Biochemical Pharmacology, 1964, Vol. 13, pp. 159–164. Pergamon Press Ltd., Printed in Great Britain.

THE EFFECTS OF ANTIOXIDANTS ON HIGH PRESSURE OXYGEN TOXICITY

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(*Received* 1 *July* 1963; *accepted* 19 *September* 1963)

Abstract—Several commonly used antioxidants have been tested for their effect against poisoning due to high pressures of oxygen (OHP). The tests used were preconclusive period and survival time of mice at 5 atm absolute oxygen, lung damage in rats exposed to 5 atm oxygen for one hour, and post OHP paralysis in rats following deep pentobarbital sodium anaesthesia and OHP at 4 atm for 30 min. 2,5-bis (1,1-dimethyl propyl) hydroquinone gave excellent protection against OHP toxicity in all tests, and several other antioxidants also protected against OHP toxicity but their potency and effectiveness varied for the different criteria of oxygen poisoning tested in the experiments.

PROLONGED exposure of animals to high pressures of oxygen (OHP) causes convulsions, lung damage, and eventually death; events collectively described as oxygen poisoning. Spastic paralysis following sub-lethal exposures to OHP is another feature of oxygen poisoning in rats,[1] especially when such animals are anaesthetized during the OHP exposure.[2] The mode of action of oxygen poisoning remains unknown; however, direct oxidation of essential tissue components has been postulated, and oxidation of sulphydryl groups in rat lungs has been reported.[3] As oxidation may contribute to the toxicity of high pressures of oxygen, the effectiveness of antioxidants in modifying OHP toxicity was examined. Several antioxidants were tested for their effects against OHP induced convulsions and lethality in mice, and against lung damage and port-OHP paralysis in rats.

A series of non-specific antioxidants was investigated by Mertz and Schwarz[4, 5] and Schwarz[6] for their action against necrotic liver degeneration caused by dietary insufficiencies and these authors divided the compounds into groups according to their effectiveness. In our experiments compounds were chosen from each group, to determine whether antioxidants in general protected against oxygen poisoning, and whether their effectiveness against liver necrosis and liver respiratory decline[5] could be correlated with effectiveness against OHP.

METHODS

Female Canberra black rats, 150–200 g, and Walter and Eliza hybrid strain mice, 25–40 g were used. The six compartment pressure chamber used in these experiments has been previously described.[7] Preconclusive and survival times in mice were determined by placing three mice in a divided container in each compartment of the pressure chamber. For each pressurization nine mice were used as controls and nine were pretreated with the antioxidant under investigation. Control mice were included

in each pressurization to reduce error due to temperature fluctuations since the ambient temperature was not constant during the time of year these experiments were performed. Preconvulsion time was taken to be the time from attaining 5 atm absolute (60 lb/in^2) in the chamber to the first generalized tonic seizure; preliminary clonic twitchings were ignored. Death was recorded as cessation of visible respiration.

The severity of paralysis in anaesthetized rats following exposure to OHP was scored by grading paralysis from 0 for unaffected rats to 5 for rats severely paralysed.[8] All such rats were anaesthetized with pentobarbital sodium (38 mg/kg) and exposed to 4 atm oxygen for 30 min and scored for paralysis 24 hr later.

The doses of compounds used were large, being approximately the maximum sublethal concentrations ($\frac{1}{2}$ LD_{50} dose) tolerated by our animals except in the case of N N'diphenyl-*p*-phenylenediamine (DPPD) and antabuse where insolubility precluded the use of such high doses.

Methylene blue, ascorbic acid and d-alpha tocopherol polyethylene glycol 1000

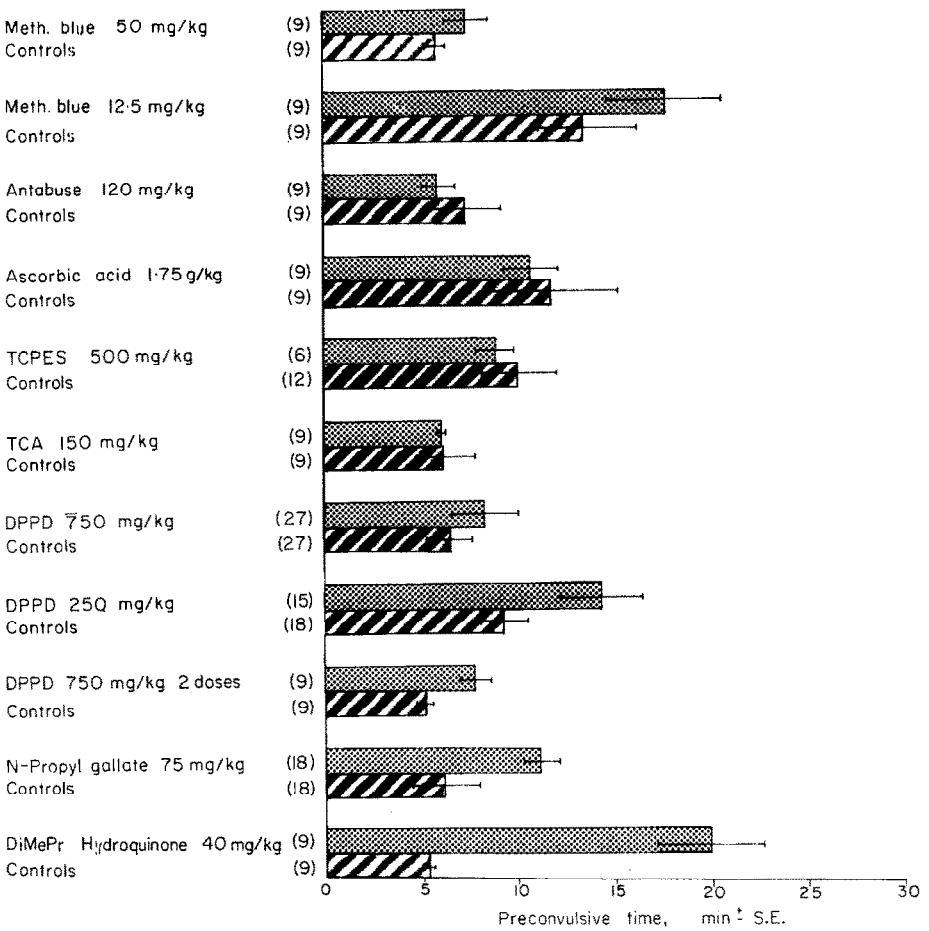

FIG. 1. Effect of antioxidants on the preconclusive period in mice (25–40 g wt.) exposed to 5 atm absolute of oxygen. Standard errors are shown, and the number of animals in each group indicated in brackets.

succinate (TCPES, 1 g = 358 international units) were disolved in water; 2,5-bis (1,1 dimethylpropyl) hydroquinone (diMePr hydroquinone) and n-propyl gallate were dissolved in propylene glycol while DPPD, α tocopherol acetate (TCA, 1 mg = 1 I.U.) and tetra ethylthiuram disulphide (antabuse) were dissolved in peanut oil. Water soluble antioxidants were injected intraperitoneally 10 min before exposure to OHP, while other compounds were injected intraperitoneally approximately 30 min before OHP with the exception of the α-tocopherol esters and DPPD.

α-tocopherol acetate was injected 5 hr prior to OHP exposure, while TCPES was injected in two doses 40 hr and 5 hr prior to OHP exposure. DPPD was injected 30 min before OHP or in two doses, 24 hr and 30 min before OHP. These dosage schedules were similar to those used and found effective in rats against lethal doses of carbon tetrachloride.[9]

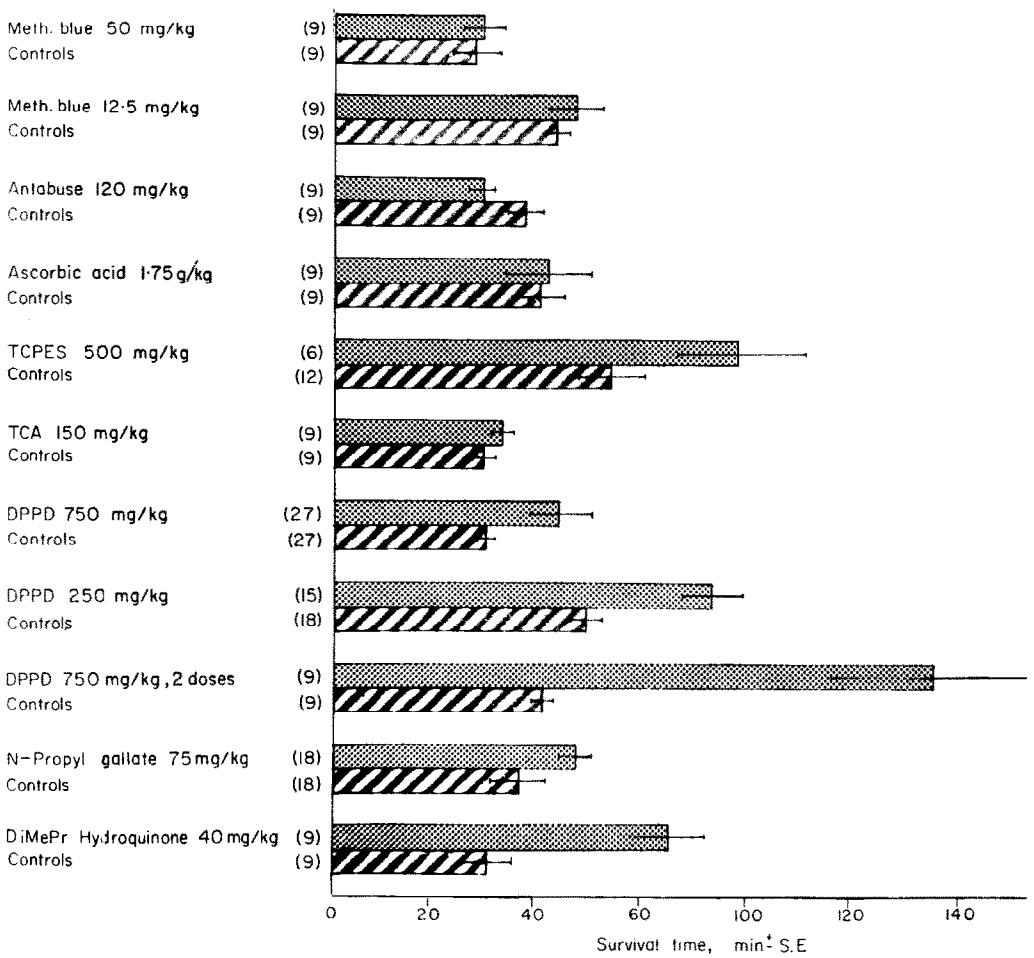

FIG. 2. Survival times of mice pretreated with various antioxidants, and exposed to 5 atm absolute of oxygen.

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RESULTS

The results are shown in Figs. 1 to 4. DiMePr hydroquinone gave the greatest prolongation of the preconvulsive period in mice ($P < 0{\cdot}001$). N-propyl gallate and DPPD had a slight effect against OHP in this respect ($P < 0{\cdot}05$) while the other antioxidants were without effect (Fig. 1). The hydroquinone derivative also markedly lengthened the survival time of mice exposed to OHP ($P < 0{\cdot}01$). DPPD prolonged survival time (Fig. 2) especially when given in two doses of 750 mg/kg, 24 hr and 30 min before pressurization ($P < 0{\cdot}001$). TCPES given 40 hr and 5 hr before pressurization also lengthened the survival time of mice in OHP ($P \simeq 0{\cdot}05$) but TCA given in a single dose 5 hr before OHP was ineffective (Fig. 2).

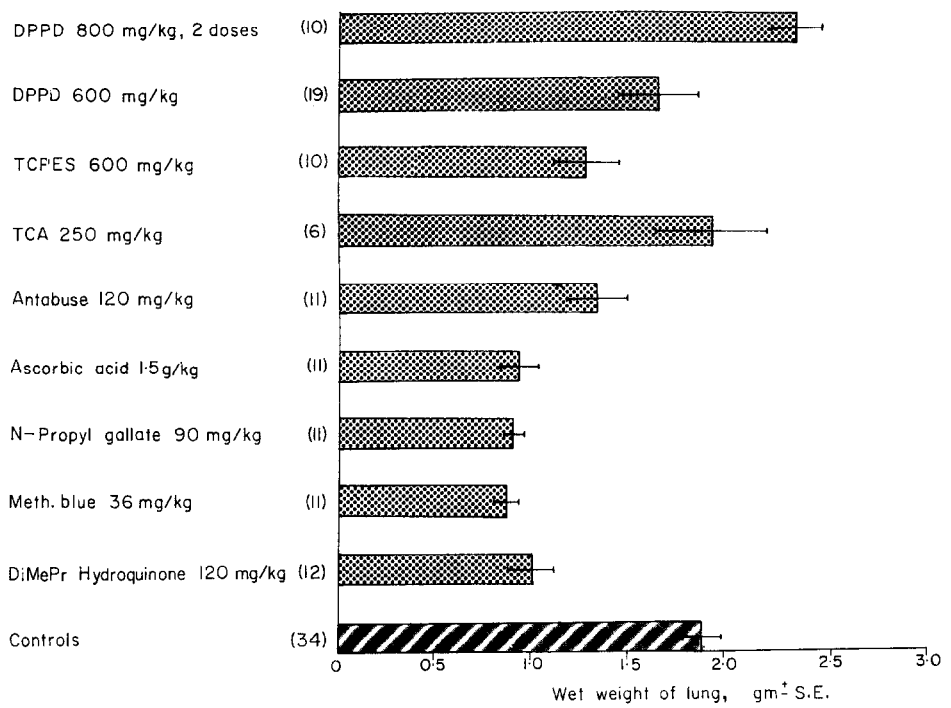

FIG. 3. Effect of antioxidants on lung damage in rats (mean weight in each group, 180 g) following exposure for one hour at 5 atm absolute of oxygen. Mean normal-lung weight for untreated rats was 0·85 g.[11]

The effectiveness of these antioxidants against OHP toxicity varied between rats and mice. Thus methylene blue, ineffective in mice, protected rats against OHP induced lung damage ($P < 0{\cdot}01$). N-propyl gallate and ascorbic acid were also more effective against OHP in this test in rats ($P < 0{\cdot}01$) than in mice tests. The hydroquinone derivative, which gave excellent protection in mice also protected rats against lung damage ($P < 0{\cdot}02$) and the α-tocopherol esters were about equally effective in both species ($P < 0{\cdot}05$ for TCPES). On the other hand DPPD was completely ineffective in protecting rats against lung damage (Fig. 3).

Several antioxidants protected rats from paralysis following anaesthesia and exposure to 4 atm of oxygen for 30 min. The only substances which failed significantly to modify the paralysis were antabuse, and tocopherol acetate in a single dose, although ascorbic acid also was not very effective (Fig. 4). Antabuse, in the doses used in these experiments failed to protect either rats or mice against the toxic effects of OHP.

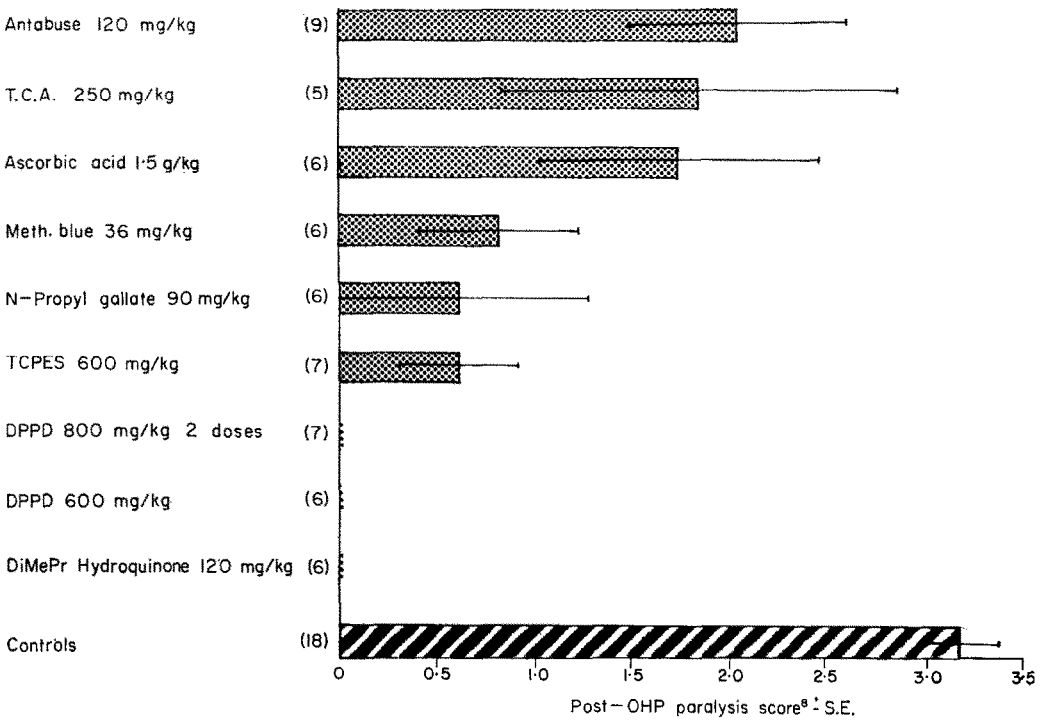

FIG. 4. Effect of antioxidants on paralysis in rats anaesthetized with 38 mg/kg pentobarbital sodium and exposed to 4 atm absolute of oxygen for 30 min.

DISCUSSION

The results reported show that strong antioxidants are effective against OHP toxicity, but their potency and relative effect against the different manifestations of OHP poisoning varies. Similarly, Schwarz[6] found that some antioxidants, including N-propyl gallate, though widely and effectively used for dietary fat stabilization, were without effect on necrotic liver damage in rats, yet other antioxidants, such as DPPD, were extremely effective as Vitamin E substitutes, in preventing such liver damage. Schwarz concluded that it was not possible to define the specific chemical properties of the antioxidants which afforded protection against dietary liver damage. The same would appear to be true for antioxidant action against OHP toxicity. Some of the antioxidants which protected against dietary and CCl_4 induced liver damage[6, 9] were also effective against oxygen poisoning, particularly 2,5-bis (1,1 dimethylpropyl) hydroquinone 3. However, DPPD, which was found by Schwarz[6] and Gallagher[9] to be the most effective antioxidant against liver necrosis in the rat was less effective against

oxygen poisoning except in the prolongation of mouse survival time where it was found as active as any substance as yet tested by us.[10] Conversely N-propyl gallate, inactive in Schwarz's experiments was effective in rats against OHP induced lung damage and post OHP paralysis. Thus there appears to be little correlation between the biological effectiveness of antioxidants on dietary liver necrosis and OHP damage. DPPD and DiMePr hydroquinone gave complete protection against paralysis following anaesthesia and OHP exposure, making these compounds comparable in activity with the *in vivo* buffer THAM, and paraminopropriophenone, in this test. Thus it would appear that some of the antioxidants do give excellent protection against OHP toxicity, but this protective effect does not appear to relate solely to antioxidant action, and the biochemical mechanisms involved which enable them to protect against the toxic actions of OHP are unknown.

Probably the differences in concentration of the various antioxidants in different tissues and organs contributes to the variation in their effectiveness against OHP toxicity, and may also account for differences in their effectiveness against OHP and liver damage.

Acknowledgements—We would like to thank Miss V. Ferguson and Miss J. Symons for their excellent technical assistance, and Miss D. O'Reilly of the Photographic Department of the Cancer Institute Board for preparation of the figures.

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