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0014-4754/89/010033-09$1.50 + 0.20/0
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## Modifiers of radiosensitivity

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*Key words.* Radiosensitivity; radioprotection; chemical modifiers; biochemical modifiers; physical modifiers; physiological modifiers; synchronisation therapy; tumour radiosensitizers; thiol depletion; tissue hypoxia; blood flow modification.

### *Introduction*

Biochemical and chemical modifiers of radiation response have developed partly because of the interest in differentially sensitizing tumour cells or protecting normal cells. The important concept for their cancer therapy application is that a therapeutic gain is being sought which has an absolute prerequisite of differential effects

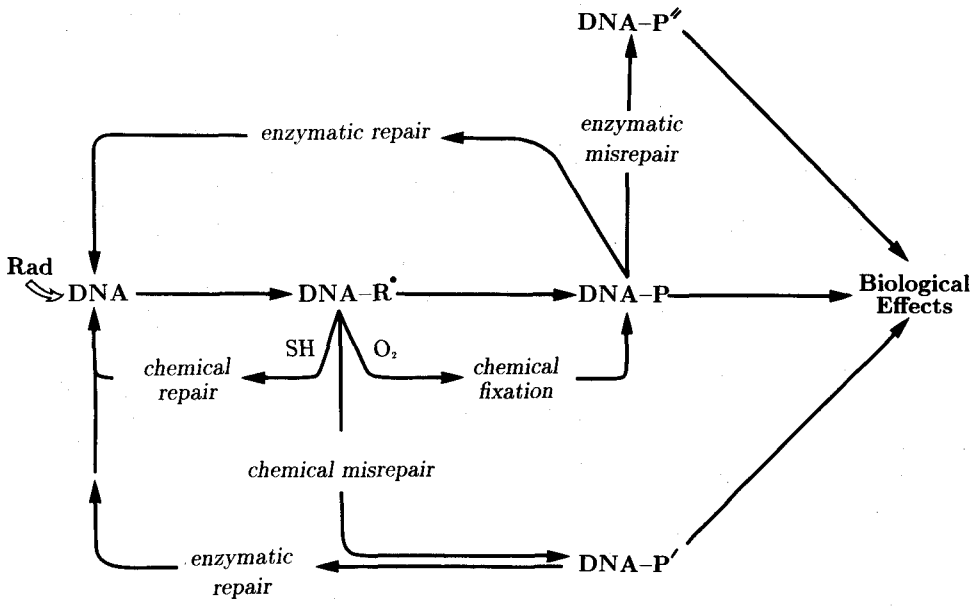


Figure 1. Scheme to illustrate that radiation-induced DNA radicals (DNA-R•) can be repaired chemically, or enzymatically, or can be fixed into altered DNA products (DNA-P, DNA-P′ or DNA-P″) by chemical fixation or chemical or enzymatic misrepair.

in tumours and normal tissues. Because of the known differences in oxygenation, resulting from the poor vascularity of tumours, chemicals which involve redox mechanisms have featured strongly. These include oxygen and oxygen-mimetic compounds, such as the nitroimidazoles. Most of the chemical radioprotective agents are thiols, which are believed to compete with endogenous sensitizers for fixation or restitution of the radiation-induced molecular damage (fig. 1)[1, 4, 38]. In recent years, the field has expanded to include modifiers of blood flow, proliferation rates and biochemical repair pathways. In addition, the radiation sensitivity of cells can be altered by changing the physical dose distribution in time and space, by varying the radiation type, the fractionation pattern, or the dose rate. All of these classes of modification of radiation sensitivity also have an intrinsic interest for the basic understanding of radiation interaction with living cells.

However, we will concentrate mainly on the areas showing clinical promise.

These areas can briefly be summarised as:

1) Physical – fractionation into small doses
   – dose rate
   – microdistribution of dose within targets (i.e. LET)
2) Chemical – redox competition (sensitizers, protectors)
3) Biochemical – repair enzyme inhibitors
4) Physiological – alterations of blood flow and oxygen availability, cellular respiration rates, cell cycle phase distribution and proliferation rates.

Although the processes can be thought of separately, particularly because the time scales are different, they are often interconnected because oxygen manipulation is frequently a common pathway towards the desired effect.

*Physical modifiers*

The influence of the physical distribution of the ionizing events in both time and space have been dealt with elsewhere in this issue (reviews by Blattman, Alper, Kellerer, Denekamp and Rojas). Briefly, when conventional X-rays or $\gamma$-rays are used to irradiate cells the resulting survival curve is shouldered, showing less efficient killing at low doses than at higher doses. This is often attributed to two different biophysical modes of causing a double-strand break in the DNA (e.g. by a single track causing two breaks or by two independent tracks intersecting the same small target volume, or alternatively by inducing 'clean' double-strand breaks or complex breaks associated with much other structural damage). The resultant curve can be described by a linear quadratic equation, with a linear term (1 hit) and a quadratic term (2 hits). Only the quadratic term would be modified by varying the rate at which damage was inflicted (e.g. by varying dose rate). An alternative biochemical explanation is that

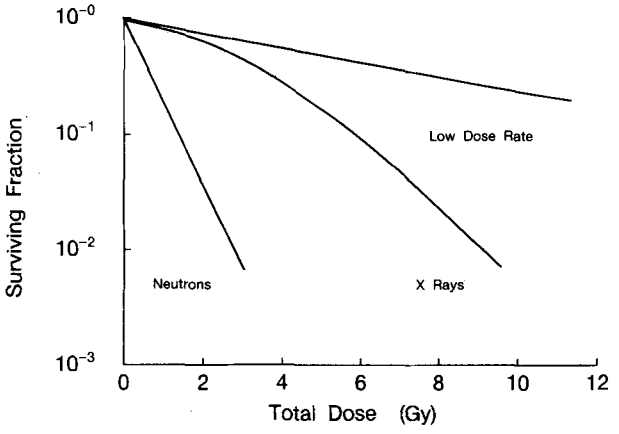


Figure 2. Survival curves illustrating the curvilinear response to acute X-ray doses, in contrast with the linear (more resistant) response to low dose-rate X-rays, or the steep linear response to high LET radiation (neutrons).

most of the DNA lesions can be repaired quite efficiently by enzymes, but after large doses the number of lesions is so great that the repair enzymes become saturated. In this model the initial slope represents the final yield after repair has occurred, of irreparable or misrepaired lesions, and the subsequent curvature represents the transition from the 'repaired' to the initially inflicted 'unrepaired' lesions[23].

If the critical dose to a target is given very slowly (at a low dose rate or as a series of individual small doses separated by several hours) the repair capacity is improved and the radiation is less damaging. Conversely, if the dose deposition is very localised and intense, as with high LET radiations, e.g. neutrons, pions and heavy ions, the initial slope is much steeper, repair is less effective and the cells are more easily killed (fig. 2).

Whatever the biochemical or biophysical reasons underlying the shouldered survival curve, the influence of chemical radiomodifiers may be reduced for the linear component of damage (i.e. the low-dose initial slope)[9,42].

*Biochemical modifiers*

The influence of drugs that modify repair pathways have been considered in detail in the chapters by Hagen and Alper in this issue. Any chemical which binds to DNA, intercalates between the bases, or substitutes for normal bases, can act as a radiation sensitizer. It is not clear whether this is a simple interaction of a sublethal lesion from the drug with a sublethal lesion from the radiation, or whether there is a supra-additive synergism, i.e. true radiosensitization. The presence of alkylating or intercalating drugs may also reduce the accessibility of radiation induced lesions to repair enzymes.

Studies with genetic mutants that show varying degrees of increased radiosensitivity are leading to an understanding of the repair processes in mammalian cells. Such lines have been found to occur spontaneously in man, e.g. Ataxia telangiectasia and Xeroderma pigmentosum patients, or can be induced by mutagenic treatment of established cell lines[12,49,54].

A complex family of repair enzymes are needed in order to remove lesions from damaged DNA. A correspondingly large series of compounds have been developed which inhibit or compete with the DNA repair enzymes, and hence increase the cell's apparent radiosensitivity. A few examples of these are illustrated in figure 3[12,54]. In most instances the drugs are only effective if they are given before irradiation, or very shortly thereafter. Few strategies have been proposed that would make these drugs tumour specific. However, a sophisticated combination of drugs may be used in a way that takes advantage of known differences in drug access, resulting from the altered biochemistry of tumour endothelium[63].

*Physiological modifiers*

It has been recognised for many years that cellular radiosensitivity, after conventional low LET radiations, depends heavily on the proliferative state of the cells, and on the levels of oxygen and of thiols. Examples of this dependence are shown in figure 4. The left hand panels show the great difference in radiation sensitivity of cells in different phases of the cell cycle. V79 cells are most resistant in late S and most sensitive in mitosis[55]. The middle panels show the sensitizing effect of oxygen, which corresponds to a factor of 3 for the dose needed to achieve a particular level of cell kill. The hypoxic radioresistance is not very pronounced until the oxygen content falls below 1%[24,51]. The right hand panels show that the radiosensitivity of mutant cells increases as the mutants show a progressive decrease in glutathione, the endogenous protective thiol[35].

*Synchronisation therapy*

In most normal tissues a stable population size is maintained by an exact balance between cell production and cell loss. In tissues exposed to a lot of wear and tear the turnover of cells is high. Thus extensive cell loss is balanced by rapid cell proliferation[14]. In the intestine, which has a rapidly cycling compartment in the crypts, at least 50% of those cells are in the S phase at any moment and 5–10% are in mitosis. By contrast in skin only 5–10% are in the S phase, and less than 1% in mitosis; 80% of the cells are in the $G_1$ or $G_0$ phase. In other quiescent tissues the fraction of cells in the S phase is very low (below 1%) and cells in mitosis are rarely seen. The vast majority of cells (95–99%) are in the $G_1$ phase, or a quiescent $G_0$ phase. Thus the mixture of subpopulations of varying sensitivity will differ from one tissue to another. In addition, the rate at which surviving resistant cells reassort themselves into sensitive phases between fractions will depend upon the cell cycle time, being rapid in intestinal epithelium and very slow in lung or kidney.

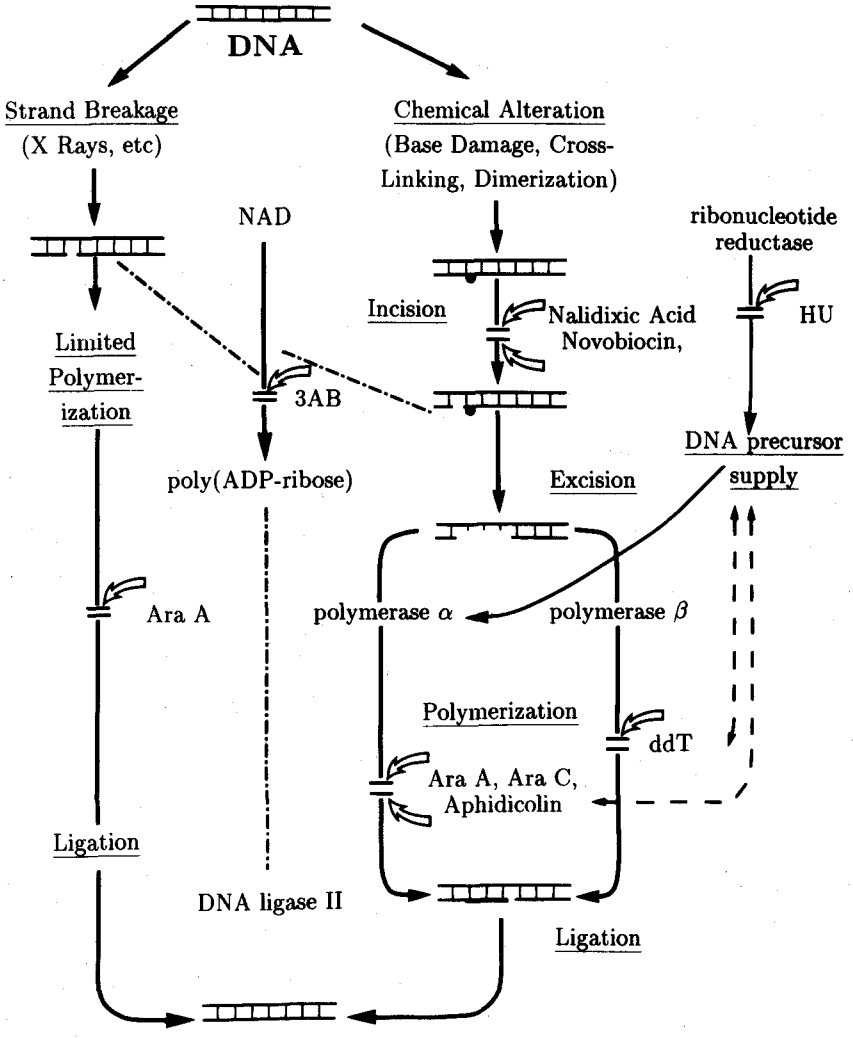


Figure 3. Scheme to illustrate how various drugs can be used to interrupt the biochemical repair of radiation-induced lesions in DNA. Ara A, Ara C, Aphidicolin, 3AB, Hydroxyurea, Nalidixic Acid and Novobiocin are some of the blocking agents which influence different parts of the repair pathways. (Redrawn from Collins et al.[11]). → normal repair pathways; -·- activation; —— competition.

Furthermore the rate at which cells repopulate to replace injured cells between successive treatments will vary[14]. In the 1960's there was a hope that synchronisation therapy would allow the cell cycle variations in sensitivity to be used for clinical advantage. The first dose of radiation would synchronise the populations, which would then move in synchrony through sensitive and resistant phases. It was shown by computer modelling that even a partial synchrony could greatly influence the response to repeated fractions[26]. If the tumour proliferation characteristics were known, and the timing of each fraction was tailored to that patient, it appeared that a gain could result. However, the variation in the cell cycle time of individual cells within each tumour[57], the variations in the exact phase sensitivities in different cell lines[14, 55] and the changes in cell kinetics as a result of the first few fractions all conspired to make this impossible in reality[14].

Two other alternatives have been considered. The first makes use of the natural diurnal fluctuations in proliferation patterns throughout each day[10]. These have been well documented for a number of normal tissues. It has therefore been postulated that therapy should be given when the maximum number of normal tissue cells are in a resistant phase[27]. Whilst this has been shown in experimental studies to be important for sparing the toxicity of cytotoxic chemotherapy, there is no evidence that it is effective with radiation[27, 28]. The second approach is to use a cytotoxic drug as a synchronising agent, to accumulate cells at the $G_1$/S boundary e.g. with hydroxyurea, and then time the radiation to coincide with a period of tumour sensitivity or normal tissue resistance as the cells are released from the block. Many synchronisation attempts were made in clinical radiotherapy, but with no apparent therapeutic benefit.

Methods of stimulating cells into more active proliferation to allow repopulation between treatments have also been postulated as a means of protecting critical normal tissues during a protracted 4–8-week series of fractionated treatments. Unfortunately, most of the agents which

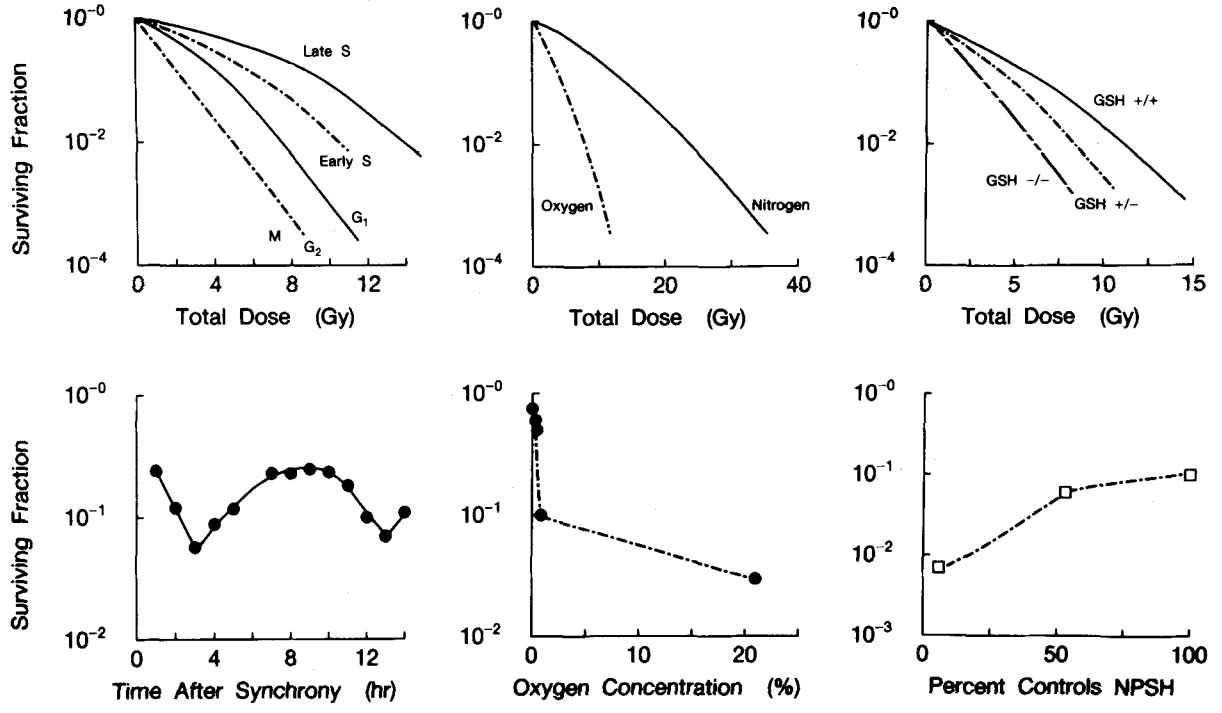


Figure 4. Illustration of the influence on radiosensitivity of cell cycle position (left hand panels), oxygen tension (middle panels) and thiol concentration (right hand panels). These three parameters all have profound influences on the radiation response of cells in vitro (data from Sinclair[55], Schrieve et al.[51] and Malaise[35]).

stimulate proliferation do so by inflicting additional cell damage, since it is cell depletion that is the main stimulus for compensatory proliferation. Therefore, there is no nett protection of the tissue.

*Modification of blood flow*

There are many differences between the microvasculature in tumours and in normal tissues[13,64]. Every solid tumour evokes a neovasculature by its products of anaerobic glycolysis (especially lactic acid) and by production of biochemical tumour angiogenesis factors[22]. The new vessels are fragile, lack innervation, and are inadequate in their three dimensional array. This gives rise to nutritionally deprived and hypoxic cells at 4–10 cell layers from each nutritional vessel, which are radioresistant (fig. 5). This is a great disadvantage for therapy since most normal tissues are well vascularised, well oxygenated and hence radiosensitive[24]. In order to reduce this disadvantage of tumour hypoxia, a number of different approaches have been considered, either to increase the oxygen supply to tumours, or to decrease the oxygen supply to the surrounding normal tissue, rendering the normal cells hypoxic and radioresistant.

*Increasing tumour oxygenation*

The most obvious approach to improve tumour oxygenation is to increase the content of oxygen in the inspired gas. Initial experimental studies with oxygen and carbogen were promising[52,61] – and even led to some clinical trials. However, the potential advantage seemed to be greater if the patient could be enclosed in a pressurised chamber and given oxygen or carbogen at elevated atmospheric pressures. This would force more oxygen into the plasma, thereby supplementing the reservoir carried in the haemoglobin. Again many clinical trials were undertaken which superseded the normobaric studies[19]. A distinct improvement was seen in certain patient subgroups, especially those presenting with anaemia, but the rewards did not seem to justify the continuance of this difficult, time-consuming addition to routine radiotherapy.

Recently, interest in normobaric oxygen and carbogen has been revived, because of the possibility that tumour oxygenation was compromised by peripheral vasoconstriction under hyperbaric conditions resulting from the prolonged exposure to oxygen during the pressurisation and 'soaking' time. Animal studies have shown that even with normal pressure oxygen or carbogen, the sensitizing effect is greatest if a short time elapses between inhaling the gas and irradiation[52,61].

Recent developments in blood transfusion technology have added another element to this approach. Chemical substitutes have been developed as alternatives to fresh or frozen blood for transfusion[37,43,62]. These consist of fine organic micelles containing a mixture of perfluorochemicals. These compounds can dissolve large amounts of oxygen: water and plasma dissolve about 2% oxygen by volume, whole blood 20%, and perfluorochemicals

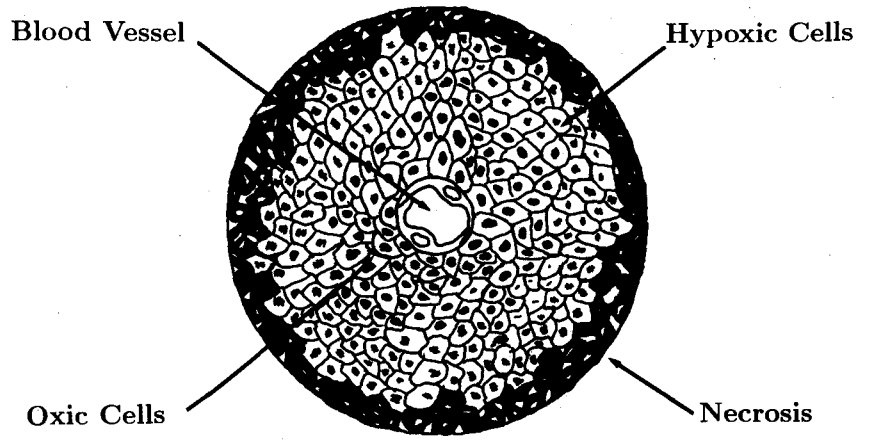


Figure 5. Diagram to show the corded structure seen in many tumours because of the progressive failure of nutrients at increasing distances from the central blood vessel. Oxic cells metabolise the oxygen, leading to hypoxic radioresistance, and finally to necrosis at 100–150 μm.

can dissolve 40% or more. However, they have a limited oxygen transport capacity at normal pressure. The volume of dissolved oxygen changes linearly with oxygen partial pressure; therefore, to fully exploit their use in vivo high partial pressures of oxygen are being used. Several animal studies have shown an increased tumour response using perfluorochemicals and high oxygen concentration[37,43,62].

Interest has also developed in chemicals which influence the affinity of haemoglobin for oxygen, thus altering its ability to give up the oxygen in the tumour microvasculature[7,29,36]. Groups of agents are under study which shift the oxygen haemoglobin dissociation curve to the left, increasing its affinity for oxygen[2] or to the right, making oxygen more readily available to cells[31,53]. Alterations in the blood viscosity, e.g. by reducing the haematocrit, could also anomalously increase the tumour oxygenation if it encouraged blood flow through small vessels that are poorly perfused by erythrocytes[30,48].

Pharmacological agents which influence cardiac output, vascular tone and differential blood flow to different regions of the body are receiving increasing attention[7,36]. Most of these agents are incapable of causing tumour blood flow to increase because of the passive nature of the non-innervated tumour blood supply. Indeed most vasodilators and antihypertensive agents cause a *reduction* in tumour blood flow as the 'steal' effect of increased flow to other regions diminishes the blood available to the tumour.

### *Induction of normal tissue hypoxia*

Several clinical studies have been undertaken in which normal tissue radioprotection was attempted by inducing local or systemic hypoxia. Local hypoxia for treatment of peripheral sarcomas was attempted using tourniquets[60]. Double the normal radiation doses were used with preservation of normal tissue integrity, but the overall therapeutic outcome was not improved. Recently trials of systemic hypoxia induced by breathing reduced oxygen tensions have been undertaken in the Soviet Union[67] and East Germany[40]. Whilst reduced oxygen tensions in the inspired gas can have a very marked effect on some normal tissues (e.g. 5% oxygen protects mouse skin by a factor of 2 or more), the same inspired gas may have little effect on other dose-limiting normal tissues, such as kidney or lung. Furthermore, it increases the radioresistance of tumours, particularly in response to low doses[58]. Thus hypoxy-radiotherapy, e.g. breathing 10% oxygen, may be useful in certain limited circumstances but is unlikely to produce a general therapeutic advantage.

### *Chemical modifiers*

The effect of oxygen as a potent radiosensitizer is believed to relate to its ability to interact with radiation induced radicals, acting as an electron acceptor. This mechanism of damage fixation is in competition with chemical restitution of the damage, in which thiols play an important role, presumably by proton donation or electron transfer (fig. 1). There is still considerable debate about the precise mechanism by which oxygen and thiols influence radiosensitivity, since they may influence direct damage inflicted by ionisation events in the DNA, or indirect damage caused by the products of water radiolysis, particularly hydroxyl radicals and the hydrated electron[3,6,33,38]. Whatever the basic mechanism it has been

clearly demonstrated that oxygen can influence the radiosensitivity of all living organisms, usually by a factor of 2.5–3.0[1,24]. This seems to be independent of the intrinsic radiosensitivity of the cells which can range from Do values of less than 1 Gy in some mammalian cells to 100 Gy in radioresistant bacteria and yeast. The critical target seems to be DNA within the nucleus, perhaps especially when it is in close association with the nuclear membrane[21].

Basic studies of the 'oxygen effect' led to the development of drugs which would mimic oxygen in its sensitizing action. It was quickly recognised that such compounds might be useful clinically if they were non-toxic and not metabolised and hence could diffuse to tissue regions that oxygen could not reach. The first of these, a 5-nitroimidazole in routine clinical use – metronidazole (trade name 'Flagyl' – May and Baker) – was shown to be effective in several systems in vitro and in vivo[5,15,65]. As predicted, it had a sensitizing effect only in the *absence* of oxygen. The search for analogues led to the more active 2-nitroimidazoles, especially misonidazole (Roche compound Ro 07-0582). This was extremely effective when used with a single dose of radiation on hypoxic skin or on tumours in normal air breathing mice (fig. 6). No effect was seen on skin in mice breathing oxygen. These experimental studies led to clinical trials throughout the world[20].

A large number of analogues of misonidazole have since been designed, aimed at 1) increasing potency, by including alkylating side chains (RSU 1069); 2) reducing its uptake in the central nervous system and shortening plasma half-life by lowering lipophilicity (etanidazole) or 3) inducing concentration of basic compounds in acidic microenvironments (pimonidazole). Two of these, etanidazole and pimonidazole, have recently entered clinical trials, with the promise of a fivefold greater effectiveness than misonidazole[7,36].

Cumulative toxicity of the drugs, particularly peripheral neurotoxicity with miso, limits the dose that could be given with a conventional 30 fraction radiotherapy regime to a suboptimal ineffective level. Nevertheless, several of the clinical trials have demonstrated a clinical gain, albeit for a limited subset of patients[20].

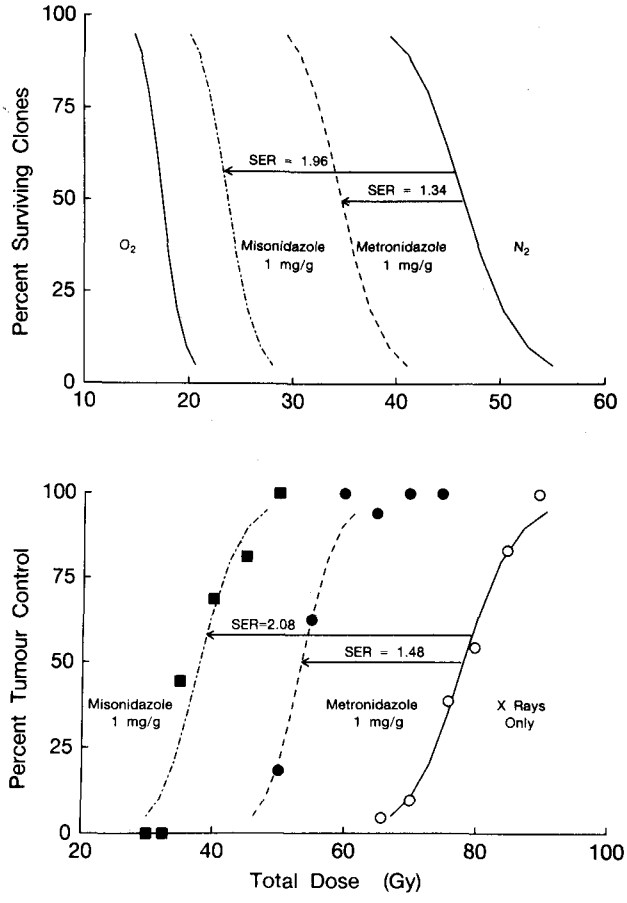


Figure 6. Figure to illustrate the sensitization by metronidazole and misonidazole. Upper panel shows sensitization of epidermal cells if irradiated in nitrogen-breathing animals. No effect was seen in oxygen. Lower panel shows the altered sensitivity of tumours in air breathing mice, reflecting the sensitization of naturally hypoxic tumour cells. (Data from Denekamp et al.[15], Sheldon and Hill[50]).

*Enhancing sensitizer efficiency by thiol depletion*

An increased efficiency of miso itself was shown to be associated with thiol depletion if cells were subjected to a prolonged exposure prior to irradiation[66]. This resulted in a loss of the 'shoulder' on the cell survival curve, and hence an increased effectiveness at low, clinically-relevant X-ray dose levels. This finding led to an interest in alternative means of decreasing the thiol content in order to enhance sensitizer efficiency. Glutathione (GSH) binding agents, such as diethyl-maleate and dimethyl-fumarate, combined with misonidazole, did increase the efficiency of the radiosensitizer, as illustrated in figure 7[8]. The development of a specific biochemical inhibitor of glutathione $\gamma$-synthesis buthionine sulphoximine (BSO), which interferes with glutamyl synthetase[25] provided an added stimulus for this area of research[7,32,51]. Subsequent studies, using prolonged exposure of animals to repeated doses of BSO have shown that it is relatively easy to severely deplete glutathione, the main intracellular non-protein thiol, in several normal tissues, but it is extremely hard to deplete tumour GSH levels below 10%[39]. Since the object is to increase the effectiveness of sensitizers in the poorly perfused regions of tumours, this is a distinct disadvantage. It is possible to deplete tumour GSH more effectively by combining BSO and a binding agent e.g. – DEM, but the combination of this drastic pretreatment with miso administration is extremely toxic, and therefore does not effectively lead to a therapeutic advantage.

*Normal tissue radioprotection*

The ability to protect cells against radiation injury by addition of thiols has been recognised for some 30

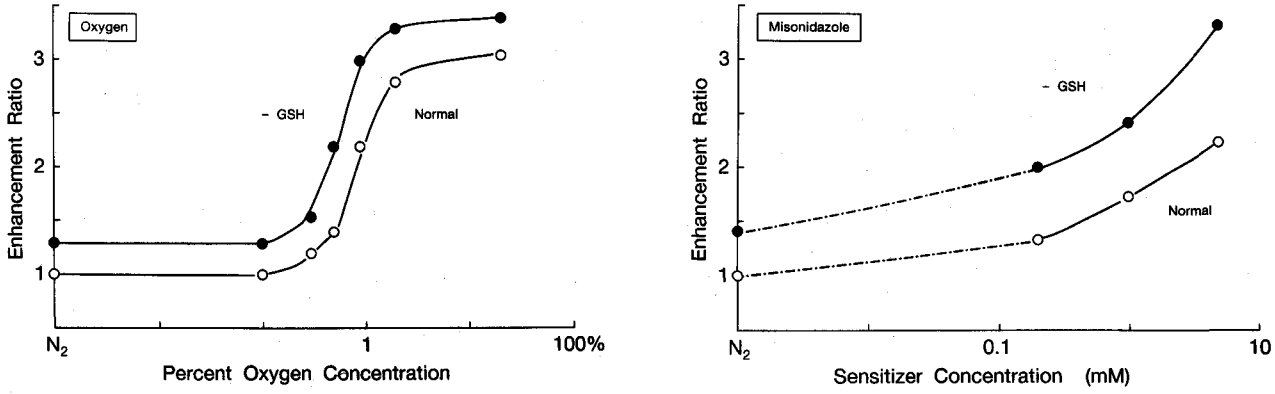


Figure 7. The change in sensitivity with varying oxygen concentration (upper panel) or varying misonidazole concentration given to V79 cells irradiated under anoxic conditions. The sensitizer concentration needed to give half the maximum effect is lower in cell lines depleted of glutathione (data from Shrieve et al.[51]).

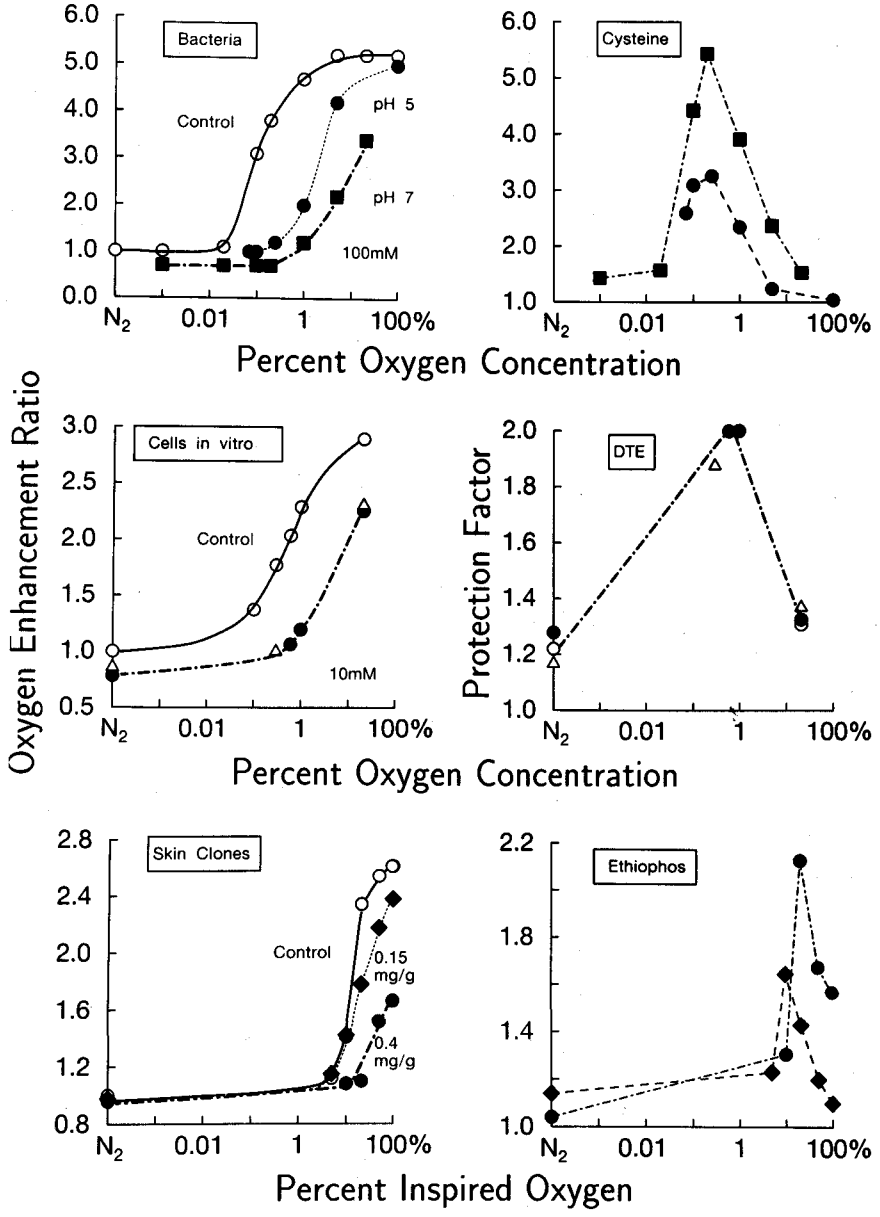


Figure 8. Data from bacteria and mammalian cells in vitro and epidermal cells in vivo to illustrate the displacement to higher oxygen concentrations of the 'K curve' in the presence of added thiols[17]. Upper panels: *Serratia marcescens* with added cysteine. Middle panels: Ehrlich ascites cells in vitro with dithioerythreitol. Lower panels: mouse skin with WR-2721.

years[4]. However, addition of nonprotein thiols is not easy to achieve. Cysteine and cysteamine are relatively toxic, whilst glutathione itself is not readily transported into cells. The American military recognised the potential value of radioprotection of their personnel when under nuclear attack and dedicated an enormous research programme at the Walter Read Hospital to developing various thiol radioprotectors. One of these, a phosphorylated pro-drug, is the aminothiol WR-2721, which is less toxic in its phosphorylated form and needs dephosphorylating before entering cells. It has been shown to be an effective radioprotector, especially against bone marrow death. Yuhas and Storer[69] tested this compound in normal tissues and tumours and deduced that it might be useful in cancer radiotherapy because it showed little protection of the tumour. In subsequent work, Yuhas et al.[68] claimed that the drug was excluded from tumour cells in 16/17 tumour types. This led to widespread interest in the clinical application of radioprotectors both alone and in combination with tumour radiosensitizers. This work has been reviewed in detail in several publications[7, 17, 18, 45]. Briefly, the level of radioprotection varies from tissue to tissue, being much lower in many other normal tissues than that seen in bone marrow. It does not correlate well with the measured drug concentration in the tissues, mainly because of the variable levels of oxygen in the critical target cells and, perhaps, due to differences in the endogenous levels of thiols[16, 17]. The addition of thiols effectively reduces the efficiency of oxygen, thereby shifting the concentration needed to achieve half maximal sensitization to higher oxygen tensions. This is the obvious corollary of the attempts to increase sensitizer efficiency by depleting thiol levels. Figure 8 shows examples of oxygen K curves derived from clonogenic assays of bacterial and mammalian cells in culture and rodent skin cells in vivo[17]. All three systems show that when thiols are added the 'K curves' are shifted to higher oxygen concentrations. The protective effect of the thiol therefore varies depending on the oxygen level that is present[16]. In anoxic cells and in very well-oxygenated cells, adding thiols has little or no effect. In marginally oxygenated cells, adding thiols makes a very large difference in the radiosensitivity, because the balance between reducing and oxidising species can be easily tipped. The protection factors (PF) have been plotted in the right hand panels and they show very convincingly that PF is critically dependent on oxygen.

Since tumours and normal tissues have cells at a range of oxygen tensions, because of their position relative to each capillary and different subpopulations become dominant

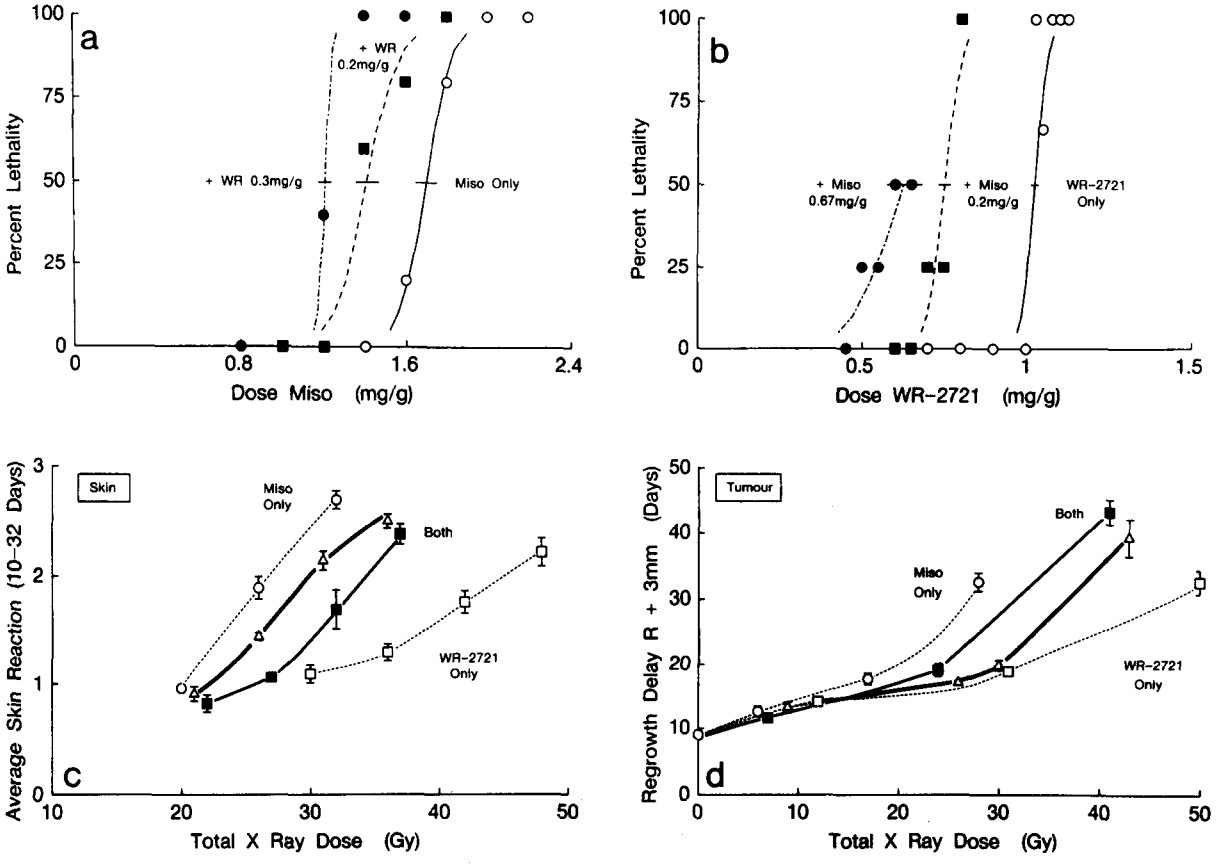


Figure 9. Data to illustrate the influence of WR-2721 on miso sensitization and toxicity (panels a, d) and of miso on WR-2721 radioprotection and toxicity (panels b and c). (Data from Rojas et al.[44, 46, 47]).

as the dose increases, the effect of thiol radioprotectors can vary with radiation dose level (i.e. level of effect)[45, 59]. Careful analysis of both tumour and normal tissue data indicates a complex dependence of the protection factor on the size of each X-ray dose. At equivalent low X-ray doses the clear advantage of WR-2721 in giving normal tissue protection but no tumour protection disappears. At clinically relevant doses of a few grays it is quite unpredictable whether the protective effect is more marked in any tumour than in any normal tissue. These findings have led to less clinical interest, though WR-2721 may still be useful for local or intracavitary administration. It is currently being tested with a few large fractions in palliative radiotherapy[34].

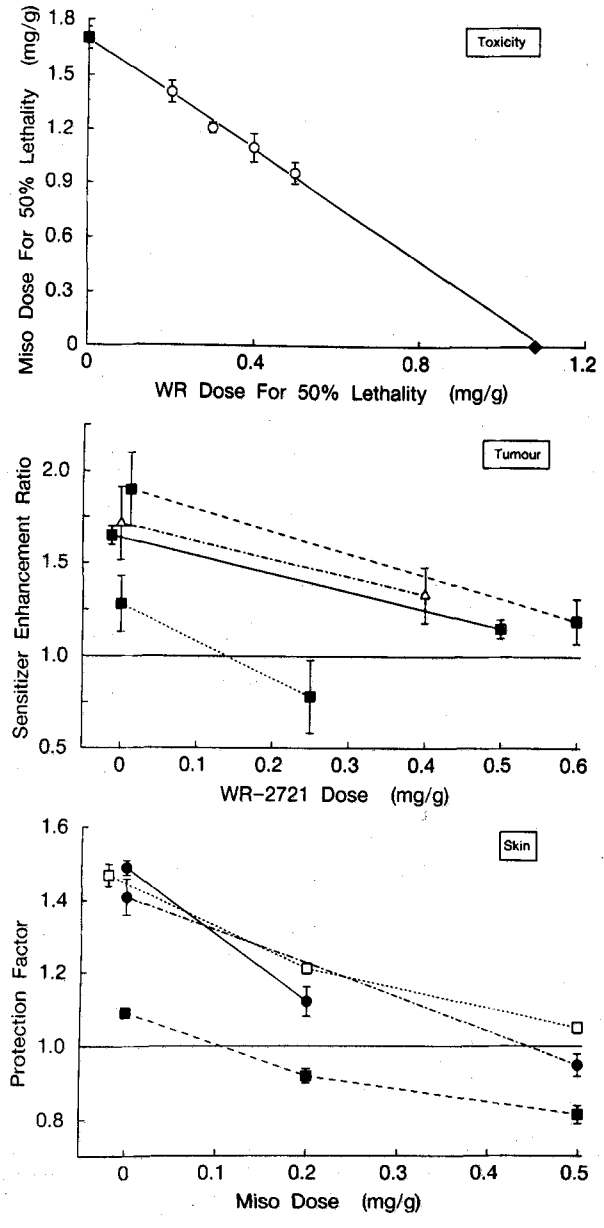


Figure 10. Data to show the competition between misonidazole and WR-2721 in toxicity, tumour sensitization and skin radioprotection. (Data from Rojas et al.[44, 46, 47]).

*Combined tumour radiosensitizers and normal tissue radioprotectors*

Since the toxicities of WR-2721 and of miso are quite different it has been postulated that the benefit of both drugs could be obtained by combined administration. This would be true if there was no additive toxicity, and no reason for an interaction in the mode of action of the two drugs. Early studies indicated that these two requirements might be met[56, 70].

Unfortunately, more detailed studies[18, 44, 46, 47] showed that the toxicity of miso was greatly modified by the addition of WR-2721 (fig. 9a). Likewise the toxicity of WR-2721 was directly influenced by the addition of miso (fig. 9b). The radioprotective effect of WR-2721 on skin could also be reduced by adding miso (fig. 9c). Furthermore, the enhancement of tumour sensitivity by miso could be reduced by thiol addition (fig. 9d). Indeed as might be predicted from a simple competition model, the degree of sensitization of anoxic cells by miso can be titrated against protection induced by the thiol[18]. Likewise the protective effect of the thiol can be titrated against sensitization either from added miso (fig. 10) or from added oxygen[17].

*Conclusion*

Many different methods exist by which the radiosensitivity of cells, and hence of normal tissues and tumours, can be manipulated. Several of these can be traced to a common redox pathway involving competition between oxidising and reducing species. Others involve the biochemical enzyme systems required for removal of DNA lesions. At the present time most of these approaches are still actively undergoing basic research studies and have not found full application in cancer clinics. For any such approach to be of therapeutic use there must be a rationale for a differential effectiveness in tumours and normal tissues.

Acknowledgments. We are grateful to Ms H. Johns for technical assistance in preparing the diagrams. This work was funded entirely by the Cancer Research Campaign.

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0014-4754/89/010041-12$1.50 + 0.20/0
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## The production of chromosome structural changes by radiation

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*Summary.* This paper attempts an update and comment upon some of the topics of chromosome aberration formation which Lea raised in Chapter VI of his classic work 'Actions of Radiations on Living Cells'[24]. Only the first nine sections of this chapter are covered, which deal primarily with the qualitative aspects of aberrations, their formation, classification and interrelationships. In commenting upon these topics, pertinent references are made to work with mammalian and human cells.

Increased knowledge of the importance of DNA as a fundamental target and the integral part it plays in the complex structure of the chromosome, coupled with cellular techniques not available to these earlier workers necessitate some revision and modification of early ideas. However, inspite of the enormous accumulation of data and ideas since the original work was published in 1946, the foundation that these early workers laid is still very solid. Surprisingly, we are still puzzled by many of the problems that perplexed them.

*Key words.* Chromosomes; chromosome aberrations, ionizing radiation; radiation effects; cells.

One of my valued possessions is a 1946 first edition of Lea's book, 'Actions of Radiations on Living Cells'[24]. No one who has read this book and the papers of those early radiation biologists which underly it, can fail to be impressed, almost awed, by the exhaustive and penetrating analysis to which they subjected available data. Every possible avenue was explored and they saw and discussed topics which are still in vogue today, though with a somewhat changed terminology.

The title of this paper is of that of Chapter VI of Lea's book. Limitations of space preclude a full-scale review of this enormous field, so I propose instead an update/annotation on some of the facets raised in the early parts of that Chapter. I shall use the section headings he used (with page reference numbers), but select items for comment which are pertinent to present day interests.

### *Experimental materials* (p. 189)

"For the detailed study of structural changes it is necessary to use nuclei in which chromosomes are large and few in number." There is no doubt that aberration structure can be visualized better in such cells, and plant materials offer a much wider range of cytological fixatives to produce crisper chromosomes. Equivalent mammalian cell cytogenetics was nonexistent in Lea's day but the plethora of work since has produced no surprise aberra-