The Effect of Radioprotective Chemicals on Phosphorescent Emission of Riboflavine in Oxygenated and Deoxygenated Solutions

Recent studies, reviewed by Gray\(^1\) suggest that the role of oxygen in radiation damage may be connected with metabolic events in the cell, which are oxygen dependent. Furthermore it appears that at the site of radiation damage consumption and depletion of oxygen occurs in vivo, as demonstrated by Boag and Dewey\(^2\) with Serratia marcescens; using a single 2 μ sec pulse of irradiation in the presence of oxygen, the bacteria were almost as insensitive as if irradiated continuously with the same dose under anaerobic conditions. Also, the studies of Hutchinson\(^3,4\) have shown that for monotypic action, energy transfers mit einem direkten arterenergischen Wirkungsmechanismus, das heisst Mimetika der Katecholamine (zum Beispiel Benzedrin und Pervitin); Klasse II: Substanzen mit einem indirekten arterenergischen Wirkungsmechanismus, das heisst Wirkung durch Freisetzung der Katecholamine (zum Beispiel Kokain und N-Benzyl-Benzedrin).

J. M. VAN ROSSUM, J. B. VAN DER SCHOOT, and J. A. TH. M. HURKMANS


---

2. J. W. BOAG and D. L. DEWEY, quoted by L. H. GRAY\(^1\).
over distances of the order 10–30 Å around the target must take place to alter radiosensitivity, and the smallness of this diffusion zone is difficult to reconcile with the concept that radicals formed by irradiation spread widely through-out the cell, and that chemical radioprotective action is a scavenger process. Furthermore the addition of –SH groups to dilute solutions of DNA or enzymes in vitro makes an oxygen effect manifest, which is not elicited when DNA in solution is irradiated in the absence of –SH groups.

Whilst the radioprotective action of many amines in mammals seems adequately explained on a basis of tissue anoxia, this is not the case with certain other agents such as cysteamine, cystamine and AET which appear to exert substantial if different actions on the oxygen requirements of cells. On the assumption that the repair of an electron gap (direct hit) in a cell macromolecule depends on a transfer of energy, and this energy may possibly be transferred by a process of resonance from an energy rich system such as the excited heterocyclic prosthetic group of an enzyme, it was decided to determine the behaviour of certain fluorescent dyes in frozen solution as regards the stability of the triplet state (E*) in the presence of certain radioprotective and other compounds, tested under both aerobic and anaerobic conditions. The simple visual test of phosphorescent light emission of the spot frozen dye solution in response to ultraviolet light exposure, described by Szent-Györgyi was followed. Of the three fluorescent substances studied —riboflavin, acridine and rhodamine—the first is of particular biological interest. Riboflavin gives off an orange phosphorescence in the presence of oxygen. This is due to the paramagnetic properties of oxygen causing a perturbation which alters the reactivity of this biological catalyst, and makes the triplet state unstable and a new electronic transition (E* → E) allowable with a return to the ground state. This behaviour of riboflavin towards oxygen is specific; absence of oxygen causes quenching of the phosphorescence. This compound is a key structure in the catalytic activity of aerobic enzyme systems, particularly in effecting the transfer of hydrogen from food-stuffs to the cytochrome system and in stabilising the energy released by oxidative phosphorylation.

The three dyes in 10⁻⁴ M solution of the free base were tested either in the presence or absence of oxygen. All solutions were made up with deionised distilled water, and the test agents were added to give final concentrations of 10⁻⁴, 10⁻³, or 10⁻² M and the solutions were maintained at a pH of 7.4. Oxygen was removed by bubbling oxygen-free nitrogen gas through each of four solutions simultaneously for 15 min. The tubes were immediately corked and placed in a freezing mixture of CO₂-methanol to ensure rapid freezing of the lower part of the solution in each tube. In each experiment oxygenated and deoxygenated control tubes were included. The fluorescent and phosphorescent emission of the upper liquid and lower frozen zone respectively for each solution was recorded. It was found that the absence of oxygen had little influence on the responses of rhodamine and acridine to the various agents tested. For rhodamine, there was a slight increase in wavelength of the emitted light with cysteamine (10⁻³ M), cystamine (10⁻³ M), cysteine (10⁻³ M) and 2-aminoethyl isotheiouronium bromide (10⁻⁴ M) and 2 methylallyl isothiouronium chloride (10⁻⁴ M) only. For acridine, oxygen depletion also caused no significant changes in wavelength of the emitted photon for the various agents tested.

For riboflavin, it was found that the quenching of phosphorescence caused by the exclusion of oxygen from the solution could be reversed by cysteamine (10⁻³ M), cystamine (10⁻³ M), cysteine (10⁻³ M), 2 aminoethyl isotheiouronium bromide (10⁻⁴ M), aminoethyl thiosulphuric acid (10⁻⁴ M), 2 methyl allyl isothiouronium chloride (10⁻⁴ M) and glutathione (10⁻⁴ M), while sodium thioglycollate, thiourea, orthomercaptopentoic acid, methionine, arginine and KCl were ineffective. With the exception of cystine, all of the active compounds are radioprotective agents in vivo, and show residual radioprotection in rats even if the treated animals are irradiated in a chamber containing pure oxygen at pressures of 4–5 atmospheres absolute—conditions which were found to annul the radioprotective action of 5-hydroxymethyl acetoxime (5 HT) and other physiological amines. One other compound tested, the 5 HT antimetabolite, UML 491, gave a milky yellow phosphorescence for riboflavin in the absence of oxygen, but was itself strongly fluorescent in the presence or absence of oxygen.

Whilst these results are difficult to interpret, and further studies may show more striking anomalies, the pattern so far obtained suggests that it is worthwhile to pursue the hypothesis, that radioprotective chemical action in vivo may result from alterations in the excited state of certain key prosthetic enzyme groups and energy transfers with other SH-SS and redox systems. That energy reserves partake in radiation damage is suggested by the finding that a rapid decrease of ATP occurs in irradiated cells and that administration of pyridoxal-5-phosphate, ATP and AMP increases radioresistance. Riboflavin, in the form riboflavin mononucleotide, is not only a constituent for the Warburg yellow enzyme, cytochrome c reductase and the amorphous oxidase for the naturally occurring f-amino acids, but as flavin adenine dinucleotide, forms the prosthetic group of diaphorase, the d-amino acid oxidases, glutamic oxidase and xanthine oxidase, and is an integral part of the prosthetic group of butyryl-coenzyme A-dehydrogenase, the enzyme which mediates the first oxidative step in the oxidation of lower fatty acids. If irradiation causes a zone of oxygen depletion, the excitability of the isalloxazine structure of flavine is altered and the transition E* → E is quenched. In these circumstances, the presence of a protective agent such as cysteamine, would render the triplet state unstable and allow the transition E* → E to provide energy for the repair of radiation damage in a reaction where local consumption of oxygen has taken place. The close relationship of oxidative phosphorylation with changes in permeability of biological membrane barriers and selective toxicity and the proposition that a breakdown in such intracellular barriers by irradiation may be a primary event in cell damage also may possibly be linked to per-