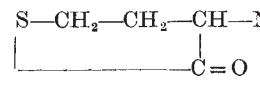
RADIOBIOLOGY

Electron Spin Resonance Investigations of Ultra-violet and X-irradiated Homocysteine-thiolactone

Some time ago we discussed the formation of radicals in irradiated samples of methionine[1] and bovine serum albumin[2]. In the case of methionine, in which the bivalent sulphur is present in the form of a thioether, X-irradiation forms radicals of the alkyl-type, but ultra-violet irradiation (254 mμ) localizes them at the sulphur atom. Moreover, ultra-violet light is able to quench the alkyl-type radicals. In the case of bovine serum albumin, X-irradiation as well as ultra-violet light forms a complex electron spin resonance spectrum consisting of a glycylglycine- and a sulphur-radical[3]. The portion of the sulphur radical part becomes bigger after ultra-violet irradiation. By ultra-violet irradiation of longer wave-length ($\lambda > 300$ mμ) the X-ray conditioned radical state at the carbon atom is transferred to the SS- or SH-groups of the albumin molecule. In the following investigations we controlled the behaviour of the bivalent sulphur in the form of a thiolactone ring compound, that is, a thioester, homocysteinethiolactone (HCT):



Polycrystalline, *d,l*-homocysteinethiolactone (HCT)-HCl (Degussa, Konstanz/Germany) with a bulk volume of about 220 cm³/100 g was sealed under vacuum ($< 10^{-3}$ torr) and irradiated at room temperature. The electron spin resonance measurements were carried out under the same conditions as previously described[1]. The X-irradiation was carried out with a 200 kV source (half value layer: 0·64 mm copper, dose-rate 920 r./min, dose 100 kr.). The irradiations with ultra-violet were performed with a mercury low-pressure lamp (*NN* 15/44, Quarzlampengesellschaft, Hanau, Germany) which emits mainly in the 254 mμ line. The intensity of the ultra-violet irradiation at the sample tube amounted to 9×10^3 ergs cm^{-2} sec^{-1}. The other technical details have been reported in a former publication[1].

After X-irradiation a radical yield of 10·8/100 eV is obtained. The electron spin resonance signal of the X-irradiated HCT is demonstrated in Fig. 1 (solid line). Though it is not possible to differentiate the obtained spectrum of the polycrystalline material, it is possible to ascertain that there exists no sulphur radical state. The different behaviour of saturation effects of the micro-wave energy on the different parts of the spectrum includes at least two different radicals. We suppose that the unpaired electrons are localized at a carbon and/or nitrogen atom of the molecule. The radicals are very stable not only in vacuum but also in air. In vacuum and at room temperature the decay of the radical concentration is only about 1 per cent/h.

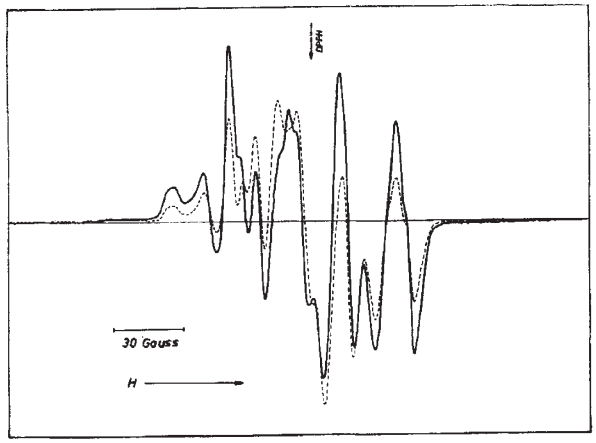


Fig. 1. Electron spin resonance spectra of irradiated homocysteinethiolactone. The spectra represent the first derivatives of the actual absorption curves. ——, 100 kr; – – –, 100 kr + 7 h ultra-violet ($\lambda = 254$ mμ)

In contrast to methionine and bovine serum albumin, ultra-violet light of 254 mμ has no influence on the X-ray signal of homocysteinethiolactone. The dotted line in Fig. 1 shows the change of the X-ray spectrum after an ultra-violet irradiation of 7 h. This dotted line spectrum demonstrates clearly that only one part of the former spectrum is influenced by quenching. The same change is obtained by warming up the X-irradiated sample of HCT. In comparison with the solid line spectrum (X-ray alone) the radical concentration was found to be diminished after ultra-violet irradiation to nearly 88 per cent. It is very interesting that no sulphur radical is detectable although the lactone ring is opened by the ultra-violet irradiation. The latter process is certain because hydrogen sulphide gas is liberated. Perhaps the yield is not big enough to detect the sulphur radicals by the sensitivity of the electron spin resonance spectrometer.

If HCT is irradiated only with ultra-violet light of the wave-length of 254 mμ for 8 h, a radical concentration of only 5 per cent is obtained corresponding to that of an X-irradiation with 100 kr. This ultra-violet signal is not decomposed, it might be a quintet and centred at $g = 2{\cdot}0046$. In contrast to sulphydryl-, disulphid- and thioether-compounds, there is no sulphur radical to be found for the thiolactone.

The results we obtained from bivalent sulphur containing compounds demonstrate quite clearly that not only the primary formation of sulphur radicals but also the transfer of the radical state to the sulphur atom depends on the chemical binding of the bivalent sulphur. In our next publication we hope to describe the influence the whole molecule has on the character of the sulphur radical.

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[1] Mönig, H., and Koch, R., *Nature*, **202**, 289 (1964).
[2] Koch, R., and Mönig, H., *Nature*, **203**, 859 (1964).
[3] Gordy, W., and Shields, H., *Rad. Res.*, **9**, 611 (1958).

Use of Technetium-99m in Hepatic Scintillography

Harper *et al.*[1] have recently pointed out the excellent possibilities of technetium-99m in medical scintillography.

Tc-99m is a nucleid with a period of semidisintegration time of 6 h, emitting γ-radiation of 140 keV, obtained by acid elution of molybdenum-99 adsorbed on an aluminium column. It is obtained as a pertechnetiate (TcO_4^-), which has a behaviour similar to the I^- ion in the body. We have used it with very good results in thyroid scintillography[2,3]. Harper has also used this compound combined with a colloid which is retained by the reticulo-endothelium in liver scintillography[1]. The Production Department of the Comisión Nacional de Energía Atómica, Argentina[4], has obtained a colloid of antimonium sulphide which has a molecular size of 100 to 400 mμ, an adequate take-up of ^{99m}Tc, and appears to be very stable. We have been able to obtain hepatic pictures of excellent resolution by intravenous injection of this substance (Fig. 1). Its concentration in the liver allows us to perform tracing only 10 min after injection and its activity is still at adequate levels after 180 min.

After 24 h the activity eliminated by urine was 10–15 per cent. We have used activities of 0·4–1·0 mc.