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

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The nucleosides deoxycytidine · HCl, thymidine, deoxyadenosine, deoxyguanosine, deoxyuridine, bromodeoxyuridine and iododeoxyuridine have been irradiated as dry crystalline powders and investigated by electron-spin-resonance spectrometry. First derivative spectra of each substance were recorded at 9·4 kMc/sec after x- and gamma-irradiation with a whole range of doses up to 10 Mr. Qualitative results obtained at about 100°K and 300°K include new structural details of spectra.

Emphasis has been placed on quantitative determinations. When obtained from the initial linear parts of dose-effect curves, *G*-values range from 0·4 to 1·4 after irradiation at room temperature. No considerable differences from these values are found at low temperature but on warming up to room temperature about half of the radicals disappear irreversibly. Departure of radical-concentration from a linear dependence on radiation dose occurs above 1 Mr at all temperatures for the nucleosides investigated.

1. INTRODUCTION

To obtain information on the rôle which radicals induced in nucleic acids play in the biological actions of ionizing radiation, nucleic acids and their components have been studied by E.S.R.-spectrometry. While the results on nucleotides, nucleic-acid bases and pentoses are dealt with in accompanying publications (Müller 1964, Köhnlein and Müller 1964), the present paper is concerned with quantitative measurements at room and liquid-nitrogen temperatures on radiation-induced radicals in nucleosides. Early investigations on radiation-induced radicals in nucleosides contain only scanty quantitative information, and this has usually been obtained from samples irradiated with excessively-high doses of radiation, at which saturation of radical-concentration occurs (Shields and Gordy 1959, Shen Pei-Gen, Blyumenfeld, Kalmanson and Pasynskii 1959).

2. EXPERIMENTAL

Two different E.S.R.-spectrometers have been used in our work: a Varian 4500, and a Hilger & Watts Microspin. Both were operated at a frequency of about 9400 Mc/sec and equipped with rectangular H_{104} double-sample cavities of our own construction (Köhnlein and Müller 1960). The magnetic field was modulated at 100 kc/sec and first derivative spectra of absorption were recorded. For E.S.R.-measurements sample temperatures could be varied continuously between room and liquid-nitrogen temperatures. The microwave power-output

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from the V 153 klystron in the Varian spectrometer was 75 mW, whereas the KS 9–20 A klystron in the Microspin spectrometer supplied 30 mW. Although only about half this power goes into the cavity, the resulting microwave field at the usual Q-values still produces microwave saturation in radiation-produced organic radicals, especially at low temperatures. The Microspin spectrometer was therefore used for measurements at low temperatures, where the microwave power-level had to be kept low to avoid saturation. The power-level at which this condition was satisfied was found by attenuating the microwave power in successive steps until the ratio of signal amplitudes from the sample and the carbon standard in the double-sample cavity remained constant. This sensitive test is possible because the carbon spectrum saturates only at higher power-levels than do the radiation-produced organic radicals. Radical-concentrations were obtained by determinations of the first moments of the spectra following the procedure described previously (Köhnlein and Müller 1962). The integrations were performed partly with a moment balance and partly with a moment planimeter (Amsler).

We obtained the compounds investigated in the form of crystalline powders of the highest purity available from Schwarz Bioresearch (thymidine, deoxyadenosine, deoxyguanosine) and from the Nutritional Biochemicals Corporation (all other compounds) and used them without further processing. Samples of 10–100 mg weight were filled into quartz tubes of 3·5 mm inner diameter. After evacuation on a high-vacuum line for at least 10 hours, the sample tubes were sealed off.

Most irradiations were performed with an x-ray tube operating at 100 kv and 25 mA and emitting soft x-rays through a beryllium window of 1·5 mm thickness. This radiation was filtered by the walls of the quartz tubes (0·5 mm thickness) before being absorbed by the material under investigation. The dose-rate delivered to the samples under these conditions was 50 kr per minute. Because of the difficulties encountered in exact dose-measurements of soft x-rays absolute doses of these irradiations are not known with great accuracy. However, the relative values of the successive dose-fractions given to one sample with E.S.R.-measurements interposed between irradiations, when determining the dependence of radical-concentration on radiation dose, could be controlled precisely by an ionization-chamber monitor. In addition some irradiations were carried out with a ^{60}Co γ-source delivering about 15 kr per minute. This value was checked by two methods: A calibrated ionization chamber and a Fricke dosimeter solution, both methods giving satisfactory agreement. G-values for radical-production are based solely on irradiations with the Co-source because of the greater reliability of the applied doses.

For irradiations at different temperatures both x- and γ-rays were used. x-irradiated sample tubes could be cooled by a stream of nitrogen gas which had passed through a metal coil immersed in liquid nitrogen. The sample temperature could be reduced to about 100 °K in this manner. For E.S.R.-measurements the irradiated material was transferred to one end of the sample tube shielded from x-rays during exposure. During γ-irradiations the sample tubes could be immersed in liquid nitrogen. Following the γ-ray exposure F-centres were annealed from one end of the sample tube by heating this end, while the other end containing the irradiated sample remained under liquid nitrogen. The annealed part was cooled again before the sample was transferred to it for E.S.R.-measurements.

3. Results

3.1. *Qualitative observations*

First derivative spectra of deoxycytidine·HCl, thymidine, deoxyadenosine and deoxyguanosine observed after irradiation at room temperature are shown in figure 1 (*a*)–(*d*). It may be noted that in no case does the spectrum change considerably with time when the sealed sample tube is stored after irradiation.

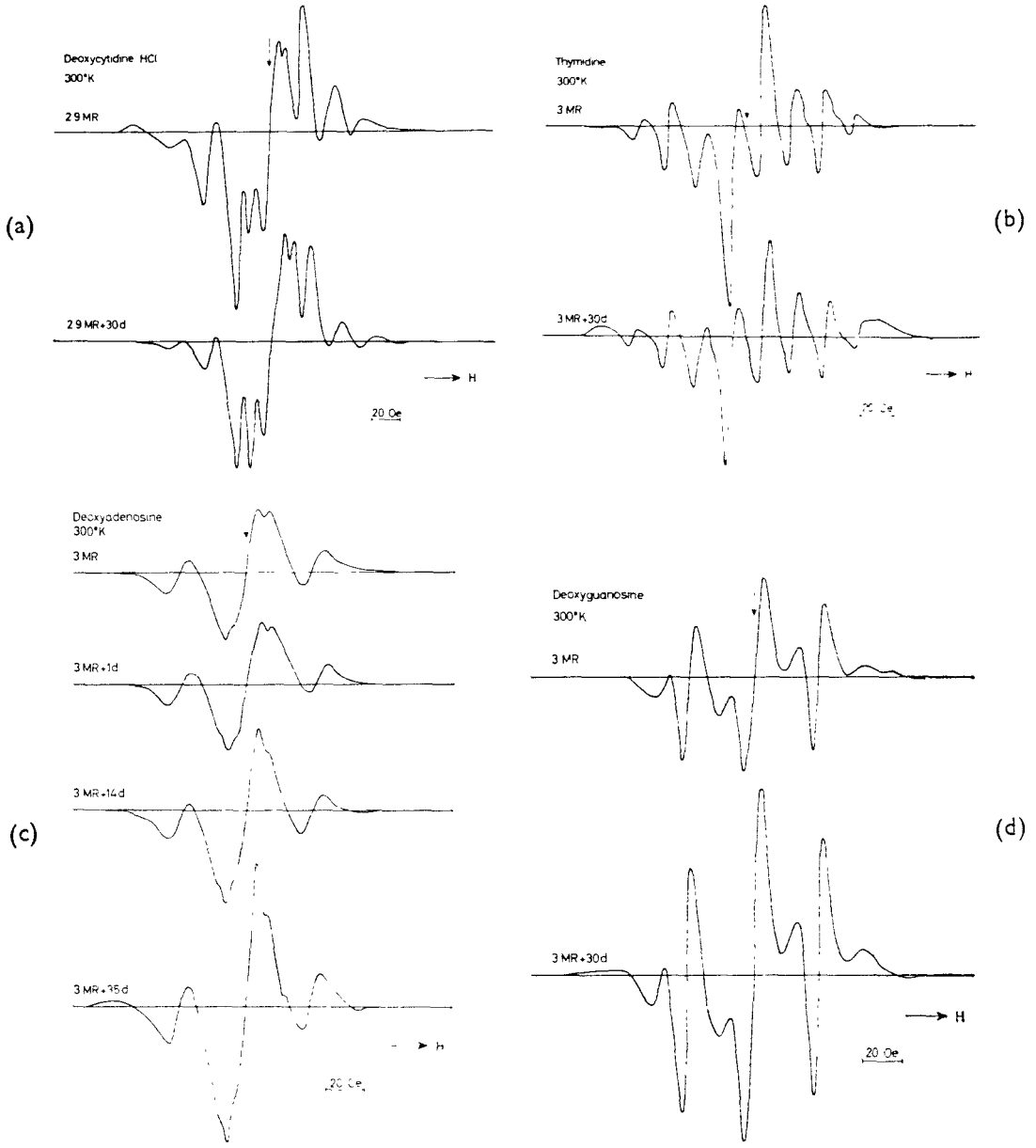

Figure 1. First derivative spectra of deoxycytidine HCl, thymidine, deoxyadenosine and deoxyguanosine *in vacuo* at room temperature immediately after irradiation and after subsequent storage. Arrow indicates $g = 2{\cdot}0036$.

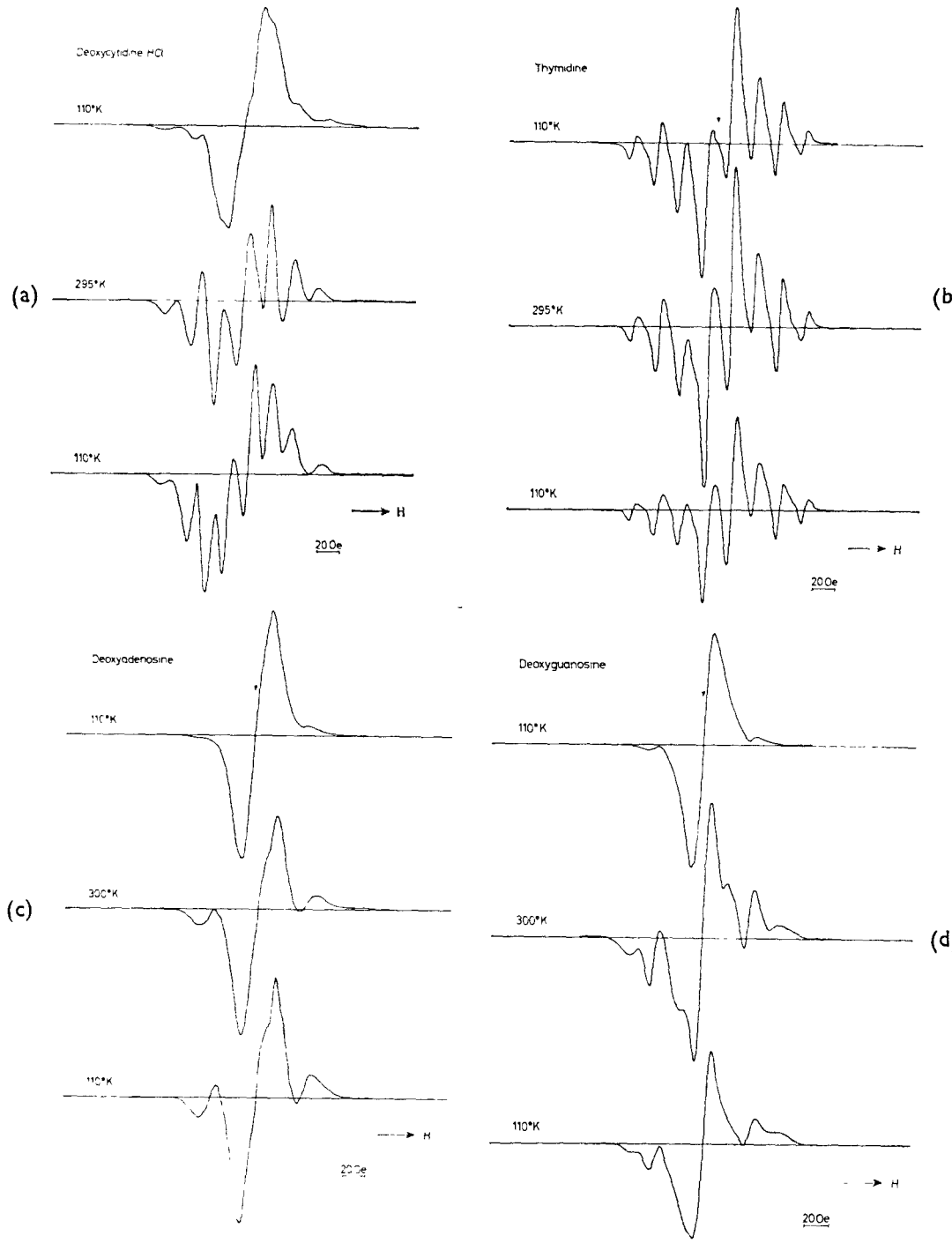

Figure 2. First derivative spectra of deoxycytidine·HCl, thymidine, deoxyadenosine and deoxyguanosine *in vacuo* at about 100°K immediately after irradiation at low temperature, after warming up to room temperature and after cooling again to 100°K. Arrow indicates $g = 2{\cdot}0036$.

The asymmetric spectrum of deoxycytidine·HCl consists of at least nine lines, which appear to belong to three groups: one group of two lines near the centre of the spectrum, a quartet and a triplet of larger spacing. The spectrum of thymidine is only slightly asymmetric and is composed of at least 15 lines, eight of which are quite prominent, whereas five more lines apparently form another group with slightly different g-factor. The structure in the spectrum of deoxyadenosine is much less resolved. It is also asymmetric and allows the identification of at least seven lines, three of which are fairly well resolved. Although the spectrum of deoxyguanosine appears to be different from that of deoxyadenosine on first sight, a careful comparison reveals a considerable similarity: three main lines and at least four satellites with corresponding spacings are found in both spectra.

Nucleosides irradiated at strongly-reduced temperatures show, after warming up to room temperature, spectra quite similar to those observed after irradiation at room temperature. This is illustrated in figure 2 (*a*)–(*d*), which gives the spectra obtained at low temperature immediately after irradiation, after warming up to room temperature, and after cooling down again. Usually the first spectrum obtained at low temperature shows less structure than those recorded after warming up, the structure of the latter being essentially retained on recooling. Small differences between spectra recorded at room temperatures after irradiation at about 100 °K and 300 °K are observed with deoxycytidine·HCl and deoxyguanosine. In both cases the relative intensity of the hyperfine lines has changed, but their number and spacing have not. As a result the spectrum of deoxyguanosine irradiated at low temperature and recorded at room temperature is very similar to the spectrum of deoxyadenosine in figure 1 (*c*). This confirms the resemblance between the spectra of deoxyadenosine and deoxyguanosine. The spectra of thymidine and deoxyadenosine do not show any significant dependence of the temperature of irradiation.

In addition to the four nucleosides that are the normal constituents of deoxyribonucleic acid, deoxyuridine, bromodeoxyuridine and iododeoxyuridine have also been irradiated at low temperature, and the resulting E.S.R.-spectrum recorded (figure 3 (*a*)–(*c*)). Characteristic differences are visible, especially with the bromine-substituted analogue. Here, several lines are found at field-strengths lower than those which occur in most other spectra, corresponding to a larger g-factor. This result is similar to that on bromouracil obtained by Köhnlein (1963) and is attributed to interaction of the unpaired electron with the bromine nucleus. When compared with deoxyuridine, an additional component is also found in the spectrum of irradiated iododeoxyuridine but this consists of a narrow line only which is superimposed on the centre of the former spectrum.

3.2. *Quantitative observations*

Radical-concentrations in irradiated nucleosides are represented in figures 4–6. The curves drawn in these figures can all be described by the function:

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

where C is the radical-concentration, C_∞ the saturation-concentration, D the radiation dose, the D_{37} the dose at which the radical-concentration is 37 per cent less than the saturation-concentration. Within the experimental errors, the above equation gives the best fit for the majority of organic compounds investigated, as has been noted previously (Müller 1963, Rotblat and Simmons 1963,

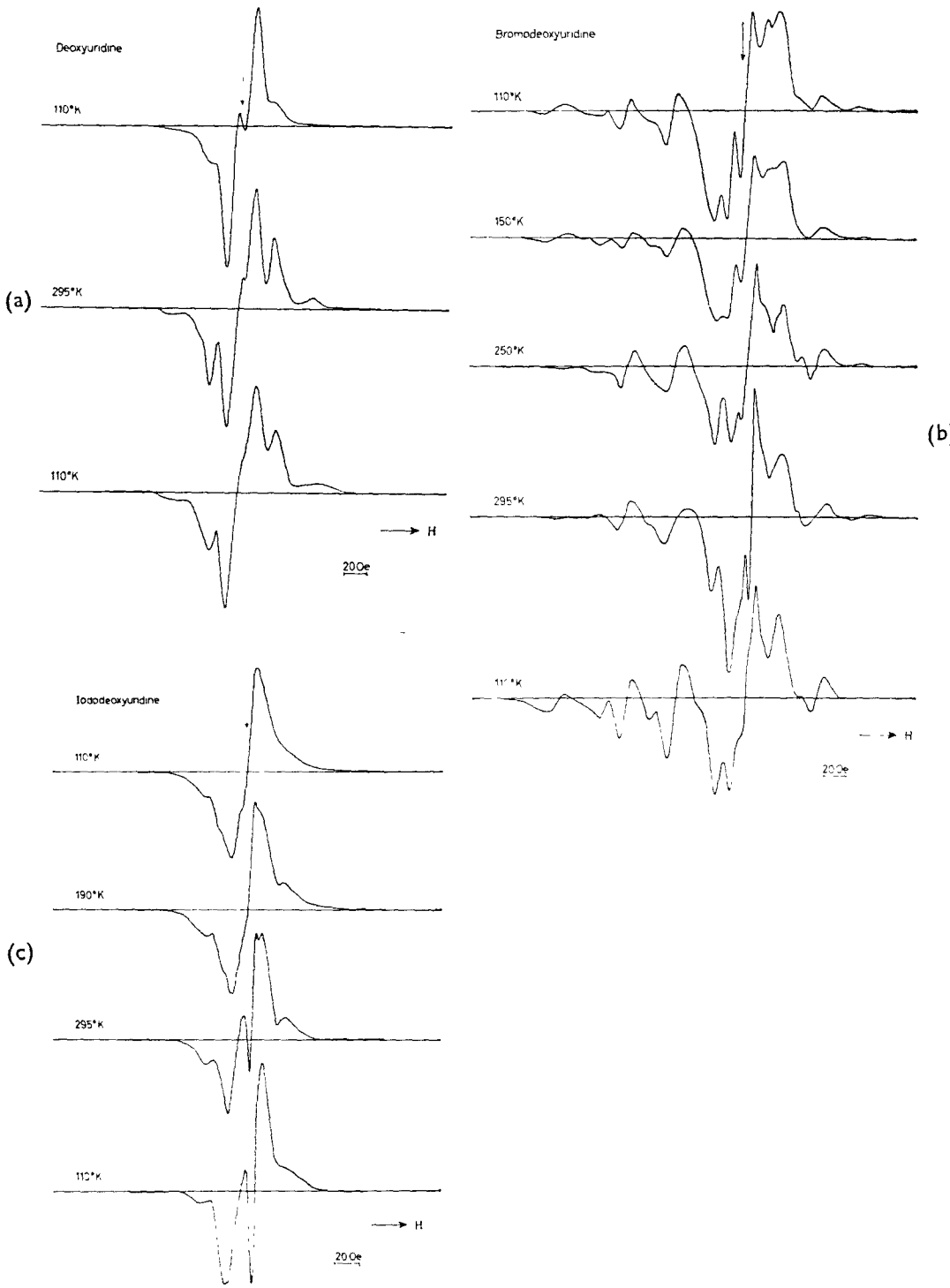

Figure 3. First derivative spectra of deoxyuridine, bromodeoxyuridine and iododeoxyuridine *in vacuo* at about 100°K, at successively increased temperatures and after cooling again to 100°K. Arrow indicates $g = 2{\cdot}0036$.

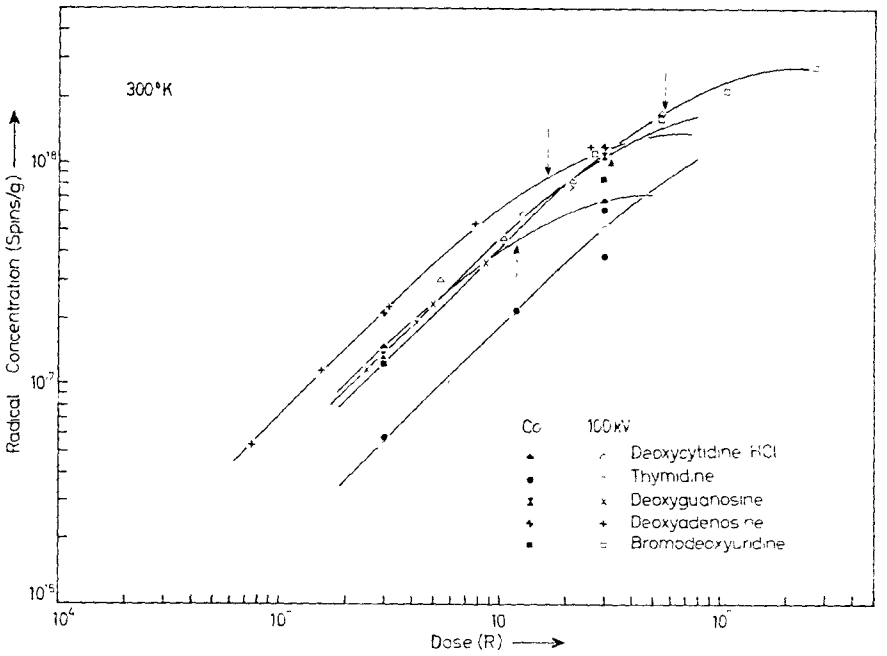

Figure 4. Radical concentrations in nucleosides *in vacuo* at room temperature against dose of radiation. The lines represent equation (1). Arrows indicate D_{37}-values.

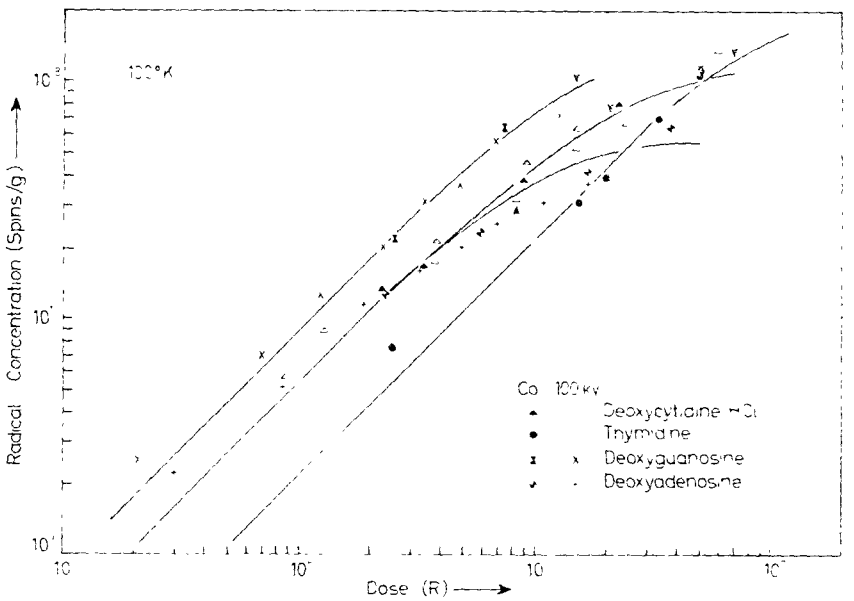

Figure 5. Radical concentrations in ' natural ' nucleosides *in vacuo* at low temperature against dose of radiation. The lines represent equation (1). Arrows indicate D_{37}-values.

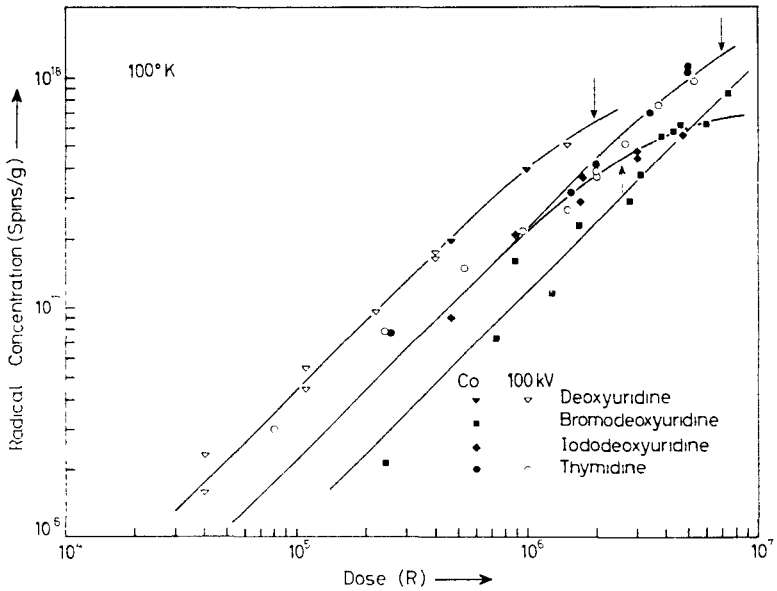

Figure 6. Radical concentrations of deoxyuridine and its brominated, iodated, and methylated derivatives *in vacuo* at low temperature against dose of radiation. The lines represent equation (1). Arrows indicate D_{37}-values.

Müller, Schambra and Pietsch 1964). At sufficiently small doses, the slope of the curve represented by equation (1) approaches the value C_∞/D_{37} which is a measure of the initial radical-yield. This is usually expressed as the G-value, i.e. yield per energy-input of 100 ev. Resulting G-values and D_{37}-values are listed in the table, together with the temperature at which irradiations and measurements

Compound	G-values			D_{37}	
Irradiated at	300°K	100°K	100°K	300°K	100°K
Observed at	300°K	100°K	300°K	300°K	100°K
Deoxycytidine · HCl	1·0	1·0	0·4	2 Mr	2 Mr
Thymidine	0·4	0·4	0·4	> 5 Mr	> 5 Mr
Deoxyadenosine	1·4	1·0	0·5	1·6 Mr	0·8 Mr
Deoxyguanosine	0·9	1·8	0·4	3 Mr	> 1·0 Mr
Deoxyuridine	—	0·9	0·4	—	> 2 Mr
Bromodeoxyuridine	0·8	0·2	0·2	6 Mr	> 10 Mr
Iododeoxyuridine	—	0·4	0·2	—	2·5 Mr

Table 1. Quantitative results on nucleosides.

have been made. G-values vary between 0·2 and 1·8. G-values at room temperature are generally equal to or smaller than those found at low temperature. However, when samples irradiated at low temperature are warmed up to room temperature, the radical-concentration always drops by a factor of between 2 and 5.

Hence, the radical-concentration measured at room temperature is always lower if the irradiation was at low temperature followed by warming than if the irradiation was performed at room temperature. This is in accordance with earlier findings on proteins (Henriksen, Sanner and Pihl 1963, Müller 1962). D_{37}-values are scattered mostly between 1 and 10 Mr, but are generally higher at low temperature.

4. Discussion

All nucleosides used in the present work are composed of the pentose D-2-deoxyribose and a nucleic acid base. Among these nucleosides are those contained in DNA. The pentose and the bases are of fairly similar molecular weight as long as the base does not contain a heavy halogen atom. From the accompanying paper (Köhnlein and Müller 1964) it may be seen that the *G*-value for radical-production in D-2-deoxyribose is as high as 4 at room temperature, but the *G*-values obtained with the bases are much smaller. Hence, one would expect most radicals to be induced in the pentose moiety, unless the formation of a chemical link between the two components affects their response to irradiation. Also, the spectra of all nucleosides should be rather similar to the spectrum of the sugar. This, however, is not what is actually found. On the contrary, the spectra display large differences as is obvious from figure 1 (*a*)–(*d*). In fact, a detailed comparison of these with the spectra obtained with pure bases reveals striking analogies. These are especially clear in the case of thymidine. The numerous lines contained in the spectrum of thymidine, their spacing and relative intensities are found almost exactly in the spectrum of thymine also. If the thymine spectrum is caused by a radical located on the methyl group of thymine, as has been suggested (Shields and Gordy 1959, Köhnlein 1963), this should be true also for the radical produced in thymidine. The other nucleoside spectra are less similar to those of the constituent bases, but nevertheless show certain analogies. Thus, the triplet found with deoxyguanosine and deoxyadenosine is also found in the purine spectra, although much less resolved. Similarly, the well-resolved lines in the spectrum of deoxycytidine can be related to lines in the cytosine spectrum, which again shows much less resolution.

It is interesting to supplement these findings by a comparison of the quantitative results. As already mentioned the *G*-value for radical-production in nucleosides should be about 2, if radicals were induced in the pentose moiety in the same way as in the free compound. However, yields measured at room temperature are lower by at least a factor of 2. The further consequences of this result are discussed in connection with our studies on nucleotides (Müller 1964) as similar conclusions can be drawn from the experiments with these compounds. Measurements of the *G*-value for radical-production in nucleosides were also reported by van de Vorst and Williams-Dorlet (1963), who give the following *G*-values measured at room temperature after ^{60}Co gamma-irradiation *in vacuo* at room temperature: adenosine: 0·03; deoxyadenosine: 0·04; and thymidine: 0·02. These figures are substantially smaller than those reported here. As the authors had to use a rather high radiation dose to obtain spectra (at least 7 Mrad) their results are certainly affected by dose-saturation, which easily brings down the *G*-value by a factor of 10. This applies even more strongly to the *G*-values reported earlier by another group, as doses of approximately 20 Mr were used by these workers (Shen Pei-Gen *et al.* 1959).

Les nucléosides déoxycytidine · HCl, thymidine, déoxyadénosine, déoxyguanosine, déoxyuridine, bromodéoxyuridine et iododéoxyuridine ont été irradiés à l'état de poudres cristallines sèches, puis étudiées par résonance de spin électronique. Chaque substance est soumise à irradiation x et gamma (doses allant jusqu'à 10 Mr) et son spectre dérivée première enregistré à 9,4 kMc/sec. Les résultats qualitatifs obtenus pour des températures voisines de 100°K et 300°K révèlent dans ces spectres de nouveaux détails structuraux.

Cependant on s'est surtout attaché à l'étude quantitative des résultats. Pour l'irradiation effectuée à température ambiante, les valeurs de *G*, obtenues d'après la partie rectiligne initiale de la courbe représentative des concentrations en radicaux en fonction des doses irradiantes, sont comprises entre 0,4 et 1,4. Ces valeurs ne subissent pas de variations importants lorsque l'irradiation a lieu à basse température ; cependant alors, par réchauffement à température ambiante, environ la moitié des radicaux disparaissent irréversiblement. Pour les nucléosides étudiés, la courbe ne montre pas d'écart à la linéarité lorsque les doses irradiantes restent inférieures à 1 Mr, quelle que soit la température.

Die Nucleoside Deoxycytidine · HCl, Thymidin, Deoxyadenosin, Deoxyguanosin, Deoxyuridin, Bromdeoxyuridin und Joddeoxyuridin wurden in Form trockener, kristalliner Pulver bestrahlt und mittels Elektronen-Spin-Resonanz-Spektrometrie untersucht. Erste Ableitungen der Spektren jeder Substanz wurden nach Röntgen- und Gammabestrahlung mit einer ganzen Serie von Dosen bis zu 10 Mr bei 9,4 GHz aufgenommen. Die qualitativen Ergebnisse, die bei 100°K und 300°K gefunden wurden, enthalten neue strukturelle Einzelheiten der Spektren.

Das Hauptgewicht der Untersuchung wurde auf quantitative Messungen gelegt. Die *G*-Werte, die von den anfänglichen linearen Teilen der Dosis-Effektkurven abgeleitet wurden, liegen nach Bestrahlung bei Zimmertemperatur zwischen 0,4 und 1,4. Bei tiefer Temperatur wurden keine großen Abweichungen von diesen Werten beobachtet, jedoch verschwindet etwa die Hälfte der Radikale irreversibel bei Erwärmung auf Zimmertemperatur. Bei den untersuchten Nucleosiden treten Abweichungen von der linearen Abhängigkeit der Radikalkonzentration von der Strahlendosis bei allen Temperaturen oberhalb 1 Mr auf.

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