Br. J. Cancer (1987), **55**, Suppl. VIII, 87–95

Role of superoxide dismutase in modification of radiation injury

A. Petkau

Medical Biophysics Branch, Atomic Energy of Canada Limited Research Company, Whiteshell Nuclear Research Establishment, Pinawa, Manitoba, Canada R0E 1L0

Summary The effects of superoxide dismutase on radiobiological end-points are discussed in terms of the time scale of primary radiation chemical reactions and later cellular and physiological processes. The effectiveness of SOD on responsive subpopulations of bone marrow progenitor cells is shown to be temperature- and dose-dependent. Under certain conditions, the protective actions of superoxide dismutase and catalase compliment each other in a sequential fashion. Finally, cellular levels of endogenous superoxide dismutase in atomic radiation workers are compared with those in a control population.

Since the initial demonstration that superoxide dismutase (SOD) inhibited the toxicity of superoxide radicals in biological systems (Lavelle *et al.*, 1973), the enzyme has been found to modify radiation damage in a variety of systems (Figure 1). These include enzymes (Armstrong *et al.*, 1978; Hexum *et al.*, 1979; Jozwiak *et al.*, 1981), various membrane structures (Bartosz *et al.*, 1977; Ogura *et al.*, 1981; Petkau *et al.*, 1976; Petkau, 1980; Stone *et al.*, 1978), mitochondria (Jozwiak *et al.*, 1981), DNA (Grabowska *et al.*, 1982), pleuropneumonia-like organisms (PPLO) (Petkau *et al.*, 1974) and a variety of mammalian cells, including primary cultured calf myoblasts (Michelson *et al.*, 1974), Chinese hamster lung cells (Goodchild *et al.*, 1981), C3H 10T1/2 fibroblasts (Miller *et al.*, 1982), and the murine haematopoietic system (Petkau *et al.*, 1978; Petkau, 1978; Petkau *et al.*, 1984). As well, intravenous doses of SOD have been found to improve the 30-day survival of irradiated mice (Petkau, 1978) and significantly decreased the lifetime incidence of X-ray-induced leukaemia in such animals (Petkau, 1985).

In man, SOD has been found to inhibit a clastogenic factor, present in sera of subjects irradiated during industrial accidents, that otherwise produces chromosomal aberrations (Emerit *et al.*, 1980). This anti-clastogenic effect of SOD on irradiated human chromosomes has also been repeatedly demonstrated *in vitro*, especially with lymphocytes in G_0 or G_2 of the cell cycle (Nordenson *et al.*, 1976; Nordenson, 1978). In this cellular system, catalase usually produces an additive protective effect.

Clinically, SOD has been used to ameliorate side effects in patients subjected to radiation therapy (RT) for tumours (Figure 1). Thus, 4–8 mg of SOD, given systemically 15–30 min after the completion of each daily irradiation, significantly reduced the morbidity associated with the treatment (Edsmyr *et al.*, 1976; Menander-Huber, 1980; Edsmyr, 1982). As well, tissue necrosis and myelosuppression have been found to respond to SOD, given months after the course of radiation therapy was completed (Emerit *et al.*, 1981; Villasor, 1982). These benefits would seem to be

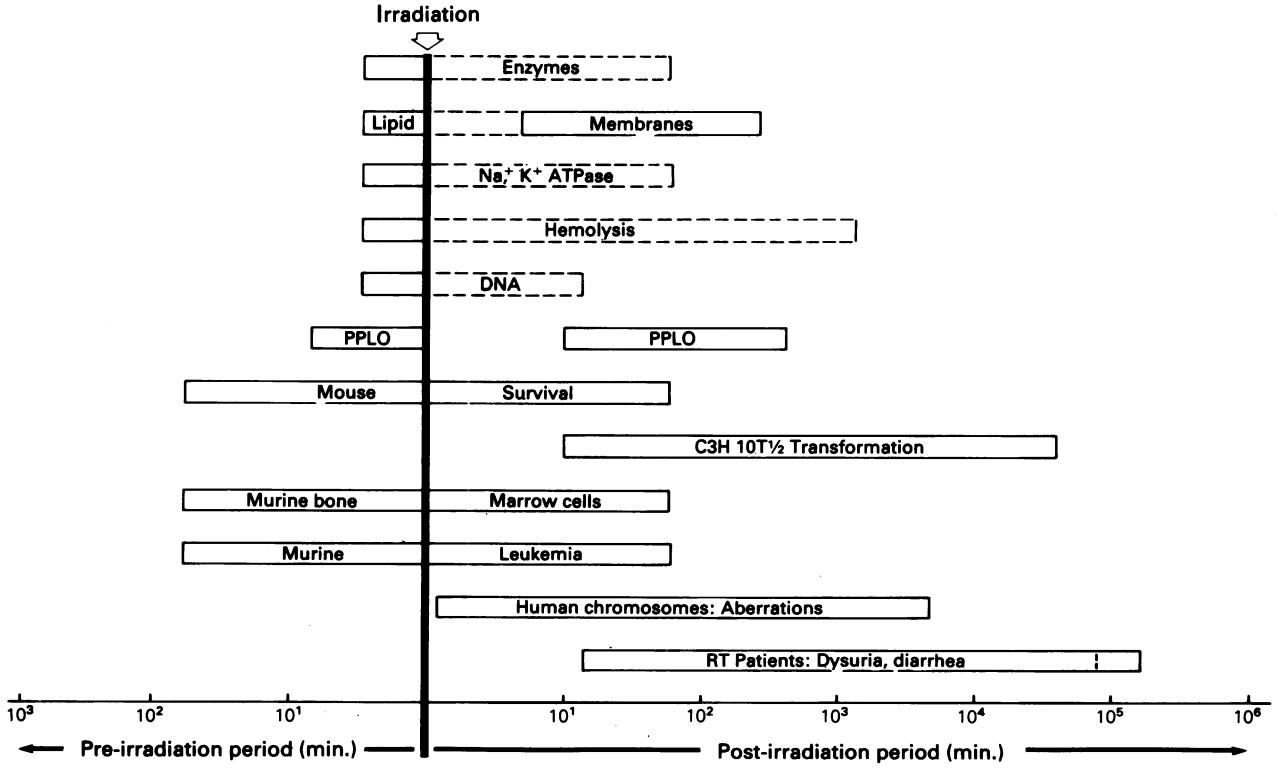

Figure 1 Time scale of radiation protection by superoxide dismutase (SOD) in the pre- and post-irradiation periods for various radiobiological systems or endpoints. Abbreviations used: Pleuropneumonia-like organisms (PPLO); Radiation therapy (RT).

directly relevant to the management of patients over-exposed to ionizing radiation in the aftermath of the nuclear reactor accident at Chernobyl.

The time during which radioprotection with SOD occurred for the various radiobiological endpoints, briefly summarized above, is given in Figure 1, where the logarithmic time scale increases to the left (pre-irradiation period) and to the right (post-irradiation period) of the irradiation, indicated by the heavy vertical line. In the figure, the portion of the horizontal bars, given by the solid lines, indicate when exogenous SOD was introduced into the system, while the portions given by the broken lines indicate the extended periods during which the enzyme may have acted under the conditions of the particular experiments. For some radiobiological endpoints, such as survival of PPLO, malignant transformation of C3H 10T1/2 fibroblasts, human chromosomal aberrations and amelioration of side effects in radiation therapy patients, protection by SOD is clearly identifiable with its action in the post-irradiation period. This period varies from 72 h for inhibition of human chromosomal aberrations to 4 months for amelioration of late radiation side effects (Figure 1). The extent of this period is noteworthy for its implications concerning the underlying processes involved.

Role of radiation chemical species

The protection efficiency of exogenous SOD in irradiated mammalian cells has been estimated at 172 ion pairs/molecule of enzyme (Petkau *et al.*, 1982), a value too high to be attributed to a selective interaction with ionizing radiation during its physical deposition. Furthermore, a 0.1% increase in exogenous SOD, relative to the $\sim 0.8\,\mu mol\,l^{-1}$ of endogenous SOD (Petkau *et al.*, 1978), provided significant radioprotection. Therefore, it is concluded that the enzyme does not inhibit damage by a physical mechanism.

A radiation chemical mechanism of SOD action derives from the fact that irradiation of water in cells creates primary radiation chemical species that either react directly with cellular constituents or, if not, generate secondary species that do (Figure 2). However, the reactions in this scheme that might cause SOD-inhibitable damage in the post-irradiation period appear limited. Thus, oxygen fixation of secondary radicals, created by reactions of the hydroxyl radical ($^{\cdot}OH$) with cellular constituents, has not been shown to be affected by SOD. On the other hand, dismutation of superoxide radicals ($O_2^{\cdot -}$) by SOD may facilitate formation of secondary $^{\cdot}OH$ and thus promote cellular damage that is fixed by oxygen. Alternatively, through interaction with H_2O_2, SOD may catalyze peroxidation of cellular constituents, as has been shown with ferrocytochrome c and linoleic acid (Hodgson *et al.*, 1975). Either process could account for the loss of protection associated with excess SOD, as in the case of Trisomy 21 (Sinet, 1982) and irradiated bone marrow stem cells *in vivo* (Petkau *et al.*, 1975). In normally functioning living cells, however, the potential for SOD to catalyze peroxidation reactions or to facilitate the formation of secondary $^{\cdot}OH$ radicals is probably low because of the presence of catalase, myeloperoxidase and glutathione peroxidase (GSH-Px) to ensure that H_2O_2 levels are controlled. In this regard, it would be of interest to obtain evidence for the juxtaposition of SOD, catalase and GSH-Px in cells and for cooperativity between these anti-oxidant enzymes on the basis of their structural relationship to one another.

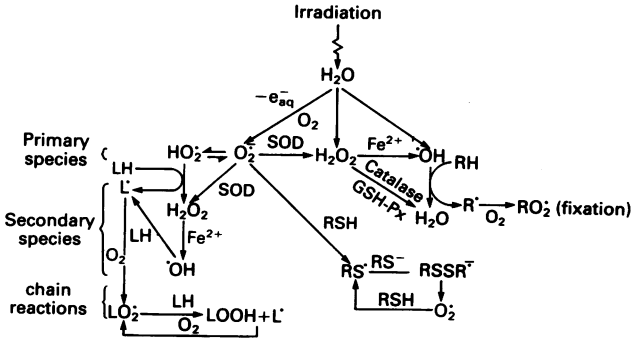

Figure 2 Relationships between primary radiation chemical species, secondary free radical species and chain reactions affecting cellular proteins (RSH) and phospholipids (LH). Symbols and abbreviations defined in text.

Another possibility for SOD to inhibit radiation damage in cells involves oxidation of -SH groups by $O_2^{\cdot -}$, as shown in Figure 2 (Armstrong *et al.*, 1978; Hexum *et al.*, 1979). The $O_2^{\cdot -}$ elaborated by reaction of oxygen with the disulphide radical anion ($RSSR^{\cdot -}$) may continue the chain reaction or react with lipids (vide infra), thus extending the scope of free radical mediated damage. Also, oxidation of -SH groups to thiyl radicals ($RS^{\cdot}$) by $O_2^{\cdot -}$ may result in the formation of H_2O_2 as follows (Asada *et al.*, 1976):

$$RSH + O_2^{\cdot -} \rightarrow RS^{\cdot} + H_2O_2$$

Published yields suggest, however, that chain oxidation of -SH groups in amino acids and proteins is of short duration and subject to chemical repair (Sutherland *et al.*, 1968; Kollmann *et al.*, 1969). The oxidation process is also inhibited by SOD (Armstrong *et al.*, 1978; Petkau *et al.*, 1974). Therefore, this mechanism is unlikely to extend radiation damage over the full post-irradiation time scale depicted in Figure 1.

Progression of radiation damage in cells may involve lipid peroxidation. As shown in Figure 2, peroxidation of lipids (LH) is initiated by $^{\cdot}OH$ and $HO_2^{\cdot}$, the protonated form of $O_2^{\cdot -}$ (pK = 4.69; Bielski, 1978). Formation, isomerization and oxygen fixation of the pentadienyl radical ($L^{\cdot}$) results in production of diene conjugated peroxy radicals ($LO_2^{\cdot}$) that, on reaction with neat lipids, form hydroperoxides (LOOH) and the pentadienyl radical ($L^{\cdot}$). The latter species then propagates the lipid peroxidation process via the $L^{\cdot} \rightarrow LO_2^{\cdot} \rightarrow LOOH \rightarrow L^{\cdot}$ loop, as shown in Figure 2. This chain process may in principle be long with no apparent potential for SOD to inhibit it. Alternatively, the hydroperoxides may undergo branching reactions, resulting in the release of alkoxy radicals ($LO^{\cdot}$) and/or $^{\cdot}OH$, two species that oxidize LH to $L^{\cdot}$. The alkoxy radical may also be formed by $O_2^{\cdot -}$ reacting with LOOH (Sutherland *et al.*, 1982; Gutteridge *et al.*, 1984; Kellogg *et al.*, 1975). Thus, by scavenging $O_2^{\cdot -}$, SOD is expected to inhibit the branching portion of the lipid peroxidation process, resulting in less lipid alcohol (LOH) being formed (Petkau, 1986). As well, the enzyme should decrease the amount of $LO^{\cdot}$ decomposition products (hydrocarbon gases) expired by living animals in which lipid peroxidation is activated (Dumelin *et al.*, 1977; Dillard *et al.*, 1977). Therefore, the effect of SOD and other anti-oxidants on expiration of hydrocarbons gases such as ethane, pentane, etc. by irradiated animals should be tested as it has been for ozone (Dumelin *et al.*, 1978). Such data might delineate the length of the post-irradiation period in which the peroxidative index was elevated above the normal level in the test animals while concurrently defining in a functional way the period in which exogenous SOD has a beneficial effect. In relationship to Figure 1, such information may have interpretative value, particularly for the more complex radiobiological endpoints such as cell transformation, chromosomal aberrations and tissue damage.

By virtue of the mechanisms and duration of lipid peroxidation, this process seems most likely to progress the furthest into the post-irradiation period. Moreover, because of its radiomimetic nature toward enzymes (Tappel, 1975), it may

damage functional proteins well after direct and indirect radiation chemical reactions have run their course and affected them. Thus, in contrast to their separation in Figure 2, lipid peroxidation and protein inactivation are intertwined, especially in the post-irradiation period. This may partially explain why minimum SOD levels in irradiated tissues occur several days after acute radiation exposures (Petkau, 1978). However, the period of decline in tissue SOD levels falls well short of the times in the post-irradiation period where exogenous SOD gave therapeutic benefit. Thus, the optimum therapeutic use of exogenous SOD is not simply a matter of matching its administration with the decline in endogenous enzyme and needs to be better defined.

Radioprotection by SOD of subpopulation of cells

Previously, it was shown that SOD protected murine bone marrow cells irradiated *in vivo* to 3.5 Gy (Petkau *et al.*, 1975). More recent data on cells suspended in phosphate buffered saline (PBS) suggest that the *in vivo* result may be due to protection of subpopulations of bone marrow progenitor cells (Figure 3). The X-ray sensitive subpopulation of murine bone marrow macrophage progenitor cells was shown to have a control D_0 of 0.96 ± 0.3 Gy (Figure 3a, inset) over the 0 to 0.5 Gy dose range, with no shoulder (Petkau *et al.*, 1984). SOD, when present during the X-ray treatment, increased the D_0 to 2.68 ± 0.59 Gy, giving a dose modifying factor (DMF) of 2.8 ± 0.7 (Petkau *et al.*, 1984). Catalase and the apo-form of SOD did not protect, indicating that the sensitive subpopulation was inactivated by $O_2^{\cdot -}$ *per se*. A similar conclusion has recently been drawn regarding oxygen toxicity in yeast mutants, deficient in SOD and catalase (Bilinski *et al.*, 1985). The DMF of 2.8 ± 0.7 suggests that SOD was able to neutralize most of the radiation sensitizing effect of oxygen on the sensitive subpopulation. Further, the DMF varied with the length of time that the cells were incubated with SOD, being 1.3 ± 0.3 when SOD was added to the cells immediately before irradiation, and increasing to 2.8 ± 0.7 when a 40 min incubation was allowed. The greater effectiveness of SOD following a 40 min incubation is consistent with cellular uptake of the enzyme as previously demonstrated (Petkau *et al.*, 1982).

For intracellular uptake of exogenous SOD to occur, the enzyme must first partition in and out of the plasma membrane. In the process, it can interact with phospholipids, disordering the lipid packing as determined on liposomes by spin labelling and differential calorimetry measurements (Lepock *et al.*, 1981). This interaction may explain why in SOD-treated bone marrow progenitor cells there was no inflection at 23°C in the plot of cell survival at 0.3 Gy (S/So) *versus* 1/T, the reciprocal of the temperature, (Figure 3a). The motion of proteins in membranes is known to be affected by phase transitions in the 20–25°C range, depending on the chemical composition of the membranes (Hidalgo, 1985; Quinn, 1981). Further, the slope of the line of S/So *vs* 1/T for the SOD-treated cells corresponds to an inactivation energy of $\sim 13\,kJ\,mol^{-1}$, well below the $41.8\,kJ\,mol^{-1}$ required for conversion of $O_2^{\cdot -}$ to $^{\cdot}OH$ by the reaction (Fielden *et al.*, 1974):

$$O_2^{\cdot -} + H_2O_2 \rightarrow {}^{\cdot}OH + OH^- + O_2$$

In the absence of exogenous SOD, the inactivation energy is even lower. Thus, it is concluded that the foregoing reaction, by which $O_2^{\cdot -}$ and H_2O_2 might be converted to $^{\cdot}OH$ as the proximal lethal radical, does not account for the inactivation

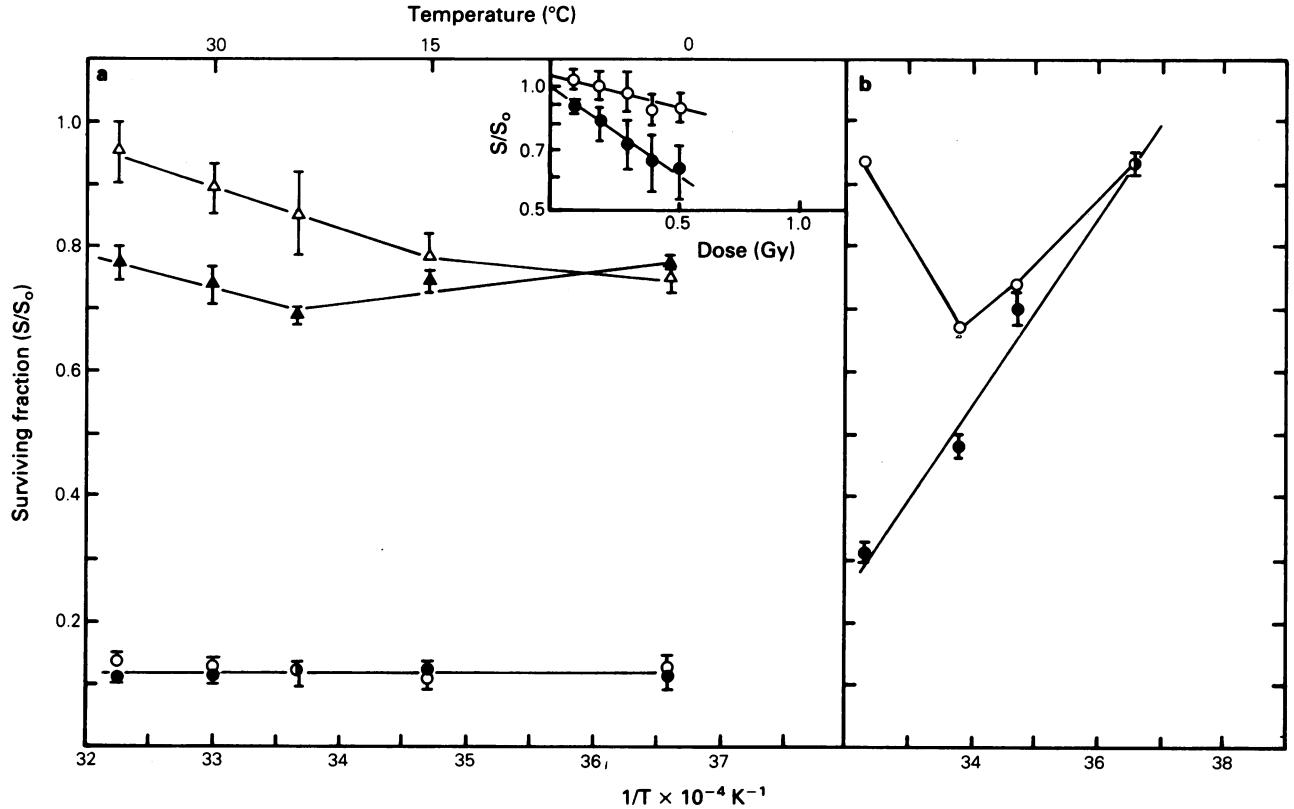

Figure 3 Panel (a) Surviving fractions (S/So) of macrophage progenitor cells from mouse bone marrow *versus* the reciprocal of the temperature (1/T) at which the cells were X-irradiated to 0.3 Gy (▲, △) or 3.0 (●, ○) in the absence (▲, ●) and presence of a 40 min treatment with $100\,\mu g\,ml^{-1}$ of SOD (△, ○). Inset: S/So *versus* X-ray dose in the absence (●) and presence of $100\,\mu g\,ml^{-1}$ SOD.

Panel (b) Depdendence on temperature of S/So, the photochemically defined surviving fraction of macrophage progenitor cells from mouse bone marrow in PBS after 10 min illumination in the absence (●) and presence (○) of $100\,\mu g\,ml^{-1}$ SOD from human red blood cells. In both panels, error bars represent $\pm$ 1 s.d.

in PBS of the sensitive subpopulation of macrophage progenitor cells in mouse bone marrow. At 3 Gy, SOD did not protect what was left (~12%) of the macrophage progenitor cells, nor was their response to X-rays dependent on the temperature (Figure 3a). Thus, in this *in vitro* system, SOD is only effective as a radioprotector at doses ≦0.5 Gy and on cells whose radiation sensitivity depends on the temperature in a manner not inconsistent with metabolic repair processes. Perhaps cooperativity exists between the protective action of SOD and radiation repair of the type that does not produce a shoulder in the survival curve (Chelack *et al.*, 1983). A study of the effect of tumour promoters on single strand breaks in the DNA of human neutrophils suggests that such lesions, when inflicted by $O_2^{\cdot -}$, are inhibited by SOD (Birnboim, 1986). This finding suggests that defining the role of SOD in DNA repair may be a fruitful area of research, especially when focussed on superoxide-sensitive subpopulations of cells. However, recent work indicates that regulation of Mn-SOD in *E. coli* occurs independently of the inducible DNA repair system (Hancock *et al.*, 1985).

Identification of superoxide-sensitive subpopulations of cells may also be done photochemically using reduced riboflavin (Petkau *et al.*, 1983). Figure 3b illustrates the temperature-dependent inactivation that occurs when macrophage progenitor cells from mouse bone marrow are exposed for 10 min to the photochemical source of $O_2^{\cdot -}$ in the absence and presence of human cupro, zinc-SOD (100 $\mu g\,ml^{-1}$). The linear decrease in survival from 95% at 0°C to 33% at 37°C in the absence of SOD corresponds to an inactivation energy of 27 $kJ\,mol^{-1}$. This value again is well below the 41.8 $kJ\,mol^{-1}$ required for $^{\cdot}OH$ formation from $O_2^{\cdot -}$ and H_2O_2 (*vide supra*). Therefore, this conversion is ostensibly not the underlying reaction of the inactivation process, a conclusion consistent with the observation that catalase, when present during the illumination period, is without effect (Petkau *et al.*, 1983). On the other hand, SOD, when present during the illumination period, protects 94% of the cells at 37°C but less well at intermediate temperatures above 0°C. At 0°C, SOD does not protect at all (Figure 3b). Between 0°C and 23°C, SOD reduced the inactivation energy to ~18 $kJ\,mol^{-1}$, in close agreement with ~19.2 $kJ\,mol^{-1}$ calculated for the process by which cupric ions in SOD are oxidized by $O_2^{\cdot -}$ in aqueous solution (Fielden *et al.*, 1974). The change in slope of the Arrhenius-type plot above 23°C, caused by the increased protection by SOD, again suggests a temperature-dependent transition in the interaction of the enzyme with the cells. This change in interaction of the enzyme with cells probably also involves the plasma membranes (*vide supra*). Studies of the interaction of SOD with cell membranes as a function of temperature are needed.

Although SOD protects the macrophage progenitor cells while superoxide radicals are generated photochemically, the enzyme is totally ineffective after 30 min into the post-illumination period (data not shown). By contrast, catalase is not protective during the illumination period (Petkau *et al.*, 1983) but increases cell survival later as shown in Figure 4. This figure demonstrates the long-term toxicity resulting from a 10 min illumination of the photochemical reagents (24 $\mu mol\,l^{-1}$ riboflavin and 10 $mmol\,l^{-1}$ methionine) in PBS. After the illumination, the macrophage progenitor cells were added at various times and incubated at room temperature for 10 min. The cell survival fraction (S/So), measured in terms of the ability to form macrophage colonies, increased from 30 to 70% at 0.1 and 100 h, post-illumination time, respectively. When catalase at 200 $\mu g\,ml^{-1}$ was added immediately after illumination had ceased, it also increased the surviving fraction from 30 to 70%. However, catalase added 24 h later was ineffective, leaving a residual longer term toxicity that inactivated ~30% of the cells. Thus, in this system, the cellular toxicity of $O_2^{\cdot -}$ and H_2O_2 occur separately but in the sequence given. This sequential

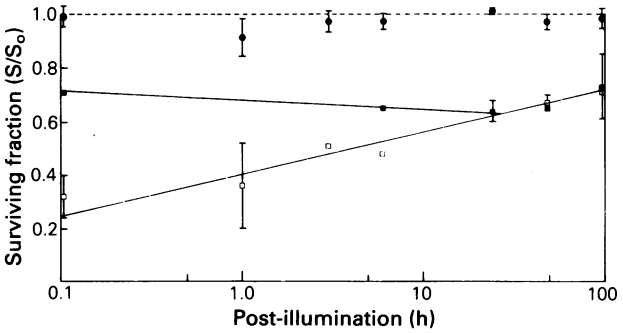

Figure 4 Long-term persistence of post-illumination toxicity of the photochemical generator of $O_2^{\cdot -}$ to macrophage progenitor cells from mouse bone marrow. Photochemical reagents in PBS were illuminated for 10 min after which the progenitor cells were added at various times and incubated at room temperature for 10 min. Legend: Cells in PBS in the dark (---); PBS plus 24 $\mu mol\,l^{-1}$ riboflavin and 10 $mmol\,l^{-1}$ methionine in the dark (●), post-illumination toxicity in the absence (□) and presence of 200 $\mu g\,ml^{-1}$ catalase (■), added to the photochemical generator after the 10 min illumination period but 10 min before the cells were added.

separation of their toxicities is consistent with an earlier conclusion, namely, that secondary $^{\cdot}OH$ from $O_2^{\cdot -}$ and H_2O_2 is not the putative radical that inactivates the superoxide-sensitive, X-ray-sensitive subpopulation of macrophage progenitor cells in mouse bone marrow.

The nature of the residual toxicity, lasting longer than 24 h (Figure 4), has not been identified. A likely agent is methional, the decarboxylation, deamination product of the methionine radical formed during the photoreduction of riboflavin (Petkau *et al.*, 1986). Another possible agent is singlet oxygen, formed via photosystem 2 by which methionine is transformed to methionine sulfoxide (Yang *et al.*, 1967; Spikes *et al.*, 1978). Methionine sulfoxide in solution at the expected concentration is not toxic to the macrophage progenitor cells (Petkau *et al.*, 1986). However, the situation may be different when singlet oxygen or N-chloramines react(s) with incorporated methionine to form methionine sulfoxide residues in proteins. Recent studies have shown that such oxidative attacks on methionine residues in functional peptides leads to their inactivation (Clark, 1982; Weiss *et al.*, 1983). As well, it has been proposed that methionine residues in nascent polypeptides are more susceptible to oxidation than in completed proteins (Fliss *et al.*, 1983). This property may partly explain the unusual sensitivity of subpopulations of bone marrow progenitor cells whose progress through the differentiation and maturation pathways implies the presence of nascent polypeptides and proteins at different stages. More research on the extent and sequelae of oxidation of methionine in nascent protein is needed and may be facilitated by current efforts to identify and separate subpopulations of cells in which they are most likely present.

Role of endogenous SOD at low radiation doses

As discussed in the previous section, exogenous SOD can modify radiation injury in subpopulations of superoxide-sensitive cells when exposed to relatively low doses (≦0.5 Gy). Endogenous SOD in mouse bone marrow cells, although ostensibly much less efficient in detoxifying radiation-induced $O_2^{\cdot -}$, also improves cell survival, increasing the D_0 value in the proportion of 1 μSv/molecule of enzyme. (Petkau *et al.*, 1982). This observation prompted a study in which endogenous SOD activity in peripheral blood leucocytes of volunteer blood donors and atomic radiation workers was compared. The volunteer blood donors lived in Winnipeg, MB, whereas the atomic radiation workers were

drawn from the Whiteshell Nuclear Research Establishment, Pinawa, MB. The table gives the mean total SOD activity/leucocyte in the volunteer blood donor groups according to sex and age. No differences between groups are noted, consistent with other reports (Saito *et al.*, 1982; Vanella *et al.*, 1982). The corresponding data for the atomic radiation workers (males only) are given in Figure 5, plotted as a function of the occupational radiation dose as measured by externally worn lithium fluoride dosimeters over a 6-month period prior to the blood samplings. The data suggest a trend toward higher levels of SOD activity as a function of the occupational dose. In fact, 25% (30/119) of the entries exceed the upper range limit of 0.23×10^{-6} units/cell, as defined by the mean $+\sigma$ (Table). Only 4% (5/119) of the entries fall below the mean $-\sigma$ (Table). Thus, the data are skewed to higher values, particularly at the upper occupational doses. However, among many of the 119 cases there is no apparent change in SOD activity with occupational dose. The question then arises as to why leucocytes in a subpopulation of atomic radiation workers are higher than expected. Clearly, this matter requires further study.

The elevation in SOD activity with occupational radiation exposure appears as an appropriate response in terms of radiation protection, the free radical theory of aging (Harman, 1981) and the correlation between life span potential and tissue SOD levels (Tolmasoff *et al.*, 1980). This appropriate response may be a contributing factor to the increased life span of workers associated with the nuclear industry. The question is why is SOD elevated in the cells of some individuals? Several possibilities are suggested: First, occupational doses may raise the peroxidative activity in tissues. Second, peripheral blood leucocytes may retain memory, in molecular terms, of their earlier states as radiation-sensitive progenitor cells in which activation of the SOD gene(s) may have occurred in response to radiation exposure. Third, cell selection could account for the elevated SOD activity in leucocytes of some atomic radiation workers. Fourth, the higher SOD activity in leucocytes from some atomic radiation workers is simply coincidental with the higher occupational doses. An epidemiological study, that might settle this matter, could be launched using sensitive immunoassays. All four possibilities provide interesting research opportunities.

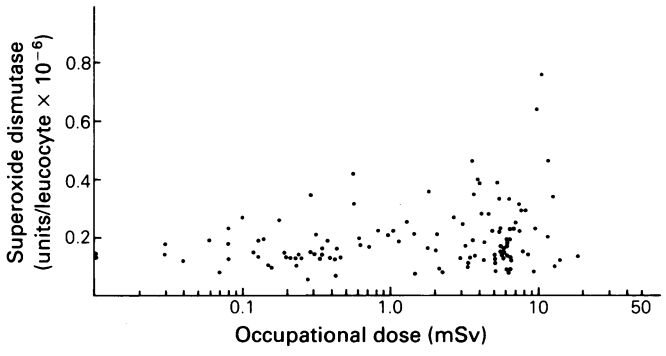

Figure 5 Total SOD activity per leucocyte in the peripheral blood of 119 atomic radiation workers *versus* the integrated dose accumulated by them individually over a 6-month period immediately prior to the blood samplings. Doses were determined by lithium fluoride dosimetry (courtesy H.M. Johnson).

Table Superoxide dismutase activity in peripheral blood leucocytes from adult volunteer blood donors

	Superoxide dismutase/ leucocyte ($\times 10^{-6}$ *units/cell*)			
	Male		*Female*	
Age (*y*)	*n*	*Mean* $\pm\sigma$	*n*	*Mean* $\pm\sigma$
<20	5	0.16±0.09	4	0.14±0.07
20–29	25	0.17±0.08	16	0.17±0.06
30–39	24	0.16±0.08	8	0.18±0.10
40–49	25	0.15±0.06	9	0.21±0.09
50–59	16	0.19±0.08	3	0.15±0.06
60–65	9	0.14±0.06	4	0.18±0.07
All ages	104	0.16±0.07	44	0.17±0.08

Concluding remarks

In summary, the use of SOD as a radioprotective agent has highlighted the fact that radiation damage progresses during the post-irradiation period, long after the primary and secondary radiation chemical species have disappeared. Radiation-induced chain reactions of lipids, and to a lesser extent proteins, seem to be involved but their lifetime may be less than the period during which SOD has been shown to be effective, especially on cells and therapeutically in man (Figure 1). This suggests that other mechanisms of long-term toxicity in the post-irradiation period must be sought. Evidence for the involvement of compounds such as H_2O_2, methional, methionine sulfoxide residues in polypeptides, and N-chloramines has been discussed. Their possible role may be potentiated by activation of the respiratory burst in phagocytic cells (Clark, 1982; Fliss *et al.*, 1983; Weiss *et al.*, 1983). Thus, phagocytosis of cellular debris may trigger a positive feedback mechanism by which radiation damage progresses. SOD is expected to intervene in such a process, acting as an anti-inflammatory agent at the same time (Weiss *et al.*, 1983). Thus, it is not surprising that SOD ameliorates radiation-induced inflammatory lesions in radiation therapy patients (Edsmyr *et al.*, 1976; Menander-Huber, 1980; Edsmyr, 1982).

Not all cells are radioprotected by SOD and it behooves researchers to identify the subpopulations that are. As I have attempted to show, this may require the application of small doses, characteristically in the shoulder region of typical cell survival curves. As well, alternative sources of $O_2^{\cdot -}$ can lead to useful data (Figure 3b).

The fact that SOD appears to be an effective radioprotector of responsive subpopulations, only at low doses, does not necessarily mean that its use is without benefit at higher radiation doses. The cellular effect obtained may be amplified biologically in living systems. Thus, intravenous doses of SOD to X-irradiated mice increased the $LD_{50(30)}$ by a factor of 1.56±0.04 (Petkau, 1978). Hematopoietic death was, therefore, significantly inhibited by SOD. Clinically, bone marrow depression in radiation therapy patients has also been treated with SOD (Villasor, 1982). These results suggest that the use of SOD in the management of over-exposed atomic radiation workers should be considered.

References

ARMSTRONG, D.A. & BUCHANAN, J.D. (1978). Reactions of $O_2^{\cdot -}$, H_2O_2 and other oxidants with sulfhydryl enzymes. *Photochem. Photobiol.*, **28**, 743.

ASADA, K. & KANEMATSU, S. (1976). Reactivity of thiols with superoxide radicals. *Agr. Biol. Chem.*, **40**, 1891.

BARTOSZ, G., FRIED, R., GRZELINSKA, E. & LEYKO, W. (1977). Effect of hyperoxide radicals on bovine-erythrocyte membrane. *Eur. J. Biochem.*, **73,** 261.

BIELSKI, B.H.J. (1978). Reevaluation of the spectral and kinetic properties of HO_2 and O_2^- free radicals. *Photochem. Photobiol.*, **28,** 645.

BILINSKI, T., KRAWIEC, Z., LICZMANSKI, A. & LITWINSKA, J. (1985). Is hydroxyl radical generated by the Fenton reaction *in vivo*? *Biochem. Biophys. Res. Commun.*, **130,** 533.

BIRNBOIM, H.C. (1986). Mechanisms of superoxide anion-induced DNA strand breakage in tumor promoter-treated human neutrophils. *Proc. AACR*, **27,** 146. (*Abstract*).

CHELACK, W.S. & PETKAU, A. (1983). Identifying superoxide-sensitive progenitor cells in mouse bone marrow. *Int. J. Radiat. Biol.*, **44,** 523.

CLARK, R.A. (1982). Chemotactic factors trigger their own oxidative inactivation by human neutrophils. *J. Immunol.*, **129,** 2725.

DILLARD, C.J., DUMELIN, E.E. & TAPPEL, A.L. (1977). Effect of dietary Vitamin E on expiration of pentane and ethane by the rat. *Lipids*, **12,** 109.

DUMELIN, E.E., DILLARD, C.J., TAPPEL, A.L. (1978). Effect of Vitamin E and ozone on pentane and ethane expired by rats. *Arch. Envir. Health*, **33,** 129.

DUMELIN, E.E. & TAPPEL, A.L. (1977). Hydrocarbon gases produced during *in vitro* peroxidation of polyunsaturated fatty acids and decomposition of preformed hydroperoxides. *Lipids*, **12,** 894.

EDSMYR, F. (1982). Superoxide dismutase efficacy in ameliorating side effects of radiation therapy: Double-blind, placebo-controlled trials in patients with bladder and prostate tumors. In *Pathology of Oxygen*, Autor, A.P. (ed) p. 315. Academic Press: New York.

EDSMYR, F., HUBER, W. & MENANDER, K.B. (1976). Orgotein efficacy in ameliorating side effects due to radiation therapy. 1. Double-blind, placebo-controlled trial in patients with bladder tumors. *Curr. Therapeut. Res.*, **19,** 198.

EMERIT, J., LOEPER, J. & CHOMETTE, G. (1981). Superoxide dismutase in the treatment of post-radiotherapeutic necrosis and of Crohn's disease. *Bull. europ. Physiopath. resp.*, **17,** 287.

EMERIT, I. & MICHELSON, A.M. (1980). Chromosome instability in human and murine autoimmune disease: anticlastogenic effect of superoxide dismutase. *Acta Physiol. Scand.* (Suppl.), **492,** 59.

FIELDEN, E.M., ROBERTS, P.B., BRAY, R.C., LOWE, D.J., MAUTNER, G.N., ROTILIO, G. & CALABRESE, L. (1974). The mechanism of action of superoxide dismutase from pulse radiolysis and electron paramagnetic resonance. *Biochem. J.*, **139,** 49.

FLISS, H., WEISSBACH, H. & BROT, N. (1983). Oxidation of methionine residues in proteins of activated neutrophils. *Proc. Natl Acad. Sci. USA*, **80,** 7160.

GOODCHILD, N.T., KWOCK, L., LIN, P.S. & CIBOROWSKI, L. (1981). Effect of superoxide anion (O_2^-) on the survival of Chinese hamster cells. In *Oxygen and Oxy-Radicals in Chemistry and Biology* Rodgers *et al.* (eds) p. 649. Academic Press: New York.

GRABOWSKA, B., WISZKOWSKA, H. & BIALEK, H. (1982). Influence of ionizing radiation on DNA molecules in the presence of radioprotectors. I. Investigations of postradiation changes in DNA structure by means of the spin-label and spectrophotometric methods. *Studia Biophys.*, **89,** 221.

GUTTERIDGE, J.M.C., HILL, C. & BLAKE, D.R. (1984). Copper stimulated phospholipid membrane peroxidation: antioxidant activity of serum and synovial fluid from patients with rheumatoid arthritis. *Clin. Chim. Acta* **139,** 85.

HANCOCK, L.C. & HASSAN, H.M. (1985). Regulation of the manganese-containing superoxide dismutase is independent of the inducible DNA repair system in Escherichia coli *J. Biol. Chem.*, **260,** 12954.

HARMAN, D. (1981). The aging process. *Proc. Natl Acad. Sci. USA*, **78,** 7124.

HEXUM, T.D. & FRIED, R. (1979). Effects of superoxide radicals on transport (Na+K+) adenosine triphosphatase and protection by superoxide dismutase. *Neurochem. Res.*, **4,** 73.

HIDALGO, C. (1985). Membrane fluidity and the function of the Ca^{2+}-ATPase of sarcoplasmic reticulum. In *Membrane Fluidity in Biology*, **4,** Aloia *et al.* (eds), Cellular aspects. p. 51. Academic Press: New York.

HODGSON, E.K. & FRIDOVICH, I. (1975). The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: Chemiluminescence and peroxidation. *Biochemistry*, **14,** 5299.

JOZWIAK, Z. & HELSZER, Z. (1981). Participation of free oxygen radicals in damage of porcine erythrocytes. *Radiat. Res.*, **88,** 11.

KELLOGG, E.W. & FRIDOVICH, I. (1975). Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. *J. Biol. Chem.*, **250,** 8812.

KOLLMANN, G., SHAPIRO, B. & MARTIN, D. (1969). The mechanism of radiation hemolysis in human erythrocytes. **37,** 551.

LAVELLE, F., MICHELSON, A.M. & DIMITRIJEVIC, L. (1973). Biological protection by superoxide dismutase. *Biochem. Biophys. Res. Commun.*, **55,** 350.

LEPOCK, J.R., ARNOLD, L.D., PETKAU, A. & KELLY, K. (1981). Interaction of superoxide dismutase with phospholipid liposomes: An uptake, spin label and calorimetric study. *Biochem. Biophys. Acta*, **649,** 45.

MENANDER-HUBER, K.B. (1980). Double-blind controlled clinical trials in man with bovine copper-zinc superoxide dismutase (Orgotein). In *Biological and Clinical Aspects of Superoxide and Superoxide Dismutase*, Bannister *et al.* (eds) p. 408. Elsevier/North-Holland: New York.

MICHELSON, A.M. & BUCKINGHAM, M.E. (1974). Effects of superoxide radicals on myoblast growth and differentiation. *Biochem. Biophys. Res. Commun.* **58,** 1079.

MILLER, R.C., OSMAK, R., ZIMMERMAN, M. & HALL, E.J. (1982). Sensitizers, protectors and oncogenic transformation *in vitro*. *Int. J. Radiat. Oncol. Biol. Phys.*, **8,** 771.

NORDENSON, I. (1978). Effects of superoxide dismutase and catalase on radiation-induced chromosome aberrations: Dose and cell cycle dependence. *Hereditas*, **89,** 163.

NORDENSON, I., BECKMAN, G. & BECKMAN, L. (1976). The effect of superoxide dismutase and catalase on radiation-induced chromosome breaks. *Hereditas*, **82,** 125.

OGURA, R., MURAKATA, M., SAKATO, T. & CHIBA, R. (1981). Effect of superoxide dismutase on the surface potential disorders of mitochondria treated with ultraviolet light exposed to methyl linoleate. *Kurume Med. J.*, **28,** 1.

PETKAU, A. (1978). Radiation protection by superoxide dismutase. *Photochem. Photobiol.*, **28,** 765.

PETKAU, A. (1980). Radiation carcinogenesis from a membrane perspective. *Acta Physiol.*, Suppl. **492,** 81.

PETKAU, A. (1985). The role of superoxide dismutase in radiation injury. In *Superoxide Dismutase, Pathological States*, **3,** Oberley, L.W. (ed), p. 99. CRC Press, Inc: Boca Raton.

PETKAU, A. (1986). Protection and repair of irradiated membranes. In *Free Radicals, Aging and Degenerative Diseases*, Johnson *et al.* (eds) Alan R. Liss, Inc: New York (in press).

PETKAU, A. & CHELACK, W.S. (1974). Protection of Acholeplasma laidlawii B by superoxide dismutase. *Int. J. Radiat. Biol.*, **26,** 421.

PETKAU, A. & CHELACK, W.S. (1976). Radioprotective effect of superoxide dismutase on model phospholipid membranes. *Biochim. Biophys. Acta*, **443,** 445.

PETKAU, A. & CHELACK, W.S. (1983). Protection of superoxide-sensitive bone marrow cells by superoxide dismutase. In *Oxy Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, **2,** Greenwald *et al.* (eds), p. 167. Elsevier Science: New York.

PETKAU, A. & CHELACK, W.S. (1984). Radioprotection by superoxide dismutase of macrophage progenitor cells from mouse bone marrow. *Biochem. Biophys. Res. Commun.*, **119,** 1089.

PETKAU, A. & CHELACK, W.S. (1986). Photochemical inactivation of macrophage progenitor cells in mouse bone marrow. In *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Rotilio, G. (ed) p. 370. Elsevier Science: Amsterdam.

PETKAU, A., CHELACK, W.S., KELLY, K. & FRIESEN, H.G. (1982). Superoxide dismutase and radiosensitivity: Implications for human mammary carcinomas. In *Pathology of Oxygen*, Autor, A.P. (ed), p. 223. Academic Press: New York.

PETKAU, A., CHELACK, W.S. & PLESKACH, S.D. (1978). Protection by superoxide dismutase of white blood cells in X-irradiated mice. *Life Sci.*, **22,** 867.

PETKAU, A., KELLY, K., CHELACK, W.S., PLESKACH, S.D., BAREFOOT, C. & MEEKER, B.E. (1975). Radioprotection of bone marrow stem cells by superoxide dismutase. *Biochem. Biophys. Res. Commun.*, **67,** 1167.

QUINN, P.J. (1981). The fluidity of cell membranes and its regulation. *Prog. Biophys. molec. Biol.*, **38,** 1.

SAITO, T., ITO, K., KURASAKI, M., FUJIMOTO, S., KAJI, H. & SAITO, K. (1982). Determination of true specific activity of superoxide dismutase in human erythrocytes. *Clin. Sci.*, **63,** 251.

SINET, P.M. (1982). Metabolism of oxygen derivatives in Downs syndrome. *Ann. N.Y. Acad. Sci.*, **396,** 83.

SPIKES, J.D. & SWARTZ, H.M. (1978). International conference on singlet oxygen and related species in chemistry and biology. Review and general discussion. *Photochem. Photobiol.*, **28,** 921.

STONE, D., LIN, P.-S. & KWOCK, L. (1978). Radiosensitization of human erythrocytes by diethyldithiocarbamate. *Int. J. Radiat. Biol.*, **33,** 393.

SUTHERLAND, M.W. & GEBICKI, J.M. (1982). A reaction between the superoxide free radical and lipid hydroperoxide in sodium linoleate micelles. *Arch. Biochem. Biophys.*, **214**, 1.

SUTHERLAND, R.M. & PHIL, A. (1968). Repair of radiation damage to erythrocyte membranes: The reduction of radiation-induced disulfide groups. *Radiat. Res.*, **34**, 300.

TAPPEL, A.L. (1975). Lipid peroxidation and fluorescent molecular damage to membranes. In *Pathobiology of Cell Membranes*, Trump *et al.* (eds) p. 145. New York.

TOLMASOFF, J.M., ONO, T., CUTLER, R.G. (1980). Superoxide dismutase: Correlation with lifespan and specific metabolic rate in primate species. *Proc. Natl Acad. Sci. USA*, **77**, 2777.

VANELLA, A., GEREMIA, E., TIRIOLO, P. & 4 others (1982). Superoxide dismutase activity and reduced glutathione content in red blood cells as a function of age. *IRCS Med. Sci. Libr. Compend.* **10**, 235.

VILLASOR, R.P. (1982). Superoxide dismutase treatment of myelosuppression resulting from cancer chemotherapy. In *Pathology of Oxygen*, Autor, A.P. (ed) p. 303. Academic Press: New York.

WEISS, S.J., LAMPERT, M.B. & TEST, S.T. (1983). Long-lived oxidants generated by human neutrophils: Characterization and bioactivity. *Science*, **222**, 625.

YANG, S.F., KU, H.S. & PRATT, H.K. (1967). Photochemical production of ethylene from methionine and its analogues in the presence of flavin mononucleotide. *J. Biol. Chem.*, **242**, 5274.

Discussion

Willson: There seem to be two areas that need discussing: first the chemistry and the biochemistry of superoxide and as to how the enzyme might protect; second the implication that the enzyme may be induced by radiation and how this might affect the various risk factors involved. First, with respect to the so-called 'fixation reaction' you mentioned, the peroxy radical formed in this reaction may in fact react further and these reactions have to be taken into account. For example they may inactivate proteins. We have shown that superoxide dismutase can protect against such reactions. Furthermore if you dialyse the enzyme against cyanide it no longer possesses its protective activity (Gee *et al.*, 1985). Can I say at this point that the term 'fixed' may be very ambiguous to free radical biochemists. To a lot of people 'fixed' means repair – if you fix a car you repair it – but the opposite is meant in radiobiology.

Petkau: Right. Back in 1977 at the first International Conference on Singlet Oxygen and Related Species in Chemistry and Biology, Dr Fielden suggested that superoxide dismutase might have an additional role. That it might in fact react with peroxides.

Willson: With peroxides or peroxy radicals?

Petkau: As I remember it peroxy radicals.

Willson: Armstrong in Canada has also shown that it can protect some enzymes.

Petkau: Well I was looking for that kind of information and am pleased that it is now coming to hand.

Cramp: With SOD and the inhibition of SOD if you look at the literature, there is a mine field, some results swinging one way and some swinging the other. Can you be more specific in identifying the long lived radiation species in cells that interact with SOD after the radiation, exposure both under anoxia as well as under aerobic conditions. Have you had any experience with adding SOD to cells under anoxia? What is the long lived species or is it a secondary process that produces peroxy radicals that react with SOD?

Petkau: Mouse bone marrow cells irradiated under N_2 are not protected by SOD. In the mouse system *in vivo*, we found that hypoxia produced an increase in the endogenous SOD, leading to radiotolerance when irradiated under re-oxygenated condition.

Cramp: Whole mice or cells?

Petkau: The whole animal was put into hypoxia, 4.5% oxygen for two days. This presumably reduced the catabolism of the enzyme, resulting in a sparing effect on superoxide dismutase. Contrary to what I had anticipated we found that the endogenous SOD levels in the mononucleated bone marrow cells were in fact elevated. I was expecting a depression. Likewise, at 8.0% oxygen for two weeks, the levels of endogenous SOD were increased relative to the controls. This allowed me to generate the curve that I showed. The bone marrow cells were extracted, prepared and the radiobiology done under standard oxygenated conditions (Petkau *et al.*, 1982, see References).

Cramp: But what is the long-lived species, which is produced by radiation and interacts with SOD or is it a secondary process which produces the effect?

Petkau: I tend to favour a secondary or tertiary species such as lipid peroxidation products, including malonaldehyde.

Cramp: But we can't find lipid peroxidation products.

Petkau: Look for changes in absorbance at 193–197 nm where OH adducts in anoxia should have a sparing effect. This has been shown to occur in ozone-treated animals (Petkau, 1986). Rarely, is formation of pentadienyl and peroxy radicals measured. The usual practice is to measure LOOH at 232 nm.

Cramp: But what about irradiated animals?

Petkau: There is one study where peroxidation in the post-irradiation period is implicated by the increase in peroxidase – positive glial cells in the brain. These glial cells contain thiol rich peroxisomes that trap and decompose peroxides (Srebo *et al.* (1972), *Radiat. Res.*, **501**, 65). This response, I think, indicates that lipid peroxidation occurred.

Superoxide dismutase given in such a situation leads to a decrease in the expiration of volatile hydrocarbons such as pentane and ethane resulting from peroxidation. Other studies have found increased lipase activity and incorporation of radiolabelled fatty acids into cell membranes after whole body irradiation to 5–8 Gy: repair in the post irradiation period is rapid and over within an hour. If you wait longer than that the signal to noise ratio becomes confusing.

Garner: The problem is that if you irradiate anything that contains lipids you get peroxides, certainly *in vitro* and probably *in vivo*. But the accumulation of these products depends on the metabolic activity of the particular system. The fact that it is not measured doesn't mean it hasn't happened.

Cramp: That is not an answer I can accept. You can't say it goes so fast that you can't see it.

Garner: It is just that there are other routes that are removing the products.

Cramp: So fast that you can't detect them?

Garner: Yes.

Brown: Can I ask whether SOD makes any difference to radiation response. Some of us have tried it and we didn't find any.