Effects of Hyperbaric Oxygenation on Metabolism VI. Efficacy of Protective Agents at 5, 7, 9, and 11 Atmospheres of 100% Oxygen[1] (34417)

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Studies on the protective effects of various compounds against high pressure oxygen (HPO) toxicity were performed at 5, 7, 9, and 11 ATA of 100% O_2. In the evaluation of protective agents it is imperative that comparisons be made at several oxygen pressures or various levels of stress since a compound may be effective as a protectant at a low stress level and be ineffective at a higher pressure. Currie *et al.* (1) previously compared the effectiveness of these same compounds against O_2 toxicity at 5 ATA O_2. The present paper reports the relative protective ability at 5, 7, 9, and 11 ATA of a group of compounds including sulfhydryl compounds, an acid–base buffer, a neuronal inhibitor, and some substrates of oxidative phosphorylation.

Methods. Male Sprague-Dawley rats (150–200 g), fasted (16–18 hr), were given intraperitoneal (ip) injections of the specific compound(s) 50 min prior to exposure to 5, 7, 9, or 11 ATA of 100% O_2. The animals had free access to water before they were placed in the high pressure oxygen chamber. The compounds listed in Table I were used. Twelve rats in separate, restricted cages were placed in the 18-in diameter, 42-in. long, Bethlehem Corporation high pressure chamber for each experiment. Soda lime was placed in the chamber to absorb CO_2. After flushing the chamber with 100% oxygen to displace all nitrogen, the chamber was closed and oxygen pressure was increased at the rate of 1 ATA/min until the desired pressure was reached, at which moment time zero was recorded. Temperature in the chamber was maintained at 21 ± 0.5° and oxygen flow at 1 liter/min per animal throughout the experiment. Animals were observed continuously through two viewing ports, and the time elapsed prior to the onset of major motor seizures was recorded for each animal. A minimum of two control animals (which had no ip injection) was included in each experiment.

TABLE I. Compounds Used.

Compounds (0.4 *M*, pH 6.4)	Dosage (mmoles/kg ip)
Controls	—
Glutathione	4 or 12
Sodium succinate	12
α-Glycerophosphate	12
GABA	12
Tris	10
Sodium glutamate	12
Cysteine	4
Sodium malate	12
Cysteine + succinate	4 + 12

Results. The results of these experiments are shown in Table II. Each column of Table II has the protective agents listed in decreasing order of effectiveness, dosage in mmoles/kg (4, 10, or 12), the number of animals in the group, and the average time to convulsion (min ± 1 SD). (min + 1 SD). Our observations at 5, 7, 9, and 11 ATA O_2 show that the combination of sulfhydryl group protection and metabolic substrate offers the greatest protection against O_2 toxicity.

[1] Supported in part by Public Health Service Research Grant GM-14226-03, from the National Institute of General Medical Sciences; and by Contract N00014-67-A-0251-0002, between the Office of Naval Research, Department of the Navy and Duke University.

TABLE II. Relative Protective Ability of Different Compounds at 5, 7, 9, and 11 ATA O_2 (time to convulsions expressed as percentage of controls for different compounds).

5 ATA		7 ATA		9 ATA		11 ATA	
Controls:	Time to convulsions 62 ± 31.6 min (80)		Time to convulsions 16.9 ± 4.0 min (10)		Time to convulsions 7.4 ± 3.0 min (17)		Time to convulsions 4.4 ± 0.9 min (9)
Compound (mmoles/kg)	% of controls	Compound (mmoles/kg)	% of controls	Compound (mmoles/kg)	% of controls	Compound (mmoles/kg)	% of controls
GSH_{12}	467 ± 45 (8) $p < 0.001$	$Cysteine_4$ + $succinate_{12}$	475 ± 118 (8) $p < 0.001$	$Cysteine_4$ + $succinate_{12}$	505 ±107 (8) $p < 0.001$	$Cysteine_4$ + $succinate_{12}$	468 ±100 (8) $p < 0.001$
$Cysteine_4$ + $succinate_{12}$	437 ± 82 (15) $p < 0.001$	$Succinate_{12}$	315 ± 121 (9) $p < 0.001$	$Succinate_{12}$	253 ± 72 (15) $p < 0.001$	$Succinate_{12}$	286 ± 86 (8) $p < 0.001$
α-Glycero-$phosphate_{12}$	363 ± 47 8 $p < 0.001$	GSH_{12}	281 ± 153 (8) $p < 0.005$	GSH_{12}	214 ± 36 (8) $p < 0.001$	α-Glycero-$phosphate_{12}$	277 ± 52 (8) $p < 0.001$
$Succinate_{12}$	336 ± 70 (8) $p < 0.001$	α-Glycero-$phosphate_{12}$	174 ± 66 (9) $p < 0.005$	α-Glycero-$phosphate_{12}$	192 ± 50 (8) $p < 0.001$	GSH_{12}	230 ± 66 (8) $p < 0.001$
GSH_4	320 ± 74 (19) $p < 0.001$	$Cysteine_4$	140 ± 40 (8) $p < 0.01$	$Glutamate_{12}$	153 ± 47 (8) $p < 0.01$	$Cysteine_4$	139 ± 30 (8) $p < 0.005$
$GABA_{12}$	241 ± 42 (15) $p < 0.001$	GSH_4	132 ± 40 (10) $p < 0.015$	$Cysteine_4$	143 ± 24 (8) $p < 0.01$	$Glutamate_{12}$	136 ± 30 (8) $p < 0.01$
$Tris_{10}$	202 ± 65 (13) $p < 0.001$	$Malate_{12}$	129 ± 21 (6) $p < 0.01$	GSH_4	142 ± 51 (9) $p < 0.01$	$GABA_{12}$	132 ± 61 (8) $p = NS$
$Glutamate_{12}$	200 ± 24 (15) $p < 0.001$	$Tris_{10}$	127 ± 34 (15) $p = NS$	$Malate_{12}$	141 ± 31 (6) $p = NS$	GSH_4	130 ± 52 (10) $p = NS$
$Cysteine_4$	172 ± 51 (23) $p < 0.001$	$GABA_{12}$	111 ± 37 (8) $p = NS$	$Tris_{10}$	116 ± 22 (16) $p = NS$	$Tris_{10}$	123 ± 30 (8) $p = NS$
$Malate_{12}$	120 ± 66 (14) $p = NS$	$Glutamate_{12}$	81 ± 18 (8) $p = NS$	$GABA_{12}$	114 ± 43 (8) $p = NS$	$Malate_{12}$	91 ± 39 (6) $p = NS$

Discussion. Sanders *et al.* (2) previously proposed that GSH protection against O_2 toxicity at 5 ATA was due to –SH group protection plus metabolic substrate protection which results from the glutamyl moiety of GSH going to succinate via the glutamate→ GABA → succinic-semialdehyde → succinate pathway. The ability of succinate to increase ATP concentration then serves to meet the increased energy needs of the cell in the HPO environment. Table II indicates these results are consonant with this proposal. The greatest protection at 7, 9, and 11 ATA was achieved with cysteine + succinate which would be expected if succinate is the compound which ultimately yields protection along with –SH group protection when GSH is used as a protectant. Similarly, it might be expected that protection with glutamate, GABA, and GSH would decrease or disappear at 7, 9, and 11 ATA due to the time necessary to convert glutamate to GABA via glutamic decarboxylase, and GABA to succinic-semialdehyde via GABA transaminase, exceeding the time in which protection must be rapidly provided at the higher oxygen tensions. Cysteine, a sulfhydryl group-containing amino acid, yielded very nearly the same degree of protection at 7, 9, and 11 ATA. This might be predicted if the mode of protection was simply that of providing a reducing atmosphere to counteract oxidation reactions. Tris, an acid–base buffer, gave results not significantly different from controls at 7, 9, and 11 ATA.

The role of succinate, an FAD-linked substrate, in ATP production and protection against oxygen toxicity was reported by Sanders *et al.* (3–5). Alpha-glycerophosphate is another FAD-linked substrate and as shown in Table II, gave only slightly less protection than succinate at 7, 9, and 11 ATA. The NAD link to the electron transport chain is adversely affected along with associate enzyme systems by hyperbaric oxygenation (6, 7). The protection obtained with these two FAD-linked substrates coupled with the lack of protection with malate (a NAD-linked molecule, but also a Kreb's cycle intermediate and a dicarboxylic acid) supports the concept that maintenance of normal ATP concentrations or energy levels is of prime importance in protecting organisms subjected to hyperbaric oxygenation.

Summary. At 5, 7, 9, and 11 ATA of 100% O_2: Cysteine + succinate offers the greatest protection against O_2 toxicity. Succinate is the most effective single compound for protection against O_2 toxicity. The failure of GSH to sustain its degree of protection at 7, 9, and 11 ATA is thought to be due to insufficient time available to permit conversion of GSH to succinate due to the increased severity of stress. The acid–base buffer, Tris, was less effective at 7, 9, and 11 ATA than –SH group protectants. Malate, a NAD-linked substrate offers no protection against O_2 toxicity.

We thank Marvin and Julie Nunn for valuable technical assistance.

1. Currie, W. D., Gelein, R. M., and Sanders, A. P., Proc. Soc. Exptl. Biol. Med., in press.

2. Sanders, A. P., Currie, W. D., and Woodhall, B., Proc. Soc. Exptl. Biol. Med. **130**, 1021 (1969).

3. Sanders, A. P., Hall, I. H., and Woodhall, B., Science **150**, 1830 (1965).

4. Sanders, A. P., Hall, I. H., Cavanaugh, P. J., and Woodhall, B., Natl. Acad. Sci.—Natl. Res. Council, Publ. **1404**, 73 (1966).

5. Sanders, A. P., Lester, R. G., and Woodhall, B., J. Am. Med. Assoc. **204**, 241 (1968).

6. Chance, B., Jamieson, J., and Coles, H., Nature **206**, 257 (1965).

7. Thomas, J J., Neptune, E. M., and Sudduth, H. C., Biochem. J. **88**, 31 (1963).

Received July 22, 1969. P.S.E.B.M., 1970, Vol. 133.