RADIATION PROTECTION AND RECOVERY

Edited by

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Operated by the Union Carbide Corporation
for the United States Atomic Energy Commission

PERGAMON PRESS

OXFORD · LONDON · NEW YORK · PARIS

PERGAMON PRESS LTD.
Headington Hill Hall, Oxford
4 & 5 Fitzroy Square, London W.1
PERGAMON PRESS INC.
122 East 55th Street, New York 22, N.Y.
P.O. Box 47715, Los Angeles, California
PERGAMON PRESS S.A.R.L.
*24 Rue des Écoles, Paris V^e^*
PERGAMON PRESS G.m.b.H.
Kaiserstrasse 75, Frankfurt am Main

Library of Congress Card Number 60–14942

PRINTED IN GREAT BRITAIN BY J. W. ARROWSMITH LTD., BRISTOL

CONTENTS

1

Introduction

THIS volume contains discussions of the biological, physiological, and biochemical methods that have been developed for protecting living organisms against radiation damage. It contains no material on physical aspects of radiation protection, such as shielding and other means used by health physicists for reducing the exposure to radiation. As soon as it was noticed that radiation had damaging effects, investigators began to seek ways of reducing this damage. Many of the approaches discussed are in reality based on work developed very early in this century—as soon as some of the biological aspects of radiation effects were recognized.

Accidental observations, around 1910 or even earlier, revealed that certain tissues become more resistant to radiation when the oxygen supply is reduced; e.g., pressing a piece of wood against the skin gives it greater resistance to x rays. Relatively few systematic investigations on the oxygen effect have been conducted, but some important papers came out in the 1920s and 1930s in connection with the use of radiation in treatment of malignancies. But the newer developments in this field are based on the observation, in the 1940s, that cytological effects can be considerably reduced by reducing the oxygen concentration. Somewhat later it was found that chemicals can simulate the effect of oxygen reduction. In these publications, it is obvious that the oxygen tension should be reduced *during* irradiation to have an effect on survival and resistance of living organisms and on chemical protection.

The detailed, step-by-step development of the work in radiation protection and recovery cannot be reviewed in one volume, since the literature on the subject has grown to enormous proportions and now invades other fields. The main difficulty is that the study of the effects of radiation is integrated so deeply with the study of the basic biological phenomena, such as cell division, mitosis, genetic effects, and physiological function, that it is not always possible to separate radiation damage from natural biological phenomena. As a matter of fact, our lack of understanding of fundamental biological phenomena is probably the basis for our failure to understand the minute details of how radiation works. It has also become obvious in these studies that radiation damage is often not very different

from damage produced by chemicals and other physical agents. Information on the mechanism of chemical protection at the cellular level is limited at this time.

The recovery of individual cells observed after exposure to radiation has found few practical applications to survival of higher organisms, especially after massive exposures to ionizing radiation. That individual cells in higher organisms do actually recover is obvious to anyone who has worked with moderate amounts of radiation, i.e., less than 500 roentgens. The newer work in developing ways of promoting recovery is the transplantation of blood-forming cells from a nonirradiated animal to an irradiated one; i.e., replacing radiation-damaged cells with undamaged ones from nonirradiated organisms. In this, many difficult and involved problems of immunological compatibility are encountered. The implications of this work to biology and medicine are obvious.

The material discussed in this volume illustrates the close cooperation between the basic biologists and chemists and the clinicians who apply the information to counteracting radiation damage or preventing damage by certain chemical compounds being investigated for their ability to counteract formation of malignant growth. This cooperation is important and encourages the laboratory investigator to direct his efforts along lines possible only with the support and interest of the clinicians.

The field is developing so rapidly that a major proportion of the information is being presented at many conferences and meetings and therefore being published in the proceedings of these meetings. This volume could not be completely up to date, but it does take a kind of inventory of the present status and potential advances in radiation protection and recovery. The wide attendance by biologists, radiologists, and clinicians at the many conferences both in this country and abroad attest to the extensive interest in the subject. We hope that this book will serve to prepare those attending the conferences by giving them some of the background that will enable them to follow the new developments in this field.

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2

Protection of macromolecules *in vitro* against damage by ionizing radiations

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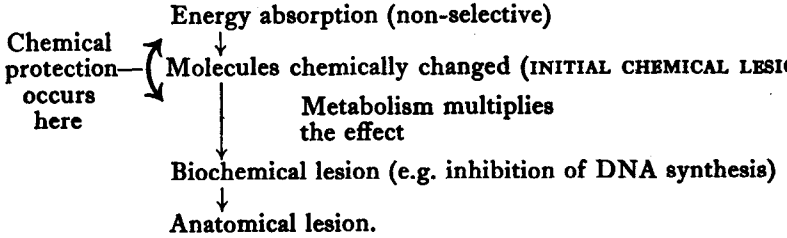
I. INTRODUCTION

WHEN a cell is exposed to ionizing radiations there are a number of different steps between the initial uptake of the energy and the final biological injury. In no case have these different stages been worked out in detail but the available evidence suggests a general scheme of this type:



Protection against the harmful end-effects of radiation can in theory be provided at each of the steps except the first. Once a material is exposed to atomic radiation there is no way of preventing the deposition of energy which follows precise and well-understood physical laws. There is essentially no selection in this initial process and as a first approximation it is adequate to consider that the energy is deposited at random in all the different cell components.

Within the space of a very short time, usually a millionth part of a second or less, some of the energy is used up to bring about chemical changes.*

* Almost all of the energy not so used—probably about three-quarters of the total amount put in—is dissipated completely harmlessly in raising the temperature of the irradiated object. With the doses necessary to affect biological objects the amount of heating is minute, e.g. if *all* the energy from 1000 r were used up as heat the temperature of a cell would go up by 0.0025°C.

Since the amount of radiation required to injure most cells is extremely small, the total number of chemical changes brought about in the sensitive organelle is of the order of some hundreds* and even the majority of these will be quite harmless.

Radiation induced changes in low molecular weight substances vital to the cell, such as ATP, cofactors, essential metabolites, cannot contribute to the radiation lesion† since the fraction changed will be quite minute. For a molecule of molecular weight of 500 approximately one out of every million present will be destroyed by 100 r.

Even the majority of the radiochemical changes in vital macromolecules are likely to be without effect and the destruction of one or two molecules of a particular enzyme of which there are hundreds present will in most cases leave the cell entirely unaffected.

The number of enzyme molecules present is usually in excess of requirement so that the loss of a few of these—and this is the most that could be produced by a biologically effective dose of radiation—would be without significance. Only if the enzyme happens to be, in the words of Krebs, a "pace-maker"[57], which means that it acts as a bottleneck in a metabolic chain, is a small eduction in enzyme activity likely to be harmful.

The most vulnerable types of molecule are those where everyone fulfils a unique role so that the destruction of a few of these has a reasonable probability of impairing an essential function of the cell. From what we know of the molecular basis of genetics the DNA in the cell nuclei could meet these requirements, but there are still formidable difficulties in the way of identifying radiochemical changes in DNA with the initial chemical lesion.

Bacq and Alexander[28] have suggested that the reaction responsible for some types of cell damage is the breakdown of an intra-cellular barrier leading to the release of enzymes which can then damage organelles to which they normally have no access. The chemical requirement for this mechanism is a change in some structural macromolecules which make up the susceptible barriers.

* 100 r will kill some mammalian cells; this dose will deposit within the nucleus (assumed to be 1 μ^3) 6×10^3 eV of energy. For most radiochemical reactions involving organic substances one molecule is destroyed for every 10–20 eV of energy (i.e. *G* value between 5–10). Hence a reasonable estimate is that some 600 molecules *in all* will be chemically changed within the nucleus of volume 1 μ^3, which contains hundreds of millions of molecules.

† The possibility that radiochemical changes may lead to the formation of toxic products has often been considered and some support has been found[73] for the suggestion that peroxides of fats, which are undoubtedly formed when animals are irradiated, are responsible for radiation sickness. Since a poison theory runs counter to the bulk of research of radiobiology it will not be considered in this review.

All these considerations suggest that the primary point of attack is a macromolecule and studies on the mechanism by which macromolecules can be protected *in vitro* against ionizing radiations may therefore provide useful clues on the nature of protection *in vivo*.

A. Direct and Indirect Action

The investigations of Risse, Fricke and Dale on the effects of ionizing radiations of a large variety of substances dissolved in water established quite clearly that at the chemical level a dissolved substance can be affected by radiation in two ways; directly when the initial process of energy deposition occurs within the molecule affected or indirectly by the reactive products formed from irradiated water.

Already in 1929, Risse[77] had realized that $OH^{\cdot}$ and $H^{\cdot}$ radicals were the principal components of activated water but subsequent work revealed that the simple reaction

$$H_2O \rightarrow H^{\cdot} + OH^{\cdot}$$

was not sufficient to explain all the facts and Allen[23] showed that it is necessary to consider this pair of reactions

$$H_2O \rightarrow H^{\cdot} + OH^{\cdot} \qquad \text{(called the radical reaction)}$$

$$2H_2O \rightarrow H_2 + H_2O_2 \qquad \text{(called the molecular reaction)}$$

the relative proportions of which depend on conditions.

From a biological point of view the "molecular reaction" can probably be disregarded since hydrogen peroxide[56] is unlikely to be important. The damaging components of "activated water" are the highly reactive radicals and perhaps also the peroxy radical formed in the presence of oxygen

$$H^{\cdot} + O_2 \rightarrow HO_2^{\cdot}$$

While a great deal is known of the nature of the reactions between organic molecules and the radicals derived from water, the chemical changes which follow direct action have been studied very much less. Many calculations have been based on the assumption that the fundamental processes of energy loss are the same in solids and liquids as in gases (e.g. air) where one ionization occurs for every 30 to 35 eV* of energy

* Only about half of this energy is required to bring about the ionization, the remainder produces excitations of the molecules. The excitations brought about by ionizing radiations are similar to those which follow irradiation with ultra-violet light. When compared on an energy basis a much larger dose (100–1000 times) of ultra-violet than ionizing radiation is needed to induce similar cellular lesions. This suggests that chemical changes due to ionizations are much more important than chemical changes following excitation.

deposited. Using this hypothetical figure the number of ionizations brought about by a given dose have been computed. So-called target sizes are calculated on the assumption that one ionization anywhere within the "target volume" destroys the biological activity of the material studied such as enzymes, viruses, or sub-cellular structures[74].

Recent chemical studies on the changes which follow direct action (i.e. when a material is irradiated by itself and not in solution) show clearly that the simple concept of "one ionization one reaction" is extremely misleading and the number of reactions (or molecules changed) can be both smaller or greater than the number of hypothetical ionizations. Since usually the experimentally determined value is within a factor of 2 or 3 of the number of postulated ionizations the target volume calculations come out approximately correctly and the molecular weight of a number of enzymes[42, 2] is about half the so-called target volume.* This approximate agreement however provides no proof of the assumptions made in these "target" calculations and at present time the most useful way to express the data is in terms of the total energy that has to be put in for a given event to occur. The *G* value is the reciprocal of this figure and gives the number of specified reactions that have occurred for every 100 eV of energy taken up by the system as a whole.

B. How to Distinguish between "Direct" and "Indirect" Action?

When an enzyme or virus is irradiated in solution the dilution test first used by Dale[38] provides a simple and unambiguous method of estimating the relative contribution of the two processes.† If the action is wholly direct (i.e. activated water molecules play no part) then the same fraction of molecules present will be inactivated by the same dose whatever the concentration. That is, the percentage inactivation of the solution will be independent of concentration, although of course the actual number of molecules affected increase with increasing concentration. When the action is wholly "indirect" then the converse applies. A given dose of radiation produces a certain number of free radicals and these will destroy a number of dissolved molecules which to a first approximation is independent of the

* Protection against direct action also invalidates this approach when applied to complex structures (see p. 32).

† Some uncertainty is introduced into the definition of direct and indirect action by firmly bound water which is found associated with all proteins and nucleic acids. Does an ionization within this hydration shell represent direct or indirect action? I believe that it should be counted as direct since the water forms an integral part of the molecule and free diffusion does not occur within it. Indirect action requires the diffusion of a radical to the site of action. By the "dilution test" a process which requires an ionization within a hydration shell will behave as "direct" action.

total number present.* Therefore if the percentage inactivation becomes greater the more dilute the solution then the action is indirect.

Unfortunately, this decisive test cannot be applied *in vivo* as it is not possible to dilute the interior of the cell at will and there is no way of deciding which of the two processes is more important except in the case of seeds and spores where the virtual absence of water makes an indirect effect extremely improbable. In mammalian systems which contain 80 per cent of water one might expect indirect action to predominate since the bulk of the energy is deposited in the water but the relatively much greater effectiveness of direct processes (see pp. 37 and 40) makes this deduction far from certain.

Based on the simple concept of the primitive form of the target theory that an ionization will inevitably inactivate, the beliefs[60] sprang up that effects due to direct action cannot be influenced by changes in external conditions. That is, if the action is predominantly direct then it should be independent of the oxygen tension, the temperature or the presence of chemical protective agents. On the other hand, indirect action would be affected by the presence of oxygen because of the formation of peroxide radicals; by temperature because if the water is frozen the radicals cannot diffuse; and by protective agents which could capture the free radicals and thereby render them harmless. Moreover, it can be predicted that the role of oxygen and protective agents would be much less important with densely ionizing radiations like α particles since the concentration of radicals along the particle track will be high so that the probability of capture by oxygen or a protective agent is much smaller than in the case of sparsely ionizing radiations like therapy x rays or γ rays.

Now this is exactly the situation encountered *in vivo*. The effects of x rays are enhanced by the presence of oxygen and reduced by the presence of protective agents or by lowering the temperature, while the biological effects of α rays are influenced to a much smaller extent. Since there are convincing reasons for believing that the oxygen effect[49] and protective agents [72] enter into the chain of events at the molecular level, it was concluded that free radicals formed in water played a predominant role in initiating radiation injury. This chain of reasoning however breaks down entirely if the chemical changes brought about by "direct" action are also affected by external factors and current radiochemical studies have provided ample evidence that this is the case. Nathalie Bach[24] reported that entirely different products were formed from a variety of simple organic molecules (no water being present) following irradiation in the presence or absence of oxygen. Alexander and Toms[21] found that the cross-linking

* In very dilute solutions the number of solute molecules changed falls off since some of the radicals are wasted in mutual interaction.

of polythene was affected by the presence of oxygen. But the most decisive proof of the role of oxygen when the action is direct is provided by the inactivation of dry films of enzymes (see Fig. 1); it is noteworthy that with α rays there is no oxygen effect. This oxygen effect has been observed with trypsin[2], lysosyme[55a] and ribonuclease[80a].

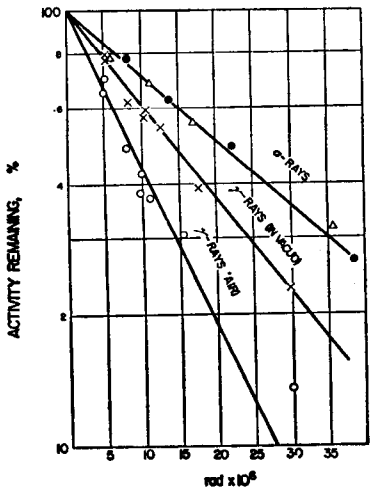


FIG. 1. Effect of oxygen on radiosensitivity of solid trypsin[2] $-\triangle-$ Po α-rays in air; $-\times-$ Co^{60} γ rays in air $-\bullet-$ Po α rays in nitrogen; $-\circ-$ Co^{6} γ rays *in vacuo*.

The effectiveness of direct action (i.e. energy needed to produce a given effect) was first shown to increase with temperatures by Bachofer and Powers[25] in the case of bacteriophage and subsequently the changes brought about in polymers[7, 5, 31] were shown to be similarly affected (see Fig. 2). Protection by added substances against direct action has been amply demonstrated and will be discussed in detail on page 32. The deduction that indirect action is an important factor *in vivo* can therefore not be made merely because external factors modify the primary effects of radiation and the relative importance of direct and indirect action can only be determined by studying their relative efficiencies in bringing about the primary chemical radiation lesion[3]. Although the efficiencies of the two processes are comparable for simple molecules, Lea[61] showed that viruses were inactivated a thousand times more efficiently by direct than by indirect action presumably because the radicals cannot penetrate to the vulnerable centre (see Table 3, page 23). When broth was added to the

solution of virus the indirect effect was completely eliminated because the radicals were captured by the added substances. The same observation was made by Laterjet *et al.*[58] and by Miss Drew[44] for the inactivation of solutions of pure DNA which possess activity as pneumococcus transforming principle. All this emphasizes that direct action can by no means be disregarded even when systems consisting predominantly of water are irradiated.

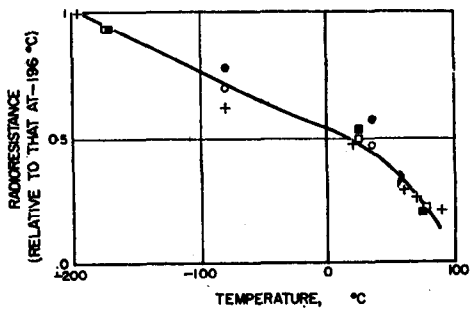


FIG. 2. Effect of temperature on effectiveness of ionizing radiations when the action is direct. +, Polyisobutylene energy per break γ-radiation; ○, inactivation of bacteriophage (vacuum dried) 50 kv x rays; ●, inactivation of bacteriophage, lyophilized; □, red cell catalase inactivation by 3.7 MeV deuterons; ■ red cell catalase inactivation by 1 MeV deuterons[5].

II. POSSIBLE MECHANISMS BY WHICH PROTECTION CAN BE BROUGHT ABOUT

The term protection has acquired a number of different meanings and in many cases the problem of whether a particular effect is defined as protection depends on the attitude of the observer. From a strictly chemical point of view protection requires an interaction with the initial excited or ionized molecules that reduces the total amount of change that occurs in the subsequent chemical reactions (i.e. transfer of energy from a radiosensitive to a radioresistant molecule). This is, however, too narrow a definition for radiobiological problems where protection may be defined as reducing the extent of the "initial chemical lesion" (see scheme outline on page 3) and it does not matter whether this is achieved by reducing the total amount of chemical change, by diverting the energy from a vital molecule to one which is not critical for the cell or even by repair of the damaged molecule at an early stage in the sequence of chemical reactions. These different processes will now be considered in outline.

A. Diverting the Energy from One Molecule to Another

1. *Indirect Action*

An added substance can protect by competitively removing (scavenging) the free radicals formed by the radiolysis of water before these have a chance to damage the molecules of key substance.*

While most organic substances will be attacked by the $OH^{\cdot}$, $H^{\cdot}$ (or $HO_2^{\cdot}$) radicals produced from water, the rate at which they react varies widely. An added substance will act as a protector if it reacts more quickly than the molecule whose fate is being followed. If the rate is very much greater as in the case of protection of serum albumin by β-mercapto ethylamine (see page 21, Fig. 5b) then no change in the protein will be detected until all the protector has been decomposed and the dose response curve will show a threshold below which no change can be detected. The magnitude of the threshold dose will to a first approximation be proportional to the concentration of protector (see Fig. 3 and an experimental example on page 21, Fig. 5). In general, however, protection is not so effective as to be so near to 100% as to give a threshold and usually the rate of radiation damage is reduced.

2. *Direct Action*

A number of instances have now been encountered where the dose needed to inactivate the "target" molecule is reduced by the presence of another substance even when the action is direct as, for example, when the substances are irradiated in the dry state. The essential feature of such reaction is that a given dose of radiation will produce less damage to the target molecule in the presence of the protector than in its absence. Conversely, a greater proportion of protector molecules will be destroyed if they are irradiated when mixed with "target" macromolecules. Formally, this protective process can be considered as a transfer of energy, originally taken up in the "target", to a neighbouring molecule and has been called energy transfer[6, 10] without however, implying any particular mechanism. Arithmetically, protection by energy transfer can be treated in exactly the same way as protection by competition. Probably the most convenient way of expressing the results is as per cent protection (see page 13) given by $[(R_p - R_c)/R_c] \times 100$ where R_p and R_c are the radiation doses required to produce the same damage with and without the protector present.

3. *Quantitative Aspects*

The effect of dose on the extent of protection depends on the system

* In the strictest sense this is not protection at all since the number of water molecules affected is the same and only the course of subsequent reactions is changed by the protector.

being studied. The usual situation is that the macromolecule being protected (e.g. enzyme) is inactivated after reaction with a free radical but this reaction by itself does not reduce the affinity of the macromolecule for further reaction with radicals. This gives rise to the so-called *self protection* effect since the macromolecule after inactivation continues to compete for

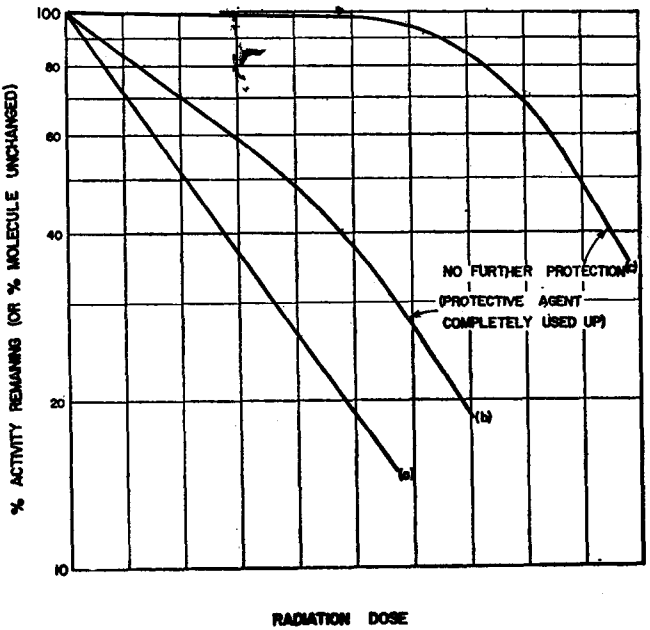
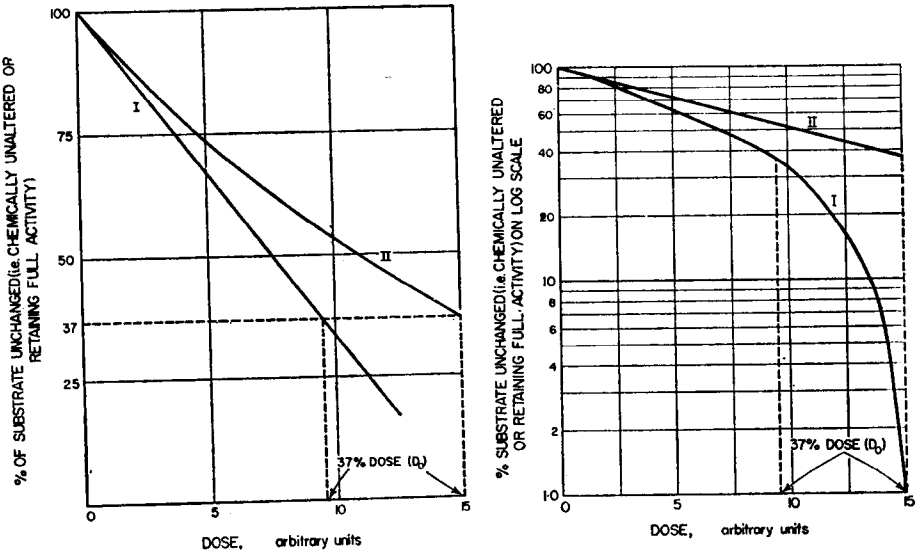


FIG. 3. Theoretical curves showing protection by competition:
(a) Test material dissolved in solution containing no protective agent.
(b) Protective agent added with an affinity for inactivating free radicals which is approximately the same as that of the test material.
(c) Protective agent with 30 times greater affinity for free radicals than the test material.

free radicals and therefore protects those molecules which have not yet been inactivated. One consequence of this is that the number of macromolecules inactivated is not proportional to radiation dose but is an exponential function (see Fig. 4). In radiochemical reactions in solution where the product no longer reacts with the free radicals the number of molecules affected is proportional to dose. An example of this behaviour is the oxidation of ferrous sulphate to ferric sulphate (Fig. 4). The ferrous salt has a great affinity for the radicals while the ferric form has not. However, this situation has not so far been encountered with biological macromolecules all of which show the self protection effect. Most

of the protective agents on the other hand fall into the second category and their high affinity for free radicals (in other words their capacity to protect) is lost after they have combined with a radical. Hence the amount of protection decreases with increasing dose due to destruction of the protective



4a. 4b.

FIG. 4. Relationship between radiation dose and radiation effect (e.g. molecules inactivated or chemically changed).

Curve I: Where the product formed by the radiation, e.g. ferric from ferrous does not react (i.e. compete for) further radicals.

Curve II: Where product has the same affinity for radical as starting material (e.g. enzymes).

(a) Plotted linearly. (b) Plotted on semilogarithmic scale.

agent. In most experiments the dose range is such that the proportion of protective agent destroyed is very small and consequently is independent of dose.

Quantitatively the amount of protection by competitive removal of free radicals can be expressed in a number of ways:

$$\frac{\text{Number of radicals reacted with protector}}{\text{Number of radicals reacted with "target molecule"}} = \text{Competition factor.}$$

This can be obtained experimentally by measuring the fraction of "target molecules" destroyed (e.g. % enzyme activity lost) by a given

dose of radiation in the absence of protector (I_c) and in the presence of protector (I_p). Then

$$\frac{\log I_c - \log I_p}{\log I_c} = \text{Competition factor}^*$$

Use is often made of the *per cent protection* given by the relationship

$$\frac{I_c - I_p}{I_c} \times 100 = \text{protection}$$

or its equivalent $[(R_p - R_c)/R_c] \times 100$, where R_p and R_c are the radiation doses to produce the same effect with and without the protector present.

This relationship differs from the competition factor in that 100% represents complete protection which in theory would require an infinite concentration of protective agent and "per cent protection" is therefore not proportional to concentration of protective agent. Particularly in biological experiments, protection is often expressed as a "dose reduction factor" (DRF), defined as:

$$\frac{\text{Radiation dose to produce a given effect in presence of protector}}{\text{Radiation dose to produce a given effect without protector}}$$

In the simplest case discussed here, DRF and competition factor are the same thing and numerically for low doses:

$$\text{DRF} = \text{Competition factor} + 1.$$

The "protective power" of a substance is the

$$\text{Competition factor} \times \frac{\text{Concentration of "target molecule"}}{\text{Concentration of protective agent}}$$

If the presence of protection occurs by the competition mechanism and there are no complicating features, then the competition factor should be directly proportional to the concentration of "target molecule". Consequently, the protective power should be a constant for any one system and independent of the concentration of both protector and target molecule.

In practice this is only seldom found and the protective power usually falls as the concentration of the protector is increased. Examples of this will be given on page 28. The reason for this behaviour is not known.

* The logarithm of the activity has to be used because there is a self-protection effect and, consequently, the inactivation is exponentially related to dose (see Fig. 4). This means there is a linear relationship between the log of the inactivation and dose.