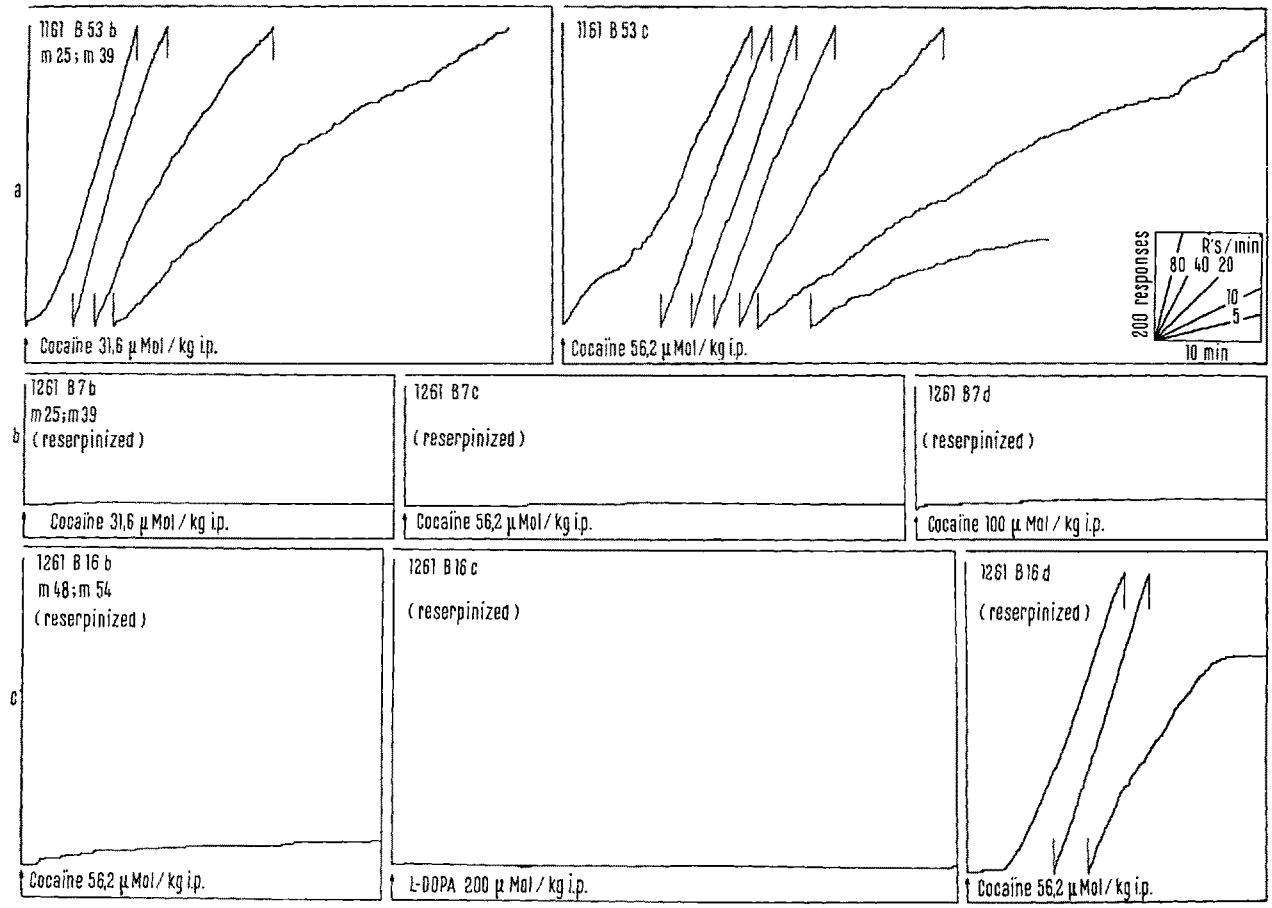

Fig. 2. (a) Cumulative record of the action of *l*-cocaine (31.6 and 56.2 µMol/kg) on the motor-activity in a group of two mice in a similar experiment as in Figure 1a. Note that *l*-cocaine causes a similar increase in locomotor activity as amphetamine though of less duration. (b) Cumulative record of the action of *l*-cocaine in the same group of two mice after they had been reserpinized in a similar way as those of Figure 1b. Note that the action of cocaine is completely abolished by pre-treatment with reserpine and that even higher doses remain ineffective. It must be concluded that cocaine can only act when CA-stores are sufficiently filled and that therefore cocaine acts by releasing CA from the stores and so causes the free CA concentration to rise in the brain. (c) Cumulative record of the action of *l*-cocaine on the motor-activity in a group of two reserpinized mice (0.5 mg/kg twice a day over 4 days). Cocaine is ineffective. After 3 h rest the mice are loaded with 200 µMol/kg L-DOPA. DOPA as such does not increase motor-activity, however L-DOPA may be converted into catecholamines in the brain so that stores now become refilled. A test dose of 56.2 µMol/kg *l*-cocaine 1 h after the application of L-DOPA does exert a practically normal increase in motor-activity. It must be concluded that cocaine has an indirect arterenergic action by releasing CA from stores.

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).

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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 monotopic action, energy transfers

[1] L. H. Gray, Rad. Research Suppl. 1 (Academic Press Inc., New York 1959), p. 73.
[2] J. W. Boag and D. L. Dewey, quoted by L. H. Gray[1].
[3] F. Hutchinson, Radiation Res. 7, 473 (1957).
[4] F. Hutchinson, Science *134*, 533 (1961).

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 throughout 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[4].

Whilst the radioprotective action of many amines in mammals seems adequately explained on a basis of tissue anoxia[5], this is not the case with certain other agents such as cysteamine, cystamine and AET which appear to exert substantial protection *in vivo* in the presence of adequate levels of oxygen tension in tissue fluids. 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[6] was followed. Of the three fluorescent substances studied –riboflavine, acridine and rhodamine– the first is of particular biological interest. Riboflavine 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 ($E^* \rightarrow E$) allowable with a return to the ground state. This behaviour of riboflavine towards oxygen is specific[6]; 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 foodstuffs to the cytochrome system and in stabilising the energy released by oxidative phosphorylation[6].

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^{-5}, 10^{-4}, or $10^{-3}M$ and the solutions were neutralised if necessary. 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 CO_2-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 ($10^{-5}M$), cystamine ($10^{-4}M$), cysteine ($10^{-5}M$) 2-aminoethyl isothiouromium bromide ($10^{-4}M$) and 2 methylallyl isothiouromium 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 riboflavine, it was found that the quenching of phosphorescence caused by the exclusion of oxygen from the solution could be reversed by cysteamine ($10^{-4}M$), cystamine ($10^{-4}M$), cysteine ($10^{-4}M$), cystine ($10^{-3}M$), 2 aminoethyl isothiouromium bromide ($10^{-4}M$), aminoethyl thiosulphuric acid ($10^{-4}M$), 2 methyl allyl isothiouromium chloride ($10^{-4}M$), and glutathione ($10^{-3}M$). while sodium thioglycollate, thiourea, orthomercapto benzoic 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[5,7]—conditions which were found to annul the radioprotective action of 5-hydroxytryptamine (5 HT) and other physiological amines. One other compound tested, the 5 HT antimetabolite, UML 491, gave a milky yellow phosphorescence for riboflavine 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[8,9]. Riboflavine, in the form riboflavine mononucleotide, is not only a constituent for the Warburg yellow enzyme, cytochrome c reductase and the aminoacid oxidase for the naturally occurring *l*-amino acids, but as flavin adenine dinucleotide, forms the prosthetic group of diaphorase, the *d*-amino acid oxidases, glycine 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[10]. If irradiation causes a zone of oxygen depletion, the excitability of the isoalloxazine structure of flavine is altered and the transition $E^* \rightarrow E$ is quenched. In these circumstances, the presence of a protective agent such as cysteamine, would render the triplet state unstable and allow the transition $E^* \rightarrow E$ 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[11,12] and the proposition that a breakdown in such intracellular barriers by irradiation may be a primary event in cell damage[13] also may possibly be linked to per-

[5] H. A. S. VAN DEN BRENK and RUTH MOORE, *Radiation Biology* (Butterworth Scientific Publications, London 1959), p. 179; Nature (Lond.) *183*, 1530 (1959).
[6] A. SZENT-GYÖRGYI, *Bioenergetics* (Academic Press Inc., New York 1957).
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[10] H. A. HARPER, *Review of Physiological Chemistry*, 7th ed. (Lange Medical Publications, Los Altos, California 1959), p. 79.
[11] A. ALBERT, *Selective Toxicity* (Methuen, London 1951).
[12] A. ALBERT, Nature (Lond.) *182*, 421 (1958).
[13] P. ALEXANDER, *Radiobiology* (Butterworths, London 1961), p. 13.

turbations caused by oxygen and protective chemicals in π electron orbitals of enzyme constituents, and the supply of energy for molecular repair processes.

Zusammenfassung. Einige Strahlenschutzsubstanzen und chemisch verwandte Verbindungen wurden in ihrer Wirkung bzw. Veränderung der Wellenlänge, die durch phosphoreszierendes Licht von Riboflavin-, Rhodamin- und Akridinlösungen auftrat, untersucht. Es wurde gefunden, dass nur die Strahlenschutzsubstanzen optisch aktiv waren.

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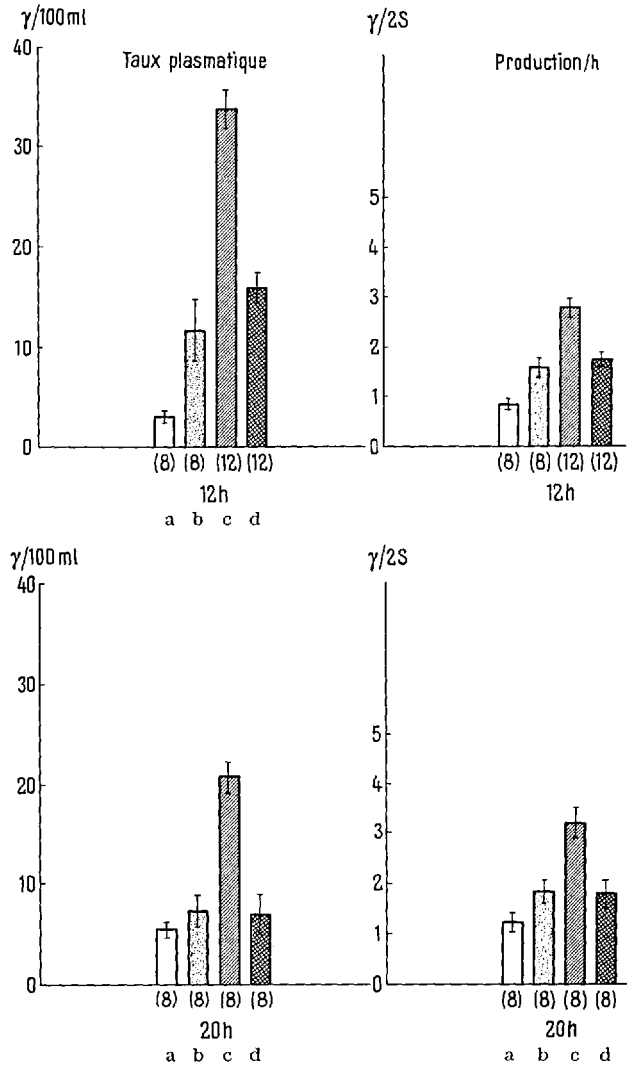
Effet inhibiteur de la tétrabénazine sur l'influence corticosurrénalienne de la réserpine

Certaines benzoquinolizines telle que la tétrabénazine (Nitoman ®), produisent des phénomènes d'origine centrale comparables à ceux provoqués par la réserpine, mais dont la durée est beaucoup plus brève[1,2]. Chez le lapin et le rat l'effet prolongé de sédation observé après réserpinisation est inhibé par l'administration préalable de tétrabénazine[3,4]. Un mécanisme d'inhibition compétitive pourrait être à la base de ce phénomène. Il semble en effet, que si la réserpine n'est pas «fixée» rapidement sur les «récepteurs», elle est éliminée de l'organisme dans les premières heures qui suivent son administration. Dans ces conditions, l'injection préalable de tétrabénazine en «occupant» les mêmes «récepteurs» empêcherait la fixation de la réserpine qui serait ainsi éliminée par l'organisme avant la fin de l'effet de la tétrabénazine et sans avoir pu agir.



Influence de l'administration de réserpine, de tétrabénazine et de tétrabénazine suivie de réserpine sur le taux plasmatique de corticostérone libre et sur la production de corticostérone par les glandes surrénales isolées et incubées *in vitro*. Déterminations 12 et 20 h après la dernière injection. Valeurs moyennes $\pm$ l'erreur moyenne de la moyenne. Entre parenthèses le nombre de rats utilisé dans chaque groupe. a) rats témoins; b) rats ayant reçu une dose de 50 mg/kg de tétrabénazine; c) rats ayant reçu une dose de 5 mg/kg de réserpine; d) rats ayant reçu une dose de 5 mg/kg de réserpine 1 h après l'injection d'une dose de 50 mg/kg de tétrabénazine.

D'autre part, la réserpinisation stimule longuement l'activité corticosurrénalienne[5]. Il nous a paru intéressant, dans ces conditions, de déterminer si le pouvoir inhibiteur de la tétrabénazine envers l'effet sédatif de la réserpine se reflète également au niveau de son action corticosurrénalienne.

Nos expériences ont été effectuées sur 72 rats mâles adultes provenant de l'élevage du laboratoire. Plusieurs jours avant l'expérience les animaux étaient acclimatés à la salle d'expérience et isolés par groupes de quatre. La tétrabénazine et la réserpine étaient injectées par voie intrapéritonéale aux doses respectives de 50 et de 5 mg/kg. La tétrabénazine était administrée 1 h avant la réserpine et les rats étaient sacrifiés 12 ou 20 h après l'injection de réserpine. Le taux plasmatique de corticostérone libre et la production de corticostérone par les surrénales *in vitro* étaient déterminés selon la technique décrite précédemment[6].

Les résultats de cette expérience, qui sont groupés dans la Figure, montrent la longue stimulation de l'activité corticosurrénalienne par la réserpinisation. 12 h après l'injection de réserpine, le taux plasmatique de corticostérone passe d'une moyenne de $2,6 \pm 0,16$ à $35,2 \pm 3,0$ γ%, tandis que la production horaire pour les deux surrénales est accrue d'une moyenne de $0,84 \pm 0,16$ à $2,85 \pm 0,21$ γ. Cet effet est très significativement ($P < 0,001$) diminué par l'administration préalable de tétrabénazine, le taux plasmatique et la production surrénalienne n'atteignant dans ces conditions, que respectivement $16,3 \pm 1,5$ γ% et $1,83 \pm 0,10$ γ en moyenne. Que l'inhibition de l'effet n'est apparemment pas totale doit probablement être at-

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