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I. Mechanism of Radical Formation in Irradiated Purine Bases and Derivatives: an E. P. R. Study Using Computer Technique

II. Radical Transformation in Irradiated Cytosine and Derivatives as Studied by E. P. R.

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Mechanisms of radical formation in irradiated purine bases and derivatives: an E.P.R. study using computer technique

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The formation and transformation of radicals induced in dry guanine and adenine and their deoxyribonucleosides and nucleotides by electron irradiation have been studied with Q-band E.P.R. at 77 and 300°K. Two basic mechanisms have been derived: (i) decay of the purine ion radicals with release of atomic hydrogen and (ii) reaction of the hydrogen atoms with the base moieties to yield the adducts at the positions C_2 and C_8, respectively.

1. Introduction

Radical formation in DNA and its constituents by ionizing radiation have been extensively studied with electron paramagnetic resonance (E.P.R.). Different approaches, e.g. quantum mechanical calculations (see Pullman 1965, Sevilla 1971), single-crystal studies (Pruden, Snipes and Gordy 1965, Hüttermann 1970), frozen solutions (Holroyd and Glass 1968, Henriksen and Jones 1970), as well as the use of gas discharges (Herak and Gordy 1965, Dertinger and Carpy 1971) have contributed to the identification of various types of radical. The present stage of work is characterized by the endeavour to find out the pathways of radical formation. Thymine and its derivatives may be regarded as an example of successful research in this respect, since it is now established that protonization and deprotonization of the ion radicals are the most significant mechanisms accounting for the radical transformation processes observed in these compounds (Sevilla 1971, Hartig and Dertinger 1971). However, experiments giving a similar insight into the mechanisms of radical transformation in the purines are lacking. It appears that the absence of pronounced hyperfine structure in most of the E.P.R. spectra of these compounds is, in fact, a serious difficulty for the unequivocal identification of the processes of radical formation involved. Thus, only the radicals originating from addition of atomic hydrogen to the C_8 position of guanine and to the positions C_2 and C_8 of adenine have been adequately analysed from their 1 : 2 : 1 triplet spectra (e.g. Dertinger 1967, Alexander and Gordy 1967, Lichter and Gordy 1968). However, these studies yielded no information concerning the molecular sites of radiation-induced release of hydrogen responsible for this adduct formation. Schmidt and Borg (1971) subjected selectively deuterated adenine derivatives to γ-irradiation and to bombardment with H and D atoms from a gas discharge. These experiments permitted to characterize the sites of hydrogen release. But these authors did not analyse the other radicals observed in the purine spectra. The nature of

the precursor radicals involved in the production of hydrogen atoms remained, therefore, obscure. Other attempts to analyse the typical broad singlet-like purine resonances, unfortunately, did not lead to conclusive results (Lacroix, Depireux and Van de Vorst 1967, Hüttermann 1969).

The present experiments were designed to fill up as far as possible these gaps in our knowledge. The aim was to identify the primary radical species, to search for trapped atomic hydrogen at low temperature, and to derive a rough scheme of radical formation and transformation.

2. Experimental methods

Dry powders of the A Grade compounds adenine, deoxyadenosine, deoxyadenosine-5′-monophosphate, guanine, deoxyguanosine, and deoxyguanosine-5′-monophosphate (Schuchardt and Serva products) were subjected to irradiation with 2 MeV electrons from a Van de Graaff accelerator the dose being approximately 3 Mrads. Irradiation was performed at 77°K in a cryostat which also contained the microwave cavity. The sample and the cavity were cooled by an intense stream of cold nitrogen gas. On irradiation, the E.P.R. spectra were taken immediately. The cryostat was then warmed up to room temperature, after which the 300°K spectra were recorded.

A modified Q-band spectrometer was used for the E.P.R. measurements. The application of computer technique enabled the spectra to be stored on magnetic tape with preservation of the g-factor, thus allowing subtraction or addition of the recorded spectra. Details of irradiation, spectrometer, and computer assembly have been given in an earlier paper (Hartig and Dertinger 1971).

3. Results

3.1. *Guanine*

Figure 1 (*a*) shows the E.P.R. spectrum of guanine obtained upon irradiation at 77°K which is a typical one-radical signal centred at $g = 2{\cdot}0043$. From its

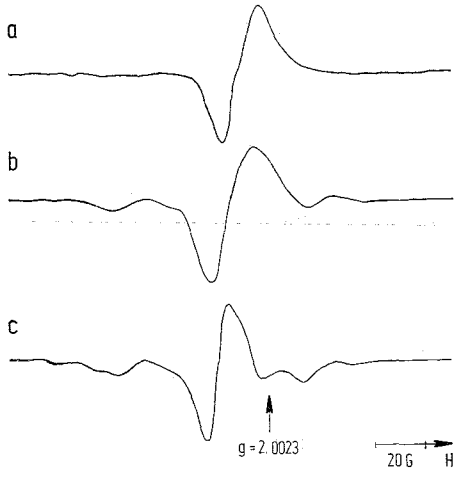


Figure 1. First derivative of the guanine E.P.R. spectrum (*a*) after irradiation at 77°K, measured at 77°K (gain 6), and (*b*) after irradiation at 300°K, measured at 300°K (gain 60). (*c*) Spectrum obtained by subtracting (*a*) from (*b*).

shape a doublet-like structure can be inferred rather than a singlet. A poorly-resolved doublet is to be expected for the guanine anion radical from quantum mechanical calculations predicting a spin density of approx. 0·25 at the position C_8 of the guanine anion (see Pullman 1965). The hydrogen bound at this position—according to $A=\rho\cdot Q$ ($\rho=0{\cdot}25$; $Q_{CH}=24$ G ; cf. Sevilla 1971)—should produce a doublet splitting of $A=6$ G. Since the remaining 75 per cent of spin density are highly delocalized, no significant additional hyperfine structure from other positions is to be expected. In view of an estimated line-width of 6 G, the doublet structure should be poorly resolved, thus supporting the association of the guanine 77° spectrum (figure 1 (*a*)) with the guanine anion.

Besides the guanine anion spectrum, the 512 G doublet of trapped atomic hydrogen could be observed at 77°K, the line-width of the individual signals being 0·4 G. An estimate of the second integrals of the anion spectrum and the hydrogen lines at 77°K revealed that both species are present in approximately stoichiometric concentration. (Precise integration of the spectra is in most cases impossible. Power saturation occurs in Q-band spectroscopy especially at low temperatures even at very reduced microwave power levels, causing deviation of the E.P.R. lines from the first derivative character. Furthermore, we believe that the saturation phenomena compensate the sensitivity gain at low temperature to a large extent. Spectrometer gain settings given in the legends of the figures should therefore be understood to mean rough approximations.)

The production of the 300° spectrum of guanine required massive irradiation (~50 Mrads) at room temperature. The spectrum observed thereafter is reproduced in figure 1 (*b*). It consists of a broad asymmetric central part with satellites occurring at both sides, the latter ones belonging to the 1 : 2 : 1 triplet (37 G coupling ; $g=2{\cdot}0051$) of the guanine C_8-adduct of atomic hydrogen (Alexander and Gordy 1967). The central triplet line does not, of course, contribute very significantly to the central portion of the total spectrum in figure 1 (*b*) which is considered to be mainly a super-position of the guanine anion spectrum and another signal. The subtraction of the anion resonance (figure 1 (*a*)) from the 300° spectrum (figure 1 (*b*)) yields the spectrum shown in figure 1 (*c*) which is essentially a singlet at $g=2{\cdot}0055$ embedded in the triplet spectrum. Again the spin-density calculations (see Pullman 1965) can be used for identification. They predict for the guanine cation radical a spin concentration of 17 per cent at the C_8 position. The resulting 4 G doublet splitting of the hydrogen attached to C_8 is, of course, too small to be resolved at 6 G line-width. Consequently the assignment of the singlet in figure 1 (*c*) as being due to the guanine cation radical is justified. Further support for the correct identification of the guanine ions stems from the *g*-factors of the individual spectra. In the course of these studies, we have observed that the anions of the DNA bases always have smaller *g*-factors than the cations (cf. Hartig and Dertinger 1971), although this may not be expected generally for organic compounds on the basis of theoretical considerations (Stone 1963).

3.2. *Deoxyguanosine*

The spectrum of deoxyguanosine at 77°K is plotted in figure 2 (*a*). In contrast to guanine the triplet of the C_8-adduct is observed. Since the adduct formation requires the consumption of trapped atomic hydrogen, one expects the

concentration of this species to be reduced. In fact we observe less hydrogen trapped than in the free base (about 10 per cent of the base figure). Furthermore a detailed analysis of the central portion of the 77° spectrum (figure 2 (*a*) revealed that both guanine ions are present. (As base ions in the case of nucleosides and nucleotides, we denote the corresponding compounds with positively or negatively ionized base moieties.) After warming-up, the spectrum in figure 2 (*b*) is obtained, which differs from the 77° spectrum only in so far as it shows a reduced ion contribution. When the remaining small ion signals are eliminated by subtracting therefrom the 77° spectrum (figure 2 (*a*) the pure C_8-adduct spectrum is obtained (figure 2 (*c*). The apparent doublet splitting of each triplet line is remarkable and can be explained by additional proton coupling and/or nitrogen hyperfine structure from N_7 (Alexander and Gordy 1967) or, alternatively, by 'site-splitting' due to the axially symmetric *g*-tensor which gives a pronounced effect in Q-band spectroscopy (Dertinger 1967). This question is, however, of minor significance for the present investigation.

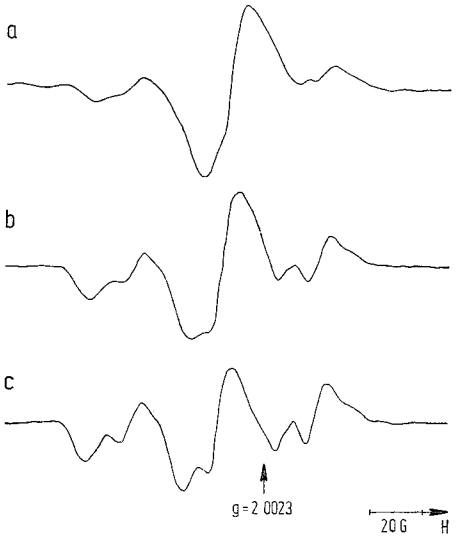


Figure 2. First derivative of the deoxyguanosine E.P.R. spectrum after irradiation at 77°K, measured at (*a*) 77°K (gain 25), and (*b*) 300°K (gain 40). (*c*) Spectrum obtained by subtracting (*a*) from (*b*).

3.3. *Deoxyguanosine-5'-monophosphate (dGMP)*

The spectra of dGMP at 77°K and after warming-up are shown in figure 3 (*a*) and (*b*). The same statements can be made as in the case of deoxyguanosine, the only significant modification at 77°K being less triplet contribution and, in turn, stronger ion signals in dGMP. Again the C_8-adduct triplet (figure 3 (*c*)) is obtained on subtraction of the 77° spectrum (figure 3 (*a*)) from the room temperature spectrum (figure 3 (*b*)), which is identical with the corresponding spectrum in deoxyguanosine (figure 2 (*c*)). The relative concentration of atomic hydrogen trapped at 77°K is about 3 per cent of the amount found in the irradiated base.

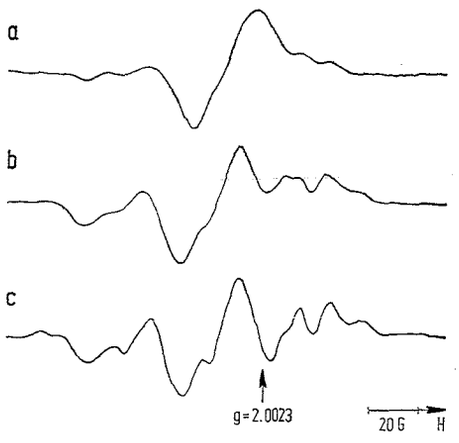


Figure 3. First derivative of the dGMP E.P.R. spectrum after irradiation at 77°K, measured at (*a*) 77°K (gain 25), and (*b*) 300°K (gain 50). (*c*) Spectrum obtained by subtracting (*a*) from (*b*).

3.4. *Adenine*

Observations made with adenine are similar to those with guanine. Again massive irradiation at room temperature was required to produce the 300° spectrum (figure 4 (*b*)) since the low temperature spectrum (figure 4 (*a*)) disappeared after warming-up. However, there is a profound difference between adenine and guanine concerning the interpretation of the 77° spectrum representing a broadened single line at $g=2{\cdot}0040$ (figure 4 (*a*)). As in the case of guanine, the theoretical E.P.R. spectra of the adenine ions can be predicted on the basis of molecular orbital calculations yielding spin concentrations at the C_8-position of 51 and 23 per cent for the adenine anion and cation, respectively (see Pullman 1965), the rest of spin being delocalized over the ring skeleton. Therefrom in analogy to guanine (see § 3.1) doublet splittings of 12 and 5 G should result for the anion and cation spectra, respectively, the latter one being unresolvable in view of an estimated line-width of 6 G. Therefore, we may justifiably associate the 77° signal of adenine (figure 4 (*a*)) with the cation radical in contrast to guanine. Trapped atomic hydrogen is also observed at 77°K. Again its concentration is approximately stoichiometric to the cation radical. However, the width of the hydrogen lines is much greater than in guanine ($\sim 1{\cdot}5$ G). It is interesting to note that the 'life-time' of the trapped hydrogen atoms is relatively small. For adenine we measured a decay of the hydrogen signals to 50 per cent of the initial amplitude to take place in approx. 9 min at 77°K. This means that rapid measurement without prolonged sample handling after irradiation is required to detect these lines.

The 300° spectrum of adenine shown in figure 4 (*b*) is similar to the corresponding guanine spectrum and will therefore be subjected to an analogous analysis. Subtraction of the 77° spectrum (figure 4 (*a*)) therefrom yields the signal plotted in figure 4 (*c*). Before discussing this spectrum some remarks are required concerning the 1 : 2 : 1 triplet whose outer lines are seen in figure 4 (*b*). Several investigations have been performed on the corresponding radical (e.g. Lichter and Gordy 1968, Schmidt and Borg 1971). From the results obtained

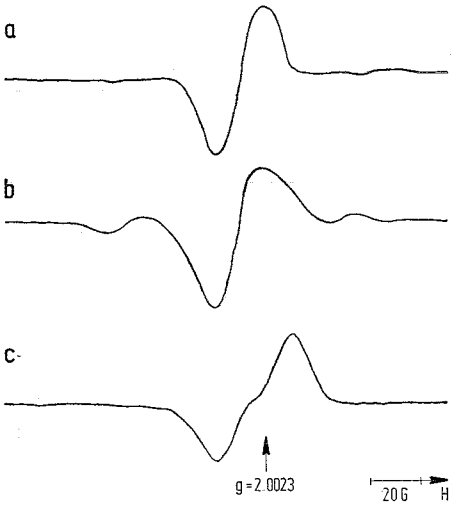


Figure 4. First derivative of the adenine E.P.R. spectrum (*a*) after irradiation at 77°K, measured at 77°K (gain 20), and (*b*) after irradiation at 300°K, measured at 300°K (gain 80). (*c*) Spectrum obtained by subtracting (*a*) from (*b*) and by elimination of the triplet lines using the spectrum of moist adenine (see text).

it must be concluded that both the C_2-adduct and the C_8-adduct of atomic hydrogen (see table 2) can be formed in adenine and derivatives depending on irradiation conditions. In polycrystalline samples the triplet spectra of these two adducts cannot be separated due to their close resemblance. Therefore, we cannot specify the triplet spectrum further. It is interesting to note that irradiation of moist adenine (and also deoxyadenosine) at 300°K enhances adduct formation to such an extent that the triplet becomes the predominant spectral component. We made use of this fact to eliminate the triplet from figure 4 (*b*) before subtracting the 77° spectrum to obtain the spectrum plotted in figure 4 (*c*). This procedure turned out to be very helpful, since now it became evident that figure 4 (*c*) represents in fact a doublet of approx. 12 G splitting centred at $g = 2{\cdot}0032$. As has been shown above, such a spectrum is to be expected for the adenine anion radical. Since in addition the *g*-factor of this doublet is lower than the value of the adenine cation signal (figure 4 (*a*), table 2) the 12 G doublet is considered to be the anion spectrum.

3.5. *Deoxyadenosine*

In figures 5 (*a*) and (*b*), the 77° and 300° spectra of deoxyadenosine are plotted. They are characterized by a 'new' signal structure, namely a broad doublet. The analysis of the 77° spectrum (figure 5 (*a*)) showed that it is composed of this doublet and of the adenine cation (figure 4 (*a*)). On warming-up, the doublet becomes more prominent owing to the decay of the cation signal. At the same time the triplet spectrum appears. Two possibilities exist to present the pure doublet spectrum leading to identical results. In figure 5 (*c*) we show the doublet resonance resulting from subtraction of the triplet spectrum from figure 5 (*b*), the former being obtained by irradiation of moist deoxyadenosine at 300°K (cf. § 3.4). The doublet is centred at $g = 2{\cdot}0040$ and has a splitting of

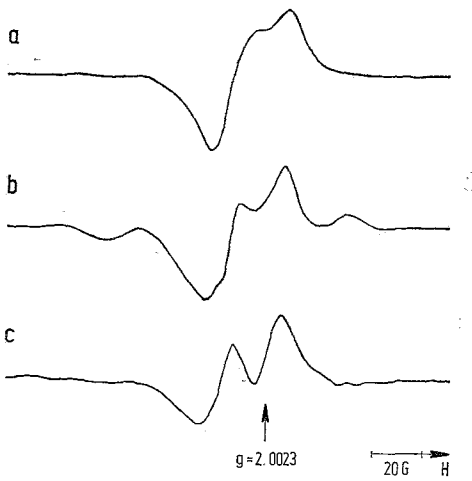


Figure 5. First derivative of the deoxyadenosine E.P.R. spectrum after irradiation at 77°K, measured at (*a*) 77°K (gain 25), and (*b*) 300°K (gain 40). (*c*) Spectrum obtained by subtracting from (*b*) the triplet spectrum resulting from irradiation of moist deoxyadenosine (see text).

17 G (cf. table 2). Since similar doublets have been observed in β2-deoxy-D-ribose at low temperature (Müller 1964) and also in deoxycytidine and deoxycytidine-5′-monophosphate (Hartig, Weibezahn and Dertinger 1972), we ascribe this spectrum to a deoxyribose radical whose structure, however, cannot be inferred from our measurements. Quartet-like spectra have been obtained in single crystals of deoxyadenosine (Alexander and Franklin 1971) and in cytidine-3′-monophosphate (Bernhard and Snipes 1968), which have been ascribed to sugar radicals. It may be that these quartets appear as doublets in the polycrystalline state owing to their anisotropic *g*-factor and hyperfine coupling, although this point still requires more rigorous examination.

From the present investigation the mechanism of the formation of the deoxyribose radical cannot be inferred directly. Yet there are indications that this radical cannot be generated by a primary event in the sugar moiety : (i) it is not formed in deoxyguanosine, (ii) only about 10 per cent of the hydrogen observed in adenine at 77°K is trapped in deoxyadenosine, (iii) the lack of hydrogen is not due to adduct formation at 77°K as in deoxyguanosine (cf. figure 2 (*a*). It may thus be concluded that the deoxyribose radical is generated via a mechanism analogous to the one leading to atomic hydrogen. This point will be outlined in § 4.

3.6. *Deoxyadenosine-5′-monophosphate (dAMP)*

In figures 6 (*a*) and (*b*) the 77° and 300° spectra of dAMP are plotted. An analysis of these spectra revealed that again the adenine ions (predominantly the cation) contribute to their central portion. Yet there are two lines at the right-hand side that have not been detected in other compounds. At 300°K these lines are more pronounced, owing to the decay of the ions. The elimination of the ion contribution by subtraction of the 77° spectrum (figure 6 (*a*)) from the room temperature spectrum (figure 6 (*b*)) yields the spectrum shown in figure 6 (*c*)).

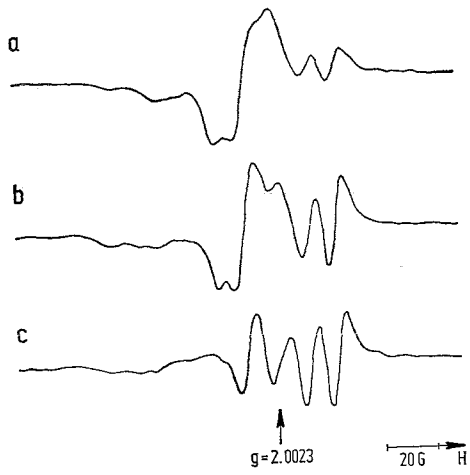


Figure 6. First derivative of the dAMP E.P.R. spectrum after irradiation at 77°K, measured at (*a*) 77°K (gain 10), and (*b*) 300°K (gain 20). (*c*) Spectrum obtained by subtracting (*a*) from (*b*).

It can be seen that the two lines mentioned above belong to a 1 : 1 : 1 : 1 quartet of 36 G total splitting centred at $g = 2{\cdot}0012$ (cf. table 2). Although hyperfine coupling of two non-equivalent protons is reflected in the quartet, its significance is not quite clear. Certainly it is not identical to the quartets identified in single-crystal studies mentioned above (cf. § 3·5.) since neither its hyperfine coupling nor its *g*-factor are compatible with those spectra. On the other hand the spectral data cannot be associated with a radical at the base moiety. We assume that figure 6 (*c*) represents a radical of the nucleotide part centred somehow in the vicinity of the negatively charged phosphate group. This assumption would help to understand the extremely low *g*-factor of the quartet. Probably, transformation of the 'normal' deoxyribose doublet, owing to intramolecular radical site migration into the quartet, occurs in dAMP. Finally the satellite lines on the left-hand sides in figure 6 (*a*) and (*b*) indicate H-adduct formation. Atomic hydrogen is found to be trapped at 77°K in low concentrations.

4. Discussion

The main question is whether the results described in § 3 can be used to establish a scheme of radical formation in irradiated purines. It is evident from the results that the discussion has to be based on the ion radicals of the base moieties, which have been identified as the primary paramagnetic species. Since no direct dissociation products of the bases are found (e.g. of the $X^{\cdot}$ type, if XH denotes the undamaged base moiety) the hydrogen trapped at 77°K must originate from the decay of the ions rather than from the radiation-induced dissociation of an undamaged base. To begin with the free base situation at 77°K, it has been shown in §§ 3.1 and 3.4 that the corresponding spectra are indicative of the guanine anion and the adenine cation, respectively (figures 1 (*a*) and 4 (*a*)). It must be concluded from this that the counter-ions have already decayed at 77°K, releasing the hydrogen atoms trapped and observed at this

temperature in approximately stoichiometric concentration. Thus, we can postulate the following decay process to account for the 77° situation of the free bases (using XH as the symbol for the undamaged bases)

$$XH^{\pm} \longrightarrow X^{\pm} + H^{\bullet} \qquad (1)$$

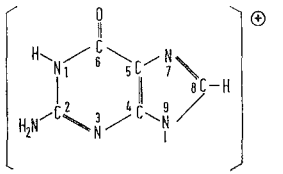
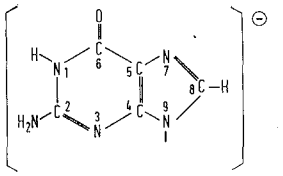
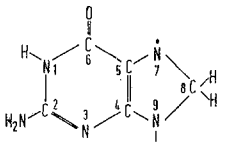
where the positive and negative signs are valid for guanine and adenine, respectively, and where $X^{\pm}$ are diamagnetic products. (For the sake of completeness it should be noted that the cations are formed by direct ionization, e.g. by the loss of an electron, whereas the anions represent the primary reaction products of thermalized electrons with the bases.) On warming-up the ions that are stable at 77°K also decay. Although the mechanism of this decay cannot be followed directly by E.P.R. we may conclude that it is again the basic process expressed by equation (1). In other words, the ions of both signs decay consecutively, one at temperatures below 77°K (delivering hydrogen that can be trapped at 77°), the other at temperatures above 77° (probably delivering hydrogen that cannot be detected directly by E.P.R.). In support of this assumption, the work of Schmidt and Borg (1971) demonstrates that two different sites of hydrogen release (at least for adenine) exist that can be associated with the ions of both signs in the light of our results. Additional support for this conclusion stems from the different line-widths of the hydrogen signal observed in guanine and adenine at 77°K. This phenomenon indicates that in the pathways of cation and anion decay hydrogen is released from different bonds and consequently trapped in magnetically distinct positions. This in turn leads, according to Schmidt and Borg, to the independent formation of the C_2- and the C_8-adduct in adenine and derivatives.

Formation of the adducts ($XH^{\bullet}_2$ see tables 1 and 2) in the free bases is observed only after massive irradiation at room temperature (figures 1 (*b*) and 4 (*b*)). This phenomenon is best explained by taking the fact into consideration that the activation enthalpy ($\sim$1 kcal/mole) necessary for the reaction of $H^{\bullet}$ with the purine bases prevents the corresponding adducts to be formed at lower temperatures. Consequently on warming-up, reactions (1) take place before the reaction

$$XH + H^{\bullet} \longrightarrow XH^{\bullet}_2 \qquad (2)$$

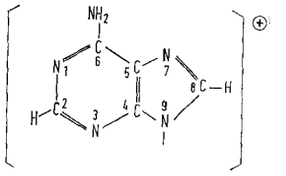
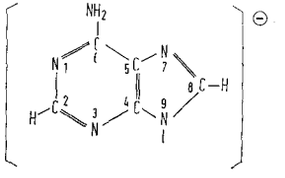
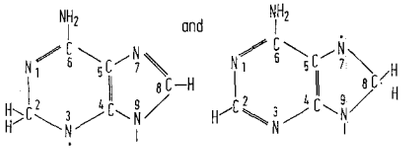
can occur to an appreciable amount. The discussion of the results obtained with the purine nucleosides and nucleotides requires a separate treatment of the guanine and adenine compounds. Deoxyguanosine (figure 2) indicates some slight but not substantial modifications as compared with guanine (figure 1): (i) at 77°K both ions contribute to the central part of the E.P.R. spectrum; (ii) some C_8-adduct formation according to equation (2) has already taken place at 77°K; (iii) correspondingly less atomic hydrogen is trapped compared with the free base; and (iv) only after warming-up is the decay of the ions observed, leading to a strong adduct formation. These modifications are easily explained by assuming higher stability of the ions (probably due to the attachment of the deoxyribose) and a higher rate constant of reaction (2) in the nucleoside facilitated by structural reasons. As can be seen by comparing figures 2 and 3 these statements are also valid for dGMP.

From §§ 3.5 and 3.6 it is evident that the reactions (1) and (2) also proceed in deoxyadenosine and dAMP. However, as opposed to the guanine derivatives, deoxyribose radicals were shown to be formed in these compounds. Although

Type of radical and spectrum	Hyperfine splitting (G)	*g*-factor	Symbol†	Formula
Cation : singlet/doublet	Theoretical : 4 Experimental : unresolved	2·0055	XH^+	
Anion : doublet	Theoretical : 6 Experimental : poorly resolved	2·0043	XH^-	
C_8-adduct of $H^\bullet$: 1 : 2 : 1 triplet (Alexander and Gordy 1967)	Approximately 37	2·0051	$\dot{X}H^\bullet_2$	

†Guanine moiety = XH.

Table 1. Radicals identified in guanine and derivatives.

Type of radical spectrum	Hyperfine splitting (G)	*g*-factor	Symbol†	Formula
cation : singlet/doublet	Theoretical : 5 Experimental : unresolved	2·0040	XH^+	
Anion : doublet	Approximately 12	2·0032	XH^-	
C_2 and C_8 adducts of $H^{\bullet}$: 1 : 2 : 1 triplet (cf. Dertinger 1967, Lichter and Gordy 1968)	Approximately 41	approximately 2·0045	$XH^{\bullet}_2$	

† Adenine moiety = XH.

Table 2. Radicals identified in adenine and derivatives. *(continued)*

Table 2—(*continued*)

Type of radical spectrum	Hyperfine splitting (G)	*g*-factor	Symbol†	Formula
Deoxyribose radical in deoxyadenosine; doublet	17	2·0040	?	?
Deoxyribose radical in dAMP : 1 : 1 : 1 : 1 quartet	36 (total)	2·0012	?	?

† Adenine moiety = XH.

Table 2. Radicals identified in adenine aud derivatives.

there is no indication of a direct interference of these species with the processes (1) and (2) one could argue in favour of a formation of these radicals in the pathway of ion decay. In view of the two sites of hydrogen release from irradiated adenine compounds proposed by Schmidt and Borg (1971), and our conclusion that these can be associated with the two ions, the following hypothesis appears attractive. If the N_9 position of the anion were the predominant site of hydrogen release in adenine under the present experimental conditions, then in deoxyadenosine and dAMP instead of atomic hydrogen a deoxyribose radical would be released from this position. This assumption is justified since we have given evidence that the anion spectrum itself is not present in figures 5, 6, and 4 (*a*) but rather the spectra of its ' decay products ', i.e. atomic hydrogen and the deoxyribose radicals. The contribution of the anionic decay is, however, of minor importance in the guanine compounds since the anion is much more stable than in the case of the adenine compounds. This is reflected in the presence of the anion in figures 2 and 3 as discussed in §§ 3.2 and 3.3. Therefore, a release of hydrogen or deoxyribose radicals from the N_9 position of the anion in analogy to the adenine case is much smaller. Concerning the trapped hydrogen, this conclusion can be confirmed directly, since the reduced E.P.R. line-width in guanine is indicative of a site of release and trapping being different from the anion (namely the cation). Thus it is to be expected from this hypothesis that deoxyribose radicals should not be formed in deoxyguanosine and dGMP, or at least in concentrations much lower than in deoxyadenosine and dAMP. Although, this is, in fact, observed in our experiments, the evidence for such a mechanism is not yet conclusive. A study of base liberation on irradiation of dry nucleosides, nucleotides, and DNA is desirable, since it could prove our hypothesis directly. Unfortunately investigations of this type have so far always been carried out in aqueous solutions where the indirect effects are more dominating (Hems 1960, Ullrich and Hagen 1971).

The fact that such insufficient paramagnetic information on radiation-induced direct sugar destruction is gained from the E.P.R. studies is, of course, remarkable. It is probably incorrect to conclude therefrom that in the dry state the deoxyribose moiety is not ionized at all. But it is imaginable that the attached bases, which have pronounced electron donor and acceptor properties, compensate most of the charge alterations resulting from an ionizing event in the sugar by some kind of charge transfer. This would mean that the ionized sugar generally does not attain stable paramagnetism in the nucleosides and nucleotides but that this paramagnetism ultimately occurs in the base.

In conclusion, the results obtained from these experiments have given evidence for hydrogen atom formation originating from the decay of the purine base ions and the reaction of these atoms with undamaged base moieties. It would be interesting to see if these processes can be identified in DNA itself. In a recent paper (Hartig and Dertinger 1971), we have shown that paramagnetic reaction products resulting from hydrogen atom attack are not observed in significant amount in the E.P.R. spectra of DNA, although atomic hydrogen, probably resulting from reaction (1), is trapped at 77°K. At room temperature thymine radicals contribute predominantly to the hyperfine structure of the DNA spectra whose formation does, however, not require atomic hydrogen, but rather protonization and deprotonization processes (Hartig and Dertinger 1971). This means that hydrogen atoms have little or no chance of reaction with the bases in the dry state, probably due to sterical reasons.

L'apparition et la transformation de radicaux de guanine, d'adénine, ainsi que de leurs désoxyribonucléosides at nucléotides, a été induite à l'état sec par irradiation électronique. Ces transformations ont été ensuite étudiées au moyen de la R.P.E. (36 GHz) à 77 et 300°K. Deux phénomènes principaux ont pu être mis en évidence : (i) la désintégration des ions puriniques avec détachement d'hydrogène atomique, (ii) la réaction de ces atomes d'hydrogène avec des bases encore intactes, ce qui conduit à des produits d'addition en C_2 et C_8, respectivement.

Die durch Elektronenbestrahlung hervorgerufene Bildung und Umwandlung von Radikalen in trockenem Guanin, Adenin sowie in deren Desoxyribonucleosiden und Nucleotiden wurde mittels E.S.R. (36 GHz) bei 77 und 300°K untersucht. Es konnte gezeigt werden, daß folgende Prozesse ablaufen : (i) der Zerfall der Purin-Ionenradikale unter Abspaltung von atomarem Wassestoff und (ii) die Reaktion dieser Wasserstoffatome mit noch ungeschädigten Basen, was zur Adduktbildung an den Positionen C_2 bzw. C_8 führt.

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CORRESPONDENCE

Radical transformation in irradiated cytosine and derivatives as studied by E.P.R.

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A systematic computer-controlled E.P.R. study at 36 GHz devoted to the elucidation of radical reactions after electron irradiation of thymine, purine bases, and their derivatives enabled us to identify the pathways of some radical transformation processes (Hartig and Dertinger 1971, Dertinger and Hartig 1972). In the present note we communicate the results obtained with dry cytosine, deoxycytidine . HCl, and deoxycytidine-5′-monophosphate (A Grade Serva products). Details of irradiation technique, E.P.R. spectrometer, and computer section have already been described (Hartig and Dertinger 1971, Dertinger and Hartig 1972).

Figure 1 (*a*) shows the E.P.R. spectra of cytosine irradiated at 77°K with 3 Mrads. Since no signals of trapped atomic hydrogen could be observed at this

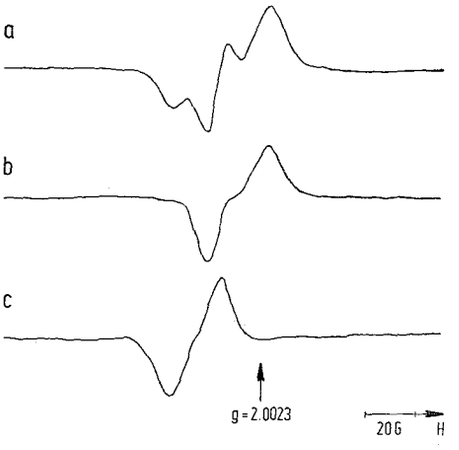

Figure 1. First derivative of the cytosine E.P.R. spectrum after irradiation with 2 MeV electrons at 77°K, measured at (*a*) 77°K (gain 20), and (*b*) 300°K (gain 30), (*c*) Spectrum obtained by subtracting (*b*) from (*a*).

temperature figure 1 (*a*) should represent a primary radical pair. After warming-up to room temperature (300°K), only the signal depicted in figure 1 (*b*) persists. This doublet spectrum of 12 G splitting centred at $g=2{\cdot}0042$ is interpreted to represent the cytosine anion radical on the basis of spin-density calculations (Baudet, Berthier, and Pullman 1962) which predict a maximum spin concentration of 54 per cent at the C_6 position of this species. The hydrogen attached to this position produces according to $A=\rho\,.\,Q(\rho=0{\cdot}54, Q_{CH}=24\,G;$

cf. Sevilla 1971) an isotropic hyperfine splitting of $A=13$ G which is in good agreement with the measured value of 12 G. Since this doublet is obviously also present in the 77° spectrum (figure 1 (*a*)) its subtraction therefrom can be performed yielding the spectrum in figure 1 (*c*) which is a narrow doublet of approximately 8 G splitting centred at $g=2{\cdot}0062$. This spectrum can be associated with the cytosine cation radical since Baudet *et al.* (1962) calculated a spin density of 0·37 at the C_5 position in this species. Thus, in analogy to the anion (see above) a doublet of approximately 9 G is expected for the cation which is in fair agreement with the observed value of 8 G. These conclusions agree with those derived from single-crystal studies from which additional information can be gained concerning the details of the hyperfine pattern (Herak and Galogaza 1969 a). Thus we find both cytosine ions to be present at 77°K with decay of the cation after warming-up. However, the mechanism of this decay cannot be inferred from the free base investigation but rather from the nucleoside and nucleotide to be discussed next.

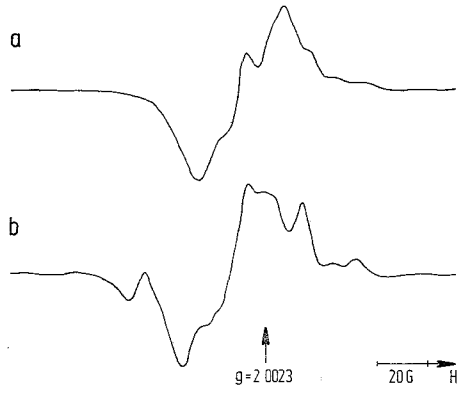

Figure 2. First derivative of the deoxycytidine E.P.R. spectrum after irradiation at 77°K, measured at (*a*) 77°K (gain 10) and (*b*) 300°K (gain 20).

Figure 2 (*a*) and (*b*) shows the spectra of deoxycytidine · HCl after irradiation at 77°K and after warming-up. From visual inspection of these spectra, it can be concluded that a doublet, the most significant structure at 77°, also contributes to the room temperature spectrum. Subtraction of the spectrum in figure 2 (*b*) from figure 2 (*a*) yields the pure doublet (18 G splitting, $g=2{\cdot}0041$) as shown in figure 3 (*a*). This doublet is similar to those found in β2-deoxy-D-ribose at low temperature (Müller 1964) and in deoxyadenosine (Dertinger and Hartig 1972). It is attributed to a deoxyribose radical. On the other hand, substraction of figure 2 (*a*) from 2 (*b*) yields a 1 : 2 : 2 : 2 : 1 quintet at $g=2{\cdot}0036$ shown in figure 3 (*b*). Since this spectrum is identical with the spectrum (figure 3 (*c*), obtained upon exposure of cytidine to bombardment with discharge-excited argon (Weibezahn and Dertinger, in preparation) it cannot representa radical at the sugar moiety. We ascribe it to the adduct of atomic hydrogen at the C_5 position of the cytosine moiety (see Herak and Gordy 1966) the splitting constants being $A_1=20$ G (isotropic coupling of the α-hydrogen at C_6), $A_2=20$ G (coupling of one β-hydrogen at C_5), and $A_3\approx 40$ G (coupling of the other β-hydrogen). Since the adduct formation requires atomic hydrogen one expects atomic hydrogen to be trapped at 77°K where only a small adduct

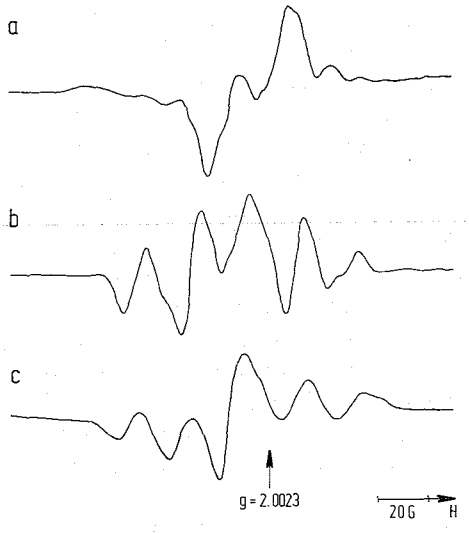

Figure 3. Components of the deoxycytidine E.P.R. spectrum (cf. figure 2) obtained (*a*) after subtraction of the spectrum in figure 2 (*b*) from 2 (*a*) and (*b*) after subtraction of 2 (*a*) from 2 (*b*). (*c*) Spectrum of cytidine bombarded with discharge-excited argon. (The resonance superimposed on the central quintet line is due to another cytosine radical.)

contribution is observed (figure 2 (*a*)). This is actually observed. On the other hand, a more detailed analysis of figure 2 revealed that the ion lines (cf. figure 1 (*b*) and (*c*) are not present, suggesting that the ions have decayed releasing the atomic hydrogen. We believe that the deoxyribose doublet (figure 3 (*a*)) is also produced by such a mechanism, i.e. by decay of the nucleoside upon an ionizing event in the base as outlined by Dertinger and Hartig (1972).

In deoxycytidine-5′-monophosphate (dCMP), the situation is more complex. The spectra obtained at 77°K (figure 4 (*a*)) and after warming-up (figure 4 (*b*)) differ significantly only with respect to the central portion. Again subtraction of the 300° spectrum from the 77° spectrum yields the deoxyribose doublet shown in figure 4 (*c*). Further analysis is not possible, since three radicals appear to be

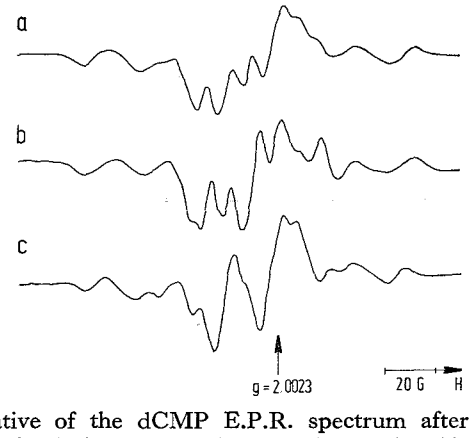

Figure 4. First derivative of the dCMP E.P.R. spectrum after irradiation at 77°K, measured at (*a*) 77°K (gain 20) and (*b*) 300°K (gain 30). (*c*) Spectrum obtained by subtracting (*b*) from (*a*).

involved in the two spectra of figure 4 (*a*) and (*b*) in different relative concentration. However, from comparison with deoxycytidine there is strong evidence that the quintet of the C_5 adduct is also present in the dCMP spectra with slightly modified coupling constants. Furthermore a 1 : 1 : 2 : 2 : 1 : 1 sextet appears to be formed (approximately 122 G total splitting) indicated by its outer lines in figure 4 (*a*) and (*b*). According to the results obtained after bombardment of uracil with atomic hydrogen from a discharge (Herak and Gordy 1965) we ascribe this sextet to the adduct of atomic hydrogen at the position C_6 of the base moiety. Thus, in dCMP we have two adducts of atomic hydrogen even at 77°K which indicates that its reaction is facilitated in this compound and that even the selectivity of the attack (C_5 or C_6 position) is removed, probably for sterical reasons. In agreement with this conclusion, only insignificant concentrations of hydrogen atoms are found to be trapped at 77°K in dCMP, and furthermore no hints of the presence of ions exist. In summary we may conclude that atomic hydrogen originating from the decay of the base ions is involved in the formation of the secondary radicals (e.g. the adducts) the reaction of the hydrogen being facilitated in the direction cytosine ⟷deoxycytidine⟶dCMP. A mechanism of this type has also been suggested by Herak and Galogaza (1969 b) and Dertinger and Hartig (1972) to account for radical transformation processes in other DNA constituents.

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