radical (I) is overwhelmingly large (Pruden *et al.* 1965), it must be concluded that the main source of hydrogen atoms in irradiated DNA constituents is the deoxyribose. This is in agreement with recent findings in irradiated molecular mixtures of thymine and deoxyribose (Müller 1968), and with a study of irradiated deoxyribose single crystals (Hüttermann and Müller 1969).

Note.—While this work was being written, a report (UCRL-19298) of an ESR study of irradiated single crystals of thymine monohydrate by Henriksen and Snipes (1969) appeared. These authors attribute the spectra to three distinct radicals. Besides the 5,6-dihydro-5-thymyl radical they claim that the 5,6-dihydro-6-thymyl and the 2-oxo-4-hydroxy-5-methyl-4-pyrimidinyl radicals are formed. This is at variance with the conclusion reached in the present paper, where the spectra are attributed to only two radicals.

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On a étudié les structures moléculaires des radicaux libres produits dans des monocristaux de thymine monohydratée irradiés à l'aide de la spectroscopie à résonance paramagnétique électronique. Deux différentes espèces de radicaux ont été observées, leur contribution relative dans le spectre dépendant des conditions expérimentales. Un des radicaux est déjà connu, c'est le 5,6,-dihydro-5-thymyle dont la conformation est discutée brièvement. Il est proposé que l'autre radical est formé par la perte d'un atome d'hydrogène du groupement méthyle, laissant un électron célibataire dans l'orbite $2p_z$ de l'atome de carbone méthylique. Le spectre hyperfin de ce radical provient de l'interaction de l'électron célibataire avec les protons α restant du groupement méthyle et, via délocalisation par résonance, avec le proton α attaché à l'atome de carbone C_6 de la thymine. Les tenseurs hyperfins de ces accouplements sont donnés, ainsi que le tenseur g de ce radical.

Mit Hilfe der Elektronenspin Resonanz (ESR)-Methode wurde die molekulare Struktur der in bestrahlten Einkristallen von Thymin-Monohydrat gebildeten freien Radikale untersucht. Es wurden zwei verschiedene Radikale beobachtet, deren relativer Anteil in den Spektren von den experimentellen Bedingungen abhängt. Eines der Radikale ist das schon bekannte 5,6-Dihydro-5-Thymyl-Radikal, dessen Konformation kurz diskutiert wird. Es wird vorgeschlagen, dass das zusätzliche Radikal durch Entfernung eines Wasserstoffatoms von der Methyl-Gruppe gebildet wird, wodurch ein ungepaartes Elektron in einem $2p_z$ -Orbital des Kohlenstoffs der Methyl-Gruppe verbleibt. Das Hyperfeinspektrum dieses Radikals kommt durch Wechselwirkung des unpaarigen Elektrons mit den zwei verbleibenden α -Protonen der Methyl-Gruppe und, über Resonanz-Delokalisierung, mit dem am Kohlenstoff C_6 des Thymin-Moleküls befindlichen α -Proton zustande. Die Tensoren dieser Hyperfein-Kopplungen sowie der g -Tensor dieses Radikals werden aufgeführt.

REFERENCES

- EISINGER, J., and SHULMAN, R. G., 1963, *Proc. natn. Acad. Sci., U.S.A.*, **50**, 694.
GERDIL, R., 1961, *Acta crystallogr.*, **14**, 333.
HEITKAMP, D., MERWITZ, O., and SPÄTH, H., 1968, *Z. Naturf. B*, **23**, 403.
HENRIKSEN, T., and SNIPES, W., 1969, Report UCRL-19298, Lawrence Radiation Laboratory, University of California, Berkeley.
HÜTTERMAN, J. and MÜLLER, A., 1969, *Radiat. Res.*, **38**, 248.
LUND, A., and VÄNNGÅRD, T., 1965, *J. chem. Phys.*, **42**, 2979.
MCCONNELL, H. M., and CHESTNUT, D. B., 1968, *J. chem. Phys.*, **28**, 107.
MÜLLER, A., 1964, *Akad. d. Wiss. u.d. Literatur, Math. Nat. Klasse*, Nr. 5, Franz Steiner Verlag, Wiesbaden; 1967, *Prog. Biophys. molec. Biol.*, **17**, 99; 1968, *Molekulare Struktur und Strahlenwirkung* (edited by H. Glubrecht *et al.*) (Stuttgart: G. Thieme).
PIETSCH, E., 1967, Thesis, University of Heidelberg.
PRUDEN, B. L., SNIPES, W., and GORDY, W., 1965, *Proc. natn. Acad. Sci., U.S.A.*, **53**, 917.
SCHONLAND, D. S., 1959, *Proc. phys. Soc.*, **73**, 788.
SNIPES, W., and SCHMIDT, J., 1968, *J. chem Phys.*, **49**, 1443.

