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X-ray-Induced Unpaired Spins in Nucleic Acid Bases and in 5-Bromouracil

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Some years ago, the production of unpaired spins by ionizing radiation was shown to occur in living systems (Zimmer, Ehrenberg & Ehrenberg, 1957). The electron spin resonance (e.s.r.) spectra observed indicate the presence of radiation-induced free radicals which are likely to form an important link in the chain of events started by the absorption of radiation and leading to observable biological effects (Zimmer, 1959, 1961). Many investigations have since been carried out using the e.s.r. technique on X-rayed biological materials. Quantitative measurements, however, are not numerous (for a summary of results and bibliography of relevant papers cf. Zimmer, Köhnlein, Hotz & Müller, 1963).

The present note describes quantitative determinations of the energy required for the formation by X- or gamma-rays of a free radical (unpaired spin) in nucleic acid bases and in one analogue: 5-bromouracil. The materials investigated were obtained from California Corporation for Biochemical Research, Los Angeles, and irradiated in the form of dry powders with 100 kv X-rays (Be-window tube, no additional filter), with 150 kv X-rays (filtered by the glass of the X-ray-tube window and by the glass of the tubular container enclosing the specimen under vacuum) or with gamma-rays from a Co^{60}-source. The two X-ray tubes deliver a wide and badly defined Bremsstrahlen spectrum, whereas the output of the Co^{60}-source consists mainly of two narrow lines of about 1·2 Mev. The determinations of the radiation energy absorbed in the specimen (dosimetry) was carried out using three independent methods: two different types of ionization chambers calibrated by the British and German National Physical Laboratories respectively and chemical actinometry ($Fe^{2+} \rightarrow Fe^{3+}$). The techniques used for recording the spectra and for obtaining absolute numbers of spins include a double cavity, a momentum balance and the calibration of a secondary standard by five different and independent methods as described in detail before (Köhnlein & Müller, 1960, 1961, 1962; Köhnlein, 1962). For all the materials, e.s.r. spectra were taken at various levels of radiation dose, of microwave power and at various time intervals after irradiation in order to avoid errors by dose saturation, by microwave saturation or by decay of radicals after irradiation.

As shown in Table 1 the energies required for the production of one unpaired spin in nucleic acid bases range from 120 ev to 1400 ev. These values can be compared to those obtained for the amino acids containing an aromatic ring (170 ev to 2500 ev), whereas the values for aliphatic amino acids fall between 20 ev and 80 ev (Köhnlein & Müller, 1962; Zimmer *et al.*, 1963). The results obtained for the nucleic acid bases appear, therefore, quite reasonable. The following findings seem to be of particular interest to radiation biology.

(i) The pair cytosine–guanine (C: 350 ev and G: 150 ev) require considerably less energy for the formation of an unpaired spin than the pair thymine–adenine (T: 1400 ev and A: 1200 ev). This difference may well be of importance for explaining the observation that in bacteria the mean lethal dose for X-rays decreases with increasing ratio CG/TA (Kaplan & Zavarine, 1962).

(ii) The energy required for the formation of one unpaired spin in 5-bromouracil is 160 ev as compared to 1400 ev for thymine. The difference observed between the "natural" base and its analogue has certainly a bearing on the much discussed sensitization towards X-ray damage after incorporation of the analogue into phage,

TABLE I

Energy required for the formation of one unpaired spin (free radical) in dry nucleic acid bases by X- or gamma-rays

Material	Radiation	Condition of irradiation and measurement	Energy absorbed/ unpaired spin
Cytosine	X-rays 100 kv	air	350 ev
	X-rays 150 kv	vacuum	350 ev
5-Methylcytosine	X-rays 100 kv	air	750 ev
	γ-rays Co^{60}	vacuum	400 ev
Uracil	X-rays 100 kv	air	190 ev
	X-rays 150 kv	vacuum	190 ev
5-Bromouracil	γ-rays Co^{60}	air	160 ev
	γ-rays Co^{60}	vacuum	220 ev
Thymine	X-rays 100 kv	air	1400 ev
	γ-rays Co^{60}	vacuum	1400 ev
Adenine	X-rays 100 kv	air	1200 ev
	X-rays 150 kv	vacuum	1200 ev
Hypoxanthine	X-rays 100 kv	air	190 ev
	X-rays 150 kv	vacuum	190 ev
Guanine	X-rays 100 kv	air	150 ev
	X-rays 150 kv	vacuum	150 ev
Xanthine	X-rays 100 kv	air	120 ev
	X-rays 150 kv	vacuum	120 ev

micro-organisms or mammalian cells. Our results are definitely not due to an increase of total energy absorption by photoelectric absorption in bromine which is negligible, if not absent, with Co^{60}-gamma rays. With relatively soft X-rays the additional effect of photoelectric absorption is, of course, very noticeable in pure bromouracil (42% Br by weight, Table 2), though in "heavy" T-phage (usually

TABLE 2

Influence of photoelectric absorption on the formation of unpaired spins (free radicals) in 5-bromouracil by X- and gamma-rays

Radiation	Fraction of molecules showing an unpaired spin after an exposure to 10^4 röntgen
X-rays 150 kv	20×10^{-6}
γ-rays Co^{60}	1×10^{-6}

containing about 1% Br by weight) the additional effect of photoelectric absorption would be much less pronounced even with X-rays. Consequently, the sensitization towards radiation damage after incorporation of 5-bromouracil into biological entities is, even with soft X-rays, most likely due to the smaller energy needed for radical formation and not to additional absorption of energy by photoelectric processes.

(iii) The energy required for the formation by X- or gamma-rays of one unpaired spin in the bases (120 ev to 1400 ev) is rather higher than the energy needed for the same effect in whole nucleic acid. The latter was measured to be 160 ev in calf thymus DNA, 110 ev in yeast RNA and 8 ev to 30 ev in different preparations of DNA from T-phage (Müller, 1962, 1963). One would not, therefore, expect free radicals formed in the bases to be the most frequent radiation damage to DNA. In view of what has been said in sections (i) and (ii) free radicals formed in the bases may, nevertheless, be of preponderant importance for the biological end result, though the possible occurrence of entirely different mechanisms should always be kept in mind (cf. Zimmer, 1961).

Additional information on these problems obtained from measurements at low temperature, from similar experiments with nucleotides and nucleosides and from analysing the hyperfine-structure of the e.s.r. spectra will be reported in other more detailed papers.

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