KERNFORSCHUNGSZENTRUM

KARLSRUHE

September 1964

KFK 271

Institut für Strahlenbiologie

Quantitative E.S.R.-measurements of radiation-induced radicals in nucleotides

Adolf Müller

GESELLSCHAFT FUR KERNFORSCHUNG M.B.H.

KARLSRUHE

Reprinted from
INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, Vol. 8, No. 2, p. 131, 1964

Quantitative E.S.R.-measurements of radiation-induced radicals in nucleotides

ADOLF MÜLLER
Institut für Strahlenbiologie, Kernforschungszentrum, Karlsruhe, Germany

(*Received* 17 *April* 1964)

The nucleotides deoxycytidine-5′-monophosphate, thymidine-5′-monophosphate · Ca, deoxyadenosine-5′-monophosphate · NH_4 and deoxyguanosine-5′-monophosphate · Na_2 have been irradiated as dry crystalline powders at room temperature and subsequently investigated by electron-spin-resonance spectrometry. First derivative spectra of each substance were recorded at 9·4 kMc/sec after doses of x- and gamma-rays up to 10 Mr had been given. Qualitative results include new structural details of nucleotide spectra, and these exhibit a close resemblance to spectra of nucleosides containing the same base.

G-values determined from the initial linear parts of curves showing radical-concentration against radiation dose-range from 2 to 5. Departure from linearity occurs at about 1 Mr. Results on nucleotides, nucleosides, nucleic acid bases and D-2-deoxyribose are compared. While the spectral hyperfine structure is characteristic of the nucleic acid bases, *G*-values and dose-effect curves for radical-concentration are typical of the pentose moiety. This result is tentatively explained by fast radical-transfer from the ribose to the base.

1. INTRODUCTION

Soon after the first application of electron-spin-resonance (E.S.R.) spectrometry to studies of radiation damage in organic compounds, this method was used for studies on nucleic acids and their constituents (Shields and Gordy 1959, Shen Pei-Gen, Blyumenfeld, Kalmanson and Pasynskii 1959, Kirby-Smith 1959, Boag and Müller 1959). These early studies were, however, directed mainly towards the qualitative aspects of the observed E.S.R.-spectra. Determinations of radical-concentrations were rarely even attempted and only after irradiation to high doses, frequently under ill-defined conditions. Although radiation doses are stated more accurately in recent E.S.R.-measurements on irradiated nucleotides (Alexander, Lett and Ormerod 1961), they are restricted to a single and very high dose. However, it will be shown later in this paper that measured yields are strongly influenced by radical saturation at high doses. Some of the earlier results may, therefore, be of little value for comparing radical yield with biological damage. To obtain quantitative information on radical production and to correlate such knowledge with radiation damage to the molecular carriers of genetic information (Zimmer 1961), work on nucleic acid and its constituent compounds was taken up with the aim of reinvestigating the earlier work and extending it by methods developed recently (Köhnlein and Müller 1962). Apart from the introduction of these new methods, spectra had to be recorded and evaluated over a wide range of radiation doses, since it had already been shown that in some materials the radical-concentration increases non-linearly with radiation dose (Zimmer, Ehrenberg and Ehrenberg 1957). In view of the intended comparison with biological radiation damage, measurements at moderate doses

were of special interest to us. In fact, at sufficiently low doses, an initial linear rise of radical-concentration with dose was found for almost all substances investigated. The *G*-values given are those obtained from this initial linear part of the dose-effect curve unless stated otherwise. Since many actions of radiation on elementary biological entities also show a linear dependence on radiation dose-it is essential to know these initial *G*-values for any application of E.S.R., measurements to biological objects.

In the present paper, results of measurements on radiation-induced radicals in nucleotides will be reported and discussed. These results will be compared with those obtained on nucleosides and their constituent nucleic acid bases and pentoses (Müller and Köhnlein 1964, Köhnlein and Müller 1964).

2. Experimental

The same experimental procedure has been adopted in this work as described in a corresponding paper on nucleosides. The nucleotides investigated were deoxycytidine-5′-monophosphate (pdC), thymidine-5′-monophosphate · Ca (pT), deoxyguanosine-5′-monophosphate · Na_2 (pdG), and deoxyadenosine-5′-monophosphate · NH_4 (pdA). These substances were supplied by Schwarz Bioresearch as crystalline powders of the highest purity available and were used without further processing.

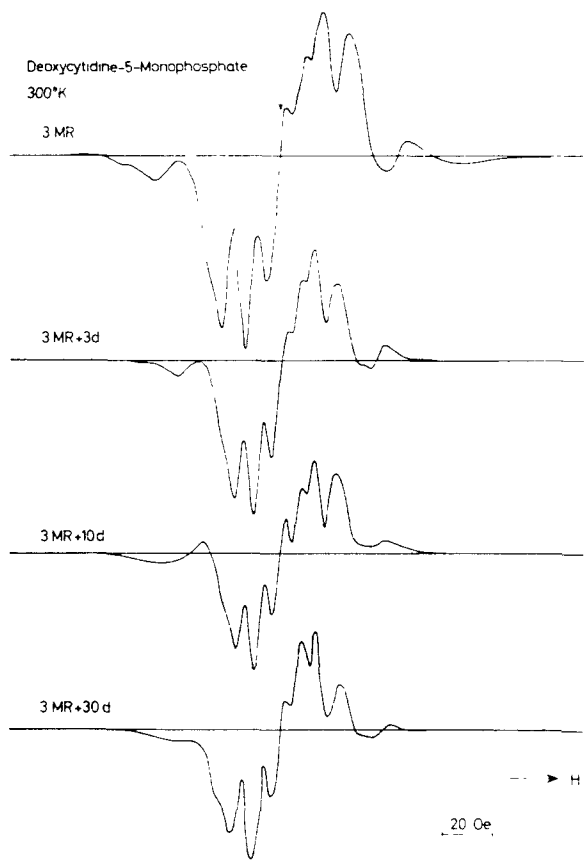

Figure 1. First derivative spectra of deoxycytidine-5′-monophosphate *in vacuo* at room temperature immediately after irradiation and after subsequent storage. Arrow indicates $g = 2{\cdot}0036$.

3. Results

First derivative spectra of pdC, pT, pdG and pdA irradiated and observed *in vacuo* at room temperature are presented in figures 1–4. The only spectrum which changed with time of storage after irradiation is that of pT. This spectrum initially consists of numerous lines which can be divided into at least two groups. Group one contains eight well-resolved lines and a number of smaller satellites and is very similar to the spectrum of thymidine. While this octet spectrum progressively diminishes with time of storage, the second group of lines, which is essentially a doublet, remains unaffected. A simultaneous decrease of radical-concentration is observed which however reduces the initial concentration by only some 20 per cent. In view of the resemblance of the doublet to some spectra obtained with nucleic acid, pT was irradiated and observed at about 100 °K also. In this experiment after irradiation at low temperature a doublet spectrum was obtained, most of which changed to an octet on warming up to room temperature in a manner reminiscent of thymine irradiated at low temperature (Müller and Köhnlein 1964).

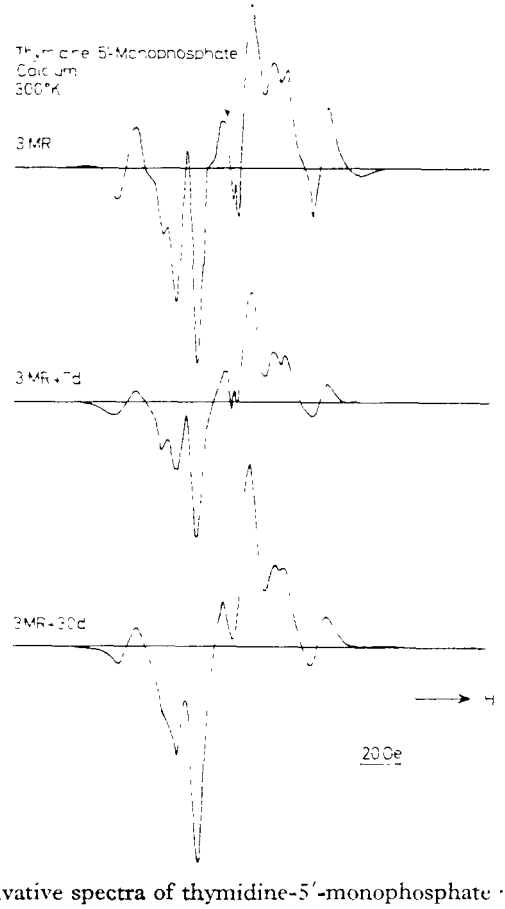

Figure 2. First derivative spectra of thymidine-5′-monophosphate · Ca *in vacuo* at room temperature immediately after irradiation and after subsequent storage. Arrow indicates $g = 2{\cdot}0036$.

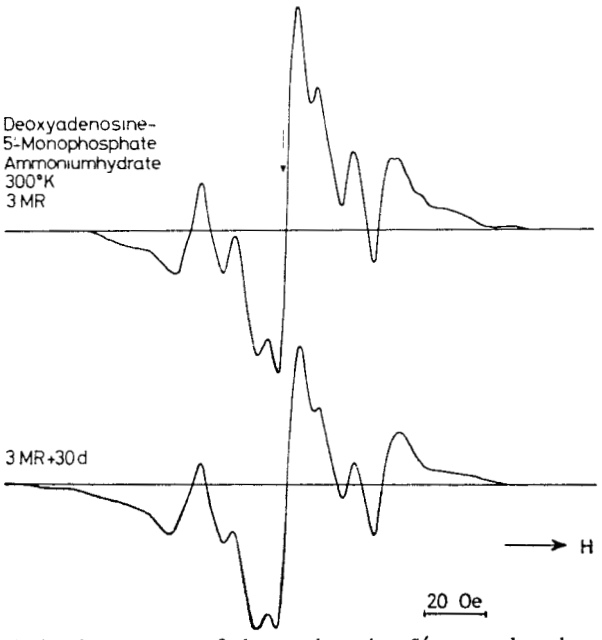

Figure 3. First derivative spectra of deoxyadenosine-5′-monophosphate NH_4 *in vacuo* at room temperature immediately after irradiation and after storage for 30 days. Arrow indicates $g = 2{\cdot}0036$.

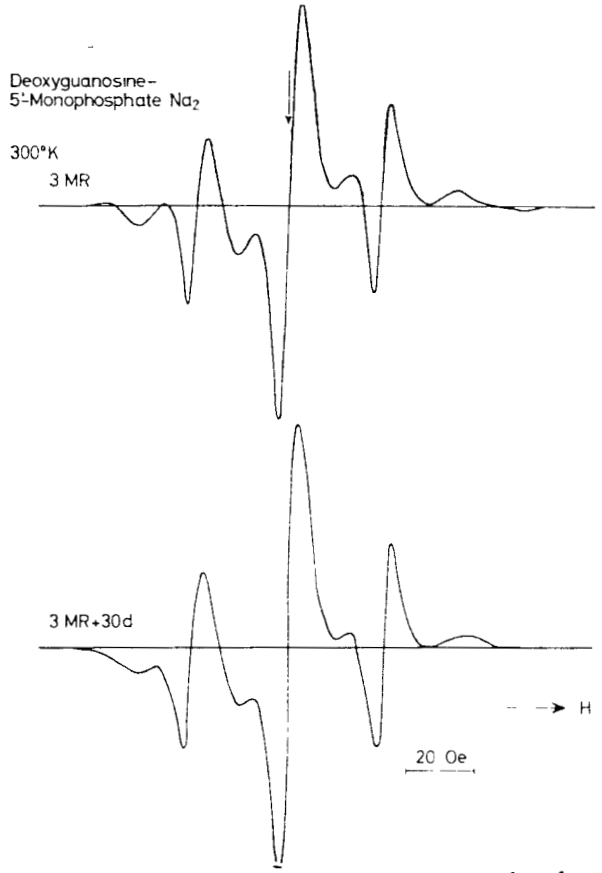

Figure 4. First derivative spectra of deoxyguanosine-5′-monophosphate · Na_2 *in vacuo* at room temperature and after storage for 30 days. Arrow indicates $g = 2{\cdot}0036$.

Nucleotide	$D_{.17}$(Mr)	G-value	s(pN)	Nucleoside	G-values		s(N)		Base	G-values	
	300 °K	300 °K			300 °K	100°K	300°K	100°K		300°K	100°K
pdC	0·8	5	4	dC	1·0	1·0	1·5	0·2	Cytosine	0·4	0·8
pT	7·0	2	4	T	0·4	0·4	3	−0·6	Thymine	0·1	1·5
pdA	1·0	2	0·4	dA	1·4	1·0	13	6	Adenine	0·1	0·14
pdG	1·6	3	2	dG	0·9	1·8	0·1	0·4	Guanine	0·8	1·3
									D-2-deoxyribose	4	2

Quantitative results on nucleotides in comparison with G-values of corresponding nucleosides, nucleic acid bases and D-2-deoxyribose.

With pdC at least nine lines are found, forming a partly-resolved asymmetric spectrum. An even less-resolved asymmetric spectrum of at least seven lines is displayed by pdA, whereas with pdG a symmetric spectrum of three well-resolved main lines and at least four satellites is observed.

The dose-effect curves for radical concentration are plotted in figure 5. All curves in this figure can be described by the function :

$$C = C_\infty [1 - \exp(-D/D_{37})] \quad (1)$$

where C is the radical-concentration, D the radiation dose, and D_{37} the dose at which the radical concentration is 37 per cent less than the saturation concentration C_∞. Equation (1) is also found to apply within the limits of error to many other compounds (Müller 1963, Rotblat and Simmons 1963, Müller, Schambra and Pietsch 1964, Müller and Köhnlein 1964, Köhnlein and Müller 1964). At sufficiently low doses the slope of the curve represented by equation (1) approaches the limiting value C_∞/D_{37} which is a measure of the initial radical yield. Initial yields expressed as G-values are listed in the table together with the D_{37}-values. All G-values of nucleotides are seen to be larger than unity. The D_{37}-values are scattered around 1 Mr except for pT where the D_{37}-value is as high as 7 Mr.

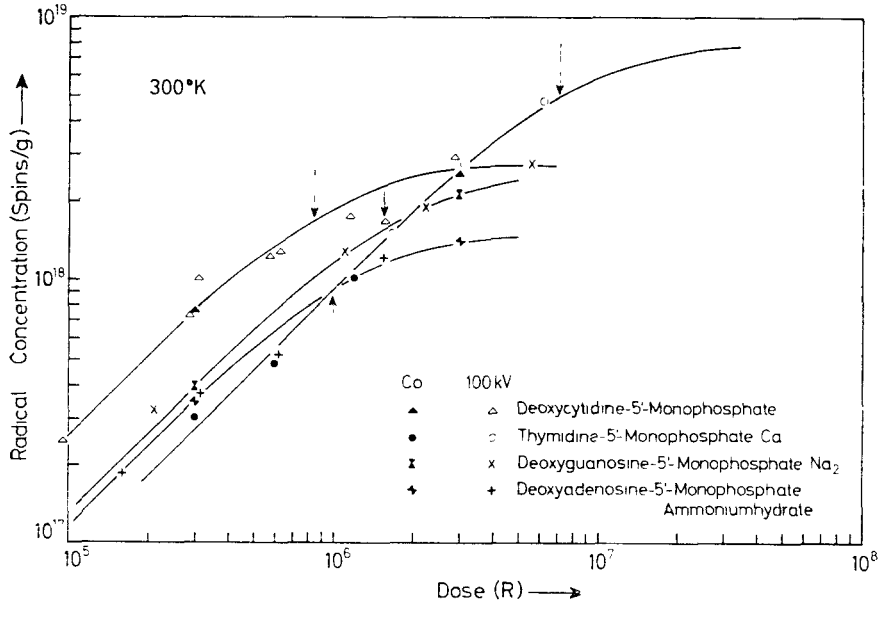

Figure 5. Radical concentrations in nucleotides *in vacuo* at room temperature against dose of radiation. Arrows indicate D_{37}-values.

4. Discussion

Our qualitative results on nucleotides are substantially in agreement with those of Shields and Gordy (1959). However, less hyperfine structure was generally recorded, although second derivative spectra were plotted by Shields and Gordy. This is also true for the observations on nucleosides and other compounds, as evidenced in the corresponding papers (Müller and Köhnlein 1964, Köhnlein and Müller 1964). The lack of resolution in the spectra of Shields and Gordy may

well have been due to over-modulation or to saturation of microwave power. Even less hyperfine structure is found in the spectra published by Alexander *et al.* (1961) probably for the same reasons. The latter authors have determined *G*-values which are between 10 and 20 times lower than those given in the table. However, the *G*-values published by Alexander *et al.* are not initial *G*-values as defined above, but have been determined after delivering radiation doses of 10 Mrad. It can be seen from figure 5 that the radical-concentration has already reached saturation level at such a dose. The resulting reduction of the *G*-value calculated from figure 5 amounts to a factor of 10 and so is in substantial agreement with the figures given by Alexander *et al.* (1961). Both spectra and radical yields in ribonucleotides were reported by Shen Pei-Gen *et al.* (1959) after even higher radiation doses of about 20 Mr. The spectra published in this work show good resolution of hyperfine structure, especially with guanylic acid and cytidylic acid, and closely resemble the corresponding spectra of the deoxyribonucleotides observed by us. The reduction in *G*-value computed from their radical-concentrations and doses is even larger than in the work of Alexander *et al.* (1961). Radical-concentrations given by Shen Pei-Gen *et al.* (1959) are of the same order as our concentrations at saturation. In addition to the results on nucleotides, *G*-values for radical production in nucleosides and nucleic-acid bases are given in the table. Evidently, the radical yields increase when the pentose and the phosphate group are bound to the bases. However, the resulting *G*-values cannot be arrived at by simple addition from the *G*-values and mole fractions of the components. As the pentose moiety contributes approximately half of the weight of a nucleoside molecule, the *G*-value should exceed 2 at room temperature and 1 at low temperature, if radicals were induced in the same way as in the separate compounds. Actually, the only *G*-value which satisfies this condition is the one measured for deoxyguanosine at low temperature. All other nucleoside yields are substantially lower, but still considerably higher than the yield determined for the free bases.

Information about the site of radical-production is obtained by a comparison of the spectra of nucleotides, nucleosides (Müller and Köhnlein 1964), bases, and pentoses (Köhnlein and Müller 1964). Obviously, none of the spectra of nucleotides and nucleosides is the same as the spectrum of their constituent pentose: D-2-deoxyribose. In view of the high radical yield of the free pentose, this result is quite unexpected, especially as it is found not only at room temperature but also at low temperature. The supposition that the spectrum of radicals induced in the free pentose might possibly be changed by binding the latter to a base is excluded by the observation that nucleotides and nucleosides containing different bases yield very different spectra, while there are strong analogies between those of compounds containing the same base. Practically identical spectra are observed after irradiation at room temperature with nucleotides and nucleosides composed of the same base, when this is cytosine, adenine or guanine. Spectra obtained from these three bases as free compounds are significantly different from those of the more complex substances containing the bases, but strong structural analogies are still observed at room and at low temperatures. The spectra of free thymine and the compounds containing thymine also show remarkable resemblances: spectra of free thymine after irradiation at room temperature display either hyperfine splitting attributed to a methyl group, or a doublet presumably due to a single hydrogen atom, depending on the origin of the thymine.

The hydrogen-type spectrum is also found after irradiation at low temperature (Köhnlein and Müller 1964). The spectra of thymidine show the methyl-type pattern at all temperatures (Müller and Köhnlein 1964), whereas in thymidine-5′-phosphate a spectrum composed of both patterns is found immediately after irradiation at room temperature but is transformed slowly into the hydrogen-type spectrum on storage. At low temperature thymidine-5′-phosphate shows the same behaviour as thymine: immediately after irradiation the doublet spectrum is dominating, but decreases in favour of the octet spectrum on warming up. It appears inconceivable that all the different spectra, which have been shown to be largely characteristic of the constituent base, could be due to radicals in the pentose in all the compounds investigated. A further indication for the validity of our argument is seen in the fact that corresponding D_{37}-values are also found for homologous substances, e.g. those containing the same base. Especially conspicuous is the high value for thymine-containing compounds. However, as long as the reasons for radical saturation are not well established, no more can be said about this parallelism.

Consequently, we deduce that sensitization of radical production in the bases by the pentose has not only been shown to occur in some instances, but is the only process compatible with all the observations. Our conclusions are further corroborated by the spectra published by Shen Pei-Gen *et al.* (1959). These spectra obtained from irradiated nucleotides composed of ribose are identical with those observed by us on nucleotides and nucleosides containing D-2-deoxyribose. Numerical values of the sensitization factors for nucleotides (pN) and nucleosides (N) can be defined as following :

$$s(pN) = G(pN)/G(N) - 1,$$
$$s(N) = G(N)/G(Base) - 1.$$

The resulting factors are included in the table. Sensitization factors at room temperature are distributed between 0 and 13. No sensitization is found with deoxyguanosine, and the highest sensitization with deoxyadenosine. The values for nucleotides show somewhat smaller scatter, lying between 0·4 and 4. If the overall sensitization of the nucleic-acid bases by D-2-deoxyribose and the phosphate group is considered, pT and pdA yield high values (19), pdG the lowest (3), and pdC an intermediate value (12). At low temperature some sensitization is still observed, although the numerical factors are always reduced. For thymidine a decrease of radical yield is found in comparison with thymine. However, the G-value for thymine at low temperature may be too high because of unknown systematic errors. This possibility is suggested by the comparison with the values reported by Henriksen (1963), contained in table 2 of the accompanying paper (Köhnlein and Müller 1964).

The question which mechanism is responsible for the observed sensitization cannot at present be answered satisfactorily. Several mechanisms of radical-transfer which could cause sensitization or protection have been discussed previously (Zimmer and Müller 1964). Diffusion-controlled intermolecular reactions were proposed by Henriksen (1963) to be responsible for the accretion of sulphur-radicals in sulphur-containing substances. However, the reaction-rates and temperature-dependence reported by Henriksen suggest that, in the substances studied here, reactions occur which are faster by many orders of magnitude and

therefore are not diffusion-controlled. Intramolecular migration of the unpaired electron to the aromatic-ring system is a very fast process that may be responsible for the observed effects. Actually the stabilization of radicals at the aromatic-ring system in compounds consisting of aromatic and aliphatic constituent groups was already deduced by Molin, Chkheidze, Buben and Voevodskii (1960, 1961) from their work on radiation-produced radicals in organic compounds. Similar conclusions were drawn by Depireux and Müller (1963) from work on dipeptides composed of aromatic and aliphatic amino acids. No energy-transfer could be found with these substances in mixed crystals, and this suggests the absence of intermolecular transfer of energy.

Whether electron vacancies of excitons (e.g. electron–hole pairs) are the carriers of intramolecular energy migration is an open question. From the fact that ionizing radiation is the primary agent, no conclusion can be drawn about the preponderance of ionization over excitation, as it has been shown in large molecules that pairs of radicals may be formed from superexcited states without intermediate ionization (Platzman 1962).

Les nucléotides déoxycytidine-5′-monophosphate, thymidine-5′-monophosphate · Ca, déoxyadénosine-5′-monophosphate NH_4 et déoxyguanosine-5′monophosphate · Na_2 ont été irradiés à l'état de poudres cristallines sèches à température ambiante, puis examinés par résonance de spin électronique.

Chaque substance est soumise à une irradiation aux rayons x ou gamma (doses allant jusqu'à 10 Mr) et son spectre dérivée première enregistré à 9,4 kMc/sec. L'analyse qualitative des spectres détectés dans les nucléotides irradiés révèle de nouveaux détails structuraux en ressemblance étroite avec ceux des spectres des nucléosides renfermant la même base.

Les valeurs de G déterminées sur la partie rectiligne initiale de la courbe représentant la concentration des radicaux en fonction des doses irradiantes sont comprises entre 2 et 5. La courbe s'écarte de la linéarité au voisinage de la valeur 1 Mr. Les résultats obtenus avec les nucléotides, nucléosides, bases d'acides nucléiques et D-2-déoxyribose sont comparés entre eux ; la structure spectrale hyperfine étant caractéristique des bases nucléiques, les valeurs de G et les courbes dose-effet pour la concentration en radicaux sont typiques du groupement pentose. On tend à expliquer ce résultat par un transport rapide des radicaux du ribose sur la base.

Die Nucleotide Deoxycytidin-5′-Monophosphat, Thymidin-5′-Monophosphat · Ca, Deoxyadenosin-5′-Monophosphat · NH_4 und Deoxyguanosin-5′-Monophosphat · Na_2 wurden in Form trockener, kristalliner Pulver bei Zimmertemperatur bestrahlt und anschließend durch Elektronen-Spin-Resonanz-Spektrometrie untersucht Erste Ableitungen der Spektren wurden nach Bestrahlung mit Dosen von Röntgen- und Gammastrahlung bis zu 10 Mr bei 9,4 GHz aufgenommen. Die qualitativen Ergebnisse enthalten neue strukturelle Einzelheiten der Nucleotidspektren, die große Übereinstimmung mit den Spektren der Nucleoside zeigen, wenn die gleiche Base darin enthalten ist.

G-Werte wurden von den anfänglichen linearen Teilen der Kurven, die die Abhängigkeit der Radikalkonzentration von der Strahlendosis wiedergeben, bestimmt und liegen zwischen 2 und 5. Abweichungen von der Linearität treten etwa bei 1 Mr auf. Die Ergebnisse mit Nucleotiden, Nucleosiden, Nucleinsäurebasen und D-2-Deoxyribose werden verglichen. Während die Struktur der Spektren durch die Nucleinsäurebasen charakterisiert wird, sind die G-Werte und Dosis-Effektkurven der Radikalkonzentration typisch für die Pentosegruppe. Dieses Ergebnis wird vorläufig durch eine schnelle Radikalübertragung von der Ribose zur Base erklärt.

References

ALEXANDER, P., LETT, J. T., and ORMEROD, M. G., 1961, *Biochim. biophys. Acta*, **51**, 207.

BOAG, J. W., and MÜLLER, A., 1959, *Nature, Lond.*, **183**, 831.

DEPIREUX, J., and MÜLLER, A., 1963, *Compte Rendu du XII. Colloque Ampère*, Bordeaux.

HENRIKSEN, T., 1963, *Electron Spin Resonance Studies on the Formation and Properties of Free Radicals in Irradiated Sulfur-Containing Substances* (Oslo : Universitetsforlaget).

KIRBY-SMITH, J. S., 1959, *Radiat. Res.*, **11**, 448.

KÖHNLEIN, W., and MÜLLER, A., 1962, *Phys. Med. Biol.*, **6**, 599 ; 1964, *Int. J. Rad. Biol.*, **8**, 141.

MOLIN, YU. N., CHKHEIDZE, I. I., BUBEN, N. YA., and VOEVODSKII, V. V., 1960, *C.R. Acad. Sci., U.R.S.S.*, **131**, 125 ; 1961, *Kinetika i Kataliz*, **2**, 192.

MÜLLER, A., 1963, *Int. J. Rad. Biol.*, **6**, 137.

MÜLLER, A., and KÖHNLEIN, W., 1964, *Int. J. Rad. Biol.*, **8**, 121.

MÜLLER, A., SCHAMBRA, P. E., and PIETSCH, E., 1964, *Int. J. Rad. Biol.*, **7**, 587.

PLATZMAN, R. L., 1962 a, *Radiat. Res.*, **17**, 419 ; 1962 b, *The Vortex*, **23**, No. 8.

ROTBLAT, J., and SIMMONS, J. A., 1963, *Phys. Med. Biol.*, **7**, 489.

SHIELDS, H., and GORDY, W., 1959, *Proc. nat. Acad. Sci., Wash.*, **45**, 269.

SHEN PEI-GEN, BLYUMENFELD, L. A., KALMANSON, A. E., and PASYNSKII, A. G., 1959, *Biofizika*, **4**, 263.

ZIMMER, K. G., 1961, *Studies on Quantitative Radiation Biology* (Edinburgh and London : Oliver & Boyd).

ZIMMER, K. G., EHRENBERG, L., and EHRENBERG, A., 1957, *Strahlentherapie*, **103**, 3.

ZIMMER, K. G., and MÜLLER, A., 1964, *Current Topics in Radiation Research*, Vol. I, edited by M. Ebert and A. Howard (Amsterdam : North-Holland Publishing Co.).