PHARMACOLOGICAL ASPECTS OF IONIZING RADIATION AND OF CHEMICAL PROTECTION IN MAMMALS

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INTRODUCTION

Ionizing radiation, irrespective of its origin, produces immediate chemical alterations in irradiated tissues. The initial chemical changes result in metabolic derangements which in the course of days or weeks may lead to manifest cellular damage and eventually to the death of the cells and of the organism.

Although in the last analysis the action of ionizing radiation is of a chemical nature and may profitably be looked upon from a toxicological point of view, radiobiological problems previously received limited attention from pharmacologists.

With the discovery that animals can be partially protected against the deleterious effects of ionizing radiation by *prior* administration of certain specific chemical compounds a new field of investigation was opened. Due to the growing application of nuclear energy for both peaceful and military purposes the study of chemical protection has attracted increasing interest and to-day this rapidly expanding field has in fact developed into a special branch of pharmacology.

The problem of intercepting the development of radiation lesions *after* the irradiation and of treating existing radiation damage, similarly represents a challenge to pharmacology. No pharmacological agent with specific action on existing radiation damage has as yet been developed. So far, only the ordinary therapeutic agents are available for symptomatic treatment of the radiation syndrome. Progress in this field must probably await a deeper understanding of the nature of the radiobiological damage. It should be realized that the protective substances offer a unique opportunity to study this latter problem.

The available evidence indicates that chemical protection against ionizing ra-

diation is brought about by a reduction in the immediate chemical and biochemical lesions. The mechanism of chemical protection is therefore intimately connected with the mechanism of the radiobiological damage, and the two problems can profitably be discussed together. It has therefore been found necessary to consider briefly some aspects of radiophysics, radiochemistry and mammalian radiobiology.

In the present paper the emphasis will be placed on the effects of the protective substances in mammals with only brief reference to their action in the numerous other organisms and test systems studied. A more complete discussion of the mechanism of chemical protection in different test systems will be given elsewhere (78).

PHYSICAL ASPECTS OF IONIZING RADIATION

The types of radiation. Ionizing radiation comprises any electromagnetic or particulate radiation capable of producing ions, directly or indirectly, in its passage through matter. Among the many types of ionizing radiation available to the nuclear physicists, only a limited number have been employed in radiobiological studies and protection experiments have so far been carried out mainly with a few types, *viz.* X-rays, gamma-rays, and neutrons. In the majority of the experiments conventional X-ray radiation in the energy range 150 to 250 Kev has been used. It seems probable that the results obtained with X-rays and gamma-rays are applicable also to irradiation with beta-rays and electron beams, but the protection experiments of Patt *et al.* (191) with neutrons render it unlikely that the results are generally valid for the densely ionizing radiations (alpha-rays, protons, deuterons, fast neutrons, *etc.*).

The mode of absorption. The chemical alterations and destructions of tissue constituents responsible for the biological action of ionizing radiation are caused either by direct interaction with target molecules, or indirectly by the formation of chemically and pharmacologically active entities from water molecules (10, 155, 200, 236).

The incident radiation interacts almost exclusively with the electron shells of atoms and molecules of the tissue, whereby part of the radiation energy is "absorbed" (84). The greater part of the absorption results in excitations, *i.e.*, electrons of the target molecules are raised to higher energy levels. The further fate of such excited molecules in condensed systems is largely unknown. The excess energy can apparently be dissipated in a number of ways and excited molecules can occasionally dissociate to give free radicals. Evidence exists that complex organic molecules have a considerable ability to dissipate the excess energy without suffering chemical alterations (162).

Approximately one third of the absorbed radiation energy leads to the ejection of electrons from the molecules whereby positive ions are produced. Although the creation of ions forms the basis for the usual measurements of radiation dose the important fact from a biological point of view is not the formation of ions as such. The decisive fact is that these molecules are left with a lone electron in one of their orbitals, and thus have become free radicals. The subsequent fate

of such molecules is governed by the laws of radical chemistry. Although a great deal is known about free radical reactions, particularly in the gas phase, the application of this field of chemistry to biochemical systems is still in its infancy. In general, free radicals are highly reactive molecules, which will exist for fractions of seconds only. However, certain types of semi-stable and even stable radicals are known. The major part of the radicals formed in irradiated tissues have a very short lifetime, but recent experiments indicate that semi-stable and stable radicals may also be formed (244).

A free radical formed by ionization of a complex molecule will eventually become stabilized by chemical changes occurring either at the site of the original ionization or at a more susceptible point after migration of the charge within the molecule (59, 242). The chemical changes thus caused by direct ionizations of molecules are said to be produced by the *direct action* of ionizing radiation.

Due to the predominance of water in soft tissues, the greater part of the ionizing events will take place in water molecules. The radiolysis of water (4, 64) results in the formation of the molecules H_2O_2 and H_2 and a number of highly reactive radicals ($H\cdot$, $HO\cdot$, $HO_2\cdot$, O_2^-, and H_2^+). For many years the radiochemical production of H_2O_2 was assumed to be of major significance in radiobiology, but during the last 10 to 15 years the emphasis has shifted to the radicals. The above radicals have been found to be very unstable with a lifetime in tissues of less than 10^{-4} sec (163).

It is significant that the course of the radiolysis of water will depend on the presence or absence of molecular oxygen. When oxygen is absent from the solution, X-rays and gamma-rays do not give rise to H_2O_2, and the amount of $HO_2\cdot$ is considered to be substantially reduced (4). The well known fact that radiobiological damage, *in vivo* as well as *in vitro*, is markedly reduced when the irradiation is carried out under reduced oxygen pressure (*the oxygen effect*) (10), is usually ascribed to the above effect. In tissues the radicals will eventually react and lead to chemical transformations of the cellular constituents. The biological effect brought about by this mechanism is called the *indirect action* of ionizing radiation.

Dosimetry. Different types of ionizing radiation vary with respect to the nature of the incident particles, the energy, and the spatial distribution of the excitations, ionizations, and the associated chemical changes. It has turned out to be a major obstacle in radiobiology to arrive at a dose unit which from a theoretical point of view is founded on a sound physical basis and which can be measured accurately for all types of radiation. This difficulty is clearly illustrated by the abundance of dose units in current use (r, rep, rem, rad, n unit, N unit, υ unit, *etc.*).

The recommended dose unit to-day is the rad, which is the amount of any type of radiation resulting in the absorption of 100 erg/g of matter (209). The rationale behind the choice of energy as the measure of dose is the tacit assumption that for different types of radiation the same amount of energy absorbed will lead to the same number of chemical changes. The assumption presupposes that for different types of radiation the same fraction of the absorbed energy is

expended in ionizations and that the energy consumed for each chemical alteration is determined by the properties of the absorbent and not by the radiation. Although the validity of the above assumptions may be questioned, the rad unit has the advantage of being based on a fundamental physical property.

In actual fact, the direct measurement of tissue dose in rads is hardly feasible. Therefore, in most radiobiological work the dose is still measured in roentgen (r) in the case of X-rays, and in the equivalent unit rep (roentgen equivalent physical) in the case of the other types of radiation. Both units refer to the ability of the incident radiation to ionize air under standard conditions. The estimation of the tissue dose on the basis of the number of roentgens of the incident radiation, is by no means easy, as can be demonstrated by an example (97): The LD_{50} dose for rabbits was found to be 807 r (measured in air) for 250 Kev radiation and 1887 r for 80 Kev. The reason for this difference is that for each r of incident radiation the dose (in rads) deposited in deeper vital tissues is widely different in the two cases due to the lower penetrability of the softer radiation.

The tissue dose can at the present time only be obtained by calculations. More or less complete tables exist from which the dose can be obtained when the type and energy of radiation as well as the properties of the absorbent are known (5).

The radiobiological effect of different types of radiation turns out to be different, even when the tissue dose in rads is the same. This fact is ascribed to the differences in the spatial distribution of the ionizations and consequent chemical changes. The quantitative determination of the "relative biological efficiency" (RBE) of various types of radiation is an important problem in radiobiology (228). The RBE is defined as the inverse ratio of the doses of two radiations required to produce a standard amount of a given biological effect. When the biological efficiency of conventional therapeutic X-rays (250 Kev) is chosen as unity, RBE values ranging from 0.7 (20 to 30 Mev beta- and gamma-rays) to approximately 10 (cataract formation by slow and fast neutrons) have been found (47).

The bulk of the data on radioprotection have been secured in experiments with conventional X-ray radiation (150 to 250 Kev) and the doses are almost exclusively given in r. When small laboratory animals such as mice are irradiated the tissue dose will be fairly homogeneous, but with larger animals the actual tissue dose in deeper structures will vary considerably with the conditions of the irradiation (44). For X-rays the conversion factor between r and rads in soft tissues is fairly constant in the above energy range (1 r = 0.93 rad).

IONIZING RADIATION AS A PHARMACOLOGICAL AGENT

Some of the characteristic features of ionizing radiation can be illustrated by comparing its properties and actions with those of pharmacological agents.

The concept of dose. In pharmacology a dose of a compound is usually expressed in grams or moles per unit body weight. The accepted dose unit in radiobiology, the rad, is correspondingly a measure of energy absorption per gram. This accounts for the fact that for penetrating radiation the LD_{50} dose for whole body exposure is approximately the same for the mouse and the man. The radiobio-

logical equivalent to the total dose of a pharmacological agent is the "volume dose" (gram-rad), which is a measure of the total energy absorbed by the irradiated organism. Certain local and specific effects (*e.g.*, genetic effects) are, for a given type of radiation, exclusively dependent on the number of rads absorbed by the specific tissue while other more generalized radiation effects (*e.g.*, radiation sickness) are largely dependent on the "volume dose".

The toxicity. It has repeatedly been stressed that ionizing radiation is exceedingly toxic in so far as a dose lethal to mammals involves an infinitesimal energy absorption and a correspondingly low number of chemical changes per cell. In this connection it may be pointed out that certain pharmacological agents are on a molar basis approximately equally toxic. Thus, a toxic dose of 0.5 mg/kg body weight of the radiomimetic compound nitrogen mustard (methyl-bis(β-dichloroethyl)amine)(HN_2) will in an adult human give rise to a concentration of approximately 3×10^{-6} M, assuming even distribution. In man the LD_{50} dose of total body X-ray irradiation is of the order 500 r, a dose which gives rise to approximately 1.5×10^{-6} M ionizations and consequent chemical changes.

The chemical specificity of the radiation damage. In general the chemical specificity of a pharmacological agent will be expected to be inversely related to the dose required to give the typical response. The high toxicity and pharmacodynamic potency of the nitrogen mustards depends on their electrophilic activity (211) primarily towards amines, thiols, and carboxylate ions.

The problem to what extent the radiation induced chemical transformations in cells and tissues are associated with specific chemical groupings is largely unsolved. The fact that comparatively few radiochemical changes can result in a strong biological response suggests that ionizing radiation may exert a considerable degree of specificity in its chemical action.

The study of the radiochemical changes involved in the direct and indirect action of ionizing radiation is a fairly new and rapidly expanding field. On the basis of the data so far available it seems warranted to summarize the main results as follows:

The sulfhydryl groups are exceedingly sensitive to the indirect action of radiation, which is evident from the work of Barron *et al.* (20, 21) on the radiochemical inactivation of enzymes requiring free SH-groups for their activity, as well as from studies on simple thiols (22, 222). Disulfide groups of simple (212, 222, 230) as well as complex (70) organic molecules are also very sensitive to the attack of free radicals. The sulfur groups are mainly converted to oxidation products (sulfinic and sulfonic acids) (223) and are partly split off as hydrogen sulfide (54). Recently it has also been found that in the radiochemical degradation of disulfides thiols are formed to some extent (222). Evidence is now accumulating that the sulfur atoms of complex organic molecules are to a considerable extent selectively damaged also when molecules are struck by direct hits (96).

Irradiation of simple and complex organic compounds results in a series of radiochemical reactions which reflect a differential susceptibility of chemical groupings. The reactions include deamination and decarboxylation of amino acids (55, 57, 158, 224), hydroxylation of benzenoid structures (227), peroxide

formations of unsaturated fatty acids (120, 174), polymerizations (2, 52, 119), *etc.*

The radiochemistry of the nucleic acids has attracted considerable interest. Again some degree of selectivity in the chemical changes is observed. The main changes brought about by irradiation in dilute solution are: Breaking of hydrogen bonds, deamination, dehydroxylation, scission of the base-sugar bond, oxidation of the sugar, breakage of the nucleotide chain, and liberation of terminal phosphate groups (33).

From the available radiochemical data it appears that although the initial ionizations are randomly distributed among the molecules along the track of the particle, the resulting chemical changes are preferentially localized to certain susceptible chemical groupings. The evidence indicates that SH- and SS-groups occupy a unique position with regard to vulnerability. The susceptibility of other chemical groupings appears to be of a lower order of magnitude. This seems to be the case with regard to the direct as well as the indirect action of radiation.

Macroscopic and microscopic distribution. The biological action of ionizing radiation is to a large extent determined by the manner in which the ionizations and chemical lesions are distributed throughout the irradiated tissues.

From a *macroscopic* point of view the ionizations will be fairly evenly distributed throughout the soft tissues of the exposed area (except for the depth-dose variations). There will be no essential difference between different organs, part of organs, or between extra- and intracellular space with regard to the radiation dose. In this respect penetrating radiation differs radically from pharmacological agents, the action of which is influenced by factors such as permeability barriers, distribution among organs, *etc.*

From a *microscopic* point of view the deposition of radiation energy is definitely discrete (100). The ionizations and the subsequent chemical changes are all confined to the immediate vicinity of the path of the particle and they will be more or less densely spaced depending on the nature and the energy of the radiation. The ionization density along the track (the linear energy transfer) seems to be of decisive importance in determining the relative biological efficiency of the radiation. For 200 Kev X-rays, beta-rays, and alpha-rays the number of ionizations per μ along the tracks have been found to be 80, 10, and 4000, respectively (100).

A consequence of the discrete nature of the energy deposition in the tissue is that if a cell is struck it receives a certain minimum dose. It can be calculated that the minimum dose to a cell (diameter 10 μ) is of the order 50 rads for an alpha particle and of the order 1 rad for 200 Kev X-rays. It follows that if the tissue dose is further reduced below a certain minimum level, the dose received by each injured cell remains the same (the minimum dose) while the number of cells struck will decrease in proportion to the dose. This fact becomes of particular interest when the genetic and cancerogenic effects of low doses are considered.

The microscopic distribution of pharmacological agents is clearly different as

these agents usually will be uniformly distributed throughout one phase. Localisation to certain cells or subcellular structures may well be found, but will occur as the result of a specific interaction between the drug and the tissue.

In conclusion the above considerations can be summarized as follows. Ionizing radiation, like drugs, ultimately brings about its biological effect, by chemical action on tissue constituents. Whereas ionizing radiation interacts with the molecules along its path irrespective of their molecular structure, drugs frequently exhibit pronounced specificity in their interactions. This circumstance may account for the fact that pharmacodynamically active compounds usually are highly specific in their biological action, while ionizing radiation in principle may interfere with any biological function. In both instances the eventual chemical alterations seem to be preferentially localized to certain specific chemical groupings.

THE RADIOPROTECTIVE SUBSTANCES

1. The concept of chemical protection. The concept of chemical protection was developed in studies on dilute aqueous solutions. Under such conditions the indirect action of ionizing radiation will dominate, since the probability for direct hits of the solute molecules is negligible. It therefore follows that in a solution containing two substances capable of reacting with the radicals, the presence of the one solute will reduce the chemical alterations of the second one. This fundamental fact was clearly established more than twenty years ago by Fricke and his associates (88). In these early studies, careful analyses were carried out on the relative ability of a series of compounds to reduce the radiochemical alterations of an indicator molecule. Although the term protection was not introduced, these workers actually measured the relative protective ability of the substances against the indirect action of ionizing radiation.

The terms protection and protective agents were introduced by Dale (53), who carried out studies in which enzymes were used as indicator molecules. The important discovery was made that certain compounds containing sulfur (thiourea, thiosulfate, colloidal sulfur) were particularly effective protectors (56, 58).

Since the discovery in 1949 (49, 194) that animals could be partially protected against the lethal effects of ionizing radiation by prior medication of cysteine and glutathione, the concept of chemical protection has mainly been used with reference to living organisms and complex biochemical systems. Phenomena of the type described by Fricke will frequently be referred to by the neutral term "competition".

The term "chemical protection" is frequently used indiscriminately to denote any reduction in the radiation damage brought about by chemical means. It is well established that for the majority of the protective compounds an effect is only observed when the agent is present in the organism during the exposure. It therefore seems logical to restrict the term "chemical protection" to the reduction in radiation lesions attained by administration of the agent *before* the exposure (154). Although this definition is purely descriptive, most workers more or less tacitly assume that the term implies a reduction in the immediate radio-

TABLE 1A

The IN VIVO protection by thiols related to cysteine and cysteamine

Compound	Animal	Dose mg/kg	Protective effect*	References
N-Alkyl and N-aryl derivatives of cysteine and cysteamine				
Cysteine	Mice, rats	950–1200 i.p.	3	146, 192, 194
Cysteine	Rats	1900 per os	2	193
Cysteamine	Mice, rats	75–250 i.p.	3	17, 146, 184, 213, 229
Cystine	Mice, rats	240–280 i.p.	0	3, 193
Cystamine	Mice	150–300 i.p.	3	3, 9, 151, 213
Cystamine	Mice, rats	400–600 per os	2	8, 15, 140, 176
N-Monomethylcysteamine	Mice	60–120 i.p.	2	142
N-Dimethylcysteamine	Mice, rats	40–70 i.p.	2	69, 142
N, N'-Tetramethylcystamine	Rats	60 i.p.	2	142
N-Diethylcysteamine	Mice	50–60 i.p.	2	69, 142
N-Piperidylcysteamine	Mice	25 i.p.	0	142
N-Methylphenylcysteamine	Mice	250 i.p.	0	142
N-Phenylcysteamine	Rats	150 i.p.	0	142
S,2-Aminoethylisothiuronium bromide HBr (AET)	Mice	240–480 i.p.	3	67, 68
S,2-Aminoethylisothiuronium bromide HBr	Mongrel dogs	100 i.p.	0	24
S,2-Aminoethylisothiuronium bromide HBr	Macaca mulatta monkeys	200–250 i.p.	3	50
S,2-Aminoethyl-N-methyl-isothiuronium chloride HCl	Mice	150 i.p.	2	221
N-Acyl derivatives of cysteine and cysteamine				
Glutathione	Mice, rats	800–1000 i.p.	3	3, 39b, 49, 193
Glutathione	Rats	2000 per os	0	193
N-Acetylcysteamine	Mice, rats	120–250 i.p.	2	69, 142, 151
N-Acetoacetylcysteamine	Mice	240 i.p.	0	142
Aletheine	Mice	250–300 i.p.	1	69, 146
Pantetheine	Mice	350–550 i.p.	0	3, 69, 146
N-Acetylmethylcysteamine	Mice	150 i.p.	0	142
Compounds with covered sulfur function				
α-Homocysteine thiolactone	Mice	—	+	134
N,S-Diacetylcysteamine	Mice	280–320 i.p.	0–1	3, 68, 69, 146
S-Methylcysteamine	Mice	850 i.p.	0	3
S-Benzylcysteamine	Mice	160 i.p.	0	146
Methionine	Mice	500–1500 i.p.	0	146
S,2-Dimethylaminoethyl-isothiuroniumchloride HCl	Mice	350 i.p.	0	221
S,2-(1-Morpholyl) ethyl-isothiuronium bromide HBr	Mice	150 i.p.	0	221
Di(ethylaminoethyl) sulfide	Mice	140 i.p.	0	69

TABLE 1A—*Continued*

Compound	Animal	Dose mg/kg	Protective effect*	References
Compounds with branched or prolonged carbon chain				
3-Mercaptopropylamine	Mice	90 i.p.	3	69
3-Mercaptopropylguanidine	Mice	125–250 i.p.	3	221
Homocysteine	Mice	450 i.p.	2	3, 146
1-Mercapto-5-diethylamino-pentane	Mice	35 i.p.	0	142
1-Mercapto-7-aminoheptane	Mice	40 i.p.	0	142
α-Methylcysteine	Rats	100 i.p.	0	142

* *Protective effect.* The grading of the optimal protective effect has been carried out according to the following arbitrary scale: 0 = No protective effect; 1 = Slight or dubious protective effect (*e.g.*, α-ketoglutaric acid); 2 = Moderate protective effect (*e.g.*, formic acid); 3 = Strong protective effect (*e.g.*, cysteamine, AET).

chemical lesions. Latarjet and Gray (154) have suggested that an alleviation of radiation lesions accomplished by medication *after* the radiation exposure should be called "restoration".

2. The radioprotective substances. The effect of the protective agents can in principle be evaluated with regard to any radiation lesion. In general, the effect can be expressed quantitatively as the so-called "dose reduction factor", *i.e.*, the ratio between equi-effective doses in the presence and absence of the protective agent. The dose reduction factor need not necessarily be the same for different biological effects.

In Tables 1 to 3 representative examples of various types of protective substances are presented. The compounds have been arranged in three main groups. The groups and subgroups are believed to reflect different modes of action. As an introduction to the subsequent discussion some general features of the various groups of protective agents will be summarized briefly.

a. The cysteine-cysteamine group. A series of thiols and disulfides chemically related to cysteine and cysteamine possess a pronounced protective action. This is the group of compounds which has been most extensively studied. Protection against radiation death has been demonstrated in a number of animal species, such as mice, rats, guinea pigs, rabbits, dogs, and monkeys. The effective doses range from 100–1000 mg/kg body weight. The compounds are ordinarily administered by intraperitoneal injection 5 to 15 min prior to the exposure. The protective effect lasts for about one hour. In a few instances [cystamine, aminoethylisothiuroniumbromide hydrobromide (AET)] protection has also been observed after oral administration.

In most of the animal species the effect obtained is moderate. The highest degree of protection against the lethal action is obtained in mice, where a dose reduction factor of 1.7 has been observed.

TABLE 1B

The IN VIVO protection by other sulfur-containing substances

Compound	Animal	Dose mg/kg	Protective effect*	References
Thiols with alcoholic or carboxylic acid groups				
Thioglycolic acid	Mice	180 i.p.	0	3, 146
Mercaptosuccinic acid	Mice	350 i.p.	0	3
2,3-Dithiopropanol (BAL)	Mice, rats	150–200 i.p. and s.c.	0–1	10, 69, 146
Dithiopentaerythrit	Mice	75 i.p.	0	150
Thiophenols				
2-Mercaptothiazoline	Mice	100 i.p.	0	221
l(−)-2-Thiolhistidine	Mice	420 i.p.	0	150
Ergothioneine	Mice	500 i.p.	0	3
4,6-Dimethyl-2-mercapto-pyrimidine	Mice	270 i.p.	0	150
o-Aminothiophenol	Mice	50 i.p.	0	150
Miscellaneous sulfur-containing substances				
Ammoniumdithiocarbamate	Mice	500 i.p.	3	238
Diethyldithiocarbamate	Mice	600 i.p.	3	16, 238
Thiourea	Mice	2500 i.p.	2	25, 145, 146, 160, 181
Thiocyanide	Mice	200 i.p.	0	3
Thioacetamide	Mice	150 i.p.	0	3, 150
Sodium tetrathionate	Mice	150 i.p.	0	140
Sodium sulfide	Rats	5 i.v.	0	193

* *Protective effect.* The grading of the optimal protective effect has been carried out according to the following arbitrary scale: 0 = No protective effect; 1 = Slight or dubious protective effect (*e.g.*, α-ketoglutaric acid); 2 = Moderate protective effect (*e.g.*, formic acid); 3 = Strong protective effect (*e.g.*, cysteamine, AET).

b. Miscellaneous sulfur-containing protective compounds. Thiourea and various inorganic sulfur-containing compounds, such as thiosulfate and colloidal sulfur, have some effect in mammals when given in fairly large doses. As mentioned above these compounds have played an important role in the development of the theory of chemical protection, primarily in the *in vitro* experiments of Dale *et al.*, but they definitely are of minor significance *in vivo* compared with the compounds of the cysteine-cysteamine group.

Diethyldithiocarbamate and related dithio acids represent a special group of compounds with pronounced protective activity. They are effective in the dose range 100–500 mg/kg. The thiocarbamates have mainly been tested in mice. The compounds are administered by the intraperitoneal route. The optimal dose reduction factor obtained in mice is about 1.4.

c. Substances with pronounced toxicological and pharmacological activity. Cer-

TABLE 2

The protection IN VIVO by compounds with pronounced pharmacological and toxicological activity

Compound	Animal	Dose mg/kg	Protective effect*	References
Histamine	Mice	220–350 i.p.	2	3, 9
		500 i.p.	0	141
Tryptamine	Mice	75–95 i.p.	3	3, 9, 141
Serotonin	Mice	95 i.p.	3	9
		25 i.v.	3	144
DOPA	Mice	95 i.p.	2	9
Tyramine	Mice	80–275 i.p.	3	3, 9
		80 i.p.	0	141
Hydroxytyramine	Mice	50 i.p.	0	141
		75–300 i.p.	3	3, 9
Arterenol	Mice	3–5.5 i.p.	2	9
		2.75 i.p.	0	141
Epinephrine	Mice	0.7–1.4 i.p.	1	98, 239
	Chickens	5 i.m.	1	226
Amphetamine	Mice	1 i.p.	1	144
Ephedrine	Mice	78 i.p.	0	9
		6 i.p.	0	144
Oxytocin	Rats, mice	23–40 units/kg i.p.	3	98, 112
Reserpine	Mice	4 s.c.	3	153
Sodium cyanide	Mice	5 i.p.	2	3
Malononitrile	Mice	6.5 i.p.	3	3
p-Aminopropiophenone	Rats	15–30 i.p.	3	99

* *Protective effect.* The grading of the optimal protective effect has been carried out according to the following arbitrary scale: 0 = No protective effect; 1 = Slight or dubious protective effect (*e.g.*, α-keto-glutaric acid); 2 = Moderate protective effect (*e.g.*, formic acid); 3 = Strong protective effect (*e.g.*, cysteamine, AET).

tain highly toxic compounds with particular action on the cellular respiration exhibit a protective action. These substances (potassium cyanide, malononitrile) are active in molar doses less than one tenth of those of the cysteine-cysteamine group. Their protective activity appears to be modest. Although they are of theoretical interest these compounds are of no practical value due to their great toxicity.

Certain central nervous system stimulants [Benzedrine (amphetamine), tryptamine, oxytryptamine, and Pervitine (methamphetamine)] as well as depressants [reserpine (Serpasil)] have recently been found to possess protective action in mice. These compounds as well are active in extremely low doses. In general, the protective effect is moderate except for reserpine, which appears to be as effective as cysteamine. The effect of reserpine is of particular interest as it seems to have its maximum when given 12 to 24 h before the exposure.

d. Various metabolites and inert compounds. It appears from Table 3 that several metabolites and inert organic compounds may offer some degree of protection. The modest effects obtained and the relatively large doses required render these compounds of little practical significance.

TABLE 3

The protection IN VIVO by various metabolites and inert compounds

Compound	Animal	Dose mg/kg	Protective effect*	References
Fructose	Mice	13500 i.p.	2	3
		5000 i.v.	0	148, 152
Glucose	Mice	13500 i.p.	1	3
		5000 i.v.	0	152
Propylene glycol	Mice	3000 i.p.	3	3
Glycerol	Mice	185 i.p.	0	3
Formic acid	Mice	92 i.p.	2	3
Pyruvic acid	Mice	700 i.p.	1	3
	Mice	250 i.v.	2	148, 152
Lactic acid	Mice	180 i.p.	0	3
		250 i.v.	0	148, 152
β-Ketobutyric acid	Mice	250 i.v.	1	152
Caprylic acid	Mice	290 i.p.	2	3
Salicylic acid	Mice	275 i.p.	2	3
Succinic acid	Mice	950 i.p.	1	3
α-Ketoglutaric acid	Mice	250 i.v.	1	152
Ethylenediaminetetraacetic acid	Mice	580 i.p.	2	3

i.p. = intraperitoneally; i.v. = intravenously; i.m. = intramuscularly; s.c. = subcutaneously

* *Protective effect.* The grading of the optimal protective effect has been carried out according to the following arbitrary scale: 0 = No protective effect; 1 = Slight or dubious protective effect (*e.g.*, α-ketoglutaric acid); 2 = Moderate protective effect (*e.g.*, formic acid); 3 = Strong protective effect (*e.g.*, cysteamine, AET).

THE PROTECTIVE EFFECTS IN MAMMALS

The bulk of the work on chemical protection in mammals has been concerned with the protective action against radiation death. The death is the final result of a large number of radiation effects on more or less vital functions and considerable efforts have been made to assess the degree of protection attained with respect to separate organs and functions. Before the various protective effects are discussed, a brief description of the respective radiation effects in the unprotected animal will be given.

I.A. The radiation syndrome

After moderate doses of whole body irradiation (100 to 500 r) a fairly specific syndrome develops which is characterized mainly by lesions of the blood-forming organs and of the gastro-intestinal tract. Although species differences are observed, the main features seem to be the same in mice, rats, hamsters (135), guinea pigs (206), dogs (46), goats (46), monkeys (111), and in man (110).

The radiation syndrome is usually divided into the following stages (109):

1. The initial reaction, which may last up to 48 h, depending on the dose. The main symptoms are: Anorexia, nausea, vomiting, fatigue. On the basis of the experience from Hiroshima and Nagasaki, early vomiting is an indication of high exposure and is considered an ominous sign (187).

2. A latent period with comparative well being, lasting for about a week. There is an inverse relationship between the length of the latent period and the size of the dose. Although the clinical symptoms in this period are slight, a progressive intractable loss of weight may be seen. Throughout the latent period an increasing number of biochemical and physiological alterations may be revealed by laboratory examinations.

3. The fulminant clinical picture. This stage is characterized by fever, diarrheas, hemorrhages, shock, and ulcerations of the intestinal tract and of the throat. The clinical symptoms are largely due to:

Damage to the blood-forming organs. The circulating lymphocytes decrease in number in the course of hours after the irradiation. The number of polymorphous granulocytes and platelets show a biphasic response. The initial increase during the first 24 h is followed by a gradual decrease which reaches the lowest level with the onset of the severe clinical symptoms. Examinations of the bone marrow reveal that the erythropoiesis is extremely sensitive to radiation but, due to the long lifetime of the erythrocytes, anemia usually appears late and is of a moderate degree. Pronounced anemia may be seen also in the acute phase due to hemorrhages caused by thrombocytopenia and damage to the capillary walls.

Impaired defense mechanisms towards infections. The stationary as well as mobile defense mechanisms against infections are reduced in their efficiency (233). The histiocytes as well as the leukocytes show reduced migratory and phagocytic ability. Previously acquired immunity may be lost, the antibody production is reduced, and the anamnestic response is likewise impaired. Also the titer of properdine declines (161, 199).

Lesions of the gastro-intestinal tract. Desquamations of the intestinal epithelium may give rise to ulcerations with consequent diarrheas (43). Paralysis of the stomach and the intestinal tract can be seen together with disturbances in the fluid and electrolyte balance (35).

4. The recovery phase. The individuals which do not succumb pass into a long recovery phase which eventually leads to a seemingly complete restitution. In rats and mice the animals surviving 30 days will usually recover. In man the clinical picture is protracted, and radiation death may be seen up to months after the exposure.

I.B. The effect of protective agents on the radiation syndrome

Most observers agree that during the first 2 to 3 days after the irradiation, the clinical picture in protected animals resembles closely that in the non-protected ones (10, 12, 48, 190). The initial loss of body weight and the hematological changes are largely the same as in the controls, but from the second or third day on the body weight of the protected mice will start to increase, whereas that of the controls continues to decline. From the first week on there is a marked difference in the symptoms of protected and non-protected animals. The most conspicuous effect of the protective agents is the rapid and early regeneration of the blood-forming organs in protected animals. For obvious reasons the protective effects on many of the symptoms of the radiation syndrome are difficult to

evaluate in quantitative terms. Patt *et al.* (192) have concluded from careful studies on mice that for cysteine the dose reduction is equal with regard to the leukopenia, lymphocytopenia, granulocytopenia, and splenic atrophy.

The perhaps unexpected finding that the initial clinical signs and symptoms are unaffected by protective agents, has prompted several investigators to look for early histological evidence of protection (61, 93, 165). In most organs similar changes were found in the two groups during the first 2 to 3 days, except in the spleen, the intestinal epithelium, and in the liver where the initial histological disturbances, which ordinarily occur within a few hours, were less pronounced in protected animals (93).

In clinical trials cysteamine has been given also after the irradiation in attempts to reduce the symptoms of radiation sickness. In reports from Bacq and his associates (14, 113, 240) a striking effect in patients has been claimed after intravenous injection of 200 mg of cysteamine or oral administration of 600 mg. The restorative effect was gauged mainly by evaluation of more or less subjective symptoms, such as asthenia, anorexia, nausea, vomiting, diarrhea, vertigo, headache, and insomnia. Other workers (45) have failed to observe any restorative effect of cysteamine. It is still an open question whether or not cysteamine is of any value when given after the irradiation, and it has not come into general use in the clinical treatment of radiation sickness. However, under certain experimental conditions (liver shielding) cysteamine does have a definite although slight effect on radiation damage also when administered after the exposure ("the Maisin effect") (138, 170).

II.A. Radiation death

The radiosensitivity of mammals, as measured by the LD_{50} dose, differs from 250 to 800 r in different mammalian species (234).

As might be anticipated from the complex symptomatology of the radiation syndrome, radiation death may be the result of many different mechanisms. The different modes of death have been revealed in experiments where the dose-survival time relationships after single whole body exposures have been analyzed (7, 29, 83, 203, 207, 208), as well as in experiments where part of the body was shielded (167, 169, 205). Although the subsequent discussion will mainly be concerned with radiation death in mice and rats, in principle similar findings have been observed in other species, such as guinea pigs (206) and monkeys (111).

Bone marrow death. With increasing dose the survival time is at first only slightly affected (premature aging, see below). When the dose approaches the LD_{50} value (approximately 600 r) the average survival time declines rapidly from the normal lifespan down to 8 to 9 days, and then remains on this level up to about 900 r. The principal cause of death for the animals having a survival time in the range 7 to 14 days, is the depression of the blood-forming organs with concomitant infections and hemorrhages.

Intestinal death. When the dose is increased above 900 r, the survival time drops to 3 to 5 days and remains on this level when the dose is further increased even up to approximately 12000 r. Statistical analyses of the individual survival times

after doses from 500 to 1000 r reveal a number of 3 to 5 day deaths also in this dose interval (39), a fact which tends to conceal the 8 to 9 day plateau. If part of the blood-forming tissues are shielded during the irradiation, the LD_{50} dose is increased to about 800 r, and the 8 to 9 day type of death disappears.

The above early type of death which is frequently referred to as "the 3½ days death", has been found to be due mainly to the lesions of the intestinal tract.

Brain death. When the whole body irradiation dose increases beyond 12000 r, the average survival time declines to days and hours. When the animals are given doses of about 100000 r they usually die during the exposure. The death is due to damage to the central nervous system and occurs under the clinical picture of increasing apathy, drowsiness, and eventually grand mal type of seizures. Careful studies in animals given local irradiation to the head have shown (95,108) that the anatomical basis for the effects are inflammatory reactions of the meninges, blood vessels, or the brain substance proper as well as degenerations of cerebellar granule cells. In this dose range death follows before the damage to the bone marrow and other tissues can make themselves apparent.

Other types of radiation death. As the result of numerous studies involving local irradiation several other modes of death have been described. The best defined syndromes are: *Oral death* (205) which is seen after irradiation with about 1000 r to the anterior part of the mouth, and *oesophageal death* (167) which occurs after local irradiation of the chest with doses above 2500 r. In the latter case, death occurs in the course of 2 to 3 weeks.

II.B. The effect of protective agents on radiation death

1. The dose-mortality curve. The great majority of experiments on relative protective ability have been carried out with mice and rats in the LD_{50} to LD_{99} dose range. In most studies the 30-day mortality has been recorded.

Mole (180) stressed that the dose-30-day mortality curve is remarkably steep, *i.e.*, the standard deviation of the LD_{50} dose of X-rays is small, a fact which is particularly striking in view of the long interval between the radiation exposure and death. Mole has calculated, on the basis of data from the literature that if for each species the LD_{50} dose is arbitrarily set equal to unity, the standard deviations will fall in the range 0.11 to 0.12 for the mouse, the rat, the hamster, and the dog. This finding is in good agreement with recent data on mice (139, 192). These standard deviations are distinctly lower than the values given by Gaddum (92) for a number of toxicological agents (0.12 to 0.17). On this basis Mole concludes that in principle it should be relatively easy to secure data on protective ability in statistically significant terms. In reality, the testing of protective activity is beset by considerable difficulties, owing to the fact that the effects to be measured usually are small.

2. The procedures used for testing. In general, three different procedures have been used for testing protective activity against death after whole body X-ray irradiation in mammals.

a) The determination of dose-mortality curves in the presence and absence of the protective agents. This method gives the most satisfactory and complete in-

formation and permits the determination of the dose reduction factor. The procedure requires the use of a large number of animals and has so far been carried through only for a limited number of substances (cysteine, cysteamine, and AET) (38, 39, 192).

b) The use of a single standard sublethal dose. This is the method ordinarily used by Langendorff *et al.* (137–153) in their extensive studies on the cysteine-cysteamine group of compounds. These workers use a standard dose of 500 r, which is close to the LD_{50} dose in their animal strains. Fifty to 100 animals are used in each group. The data are usually expressed as the per cent of the animals surviving after 30 days. The authors state (142) that when 50 animals are used in each group, the absolute difference in per cent survival between the experimental group and the control group must exceed 15 to 19 in order to give a significant difference ($p = 0.05$). The procedure permits a semiquantitative evaluation of relative protective ability.

c) The use of a single LD_{99} dose. This method has been extensively used by Bacq and his associates (8–18). Groups of 10 animals are irradiated with a single dose of 700 r, a dose which is almost 100% lethal for their strain of animals. By stepwise increasing the radiation dose, a rough estimate of the dose reduction factor can be made.

The above procedure, which has the merit of being fairly simple, is undoubtedly adequate for the purpose of establishing qualitatively strong protective activity. However, it is apparently less suited for the study of substances with weak protective activity and some of the results obtained with this method have not been confirmed by other workers (141, 150).

An important improvement in the latter procedure has recently been reported by Kimball *et al.* (130). These workers have developed a method of sequential testing which is very economic in time and animals and appears to be ideally suited for the purpose of screening a large number of compounds with a minimum amount of time and expense. In the evaluation of protective activity early mortality is used as a measure of radiation injury on the assumption that early death patterns correlate well with 30-day mortality. The sample size is not predetermined but depends on the results.

3. The degree of protection obtained. In Tables 1 to 3 the main results with regard to protection against lethality are summarized. The bulk of these data have been secured by the use of the second or third assay method mentioned above.

The dose-effect relationship has been investigated by Patt *et al.* in the case of cysteine (192). The protective effect was found to be proportional with the logarithm of the cysteine dose. Probably, similar relationships hold true for the other protective compounds of this group.

For a given compound, the two parameters of particular interest are the optimal protective effect attainable, and the protective effect on a molar basis, respectively. The former obviously requires knowledge of the toxicity of the compound, which therefore must be determined in separate experiments. The protective effect on a molar basis is frequently given relative to that of cysteamine.

a) *The cysteine-cysteamine group.* The protective effect against radiation death has been analyzed in detail in mice with respect to cysteine, cysteamine, and AET.

Following whole body irradiation, different modes of death occur in the different dose ranges, due to differential sensitivity of the various vital tissues. Studies on the effect of protective agents in various dose intervals may therefore give information on the mode and site of action of these compounds.

Protection against different forms of death. In the case of cysteine (192) 1.2 g/kg gave a constant dose reduction of approximately 40% over the entire dose range from 100 to 1000 r of whole body irradiation. Moreover, since approximately the same degree of protection was found with regard to the radiation-induced leukopenia, granulocytopenia, lymphopenia, and splenic involution, Patt *et al.* (192) concluded that the cysteine caused a true dose reduction, *i.e.*, a deletion of a certain fraction of the radiation energy. Catsch (38), on the other hand, obtained evidence that the dose reduction actually increases with increasing radiation dose. The dose-mortality curve for the protected animals did not give a straight line when plotted on a semi-logarithmic scale. Detailed statistical analyses of the survival time of protected and non-protected animals, respectively, revealed that the dose reduction factor for cysteamine (3 mg cysteamine per 20 g mouse) was 1.36 with regard to death occurring after 1 to 5 days, and 2.13 for death occurring after 6 to 15 days. These data strongly indicate that cysteamine protects to a different degree against radiation damage of the intestinal tract and of the bone marrow. This is consistent with previous findings that cysteamine affords only limited protective activity against local irradiation of the abdomen (61, 182, 232). Since an identical dose mortality curve was obtained in experiments with 5 mg AET per mouse (38), the above conclusions apply to AET as well.

Maldague *et al.* (171) observed that cysteamine gives a certain protection against the oesophageal death occurring after local irradiation of the chest. Unprotected rats suffered oesophageal death after doses above 3500 r, while protected animals escaped this type of death after doses up to 4500 r to die subsequently from pulmonary lesions. Some protection, as evidenced by increased survival time, can be demonstrated even against the central nervous death. Thus, cysteamine prolonged the survival time of mice given 66000 r at a rate of 1500 r/min (214).

Structural requirements for protective activity. It appears from Table 1 that there is a high degree of structural specificity with regard to the protective activity of the compounds of the cysteine-cysteamine group. Minor changes in the chemical structure result in large variations in protective ability. The available data seem to permit the following generalizations with regard to the structural requirements for protective activity (13, 132, 142, 221):

The sulfur atoms must be present either as free sulfhydryl groups, or as disulfide groups. The S-alkyl compounds (the thioethers) are uniformly inactive. The compound AET, as well as the other protective isothiuronium derivatives, are only apparent exceptions, since they undergo rapid intramolecular rearrange-

ment under physiological conditions to give cysteamine derivatives possessing a free SH group (105, 221). Similarly, the protective compounds α-homocysteine thiolactone and S-acetylcysteamine are capable of being rapidly transformed to the free SH compounds.

The substances active *in vivo* all contain an *amine group*, which apparently is essential. Substitutions in the amine group strongly affects the protective ability. In general, acylation and arylation of the nitrogen will abolish the protective effect. Alkylations with aliphatic or alicyclic residues slightly increase the protective ability, although the concurrent increase in the toxicity (133, 221) makes comparisons difficult. The guanidyl group seems to add considerably to the activity as compared with the amine proper. Thus, both 2-mercaptoethylguanidine and 3-mercaptopropylguanidine were found to be more active than cysteamine in providing radiation protection in mice (221). The quaternary alkylated amine thiocholine seems to be completely devoid of protective action (62).

The structure of the *carbon chain* also influences the protective effect. The effect appears to be maximal for straight chain compounds with 2 or 3 carbon atoms. An increase in the chain length or a branching of the chain reduces the activity.

The presence in the molecule of a carboxyl group in addition to the amine leads to a decrease in the protective ability. Thus, on a molar basis cysteamine is approximately five times as active as cysteine (229).

It should be pointed out that the above requirements are valid only for protection in whole animals. In simpler systems, such as bacterial suspensions, the protection by sulfur-containing compounds is to a large extent proportional to the sulfhydryl content (116, 118).

Oral administration. The data discussed above all refer to parenteral administration of the compounds in question.

Only few observations have been reported on the effectiveness of the protective compounds after oral administration. In general, the most potent members of the cysteine-cysteamine group seem to offer a moderate degree of protection when given in this latter way. In rats a slight protective effect has been observed after oral administration of cysteine (1.9 g/kg) (193), and AET (0.3–0.6 g/kg body weight) (143). In the case of cystamine (the disulfide form of cysteamine) the results are inconsistent (8, 15, 146, 176).

Fractionation of the dose. It is well established that when the total radiation dose is fractionated with hours or days between each exposure, the total LD_{50} dose will increase materially. Patt *et al.* (192) have found that if a single dose is split into two, with an intervening injection of cysteine, the dose-reduction factor obtained with regard to the second part of the dose is not influenced by the first part of the dose. In contrast, Langendorff and Catsch (137) observed that if the interval between two successive doses is 24 h, administration of cysteamine prior to each irradiation has only a limited or doubtful effect. Moreover, Rugh and Clugston (214) found that when mice were given a daily dose of 100 r until death, daily injections of cysteamine prior to each exposure caused the mice to die earlier

than the non-protected controls. These data indicate that the protective agents are of no value against repeated or continuous irradiation.

b) Other sulfur-containing agents. The dithiocarbamates. The data on protective ability of dithiocarbamates (16, 238) seem to warrant the following conclusions (238):

The most potent member of the series, ammonium dithiocarbamate proper, offers an optimal protection to mice equal to that of cysteamine. On a molar basis, it is only about one third as active as cysteamine. Alkylations of the amine function of dithiocarbamate as well as an increase in the length of the alkylating group will increase the toxicity without changing significantly the protective ability. Of special significance is the finding that derivatives with S-substitutions also possess distinct protective activity. Diethyldithiocarbamate is nearly as potent as the parent compound dithiocarbamate, while the disulfide form (tetraethylthiuramdisulfide) is inactive.

Thiourea and some of its derivatives have been the subject of several tests for protective activity (25, 145, 160, 181). In rats, a protective effect is scarcely demonstrable due to the toxicity of thiourea in this animal species. In mice, however, a dose reduction factor of 1.1 to 1.15 may be obtained after intraperitoneal injection. Thiourea afforded protection to mice also after oral administration when the compound was given daily for 14 days (25). It should be pointed out that the doses needed for protection are on a molar basis 10 to 30 times greater than for cysteamine.

c) Compounds with pronounced pharmacological and toxicological activity. A number of compounds with pronounced effects on the central nervous system and on the cardiovascular system have been shown to possess protective activity. Different investigators agree that tryptamine, serotonin (3, 9, 141), and epinephrine (98, 226, 239) are protective. Langendorff *et al.* (144) have also found amphetamine and methamphetamine to be protective. Furthermore, tyramine, hydroxytyramine, arterenol, and histamine (3, 9) have been reported to be active, but these claims have not been confirmed by Langendorff *et al.* (141).

Attempts have been made to ascribe the protective effect of the above mentioned substances to a general protective ability of amines (3, 13). The amines were supposed to act by competing with tissue constituents for the radiation-induced active agents formed from water (3). In general, beta-substituted ethylamines were thought to be active *in vivo*, particularly when substituted with phenolic groups. Such chemical substitutions were accordingly assumed to influence the ability of the amines to inactivate free radicals.

The above explanations seem untenable for several reasons: Firstly, the available data fail to reveal any consistent relationship between chemical structure and protective ability for the great number of amines tested. Secondly, the compounds are active in very low doses (approximately one tenth to one hundredth of those of the cysteine-cysteamine group). Thirdly, several of the compounds are active in low concentrations and inactive in higher concentrations (144). It seems more likely that the protective effect of these substances in some way or other is associated with their systemic effects (153, 239).

Several compounds known to interact with the transport mechanism and the cellular utilization of oxygen exhibit some protective effect in mice when administered in toxic doses. Few attempts have been made to evaluate their protective effect in any detail. Some of the compounds (sodium cyanide, sodium azide, and malononitrile) are active in very low doses (5–10 mg/kg). In the case of succinonitrile and the methemoglobin-forming noxes (sodium nitrite and *p*-aminopropiophenone) doses of 30–100 mg/kg are needed. On the basis of their known pharmacological and toxicological actions it has been widely assumed (99) that these compounds bring about protection by reducing the intracellular oxygen tension.

d) Metabolites and inert compounds. A number of normal metabolites have been tested for protective activity. Particularly metabolites of the carbohydrate metabolism have been studied. This seemed logical in view of the clinical observation that the radioresistance appears to be increased in diabetic patients (81, 152). For several of these compounds a protective effect has definitely been established under *in vitro* conditions (3, 118). However, the available data from studies *in vivo* are inconsistent and the protective effects reported are modest. Pyruvic acid (3, 148), α-ketoglutaric acid (152), and heroic doses of fructose and glucose (3) have been reported to offer some protection. The protection has been ascribed to an effect on the cellular metabolism and oxygen consumption whereby the intracellular oxygen tension is presumed to be reduced (190).

Special emphasis has been placed on various phosphate esters. A definite protective effect seems to have been established for adenosinetriphosphate which is reported to increase the LD_{50} by approximately 30% (149). Other phosphate esters (152, 179) have been found to decrease the radioresistance of animals and thus to function as radiosensitizers [menadione (2-methyl-4-naphtho-hydroquinone diphosphate), thiamine (aneurin diphosphate), riboflavine (lactoflavine-5-phosphate)].

Various non-physiological organic compounds such as propylene glycol (3) and ethanol (189) have likewise been found to exert some protection after large doses.

III.A. The late radiation effects

Months or years after the irradiation certain late manifestations of radiation damage may be observed.

1. Neoplasia. Ionizing radiation is a very powerful carcinogenic agent, and it appears probable that all cells capable of multiplication are susceptible to induction of neoplasia by irradiation (90, 91). The incidence and sites of the tumors depend on the radiation dose, the animal species, the radiosensitivity of the exposed tissues, and on a variety of other physiological factors. Mice are particularly susceptible to thymic and lymphoid neoplasms and leukemias, whereas guinea pigs appear to be resistant to radiation-induced leukemia (131). Mice are likewise highly susceptible to the induction of ovarian tumors. In rats, radiation-induced development of adenocarcinomas of the intestinal tract, glioma, renal carcinoma, and various sarcomas, have been described (27, 30).

2. Developmental disturbances. The developmental disturbances after irradiation of the embryo represent a special type of late effects. The mouse embryo appears to be very sensitive to radiation (216), and abnormalities can be produced by doses as low as 50 r. The radiation sensitivity varies with the age of the embryo and can be divided into three phases. During the pre-implantation period, irradiation results in an all-or-none effect, *i.e.*, there is a high incidence of death, but the embryos which survive are normal at term. During the second phase, the period of major organogenesis, the sensitivity to gross abnormalities reaches a peak, whereas the susceptibility to prenatal death decreases. For each of the numerous abnormalities there appears to be a rather short time interval, the "critical period" during which it can be induced. During the last part of the gestation the sensitivity to radiation damage drops to low levels. A number of reports indicate that the human embryo is similarly subject to severe irradiation injury.

3. Radiation cataract. Numerous reports describe the occurrence of cataract in humans, as a complication to radiotherapy involving exposure of the eye. In man the latent period for this radiation sequela varies from 1 to 12 years (178). More than 600 r of X-rays are needed to produce clinically significant lesions (159). The developing eye is much more radiosensitive than the adult eye.

Detailed studies in rabbits (183, 201) have demonstrated that the actively proliferating cells of the anterior epithelium of the lens are the most susceptible to radiation damage. Abnormal fibers are produced which cause opacities in the subcapsular region. Radiation cataract has attracted renewed interest since it was discovered that fast as well as slow neutrons are exceptionally effective in producing this lesion (RBE 5 to 10) (136, 183).

4. Greying of hair. This easily recognizable effect (107) has recently been investigated in considerable detail by Chase *et al.* (41, 42). The size of the hairs and of the follicles seems to determine their resistance. Thus, the threshold dose for greying is about 200 r in the mouse and the hamster, 900 r in the rabbit, and 2000 r in the guinea pig. Mouse embryos and new-born mice are more resistant in this respect than the adults. Chase ascribes the greying to a partial or total inactivation of dendritic cells.

5. Premature aging. Acute as well as chronic irradiation will shorten the life span of mammals. In this respect ionizing radiation seems to differ from many other forms of toxic stress from which recovery is apparently complete (51). The shortening of the life span after acute or chronic exposure is moderate. Thus, Hursh *et al.* (quoted in 28) showed that rats given a single dose of 600 r lost some 20 % of their life expectancy.

The shortened life span cannot be accounted for by the increased incidence of neoplasms and leukemias (51). In fact, the mechanism responsible for the reduced life span is unknown (237) and the effect is usually referred to as premature aging. The reduced vitality is evident also from the fact that the LD_{50} dose upon renewed exposure is correspondingly reduced (28).

6. Genetic effects. The genetic effects of radiation have been extensively studied in lower forms of life (*Drosophila melanogaster* and plant material) and the basic

principles of radiation genetics are well established. In recent years, comprehensive studies have been carried out in mice and the results obtained are in principle consistent with those obtained on lower forms of life (217, 218).

III.B. The effects of protective agents on the late radiation sequelae

Most of the late radiation effects are reduced to a lesser extent by protective agents than the acute ones, and some do not seem to be influenced at all.

The incidence of tumors in rats protected against an otherwise lethal dose by glutathione or *p*-aminopropiophenone was found to be remarkably high (30) suggesting that little or no protection against tumor development had been obtained. Similar results were later obtained with cysteamine (168, 169).

Recently Mewissen and Brucer (177) have demonstrated that cysteamine and cystamine in fact increase the incidence of tumors in mice. In protected mice the incidence of tumors was significantly higher than in unprotected animals surviving an equal radiation dose. These unexpected results may possibly be ascribed to a co-carcinogenic action of cysteamine. Another possibility is that the extra survivors in the protected group may represent a selection of animals particularly susceptible to tumor induction.

Cysteamine offers protection in rats and mice against the death of the offspring after irradiation *in utero* (164, 213). The rats surviving after cysteamine treatment prior to irradiation *in utero* did not have normal life span (169), and the male but not the female offspring were found to be sterile.

Protection against cataract formation has been observed in rabbits given either cysteine, glutathione, or thiourea intravenously before local irradiation of the eye (231, 241). Thioglycolate administered intravenously and BAL applied locally were totally ineffective.

The shortened life span of animals surviving a sublethal single whole body exposure is not influenced by cysteamine pretreatment (169).

Cysteamine appears to be ineffective against the genetic effects of radiation in mammals as judged from the incidence of dominant lethality in mice exposed to 500 r (124). This finding may possibly be ascribed to a low concentration of cysteamine in the gonads (72), a view which is supported by the fact that a slight protection only is obtained against other types of radiation damage to these organs. The sterilizing dose in female rabbits (60) and mice (215) is only slightly higher in cysteamine-protected than in non-protected animals. Furthermore, cysteamine affords no protection against testicular atrophy in mice, as evidenced by the weight loss and the microscopical changes (166).

Forssberg has shown that local injection of cysteine into the skin of guinea pigs more or less prevents the X-rays-induced epilation (85). This observation is of particular significance since it provides strong evidence that the local concentration in the irradiated cells and tissues is of decisive importance. Intraperitoneal administration of protective isothiuronium compounds failed to offer protection against the greying of hair in animals surviving an otherwise lethal dose (221). Most probably the local concentration obtained in the skin by the latter mode of application was too low.

IV. Chemical protection against biochemical effects of radiation

Compounds effective in reducing the clinical symptoms of the radiation syndrome may be expected to reduce the associated biochemical alterations as well. Studies on such protective effects may possibly give information on the mechanism of action of these compounds.

A considerable body of data has been collected on the metabolic effects of ionizing radiation (71, 115, 186). It is unfortunate, however, that most of the easily detectable biochemical and physiological alterations appear relatively late after the irradiation and may be secondary in nature. In the present discussion only selected biochemical effects will be considered.

The great sensitivity of the nucleic acid metabolism to ionizing radiation is well established. With the aid of P^{32} a marked decrease in the synthesis of deoxyribonucleic acid (DNA) and to a lesser extent in ribonucleic acid (RNA), has been found in various organs during the first hours and days after moderate doses of whole body irradiation (114, 115). In the bone marrow, the total quantity of DNA and RNA decreases substantially, reaching the minimum level on the fourth day, whereupon it increases, reaching normal values after 10 to 20 days (172). Essentially similar results have been obtained in studies on regenerating livers (114, 129, 235). The damage to the DNA has been suggested to be the underlying cause of cellular radiation death (115), and to be the biochemical explanation of the relationship between radiosensitivity and mitotic activity.

The radiation effects on the nucleic acid metabolism are strongly reduced in animals treated with radioprotective agents (86, 106). Gros *et al.* (104) have shown that in irradiated rats the nucleic acid content of bone marrow and spleen diminish to the same extent in control and in cysteamine-treated animals for the first two days. From the fourth day on, the protective effect is evidenced in a higher nucleic acid content in the treated animals.

The nitrogen balance is negative in irradiated animals, as could be expected from the weight loss after irradiation. After moderate radiation doses the rate of protein synthesis, as measured by the incorporation of labeled substrates, is unaffected or even somewhat increased (34). Similar findings have recently been made in an *in vitro* system (127). The main derangement in the protein metabolism seems to be an increased catabolism with increased excretion of urinary nitrogen (creatinin, urea, and various amino acids). The extra amino acid excretion (leucine, valine, taurine, cysteic acid, and methionine) has received particular attention, since it appears to be an early and constant phenomenon even after low doses of radiation (125, 126, 128, 175).

The effect of protective agents on the radiation-induced changes in the protein metabolism has not been studied in any detail. Aebi *et al.* (1) have observed a slight reduction in the endogenous taurine production of X-ray-irradiated animals protected by cysteamine. The reduced weight loss of protected animals (12) may be considered indirect evidence that these agents also protect against the increased protein catabolism.

THE MECHANISM OF ACTION OF THE PROTECTIVE AGENTS

The requirement that the protective agents must be present in the organism during the irradiation, whereas they in general are totally inactive when administered after the exposure indicates that these substances intercept the radiation injury at an early stage in the development.

Obviously, it is an extremely difficult task to secure experimental data on the events occurring within cells during the radiation exposure. The attempts made to explain the mechanism of chemical protection rest largely on indirect evidence and inferences from model experiments *in vitro*. An interference with the early chemical and biochemical radiation changes can conceivably be brought about in several ways. There has been a widespread tendency among workers in this field to overemphasize one particular aspect and to try to explain all facts on the basis of one of the possible mechanisms. It now seems likely that *in vivo* several mechanisms may contribute to the protective effect of one specific compound and that different mechanisms may predominate for the different types of protective agents.

The various radioprotection mechanisms proposed will be considered below.

1. Protection by inactivation of free radicals. By definition a protective effect achieved by inactivation of radiation-induced free radicals is a reduction of the indirect action of ionizing radiation. For many years the science of radiobiology was divided into opposing camps with regard to the view on the relative importance *in vivo* of the direct and indirect mechanism of action. In recent years it has become increasingly evident that both mechanisms may contribute to the radiation lesions, but that in certain instances the one or the other may predominate. Recent attempts to assess the relative contributions to the radiation damage of the direct and indirect action have been made by Hutchinson *et al.* (121), who conclude that in yeast cells direct and indirect action are about equally important with respect to inactivation of certain enzymes.

The protective effect attained by inactivation of free radicals will be conditioned by the concentration of the protective agent in the tissue of interest, and by its specific reactivity towards the products of radiolysis. On this basis it seems *a priori* unlikely that the majority of the pharmacologically active compounds exert their protective effect by this mechanism. The local tissue concentration obtainable is exceedingly low and the evidence presented to show that these compounds are particularly active with regard to inactivation of free radicals is not convincing.

Since it is well known that a number of sulfur-containing protective agents react rapidly with free radicals (22, 222, 230), it was widely assumed for a number of years that the protection offered *in vivo* by these compounds could be explained by such a mechanism. Conversely, the fact that a certain radiation effect could be reduced by these protective agents was considered evidence that the effect was brought about by indirect action.

The "radical scavenger hypothesis" may conceivably account for the protective effects observed in various simple systems, under conditions where the indirect action is dominating and the concentration of the protector is high

relative to that of the target. This view is supported by the fact that several compounds, which are inactive *in vivo*, are protective in proportion to their thiol content under *in vitro* conditions (85, 116, 118). The theory fails to account for the large differences in chemical protection *in vivo* between structurally related thiols. Minor changes in chemical structure would not be expected to alter materially the reactivity of thiols with free radicals. The available experimental evidence supports the view that different thiols are approximately equally effective in this regard (87, 116). In experiments where the radical-capturing ability of cystamine was directly measured (80) strong evidence was obtained that the protection offered *in vivo* by this compound cannot be adequately explained by the "radical scavenger hypothesis".

Since any organic compound will compete with a target molecule for free radicals, there can be no doubt that the radical scavenger mechanism is operating to some extent in cells and tissues. It seems probable that the limited protection offered by large doses of various metabolites and inert compounds is mainly brought about by this mechanism.

2. Protection by removal of oxygen. The fact that most radiation lesions *in vivo* as well as *in vitro* are reduced when the irradiation is carried out under reduced oxygen pressure has played a central role in the attempts to explain the mode of action of the protective agents (31, 103, 118, 190). The view that the protective agents may exert their action by reducing the cellular oxygen tension has been supported by the finding that the degree of protection obtained is about the same for oxygen deprival and for protective agents. Furthermore, several protective thiols have been found to counteract oxygen poisoning (94).

Unfortunately, limited information is available on the actual intracellular oxygen concentration under experimental conditions, a fact which has left ample room for speculations. Several of the protective compounds may conceivably reduce the intracellular oxygen tension either by way of an action on the central nervous system (153), by peripheral action on the cardiovascular system (31, 98), by interference with the pulmonary gas exchange (147), by methemoglobin formation, or by increasing the cellular oxygen consumption (98, 118, 190).

Efforts have been made to measure the arteriovenous difference in oxygen tension in the absence and presence of protective thiols. The results are inconclusive in so far as cysteine was found to reduce temporarily the oxygen tension of the mixed venous blood in dogs (219, 220), while protective doses of cysteamine did not influence the oxygen tension (40). The few data available on the actual oxygen concentration of tissues demonstrate that administration of thiols (cysteine, cysteamine, and glutathione) reduces the oxidation/reduction potential, whereas the oxygen concentration does not seem to be influenced at all (37).

The significance of reduced oxygen tension *in vivo* can be evaluated indirectly by investigating whether the effects of protective agents and low oxygen tension are additive. Devik and Lothe (63) observed in mice an additive effect of oxygen deprival and protective agents (cysteine, cysteamine, and cystamine), indicating that two different mechanisms were operative.

Gray (102) has measured the oxygen concentration in various *in vitro* systems after the addition of protective thiols. From the data it can be concluded that in several of the *in vitro* experiments of previous authors, the protective effects might well be ascribed to a progressive lowering of the oxygen tension caused by spontaneous oxidation of the added thiol.

3. Protection by replacment. The possibility has been considered that the protective agents may function as "spare parts" capable of replacing cellular constituents before the biochemical functions have been irreversibly damaged (13, 140). Although a few protective agents (cysteine, cysteamine, and glutathione) do occur in cells and may be part of essential metabolic systems, most of the protective agents cannot be envisaged to function in this way. Moreover, in mammals *in vivo*, cysteamine given as such is not incorporated into the coenzyme A molecule (117). Altogether, there seems to be no experimental evidence in support of the latter hypothesis.

4. Protection by alteration of cellular metabolism. One of the striking facts in radiobiology is the wide variations in the radiosensitivity of various animal species, of different tissues of the same animal, and of males and females of the same species. Even the sensitivity of one specific cell varies, *e.g.*, with the mitotic phase. The underlying cause of these differences is unknown but possibly resides in metabolic differences.

It is possible to influence the radiosensitivity of an organism by a variety of procedures. Serious stress, whether caused by irradiation or by other means, leads to an increased radioresistance having its maximum at approximately the fifteenth day (26). Castration increases strongly the radioresistance of male rats and decreases that of female rats (26, 139). In experiments on bacteria (*Escherichia coli*) it has been shown that the type of media used to culture the cells prior to irradiation determines to a great extent their radiosensitivity (6, 225). In the latter case the change in radiosensitivity is clearly brought about by alterations in biochemical pathways (adaptation phenomena).

The change in radiosensitivity caused by the above procedures has the characteristic feature of lasting for a comparatively long period of time, in contrast to the short-lasting effect of the protective substances. Reserpine (Serpasil) has its optimal effect when administered 12 to 24 hours prior to the radiation exposure (153). During this time the tissues release serotonin (30a) and epinephrine and norepinephrine (34a, 118a, 136a). The protective effect parallels the degree of depletion of the amines from the tissues and probably not the concentration of reserpine. The changes in radiosensitivity obtained by altering the chemical composition and metabolism of the tissue cells appear to constitute a fruitful field of investigation.

5. Protection by chemical modification of target molecules. The possibility has repeatedly been suggested (13, 190) that the protective agents may interact, in some way or other, with cellular target molecules and thereby increase their radioresistance. Apart from a few experiments in which enzymes were partially protected by the presence of their specific substrates (66) no evidence had previously been presented in support of this hypothesis.

The mixed disulfide mechanism. Work in this laboratory has demonstrated (73, 80) that the SH- and SS-containing protective agents become attached *in vivo* to tissue constituents through the formation of mixed disulfides. Based on chemical, biochemical, and radiochemical studies the "mixed disulfide mechanism" for chemical protection *in vivo* by the compounds of the cysteine-cysteamine group was formulated (73, 195). This hypothesis has later been supported by a considerable body of data. The hypothesis may be stated as follows:

1) The protective compounds of the cysteine-cysteamine group exist in the body largely in the form of mixed disulfides with the SS- and SH-groups of tissue constituents during the period when they exert protection. This binding of protective residues to target molecules is temporary. The mixed disulfides will be reduced by the disulfide-reducing systems of the body (*e.g.*, glutathione reductase) and the protective residues thus liberated will be subject to metabolism and excretion.

2) The modification of target SH- and SS-groups by mixed disulfide formation with the protective agents represents a partial protection of the target sulfur atoms against the indirect action of ionizing radiation. When a disulfide bond is attacked by free radicals (Fig. 1), one of the sulfur atoms is oxidized to a sulfinic or sulfonic acid group, whereas the other one is reduced to an SH-group. Since an approaching radical may attack either the one or the other of the two sulfur atoms, it is assumed that in 50% of such events the original target sulfur atom will be reconstituted as an SH-group. In this way the mixed disulfide formation is assumed to reduce the chance of irreversible alteration of target molecules.

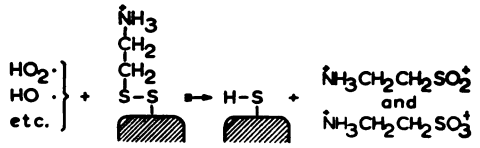

FIG. 1. The mixed disulfide hypothesis for protection against the *indirect* action of radiation.

3) The chemical combination of the protector with the target molecule in the form of a mixed disulfide represents a partial protection of the target also against the *direct action* of ionizing radiation. In an ionized organic molecule the charge can migrate until rupture of a susceptible bond occurs (59, 82, 242). The theory assumes that the mixed disulfide bond serves as the ultimate source of the electrons repairing the original ionization (Fig. 2). Again, the subsequent rupture of the mixed disulfide bond is believed to result in the formation of one SH-group. The mixed disulfide formation is thus envisaged to provide additional possibilities for dissipation of the radiation energy in a non-deleterious way.

The main experimental evidence supporting the mixed disulfide mechanism has been extensively discussed elsewhere (78). It can be summarized briefly as follows. After the administration of protective doses of S^{35}-labeled cystamine and cysteamine to mice, a substantial fraction of the blood radioactivity is chemically fixed to the proteins of serum and erythrocytes (74) and to intracellular constituents. The fixation is mainly due to the formation of mixed disul-

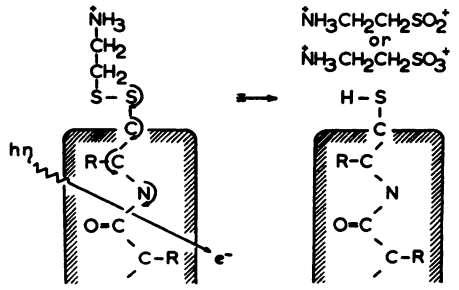

FIG. 2. The mixed disulfide hypothesis for protection against the *direct* action of radiation.

fides with the protein SH-groups (73, 74). A high degree of correlation exists between the protective potency of thiols and disulfides and their ability to form mixed disulfides with biological sulfur groups. Thermodynamic studies *in vitro* have demonstrated that a series of protective thiols (cysteine, glutathione, cysteamine, N-methylcysteamine, N-dimethylcysteamine, N-diethylcysteamine, N-morpholylcysteamine, or AET) form large amounts of mixed disulfides with cystine and oxidized glutathione, which were used as prototypes of cellular disulfides (76, 77, 195, 196). The same holds true for the interaction of the protective disulfides with cysteine and reduced glutathione. On the other hand, a number of non-protective thiols (*o*-aminothiophenol, 2-mercaptothiazoline, thiolhistidine, ergothioneine, thioacetamide, thiocyanide, or dithiopentaerythrit, 4,6-dimethyl-2-mercaptopyrimidine *etc.*) did not form mixed disulfides with the model compounds (79, 197). The apparently obligatory requirement for the presence in the molecule of an amine, in addition to the SH- or SS-group, can be given a logical interpretation as a necessary requirement for the thermodynamic stability of the mixed disulfide.

The mixed disulfide mechanism presupposes that the protective compounds react rapidly with the target molecules. This follows from the fact most protective substances are active when given immediately prior to the radiation exposure. It has been found that at pH 7.4 and 37°C the mixed disulfide formation between the protective compounds and the biological disulfides occurs within a few minutes (76, 77, 78). A series of non-protective thiols reacted sluggishly or not at all with cystine and oxidized glutathione. For a few compounds (acetylcysteamine, thioglycolic acid) the sluggish reaction rate (77, 78, 197) may explain why they possess dubious or no protective effect despite the fact that they do form substantial amounts of mixed disulfides.

The view that the temporary formation of additional disulfide bonds may protect an organic molecule against direct hits by serving as electron sources capable of repairing the ionizations is supported by paramagnetic resonance studies of Gordy *et al.* (96). These workers conclude from their studies on proteins in the dry state that: "The evidence is strong that whenever radiation knocks out an electron to create a hole or vacancy at any given point in the protein, this hole or vacancy is quickly filled by an electron borrowed from a cystine group. This mechanism prevents the breaking of bonds and probably diminishes greatly the damage by the radiation."

The observed correlation between protective ability and rate and extent of mixed disulfide formation represents the only explanation so far given of the relation between chemical structure and protective ability for the compounds of the cysteine-cysteamine group.

The fundamental role of SH-groups in biochemical processes is well established (19) and as pointed out, SH- and SS-groups are particularly vulnerable to the direct as well as indirect action of ionizing radiation (21, 70, 222).

The mixed disulfide hypothesis has the virtue of providing a mechanism for protection against the direct as well as the indirect action of ionizing radiation.

CONCLUDING REMARKS

The study of chemical protection has greatly extended our knowledge of certain aspects of radiobiology, and results applicable to practical medicine can confidently be expected. In conclusion, certain aspects of radiobiology which, in the opinion of the authors appear to be essential and promising fields of investigation, will be considered.

On the nature of the target molecules. One of the most fundamental problems in radiobiology is to reveal the nature of the immediate chemical radiation lesions and to identify the target molecules, *i.e.*, the molecules the alteration of which leads to the deleterious radiation effects. It was pointed out above that a dose lethal to mammals involves few ionizations compared with the number of molecules in the cell. Most of the chemical transformations are probably without consequences for the cellular metabolism since they will be suffered by molecules which are present in the cell in comparatively large numbers. The fact that profound biological consequences are initiated by a few chemical transformations (probably less than one thousand per cell) strongly suggests that the target molecules belong to the catalytic systems of the cells.

Since protection can be demonstrated against many radiation effects it was logical to conclude (3, 190) that the protective agents intercept the radiation lesions already in the physico-chemical phase, and to focus the attention on phenomena such as oxygen deprival and inactivation of radicals. This physico-chemical point of view is clearly reflected in the interpretation of chemical protection as a "true dose reduction" (3, 192). The experimental data in support of the mixed disulfide mechanism provide evidence for the concept that protection can be accomplished at a later stage in the sequence of events. An essential feature of this view is that the presence of the protector does not necessarily influence the initial ionizations of the target molecules. The protective action is presumed to be exerted by providing additional possibility for the target molecules to dissipate the absorbed radiation energy in a non-deleterious way. This view suggests that more attention should be devoted to radiochemical studies of tissue constituents.

On the basis of the mixed disulfide hypothesis suggestions can be made as to the chemical nature of the target molecules of cells and tissues. As a working hypothesis it has been assumed (79) that the target molecules carry in the vicinity of accessible SH- or SS-groups, negatively charged groups (carboxyl, phenol, or hydroxyl groups) capable of stabilizing the formation of mixed disulfides with the

protector. Admittedly, the target molecules cannot easily be identified on the basis of these criteria. However, the view may serve as a guide in future efforts to develop more efficient protective agents.

The physiological level of chemical protection. The striking differences with regard to the radiosensitivity of different cells as evidenced by the LD_{50} dose (varying from a few hundred to several hundred thousand r) most likely is due to essential inborn differences in metabolic pathways and cellular organization. The individual variations in radiosensitivity of one specific organism under different physiological and pathophysiological conditions may similarly be ascribed in part to alterations in metabolic pathways (adaptive phenomena). However, the major part of the individual variations in radiosensitivity may possibly reflect variations in a "physiological level of chemical protection".

Among the simple organic compounds of the cells several will possess some degree of protective activity (glutathione, cysteine, homocysteine, pyruvic acid, hexoses, *etc.*). Special significance may be attached to the sulfur-containing agents. In most mammalian cells the concentration of glutathione is of the order 1 g/kg, *i.e.*, of the same order as the protective doses used. There is strong evidence that these thiols exist in the tissues largely in the form of mixed disulfide with available SH- and SS-groups (75, 77, 195). In the light of the mixed disulfide hypothesis the latter circumstance represents a physiological level of chemical protection which would be expected to be influenced by the actual concentration of the protector in the tissue, and by the oxidation/reduction potential of the SS/SH systems. The pronounced effect on the cellular oxidation/reduction potential of several radiosensitizing and radioprotective compounds (36) may be of significance in this regard. It would seem logical to investigate the effect of the factors known to influence radiosensitivity (*e.g.*, insulin, thyroxine, steroid hormones, *etc.*) on these particular systems.

Any factor tending to strip the target molecules of their protective residues will presumably increase the radiosensitivity of the cell. Such a view may serve as a working hypothesis in attempts to explain the mechanism of action of the so-called "radiosensitizers". It is of special interest that some of the compounds found to possess radiosensitizing action are indeed thiols (132), and that the radiosensitizers of the naphthoquinone type (*e.g.*, menadione) are known to interact with thiols (89).

The pharmacology of thiols and disulfides. The study of chemical protection has made available to the pharmacologists a large number of new compounds, particularly thiols. A body of observations concerning some of their biological effects have been brought to light as well as new points of view on the chemistry and biochemistry of thiols and disulfides. Although data on the toxicity of thiols have been collected (18, 23, 133, 134, 221, 243), the general pharmacology of the radioprotective thiols has only been studied to a limited extent (65, 157, 185, 202, 210). It seems likely that systematic pharmacodynamic studies of thiols and disulfides may now prove to be rewarding.

Practical aspects. In mice and rats the optimal protection obtained by chemical agents corresponds to a dose reduction factor of 1.5 to 2. By combining the most

effective protectors with antibiotics and the use of the best restorative factors known (bone marrow injections) (122, 123), a total dose reduction factor of about 4 has been obtained in mice (32). Obviously, the practical application of these results in humans meets with considerable difficulties.

At present, the most suitable compounds for practical use are AET and cystamine, which both are chemically stable and can be given orally. AET has been found to be protective also in primates (50) and in dogs (188), but the toxicity in these species is so high that the compound had to be administered in increasing doses over a period of several days before protective dose levels were achieved. The effects in man of AET and other cysteamine derivatives are now being actively studied.

Whether greatly improved protective agents can be developed remains to be decided. However, the definite protective and restorative effects obtained by the compounds presently available, is sufficiently strong to command the attention of the medical profession, which should be prepared to take advantage of these effects in case of atomic disaster. Already in peace-time the medical profession has been confronted with problems of this nature.

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