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CHEMICAL LUMINESCENCE OF LUMINOL UNDER THE EFFECT OF IONIZING RADIATION, AND ITS INFLUENCING BY MEANS OF ANTIRADIATION AGENTS

by

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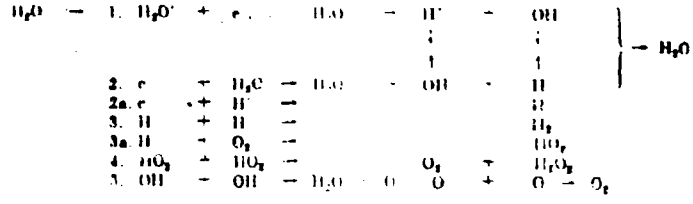
Nuclear research, with its manifold dangers of injury caused by ionizing radiation, has placed the problem of radiation biology into the forefront of scientific interest, and has directed the attention to the possibilities of limiting radiation-caused injuries by means of prophylactic and therapeutic measures. From among the numerous publications on this subject the work of Patt et al (1949) deserve particular attention. They showed that cysteine exerts a marked protection against radiation when administered in sufficient doses prior to the application of ionizing rays. When given after irradiation, it is completely ineffective.

It is known from enzyme chemistry that cysteine is an outstanding means for the reduction of S-S linkages. Enzymes inactivated through oxidation, which require a thiol group for their activity, may in many cases be reactivated with cysteine (Hopkins 1938, Bersin 1939, Bergstermann, 1948). It was therefore obvious to assume a similar action of cysteine also in radiation protection. Barron (1947) has shown that already very small X-ray doses injur, reversibly, enzymes containing sulfhydryl groups by oxidation of the latter. We ourselves have obtained in the framework of intoxication experiments with succinic acid dehydrogenase that the substrate succinic acid, and also malonic and fumaric acids, exhibit a certain protective effect against the action of X-rays, while the inactivation caused by ultraviolet cannot be prevented either by succinic- or by malonic acid. Apparently here the substrate or substrate-like malonic acid exerts, through complex formation with the enzyme, a protective effect against the oxidation of their thiol group brought about by the X-rays.

If cysteine were to reactivate enzymes and other active groups oxidatively damaged by ionizing rays solely by reduction of oxidized sulfhydryl groups, then it would be hard to understand why an unequivocal protective effect is attained only when administered before the application of X-rays. It is more probable that the detoxication effect of cysteine sets in already before vital substrates, sensitive to oxidation, had been attacked.

On the basis of the theoretical considerations of J. Weiss (1944), supported by many experimental results, we may assume that under the effect of ionising radiation water molecules are split to short-lived cleavage products, particularly H atoms as well as OH^- and H_2O radicals, as well as H_2 and H_2O_2 molecules. Some of the most important reactions and recombinations are as follows:



(Summarizing Representation, Allen, 1952)

This theory is in harmony with the radiochemical transformations in aqueous solutions which have been noted so far. It enables us to understand the observation that substances are oxidized or reduced according to their redox potential. It explains the considerable increase of toxicity of ionizing rays, by the fact that the oxygen which is dissolved in water prevents the recombination of water radicals to H_2O_2 and thereby gives rise to oxygen radicals of strong oxidizing effect. It gives a simple explanation for the protective effect of many substances added to water, including particularly those which are readily oxidisable, such as cysteine and similar compounds, by assuming that these substances are suitable to "catch" the short-lived radicals formed in the water. According to this theory a great part of the radiation effect is brought about not by means of direct action (impact) on the molecule itself, but through the intermediary of the solvent (indirect effect)(Dale, 1943, 1947; Minder, 1946, 1952). Actually the chemical transformations brought about per X-ray dose are markedly dependent on the volume of the irradiated aqueous solvent while concentration changes — within a medium concentration range — play a much smaller role. This observation can hardly have any other explanation but that the greater part of the radiation energy is captured by the water molecules and only then led to the reacting substance. To what extent the energy transport through the distance of many water molecules takes place by diffusion of water radicals or by other energy-conduction processes is as yet completely unclear. Numerous observations point to a dependence of radiochemical processes on diffusion (cf. e.g. Minder 1952, Doessauer 1954). In fluor-

escence studies using highly dil[illegible] fluorescent substances in aromatic solvents it was possible to dete[illegible] conduction of energy through a great number of aromatic molecul[illegible]ted in Allen, 1953).

More recent reviews on th[illegible]otective effect of various substances against radiation injury are giv[illegible] Patt (1953), Latarjet and Gray (1954) and Bacq and Herve (1954). According to these studies cysteamine was found, in animal experiments [illegible] be the most effective of all protective substances studied so fa[illegible]s oxidation product, cystamine, exhibits a similarly good protec[illegible]effect in the animal organism, but was ineffective in pea seedlings. Excellent radiation protection was exhibited also by cysteine, glutathione, thiourea and other thiol-group-containing substances. In addition, a protective effect was demonstrated with numerous amines, e.g. methylamine, tyramine and histamine. Even KCN is supposedly effective in the case of a variety of animal material. No noteworthy protective effect was ascertained after the application of cystine and ergothioneine. RAL has a protective action in vitro (Burnett et al, 1951), but is hardly effective in animal experiments. In our own animal experiments we, too, were able to achieve good protective effects with cysteamine, cysteine and glutathione, in the case of an irradiation with about 800 r; the order of magnitude of these effects agrees well with that described by other authors. On the other hand our studies relating to the protective effect of KCN gave no conclusive result.

In order to obtain a better insight into the mechanism of effect of various biologically effective radiation-protection agents we looked for a simple reagent for activated oxygen and believe that luminol (aminophthalic hydrazide) is particularly suitable for such model experiments.

According to Harvey (1929) luminol reacts, in the presence of activated oxygen, with the production of luminescence (cf. also Albrecht 1928, Drew 1939). According to G. Gleu and K. Pfannstiel (1936), the luminescence is considerably intensified when luminol is treated with hemin after the addition of H_2O_2. This is a result of the liberation of activated oxygen from the H_2O_2. We, ourselves, were able to note the luminescence of aqueous luminol solution at metal borders under the action of atmospheric oxygen. The physicochemical process which underlies this luminescence is still not fully explained; among the processes discussed are reversible dehydrogenation processes; formation of O_2-molecule bridges in the hydrazide ring (Faraday Soc. Disc. 1939); etc. Probably we must differentiate between a reversible and an irreversible process involving the oxidation of luminol (Druckrey 1941).

Experimental Results

When irradiated with X-rays, luminol crystals exhibited only a weak fluorescence. When, however, an aqueous luminol solution is exposed to the action of ionizing rays, marked luminescence is brought about at 100 MA, 80 kV and 70 cm tube distance. At a layer thickness of several cm of water the luminescence is detectable already at 3 MA and

80 kV. The studies were carried out in paraffin-covered aluminum dishes, since glass and most plastics exhibit fluorescence under the effect of X-rays. The starting solution contained 0.1% luminol in 5% soda, and was diluted up to 10 times in the various studies. The influencing of light phenomena was ascertained through comparisons in two equally large dishes placed next to each other and irradiated at the same time. So far we were unable to overcome the technical difficulties of an objective measurement by means of an appropria e photometer. A measurement of quantitative difference would be very valuable for the numerical ascertainment of the course of the reactions.

The luminescence phenomena occurring in the aqueous luminol solution under the effect of ionizing radiation are dependent on oxygen. Hence we are dealing with chemical luminescence. The luminescent phenomena were not intensified by the addition of hematin. Hence it may be assumed that the H_2O_2 formed in the water by the effect of ionizing rays plays no decisive role. Much rather it is to be assumed that -- according to Weiss' concepts -- there is an effect of oxygen-containing radicals which form in the water under the influence of radiation. When the thickness of the water layer is increased through the addition of distilled water or a corresponding soda solution, the luminescence becomes more intensive corresponding to the thickness of the layer. Hence we have to do predominantly with an indirect radiation effect in which the radiation energy is taken up by the aqueous medium and then conducted to the luminol under the action of oxygen.

Numerous observations exist to the effect that radiochemical processes are considerably weakened by freezing the aqueous solution; e.g. the splitting of chlorine from organic chloro compounds (Minder et al). These observations indicate that in radiochemical transformations diffusion process are of importance. On the other hand, the luminescence induced by radiation in luminol was not recognizably weakened by freezing to -10°. These luminescence phenomena are markedly independent of diffusion, even though we have to do with indirect radiation effects where the energy is taken up by water and then conducted to the luminol through the intermediary of oxygen, probably through several water molecules. The role of oxygen remains unclarified; the diffusion of oxygen in the ice phase ought to be considerably reduced in comparison to its diffusion in the aqueous solution. It is possible that certain addition compounds of oxygen and luminol (Drew 1939) form already before freezing, so that a diffusion of oxygen becomes unnecessary.

The radiation-induced luminescence phenomena are eliminated almost completely by cysteamine* and cysteine in larger concentrations -- that is, at a luminol/cysteine ratio of about 1:10 and greater. In cysteine concentrations that are biologically effective a marked weakening is detectable. The agreement of the effective concentrations in vivo and vitro

* Cysteamine was kindly placed at our disposal by Labaz Co, Brussels; cysteine by the Nordmark Werken and Chemiewerken Homburg; and cystamine by the Pharmacell Co.

speak for an identical radiochemical reaction. The idea occurred that cysteine may have extracted the oxygen from water, through its oxidation (cf. Patt 1953). Against this speaks the fact that the protective effect is dependent on concentration but little dependent on time. Immediately after the addition of cysteine the full protective effect is detectable. Since cysteine oxidizes only slowly, the removal of oxygen must have become noticeable only after some time and must have equalized again after shaking. The experiments were conducted with glass-distilled water and analytic-grade substances so as to exclude the oxidation-accelerating effect of heavy-metal traces as much as possible. It is much more likely that we have to do with an interception of radiation-induced, short-lived, oxygen-containing radicals with cysteine. As a proof, one can refer to the fact that this process is dependent on diffusion. Freezing to -10° completely eliminates the protective effect of cysteine. The radiation-induced luminescence, extinguished by addition of cysteine, is again detectable at -10° at full intensity. It is again extinguished after thawing. From this it may be concluded that the protective effect is linked to the oxidation to cystine. This reaction is diffusion-dependent and no longer takes place in the ice phase.

As was the case in biological experiments, cysteamine shows the strongest protective effect against radiation-induced luminescence of luminol. Cysteine is somewhat less effective. The weakest effect is displayed by glutathione. Histamine and NaCN show no protective effect even in the highest doses. In the case of NaCN we even think that we observed a slight intensification in some experiments. These results necessitate an accurate verification by means of an improved methodology. Apparently the protective effect of amines and NaCN described by Bacq lies on a completely different level. In the case of NaCN we might assume that the disruption of the oxygen transport and thus an accumulation of metabolic products with negative reduction potential plays a role in the area of radiation-sensitive, biologically significant cell structures, thus, the radiation protection takes place in the same manner as in the case of hypoxemia.

In contrast to the results in animal experiments, cystamine is ineffective in luminol experiments. Since according to Bacq cystamine exhibits no protective effect against radiation in the case of pea seedlings either, it may be assumed that in the animal organism cystamine is rapidly reduced at suitable reduction sites, probably by fixed SH groups which, in turn, can be only slightly protective against radiation due to their fixed position. Thus we were able to note that in the test tube cysteine is oxidized by cystamine to the difficultly soluble cystine. In regard to the transformation of SH- and S-S groups, see also Bersin (1938). The explanation given by Bacq himself that the protective effect of cystamine is to be explained by the liberation of histamine is thought by us to be less probable.

Discussion

By recalculation of the energy deposited during the action of ionizing radiation and of the gene mutations attained, Timofeeff-Ressovsky,

Zimmer and Dolbruck were the first to come to the conclusion, in 1935, that the energy deposit leading to mutation is situated in a region which is considerably larger than the gene-carrying chromomer. Further considerations, too, have led to the conclusion that the so-called effected volume is substantially larger than that corresponding to the actual domain of the radiation-sensitive, biologically important cellular element (control centers of the cells according to Jordan). These experimental results led to the assumption that the deposited energy can migrate over large areas of the cell structure. It was thought that long-chain proteins, above all, were suitable substrates for such energy conductions (Wirts 1947, Evans and Gergely 1949, cited in Dessauer 1954).

Even in the case of most radiochemical processes in dilute aqueous and organic (Minder 1952) solutions a calculation gives a considerable difference in size between the effected volume (deposit density) and the molecular density (concentration) of the reacting chemical substance. From this it is possible to derive an energy transport from the energy-absorbing solvent molecules to the site of reaction where the energy taken up can be utilised for a consequent chemical reaction.

For dilute aqueous solutions the above-discussed radical theory (Weiss) gives concrete ideas. On the basis of the available literature the described indirect reactions exhibit a more or less pronounced temperature dependence. This is attributed to a diffusion exchange during the reaction. We have to do here most likely with consequent processes, while the first step of the energy uptake under the effect of ionizing radiation is uninfluenced by temperature. Most authors seem inclined to assume, in the case of indirect radiation effects in aqueous solutions, a diffusion of energy through diffusion of water radicals (cf. e.g., Dessauer 1954).

The radiation-induced chemical luminescence of luminol, too, is an indirect radiation effect. As a proof of this one may consider the dependence of the light intensity on the volume of irradiated solvent, the oxygen dependence as well as the protective effect of cystsine and other SH-containing substances. It is noteworthy that this physiological process which may be detected, without chemical transformations, by its luminescence phenomena, is unaffected by temperature and accordingly runs its course in a markedly diffusion-independent manner. This seems to indicate that not only the uptake of irradiated energy but also the energy transport takes place without being particularly influenced by diffusion processes. Accordingly one must assume that under the action of ionising radiations on not only solid crystalline substances but also in aqueous solutions (paracrystalline structure) and probably also in organic solvents (Minder), there take place energy displacements to the reaction sites where this energy may be very readily utilised for consequent processes, due to a favorable energy gradient.

In contrast to the radiation-induced photon emission in the case of the chemical luminescence of luminol, the protective effect of cysteine takes place through a chemical process, to wit, oxidation to cystine. Probably the first step of the reaction is the removal of an H atom under

formation of a cysteine radical. [illegible] ice phase, too, cysteine radicals will form. Nevertheless the migr[illegible] of hydrogen and the formation of cystine are prevented due to the obstruction of diffusion, so that the cysteine radical and H are recombined without further conduction of the energy.

The combination of temper[illegible]-independent- and temperature-sensitive reaction courses appears to be very instructive and promising of further conclusions with regard to the understanding of the transport of radiation-induced energy depo[illegible] in aqueous solvents as well as in experimental biological materials which in most cases contain abundant quantities of water.. As mentioned above, the transport takes place in the form of electronic energy displacements through the aqueous solvent, most probably to the reaction sites with the greatest energy gradient. The initiated reactions take place in the biological material by circumvention of the physiological regulatory processes. It is obvious that labile structures, in the process of reconstruction, are particularly sensitive, and radiation injuries are most enduring at sites where duplicants are affected. It is true that energy migrations over energy-level bands of protein chains, suggested by numerous authors as the explanation of biological effects of radiation, appear possible on the basis of theoretical considerations (Wirtz 1947, Evans and Gergely 1949), nevertheless at the present time no conclusive experimental basis exists for such a hypothesis.

Summary

1. Under the influence of X-[illegible] an aqueous solution of luminol exhibits a marked chemical lumin[illegible]ce. We have to do here with an indirect radiation effect. It is highly dependent on the concentration in the concentration range of C.01-[illegible]. It is linked to the presence of oxygen and can be weakened by cysteine in concentrations corresponding to the biologically effective concentrations.

2. The radiation-induced chemical luminescence is independent on the temperature and takes place with the same intensity in the ice phase, hence it is independent of diffusion processes. An energy transport through electron displacements in the water phase is discussed.

3. By contrast, the protective effect of cysteine is temperature-dependent and is lost upon freezing.

4. Protective effect against radiation is exhibited by cystemine, cysteine and glutathione. Cystamine, histamine and KCN are ineffective.

BIBLIOGRAPHY

Albrecht, H.O., Z. physik. Chem. [illegible]rnal of Physical Chemistry) 136, (1928), 321.

Allen, A.O., Ann. Rev. Phys. C[illegible] (1952), 57

Bacq, I.M., and Herve, A. Str[illegible]r. (Radiation Therapy) 95 (1954), 2, 215

Barron, E.S.G., Dickmann, S., [illegible].A., and Singer, T.P., J. Gen. Physiol. 32 (1953), 53[illegible]39.

Bergstermann, H., Arch. exp. P[illegible]rchives of xperimental Pathology) 204 (1947), 509; Biochem. [illegible]Biochemical Journal) 1948, 120 and 439.

Bersin, T., and Steudel, J., B[illegible]ch. Chem. Ges. (Bulletin of the German Chemical Society) [illegible]1938), 1015.

Burnett, W.T., Stapleton, G.E., [illegible], M.L., and Hollaender, A., Proc. Soc. Exper. Biol. Med. [illegible]1), 636.

Dale, W.M., Brit. J. Radiol. S[illegible] (1947)

Dessauer, F., Quantenbiologie ([illegible]m Biology), Berlin, 1954

Drew, H.D.K., Trans. Farad. So[illegible] (1939), 207.

Druckrey, H., Z. physiol. Chem. [illegible]rnal of Physiological Chemistry) 269 (1941), 158.

Evans, M.G., and Gergely, J., [illegible]. Biophys. Acta 3 (1949), 188

Gleu, K., and Pfannstiel, K., J[illegible]t. Chem. (Journal of Practical Chemistry) 146 (1936), [illegible]8 (1937), 72.

Harvey, E.N., J. Phys. Chem. 33 (1[illegible]), 1456.

Hopkins, H., Gowland, and Morga[illegible], Biochem. J. 32 (1938), 611.

Latarjet, R., and Gray, L.H., Ac[illegible]diol. 41 (1954), 61

Minder, W., and Heydrich, H., Fa[illegible] Soc. Disc. 1952, 305.

Patt, H.M., Physiol. Rev. 33 (19[illegible]35.

Weiss, J., Nature, London 153 (1[illegible]), 748; 169 (1952) 460; Brit. J. Radiol. Suppl. 1 (1947), [illegible]

Wirts, K., Z. Naturforsch. (Journal of Natural Science Research) 26, (1947), 94.