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Oxygen-Dependent Sensitization of Irradiated Cells

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INTRODUCTION

Observations that oxygen increases the response of cells exposed to ionizing radiation can be traced back to the beginning of this century. The earliest of these seems to have been made by Schwartz (1909), although he did not interpret his results in terms of different oxygen concentrations. Investigations by Holthusen (1921) with Ascaris eggs, by Petry (1921) with seeds, by Crabtree and Cramer (1933) with a transplantable murine carcinoma, and by Mottram (1935) with rats are among those having special importance. However, the studies begun in the 1950's by L. H. Gray and his colleagues clearly represent a milestone in radiation biology. Gray's 1961 review is an excellent survey of information to that time.

When Gray surveyed the literature, clear evidence already showed that in very dry biological systems, for example bacterial spores (Powers et al. 1960), more than one kind of oxygen-dependent sensitizing process existed. Recent work with cells irradiated in suspension (Alper 1963, Tallentire et al. 1972, Shenoy et al. 1975, Ewing and Powers 1976) has confirmed this 20-year-old observation: oxygen operates in more than one way in affecting the radiation sensitivity of the cell.

Oxygen sensitizes all cells to irradiation. Its effects have been studied with many experimental techniques and with many

different organisms. Perhaps in part because of this diversity, definitive studies do not show whether oxygen sensitizes all organisms through identical chemical pathways. Some experimental information supports this assumption; other data contradict it. The survey below will examine the chemical mechanisms involved in oxygen-dependent sensitizations of cells irradiated in suspension.

SENSITIZATION BY DIFFERENT O_2 CONCENTRATIONS

The effects of varying O_2 concentrations have been studied in several cellular systems by a number of authors. Most of the early studies recognized a dependence of radiation sensitivity upon O_2 concentration that increases to a plateau very sharply with no microstructure in the response. The studies on Bacillus megaterium spores (Tallentire et al. 1972, Ewing and Powers 1976) demonstrated the important fact that there is an intermediate region of sensitivity in which there is a plateau at low $[O_2]$ at approximately half the sensitivity of the plateau seen at high $[O_2]$. These observations are commented on extensively below. Here we note that while previous authors have not demonstrated this intermediate effect in their studies, their results are indeed consistent with those two sets of experiments. In Figure 1 we have normalized the radiation sensitivity within several systems and have plotted the degree to which different $[O_2]$ sensitize

within the maximal O_2 effect. Note that the two spore studies are central with the Ewing and Powers (1976) study demonstrating a smooth relationship between $[O_2]$ and radiation sensitivity. As pointed out previously, the Manchester spore studies are consistent with the Austin studies, except that the plateau occurs at a different level, this being explained, perhaps, by the difference between ^{60}Co gamma rays and 50 kVp X-rays. The remarkable aspect of Figure 1 is that two different vegetative bacteria and two sets of experiments on V79 mammalian cells are consistent with the response demonstrated by the bacterial spores. So, while there may be differences in absolute sensitivity among the variety of systems, the O_2 effect, when looked at as in Figure 1, is truly the same in the several systems, perhaps indicating a unity in the mechanisms involved in the O_2 effect, whether they be observed in mammalian cells or bacterial spores. Indeed, in the most recent observations on hamster cells (Millar et al. 1979), indicated as open squares on Figure 1, the presence of a plateau at low $[O_2]$ is acknowledged. The difference between the spore and the mammalian cells in this instance is that the plateau appears at concentrations of O_2 a factor of 10 below those at which the plateau appears in the spore system.

The relative amount of sensitization seen at a particular O_2 concentration is very nearly independent of cell type or the suspending medium at the time of irradiation. This indicates to

us that the intensive investigations in bacterial systems, whether spores or vegetative cells, are proper models for construction of experiments in mammalian cells to search for similar effects.

SEPARATION OF O_2-DEPENDENT DAMAGE INTO COMPONENTS

Bacterial Spore Studies

The first recognition of oxygen's having more than one kind of action was made in a dried system. Using dried bacterial spores, Powers et al. (1960) showed that oxygen's sensitization could be experimentally resolved into at least two major classes of damage. In their experiments, the exchange of gases took several minutes, and, based on this reference time-scale, they established these definitions: (a) Class I damage is independent of oxygen; its magnitude is the same whether or not O_2 is present; (b) Class II damage is oxygen-dependent and short-lived; it can be observed only when oxygen is present during irradiation; and (c) Class III damage, the "post-irradiation" O_2-effect, is produced during either oxic or anoxic exposures; however, the development of this kind of damage is very slow in this dry biological system. There is good evidence that the kind of damage designated as Class III involves a reaction between O_2 and a radiation-induced cellular radical (Powers 1966).

Tallentire and Powers (1963) later showed that intracellular water protects against both kinds of oxygen-dependent damage.

Spores irradiated in suspension or under saturated water vapor conditions show no Class III and only a reduced amount of Class II damage; under these conditions O_2 introduced a few minutes after anoxic irradiation did not increase the sensitivity. Tallentire and Powers did propose, however, that two kinds of oxygen-dependent damage probably operate in the irradiated wet spore. The review article by Powers and Tallentire (1968) summarizes the work with dried biological systems.

Recent work has made it very clear that organisms irradiated in suspension do indeed show more than one kind of oxygen-dependent damage. Using ^{60}Co γ-rays, Tallentire et al. (1972) found evidence for at least two components of oxygen-dependent damage in bacterial spores.

Later experiments with 50 kVp X-rays showed that the sensitization of spores by oxygen could be separated into at least three components (Ewing and Powers 1976) that are O_2-concentration dependent. These results are illustrated in Figure 2. t-Butanol is an effective scavenger of OH radicals and the oxygen-dependent damage removed with this alcohol present is designated as the "'OH component." Two other components of damage are designated as the "low-O_2" and "high-O_2" components, to emphasize that different concentrations of oxygen can produce different kinds of damage (Ewing 1978a).

An important difference has not yet been resolved between the spore experiments with 50 kVp X-rays (Ewing and Powers 1976) and those with ^{60}Co γ-rays, noted just above (Tallentire et al. 1972).

In both sets of data, the changes in response occur over very similar oxygen concentrations, just as they do for most organisms (cf. Figure 1). Furthermore, the changes themselves are qualitatively the same: as the $[O_2]$ is raised, the responses increase, reach a plateau, then increase again to a maximum value. However, with γ-rays, the plateau occurs when about 30% of the maximum sensitization has been reached (cf. Figure 4, Tallentire et al., 1972). With 50 kVp X-rays, the plateau comes at 70%. The basis for this discrepancy is not known, although differences other than photon energy exist between the two sets of experiments; e.g., the γ-ray work was in phosphate buffer whereas the X-ray experiments used water-suspended spores. This "water-versus-buffer" question would not be important when γ-rays are used (Tallentire, private communication, quoted in Ewing 1975), but it would be important with 50 kVp X-rays (Ewing 1975).

Recent work with bacterial spores and 1,4-diazabicyclo[2.2.2]-octane (DABCO), a quencher of singlet oxygen ($O_2^* \, ^1\Delta_g$), suggests that an additional fourth component of damage may now have been recognized (Barber and Centilli, unpublished). Over a range of oxygen concentrations, DABCO has a protective effect which is additive to that seen through OH radical removal; this suggests a component of sensitization involving singlet O_2, although the results of further experiments are needed for confirmation.

The oxygen-dependent sensitization of bacterial spores can

also be resolved into time components if the radiation dose is delivered in a very brief interval. With pulsed techniques that allowed examination of incidents in very short times after irradiation, Weiss and his colleagues (Weiss and McDonald 1976, Weiss and Santomasso 1977, Jones and Weiss 1977) noted that in wet spores, as in dry spores, O_2 delivered to spores after anoxic irradiation can increase sensitivity. Complementary work by Tallentire and his colleagues (Tallentire et al. 1977, Stratford et al. 1977) showed that after anoxic irradiation of spores in suspension, the decay of the potentially lethal species can be resolved into two components; the radical half-lives associated with these two reactions are 9 seconds and 120 seconds. Spores irradiated in O_2 show only one first-order radical decay process ($T_{\frac{1}{2}}$ = nine seconds). Presumably, this is the faster of the two processes observed in anoxia.

Very recent results, also by Tallentire and his co-workers with this same rapid-mix method (Tallentire et al. 1979), provide additional information about these two long-lived components. When an OH radical scavenger is present during anoxic irradiation, the production and subsequent two-component decay is unaffected. Thus, $^{\cdot}OH$ appears not to be involved in either of the two O_2 actions. When these authors use N_2O (present during irradiation), the initial survival level is lowered for "zero time before O_2 introduction," and it remains at the same reduced level even when the introduction of O_2 is delayed. They sug-

gested that ·OH is able to react with the two O_2-sensitive "species" to "fix" damage by excluding the possibility that the radical sites could decay to a harmless state. Limited data, for which they used an OH radical scavenger along with N_2O, show that the effect of N_2O can be partially, but not completely, blocked. We should note that according to these observations ·OH dependent O_2 sensitization operates only in the presence of N_2O -- an agent that supposedly acts only by increasing ·OH yield. If that is the only action, we ask why ·OH scavengers do not affect O_2 sensitization processes in the absence of N_2O. The difference is the removal of e^-_{aq} in the N_2O case and its presence in N_2O absence. Their experiment with acetone, supposedly removing e^-_{aq}, does not give the answer, for it is used at 1 M, a concentration that effectively also removes all ·OH ($k_{acetone + \cdot OH} = 6.8 \times 10^7$ M^{-1} $seconds^{-1}$).

Although both laboratories whose work was cited just above worked with B. megaterium spores, their results are not entirely compatible. Weiss and Santomasso (1977) used a single three nanoseconds pulse of electrons, giving a total dose of either 400 or 600 krad to spores mounted on wet membrane filters. After anoxic irradiation, they observed the decay of an oxygen-sensitive radical having a half-life of either 10.5 seconds or 7.4 seconds respectively, depending on the radiation dose they used. In contrast, Tallentire and his co-workers irradiated spores in suspen-

sion with two microseconds pulses of electrons, giving a total dose of 600 krad at about 0.8 krad/pulse. The total exposure could last up to two seconds. After anoxic irradiation, they observed the decay of two oxygen-sensitive "species". Apparently, Weiss and Santomasso saw one but not both the radicals observed by Tallentire et al. Neither set of authors has attempted to explain the difference in results.

Vegetative Bacterial Cell Studies

Although experiments with dried vegetative bacteria showed two classes of oxygen-dependent damage (Webb 1964), tests with bacteria irradiated in suspension have not. Epp and his colleagues, whose experiments have been recently summarized (Epp et al. 1976), used a double-pulse technique with both Escherichia coli and Serratia marcescens. They irradiated oxygenated cells; then, by varying the time before a second electron pulse, they allowed different amounts of O_2 to diffuse back into the cells. Their analysis showed an upper limit of $\sim 10^{-4}$ seconds for the lifetime of the radical which can react with O_2 to cause damage. However, they did find a discontinuity in the graph of "decade spacing" (i.e. relative decrease in log fractional survival) versus interpulse time. This "bump" might be taken as evidence for more than one kind of oxygen-dependent damaging process, although this is not certain.

Michael et al. (1973) used a gas explosion technique to deliver O_2 at controlled times to anoxically irradiated cells. They found a post-irradiation O_2 effect and estimated the half-life as 500 microseconds for the radical which reacts with O_2 to cause damage. This value is different from that obtained by Epp et al. described just above, who also worked with vegetative bacteria. Michael et al. did not, however, find evidence for more than one kind of reaction involving O_2. Shenoy et al. (1975) have used the liquid rapid-mix method in somewhat similar studies. This procedure, which showed two oxic sensitization components with mammalian cells, described below, also failed to demonstrate more than one kind of oxygen-dependent damage in wet bacteria.

This difference in the measures of radical lifetimes from the two experimental methods is significant and it merits further comment. Michael et al., irradiating under anoxic conditions and introducing O_2 afterward, measured a radical half-life of ∿500 microseconds; Epp et al., who irradiated in O_2 to radiolytically bind the dissolved O_2 and thereby achieve anoxia before the second pulse of radiation, found an upper limit to the radical lifetime of about 10^{-4} seconds. The upper limit to the lifetime of the radical found by Michael et al. would be about 10x longer than that found by Epp et al. (1976). We note here that the conflict in their results could be based on the different experimental techniques that were used. The different conditions of irradia-

tion may not have produced the same radical populations, and the two laboratories may not, in fact, have been studying the same kind of O_2-dependent damage. This suggestion is based on published work with dried bacterial spores. In these spore experiments, Powers et al. (1960) irradiated in anoxia, as Michael et al. did, and produced Class III damage. When they irradiated in O_2, as Epp et al. did, they produced in spores, both Class II and Class III kinds of O_2-dependent damage. Powers and Held (1979) have, in fact, recalled an earlier suggestion of Ewing (unpublished) that may emphasize the importance of the different irradiation procedures these two laboratories used with vegetative bacteria. As they pointed out, Stratford et al. (1977), using wet bacterial spores, observed the post-irradiation decay of two O_2-sensitive radicals following anoxic exposure. However, when they irradiated in O_2, only one radical, the one with the shorter half-life, was seen. In terms of procedure, these conditions of irradiation duplicate those of Michael (anoxic) and Epp (oxic), and Epp did, in fact, observe a shorter radical half-life (based on his estimate of upper limit to the radical lifetime) than did Michael. While these experiments with vegetative bacteria have not resolved O_2-dependent damage into separate components, it may yet be possible to do so with different experimental techniques and, perhaps, different methods of analysis.

In Vitro Mammalian Cell Studies

In contrast to the results with vegetative bacteria, information collected with mammalian cells, after pulsed exposures, also shows that sensitization by oxygen can be resolved into components. However, the time scales for these effects are much shorter than those noted above for bacterial spores. This difference in radical lifetimes is a major point of conflict between those studies with bacterial spores and these with mammalian cells. Tallentire et al. (1977) proposed that the long lifetimes they observed might have resulted from a relative dryness of the spore core, the presumed site for radiation-induced damage; this state of dryness would exist even though the spores were suspended in water. Thus, we might infer that the chemical processes through which O_2 sensitizes are the same in both spores and mammalian cells, even though the reactions themselves are considerably slower in spores.

Using a liquid fast-flow, rapid-mix method with mammalian cells, Shenoy et al. (1975) found that O_2 -- at any concentration -- delivered two milliseconds before irradiation gave a constant amount of sensitization. (With this mixing technique, two milliseconds is the shortest possible time between O_2 contact and the radiation pulse.) Greater sensitization was obtained by allowing longer O_2 contact times before irradiation. They discussed their results in terms of "fast" and "slow" O_2 effects and suggested that the most

plausible interpretation was that damage was produced at two sites within the cells; the "fast" versus "slow" resolution represented the times needed by O_2 to diffuse to these different sites.

Watts et al. (1978) have recently applied the gas-explosion method to in vitro mammalian cell experiments. This procedure allows a resolution of about one millisecond between O_2 delivery time and the radiation dose, whereas in the liquid rapid-mix method, described just above, the best resolution is about three milliseconds. These two methods for delivering O_2 to the cells give different results for the shortest (pre-irradiation) O_2 contact time necessary to achieve the maximum amount of oxic sensitization. However, in these preliminary experiments, the gas explosion technique probably also shows two post-irradiation O_2 effects, just as the liquid rapid-mix method did. Their discussion does not focus on this point, however; we infer this from our examination of their Figure 2.

Michaels et al. (1978) and Ling et al. (1978) have also recently studied O_2-dependent damage in mammalian cells irradiated in vitro. Using their double-pulse method, first applied to bacteria (Epp et al. 1973), they found an upper limit of $\sim$ three milliseconds for the lifetime of the radical which can react with O_2 to cause damage. This is about 30x longer than the upper limit found with vegetative bacteria with this same technique although, as they pointed out, this is compatible with the greater size of

the mammalian cells and, logically, therefore, with the greater distance O_2 must diffuse before it can reach the target radicals, presumably located near the cells' centers. The difference in upper limits they saw with bacterial and mammalian cells does not mean that the lifetimes of the O_2-sensitive radicals actually involved are different; as they discussed, they measured the maximum lifetime these radicals could have, not the radical's half-life (Ling et al. 1978).

Some recent work with chemical model systems, where biologically important molecules are irradiated in vitro, also suggests that O_2 may have more than one action. Held and Powers (1979) and Powers and Held (1979) have extracted wild-type DNA from a strain of Bacillus subtilis, irradiated the DNA, and then measured the loss of transforming ability in a trp^- recipient cell. As they described in an earlier paper (Held et al. 1978) they again found that O_2 at high concentrations ($\sim 10^{-3}$ M) protects DNA irradiated under these conditions (relative to the response after anoxic exposures). They also noted that the sensitivity changes little over a wide range of O_2 concentrations except around 10^{-3} M, where the sensitivity drops, and in the region around 10^{-6} M, where the sensitivity peaks sharply. An important property of this peak is that it is removed by addition of $^{\cdot}OH$ scavengers, indicating $^{\cdot}OH$ involvement in the O_2 effect at low concentrations of O_2, just as in the wet spore experiment of Ewing and Powers (1976).

Studies by Michaels and Hunt (1977a,b) may be relevant to these results with transforming DNA. They irradiated single-stranded polynucleotides to study the reactions of O_2 at the radical site formed by $^{\cdot}OH$ attack. Using pyrimidines (polycytidylic acid and polyuridylic acid), they found evidence for two polynucleotide radicals which react with O_2 at different rates. They proposed that at low O_2 concentrations the absolute rate constant is about 5.8×10^8 M^{-1} $seconds^{-1}$, while at high O_2 concentrations, the rate constant for the poly $C\text{-}OH^{\cdot} + O_2$ reaction is about 1.8×10^8 M^{-1} $seconds^{-1}$. Their results with purine polynucleotides and with double-stranded polymers are considerably more complex and, as they discuss, further experimental work is needed to identify the reactions which are occurring.

The studies cited in this section provide conclusive evidence that O_2 has more than a single chemical pathway through which it effects sensitization in several biological systems. The studies discussed in the following section will summarize what has been learned concerning the chemical nature of these components of damage.

EFFECTS ADDED CHEMICALS HAVE ON OXIC SENSITIZATION: PROTECTORS & O_2

When water is irradiated, the three primary radiolytic products are the hydrated electron (e^-_{aq}), the hydrogen atom ($^{\cdot}H$), and

the hydroxyl radical ($^{\cdot}OH$). Information concerning these radicals has accumulated rapidly since the 1960's, and radiation biologists have tried to associate reactions of these transients with specific biological endpoints. Only the hydroxyl radical has been clearly implicated in causing cell death after irradiation (Johansen and Howard-Flanders 1965, Sanner and Pihl 1969, Powers and Cross 1970, Chapman et al. 1975), although, as discussed below, the information from different organisms suggests that different processes may be involved. Some years ago, Adams and Dewey (1963) noted that chemical additives which reacted well with hydrated electrons are generally radiation sensitizers. This observation, that e^-_{aq} removal increases the radiation sensitivity, suggests in itself that the e^-_{aq} might be playing a protective role in reducing the amount of radiation-induced damage. This concept is central to the electron sequestration model of Powers (1972) which deals with mechanisms of radiation damage and the chemical basis for the actions of some radiation sensitizers.

Results from studies with bacteria, bacterial spores, and mammalian cells have led to different conclusions about the involvement of $^{\cdot}OH$ in damage and sensitization. This may be due, in part, to the absence of comparative studies among the three types of cells. Careful studies are urgently needed before we can understand how to apply information from one of these biological systems to another.

Bacterial Spore Results

When bacterial spores are irradiated in anoxia or in high concentrations of O_2, the addition of a scavenger to remove OH radicals does not necessarily protect (Powers et al. 1972 and Ewing 1976a). t-Butanol, for example, will not protect spores irradiated under either of these two conditions (as long as no other sensitivity-modifying agent is present); in fact, high concentrations of t-butanol (>1 M) will increase the anoxic response (Ewing 1976a), an observation that has not yet been explained.

Some OH radical scavengers, however, have been found which do protect spores irradiated either in air (Ewing 1975) or in anoxia (Ewing 1976b). Figure 3 illustrates the results in anoxia. This protection is not a simple function of $^{\cdot}OH$ removal, but it seems to be correlated with the ability of the scavenger to form an α-carbon radical (i.e., a reducing radical) after reaction with $^{\cdot}OH$ (Ewing 1976). Results emphasize both the importance of this correlation and the fact that $^{\cdot}OH$ removal itself does not necessarily protect. CO_2 is a protector (cf. Figure 3), but it does not react with OH radicals; in fact, CO_2 is one of the very few e^-_{aq} scavengers which is not a radiation sensitizer. Formate at 2×10^{-2} M reduces the response to the minimum level reached by any of the tested compounds, a decrease in k of ∿20%. t-Amyl alcohol at 2.8×10^{-2} M and t-butanol at 9.6

x 10^{-2} M scavenge OH radicals as efficiently as that formate concentration, but neither of these two alcohols protects. This is clear evidence that ·OH removal in itself will not invariably protect spores in the absence of sensitizers.

In a set of experiments to test the importance of forming a reducing radical, methanol (a protector) and t-amyl alcohol (a non-protector) were used simultaneously. At selected concentrations, where t-amyl alcohol scavenges ·OH's more efficiently than methanol, methanol's ability to protect was reduced. This supports the hypothesis that methanol is not itself the protector; instead, the protecting agent is formed after a reaction with a water-derived radical, in this case the ·OH (Ewing 1976b).

Figure 2 shows that over a range of O_2 concentrations, t-butanol protects. In contrast to what was found after anoxic irradiation, tests have shown that this protection in low O_2 concentrations can be specifically attributed to a simple removal of OH radicals (Figure 4). Different additives used at the same ·OH scavenging efficiency protect equally well (Ewing 1978a).

Additives have also been tested for effects against the low- and high-O_2 components of damage (cf. Figure 2). The results with methanol are shown in Figure 5; those with ethanol, in Figure 6.

Under anoxic conditions, methanol reduces the response (Ewing 1976b). When spores are irradiated in 0.8% O_2 ($\sim 10^{-5}$ M

dissolved), methanol removes the O_2-dependent $^{\cdot}OH$ damage (cf. Figure 3). As shown in Figure 5, higher concentrations of methanol tested in 0.8% O_2 reduce the response further (Ewing, unpublished). But the amount of the protection in 0.8% O_2 (i.e., the $-\Delta\underline{k}$) and the methanol concentrations over which this protection occurs suggest that this is simply the same protection seen with methanol in anoxia. From this, we conclude that methanol does not affect the low-O_2 kind of damage. Methanol, tested in air (Ewing 1976a), first shows a reduction in the overall response, parallel to that seen in anoxia; higher methanol concentrations reduce the sensitivity even more. Thus, methanol is able to reduce, although not eliminate, damage attributable to the high-O_2 component.

The results with ethanol (Figure 6) are more complex. Like methanol, ethanol protects spores irradiated in anoxia, although the amount of protection (the $-\Delta\underline{k}$) is not as great as with methanol. Again like methanol, ethanol does not appear to protect against low-O_2 damage, although as the ethanol concentration increases above 2 M, the response rises sharply (Ewing, unpublished). In air, ethanol protects against the high-O_2 component, as methanol does, but high ethanol concentrations again increase the response. This increase seems to mirror the increase seen with ethanol in 0.8% O_2; that is, in air the turnabout in protection at high ethanol concentrations is probably due to ethanol's

unexpected ability to increase damage through the low-O_2 component.

Tests with ethanol have also been run at an intermediate O_2 concentration. In 5.5% O_2 ($\sim 8 \times 10^{-5}$ M dissolved), the $\cdot OH$ component is negligibly small. Presumably, the low- and high-O_2 components are both operating. In increasingly higher ethanol concentrations, the response in 5.5% O_2 is reduced; it passes through a minimum, then it increases again (cf. Figure 6). These results are qualitatively the same as those observed when ethanol was tested in air. However, two important quantitative differences are apparent. First, in 5.5% O_2, ethanol begins to protect at lower concentrations than it did when tested in air; and, second, the maximum amount of protection (the $-\Delta k$) is greater in 5.5% O_2 than it is in air. We find, in fact, that the ratio of ethanol concentrations for 50% protection (air: 5.5% O_2) is 0.6 M/0.15 M = 4.0. This is the same as the ratio of O_2 concentrations used in the two tests, 0.209/0.055 = 3.8. These results might suggest that O_2 and ethanol are competing for a single damaged cellular site. A lower O_2 concentration correspondingly reduces the ethanol concentration required for the same level of protection; it also increases the amount of protection that is possible.

Such a competition between ethanol and O_2 (specifically the high-O_2 component in these experiments) was not found in studies

with E. coli by Johansen and Howard-Flanders (1965). From their experiments, they concluded that ethanol could interfere with the formation of R· (the damaged cellular site), but ethanol and O_2 did not compete for reactions at that site. This result with bacterial cells does not complement that found by Weiss and Santomasso (1977), who pulse-irradiated spores suspended in water or pure ethanol. In ethanol, they found no decrease in the yield of the oxygen-sensitive radical (i.e., ethanol did not interfere with the formation of the damaged site), although the half-life of the radical in ethanol was considerably reduced. (No value was given for the reduced half-life.) Whether these results in pure ethanol (Weiss and Santomasso 1977) are compatible with those illustrated in Figure 6, which show an O_2-ethanol competition, is unknown. Pulse-irradiation studies at lower O_2 concentrations would clarify this point.

Glycerol is the additive which has the greatest protective ability in the spore system (Webb and Powers 1963, Ewing 1975). At sufficiently high concentrations, glycerol protects spores irradiated in anoxia, and, even when irradiated in air, glycerol reduces the response to the same protected level seen in 100% N_2. With spores, no other additive has been found which can eliminate all oxygen-dependent damage.

From these spore results, in which radiation protectors have been tested in anoxia and in various O_2 concentrations, we

may make these general observations:

(1) Although OH radicals are clearly damaging under some experimental conditions, in anoxia -- with no other sensitivity-modifying agent present -- OH radical removal per se does not protect. The same generalization holds true for spores irradiated in high $[O_2]$: $^{\cdot}OH$ removal in itself does not protect.

(2) However, over a limited range of O_2 concentrations (roughly 10^{-6} to 10^{-4} M), simple $^{\cdot}OH$ removal will reduce the response. Thus, although O_2 is not known to affect the initial yield of $^{\cdot}OH$ and although O_2 and the $^{\cdot}OH$ do not react, $^{\cdot}OH$ damage becomes "temporarily" important. And its occurrence requires the presence of O_2 within a specific concentration range; at $[O_2] > 10^{-4}$ M, $^{\cdot}OH$ removal no longer protects.

(3) Some additives have been found which protect spores irradiated under anoxic conditions. These agents probably function by forming an α-carbon radical, which is the actual protector. These agents, which can all form reducing radicals, protect in anoxia: formate ion, methanol, CO_2, ethanol, allyl alcohol, glycerol, 1-propanol, and 2-propanol. If these agents protect in anoxia through the formation of a reducing radical, a reasonable inference is that the damage being repaired or prevented arises through an oxidation reaction.

(4) Those agents which form α-carbon radicals and protect in anoxia also protect against one or both the low- and high-O_2

components of damage. (All OH radical scavengers which have been tested, including one sensitizer, Ewing 1978, can remove the ·OH component of oxygen-dependent damage and thereby protect.) Allyl alcohol (Ewing, unpublished), methanol, ethanol, and glycerol all protect against high-O_2 damage; this implies, again, following the reasoning in item (3), that an oxidation reaction is involved in the damage from this specific component of oxygen's sensitization. This same conclusion, that the high-O_2 component probably involves an oxidation reaction, has been reached through other spore studies (Simic and Powers 1974, Ewing 1978). The fact that methanol and ethanol do not protect against low-O_2 damage suggests that the process leading to sensitization here does not involve an oxidation step or process.

Bacterial Vegetative Cell Results

The vegetative cell studies most easily compared to the spore experiments described in the preceding section are those by Sanner and Pihl (1969) and by Johansen and Howard-Flanders (1965). Both investigations examined possible roles that radical scavenging agents have in reducing the radiation sensitivity.

Sanner and Pihl (1969) used *E. coli* B, suspended in distilled water and irradiated in either liquid or frozen states, with or without selected additives. They found that both ethanol and glycerol protect bacteria irradiated anoxically in liquid suspension at 0° C; a 1 M concentration of either additive will reduce

the response to about 65% of that seen without the additive. They also used acetone, an agent they found relatively poor at reducing the sensitivity of E. coli irradiated in anoxia. In contrast, Tallentire and Jacobs (1972), working with bacterial spores, found that acetone was an effective radiation sensitizer, although very high concentrations were required for an effect.

Figure 7 shows their results with these and several other compounds. They have plotted the relative sensitivity against either the $^{\cdot}OH$ scavenging efficiency (left panel) or the e^-_{aq} scavenging efficiency (right panel). If the protection they observed must arise from either $^{\cdot}OH$ or e^-_{aq} scavenging, clearly OH radical scavenging is responsible. However, the data points (right panel) do not themselves establish a well-defined relationship, although this is not strictly required since all the additives may not necessarily protect through OH radical scavenging. Perhaps more serious is the fact that the data points also show scatter around the theoretical line (shown in Figure 7), which was drawn based on the assumption that $^{\cdot}OH$ are responsible for 45% of the lethal damage in anoxia.

From these results, Sanner and Pihl concluded that $^{\cdot}OH$ removal will protect bacteria irradiated under anoxic conditions; they estimated that under these conditions about 50% of the total damage arises through reactions of hydroxyl radicals. They noted that this estimate agrees well with conclusions from Webb's work

(Webb 1964). He mounted Staphylococcus aureus cells on membrane filters and dried them; the anoxic radiation sensitivity dropped by about 50% when the equilibrium vapor pressure was reduced to about 1 Torr. Further drying had little effect on the response. However, this amount of protection, seen in either E. coli or Staphylococcus, is considerably greater than the protection observed when bacterial spores are irradiated under similar conditions. Tallentire and Powers (1963) found that drying produced only about a 25% reduction in anoxic response. (It is important to recall that in both the spore and Staph. systems, drying decreases the radiation sensitivity only under anoxic conditions; in both organisms, the two classes of oxygen-dependent damage increase greatly as water is removed.)

Sanner and Pihl (1969) also concluded that no amount of protection, including that from the sulfhydryl cysteamine, was greater than that expected from ·OH scavenging alone.

Johansen and Howard-Flanders (1965), in a slightly earlier study, used E. coli B/r, irradiated in buffered saline at 2^{o}-5^{o} C. (It is not known if the overall results would have changed if distilled water, rather than buffer, had been used; or, alternately, if buffer, rather than water, had been used in the experiments described by Sanner and Pihl 1969.) With bacterial spores, phosphate buffer is itself a slight radiation sensitizer (Ewing 1975), and the actions of p-nitroacetophenone in a low O_2 concentration

are different in water compared with buffer (Ewing 1977).

Johansen and Howard-Flanders (1965) studied several radiation protectors in some detail. They concluded that the effects of the sulfhydryl mercaptoethanol are two-fold: first, this agent could interfere with the formation of a damaged (oxygen-sensitive) site within the cell by scavenging water-derived radicals; and, second, mercaptoethanol could protect by successfully competing with O_2 for reaction at this damaged site. Figure 8 shows their results with five protective agents. Curiously, they included nitric oxide (NO) among the protectors. Although NO has complex effects on radiation sensitivity, their own results clearly show (Figure 4 of Johansen and Howard-Flanders 1965) that the sensitivity at all NO concentrations is greater than that seen in its absence, although a peak in the response may indicate two actions of NO. This is parallel to the earlier observation of Powers et al. (1960) that NO has two actions in the dry spore.

The method of analysis used by Johansen and Howard-Flanders is slightly different from that of Sanner and Pihl; but, again, if the protection the former team observed in air must arise from scavenging either $^{\cdot}OH$ or a combination of e^-_{aq} and $^{\cdot}H$, the data clearly favor an involvement of OH radicals. As was the case with the anoxic study (cf. Figure 7), the fit of the data to the expected line is not extremely good. Johansen and Howard-Flanders, in fact, called the fit "reasonably good" and suggested that

"uncertainties in the concentrations of the added substances within the cell" might account for some of the scatter (Johansen and Howard-Flanders 1965). From this analysis, they concluded that OH radicals contribute about half the lethal damage when these bacteria are irradiated under aerobic conditions.

These two investigations with vegetative bacteria, within themselves, provide a consistent view of the role played by OH radicals both in O_2 and in anoxia and they presented several chemical models that were reasonable reflections of the state of radiation chemical knowledge of that time.

The data from the two bacterial studies can be compared by plotting them on the same graph. Figure 9 shows such a plot after a recalculation of the aerobic data of Johansen and Howard-Flanders (1965), without the nitrous oxide point, to conform to the analysis method used by Sanner and Pihl (1969). More recent values of the reaction rate constants (Ross and Ross 1977) were used, and the points have shifted somewhat from their original positions. The line in this figure is the same as that from Figure 7 (Sanner and Pihl 1969). The fit of the aerobic data points to the theoretical line is no worse than the fit of the original anaerobic points.

In Vitro Mammalian Cell Results

Much of the groundwork on the oxic sensitization of mammalian cells and on the possible roles played by OH radicals comes from

the work of Chapman and his colleagues. They tested several radiation protectors over a range of concentrations, usually up to the limit set by toxicity of the added agent (Chapman et al. 1975). Dimethylsulfoxide (DMSO) is one of the very few compounds that can be used at high concentrations in cultures of mammalian cells, and for this reason, unfortunately, DMSO must be used in the studies concerning OH radical involvement when cells are irradiated in vitro. Chapman and his colleagues found that DMSO can provide considerable protection for cells irradiated in air; DMSO also protects anoxically irradiated cells, although the magnitude of the effect is much smaller. DMSO has also been tested in bacterial spores (Ewing 1978), where, in contrast, it was found to be a very potent radiation sensitizer. However, spores treated with DMSO and then washed before irradiation in water still showed virtually the same response noted if DMSO had not been removed before irradiation. Thus, this sensitization seems attributable to changes DMSO causes in spore "physiology." These unidentified changes, while clearly not toxic, seem responsible for the greatly increased responses to irradiation, both in O_2 and in anoxia, when DMSO was present. To our knowledge, this "washed out" experiment has not been done with mammalian cells.

Chapman and his co-workers (Chapman et al. 1973) have also observed that cysteamine was, in fact, a better protector of mammalian cells than DMSO. Lower concentrations of cysteamine, tested in O_2 and in anoxia, gave as much protection as higher

concentrations of DMSO; in addition, the maximum amount of protection, in both gases, was greater from cysteamine. They concluded that, like DMSO, cysteamine protected by scavenging ·OH; however, another radiation chemical process, perhaps repair through hydrogen-donation, is also possible with cysteamine. This may conflict with the results Sanner and Pihl observed with bacteria (Sanner and Pihl 1969). They found that all the protection from cysteamine could be accounted for soley by ·OH removal.

Other additives which Chapman and his colleagues tested showed only small abilities to protect at concentrations lower than the limits set by toxicity. The results of their survey (Chapman et al. 1975) are illustrated in Figure 10. (The cells were irradiated in culture medium.) To analyze and to test for an involvement of OH radicals in lethal processes, the authors implicitly assumed that these compounds would all protect to the same minimum response level -- the level seen with only DMSO -- if toxicity were not a limiting factor. They plot the reciprocal of the additive concentration for 50% of the effect DMSO had against the rate constant for ·OH scavenging by the specific additive. The result, shown in the right panel of Figure 10, is a straight line having the expected slope of +1.0. The relationship they observed provided the basis for the conclusion that, in air, OH radical removal protects against O_2-dependent damage. Based on these and earlier data (Chapman et al. 1973), they estimated that for irradiation in air about 62% of the lethal damage in mammalian cells

results from the actions of OH radicals. The small amount of protection they saw when DMSO-treated cells were irradiated anoxically (cf. Figure 10) was attributed to OH radical removal, although anoxic experiments, like those for air shown in Figure 10, were not reported. The authors estimated that in anoxia about 30% of the damage results from the effects of OH radicals, an estimate that seems somewhat high.

These data by Chapman and his colleagues, Figure 10, can be re-analyzed and compared with the results from the aerobic and anaerobic bacterial studies, which were collected in Figure 9. Table I shows the calculated values for "relative sensitivity" and "'OH scavenging efficiency" that were calculated from the original mammalian cell data (Chapman et al. 1975). Comparison with the bacterial cell data in Figure 9 shows a remarkably good agreement.

These results can also be compared with those in Figure 4, where, with bacterial spores, 'OH removal is clearly responsible for the observed protection. According to Figure 4, a $\underline{k}C$ of about 10^4 seconds^{-1} will achieve 50% of the maximum amount of protection, a scavenging efficiency almost 10^5 times lower than that needed for protection through 'OH removal in the two organisms compared in Figure 9 and Table I. (The calculated point for spores is "relative sensitivity" = 0.89; "$\underline{k}C_{50\%}$" = 10^4 seconds^{-1}.)

This comparison of data from spores, vegetative bacteria, and mammalian cells raises perplexing questions and provides few satisfactory answers. ·OH removal does not protect spores irradiated under either anoxic or well-oxygenated conditions; however, over an intermediate range of O_2 concentrations -- in spores -- simple ·OH removal will protect. In contrast, results from several studies indicate that ·OH removal will protect vegetative bacteria irradiated under either anoxic or well-oxygenated conditions. The amount of protection seen with vegetative bacteria under these two conditions seems to be a simple function of the efficiency for OH radical removal; equal ·OH scavenging efficiencies will produce equal amounts of protection. In spores, ·OH damage is clearly oxygen-dependent (or, more generally, "sensitizer-dependent"). In spite of this apparently unique origin for an ·OH involvement, we do not understand why the ·OH scavenging efficiency needed to protect vegetative bacteria is about 10^5 times greater than that needed in spores for the same relative amount of protection.

The same conclusion regarding the importance of OH radical removal has also been reached from studies with mammalian cells: it seems that simple OH radical removal protects. In this case, however, the maximum amount of protection seen in the anoxic studies is much less than that protection seen in experiments which used air-equilibrated cells. We do not understand why

these two experimental conditions would give similar results with vegetative bacteria but different results with mammalian cells, especially since, in both kinds of cells, the role played by OH radicals is believed the same. On the other hand, under aerobic conditions, the same OH scavenging efficiency does give about the same relative amount of protection with either vegetative bacteria or mammalian cells.

With the information presently available from these three biological systems, it appears that O_2 need not sensitize these cells through the same chemical pathways. However, before accepting this conclusion, we should carefully re-examine the information on which it is based. We should remember that there have been no comparative studies where the same additives were rigorously tested in all three systems. This is partly due, of course, to toxicity problems with vegetative bacteria and mammalian cells. We urgently need this kind of experimental data. With spores, we found that various OH radical scavengers did not always have the same effects, and consequently, they could not be used interchangeably. In spite of the correlation drawn between protection and OH radical scavenging in both vegetative bacteria and mammalian cells, there is surely enough experimental uncertainty to emphasize the need for additional data.

SUMMARY

This survey has focused primarily on O_2 effects in three biological systems, all tested in suspension: bacterial spores, vegetative bacterial cells, and mammalian cells. We have examined information from these systems which shows that O_2 has more than one process through which it can act, and we have looked at the effects various protectors have on oxygen's ability to sensitize. While selecting from among the many studies within these guidelines, we have largely ignored the studies which test O_2 in combination with other radiation sensitizers. There is considerable information from these studies, but, within the intentionally limited scope of this survey, we cannot cover this information here.

Studies with bacterial spores provide clear evidence that multiple components to oxygen-dependent radiation sensitization exist. Studies with mammalian cells also show that at least two oxygen-dependent sensitization processes can be distinguished, although we have not yet learned how to relate the components of sensitization from these two very different organisms. Similar studies with vegetative bacteria in suspension have not resolved oxic sensitization into components, although different experimental techniques may yet do so. It is essential to emphasize that the observation noted almost 20 years ago with very dry bacterial spores

now clearly applies to mammalian cells irradiated in vitro: there is more than one kind of oxygen-dependent damage.

We have examined the roles water-derived radicals might play in radiation sensitivity and, specifically, in sensitization by O_2. We find that, among the primary radiolytic products, OH radicals are clearly implicated in damage in all three biological test systems. However, we must exercise great care in drawing conclusions here, since the specific roles proposed for OH radicals are different in these organisms.

In bacterial spores, $^{\cdot}OH$ removal in itself does not protect in anoxia or in high concentrations of O_2 if there is no other sensitivity-modifying agent present. (Many organic and inorganic sensitizers have effects which can be partially or completely removed by agents which scavenge OH radicals.) With spores, $^{\cdot}OH$ removal over a limited intermediate range of O_2 concentrations will, however, protect. Results of tests with those agents which protect in anoxia and in air show that, although these agents scavenge OH radicals, that is, in itself, not the protecting step; instead, $^{\cdot}OH$ scavenging probably results in the formation of the actual protector.

In bacteria, results of surveys to test the effects various radical scavengers have on radiation sensitivity and on the sensitization by O_2 have provided the basis for the supposition that $^{\cdot}OH$ removal will protect both in anoxia and in the presence of O_2.

Many authors have suggested that OH radicals react with a cellular target molecule and leave a radical site; this is the site which can then react with O_2 to cause damage. It is widely believed and often expressly stated that DNA is the likely cellular target for OH radical attack.

In mammalian cells, there are severe difficulties in using radical scavenging agents at the necessary high concentrations. Nevertheless, based on the information which can be obtained, a reaction scheme, similar to that proposed for bacteria, has been suggested for O_2-dependent sensitization; again, it is expressly stated that DNA is the likely target for cellular damage.

A re-analysis of the data from these biological systems suggests that these conclusions may not be as firm as we had thought. From the results with the different kinds of cells, we see that the proposed roles for oxygen-dependent sensitization, and especially for the involvement of OH radicals, are not complementary. Before we accept the conclusion that O_2 operates through different chemical pathways in these organisms, we should carefully re-examine the data on which our conclusions have been based.

We must remember when we use an OH radical scavenger and observe protection that we have not proved that $^{\cdot}OH$ removal is the specific reaction responsible for the protection; neither have we proved that OH radicals are damaging to irradiated cells.

These conclusions may be entirely true, but our simple observation of protection has not established them. We must also remember that for many years most analyses were based on the assumption that only one kind of O_2-dependent sensitization process exists. Clearly O_2 can sensitize bacterial spores, mammalian cells, and very likely bacterial cells as well, through more than one chemical or physical process. Our taking the simplest case of assuming only one effect of O_2, building models, and drawing conclusions may not have been as profitable as we had hoped. Perhaps it is time to discard this simplest case in our model-building and take a more realistic, although necessarily more complex, view of how O_2 acts to sensitize cells.

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Adams, G. E., and D. L. Dewey. 1963. Hydrated electrons and radiobiological sensitization. Biochem. Biophys. Res. Commun. 12: 473-477.

Alper, T. 1963. Lethal mutations and cell death. Physics Med. Biol. 8:365-385.

Alper, T. 1969. Mechanisms of lethal radiation damage to cells, in Proceedings of Second Symposium on Microdosimetry, Stresa (Italy), October 20-24, 1969. pp. 5-36.

Anbar, M., M. Bambenek, and A. B. Ross. 1973. Selected Specific Rates of Reactions of Transients from Water in Aqueous Solution. I. The Hydrated Electron. U.S. Department of Commerce, NSRDS-NBS 43.

Chapman, J. D., A. P. Reuvers, J. Borsa, and C. L. Greenstock. 1973. Chemical radioprotection and radiosensitization of mammalian cells growing in vitro. Radiat. Res. 56:291-306.

Chapman J. D., D. L. Dugle, A. P. Reuvers, B. E. Meeker, and J. Borsa. 1974. Studies on the radiosensitizing effect of oxygen in chinese hamster cells. Int. J. Radiat. Biol. 26:383-389.

Chapman J. D., D. L. Dugle, A. P. Reuvers, C. J. Gillespie, and J. Borsa. 1975. Chemical radiosensitization studies with mammalian cells growing in vitro, in Radiation Research: Biomedical, Chemical, and Physical Perspectives, O. F. Nygaard, H. I. Adler, and W. K. Sinclair, eds. Academic Press, New York, pp. 752-760.

Crabtree, H. G., and C. Cramer. 1933. The action of radium on cancer cells. II. Some factors determining the susceptibility of cancer cells to radium. Proc. Roy. Soc. B 113:238-250.

Dewey, D. L. 1963. The x-ray sensitivity of Serratia marcescens. Radiat. Res. 19:64-87.

Elkind, M. M. 1975. Cellular and subcellular biology, in Radiation Research: Biomedical, Chemical and Physical Perspectives, O. Nygaard, H. I. Adler, and W. K. Sinclair, eds. Academic Press, New York, pp. 1349-1361.

Epp, E. R., H. Weiss, N. D. Kessaris, A. Santomasso, J. Heslin, and C. C. Ling. 1973. Oxygen diffusion times in bacterial cells irradiated with high-intensity pulsed electrons: New upper limit to the lifetime of the oxygen-sensitive species suspected to be induced at critical sites in bacterial cells. Radiat. Res. 54: 171-180.

Epp, E. R., H. Weiss, and C. C. Ling. 1976. Irradiation of cells by single and double pulses of high intensity radiation: Oxygen sensitization and diffusion kinetics, in Current Topics in Radiation Biology Quarterly, M. Ebert and A. Howard, eds. North-Holland Publishing Company 11:201-250.

Ewing, D. 1975. Two components in the radiation sensitization of bacterial spores by p-nitroacetophenone: The ·OH component. Int. J. Radiat. Biol. 28:165-176.

Ewing, D. 1976a. Effects of some ·OH scavengers on the radiation sensitization of bacterial spores by p-nitroacetophenone and O_2 in suspension. Int. J. Radiat. Biol. 30:419-432.

Ewing, D. 1976a. Anoxic radiation protection of bacterial spores in suspension. Radiat. Res. 68:459-468.

Ewing, D. 1977. Sensitization of bacterial spores by p-nitroacetophenone (PNAP) and 0.8% O_2. Radiat. Res. 72:291-295.

Ewing, D. 1978a. Additive sensitization of bacterial spores by oxygen and p-nitroacetophenone. Radiat. Res. 73:121-136.

Ewing, D. 1978b. Effects of dimethylsulfoxide (DMSO) on the x-ray sensitivity of B. megaterium spores. (Abstract) Radiat. Res. 74:516.

Ewing, D., and E. L. Powers. 1976. Irradiation of bacterial spores in water: Three classes of oxygen-dependent damage. Science 194:1049-1051.

Fielden, E. M., O. Sapora, and P. S. Loverock. 1978. Rapid lysis III. The effect of radiosensitizers on the production of DNA damage and the time course of its repair. Radiat. Res. 75: 54-65.

Gray, L. H. 1961. Mechanisms involved in the initiation of radiobiological damage in aerobic and anaerobic systems, in The Initial Effects of Ionizing Radiations on Cells, R. J. C. Harris, ed. Academic Press, New York, pp 21-44.

Held, K. D., and E. L. Powers. 1979. Effects of varying O_2 concentration on the x-ray sensitivity of transforming DNA. Int. J. Radiat. Biol. (Submitted).

Held, K. D., R. W. Synek, and E. L. Powers. 1978. Radiation sensitivity of transforming DNA. Int. J. Radiat. Biol. 33:317-324.

Holthusen, H. 1921. Beiträge zur biologie der strahlenwirkung. Untersuchungen an askarideneiern. Pfluger Arch. 187:1-24.

Howard-Flanders, P., and T. Alper. 1957. The sensitivity of microorganisms to irradiation under controlled gas conditions. Radiat. Res. 7:518-540.

Johansen, I. 1974. Competition between tetramethylpiperidinol N-oxyl and oxygen in effects on single-strand breaks in episomal DNA and in killing after x-irradiation in Escherichia coli. Radiat. Res. 59:398-408.

Johansen, I., R. Gulbrandsen, and R. Pettersen. 1974. Effectiveness of oxygen in promoting x-ray induced single-strand breaks in circular phage λ DNA and killing of radiation-sensitive mutants of Escherichia coli. Radiat. Res. 58:384-397.

Johansen, I., and P. Howard-Flanders. 1965. Macromolecular repair and free radical scavenging in the protection of bacteria against x-rays. Radiat. Res. 24:184-200.

Jones, W. B. G., and H. Weiss. 1977. An ESR study of the transient signals produced in wet spores irradiated by intense 3 nsec pulses of electrons. (Abstract) Radiat. Res. 70:657.

Ling, C. C., H. B. Michaels, E. R. Epp, and E. C. Peterson. 1978. Oxygen diffusion into mammalian cells following ultrahigh dose rate irradiation and lifetime estimates of oxygen-sensitive species. Radiat. Res. 76:522-532.

Michael, B. D., G. E. Adams, H. B. Hewitt, W. B. G. Jones, and M. E. Watts. 1973. A posteffect of oxygen in irradiated bacteria: A submillisecond fast mixing study. Radiat. Res. 54:239-251.

Michaels, H. B., E. R. Epp, C. C. Ling, and E. C. Peterson. 1978. Oxygen sensitization of CHO cells at ultrahigh dose rate: Prelude to oxygen diffusion studies. Radiat. Res. 76:510-521.

Michaels, H. B., and J. W. Hunt. 1977a. Reactions of oxygen with radiation-induced free radicals on single-stranded polynucleotides. Radiat. Res. 72:18-31.

Michaels, H. B., and J. W. Hunt. 1977b. Radiolysis of the double-stranded polynucleotides poly(A+U) and DNA in the presence of oxygen. Radiat. Res. 72:32-47.

Michaels, H. B., and J. W. Hunt. 1978. Determination of peroxides and hydroperoxides in irradiated solutions of nucleic acid constituents and DNA. Analyt. Biochem. 87:135-140.

Millar, B. C., E. M. Fielden, and J. J. Steele. 1979. Radiosensitizers and the oxygen effect in mammalian cells. Presented at the Association of Radiation Research (Great Britain), North East Wales Institute in Wrexham, Clwyd, Wales. Elsevier, Amsterdam. (in press).

Mottram, J. C. 1935. On the alteration in sensitivity of cells towards radiation produced by cold and by anaerobiosis. Brit. J. Radiol. 8:643-651.

Petry, E. 1921. The conditions for the biological activity of Röntgen Rays. Biochem. Z. 119:23-44.

Powers, E. L. 1966. Contributions of electron paramagnetic resonance techniques to the understanding of radiation biology, in Electron Spin Resonance and the Effects of Radiation on Biological Systems, NAS-NRC Nuclear Science Series, pp. 137-159.

Powers, E. L. 1972. The hydrated electron, the hydroxyl radical, and hydrogen peroxide in radiation damage in cells. Isr. J. Chem. 10:1199-1211.

Powers, E. L., and M. Cross. 1970. Nitrous oxide as a sensitizer of bacterial spores to x-rays. Int. J. Radiat. Biol. 17:501-514.

Powers, E. L., and K. D. Held. 1979. The oxygen effects in radiation biology and radiation chemistry. Presented at the Association of Radiation Research (Great Britain) at the North East Wales Institute in Wrexham, Clwyd, Wales. Elsevier, Amsterdam. (in press).

Powers, E. L., R. C. Richmond, and M. Simic. 1972. OH radicals in radiation sensitization. Nature New Biology 238:260-261.

Powers, E. L., and A. Tallentire. 1968. The roles of water in the cellular effects of ionizing radiations, in Actions Chimiques et Biologiques des Radiations, M. Haissinsky, ed. Paris: Masson et Cie, 12:3-67.

Powers, E. L., R. B. Webb, and C. F. Ehret. 1960. Storage, transfer, and utilization of energy from x-rays in dry bacterial spores. Radiat. Res. (Suppl. 2):94-121.

Roots, R., and K. C. Smith. 1974. On the nature of the oxygen effect on x-ray-induced DNA single-strand breaks in mammalian cells. Int. J. Radiat. Biol. 26:467-480.

Ross, A. B., and F. Ross. 1977. Selected Specific Rates of Reactions of Transients from Water in Aqueous Solution. III. Hydroxyl Radical and Perhydroxyl Radical and their Radical Ions. U.S. Department of Commerce, NSRDS-NBS 59.

Sanner, T., and A. Pihl. 1969. Significance and mechanism of the indirect effect in bacterial cells. The relative protective effect of added compounds in Escherichia coli B, irradiated in liquid and in frozen suspension. Radiat. Res. 37:216-227.

Schwarz, G. 1909. Desensibilisierung gegen Röntgen- und radiumstrahlen. Muenchener Medizinische Wochenschrift 56:1217-1218.

Shenoy, M. A., J. C. Asquith, G. E. Adams, B. D. Michael, and M. E. Watts. 1975. Time-resolved oxygen effects in irradiated bacteria and mammalian cells: A rapid-mix study. Radiat. Res. 62:498-512.

Simic, M., and E. L. Powers. 1974. Correlation of the efficiencies of some radiation sensitizers and their redox potentials. Int. J. Radiat. Biol. 26:87-90.

Stratford, I. J., R. L. Maughan, B. D. Michael, and A. Tallentire. 1977. The decay of potentially lethal oxygen-dependent damage in fully hydrated Bacillus megaterium spores exposed to pulsed electron irradiation. Int. J. Radiat. Biol. 32:447-455.

Tallentire, A., D. J. W. Barber, R. L. Maughan, B. D. Michael, and I. J. Stratford. 1979. Manipulation of long-lived free radical damage in wet bacterial spores. Presented at the Association of Radiation Research (Great Britain) at the North East Wales Institute in Wrexham, Clwyd, Wales. Elsevier, Amsterdam, (in press).

Tallentire, A., and G. P. Jacobs. 1972. Radiosensitization of bacterial spores by ketonic agents of differing electron-affinities. Int. J. Radiat. Biol. 21:205-213.

Tallentire, A., A. B. Jones, and G. P. Jacobs. 1972. The radiosensitizing actions of ketonic agents and oxygen in bacterial spores suspended in aqueous and non-aqueous milieux. Isr. J. Chem. 10:1185-1197.

Tallentire, A., R. L. Maughan, B. D. Michael, and I. J. Stratford. 1977. Radiobiological evidence for the existence of a dehydrated core in bacterial spores, in Spore Research 1976, A. N. Barker, J. Wolf, D. J. Ellar, G. J. Dring, and G. W. Gould, eds. Academic Press, New York, pp. 649-659.

Tallentire, A., and E. L. Powers. 1963. Modification of sensitivity to x-irradiation by water in Bacillus megaterium. Radiat. Res. 20:270-287.

Watts, M. E., R. L. Maughan, and B. D. Michael. 1978. Fast kinetics of the oxygen effect in irradiated mammalian cells. Int. J. Radiat. Biol. 33:195-199.

Webb, R. B. 1964. Physical components of radiation damage in cells, in Physical Processes in Radiation Biology, L. Angenstein, R. Mason, and B. Rosenberg, eds. Academic Press, New York, pp. 267-285.

Webb, R. B., and E. L. Powers. 1963. Protection against actions of x-rays by glycerol in the bacterial spore. Int. J. Radiat. Biol. 7:481-490.

Weiss, H., and J. C. McDonald. 1976. Response of anoxic and oxic wet spores to intense 3 nsec pulses of electrons. (Abstract) Radiat. Res. 67:535-536.

Weiss, H., and A. Santomasso. 1977. A post irradiation sensitizing effect of oxygen in anoxic wet spores exposed to intense 3 nsec duration pulses of electrons. (Abstract) Radiat. Res. 70: 656-657.

Willson, R. L. 1970. The reaction of oxygen with radiation-induced free radicals in DNA and related compounds. Int. J. Radiat. Biol. 17:349-358.

Table I. $^{\cdot}OH$ Scavenging Efficiency for half maximal protection of Chinese hamster cells irradiated in vitro.

AIR/ANOXIA	ADDITIVE	RELATIVE SENSITIVITY	$\underline{k}_{\cdot OH} C$ ($\times 10^{-8}$)
Air	DMSO	0.71	8.4
Air	Iso-butanol	0.71	7.2
Air	Ethylene glycol	0.71	11.0
Air	t-Butanol	0.71	6.6
Anoxia	DMSO	0.91	8.4

Figure 1. Radiation sensitivity at particular [O_2] relative to the maximal sensitivity seen in O_2 for the particular system. The symbols are as follows: B. megaterium spores (AUS) -- spores irradiated in H_2O with 50 kVp x-rays (Ewing and Powers 1976); V-79 -- Chinese hamster cells irradiated in culture medium with 250 kVp x-rays (Chapman et al. 1974); B. megaterium spores (MAN) -- spores irradiated in phosphate buffer with ^{60}Co γ-rays (Tallentire et al. 1972); Shigella -- irradiated in phosphate buffer with 200 kVp x-rays (Howard-Flanders and Alper 1957); Serratia -- irradiated in buffer with 200 kVp x-rays (Dewey 1963); V-79-753B -- Chinese hamster cells irradiated in culture medium with ^{60}Co γ-rays (Millar et al. 1979). The solid line is the response for spores as presented by Ewing and Powers 1976.

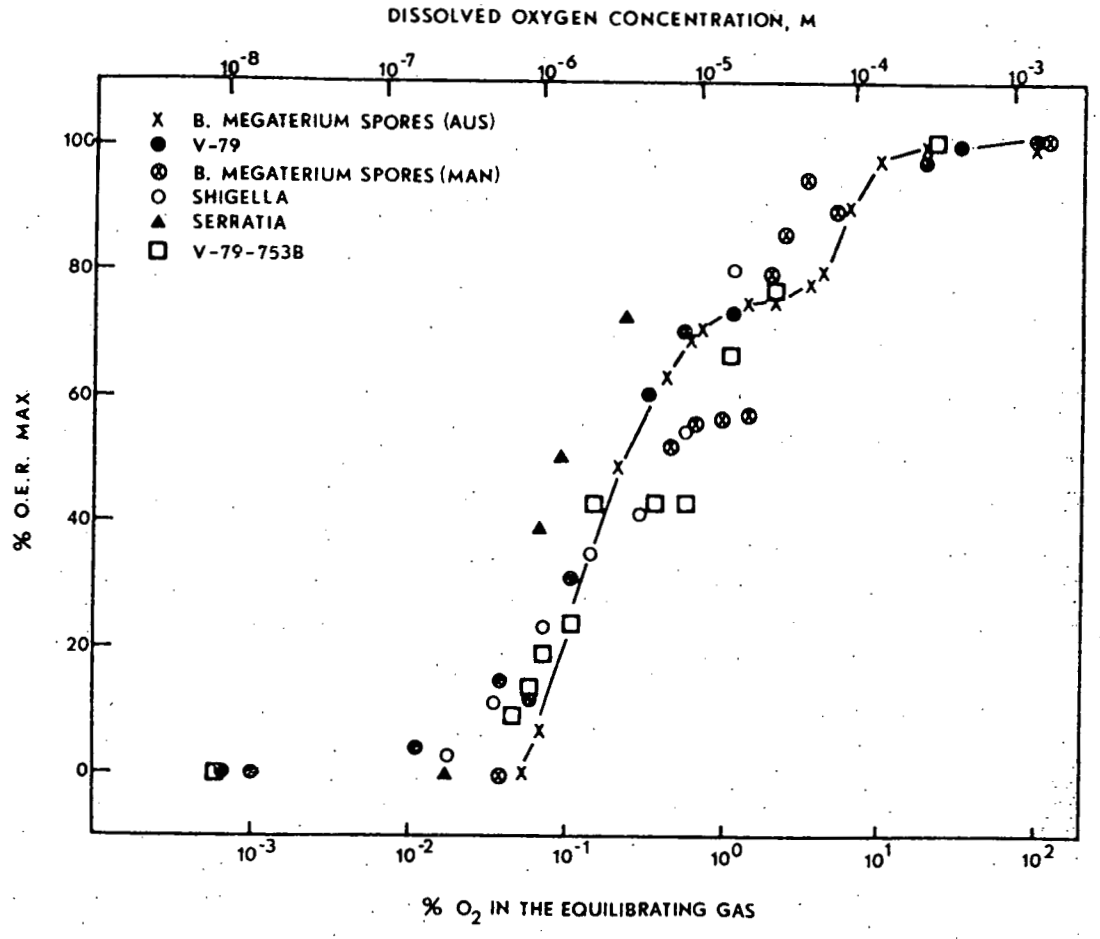

DISSOLVED OXYGEN CONCENTRATION, M
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
X B. MEGATERIUM SPORES (AUS)
● V-79
⊗ B. MEGATERIUM SPORES (MAN)
○ SHIGELLA
▲ SERRATIA
□ V-79-753B
100
80
60
40
20
0
% O.E.R. MAX.
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
% O_2 IN THE EQUILIBRATING GAS

Figure 2. The radiation sensitivity of B. megaterium spores as a function of O_2 concentration (Ewing and Powers 1976). Irradiation was with 50 kVp x-rays. Different $[O_2]$ were prepared by adding measured amounts of N_2 to a cylinder containing O_2. The resulting $[O_2]$ was measured with a gas chromatograph. (See Powers and Cross 1970 for procedural details.)

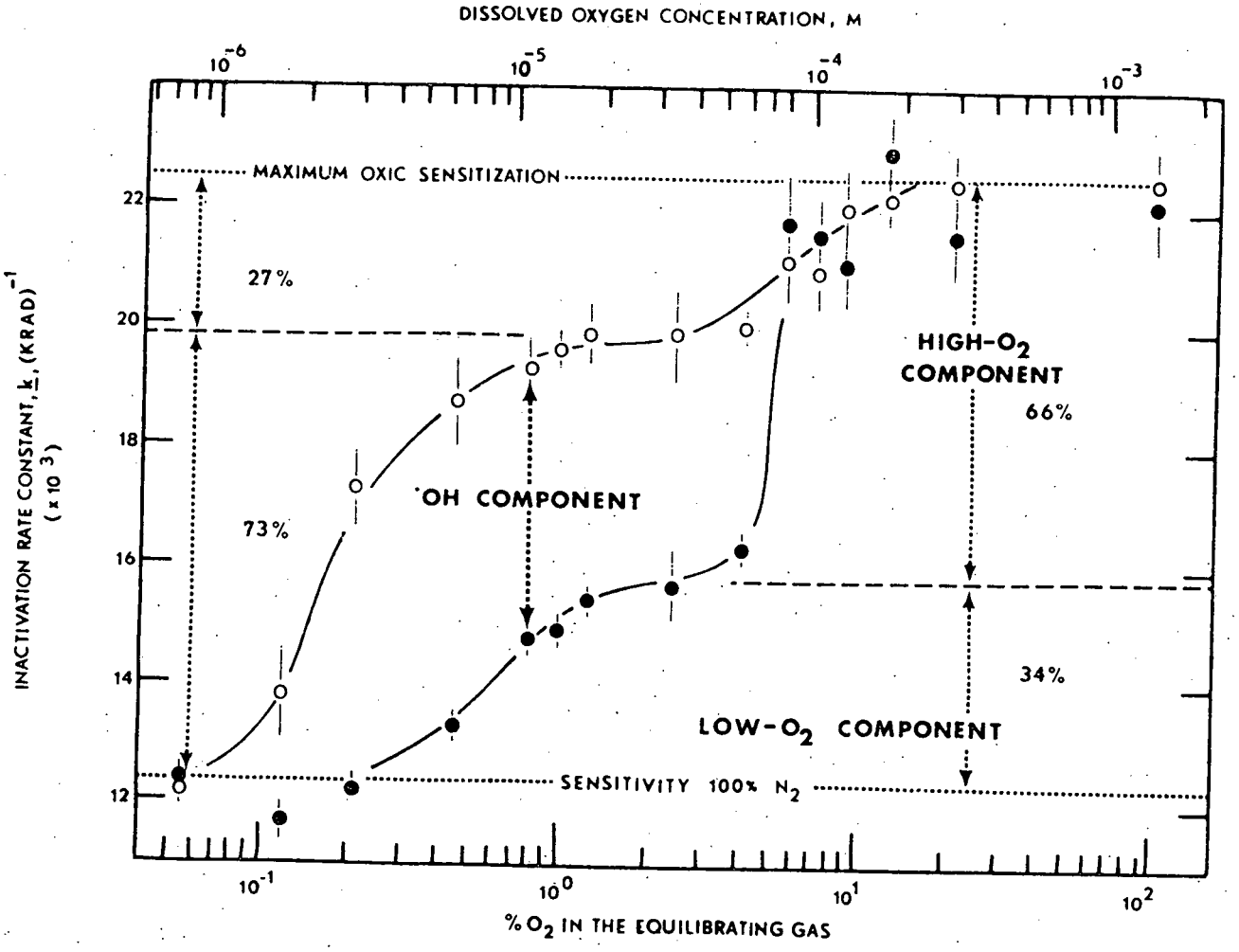

DISSOLVED OXYGEN CONCENTRATION, M
10^{-6}
10^{-5}
10^{-4}
10^{-3}
MAXIMUM OXIC SENSITIZATION
22
20
18
16
14
12
27%
73%
·OH COMPONENT
HIGH-O_2 COMPONENT
66%
34%
LOW-O_2 COMPONENT
SENSITIVITY 100% N_2
INACTIVATION RATE CONSTANT, $\underline{k}$, $(KRAD)^{-1}$ ($\times 10^3$)
10^{-1}
10^{0}
10^{1}
10^{2}
% O_2 IN THE EQUILIBRATING GAS

Figure 3. Anoxic radiation sensitivity of B. megaterium spores, suspended in various concentrations of the additives noted. Irradiation was with 50 kVp x-rays. (Reproduced from Ewing 1976b.)

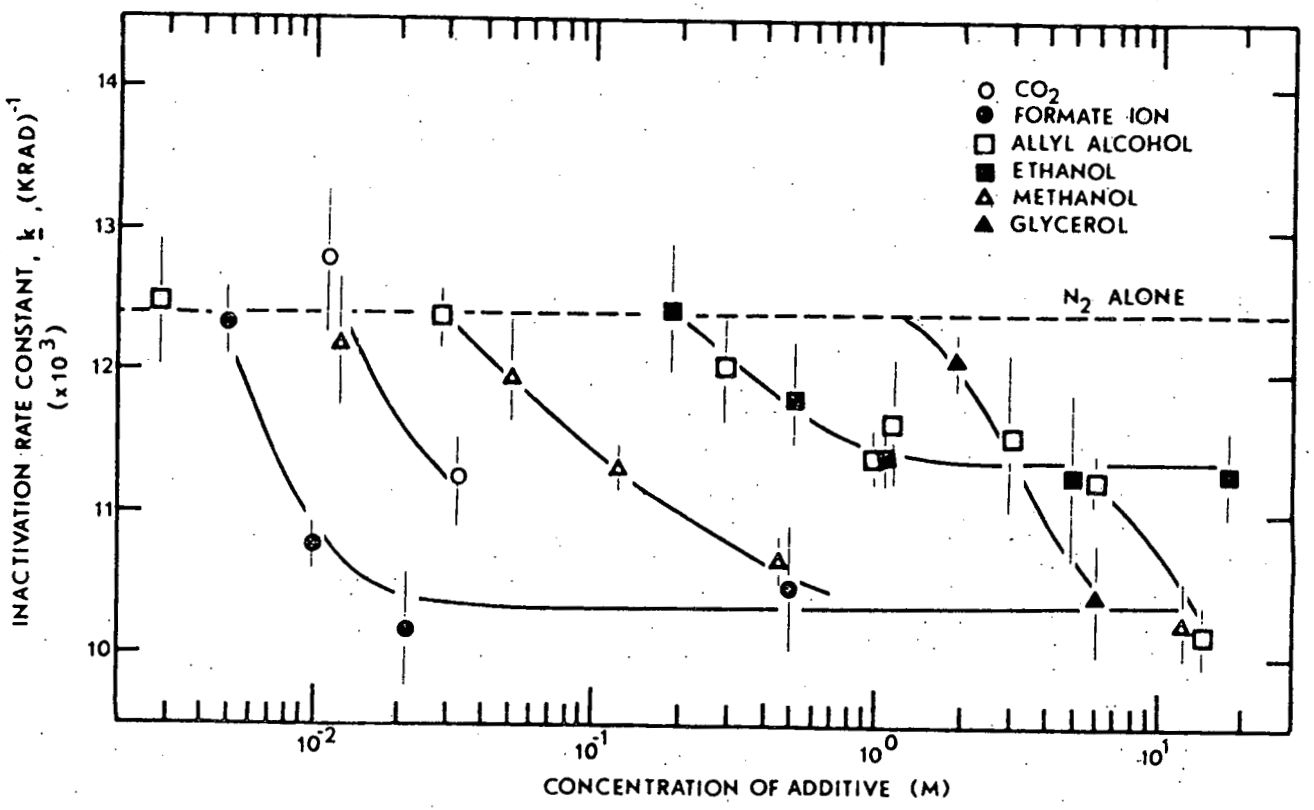

INACTIVATION RATE CONSTANT, k, (KRAD)$^{-1}$ (x10^{-3})
14
13
12
11
10
10^{-2}
10^{-1}
10^{0}
10^{1}
CONCENTRATION OF ADDITIVE (M)
CO_2
FORMATE ION
ALLYL ALCOHOL
ETHANOL
METHANOL
GLYCEROL
N_2 ALONE

Figure 4. Changes in the radiation sensitivity of B. megaterium spores irradiated in 10^{-5} M O_2 with various concentrations of several alcohols also present. The abscissa shows the ·OH scavenging efficiency, the product of the specific alcohol concentration and the bimolecular rate constant for its reaction with ·OH. (Reproduced from Ewing 1978a.)

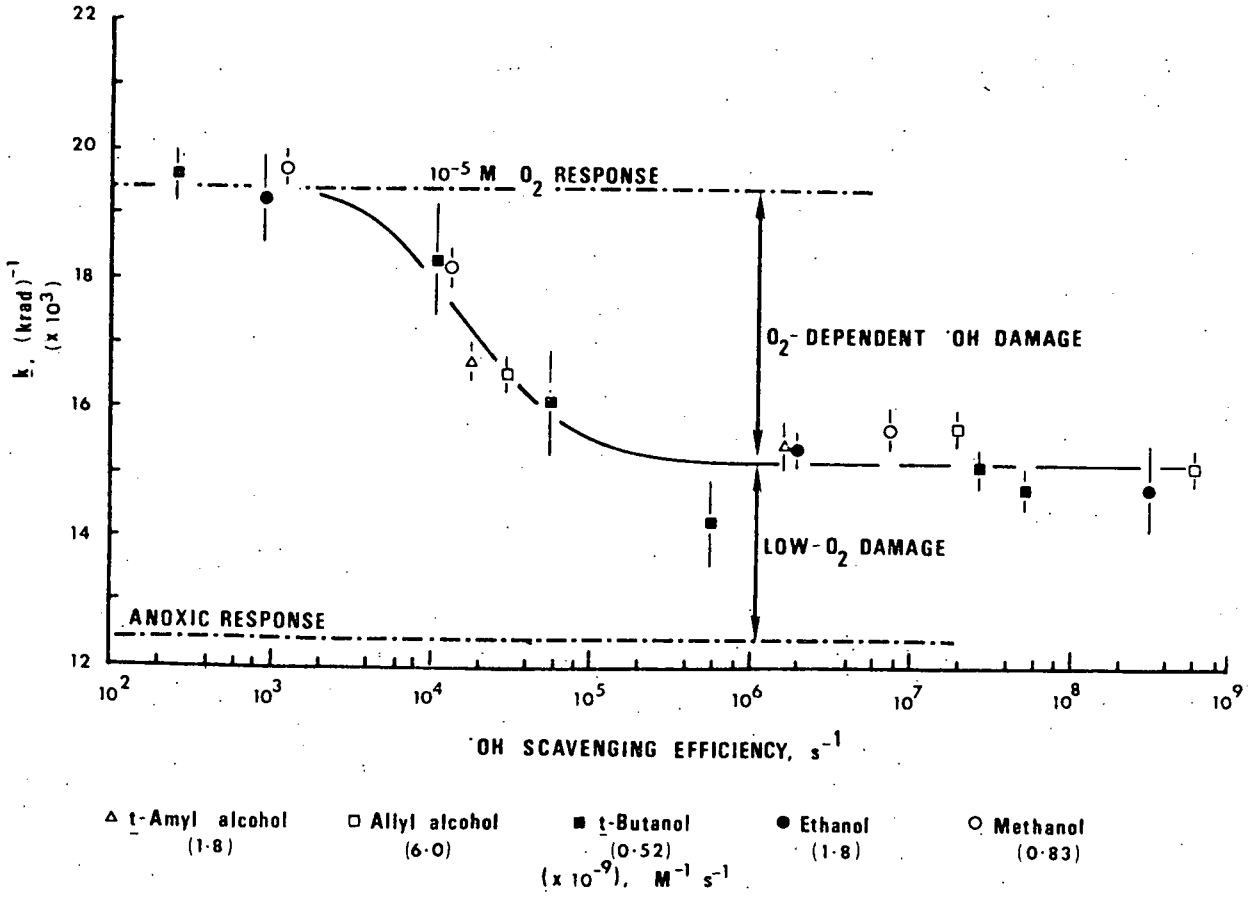

22
20
18
16
14
12
k_1 (krad)$^{-1}$ (x 10^3)
10^{-5} M O_2 RESPONSE
O_2-DEPENDENT OH DAMAGE
LOW-O_2 DAMAGE
ANOXIC RESPONSE
10^2
10^3
10^4
10^5
10^6
10^7
10^8
10^9
OH SCAVENGING EFFICIENCY, s^{-1}
△ t-Amyl alcohol (1·8)
□ Allyl alcohol (6·0)
■ t-Butanol (0·52)
● Ethanol (1·8)
○ Methanol (0·83)
(x 10^{-9}), M^{-1} s^{-1}

Figure 5. Changes in the radiation sensitivity of B. megaterium spores, irradiated in water with 50 kVp x-rays, under three reference conditions when different concentrations of methanol (MeOH) are present. The lower horizontal line shows the response in anoxia with no additive present; the symbols O show the protective effects MeOH has in anoxia (Ewing 1976b). The middle horizontal line shows the response from the Low-O_2 Component of damage; in the [O_2] used for these tests, MeOH has already removed the $^{\cdot}OH$ Component of damage (cf. Figure 4) at concentrations lower than those shown in this figure. The symbols Δ show the reduction in the response with higher [MeOH] (Ewing, unpublished). The upper horizontal line shows the response in air with no additive present; the symbols □ show the reduction in this response when MeOH is added at higher and higher concentrations (Ewing 1976a). Refer to the text for a discussion of these results.

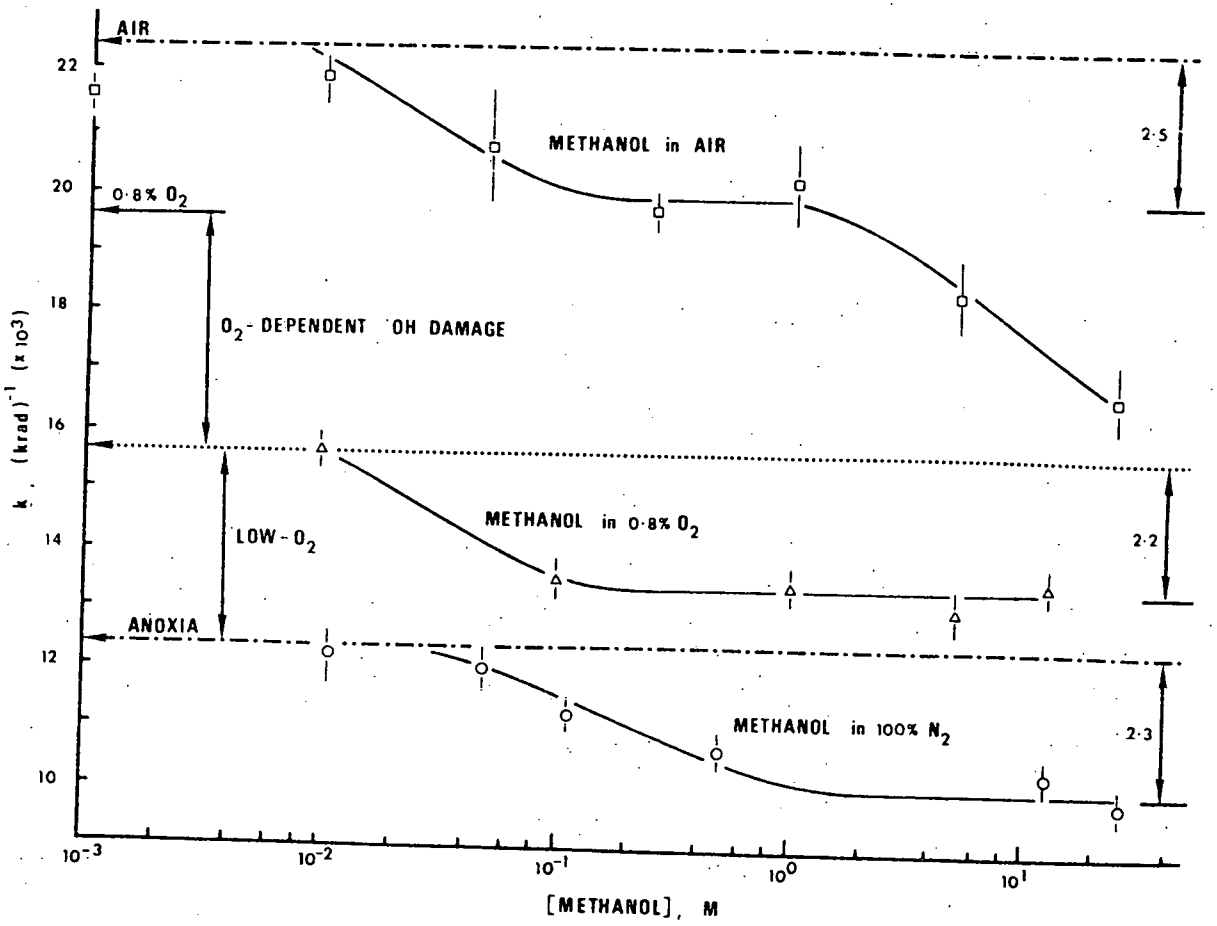

AIR
0·8% O_2
ANOXIA
METHANOL in AIR
METHANOL in 0·8% O_2
METHANOL in 100% N_2
O_2-DEPENDENT OH DAMAGE
LOW-O_2
2·5
2·2
2·3
22
20
18
16
14
12
10
k_s (krad)$^{-1}$ (x 10^3)
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
[METHANOL], M

Figure 6. Changes in the radiation sensitivity of B. megaterium spores, irradiated in water with 50 kVp x-rays, under four reference conditions when different concentrations of ethanol (EtOH) are present. The lower horizontal line shows the response in anoxia with no additive present; the symbols ● show the protective effects EtOH has in anoxia (Ewing 1976b). The middle horizontal line shows the response from the Low-O_2 Component of damage; in the [O_2] used for these tests, EtOH has already removed the $^{\cdot}OH$ Component of damage (cf. Figure 4) at concentrations lower than those shown in this Figure. The symbols ▲ show the effects on the Low-O_2 Component of oxygen-dependent damage when different [EtOH]'s are used (Ewing, unpublished). The uppermost horizontal line shows the response in air with no additive present; the symbols ■ show the changes in radiation sensitivity when different [EtOH]'s are present. Arrows pointing to the ordinate show the response in 5.5% O_2 with no additive present and also the response in 5.5% O_2 when 10^{-1} t-butanol is added. The symbols □ show the changes in response when different [EtOH]'s are tested in 5.5% O_2. Refer to the text for a discussion of these results.

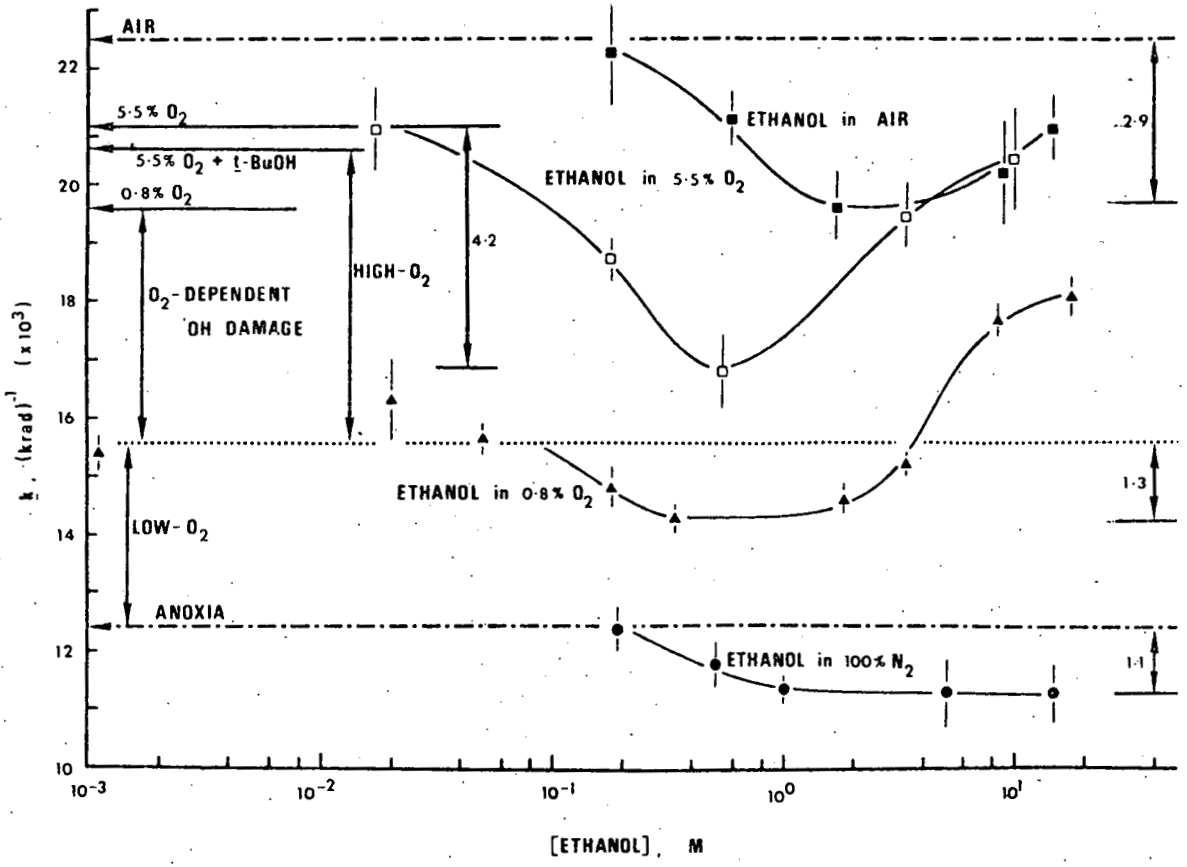

AIR
5·5% O_2
5·5% O_2 + t-BuOH
0·8% O_2
O_2-DEPENDENT OH DAMAGE
HIGH-O_2
LOW-O_2
ANOXIA
4·2
2·9
1·3
1·1
ETHANOL in AIR
ETHANOL in 5·5% O_2
ETHANOL in 0·8% O_2
ETHANOL in 100% N_2
k_1, $(krad)^{-1}$ ($\times 10^3$)
[ETHANOL], M
22
20
18
16
14
12
10
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}

Figure 7. Ability of different compounds to protect E. coli B at 0^{o} C, as a function of their rate of interaction with $^{\cdot}OH$ (A) and with e^{-}_{aq} (B), respectively (Sanner and Pihl 1969). The relative sensitivity observed in the presence of the compounds is plotted versus the product of the protector concentration and the respective second-order rate constants for the interaction of the different protectors with $^{\cdot}OH$ and with e^{-}_{aq}. The fully drawn curve in A is a theoretical curve calculated as described in the text. The radiation sensitivity in the absence of added compounds was set equal to 1. All values are based on dose-effect curves. Abbreviations: Ad, adenine; Cyt, cytosine; EtOH, ethanol; Form, sodium formate; Glu, glucose; Gly, glycerol; Glygly, gylcylglycine; MeOH, methanol; RSH, cysteamine; Th, thymine.

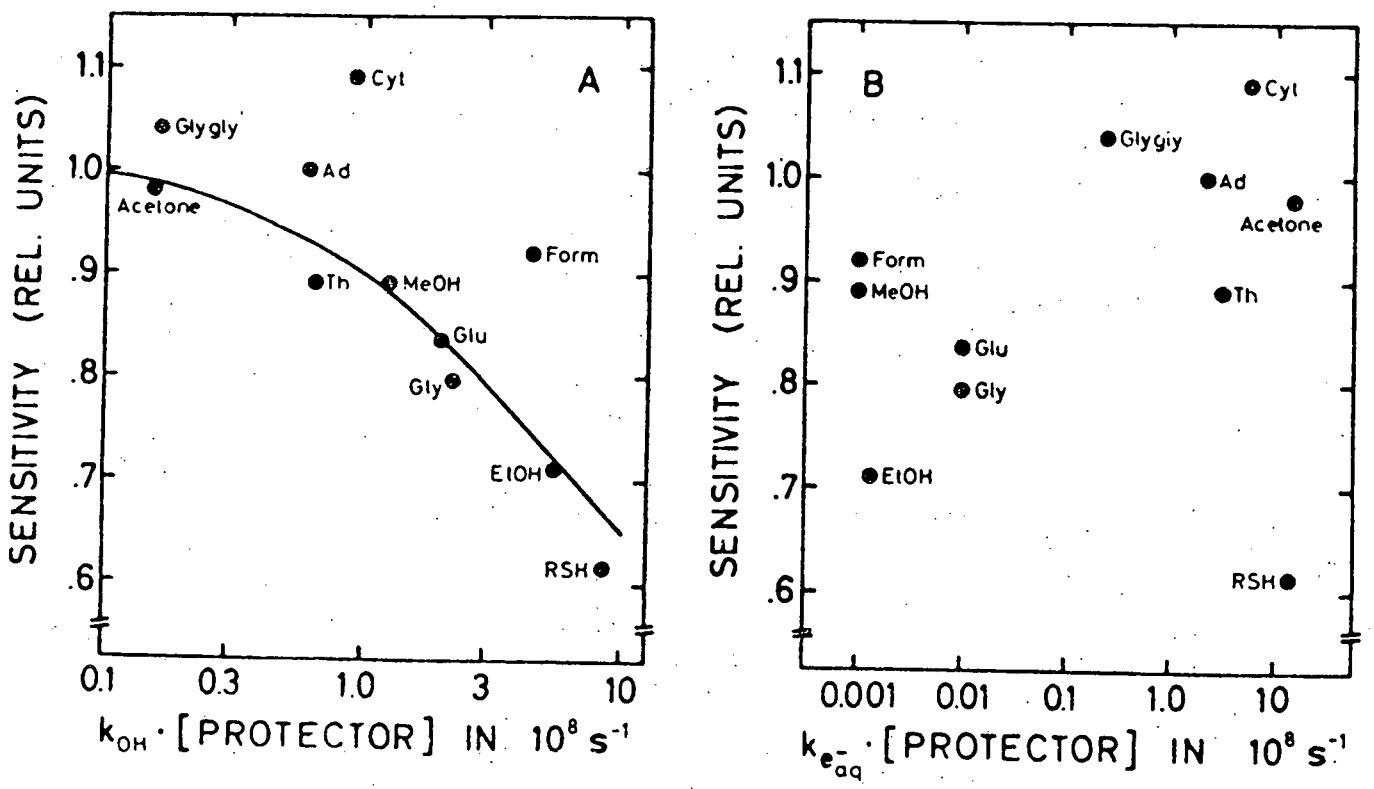
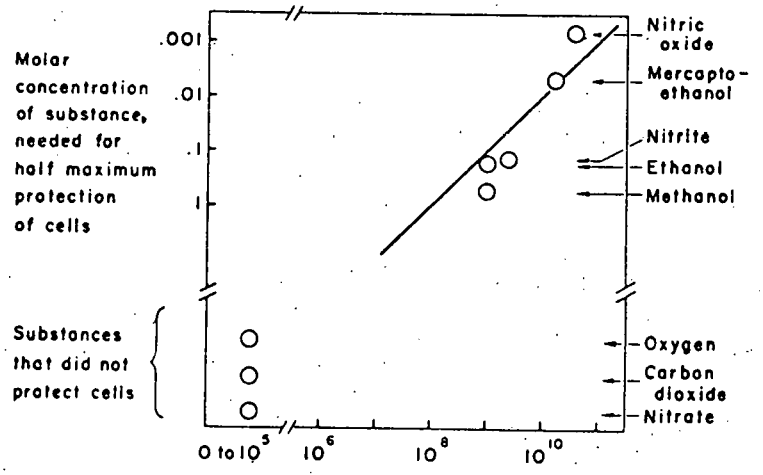

A
B
SENSITIVITY (REL. UNITS)
1.1
1.0
.9
.8
.7
.6
Cyt
Glygly
Ad
Acetone
Form
Th
MeOH
Glu
Gly
EtOH
RSH
0.1
0.3
1.0
3
10
$k_{OH} \cdot$ [PROTECTOR] IN 10^8 s^{-1}
0.001
0.01
0.1
1.0
10
$k_{e^-_{aq}} \cdot$ [PROTECTOR] IN 10^8 s^{-1}

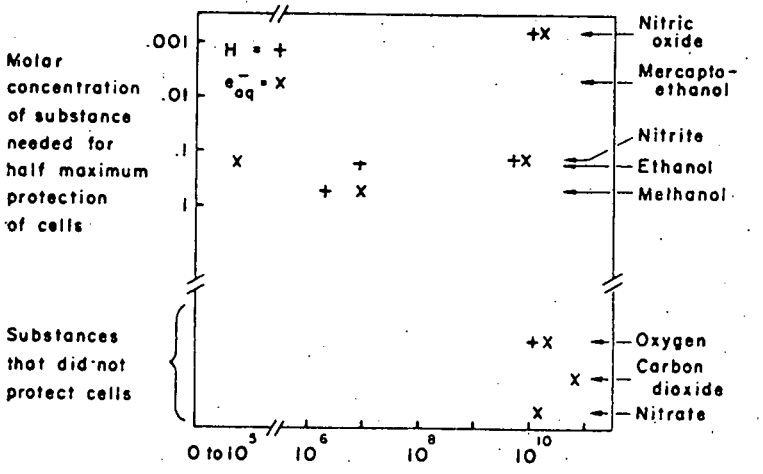
Figure 8. Protection of E. coli B/r (Johansen and Howard-Flanders 1965). The effective concentrations of various substances in protecting bacteria against x-irradiation are plotted against data for the reaction rates of these substances with hydroxyl radicals (top panel) and with the reducing species (bottom panel). Oxygen at 2×10^{-3} M, carbon dioxide at 7×10^{-2} M, and sodium nitrate at 8×10^{-1} M did not protect bacteria; these substances are plotted below the intercept in both figures.

PROTECTION OF E coli $B/_r$ AGAINST X-IRRADIATION



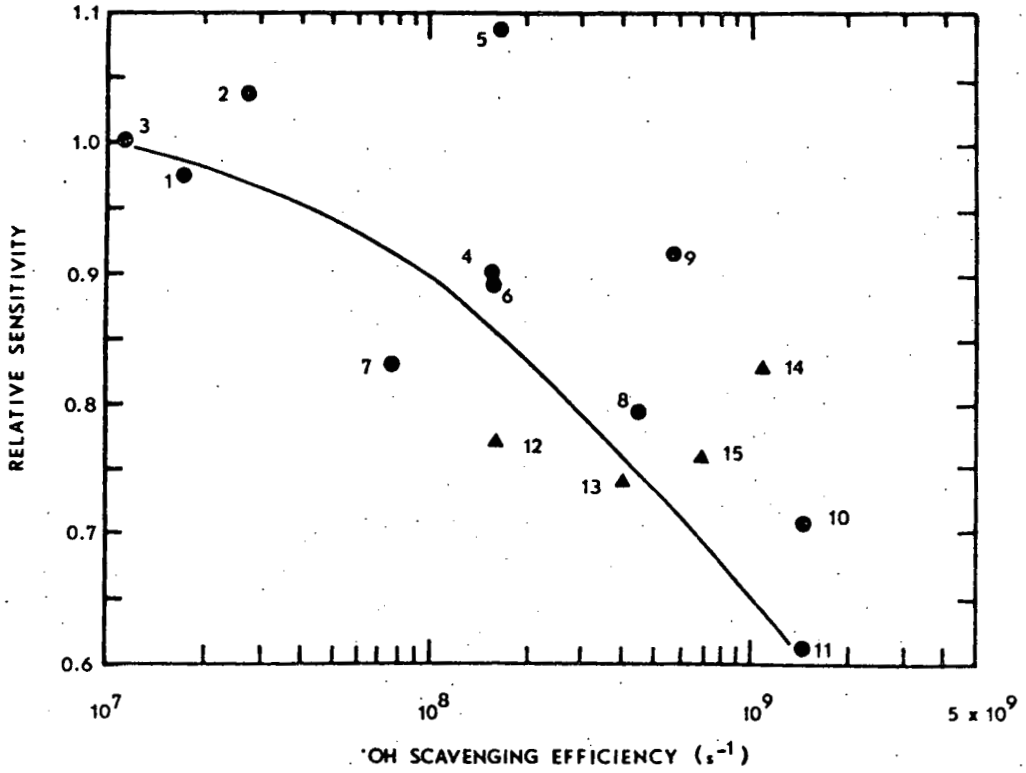
Reaction rates with OH in aqueous solutions, liters/mole/sec

PROTECTION OF E coli $B/_r$ AGAINST X-IRRADIATION



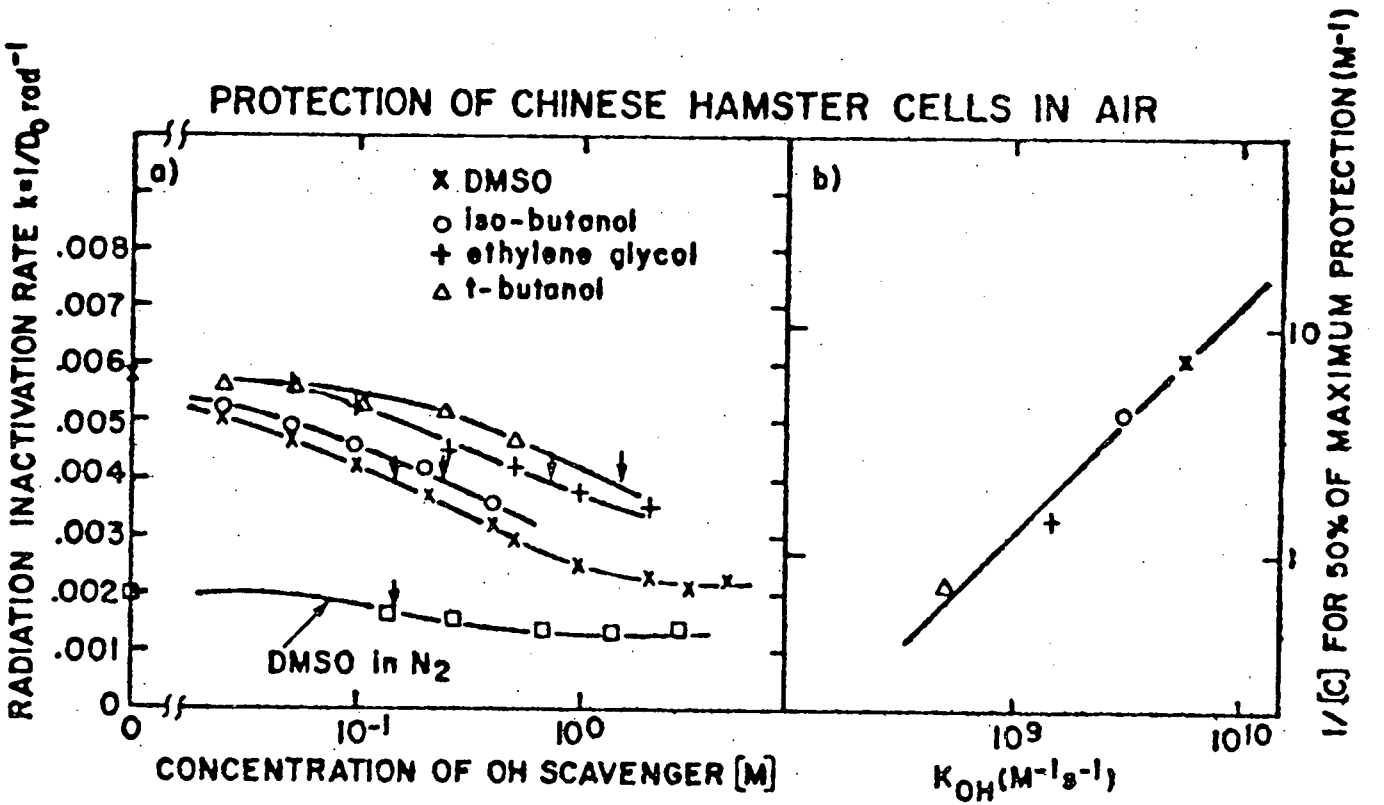
Reaction rates with H and e^- in aqueous solutions, liters/mole/sec

Figure 9. A re-calculation and comparison of the effects added OH radical scavengers have on the anoxic (Sanner and Pihl 1969) and aerobic (Johansen and Howard-Flanders 1965) radiation sensitivity of E. coli. More recent values of the scavengers' reaction rate constants with OH radicals were used (Ross and Ross 1977) for this comparison, and some of the data points are shifted from their original sites (cf. Figures 7 and 8). More recent values for e^-_{aq} scavenging (Anbar et al. 1973) were not sufficiently different from those originally used to warrent our replotting those data. As the text explains, the aerobic test with NO was omitted from this comparison.



E. COLI (ANOXIC): 1--ACETONE 2--GLYCYLGLYCINE 3--ADENINE 4--THYMINE 5--CYTOSINE 6--METHANOL 7--GLUCOSE 8--GLYCEROL 9--FORMATE 10--ETHANOL 11--CYSTEAMINE. E. COLI (AEROBIC): 12--METHANOL 13--ETHANOL 14--MERCAPTOETHANOL 15--NO_2^-

Figure 10. a) The chemical radioprotection by various ·OH scavengers of the multi-target inactivation rate of air-saturated Chinese hamster cells; b) The correlation between the reciprocal of protector concentration effecting 50% of the maximum radioprotection and the absolute rate constants of ·OH with the specific chemical protector (Chapman et al. 1975).


PROTECTION OF CHINESE HAMSTER CELLS IN AIR
a)
b)
x DMSO
o iso-butanol
+ ethylene glycol
△ t-butanol
DMSO in N2
RADIATION INACTIVATION RATE k=1/D0 rad-1
.008
.007
.006
.005
.004
.003
.002
.001
0
10-1
100
CONCENTRATION OF OH SCAVENGER [M]
109
1010
KOH(M-1s-1)
10
1/[C] FOR 50% OF MAXIMUM PROTECTION (M-1)