CHEMICAL PROTECTION AGAINST IONIZING RADIATION[1,2]

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Because of the ubiquitous nature of the energy absorption, each cell is at risk during exposure to ionizing radiations. In consequence, radiation effects in an organism such as a mammal are widely distributed in space and time. In this fact lies perhaps the major difference between the effects of radiations and of other physical and chemical agents. Since the ionizing or high-energy radiations can lead to a multiplicity of injury or disease states, protection may be a rather nebulous concept. Aside from the obvious application of physical shielding, the possibility of a more or less general protection against radiation effects depends upon an appropriate diversion of the pathways by which the radiant energy is absorbed and dissipated. The final common pathway persists for perhaps a millionth of a second after the passage of an ionizing particle, and this imposes a rather severe constraint on the probability of over-all protection. The formation of long-lived organic intermediates may prolong this interval somewhat, but even under these conditions, some irreparable damage probably occurs within a fraction of a second. Protection against one or another radiation effect is, of course, another matter, and this possibility is not necessarily restricted by the rapidity of the primary interactions.

In this paper, we shall review recent work on chemical protection against radiation effects in mammals; this will be concerned largely with whole body exposure to external radiation. There is, of course, a great interest in this general area for theoretical as well as practical reasons. It may be noted that there has been a rather sharp decrease in the publication of new information during the last few years. This may be attributed, in part at least, to the need for new ideas and approaches to the problem.

In a way, ionizing radiation may be thought of as a "general protoplasmic poison" and the search for protective agents is, in essence, an attempt to develop antidotes to such a poison. Radiation effects may be acute or chronic, depending upon the radiation dose and the time over which it is administered. The so-called latent period between the absorption of the radiant energy and its ultimate biological expression, for example, as lethal action, is generally

[1] The survey of the literature pertaining to this review was concluded in March, 1962.

[2] Abbreviations used in this chapter include: AET (s-(2-aminoethyl) isothiouronium); APT (3-mercaptopropyl-isothiouronium); DNA (deoxyribonucleic acid); ESR (electron spin resonance); MEA (2-mercaptoethylamine); MEG (mercaptoethyl guanidine).

inversely proportional to the total dose (1). Doses of the order of 50,000 rads at high dose rates are followed by immediate injury and death, presumably as the result of damage to the central nervous system (2, 3); exposure to about 1,000 rads leads to death in several days owing to loss of the gut epithelium (4, 5, 6); irradiation with several hundred rads is followed by profound effects upon the hematopoietic system with death occurring within two to four weeks (7, 8); smaller doses of 100 r or less may produce only equivocal acute symptomatology and lead to such chronic effects as decreased life span, increased tumors, incidence of degenerative diseases, or cataracts, long after the initial radiation insult (9, 10, 11). Protraction of the dose and intermittent doses provide correspondingly slower development of milder symptomatology. The influence of dose protraction or fractionation can be understood if one assumes that a multiple injury is necessary for a given effect and that at least some of the injury is reparable. Because of the capacity for repair, a higher total dose is required at low dose rates. It has been estimated that the dosage requirement for an equivalent life shortening is about five times as great for chronic as for acute irradiation (12). By and large, cell depletion is a common denominator of the major acute radiation effects. If one excludes the immediate shock-like syndrome after high dosages, it is possible to relate differences in time of development of various changes and in modalities of acute lethality to differences in kinetics of cell renewal in the various systems. There is as yet no clear association between acute damage and the more delayed changes after a single irradiation (13). Also, the picture in regard to chronic irradiation is not completely understood.

Since the irradiated organism must contend with a series of crises, not all of which are causally related, attempts at prophylaxis have been focused mainly on the pathways of energy absorption and dissipation. Partly in consequence of such efforts, it is now clear that the initiation of many radiobiological effects is not entirely simple and proximate. There is reason to believe that biological effects are brought about by transfer of energy to certain small targets as distinct from the transfer of energy to the bulk of protoplasm. Owing to the statistical nature of the primary ionizations and excitations, certain structures can receive large amounts of energy even though the over-all energy absorption is minute. Moreover, energy can migrate from the point of original deposition, not only within a molecule, but also from molecule to molecule. Energy transfer can be quite specific. Free radicals produced in the ambient water are thought to represent an important mechanism for distribution of the absorbed energy. In point of fact, the possible biological significance of activated-water reactions led to the first successful approaches to chemical protection (14).

Protection involves, in the broad sense, the prevention or reversal of radiation effects. Since, as we have noted, radiation effects are non-specific and widely distributed in time and space, a variety of biological indices expressive of such changes may be used as criteria of protection. These include alterations in viscosity of natural and synthetic polymers (15),

membrane permeability (16), intracellular enzyme concentration (17), mitotic activity (18), organ mass or composition (19), organ function (20), and survival of the organism (21). The most convenient and frequently studied parameter is, of course, survival. Although single-cell or isolated tissue systems may offer some statistical or manipulative advantage, they are less reliable in predicting whether a given compound will prevent radiation lethality. Thus, the inability of a compound to alter a radiation-induced change in the viscosity of a nucleic acid or in the concentration of a liver enzyme would not preclude the possibility of a pharmacological action on the body leading, for example, to sparing of some hematopoietic tissue with ultimate survival.

As a generalization, protection in mammalian radiobiology is usually taken to signify a reduction in acute lethality after single doses of X or *gamma* irradiation. The slope of the dose-mortality curve is very steep and a comparatively small dose reduction in the $LD_{50/30}$ range may lead to a marked change in lethality. For this and other reasons as well, it is important to evaluate potential protective agents in terms of the dose-mortality curve. If the protective action is uniform over a given dose range, the ratio of doses for equivalent lethality, e.g., the 30-day LD_{50}, for protected and unprotected animals provides a dose-reduction factor, which is the most meaningful parameter for evaluation of protective capacity (22). As noted recently by Thomson (23), single-point mortality determinations may lead to spurious conclusions about protective activity. The importance of such considerations in the evaluation of radiation protective effects generally was also emphasized earlier by Patt (24).

Since mice can be protected by a variety of compounds that display little or no activity in other mammalian species, the use of this animal for such studies has been questioned recently (25). An attempt has been made to correlate radioprotectability with the ease of inducing hypoxia in the mouse. The possibility of such a correlation was suggested by the well-known protective effect of hypoxia, which is as great or greater than that of any known chemical agent. Because of the high ratio of surface to mass, mice consume more oxygen per unit of weight than rats or men. The oxygen saturation curve is much flatter than in most mammals and, as a result, whereas the blood of man or rat is 50 per cent saturated at a pO_2 of 35 mm Hg, that of the mouse is only 25 per cent saturated (26). Hence, any further reduction by chemical or environmental means brings the mouse to a much lower relative tissue oxygen level with a corresponding increase in radioresistance.

The importance of age at the time of irradiation to the subsequent development of radiation injury has been reemphasized. In mice, the life-shortening effect of x-irradiation shows a maximum at four weeks of age, a minimum at 40 weeks and then a rapid increase in sensitivity with age (27). Although local reactions in skin and tissue (necrosis, sloughing) are more extensive in children than in adults, repair is also more vigorous; the balance is such that the infant may require only 80 per cent of the adult dose of x-irradiation for a comparable effect. Sensitivity to local necrosis increases

to a maximum at the age of 70+ in adults (28). It is of interest to note that the hypocalorically reared rat, with its extended life-span, is more sensitive to acute radiation effects than normal controls, having an $LD_{50/30}$ of 624 r as compared to 709 r for normally fed litter mates (29).

Although some time must elapse between the causation of damage and its expression, virtually all of the radiation chemical protective agents to be effective must be given before the exposure. Because of this requirement and other considerations to be discussed later, it is believed that such agents are concerned more with the prevention of injury than with the enhancement of recovery. This distinction applies to the overt physiologic and pathologic signs of radiation exposure. Injury and recovery processes must occur at the molecular level even during the course of irradiation, and it is conceivable that some protective agents may act by promoting the restoration of damaged molecules. In this connection, it may be instructive to note that there are procedures that can be instituted after irradiation that are more obviously concerned with recovery. Without exception such radiation antidotes are quite specific and, in consequence, have a limited effect. Thus, cells of bone marrow (30), spleen (31, 32) or fetal liver (33) when injected into the irradiated animal, colonize and grow in the hematopoietic tissues of the host tiding it over the period when its own hypoplastic marrow is incapable of responding to the needs of the body. Such transplantation may have a dramatic influence on recovery of the hematopoietic system. However, it does not modify other sequelae of radiation exposure, either acute or chronic, with the exception of leukemia (34, 35, 36). Similarly, antibiotics, by suppressing bacterial growth, may tip the balance between survival and death when the integrity of the gastro-intestinal epithelium is breached (37), or when the reduced number of circulating and tissue leukocytes is incapable of coping with naso-pharyngeal invaders (38). The effect of antibiotic treatment varies widely with species, being most marked in some strains of mice (39), and less in rats and dogs (40). Antihemorrhagic agents (41), blood (42), platelets (43), and cell-free spleen extracts (44), as well as general supportive measures (45), have been used with varying success.

In contrast to such rather specific postirradiation procedures, appropriate chemical prophylaxis may be used to decrease injury in a more general sense, presumably by intercepting the chain of radiation-induced effects at a point relatively early in its development. It was only in 1940 with the work of Dale (46) on the inactivation of enzymes in dilute solutions that we became aware of the possible role of radicals derived from water in the causation of biological damage. These findings and the subsequent investigations of Barron (47) were a logical outgrowth of the studies by Fricke (48) who a decade earlier had pointed out that the effect of radiations on simple organic and inorganic compounds in solution could be due to activated water. Since the free radicals formed upon irradiation of water are mainly oxidants, it followed that reducing substances, given just prior to exposure, might protect at least against some radiation effects. This possibility was strengthened by the fact that oxygen itself was believed to be an important intermediary

in radiochemical and radiobiological reactions (49). The first such protective antidote was cysteine, which was found by Patt *et al.* (14) to protect rats and mice against the lethal effects of x-irradiation. In the same year, Herve & Bacq (50) obtained by pretreatment with sodium cyanide an increased survival of irradiated mice. It has been suggested (23) that a circular argument has arisen on the basis of these and subsequent findings; namely, it is assumed that free radicals are the primary cause of damage from x-irradiation; (*a*) since compounds such as cysteine and cysteamine prevent radiation damage, and (*b*) since cysteine and cysteamine react with free radicals, it follows, q.e.d., that free radicals are the primary causes of radiation damage.

Electron spin resonance (ESR) spectroscopy, a rapidly developing new physical tool first adapted in 1945 by Zavoisky (51) to free radical detection, may provide evidence that can be used to straighten the above-mentioned circular syllogism and establish a causal relationship that is now lacking. A good deal of data has already been accumulated in this area, but because of the difficulty in identifying and decoding the complex micro-wave messages received from biological substances, their interpretation is still open to question. Free radicals can be detected in dry irradiated zein (52); when cysteamine or 2-mercaptoethylamine (MEA) is added, there is a decrease in radical yield. Similar findings have been reported with a freeze-dried albumin preparation (53). The free radicals indicated in ESR patterns from irradiated frozen yeast are not seen when MEA is added 30 sec before irradiation (54). When dried *B. megaterium* spores are x-irradiated, radicals can also be detected, some of which are dependent on the presence of oxygen (55). Other studies have shown that cysteine can alter and reduce the type of free radicals formed in rat liver irradiated *in vitro* (56). Although micro-wave spectroscopy indicates that agents known to afford some protection to biological systems can also reduce the number of free radicals formed upon irradiation, ionizing radiation induced molecular derangements need not involve the appearance of unpaired electrons (57).

Many hundreds of compounds have been tested for radioprotective effects. Greatest emphasis has been placed on aminothiols, the most effective and thoroughly studied being relatively simple derivatives of 2-mercaptoethylamine. The results of such testing have been reviewed in detail elsewhere (58, 59) and it is sufficient for our purpose to note that compounds such as cysteine, 2-mercaptoethylamine (MEA), and S-(2-aminoethyl) isothiouronium (AET) afford, under optimal conditions, similar protection, namely a dose-reduction factor for acute lethality in mice of about 1.7. The most efficient of these aminothiols and their derivatives, i.e., the one that produces maximum effectiveness at the lowest molar dose is the propyl homologue of AET, 3-mercaptopropyl-isothiouronium (APT), whereas the most effective per unit weight is 3-mercaptopropylamine. However, these compounds are also the most toxic of this group and, as shown in Table 1, there is little difference in the ratios of the LD_{50} to the effective dose of the various compounds.

It appears that both the $-SH$ and $-NH_2$ groups are required for protec-

tion. The absence of these free groups or their substitution may sharply decrease or eliminate the protective properties (60). At the same time, a definite spatial relationship must be maintained between the $-SH$ and $-NH_2$ moieties; separation by more than three carbon atoms causes a significant loss of activity (61). Structural specificity alone does not guarantee an active protective compound. Material may be inactive for a number of reasons, for example, poor absorption from the gastro-intestinal tract, inadequate tissue distribution, or cellular penetration, or rapid detoxication.

TABLE 1

COMPARISON OF EFFECTIVENESS OF PROTECTIVE THIOLS[a]

	LD_{50} (*mg/kg*)[b]	*Effective Dose* (*mg/kg*)[b,c]	*Therapeutic Index*	*Relative Protection at Optimal Dose*		
				Intravenous	*Intraperitoneal*	*Oral*
Cysteine	1700	1200	1.4	+++	++	±
Cysteamine	200	150	1.5	+++	+++	±
Cystamine	220 (est.)	150	—	Toxic	+++	++
AET	480	350	1.4	+++	+++	++
3-mercapto-propylamine	100	75	1.3	?	+++	?
APT	320	320	1.5	?	+++	++

[a] From Thomson and Patt (90).

[b] By intraperitoneal injection except for cysteine, for which the doses given are for intravenous injection.

[c] For dose reduction factor of 1.7.

The pharmacological action of many of these compounds is only cursorily understood (23).

Even the best of the radioprotective thiols suffer serious limitation. All of these materials must be given in near toxic amounts to be effective; the therapeutic index varies from 1.3 to 1.5 for the most potent protective thiols (58). In addition, most sulfhydryls must be given shortly before irradiation (62), thus, providing a peak concentration in marrow, liver, and spleen during exposure (63). With the narrow span between an effective concentration and toxicity that characterizes all of the radioprotective agents, the animals may be in a state of severe physiological embarrassment at the time of irradiation (64, 65). If we assume similar protective action and toxicity in both mouse and man the application of such compounds on a mass basis to human populations is obviously impractical. Although the evidence is still scanty, it would appear that the protective thiols are even more toxic in the higher mammals than in rodents. AET in concentrations far below a protective level (2 to 20 mg/kg, p.o.) can lead to a number of unpleasant sequelae in man (66, 67). Many sulfhydryls are also skin sensitizers (68), and repeated

injections may provoke severe allergic manifestations in some individuals. AET in the dog leads to vagal refractoriness in the myocardium, as well as to a rapidly developing hypotension following a hypertensive peak (69). Both cysteamine and AET have been shown to be too toxic to provide protection in dogs. Nor is cysteine well tolerated; 500 mg/kg, a dose well below the maximal protective level in mice, causes vomiting in dogs regardless of the route of administration (70). The same problem of high toxicity has been seen in monkeys. However, it is reported that if lower doses are first given over a period of several days protective doses of AET can be tolerated (71).

Studies have been made of the tissue distribution of S^{35}-labeled MEA in the mouse (63). In confirmation of earlier work (72 to 74) a high concentration of S^{35} activity was found in the liver, spleen, bone marrow, and gut epithelium 20 min after injection. Because of the possibility of metabolic interactions, label distribution may not be a precise reflection of cysteamine distribution. Although a high activity was also found in the cornea, it is of interest that systemic administration of MEA does not modify the radiation-induced mitotic depression in corneal epithelium. However, when the agent is dropped directly into the eye, there is some protection (75). The failure of MEA to protect the rat testis (76) has been attributed to the high radiation dose and the correspondingly low dose of protective agent; following local irradiation with 230–460 r pretreatment with 30 mg of MEA inhibited the reduction in spermatogonia and pre-spermatocytes (77). A significant paper (78) on the comparative tissue distribution of labeled protective agents is concerned with mercaptoethylguanidine (MEG) and two less effective derivatives, the D and L isomers of mercaptobutyl-2-guanidine hydrobromide. Although their protective capacity differed by a factor of five, the tissue distribution was identical. On the other hand, the intracellular distribution in microsomes of liver (mouse, rat) and spleen (rat) appears to be correlated with the degree of protection afforded the whole animal. The data point to three general types of binding to cellular components: (*a*) a loose enzyme-substrate complex, (*b*) a mixed disulfide, and (*c*) a tight binding of unknown nature. It is proposed that the protective molecule is held mainly in a loose enzyme-substrate type complex that effectively quenches free radicals formed in the medium. Although the protective thiols may be bound to cytoplasmic particulates, in the manner described, it will be recalled that the nucleus appears to be considerably more radiosensitive than the cytoplasm. Apropos of this, radioautographic studies suggest that there is also a high concentration of protective thiols in nuclei (79); however, as noted above, without chemical identification the significance of label distribution is open to question.

In addition to the radioprotective compounds of the cysteine-cysteamine class, certain pharmacologic agents are also known to have a protective action. For the most part, these are believed to exert their effect via tissue hypoxia. Protective doses of biological amines such as epinephrine, norepinephrine, histamine, and tryptamine, despite significantly different vasomotor effects, reduce the partial pressure of oxygen in the spleen (80 to

82). The reduction is comparable to that resulting from protective levels of hypoxia. Antagonists can eliminate the vasomotor action of these compounds, but unless the polarographically determined oxygen tension in the spleen is also increased, protective action persists (83). The mode of action of serotonin or 5-hydroxytryptamine, which on a molar basis ranks among the better protective agents (84, 85), is not clear, but the fact that the protective effect in mice (85) and rats (86) can be markedly reduced by several serotonin antagonists suggests that its protective activity may be related to one or another of its several pharmacological actions, e.g., on blood vessels, or respiratory passages. Since the radioprotective effect can be markedly reduced by high oxygen pressure during irradiation, it is probably also associated with tissue hypoxia. The amino-aciduria following irradiation is markedly decreased by serotonin and it is believed (87) that this is due to an effect on the enzyme systems involved; namely, those in which pyridoxal-5-phosphate functions as a coenzyme. It has been demonstrated that protective agents, serotonin among others, can prevent the sudden drop in skin permeability after irradiation (88). Since this can occur in the absence of oxygen, it is inferred that hypoxia does not play an important role in the genesis of the protective effect (89). Serotonin is of some interest because its substantial protective capacity is associated with a wide margin of safety, the effective therapeutic dose being only a fraction of the lethal dose (85), a characteristic seldom met in protective agents. The *Rauwolfia* alkaloid, reserpine, which has a relatively small protective effect when administered 12 to 24 hr before irradiation, may act by releasing 5-hydroxytryptamine (90).

Current literature includes reports of protection afforded by a heterogeneous group of compounds. In most cases the level of protection attained is well below that of the more effective agents; sufficient data are seldom given for quantitative comparison of protective effect, for example, in terms of a dose-reduction factor. Ultraviolet irradiation 24 hr before whole-body x-irradiation is reported to prolong mean survival time in mice (91). Immunization of mice and guinea-pigs 15 days before *gamma*-irradiation with homologous serum from previously irradiated animals also prolongs survival as, to a lesser degree, does the serum of nonirradiated animals (92). Administration of *Mycobacterium tuberculosis*, and Zymosan several weeks before whole-body x-irradiation is reported to reduce subsequent mortality (93); pretreatment with bacterial endotoxin (94), urethane (95), cholesterol or cholesterol-hydroperoxide (96), and parathyroid hormone (97) are also reported to have a favorable effect. The antihistaminic drugs, referred to in the Russian literature as dimerole and dinesine, may modify leukocyte changes induced by *gamma* and x-irradiation in mice, rats, and dogs (98).

It should be emphasized that most of the chemical approaches to radiation protection in mammals have been concerned with acute manifestations of injury under fairly circumscribed radiation conditions. There is little information regarding the more chronic sequelae, and the influence of radiation dose protraction and fractionation, and radiation quality on protective effects. In general, it would seem that radioprotective substances can

influence the chronic effects of ionizing irradiation to a lesser degree than the acute effects. Cysteine delays the onset of radiation-induced cataracts in rabbits by several weeks (99); glutathione and thiourea have a similar effect (99). MEG can protect against thymic lymphoma in *gamma*-irradiated RF mice (34), but MEA does not reduce total tumor incidence or prolong life-span in irradiated rats (100). When $C_{57}B1/6$ mice are given MEA or cystamine before exposure to 550 rad of Co^{60} *gamma* radiation, there is an even higher incidence of lymphatic leukemia than in similarly irradiated controls (101).

Chronically irradiated mice (100 r per day) died sooner when each irradiation was preceded by an injection of MEA (102). When small doses of AET were used, a slight protective effect could be demonstrated in chronically irradiated mice, but repeated injections of a dose that is protective against acute lethality (100 mg/kg) decreased survival due to cumulative toxic effects (103). Hydroxyacetonitrile, serotonin, or *p*-aminopropiophenone, which apparently do not produce cumulative toxic effects, decreased lethality in mice exposed to 200 r/day for 10 days (104). A tolerance to repeatedly administered MEA could not be developed to the point of improving survival in chronically irradiated mice (105). Tolerance to serotonin can be developed in mice (106), but with increasing tolerance radioprotection is lost, even though the amount of the compound being administered is several-fold greater than the toxic dose for normal mice. In regard to protection against different radiation qualities, cysteine, AET, and serotonin afford considerably less protection to neutron-irradiated than to X- or *gamma*-irradiated mice (107). In general, the effect of hypoxia also diminishes with increase in ionization density or linear energy transfer of the radiation.

Increasing attention has been given recently to combinations of agents as a possible means of reducing toxic effects while retaining or perhaps enhancing protective action. Cysteine has been combined with MEA (108), hydroxylamine (109), cyanide (70), or serotonin and acetylcholine (110) with some increase in survival above that obtained when each is administered alone. In dogs, striking protective effects deserving further study have been reported by a combination of MEA, cysteine, dihydroxydiphenylamine, and *p*-aminopropiophenone (111). When a substance such as urethane, demecolcine (Colcemide), epinephrine, sodium arsenite, cadmium chloride or typhoid-paratyphoid vaccine is given to mice 24 to 48 hours before MEA, the protective effect is enhanced (112). Combinations of sulfhydryls given with bone marrow before irradiation and antibiotics given after irradiation, (113, 114, 115) also have increased protective effects. Interpretation of the enhanced survival with MEA and bone marrow transplantation is fairly obvious; the former has a more or less nonspecific protective effect, while the latter is concerned directly with recovery of the damaged marrow, which is a major factor in acute lethality. In most instances, however, interpretation of the effects of mixtures of protective agents is complex; the experimental conditions are seldom appropriate for distinction of additivity, synergism, or potentiation. The protective effect of cysteamine and cysteine appears to

be additive in mice (108); in contrast, hydroxylamine and cysteine seem to act synergistically (109).

A clue to the mechanisms involved in radiation damage and protection may be sought in substances that potentiate rather than attenuate the effects of ionizing radiations. Aside from obvious toxins and poisons, little is known about this aspect of the subject. The aminothiol derivatives β-homocysteine, isocysteine, and penicillamine are reported to sensitize (116); however, this has not been firmly established (58). Halogenated pyrimidines, e.g., 5-bromo- and 5-iododeoxyuridine can sensitize against certain radiation effects, presumably because of their incorporation in deoxyribonucleic acid (DNA). Halogenation is thought to increase the lability of DNA (117).

A number of explanations have been offered to account for the action of radiation protective agents. In general, the proposed mechanisms are concerned with (*a*) inactivation of radicals and other chemical intermediates, (*b*) depletion of oxygen, or (*c*) alteration of target molecules. The tendency to interpret protection as a simple competition with free radicals derived from irradiated water has been decreasing in recent years. This is due, in part, to the fact that some agents can protect nonaqueous systems (118), while others have strong pharmacological properties that seem to be related to the protective effect (119). Arguments continue to be advanced for and against a connection between protection by aminothiols and hypoxia. Unfortunately, there is practically no information about intracellular oxygen tensions, the one parameter that might give a definitive answer to this question. Technical difficulties have led to differences of opinion as to whether thiols, such as cysteine or MEA, afford a greater protection in combination with hypoxia (120). Recent work in several laboratories (121, 122, 123) indicates that cysteine, MEA, AET, and other thiols exert a protective effect without altering the partial pressure of oxygen (as determined polarographically) in target tissues such as bone marrow and spleen. However, there are basic reservations about the validity of the use of open electrodes for measurement of tissue oxygen tensions (124). There are also physiological constraints on this technique; some tissue destruction is inescapable during placement of the electrode, with the result that oxygen tension is measured in a pool of tissue fluid and blood (25). The disappearance of protective action with thiols under several atmospheres of pure oxygen as previously reported (125) has been challenged by recent investigations (25). These indicate that cysteamine protection persists under five atmospheres of oxygen in rats and can only be reduced by pretreatment with sodium cyanide to reduce oxygen consumption. Such data would tend to support the conclusion that some factor other than hypoxia is responsible for the protective action. On the other hand, it has recently been shown (126) that AET, β-mercaptopropylamine and MEA reduce the consumption of oxygen in rats, and hence irradiation of the animal presumably precedes against a background of general reduction in the intensity of oxidation-reduction processes. A decrease in the pO_2 of rabbit cortex following β-mercaptopropylamine

(126), as well as a decrease in the oxygen saturation of venous blood following intraperitoneal injection of cystamine (127) have also been reported. Other studies point to a severe hypoxia and hypotension with cystamine, but not with MEA (128).

Even if some protective agents, particularly the biological amines, act by inducing a hypoxia in critical sites, there is still the problem of explaining the oxygen effect. The sensitizing action of oxygen has been thought of in terms of interactions between oxygen molecules and the electrons, ionized and excited molecules, and free radicals that are found along the tracks of ionizing particles (129). Interaction of oxygen with free radicals may lead to the formation of more toxic agents; oxygen interaction with ionized or excited molecules may also provoke their further breakdown. A corollary to the latter is the possibility that protective agents might combine with target molecules and thereby alter their sensitivity. The concept of protection by mixed disulfide formation is an example of this (130). While the mixed disulfide hypothesis is an appealing possibility, there are reasons to question it as an inclusive explanation of the protective action of aminothiols *in vivo* (131).

Ideally, a protective agent should be nontoxic with no delayed or cumulative effect, and with a relatively long duration of protective activity. These are universal properties to be sought in an antidote to any injurious agent. In the case of ionizing radiations, the ideal radioprotective chemical could be conceived as a ubiquitously distributed energy trap, capable of absorbing and dissipating incident energy without damage to important biological molecules. How such a reactive entity could retain its integrity for long periods of time in the metabolically active cell is difficult to envisage, but if it were possible, protection might far exceed the factor of two in dose reduction which appears to be the maximum for known agents. Perhaps the two surprising things about the phenomenon of chemical protection in mammals is that some sort of biological saturation appears to be a requirement for protection in almost every instance, and that the upper limit of protection seems to be independent of chemical agent and postulated mechanism.

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