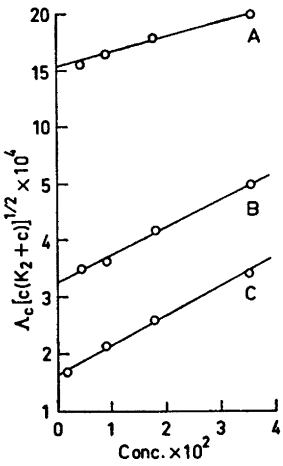

Fig. 2. Application of eqn. (3) to the system: A, diisopropylamine-50 % isopropanol-50 % methyl ethyl ketone; B, diisopropylamine-methyl ethyl ketone; C, diisopropylamine-isopropanol.

because of acetal formation, and therefore freshly prepared solutions were used throughout.

The conductometric measurements were made at a frequency of 300 Hz using a circuit containing operational amplifiers. The accuracy of the bridge was within ± 0.1 % for $\varkappa > 10^{-5}$ ohm^{-1} cm^{-1} and within ± 1 % for $\varkappa > 5 \times 10^{-7}$ ohm^{-1} cm^{-1}. A conductivity cell suitable for nonaqueous solutions was made. The electrodes, placed at a distance of 0.4 cm and with an area of 3 cm^2 each were black platinized. All measurements were carried out at 25.0°C. The conductivity of pure isopropanol was 9×10^{-8}, of methyl ethyl ketone 1.5×10^{-7} and of the mixture 2.6×10^{-7} ohm^{-1} cm^{-1}. The same solvent corrections were subtracted throughout.

1. French, C. M. and Roe, I. G. *Trans. Faraday Soc.* **49** (1953) 314.

Received December 10, 1965.

Decay of Radiation Induced Radicals and the Yield of Thermoluminescence

TORE SANNER and
ALEXANDER PIHL

Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, Oslo, Norway

The loss of biological activity of compounds exposed to ionizing radiation in the dry state is in part the end result of the secondary processes occurring after absorption of the radiation energy. Electron spin resonance (ESR) spectroscopy and thermoluminescence (TL) measurements have found increasing use in the study of these secondary processes. TL studies involve measurements of the light emitted from irradiated compounds upon subsequent heat-treatment. Since heat-treatment facilitates the decay of radiation-induced radicals, it seems of interest to study whether the yield of TL is somehow related to the concomitant disappearance of radicals, as measured by ESR spectroscopy. It is the purpose of the present communication to present an empirical relationship between results obtained with TL and ESR spectroscopy.

When substances are irradiated in the dry state at cryogenic temperatures and subsequent heat-treated, part of the radicals observable at low temperature will disappear in combination reactions (see, *e.g.*, Zimmer and Müller [1]). When attempts were made to plot our results on the extent of radical decay upon heat-treatment after irradiation at 77°K against the TL yield observed by Lehman and Wallace [2] on the same compounds, no obvious relationship was found. However, when the disappearance of ESR centers was plotted *versus* the product of the yield of TL and of ESR centers at 77°K, a straight line in a logarithmic plot, as shown in Fig. 1 A, was obtained.

The number of radicals observed by ESR spectroscopy after irradiation with increasing doses will initially increase linearly with the dose. At higher doses, however, the apparent rate of radical formation will decrease, and eventually the number of ESR centers in the sample will reach a limiting, maximum number.[3-6] In Fig. 1 B

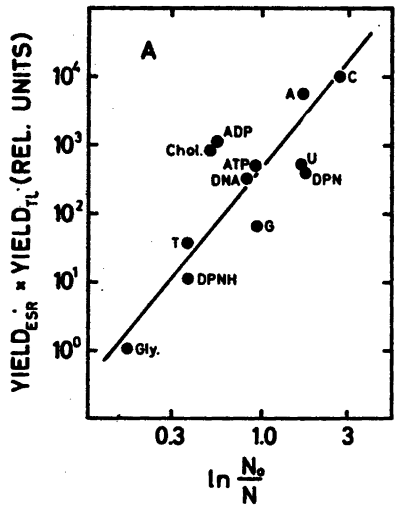

Fig. 1 A. Relationship between decay of radiation induced radicals and the product of the yield of thermoluminescence and of ESR centers at 77°K. In the ESR studies (unpublished data) the samples were irradiated at 77°K in vacuum with X-rays to a dose of 4.8×10^5 R. The number of ESR centers immediately after irradiation, N_0, and after heat-treatment for 4 min at 293°K, N, was determined according to methods previously described.[5] The yield of thermoluminescence was determined after irradition at 77°K. The TL data are taken from Lehman and Wallace.[2] Abbreviations: Gly, glycine; DPNH, reduced diphosphopyridine nucleotide; T, thymine; G, guanine; DNA, deoxyribonucleic acid; DPN, oxidized diphosphopyridine nucleotide; ATP, adenosine triphosphate; U, uracil; Chol, cholesterol; ADP, adenosine diphosphate; A, adenine; C, cytosine.

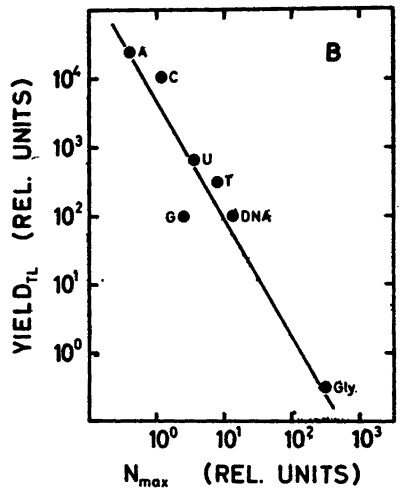

Fig. 1 B. Relationship between maximum number of radicals found after irradiation in vacuum at room temperature, N_{max}, and the yield of thermoluminescence after irradiation at 77°K. The ESR data are taken from Müller *et al.*[6-8] The TL data are taken from Lehman and Wallace.[2] Abbreviations as in Fig. 1 A.

the maximum number of radicals, N_{max}, observed for a number of compounds [6-8] is plotted *versus* the yield of thermoluminescence.[2] Again a straight line is obtained in a logarithmic plot.

Although data are presented here only for a limited number of compounds, the empirical relationships over several logarithmic units shown in Figs. 1 A and B can hardly be fortuitous. The correlation coefficients have been calculated to be 0.81 and −0.95 for the data in Figs. 1 A and B, respectively.

The significance of the relationships presented is not immediately apparent. The fact that a limiting, maximum number of radicals is obtained when a sample is irradiated with increasing doses, suggests that concurrently two sets of reactions occur during the exposure, the production of radicals, being a linear function of the dose, and a radical decay reaction. This implies that the relationships presented in Figs. 1 A and B are not independent. Attempts to analyse the underlying causes of the relationships presented will have to await further data. In particular, the results of ESR and TL experiments carried out under strictly comparable conditions are needed.

This investigation was supported by *Division of Radiological Health, Bureau of State Service, U.S. Public Health Service.* One of the authors (T.S.) is a Fellow of the *Norwegian Cancer Society.*

1. Zimmer, K. G. and Müller, A. In Ebert, M. Howard, A. *Current Topics in Radiation Research*, North Holland Publ. Co., Amsterdam 1965, Vol. 1, p. 1.

2. Lehman, R. L. and Wallace, R. In Pullman, B. *Electronic Aspects of Biochemistry*, Academic Press, New York 1964, p. 43.
3. Henriksen, T. and Pihl, A. *Nature* **185** (1960) 307.
4. Prydz, S. and Henriksen, T. *Acta Chem. Scand.* **15** (1961) 791.
5. Rotblat, J. and Simmons, J. A. *Phys. Med. Biol.* **7** (1963) 489.
6. Müller, A., Schambra, P. E. and Pietsch, E. *Intern. J. Radiation Biol.* **7** (1963) 587.
7. Müller, A. *Intern. J. Radiation Biol.* **6** (1963) 137.
8. Köhnlein, W. and Müller, A. *Intern. J. Radiation Biol.* **8** (1964) 141.
9. Henriksen, T., Sanner, T. and Pihl, A. *Radiation Res.* **18** (1963) 147.

Received December 3, 1965.

Effect of Phospholipases A, C, and D on the Iodide-Complexing Phospholipid of Thyroid

PANU VILKKI and ILMARI JAAKONMÄKI

Department of Medical Chemistry, University of Turku, Turku, Finland

An iodide-complexing factor has been previously demonstrated by one of the authors to occur in the lecithin fraction of thyroid phospholipids.[1,2] This specific factor resisted all attempts to separate it from lecithin by conventional methods employing precipitation, extraction by different solvents, countercurrent distribution, or chromatographic separation. None of the other lipid fractions showed any affinity towards iodide. From these observations it appeared obvious that the iodide-complexing factor is a lecithin. To prove this the lecithin fraction was subjected to the action of phospholipases A, C, and D, in order to elucidate whether also the enzymatic criteria for lecithin are met by the iodide-complexing factor.

Lecithin was obtained by homogenizing human thyroid tissue * in chloroform containing 1 % methanol by a Waring Blendor type homogenizer. The centrifuged extract was drawn to dryness by a rotating film evaporator. The residue was dissolved in a small volume of chloroform, and the phospholipids were precipitated three times by adding a tenfold volume of acetone. The precipitate was again dissolved in chloroform, and a tenfold volume of absolute ethanol was added to remove the bulk of non-lecithin lipids. After 20 h at −15°C the precipitate was discarded, and the supernatant was evaporated to dryness. The crude lecithin obtained was dissolved in a small volume of chloroform-methanol 1:1 v/v and chromatographically purified in a 10 mm. diameter column of basic Al_2O_3 (Merck 1076), recovering the 1:1 v/v chloroform-methanol eluate, thus leaving all the non-choline P-lipids in the adsorbent.[3] The eluate was evaporated to dryness, and a sample of the residue was analyzed for phosphorus, nitrogen, and choline. Phosphorus was determined by a modified method of Berenblum and Chain [8] using methol as reducing agent. The content of phosphorus varied from 3.6 to 3.8 %, corresponding to a mean molecular weight of 840 if one atom of P is assumed per molecule of lipid. Nitrogen was analyzed by a micro-Kjeldahl procedure, and choline by the method of Glick. The molar ratio of P:N:choline was 1:1:1 with no more than 6 % of variation. These values indicate a highly purified sample of lecithin.

A 100 mg sample of the purified lecithin was dissolved in 60 ml of diethylether. 1 ml of 0.1 % venom of *Crotalus durissus terrificus* (phospholipase A, Calibiochem) was added in 0.005 M solution of $CaCl_2$.[4,5] The mixture was kept at 30°C under reflux overnight. The precipitate formed was centrifuged off and washed three times with diethyl ether. No phosphorus was detected in the last washing. The ether supernatant and washings were pooled, drawn to dryness, and tested for their iodide-complexing power, none of which was found. The precipitate which contained lysolecithin was dissolved in chloroform-methanol, and purified with Al_2O_3 absorption procedure as described for lecithin above. The recovery was 55 mg of lysolecithin checked to be pure in thin layer chromatography.[10]

The purified lecithin was also subjected to the action of phospholipase C (from *Clostridium Welchii*, Sigma) by the method of Zeller.[6] Phospholipase C cleaves the linkage between glycerol and phosphate yielding diglyceride and phosphorylcholine as products of hydrolysis.

A further sample of lecithin was treated with phospholipase D [6] from cabbage (B grade, Calbiochem). The cleavage occurs here between phosphate and choline yielding phosphatidic acid and choline.

* Obtained from the surgical unit ,University of Turku Hospital.