Effects of Hyperbaric Oxygenation on Metabolism
V. Comparison of Protective Agents at 5 Atmospheres 100% Oxygen[1] (34282)

William D. Currie, Robert M. Gelein, Jr., and Aaron P. Sanders
Division of Radiobiology, Department of Radiology, Duke University Medical Center, Durham, North Carolina 27706

Many studies have been performed to determine the efficacy of various compounds to protect against oxygen toxicity. These studies included a wide assortment of compounds in a diverse group of animals at many different oxygen pressures. Gerschman *et al.* (1) investigated various thiolic and related compounds in mice exposed to 1–10 atmospheres absolute (ATA) oxygen pressures. Bean (2), Gottlieb and Jagodzinski (3), and Sanger *et al.* (4), used Tris in albino rats and mice at oxygen pressures up to 6.3 ATA. Taylor (5) investigated the effects of tocopherol, methylene blue, glutathione (GSH), and Vitamin E in hooded rats at 75 psig[2] oxygen pressure. Wood *et al.* (6, 7) used gamma-aminobutyric acid (GABA) in Wistar rats at oxygen pressures of 75 psig. Gottlieb and Cymeran (8) utilized ammonium chloride in mice at 5 ATA oxygen pressure. Sanders *et al.* (9–11), employed succinate and other metabolites in rats at 5 ATA oxygen pressure.

Such a variety of compounds in different species at different oxygen pressures does not permit a direct comparison of the usefulness of the various protective agents. The present paper reports a comparison of several of the protective agents used in rats exposed to 5 ATA oxygen pressures.

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 being exposed to 5 ATA 100% oxygen. The animals had free access to water before they were placed in the high pressure oxygen chamber. The solutions used are listed in Table I.

TABLE I. Solution Used.

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

After flushing the chamber with 100% oxygen to displace all nitrogen, the chamber was closed and oxygen pressure increased at the rate of 1 ATA/min until 60 psig 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. Twelve rats in separate, restricted areas were placed in the 18-in. diameter, 42-in long, Bethlehem Corporation high pressure chamber for each run. Soda lime was placed in the chamber to absorb CO_2.

Animals were observed through two viewing ports throughout the experiment. The time elapsed prior to the onset of grand mal seizures was recorded for each animal. A minimum of two control animals (which had

[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.

[2] Pounds per square inch guage.

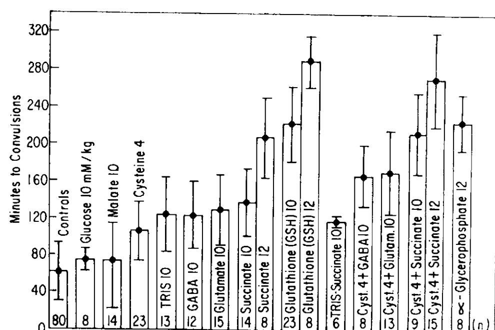

FIG. 1. Time to convulsions in 5ATA-O_2 for different protective agents (mean ± SD).

no ip injection) was included in each experiment.

Results. The results of these experiments are shown in Fig. 1. Each bar on the graph indicates the compound(s) used, dosage in mmoles/kg (4 or 10 or 12), the number of animals in the group, and the mean convulsion time of the group plus or minus the standard deviation. The effectiveness of the various compounds in delaying onset of convulsions at 5 ATA 100% oxygen is as follows: GSH > cysteine plus succinate > cysteine plus glutamate > cysteine plus GABA > succinate > GABA > glutamate > Tris-succinate > Tris > cysteine > glucose > malate.

Discussion. One of the problems in comparing the protection afforded by different compounds administered via the intraperitoneal route is the relative time and rate of absorption from the peritoneal cavity. We have found it essential to use fasted animals. In addition, it has been necessary to permit an equilibration period following ip injection, prior to placing the animal in the hyperbaric chamber. For this series of compounds, a 45–55-min equilibration interval gives the best protection in Sprague-Dawley rats of this weight range, therefore, we have arbitrarily standardized the equilibration period at 50 min for all compounds. A 30- or 75-min equilibration period prior to HPO exposure affords significantly less protection, if any, against convulsions at 5 ATA oxygen. In mouse experiments maximal protection against oxygen toxicity was obtained when an equilibration period of 18 min was used (unpublished observation). Thus, optimal time of equilibration prior to HPO exposure must be determined for each compound, for the route of entry (ip vs. iv vs. oral), and for the particular species being studied. Failure to recognize these factors may result in a compound being an effective protectant against oxygen toxicity in the hands of one investigator and ineffective in the hands of another.

The time of fasting is important as related to equilibration time after the ip injection. We found that with 16–18 hr of fasting, a 50-min equilibration time was optimal. If, however, the fasting period extended from 24 to 28 hr, a shorter equilibration interval was required to obtain the same degree of protection. We believe this reflects more rapid absorption by the animals that were fasted for the longer 24–28-hr period. To assure that fasting is limited to the desired time interval, automatic control of the animal room lighting was found necessary. (Rats, in general, stop eating when the room is well illuminated, thus manipulation of the lighting allows reasonable control of the beginning of the fasting period.)

It is recognized that fear in animals greatly increases the sensitivity to oxygen toxicity. Thus, in making group comparisons, special precautions must be taken to ascertain that all animals are handled in the same manner—with particular effort to avoid frightening the animals.

The usual rationale for utilizing GSH as a protectant against oxygen toxicity is based on sulfhydryl group protection against an oxidizing environment. However, the high level of protection obtained with GSH cannot be attributed to SH group protection alone. If we decreased the dosage of GSH to 4 mmoles/kg, the time to convulsions in the 5 ATA oxygen exposure was 198.7 ± 45.6 min (n = 19). If we used a 4 mmoles/kg dose of cysteine, which is equivalent in SH groups to the 4 mmoles/kg of GSH (glutamylcysteinylglycine), we found the time to convulsions in the 5 ATA oxygen exposure was 106.9 ± 31.4 min (n = 23). (We could not compare the 10 mmoles/kg GSH dose with an equivalent dose of cysteine since the latter is lethal at this concentration.) Thus, the

increased protection obtained with 4 mmoles/kg GSH is, in our opinion, due to GSH cleavage with formation of glutamate and cysteinylglycine, and subsequent shunting of glutamate (via GABA) to succinic-semialdehyde, thence to succinate (12). Succinate's ability to stimulate energy production serves to satisfy the increased energy demands of the cell in the HPO engironment. Note that 4 mmoles/kg of cysteine plus 10 mmoles/kg of succinate afforded approximately the same protection as 10 mmoles/kg of GSH. Similarly, the protection obtained with 12 mmoles/kg of GSH is nearly equalled by a mixture of 4 mmoles/kg of cysteine plus 12 mmoles/kg of succinate.

Summary. During 5 ATA oxygen exposures: The GSH was the best single compound for protecting against oxygen toxicity. This appears to be a combined SH protection and metabolite (glutamate → GABA → succinate) protection. The SH group protection, as evidenced by the cysteine experiments, was not as effective as the metabolites: succinate, glutamate, and GABA. The acid-base buffer Tris was less effective than the SH group protectors, or GABA, glutamate, or succinate. Glucose and malate gave no protection against oxygen toxicity.

We thank Marvin and Julia Nunn for valuable technical assistance.

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Received June 27, 1969. P.S.E.B.M., 1969, Vol. 132