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Biochemistry

Mutagenicity of oxygen free radicals

(superoxide radical/superoxide dismutase/oxygen toxicity/Ames test)

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ABSTRACT **Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) was used as an intracellular generator of oxygen free radicals and was found to be highly mutagenic for *Salmonella typhimurium*. It caused both base-pair substitution and frameshift mutations. Paraquat was much more toxic and mutagenic in a simple nutritionally restricted medium than in a rich complex medium. The mutagenicity of paraquat was dependent upon the presence of a supply of both electrons and oxygen. Cells containing high levels of superoxide dismutase (superoxide:superoxide oxidoreductase, EC 1.15.1.1) were more resistant to the toxicity and the mutagenicity of paraquat than were cells containing normal levels of this enzyme. The mutagenicity of paraquat thus appears to be due to its ability to exacerbate the intracellular production of superoxide radicals.**

Superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH·) are the intermediates formed during the progressive univalent reduction of dioxygen (1–3). These intermediates are very reactive and are normally generated in respiring cells (3) as well as during exposure to ionizing radiation (4, 5). There is also mounting evidence that the cytotoxicity of certain antitumor compounds and xenobiotic compounds is due to reduced oxygen species produced during redox cycling (6–10). Oxygen cytotoxicity is held in check by the delicate balance between the rates of generation of reduced oxygen species and the rate of their removal by the different defensive mechanisms (11); any shift in this delicate balance can lead to cellular damage. Living cells have evolved different defense mechanisms to protect against the deleterious effects of the reduced oxygen species (3, 11, 12). Superoxide dismutases protect against O_2^-, and hydroperoxidases protect against H_2O_2 (11, 12).

Hyperbaric dioxygen is known to be mutagenic to *Escherichia coli* (13, 14), and physiological concentrations of dioxygen are mutagenic to certain anaerobic mutants of *Salmonella typhimurium* (15). It has also been shown that superoxide radicals, generated by xanthine oxidase or by potassium superoxide, cause DNA strand scissions *in vitro*, and that scavengers of O_2^-, H_2O_2, or OH· provide protection (16–18). Paraquat (PQ^{2+}) can act as an *in vivo* generator of superoxide radical (6, 7, 19–21). It forms a relatively stable, yet oxygen-sensitive, paraquat radical ($PQ^{\cdot+}$) that reacts very rapidly ($k_2 = 7.7 \times 10^8\ M^{-1}\ s^{-1}$) with dioxygen to generate superoxide radical (20). PQ^{2+}, under appropriate conditions, is capable of inducing superoxide dismutase in *E. coli* (22). This investigation was undertaken to determine if oxygen free radicals, generated by PQ^{2+}, are mutagenic *in vivo*; the *S. typhimurium* Ames tester strains TA98 and TA100 were used to test for mutagenicity.

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MATERIALS AND METHODS

Chemicals. PQ^{2+} (1,1′-dimethyl-4,4′-bipyridinium dichloride, methyl viologen), mitomycin C, sodium azide, and proflavine were purchased from Sigma† and were dissolved in sterile water just prior to use.

Bacterial Strains. *S. typhimurium* histidine auxotroph strains TA98 and TA100 were kindly supplied by B. N. Ames (Berkeley, CA). Bacterial cultures were maintained and checked before use as recommended (23).

Media and Growth Conditions. Complete Vogel–Bonner (VB) minimal medium contained: glucose, 2%; L-histidine, 0.1 mM; biotin, 5 μM; $MgSO_4 \cdot 7H_2O$, 0.02%; citric acid·H_2O, 0.2%; K_2HPO_4, 1%; and $NaNH_4HPO_4 \cdot 4H_2O$, 0.35%. VB minimal salts is the same as VB minimal medium without glucose, histidine, and biotin. Minimal media with limited histidine (2.5 μM) or without histidine were used where indicated. Media for viable counts were prepared by adding 2% agar to the appropriate liquid media. Trypticase soy broth (3%) and yeast extract (0.5%) from Baltimore Biological Laboratory (Cockeysville, MD) were used in preparing the trypticase soy/yeast extract medium (TSY). Cells were grown aerobically at 37°C on a rotary shaker (New Brunswick) at 200 rpm. Cells were grown anaerobically inside a Coy anaerobic chamber where O_2 was kept at less than 5 ppm. Growth was monitored in terms of changes in turbidity at 600 nm using a Gilford spectrophotometer model 2000. Specific growth rates and generation times were calculated as described (24).

Mutagenesis. The following different test systems were used for assessing the mutagenicity of PQ^{2+}: (i) *Spot tests.* Tests were carried out according to the recommendations of Ames *et al.* (23). (ii) *Plate incorporation assays.* Assays were as recommended by Ames *et al.* (23), with the exception of 2% glucose being incorporated into the top agar layer. (iii) *Liquid incubation assay.* In this assay overnight (15- to 17-hr) cultures growing in complete VB minimal media with excess histidine (0.1 mM) were subcultured into fresh media of the same composition or as stated otherwise to an initial OD of 0.05–0.1 at 600 nm. The cultures were allowed to grow for about 2 hr to an approximate OD_{600} of 0.2 before being subdivided into 50-ml portions and reincubated with the specified concentrations of PQ^{2+} or the standard mutagens. Rat liver homogenates (S9 fraction) were not used in this study. After 5 hr of incubation in the presence of the test compounds, two 25-ml samples were collected. The

Abbreviations: SOD, superoxide dismutase; PQ^{2+}, paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride, methyl viologen); TSY, trypticase soy/yeast extract medium; VB minimal, Vogel–Bonner minimal medium; His^+, histidine-independent; CFU, colony-forming unit.

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cells were removed by centrifugation and washed once with VB minimal salts to remove the test compounds. One 25-ml sample was saved for enzyme assays. The remaining 25-ml sample was resuspended in VB minimal salts to an OD_{600} of 2.0. This step was necessary to equalize the number of cells plated on the selection media, thus minimizing the possibility of false results due to differences in the number of generations afforded by the limited concentration of histidine present in the plates (25). Aliquots (0.1- and 0.2-ml) of these cell suspensions were plated onto each of four selection plates containing limited histidine (2.5 μM) [this concentration is half that recommended by Ames *et al.* (23), in order to reduce the number of generations] and onto four plates lacking histidine to score for histidine-independent (His^+) revertants. Total viable counts were determined from the same cell suspensions by plating 0.1-ml aliquots of appropriate dilutions onto the surface of complete VB minimal agar plates containing excess histidine (0.1 mM). The plates were incubated at 37°C for 96 hr before the colonies were counted. All colonies that were scored as revertants were further checked for their true His^+ phenotype by transferring onto plates containing no histidine. The viable cells per ml and the number of His^+ revertants per ml were used to calculate the number of revertants per 10^8 cells.

Assays. Cells were harvested at 4°C by centrifugation for 15 min at 12,000 × *g* and were resuspended in 0.05 M potassium phosphate plus 0.1 mM ethylenediaminetetraacetic acid (EDTA) at pH 7.8. The cells were disrupted for 3 min by using a Heat Systems W-370 sonicator, operated at an output of 60 W. The cell suspension was kept at 4–6°C by immersion in an ice/salt bath and by application of the sonicator power for 30-sec intervals. Cell debris was removed by centrifugation at 27,000–30,000 × *g* for 30 min. Cell-free extracts were then dialyzed for 24 hr against two 4-liter batches of the phosphate/EDTA buffer. Protein was estimated by the method of Lowry *et al.* (26), using bovine serum albumin (Sigma) as a standard. Superoxide dismutase (superoxide:superoxide oxidoreductase, EC 1.15.1.1) was assayed by the cytochrome *c* assay (27). Superoxide dismutase isoenzymes were separated by electrophoresis on 10% polyacrylamide gels (28), were visualized using an activity stain (29), and were quantitated by linear scanning densitometry (30). Catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6) was assayed by measuring the disappearance of H_2O_2 at 240 nm (31).

RESULTS

Effects of Medium on PQ^{2+} Toxicity. *S. typhimurium* TA98 and TA100 were exposed to a wide range of PQ^{2+} concentrations in a simple nutritionally restricted medium (VB minimal medium) and in a rich complex medium (TSY). The cells were strikingly less sensitive to PQ^{2+} in TSY than in VB minimal medium (Fig. 1). Thus, 1 mM PQ^{2+} completely inhibited growth of TA98 and TA100 in the VB minimal medium (Fig. 1 *A* and *C* and Table 1) but caused only approximately a 50% increase in their generation times in the TSY medium (Table 1). Furthermore, 0.1 mM PQ^{2+} was highly inhibitory to the growth of *S. typhimurium* in the minimal medium but had no effect in TSY medium (Fig. 1 and Table 1). This toxic effect of PQ^{2+} in the

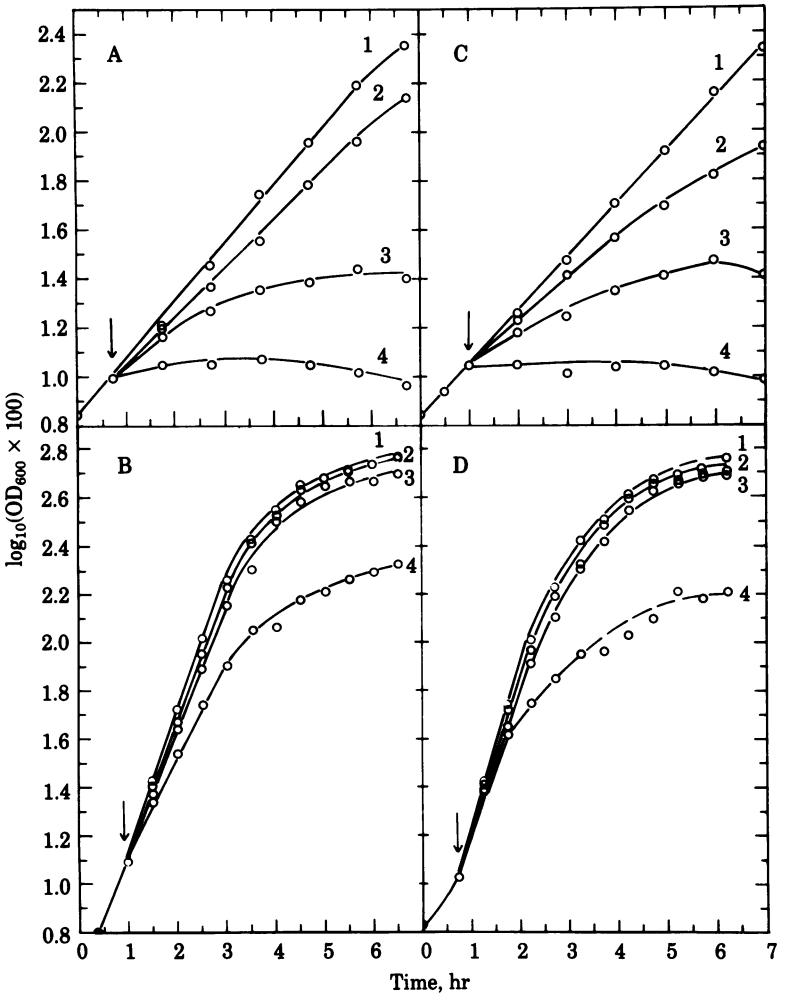

FIG. 1. Effects of PQ^{2+} on growth of *S. typhimurium* TA98 and TA100 in various media. The inocula were from 15-hr cultures grown in TSY or in VB minimal media. The inocula were introduced into fresh media and allowed to grow to an OD_{600} of 0.1 before the indicated concentrations of PQ^{2+} were added. Growth was monitored at 600 nm. (*A*) TA98 in VB minimal medium; (*B*) TA98 in TSY medium; (*C*) TA100 in VB minimal medium; (*D*) TA100 in TSY medium. PQ^{2+} concentrations were: line 1, no PQ^{2+}; line 2, 0.01 mM; line 3, 0.1 mM; and line 4, 1.0 mM. Arrows indicate the times at which PQ^{2+} was added to the cultures.

Table 1. Effects of PQ^{2+} on growth, superoxide dismutase, and catalase in *S. typhimurium**

Strain	PQ, mM	VB minimal medium: ΔOD_{600} at 5 hr	VB minimal medium: Generation time, min	VB minimal medium: SOD, units/mg	VB minimal medium: Catalase, units/mg	TSY medium: ΔOD_{600} at 5 hr	TSY medium: Generation time, min	TSY medium: SOD, units/mg	TSY medium: Catalase, units/mg
TA98	0	1.47	76	21.1	3.4	5.75	31	37.5	8.0
	0.01	0.84	92	36.0	2.1	4.98	32	43.8	6.5
	0.10	0.18	134	54.1	12.8	4.80	36	70.5	6.7
	1.0	0.01	1,122	40.8	8.9	2.06	46	133.4	9.0
TA100	0	1.33	81	18.0	2.9	5.18	25	30.1	5.0
	0.01	0.56	103	42.5	3.9	4.64	27	53.6	4.0
	0.10	0.19	129	59.6	6.4	4.58	29	74.3	4.7
	1.0	0.01	1,808	35.7	7.5	1.42	32	159.0	7.6

SOD, superoxide dismutase.

* Cells were grown for 5 hr at 37°C in the presence of PQ^{2+} at different concentrations. Growth was followed at 600 nm. At 5 hr, the cells were removed, washed, and sonicated, and the extract was clarified by centrifugation, dialyzed, and assayed for superoxide dismutase and catalase activities.

minimal medium was eliminated under anaerobic conditions (data not shown).

Effects of PQ^{2+} on Levels of Superoxide Dismutase and Catalase. We have previously seen that aerobic exposure of *E. coli* to PQ^{2+}, in a TSY medium, causes dramatic induction of the manganese-containing superoxide dismutase (22). Similar results were obtained with *S. typhimurium*. It is proposed that this induction of the O_2^--scavenging enzyme represents an important defense against PQ^{2+} toxicity. Table 1 presents the level of superoxide dismutase present in *S. typhimurium* TA98 and TA100 grown in both VB minimal and in TSY media over a range of PQ^{2+} concentrations. It is evident that the cells grown in TSY without PQ^{2+} contained higher levels of the enzyme than cells grown in VB minimal media. The cells, in both types of media, responded to PQ^{2+} by increasing their content of superoxide dismutase. In the VB minimal medium, however, the cells were not able to increase their level of superoxide dismutase to match the increase in the flux of O_2^- caused by increasing the concentration of PQ^{2+} beyond 0.1 mM. In TSY medium the cells continued to increase their superoxide dismutase level and were protected from PQ^{2+} toxicity. PQ^{2+} had no significant effect on the level of catalase in TSY medium; however, a 2- to 4-fold increase was seen in the VB minimal medium (Table 1).

Mutagenicity of PQ^{2+}. When PQ^{2+} (0.05–5.0 mM) was incorporated in the solid VB minimal medium containing limited histidine, the background growth of the test organisms was nonconfluent and minute colonies were seen that were not true His^+ revertants. At a lower concentration of PQ^{2+} (0.05 mM), the number of true revertants per plate was slightly higher than the spontaneous reversions (data not shown). At concentrations of PQ^{2+} higher than 0.1 mM, the number of revertants per plate was much lower than the controls, suggesting that PQ^{2+} was highly toxic to the cells. The mutagenicity of PQ^{2+} was also tested by the spot assay method at levels of 13–260 μg per filter disc, and the compound was found to be highly toxic as judged by large zones of inhibition (data not shown).

In order to circumvent the problems associated with the bactericidal effect of PQ^{2+}, the liquid incubation assay was used. This assay allowed the enumeration of the viable cells surviving such exposure and hence the calculation of revertants per viable cell. The tester strains (TA98 and TA100) were exposed to PQ^{2+} (0.01–1.0 mM) in the VB minimal medium containing excess histidine (0.1 mM). An exposure of 5 hr was found to be optimal (data not shown). Proflavine (30 μM) and mitomycin C (0.1 μg/ml) were used as positive control mutagens for strain TA98. Azide (2 μg/ml) and mitomycin C (0.1 μg/ml) were the positive control mutagens for TA100. Table 2 clearly shows that PQ^{2+} was highly mutagenic for both strains. PQ^{2+} at 1 mM was more mutagenic than proflavine and azide in TA98 and TA100, respectively. However, PQ^{2+} was much less mutagenic than mitomycin C (Table 2). The mutagenic effects of PQ^{2+} were equally demonstrable when selection plates containing limited histidine (2.5 μM) or lacking histidine were used. The number of revertants per 10^8 cells was higher when the selection plates contained limited histidine. We found that the amount of histidine added (2.5 μM) to the selection plates was enough to support the growth of the plated population for only one generation. Therefore, these results might indicate that some growth is required for the full expression of the mutagenic effect of PQ^{2+}.

Roles of Oxygen Free Radicals and Superoxide Dismutase. We have demonstrated that PQ^{2+} toxicity in *E. coli* is exclusively due to its ability to exacerbate the intracellular production

Table 2. Mutagenic effects of PQ^{2+} in VB minimal medium*

Strain	Treatment	Revertants/10^8 CFU: Limited histidine	Revertants/10^8 CFU: No histidine
TA98	Control	4	2
	PQ^{2+} (0.01 mM)	17	13
	PQ^{2+} (0.1 mM)	73	30
	PQ^{2+} (1.0 mM)	311	49
	Proflavine (30 μM)	124	16
	Mitomycin C (0.1 μg/ml)	1,904,800	14,881
TA100	Control	42	12
	PQ^{2+} (0.01 mM)	58	15
	PQ^{2+} (0.1 mM)	300	59
	PQ^{2+} (1.0 mM)	2,683	207
	Azide (2 μg/ml)	148	54
	Mitomycin C (0.1 μg/ml)	6,740,000	53,000

CFU, colony-forming units.

* PQ^{2+} was added to 50 ml of exponentially growing culture (OD_{600} = 0.2) contained in a 250-ml Erlenmeyer flask. Known mutagens (proflavine, azide, and mitomycin C) were included as positive controls. The cultures were reincubated at 37°C with shaking (200 rpm) for 5 hr, before the cells were washed and plated on selection plates containing limited histidine (2.5 μM) and on plates lacking histidine, in order to score for the number of His^+ revertants. Total viable counts were determined from the same cell suspensions by plating 0.1-ml aliquots of appropriate dilutions onto the surface of complete VB minimal plates containing excess histidine (0.1 mM). The plates were incubated at 37°C for 96 hr before the colonies were counted. The viable cells per ml and the number of His^+ revertants per ml were used to calculate the number of revertants per 10^8 cells.

Table 3. Effect of anaerobiosis on PQ^{2+} mutagenicity*

Strain	Treatment	Revertants/10^8 CFU: Limited histidine	Revertants/10^8 CFU: No histidine
TA98	Control	5	4
	PQ^{2+} (1 mM)	16	7
	Proflavine (30 μM)	29	12
	Mitomycin C (0.2 μg/ml)†	2,100,000	—
TA100	Control	53	13
	PQ^{2+} (1 mM)	43	20
	Azide (4 μg/ml)†	271	68
	Mitomycin C (0.2 μg/ml)†	2,615	—

* Experimental conditions were as in Table 2, except that anaerobic conditions (Coy anaerobic chamber) were used throughout.
† Notice that the concentrations of azide and mitomycin C were double the concentrations used aerobically (Table 2).

of superoxide radicals (7, 21, 32). If the mutagenicity of PQ^{2+} is due to its ability to generate superoxide radicals, then removal of oxygen should prevent this mutagenic effect. Indeed this was the case (Table 3). Furthermore, the mutagenic effect of PQ^{2+} was dependent on the availability, in the suspending medium, of a readily metabolizable electron source (Table 4). This supported the notion that the mutagenicity of PQ^{2+} is dependent on its redox cycling capacity and on its ability to augment the rate of O_2^- formation in the cells. Finally, if the causative agent in PQ^{2+} mutagenicity is related to O_2^-, then cells with higher levels of superoxide dismutase should be less susceptible to PQ^{2+} mutagenicity. Table 4 shows that PQ^{2+} was nonmutagenic in the rich medium (TSY), in which the level of superoxide dismutase was higher than that in the VB minimal medium.

DISCUSSION

The data presented above clearly demonstrated that PQ^{2+} is a mutagen capable of reverting both base-pair substitutions and frameshift mutations. The ability of PQ^{2+} to cause deletion mutations was not tested. PQ^{2+} in aerobic VB minimal medium was highly toxic to the *S. typhimurium* tester strains. This toxic effect seemed to cause erratic results in the standard Ames test (unpublished data). However, the mutagenicity of PQ^{2+} was consistently observed when tested in the modified liquid incubation assay in which the cells were exposed to PQ^{2+} for a specified length of time, washed free from the toxic PQ^{2+}, and then plated onto the selection media. It was important to express the number of revertants as a function of surviving cells, rather than as a function of the total number of cells plated.

Table 4. Effect of cultural conditions and SOD levels on PQ^{2+} mutagenicity*

Strain	Conditions	PQ^{2+}, mM	SOD,† units/mg	Revertants/10^8 CFU‡
TA98	VB minimal medium	0	18	5
		1	40	320
	TSY medium	0	33	12
		1	136	18
	VB salts	0	18	11
		1	16	9
TA100	VB minimal medium	0	17	42
		1	36	3,200
	TSY medium	0	30	103
		1	160	130
	VB salts	0	18	109
		1	15	84

* Exponential cultures growing in VB minimal medium were diluted to an OD_{600} of about 0.2 in the specified media with and without 1.0 mM PQ^{2+}, and reincubated as described for Table 2.
† After 5 hr of growth in the different media, 25-ml samples were collected, and dialyzed cell-free extracts were assayed for superoxide dismutase activity.
‡ Revertants were enumerated on plates with limited histidine (2.5 μM).

It has been shown that a supply of electrons is needed to reduce PQ^{2+} to its corresponding cation radical ($PQ^{\cdot+}$), that oxygen serves to reoxidize this radical (6, 7, 16–18), that the product of this cyclic autoxidation is O_2^- (6, 20, 32), and further that the toxicity of PQ^{2+} is related to its ability to increase the intracellular flux of O_2^- (6, 22, 32). The mutagenicity of PQ^{2+} has now been shown to be dependent upon the presence of a supply of both electrons and oxygen. Furthermore, increased levels of superoxide dismutase impart protection. Therefore, it is apparent that the toxicity (7, 21) and the mutagenicity of PQ^{2+} are related phenomena. We therefore conclude that the mutagenicity and toxicity of aerobic PQ^{2+} are largely due to its ability to exacerbate the intracellular production of O_2^-. We know of no other report showing that intracellularly generated O_2^- is mutagenic and that there is an inverse relationship between the levels of superoxide dismutase and the extent of mutagenesis. A pronounced oxygen effect was also observed with mitomycin C in *S. typhimurium* TA100 (Tables 2 and 3), which is in accord with recent findings (33) that mitomycin C generates O_2^-. A similar effect of mitomycin C was not seen in strain TA98. This apparent difference may be related to the inherent genetic differences between the two strains, which could affect the interaction between mitomycin C and the DNA.

Dioxygen is mutagenic (13–15), and ionizing radiation is both mutagenic and carcinogenic (34). Furthermore, activated phagocytes (35) have been shown to cause base-pair substitutions and frameshift mutations in the Ames test. It is interesting to note that both ionizing radiation (4, 5) and activated phagocytes (36) are known to produce O_2^- as well as other partially reduced oxygen species (i.e., H_2O_2 and $OH^{\cdot}$). It has also been reported (35) that activated phagocytes from patients with chronic granulomatous disease, that are unable to generate O_2^-, are not mutagenic. These results and our present findings suggest a common role for oxygen free radicals in the mutagenicity of dioxygen, PQ^{2+}, ionizing radiation, and activated phagocytes. It appears that the mutagenic agent is O_2^-, or products derived therefrom (i.e., H_2O_2, $OH^{\cdot}$) (37). The protective effect of superoxide dismutase seen here attests to the role played by O_2^-.

It is known that $OH^{\cdot}$ is responsible for approximately 90% of the DNA damage induced *in vitro* by ionizing radiation (38). H_2O_2 has also been shown to cause DNA strand breakage and liberation of DNA bases, as well as chemical alterations of the bases (39, 40). More recently, *in vitro* studies have shown that O_2^--generating systems cause single-strand scissions in DNA (17–19), and that superoxide dismutase, catalase, or scavengers of $OH^{\cdot}$ protect against these scissions. Oxygen free radicals have also been shown to induce oxidative modifications of DNA bases (41).

It is clear that oxygen free radicals can damage DNA. However, it is not known to what extent such oxidative damage or modifications of the DNA are repairable. DNA damage may lead to cell death or to mutations. Modified DNA or damaged DNA may lead to mutations by a direct alteration of the genetic code (misreplication) or by an error during DNA repair (misrepair). It seems logical to expect that the rate of formation and accumulation of modified or damaged DNA will depend both

on the activities of the different protective mechanisms naturally present in the cells (i.e., superoxide dismutase, catalase, antioxidants) and on the capacity of the cells to repair damaged DNA. The present study suggests an important role of superoxide dismutase in protecting against the toxicity and the mutagenicity of oxygen free radicals. Preliminary results show that *recA*$^-$ strains of *S. typhimurium* are more sensitive to the toxicity of PQ^{2+} than is the wild-type strain (unpublished data). The mutagenic species of oxygen remains to be identified, and the roles of catalase, OH· scavengers, and the DNA repair mechanisms in the protection against oxygen radical mutagenicity have yet to be assigned.

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1. Taube, H. (1965) *J. Gen. Physiol.* **49**, Suppl. 29–52.
2. Czapski, G. (1971) *Annu. Rev. Phys. Chem.* **22**, 171–208.
3. Fridovich, I. (1975) *Annu. Rev. Biochem.* **44**, 147–159.
4. Smith, K. C. & Heys, J. E. (1968) *Radiat. Res.* **33**, 129–141.
5. Van Hemmen, J. J. (1971) *Nature (London)* **231**, 79–80.
6. Hassan, H. M. & Fridovich, I. (1979) *Arch. Biochem. Biophys.* **196**, 385–395.
7. Fridovich, I. & Hassan, H. M. (1979) *Trends Biochem. Sci.* **4**, 113–115.
8. Lorentzen, R., Leska, S., McDonald, K. & Ts'o, P. O. P. (1979) *Cancer Res.* **39**, 3194–3198.
9. Lorentzen, R. & Ts'o, P. O. P. (1977) *Biochemistry* **16**, 1467–1473.
10. Cone, R., Hasan, S. K., Lown, J. W. & Morgan, A. R. (1976) *Can. J. Biochem.* **54**, 219–223.
11. Fridovich, I. (1978) *Science* **201**, 875–880.
12. Hassan, H. M. & Fridovich, I. (1980) in *Enzymatic Basis of Detoxication*, ed. W. B. Jakoby (Academic, New York), Vol. I, pp. 311–332.
13. Fenn, W. O., Gerschman, R., Gilbert, D. L., Terwilliger, D. E. & Cothran, F. W. (1957) *Proc. Natl. Acad. Sci. USA* **43**, 1027–1032.
14. Gifford, G. D. (1968) *Biochem. Biophys. Res. Commun.* **33**, 294–298.
15. Bruyninckx, W. J., Mason, H. S. & Morse, S. A. (1978) *Nature (London)* **274**, 606–607.
16. Brawn, K. & Fridovich, I. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **31**, 1945 (abstr.).
17. Brawn, K. & Fridovich, I. (1981) *Arch. Biochem. Biophys.* **206**, 414–419.
18. Leska, S. A., Lorentzen, R. J. & Ts'o, P. O. P. (1980) *Biochemistry* **19**, 3023–3028.
19. Dodge, A. D. (1971) *Endeavour* **30**, 130–135.
20. Farrington, J. A., Ebert, M., Land, E. J. & Fletcher, K. (1973) *Biochim. Biophys. Acta* **314**, 372–381.
21. Hassan, H. M. & Fridovich, I. (1978) *J. Biol. Chem.* **253**, 8143–8148.
22. Hassan, H. M. & Fridovich, I. (1977) *J. Biol. Chem.* **252**, 7667–7672.
23. Ames, B. N., McCann, J. & Yamasaki, E. (1975) *Mutation Res.* **31**, 347–364.
24. Hassan, H. M. & Fridovich, I. (1977) *J. Bacteriol.* **130**, 805–811.
25. Green, M. H. L. & Muriel, W. J. (1976) *Mutation Res.* **38**, 3–32.
26. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
27. McCord, J. M. & Fridovich, I. (1969) *J. Biol. Chem.* **244**, 6049–6055.
28. Davis, B. J. (1964) *Ann. NY Acad. Sci.* **121**, 404–427.
29. Beauchamp, C. & Fridovich, I. (1971) *Anal. Biochem.* **44**, 276–287.
30. Hassan, H. M. & Fridovich, I. (1977) *J. Bacteriol.* **129**, 1574–1583.
31. Beers, R. F. & Sizer, I. W. (1952) *J. Biol. Chem.* **195**, 133–140.
32. Hassan, H. M. & Fridovich, I. (1979) *J. Biol. Chem.* **254**, 10846–10852.
33. Lown, J. W., Begleiter, A., Johnson, D. & Morgan, A. R. (1976) *Can. J. Biochem.* **54**, 110–119.
34. Ward, J. F. (1975) *Adv. Rad. Biol.* **5**, 182–239.
35. Weitzman, S. A. & Stossel, T. P. (1981) *Science* **212**, 546–547.
36. Babior, B. M. (1978) *N. Engl. J. Med.* **298**, 659–668.
37. McCord, J. M. & Day, E. D., Jr., (1978) *FEBS Lett.* **86**, 139–142.
38. Armel, P. R., Strniste, G. F. & Wallace, S. S. (1977) *Radiat. Res.* **69**, 328–338.
39. Rhaese, H.-J. & Freese, E. (1968) *Biochim. Biophys. Acta* **155**, 476–490.
40. Massie, H., Samis, H. & Baird, M. (1972) *Biochim. Biophys. Acta* **272**, 539–548.
41. Sharma, R. C. & Yamamoto, O. (1980) *Biochem. Biophys. Res. Commun.* **96**, 662–671.