1972

# PARGYLINE, SUCCINIC ACID AND L-ASCORBIC ACID AS PROTECTIVE AGENTS AGAINST HYPERBARIC OXYGEN TOXICITY: POSSIBLE INVOLVEMENT OF ALTERED GAMMA-AMINOBUTYRIC ACID AND AMMONIA METABOLISM

Robert Alfred Schatz
*University of Rhode Island*

# PARGYLINE, SUCCINIC ACID AND L-ASCORBIC ACID AS PROTECTIVE AGENTS AGAINST HYPERBARIC OXYGEN TOXICITY: POSSIBLE INVOLVEMENT OF ALTERED GAMMA-AMINOBUTYRIC ACID AND AMMONIA METABOLISM

BY

ROBERT ALFRED SCHATZ

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PHARMACEUTICAL SCIENCES

(PHARMACOLOGY AND TOXICOLOGY)

UNIVERSITY OF RHODE ISLAND

1972

DOCTOR OF PHILOSOPHY THESIS

OF

ROBERT ALFRED SCHATZ

Approved:

Thesis Committee:

Chairman _____

_____

_____

_____

_____

Dean of the Graduate School

UNIVERSITY OF RHODE ISLAND

1972

TITLE ABSTRACT

POSSIBLE MECHANISMS OF OHP TOXICITY

# ABSTRACT

Schatz, Robert Alfred. Ph. D., University of Rhode Island, June, 1972. Pargyline, Succinic Acid and L-Ascorbic Acid as Protective Agents Against Hyperbaric Oxygen Toxicity: Possible Involvement of Altered Gamma-Aminobutyric Acid and Ammonia Metabolism.

The mechanisms whereby pargyline, succinic acid and ascorbic acid protect against hyperbaric oxygen convulsions, pulmonary damage and mortality following hyperbaric oxygen exposure were investigated in relation to the effects of these agents on hyperbaric oxygen-induced alterations in brain gamma-aminobutyric acid and ammonia metabolism.

Exposure to hyperbaric oxygen (60 pounds per square inch, gauge pressure) produced convulsions, post-exposure lethality and elevation in lung weight, lung water content and lung hemoglobin content. Pargyline, succinic acid and ascorbic acid provided partial protection against all of the above aspects of oxygen toxicity.

Comparison of the doses of pargyline, succinic acid and ascorbic acid that provided maximal protection against hyperbaric oxygen convulsions showed that all three agents were approximately equal, in the doses used, with respect to their effectiveness in decreasing the incidence of hyperbaric oxygen convulsions and increasing the latency to their onset. Pargyline and succinic acid were approximately equal, in the doses used, in their ability to reduce post-exposure lethality and hyperbaric oxygen-induced increases in lung weight, water content and hemoglobin content. Ascorbic acid was less effective in its ability to reduce the above changes induced by hyperbaric oxygen exposure.

Hyperbaric oxygen decreased brain gamma-aminobutyric acid levels. This decrease was due to inhibition of glutamic acid decarboxylase without any effect on gamma-aminobutyric acid transaminase activity. Both pargyline and succinic acid increased brain gamma-aminobutyric acid levels in room air-exposed mice and prevented the decrease in brain gamma-aminobutyric acid produced by hyperbaric oxygen exposure. Pargyline increased gamma-aminobutyric acid levels by inhibiting gamma-aminobutyric acid transaminase activity in room air- and hyperbaric oxygen-exposed mice. Succinic acid increased the activity of both glutamic acid decarboxylase and gamma-aminobutyric acid transaminase, glutamic acid decarboxylase to a greater degree than gamma-aminobutyric acid transaminase. Glutamic acid decarboxylase activity was still slightly increased in mice exposed to hyperbaric oxygen. These alterations in enzyme activity were responsible for the ability of pargyline and succinic acid to increase brain gamma-aminobutyric acid levels in room air-exposed mice and to prevent the hyperbaric oxygen-induced decrease in brain gamma-aminobutyric acid in animals exposed to hyperbaric oxygen. There was a significant correlation between hyperbaric oxygen seizure susceptibility and brain gamma-aminobutyric acid levels in pargyline- or succinic acid-treated mice.

In pargyline- or succinic acid-treated mice there was a dose-response relationship between amount of drug administered and degree of elevation in brain gamma-aminobutyric acid.

Ascorbic acid had no effect on brain gamma-aminobutyric acid levels nor did it prevent the hyperbaric oxygen-induced decrease in brain gamma-aminobutyric acid. Ascorbic acid treatment had no effect on glutamic acid

decarboxylase or gamma-aminobutyric acid transaminase activities nor did it prevent the hyperbaric oxygen-induced decrease in glutamic acid decarboxylase activity.

Hyperbaric oxygen exposure increased brain ammonia levels and decreased brain glutamine levels. All three agents reduced these hyperbaric oxygen-induced alterations. There was a correlation between brain ammonia levels and hyperbaric oxygen seizure susceptibility in mice treated with pargyline, succinic acid or ascorbic acid. The same was true for glutamine levels and hyperbaric oxygen seizure susceptibility.

These results demonstrate that decreased gamma-aminobutyric acid levels and increased ammonia levels in the brain are involved in the etiology of hyperbaric oxygen toxicity. The effectiveness of the three agents tested as protective agents against hyperbaric oxygen toxicity appears related to their ability to alter the hyperbaric oxygen-induced changes in brain ammonia and gamma-aminobutyric acid metabolism.

The relationship between hyperbaric oxygen-induced alterations in gamma-aminobutyric acid and ammonia metabolism and the effects of pargyline, succinic acid and ascorbic acid thereon are discussed with respect to mitochondrial function and consequent adenosine triphosphate production.

To: A j h

# ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his wife, Anne, for her understanding, patience and thoughtfulness.

The author also wishes to express his gratitude to his parents for their understanding, patience and thoughtfulness.

The author conveys his sincere appreciation to Drs. Harbans Lal, John J. DeFeo, George C. Fuller and Gary P. Carlson for their guidance throughout the duration of this study.

The author wishes to convey his thanks to his fellow students in the Department of Pharmacology for their many helpful suggestions during these studies.

Financial support for this investigation was provided by PHS Training Grant No. 1 T01ES 00104 from the Division of Environmental Health Sciences through the Institute of Environmental Biology at the University of Rhode Island.

## LIST OF ABBREVIATIONS USED

| | |
|---|---|
| ATP | adenosine triphosphate |
| CNS | central nervous system |
| DA | dopamine |
| GABA | gamma-aminobutyric acid |
| GABA-T | gamma-aminobutyric acid transaminase (EC 2.6.1.19) |
| GAD | L-glutamic acid decarboxylase (EC 4.1.1.15) |
| GLDH | L-glutamic acid dehydrogenase (EC 1.4.1.2) |
| 5-HT | serotonin |
| α-KG | alpha-ketoglutaric acid |
| OHP | hyperbaric oxygen |
| MAO | monoamine oxidase (EC 1.4.3.4) |
| NADH | reduced nicotinamide adenine dinucleotide |
| NADP | nicotinamide adenine dinucleotide phosphate |
| NE | norepinephrine |
| psig | pounds per square inch (gauge pressure) |
| Pyr P | pyridoxal phosphate |

TABLE OF CONTENTS

Page

# LIST OF TABLES

List of Tables (continued)



APPENDIX TABLES

# LIST OF FIGURES

List of Figures (continued)

# I. INTRODUCTION

With man's increasing quest for knowledge and search for natural resources other than those found on land, it is becoming more and more likely that man will encounter greater than normal partial pressures of oxygen in various situations such as diving operations, space travel and use of hyperbaric oxygen in medicine.

In the field of medicine, OHP is being employed in the treatment of myocardial infarction (Peter et al., 1966) and shock (DelGuercio et al., 1966). Use of OHP to extend the safe-period of circulatory arrest during surgery (Meijne, 1966; Moor et al., 1966), particularly of congenital cardiac defects (Berrhard et al., 1966), has been considered a life-saving procedure. OHP has also been used in the treatment of anaerobic infections (Hitchcock et al., 1967; Jones, 1967; Brummelkamp, 1966), in burn therapy (Taber, 1967; Nelson et al., 1966), in cancer chemotherapy (Churchill-Davidson, 1966; Nathanson et al., 1966) and in treatment of carbon monoxide and cyanide poisoning (Norman et al., 1970; Shene et al., 1966).

For these reasons, man has become increasingly aware of the possible value of OHP in many aspects of modern life. Man has, however, also become aware of the toxic effects of OHP, which include muscle fasciculations, convulsions, pulmonary damage and post-exposure lethality.

In recent years, investigators have begun to explore the mechanism(s) of this toxic syndrome by using, as tools, drugs that ameliorate one or more of the aspects of OHP toxicity. This approach will

provide us with further insight and information regarding the cellular mechanism(s) of OHP toxicity. Further, a drug capable of providing protection against OHP toxicity will be of immense value in that it will permit wider and more frequent use of OHP in a variety of clinical conditions and may also prevent any toxic effects occurring during the course of undersea and aerospace exploration.

We have selected to study the effects of three agents. The protective effects of two of these agents against some of the aspects of OHP toxicity are well documented. These two agents are pargyline (Blenkarn et al., 1969; Faiman et al., 1971) and succinic acid (Sanders et al., 1969). Since preliminary investigations revealed that L-ascorbic acid protected against OHP convulsions and since very little previous work had been done with this agent in the area of OHP toxicity, we included ascorbic acid in our investigation.

The protective effect of pargyline against OHP convulsions was originally thought to be due to the ability of this agent to elevate brain amines (NE, 5-HT and DA) since it had previously been shown that OHP exposure decreased brain NE and 5-HT (Faiman and Heble, 1966). Subsequent investigation revealed that the protective effect of the MAO inhibitors was not altered by amine precursors or amine synthesis inhibitors (Blenkarn et al., 1969; Faiman et al., 1971). The conclusion reached by both groups of investigators was that the protective effect of MAO inhibitors was unrelated to altered turnover rates or absolute brain levels of these amines (Blenkarn et al., 1969; Faiman et al., 1971).

Various MAO inhibitors have been shown to elevate brain GABA levels (Balzer et al., 1960; Popov and Mattheis, 1969) and decreased

levels of brain GABA have been shown to be involved in the etiology of OHP convulsions (Wood, 1970). There have been many reports in recent years concerning the role of GABA as an inhibitory transmitter in the CNS (Roberts, 1962; Kravitz, 1967). Aside from the fact that GABA's role as a CNS transmitter has not yet been clearly elucidated, it has been shown to have definite neuronal inhibitory properties in the CNS (Roberts, 1962; Kravitz, 1967) and by virtue of this, may be expected to play a role in the etiology of convulsive episodes. In brief, the main points that support this hypothesis are the facts that OHP decreased GABA, the decrease was specific for GABA and occurred prior to OHP convulsions and GABA administration prevented OHP convulsions (Wood, 1970).

It is now well accepted that ammonia is a convulsant agent (Wolfe and Elliott, 1962). Further, brain ammonia has been shown to be significantly elevated during OHP exposure (Gershenovich and Krichenskoya, 1954; Szam, 1969) and the greatest elevation occurred concurrently with the onset of OHP convulsions (Gershenovich and Krichenskoya, 1954). The MAO inhibitors may prevent the contribution of ammonia to the ammonia pool by preventing the enhanced catabolism of biogenic amines (Faiman and Heble, 1966) or inhibition of the Krebb's cycle by OHP (Dickens, 1962) which may reduce the flow of ammonia acceptors resulting in an elevation in brain ammonia.

This investigation was undertaken to investigate the effects of the aforementioned drugs on various aspects of OHP toxicity with respect to the possible involvement of altered brain GABA and/or ammonia metabolism in the protective phenomenon.

The hypotheses to be tested are:

1. The protective effect of pargyline, succinic acid and ascorbic acid against OHP toxicity is related to the ability of these agents to prevent the OHP-induced decrease in brain GABA levels. This effect on brain GABA is mediated by increased synthesis and/or decreased degradation of GABA.
2. The protective effect of pargyline, succinic acid and ascorbic acid is also related to the ability of these agents to prevent the OHP-induced elevation in brain ammonia and decrease in brain glutamine levels.
3. In addition to the protective effect of these agents on OHP convulsions, pargyline, succinic acid and ascorbic acid also protect against OHP-induced pulmonary damage and post-OHP mortality.

# II. LITERATURE SURVEY

## HYPERBARIC OXYGEN CONVULSIONS

The most dramatic manifestation of oxygen toxicity at pressures in excess of 2 to 3 ATA (33 to 66 ft) is the onset of convulsions. The first report concerning the effects of exposure to OHP was that of Bert (1878), in which he described in detail the incidence of convulsions in various animal species exposed to hyperbaric oxygen. This work was subsequently confirmed by numerous other investigators whose work has been reviewed by Bean (1945) and Haugaard (1968). The seizures take the form of generalized convulsions which are frequently, but not always, preceded by minor twitching of the head and forelimbs.

OHP convulsions have also been reported to occur in man (Behnke et al., 1934-5). Lambertsen (1965) described the course of progressive symptoms as follows:

> The convulsions are usually but not always preceded by the occurrence of localized muscular twitching, especially about the eyes, mouth and forehead. Small muscles of the hands may be involved and incoordination of diaphragm activity in respiration may occur. These phenomena increase in severity over a period which may vary from a few minutes to nearly an hour with essentially clear consciousness being retained. Eventually an abrupt spread of excitation occurs and the rigid tonic phase of the convulsion begins. The tonic phase lasts for about 30 secs and is accompanied by an abrupt loss of consciousness. Vigorous clonic contractions of the muscular groups of head and neck, trunk and limbs then occur becoming progressively less violent over about one minute.

## HYPERBARIC OXYGEN-INDUCED PULMONARY DAMAGE

Smith (1899) carried out the first extensive investigation on the effect of increased oxygen tensions on the lungs of animals. He showed that oxygen was a lung irritant producing inflammation and congestion and that these effects occurred at partial pressures of oxygen less than those required for the onset of convulsions. At pressures less than 0.7 ATA oxygen, damage occurred very slowly, if at all, and greater than 3 ATA, there was the overshadowing onset of convulsions. Clark and Lambertsen (1971) have recently reviewed pulmonary oxygen toxicity.

The overt sign of lung damage in OHP-exposed animals was the occurrence of dyspnea (Gesell, 1923; Paine et al., 1941). Gross examination of lungs showed pulmonary damage consisting of extensive areas of hemorrhage and edema (Karsner, 1916; Karsner and Ash, 1917). Karsner (1916) reported the occurrence of congestion, edema, epithelial degeneration and desquamation, fibrin formation and pneumonia in rabbits exposed to 80-90 percent oxygen for 24-48 hours. Thickening and hyalinization of the walls of the pulmonary arteries was observed by Smith et al (1932), and the occurrence of atelectasis in the lungs of exposed animals has also been clearly established (Behnke et al., 1934; Van Den Brenk and Jamieson, 1962).

## POSSIBLE RELATIONSHIP BETWEEN HYPERBARIC OXYGEN-INDUCED CONVULSIONS AND PULMONARY DAMAGE

The studies of Smith (1899) demonstrated that pulmonary pathology occurred in the absence of OHP convulsions. Further, pulmonary damage occurred during relatively short periods of exposure to OHP (2-3 ATA) (Bean and Johnson, 1955), although the onset of seizures was the most dramatic sign of OHP toxicity at these high pressures (Stadie et al.,

1944). The observations of Johnson and Bean (1957) and Wood et al. (1965) that lung damage occurred in animals that had suffered severe seizures raised the possibility that there may be a cause and effect relationship between seizures and this rapidly developing pulmonary damage.

This possible relationship was discounted by Shilling and Adams (1933) when they found that pentobarbital eliminated seizures but not lung damage in OHP-exposed rats and that no lung damage occurred after chemically induced (strychnine) seizures. Jamieson and Van Den Brenk (1962), however, found pentobarbital to be as effective in reducing lung damage as it was in preventing the onset of seizures. Further, severe lung damage has been reported after seizures induced by thiosemicarbazide (Tennekoon, 1954) or pentylenetetrazol (Riechert, 1941). Bean et al. (1966) found that anaesthetic agents protected against both seizures and lung damage induced by chemical agents or OHP whereas sympatholytic agents protected against only pulmonary pathology. These investigators suggested that pulmonary damage was largely the result of some neuroendocrinogenic component of the seizure.

It is still not established whether slow or rapid OHP-induced pulmonary damage are brought about by the same mechanism, but microscopic examination of both types of lung revealed little qualitative difference between the two types of lung damage (Van Den Brenk and Jamieson, 1962). It may be that development of pulmonary damage at less than 2 ATA oxygen involves the sympathetic nervous system and that onset of seizures at higher oxygen pressures stimulates this portion of the nervous system thereby hastening the onset of lung damage.

EFFECT OF ALTERATION OF BRAIN AMINES ON SEIZURE SUSCEPTIBILITY

Schlesinger et al. (1965) found significant differences in brain 5-HT and NE in mice of certain seizure-susceptible strains (DBA/2J), relative to seizure-resistant mice (C57Bl/6J), at the age when seizure incidence was maximal (21 days). These investigators (Schlesinger et al., 1968) also observed that agents that lowered brain amines (reserpine, alpha-methyl-p-tyrosine, p-chlorophenylalanine) increased the susceptibility of mice to audiogenic seizures, while agents which increase brain amines (MAO inhibitors, 5-hydroxytryptophan) had a protective effect against audiogenic seizures. MAO inhibitors have been reported to inhibit various components of audiogenic seizures (Plotinkoff et al., 1963). Lehman (1967) observed that MAO inhibitors tended to protect against audiogenic seizures in mice, while reserpine increased their severity.

Reserpine, which releases brain amines, enhanced electroshock convulsions, while MAO inhibitors, which increase brain amines, protected against these convulsions (Prockop, et al., 1959). Exposure to OHP lowered NE in both mouse (Faiman and Heble, 1966) and rat (Haggendal, 1967; Haggendal, 1968) brain. Brain 5-HT was decreased in mouse (Faiman and Heble, 1966) and increased in rat (Haggendal, 1968). Reserpine shortened the time to onset of OHP convulsions in mice (Haggendal, 1968; Oliver et al., 1970), while the MAO inhibitors nialamide (Haggendal, 1968), iproniazid and pargyline increased the latency to OHP seizures (Blenkarn et al., 1969).

From the preceding it would appear that brain amines play a role in OHP-induced convulsions, but this was not found to be the case after subsequent investigation. Inhibitors of NE synthesis (alpha-methyl-p-

tyrosine) and 5-HT (p-chlorophenylalanine) lowered brain NE and 5-HT content but had no effect on OHP tolerance in rats (Blenkarn et al., 1969) or mice (Faiman et al., 1971). The amine precursors 5-hydroxytryptophan elevated 5-HT levels and 3,4-dihydroxyphenylalanine elevated NE and DA levels without producing any significant effect on OHP tolerance in rats (Blenkarn et al., 1969) or mice (Faiman et al., 1971). Inhibition of dopamine-β-oxidase (EC 1.14.2.1) by disulfuram elevated brain DA levels and increased OHP tolerance (Faiman et al., 1971). Our preliminary investigations, however, demonstrated that the dopamine-β-oxidase inhibitor diethyldithiocarbamate did not alter OHP tolerance. Further, since disulfuram, upon absorption, has been shown to be converted to its corresponding thiol diethyldithiocarbamate (Stromme, 1965) and since diethyldithiocarbamate did not provide any significant protection against OHP convulsions, it appears that the protective effect of disulfuram is not due to inhibition of dopamine-β-oxidase or subsequent elevation of brain DA levels.

Altered turnover rates of NE and 5-HT have also been implicated in the etiology of the OHP-induced convulsion (Faiman and Heble, 1966; Haggendal, 1967, 1968; Neff and Costa, 1967; Diaz et al., 1966). Blenkarn and coworkers (1969), however, concluded from their studies that a direct relationship between OHP central nervous system toxicity and 5-HT or NE turnover rates did not exist in rats. Since the findings of Faiman et al. (1971) in mice concur with those of Blenkarn et al. (1969), the same may be said to be true in the case of mice.

It appears, therefore, that no direct correlation exists between OHP convulsions and NE or 5-HT levels or turnover rates in rat or mouse

brain. As will be seen in the following section, this may also be true in the case of electroshock and audiogenic seizures.

GAMMA-AMINOBUTYRIC ACID AND HYPERBARIC OXYGEN

Wolfe and Elliott (1962) have reviewed the evidence for the role of GABA in convulsions induced under a wide variety of conditions. Although the role of GABA in the brain is not totally understood, a variety of evidence points toward its neuronal inhibitory properties (see Roberts, 1962). There is considerable evidence for the involvement of GABA in the causation of seizures induced by OHP exposure. This evidence can be summarized as follows:

1. Exposure of animals to OHP decreased brain GABA (Wood et al., 1963; Wood et al., 1967).
2. The decreased GABA and OHP convulsions were reversible (Wood and Watson, 1963).
3. The decrease in GABA occurred prior to convulsions (Wood and Watson, 1963).
4. Of the amino acids tested, the decrease was specific for GABA (Wood and Watson, 1963).
5. Susceptibility to OHP convulsions correlated well with the rate of decline in brain GABA for different species, different pressures of oxygen and different stages of development (Wood et al., 1967; Wood et al., 1969).
6. GABA administration protected animals against OHP convulsions (Wood et al., 1963; Wood et al., 1966; Wood and Watson, 1968; Wood, 1970).

The metabolic pathway of GABA is shown in Figure 1. Glutamic acid is metabolized to GABA by GAD and GABA is then converted to succinic semialdehyde by GABA-T. The final step in the pathway is the conversion

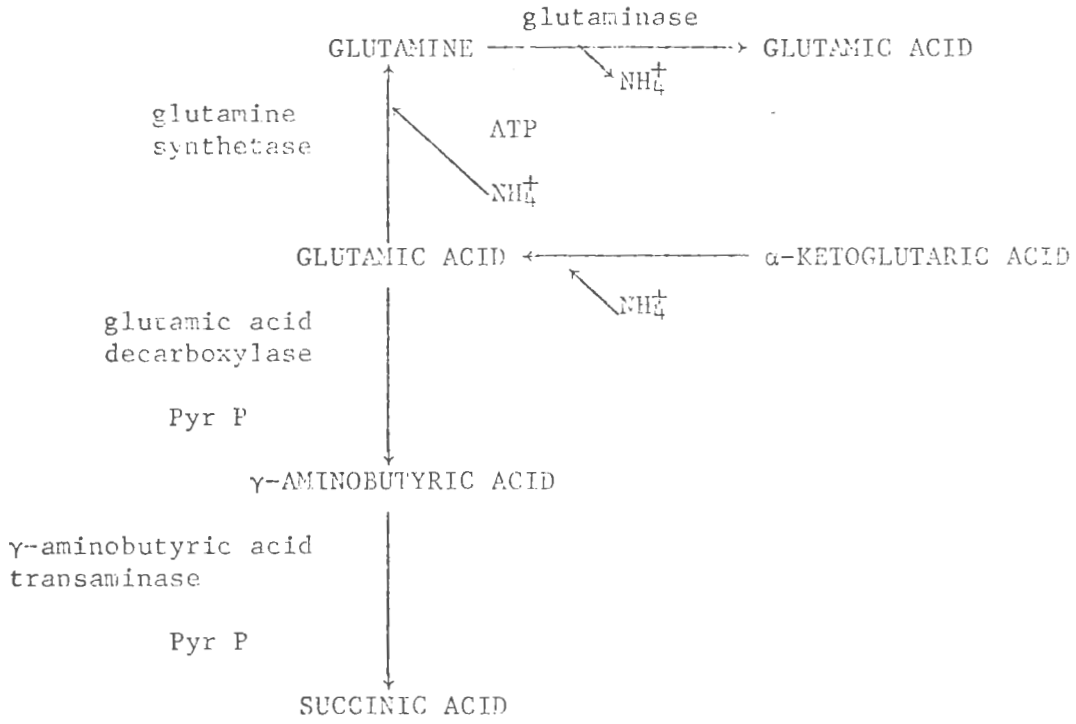


Figure 1. GAMMA-AMINOBUTYRIC ACID-GLUTAMINE PATHWAY IN BRAIN

of succinic semialdehyde to succinic acid by succinic semialdehyde dehydrogenase (EC 1.2.1.16). Both GAD and GABA-T are Pyr P dependent enzymes (see Baxter, 1970).

The decrease in brain GABA levels during OHP exposure has been shown to be due to inhibition of GAD (Wood et al., 1967; Myles and Wood, 1968). OHP had no effect on either GABA-T or succinic semialdehyde dehydrogenase (Wood et al., 1967; Myles and Wood, 1968).

Previously, phenelzine (Popov and Mattheis, 1969) and iproniazid (Balzer et al., 1960), two inhibitors of MAO structurally unrelated to pargyline, were reported to elevate brain GABA. The elevation in brain GABA after phenelzine was due to inhibition of the GABA degrading enzyme GABA-T (Popov and Mattheis, 1969). These drugs protect against OHP convulsions (Blenkarn et al., 1969; unpublished data). Reserpine decreased both brain GABA (Balzer et al., 1960) and the latency to OHP convulsions (Oliver et al., 1970). Iproniazid prevented both the decrease in the threshold to electroshock seizures and the decrease in brain GABA caused by reserpine (Balzer et al., 1960). Amino-oxyacetic acid elevated brain GABA, protected against audiogenic seizures (Schlesinger et al., 1968) and decreased the incidence of severe OHP convulsions (Wood and Watson, 1962).

From the preceding, it appears that maintenance of normal brain GABA levels is an important factor in the susceptibility of animals to audiogenic, electroshock or OHP seizures and that the protective effects of the MAO inhibitors on these three seizure types may be a consequence of their ability to maintain normal levels of brain GABA.

AMMONIA

The GABA precursor, glutamic acid, is also the precursor to

glutamine. Glutamine synthesis (see Figure 1) is the brain's ammonia detoxication mechanism and thus is important in maintaining the intracellular ammonia levels within a certain range (Katunuma et al., 1965).

As mentioned in the introduction, ammonia is a convulsant agent (see Dickens, 1962). Elevated brain ammonia levels produced by ammonium acetate (Ugarte et al., 1968) or ammonium chloride (Salvatore and Bocchini, 1961) caused convulsions and pulmonary edema (König and König, 1949). Ammonia levels were increased during OHP exposure (Gershenovich and Krichenskoya, 1954; Szam, 1969) and neutralized ammonium chloride tended to decrease the latency to OHP convulsions (Gottleib and Cymerman, 1966). Gershenovich and Krichenskoya (1954) found that the OHP-induced elevation in brain ammonia occurred concurrently with the onset of convulsions. They also noted a marked decrease in brain glutamine (Gershenovich and Krichenskoya, 1954).

The bases for increased levels of ammonia during OHP are not known. Enhanced catabolism of biogenic amines in the brain (Faiman and Heble, 1966) may contribute some ammonia to the ammonia pool. Inhibition of the citric acid cycle by OHP (see Dickens, 1962) may reduce the flow of ammonia acceptors resulting in an increase in brain ammonia. Increased brain ammonia was reported when the citric acid cycle was interrupted by fluoroacetate poisoning (Benitez et al., 1956). Inhibition of the glutamine forming enzyme, glutamine synthetase (EC 6.3.1.2), by methionine sulfoximine has been shown to result in convulsions (Warren and Shenker, 1964). These investigators and Folbergrova (1964) reported that methionine sulfoximine administration led to a marked and sustained decrease in cerebral glutamine and a corresponding rise in ammonia levels. This may also be the case in the OHP-induced increase in brain ammonia since

it has been shown that OHP inhibits glutamine synthetase (Gershenovich et al., 1963).

In the CNS, the synthesis of glutamine exhibits priority over other energy-consuming reactions and the synthesis of glutamic acid proceeds in spite of the risk of a depletion of dicarboxylic acids, suggesting that the brain must prevent intracellular accumulation of ammonia even at a high cost (see Weil-Malherbe, 1962).

Iproniazid has been shown to decrease the elevated ammonia levels in the blood of patients with severe hepatic damage (Pletscher et al., 1960). Further, isoniazid, a structural analogue of iproniazid possessing no MAO inhibitory activity, does not decrease the elevated ammonia levels in the blood of these patients (Pletscher et al., 1960) nor does it protect against OHP toxicity (Blenkarn et al., 1969).

From the preceding, it appears that there is a relationship between elevated ammonia levels and OHP convulsions. Possibly, MAO inhibitors may alleviate OHP toxicity by preventing this elevation.

SUCCINIC ACID

OHP has been shown to decrease the concentration of ATP in rat liver, kidney and brain (Sanders et al., 1966). ATP is considered to be the primary source of chemical energy in brain tissue and is necessary for maintenance of electrical activity and the "sodium pump" (Kramer et al., 1968). Consideration of these facts makes it apparent that the decrease in brain ATP levels during OHP exposure may be important in the etiology of OHP convulsions. This decrease in ATP may account for inhibition of glutamine synthetase (Gershenovich et al., 1963) referred to in the previous section which considered ammonia involvement in OHP toxicity.

The rationale for using succinic acid as a protective agent against OHP convulsions lies in this agent's ability to provide reducing equivalents for the mitochondrial electron transport chain with subsequent production of ATP. Succinic acid prevented the OHP-induced decrease in cerebral ATP and decreased the incidence of OHP convulsions (Woodhall et al., 1971) thus supporting the hypothesis that decreased ATP levels are involved in the etiology of OHP seizures.

It is, however, possible that the decreased brain ATP levels in themselves are not responsible for OHP convulsions and that the actual cause of the seizure is a decrease in brain GABA and/or elevation in brain ammonia levels secondary to the ATP decrease.

For example, the previously mentioned OHP-induced decrease in brain glutamine synthetase activity (Gershenovich et al., 1963) may be due to decreased ATP levels since this enzyme is ATP dependent. This enzyme inhibition could then result in the previously reported elevation of brain ammonia levels seen during OHP exposure (Gershenovich and Krichenskoya, 1954; Szam, 1969).

Decreased GABA levels during OHP exposure are due to inhibition of GAD (Wood et al., 1967; Myles and Wood, 1968) and GAD is a Pyr P requiring enzyme (Baxter, 1970). Since pyridoxal kinase (EC 2.7.1.35) requires ATP (Baxter, 1970), the inhibition of GAD and consequent decrease in brain GABA during OHP exposure could be due to inhibition of conversion of the Pyr P cofactor to its active form by pyridoxal kinase.

For this reason and since no one, to our knowledge, has investigated the effects of succinic acid on GABA or ammonia metabolism, we studied the effects of succinic acid on OHP toxicity in relation to GABA and ammonia metabolism.

L-ASCORBIC ACID

In an attempt to reproduce the data of Oliver et al. (1970) which showed that reserpine decreased the latency to OHP convulsions, it was found that ascorbic acid markedly decreased the incidence of OHP convulsions. A 20% solution of L-ascorbic acid had been used to dissolve the reserpine base and it was found that the vehicle-treated mice were protected against OHP seizures.

It has been shown that OHP exposure markedly decreased adrenal gland ascorbic acid content (Gerschman and Fenn, 1954) as did other types of stress such as cold or hypoxia (Sayer and Sayer, 1949). It has also been demonstrated that ascorbic acid protected against OHP-induced pulmonary damage but exhibited no protection against OHP convulsions (Jamieson and Van Den Brenk, 1964).

We, therefore, undertook to investigate the effect of ascorbic acid on OHP toxicity in relation to GABA and ammonia metabolism in the brain.

# III. EXPERIMENTAL

## ANIMALS

Swiss albino, random-bred male mice, weighing 25-35 g (Charles River Breeding Laboratories, Wilmington, Mass.) were used throughout this study. They were housed in animal quarters maintained at 21-23°C with room lights alternated on a 12 h light-dark cycle. Commercial laboratory chow and water were available ad libitum up to one hour before each experiment. Animals were used not earlier than one week after receipt from supplier via commercial shipper.

## MATERIALS

Analytical grade chemicals or equivalent were used throughout the study. Pargyline was supplied by Abbott Laboratories, North Chicago, Ill. Sodium succinic acid hexahydrate was obtained from Cal-Biochem (Los Angeles, Calif.) and L-ascorbic acid from Fisher Chemical Co. (Fairlawn, N. J.). NADP was obtained from P-L Biochemicals Inc., Milwaukee, Wisc. and NADH from Cal-Biochem. GLDH (ammonia-free), GABase and glutaminase (EC 3.5.1.2) were obtained from Worthington Biochemicals, Freehold, N. J. L-glutamic acid-1-C-14 (sp. act. 52 mCi/mM) was obtained from Cal-Atomic, Los Angeles, Calif. The cyanomethemoglobin assay system was obtained from Hycel Research Products, Houston, Texas. Pyridoxal phosphate was obtained from Cal-Biochem. Hyamine (10-X) was obtained from New England Nuclear, Boston, Mass. All reagents, buffers and dilutions were made with deionized water.

## HYPERBARIC OXYGEN CHAMBER

The hyperbaric chamber (Figure 2) was designed by Van Tassel (1965) and constructed with slight modification by the University of Rhode Island Instrument Shop. Modifications included stainless steel endplates, an inch-thick plexiglass cylinder (ten in I.D.) two feet in length and an elliptical door of one-inch-thick aluminum. The chamber contained three ft of 1/8 in copper tubing to allow circulation of water for maintenance of a constant temperature (25 ± 1°C). Soda lime and calcium chloride were placed in the bottom of the chamber to absorb carbon dioxide and moisture, respectively.

## EXPOSURE TO HYPERBARIC OXYGEN

Mice contained in individual plexiglass containers (3 x 3 x 3 in) with perforated sides and tops to allow free circulation of chamber air, were placed in the OHP chamber. The chamber was flushed with 100% oxygen (USP) for three min after which the chamber was compressed to five atmospheres (60 psig) at the rate of 30 psig/min (two min). Decompression was achieved in 15 min (three psig/min). During the studies the flow rate was maintained at three liters/min. OHP exposures were for a period of 90 min including flush, compression and decompression.

## EXPERIMENTAL DESIGN

Mice were divided into four groups: control (vehicle-treated), drug (pargyline, L-ascorbic acid or succinic acid-treated), OHP exposed plus vehicle-treated and OHP exposed plus drug-treated.

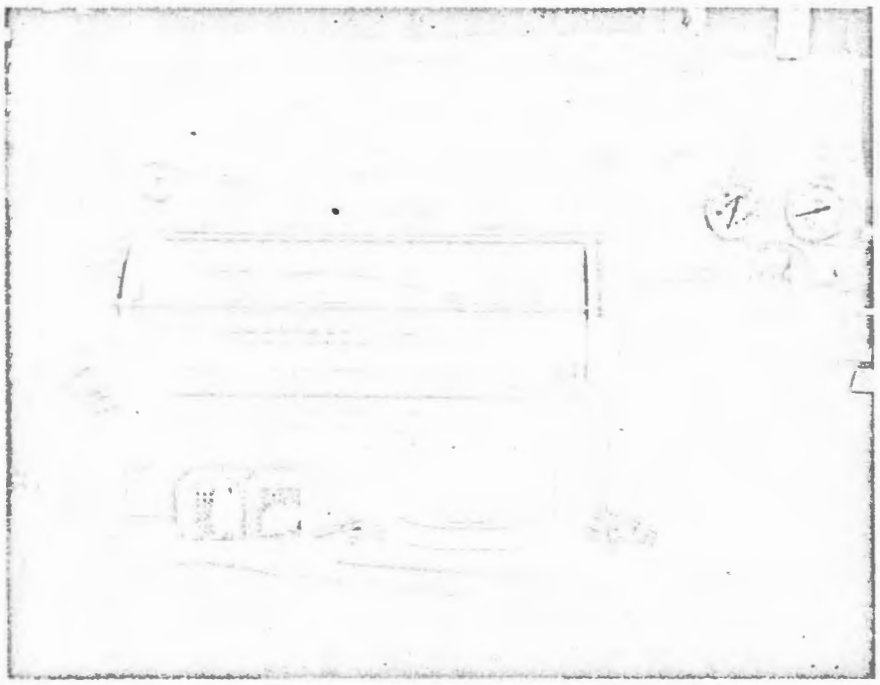

Figure 2. HYPERBARIC OXYGEN CHAMBER

PARAMETERS OF HYPERBARIC OXYGEN TOXICITY

Convulsions: Time to onset of convulsion was measured as time to onset of any convulsive movement. Time to onset of a seizure-complex was considered as time to onset of convulsions consisting of tonic posture, loss of righting reflex (supine posture on side or back) or other seizure lasting 20 sec or more.

Pulmonary damage: After OHP exposure, both lungs were excised, weighed, placed in tared containers and dried to a constant weight at 75°C. Values were reported as lung weights, as percent body weight $(\frac{\text{lung wet weight}}{\text{body weight}} \times 100)$ and percent water content of lungs $(\frac{\text{lung wet weight - lung dry weight}}{\text{lung wet weight}} \times 100)$. Lung hemoglobin was determined by the cyanomethemoglobin method of Hainline (1958). The Hycel standard contained 80 mg cyanomethemoglobin/100 ml. A preweighed vial of Hycel cyanomethemoglobin reagent was dissolved in water to make one liter of solution. Lungs were excised, weighed and homogenized in 25 volumes of ice-cold Hycel reagent. Samples were centrifuged at 10,000 rpm (Sorvall refrigerated centrifuge, rotor SS-34) for ten min as were Hycel standards. One ml of supernatant was mixed with two ml of reagent and read vs reagent at 540 mμ on a Beckman DB-G grating spectrophotometer. Values were reported as hemoglobin content (mg) of both mouse lungs.

Lethality following hyperbaric oxygen exposure: Mortality was recorded at 30 min intervals for the first four hours after OHP exposure. Mortality was then periodically recorded for up to 96 h after OHP exposure.

DRUG DOSAGE

Pargyline, succinic acid and L-ascorbic acid were administered

intraperitoneally at several doses and time intervals prior to OHP exposure. The dose and time that afforded maximal protection against OHP convulsions was used in the pulmonary toxicity and mechanistic studies. All drugs were prepared just prior to use. Drug solutions were made in saline and 10 ml/kg was administered. Drug solutions adjusted to pH 6.5 with 2N NaOH or 2N HCl were also administered at the dose and time intervals affording maximal protection in order to determine whether or not pH was a factor in the protective effect of any of the three drugs.

MEASUREMENT OF GAMMA-AMINOBUTYRIC ACID

The assay procedure was that of Graham and Aprison (1966). Mice were frozen in liquid nitrogen and kept at -40°C for 20-24 h. The heads were then removed with an ice-cold hacksaw and cut longitudinally. Whole brains were rapidly removed (45 sec), weighed and homogenized in 20 volumes of ice-cold 75% (v/v) ethanol. Samples were then centrifuged at 5,000 rpm at 0°C for 15 min. The supernates were removed and stored at 4°C while the pellet was resuspended in 20 volumes of 75% ethanol and centrifuged as above. Both supernate fractions were then combined and mixed thoroughly. Two ml samples were added to sample vials and evaporated to dryness at 60°C in a water bath with constant air flow into each vial. Samples were kept at -40°C for 24 h at which time GABA was measured. The contents of each vial were suspended in five ml of water. Two ml of sample was shaken with an equal volume of chloroform and centrifuged in a clinical centrifuge (International Equipment Co.) at 3500 rpm for 20 min.

The substrate solution[1] contained 1.4 ml pyrophosphate buffer (0.1 M, pH 8.4), 0.2 ml NADP (0.005 M in water), 0.2 ml alpha-ketoglutaric acid (0.1 M in buffer), 0.2 ml mercaptoethanol (0.06 M in buffer) and ten mg GABase enzyme. Incubation mixtures contained 0.1 ml brain sample, standard or water; 0.1 ml pyrophosphate buffer and 0.04 ml substrate solution. Tissue blanks were prepared in the same manner except that no GABase was added. Incubations were for 45 min at 37°C in a Dubnoff metabolic shaker. After incubation, 0.2 ml alkaline phosphate[2] was added to the tubes and mixed thoroughly. Tubes were heated at 60°C for 15 min, then 0.2 ml of $NaOH-H_2O_2$[3] was added, mixed thoroughly and the tubes were heated at 60°C for ten min. One ml of water was added to the tubes which were then mixed thoroughly and the fluorescence was read immediately in an Aminco-Bowman spectrophotofluorometer (excitation 360 mμ, emission 460 mμ, sensitivity setting at 50 and slit width of 1/64 in). Values were reported as μmoles GABA/g brain (wet weight).

MEASUREMENT OF GAMMA-AMINOBUTYRIC ACID TRANSAMINASE ACTIVITY

The GABA-T assay procedure was that of Salvador and Albers (1959). Brains were removed rapidly with the aid of other investigators, weighed

---

[1]Prepared by dissolving 4.46 g $Na_2P_2O_7-10H_2O$ in 80 ml water. Norit-A was added and the solution stirred for 15 min after which the solution was filtered through Celite. The pH was adjusted to 8.4 with 1N HCl and the volume adjusted to 100 ml. The buffer was stored in an amber bottle at 4°C and was prepared fresh 24 h prior to each assay.

[2]Prepared by dissolving 15.2 g $Na_3PO_4-12H_2O$ and 2.68 g $Na_2HPO_4$ in sufficient water to make 100 ml. Solution was stored in an amber bottle at room temperature.

[3]Prepared fresh just prior to use by adding 0.2 ml 3% $H_2O_2$ to ten ml 10 N NaOH.

and homogenized in three volumes of ice-cold 1.25% Triton X-100. Five ml of GABA-α-KG[1] mixture was added to 0.2 ml of homogenate. One-half ml samples of this mixture were added to 0.5 ml of DAB reagent[2] (Salganicoff and DeRobertis, 1965) and stored on ice for use as tissue blanks. The removal of brains, weighing and homogenization procedures took no longer than 45 min for 20 mice.

The succinic semialdehyde standard was prepared according to Prescott and Waelsch (1946). Twenty mg ninhydrin was mixed with 1.4 ml glutamic acid solution (0.001 g/ml in 0.5N acetic acid) after which time 2.6 ml of 0.5N acetic acid was added. Reagent blanks contained four ml of 0.5N acetic acid and 20 mg ninhydrin. Reagent blanks and standards were placed in boiling water for ten min. After the tubes were cooled in ice, the following reagents were added at five min intervals: 0.4 ml guanidine carbonate (14% in water), one ml lead acetate (12% in water, prepared fresh each day), 0.5 ml of 5N NaOH and 0.1 ml water. Tubes were centrifuged at 3,000 rpm for ten min in a clinical centrifuge. After centrifugation, 0.5 ml aliquots of reagent blank and standard were added to 0.5 ml of DAB reagent, mixed and stored on ice.

The GABA-α-KG mixture containing brain homogenate was incubated in a Dubnoff metabolic shaker under room air at 37°C for 60 min. After incubation, 0.5 ml aliquots were added to 0.5 ml DAB reagent.

---

[1]Prepared by dissolving 2.575 g GABA and 1.46 g α-KG in 50 ml water. The pH was brought to 8.4 with 5N NaOH and the volume adjusted to 100 ml with water. This mixture was prepared fresh daily and was 0.25 M in GABA and 0.1 M in α-KG.

[2]Prepared by dissolving 2.8 g 3,5-diaminobenzoic acid HCl in 25 ml 1 M potassium phosphate buffer (pH 5.9) (1 vol of 1 M $K_2HPO_4$ plus 9 vol of 1 M $KH_2PO_4$). The pH was brought to 5.9 with 5N NaOH and adjusted to a final volume of 50 ml with phosphate buffer.

Samples, tissue blanks, reagent blanks and standards were heated at 60°C for 60 min. Nine ml water was added to each tube and tubes were centrifuged at 3,000 rpm for ten min. Forty µl of supernatant was mixed with ten ml water and samples were read in an Aminco-Bowman spectrophotofluorometer (excitation 405 mµ, emission 505 mµ, sensitivity setting at 50 and slit width of 1/64 in). Values were reported as µmoles succinic semialdehyde/g brain/h.

MEASUREMENT OF GLUTAMIC ACID DECARBOXYLASE ACTIVITY

The assay procedure was that of Roberts and Simonsen (1963). The C-14-L-glutamic acid substrate solution was made by combining one ml of 1-C-14-L-glutamic acid (25 microCi), 25 ml of 0.2 M L-glutamic acid adjusted to pH 6.5 with KOH, 10 ml of 5% (v/v) Triton X-100, 36 ml of 0.4 M potassium phosphate buffer[1] (pH 6.5) and five mg pyridoxal phosphate. This solution was adjusted to a final volume of 100 ml with 0.2M potassium phosphate buffer[2] (pH 6.5). The solution was $5 \times 10^{-2}$ M in 1-C-14 glutamate (sp. act. 5 µc/mmole), $2 \times 10^{-4}$ M in pyridoxal phosphate, 0.5% in Triton X-100 and 0.2 M in phosphate. The final pH was 6.5 and the mixture was stored in 25 ml portions at -40°C until use.

The procedure for collection of brain samples was as described in the GABA-T assay except that brains were homogenized in seven volumes of ice-cold deionized water. One ml of homogenate was added to a scintillation vial that was used as an incubation flask and one ml of

---

[1]Prepared by adding 0.4 M dibasic potassium phosphate to 0.4 M monobasic potassium phosphate until pH 6.5 was reached.

[2]Prepared by adding 0.2 M dibasic potassium phosphate to 0.2 M monobasic potassium phosphate until a pH of 6.5 was reached.

Hyamine (10-X) was added to another vial. The unit[1] was assembled and the homogenates were preincubated at 37°C for ten min during which time each flask was flushed with 100% nitrogen for two min. The reaction was started by the addition of one ml of substrate solution (warmed to 37°C) by means of a hypodermic syringe. The needles were immediately removed to seal the unit and the samples were incubated at 37°C in a Dubnoff metabolic shaker.

At the end of exactly 15 min, 0.2 cc of 4N sulfuric acid was added to stop the reaction. The units were allowed to shake for another 60 min to insure complete absorption of the C-14-$O_2$ by Hyamine (10-X). The counting vial was detached and ten ml of scintillation solution[2] was added. Radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer (25% gain setting). Internal standards of C-14 toluene (New England Nuclear) were added to every other sample for determination of percent efficiency. All samples were counted for ten min. Values were reported as μmoles $CO_2$/g brain/h $\left(\frac{\text{sample cpm - blank cpm x 32}}{\text{percent efficiency x 110}}\right)$. GAD catalyzes the conversion of one mole of L-glutamic acid to one mole of $CO_2$ and one mole of GABA.

---

[1]The unit consisted of two counting vials connected by a glass U tube (1/4 in I.D.). The vials and U tube were connected by means of 1/2 in lengths of high pressure tubing (soft rubber, 1/4 in I.D., 5/8 in O.D.). Two needles (23 gauge, 1 in) per unit were inserted through the tubing and removed after addition of C-14 substrate. Sulfuric acid was introduced through the tubing by means of a needle and syringe at the end of the incubation period. During pre-incubation and after nitrogen flush, needles were stoppered with 1/8 in O.D. glass tubing sealed at both ends.

[2]Prepared by dissolving 1 g POPOP (1,4-bis [2-(5-phenyloxazolyl)] benzene) and 0.1 g PPO (2,5 diphenyloxazole) in sufficient toluene to make one liter.

MEASUREMENT OF BRAIN AMMONIA

Brain samples were collected as described in GABA methods except that brains were homogenized in three volumes of ice-cold 1N perchloric acid. Samples were centrifuged at 15,000 rpm for 15 min at 0°C. One ml of supernate was adjusted to pH 5 with 2N KOH and samples were again centrifuged at 15,000 rpm for 15 min. This supernatant was used for determination of ammonia and glutamine. The assay procedure was that of Buttery and Roswell (1971). The substrate solution was made by combining 27 ml glycerol, 0.85 ml GLDH (100 mg/5 ml), 10 mg NADH, 0.121 g α-KG (dissolved in a small amount of buffer and adjusted to pH 7.8 with 1N NaOH). The volume of this solution was adjusted to 100 ml with pH 7.8 potassium phosphate buffer[1] (0.133 M).

This mixture contained $8.3 \times 10^{-3}$ M α-KG, 0.1 M phosphate, 0.166 mg/ml GLDH, 0.1 mg/ml NADH and 0.34 g/ml glycerol and was pH 7.8. This solution was pre-incubated at 37°C for 30 min in order to remove endogenous ammonia. A 2.6 ml portion of this pre-incubated mixture was added to 0.4 ml of brain extract and incubations were resumed at 37°C for a further 45 min. Nonenzymic controls were run as follows: 0.4 ml samples were incubated at 37°C for 45 min with 2.6 ml of a pre-incubated mixture as described but with GLDH replaced by buffer. For each assay series enzymic and nonenzymic reagent blanks were also incubated in which 0.4 ml water replaced the tissue sample. All tubes were stoppered to prevent absorption of atmospheric ammonia. After the 45 min incubation period, six ml water was added to each tube, extinctions were measured at 340 mμ in a Beckman DB-G spectrophotometer and the ammonia

[1]Prepared by adding 0.133 M monobasic potassium phosphate to 0.133 M dibasic potassium phosphate until pH of 7.8 was reached.

content of each sample was calculated as follows:

| Incubation | Extinction, 340 mμ |
|---|---|
| Enzymic, water | A |
| Enzymic, sample | B |
| Nonenzymic, water | C |
| Nonenzymic, sample | D |

Ammonia content of sample = $(A - B) - (C - D) \times \frac{1}{6.22} \times 3$ μmole

The calculation utilized the molar extinction coefficient of NADH ($6.22 \times 10^3$ $cm^2$) and values were reported as μmoles ammonia/g brain (wet weight).

MEASUREMENT OF BRAIN L-GLUTAMINE

L-glutamine was measured by the method of Buttery and Roswell (1971). Measurements of glutamine utilized portions of the brain extracts prepared for the ammonia assay so that both ammonia and glutamine were simultaneously measured in each brain.

Samples of brain extract (0.2 ml) were incubated at 37°C for 90 min each with 0.4 ml of 0.1 M citrate-phosphate buffer[1], pH 4.9, and 0.2 ml glutaminase (2 mg/ml buffer). A 0.4 ml portion of each incubation was then added to 2.6 ml of pre-incubated enzymic ammonia assay mixture and maintained at 37°C for 45 min, diluted with six ml water and read at 340 mμ. This same procedure was followed in a control incubation with 0.2 ml water replacing the brain extract. The initial ammonia content of each sample was taken into account by including incubations containing 0.2 ml brain extract or 0.2 ml water in which buffer replaced glutaminase.

---

[1]Prepared by adding 0.1 M citric acid to 0.2 M disodium phosphate until a pH of 4.9 was reached (McIlvaine, 1921).

The L-glutamine content of each sample was calculated as follows:

| Initial incubation | Extinction 340 mμ after GLDH reaction |
|---|---|
| Glutaminase, water | W |
| Glutaminase, sample | X |
| No glutaminase, water | Y |
| No glutaminase, sample | Z |

$$\text{L-glutamine content of original 0.2 ml sample} = (W - X) - (Y - Z) \times \frac{2}{6.22} \times 3 \text{ μmole}$$

Again the calculation utilized the molar extinction coefficient of NADH. Values were reported as μmoles L-glutamine/g brain (wet weight).

## STATISTICAL METHODS

The Student "t" test for independent means and chi-square 2 x 2 contingency analysis (Dixon and Massey, 1969) were used to test for the significance of differences between control and experimental groups. Correlation coefficients were calculated using linear regression analysis (Dixon and Massey, 1969). Tests were performed on an Olivetti Underwood Programma 101 desk computer and the level of significance was determined by comparison of "t" and chi-square values with values from standard tables.

# IV. RESULTS

## PARGYLINE

Pilot studies were conducted to ascertain which pretreatment time and dose of pargyline produced the largest decrease in the incidence of OHP convulsions. The results of this preliminary study are found in Table 1 of the appendix. The optimal effect in decreasing OHP convulsions was provided by the intraperitoneal injection of 100 mg/kg pargyline 30 min prior to OHP exposure (apperdix Table 1). The incidence of OHP convulsions was significantly decreased by this dose of pargyline (Figure 3). Both the latency to convulsion onset and the latency to seizure complex were prolonged by prior pargyline treatment (Figure 3). Further, the percentage of animals that had convulsed at the end of the 90 min OHP exposure was 40% and 20% for convulsion onset and seizure complex, respectively, in pargyline-treated mice, whereas all vehicle-treated mice had convulsed prior to the end of the OHP test period (Figure 3). That this decreased incidence of OHP convulsions was not due to the pH of the pargyline solution (pH 3.8) is shown by the fact that a pargyline solution adjusted to a pH of 6.5 was as effective in increasing the latency to OHP seizures and decreasing their incidence as was the more acidic pargyline solution (appendix Table 1).

Pargyline also significantly decreased post-OHP mortality (Figure 4). After OHP exposure, 90% of the control mice died within four h as opposed to a ten% mortality in the case of the pargyline-treated mice (Figure 4).

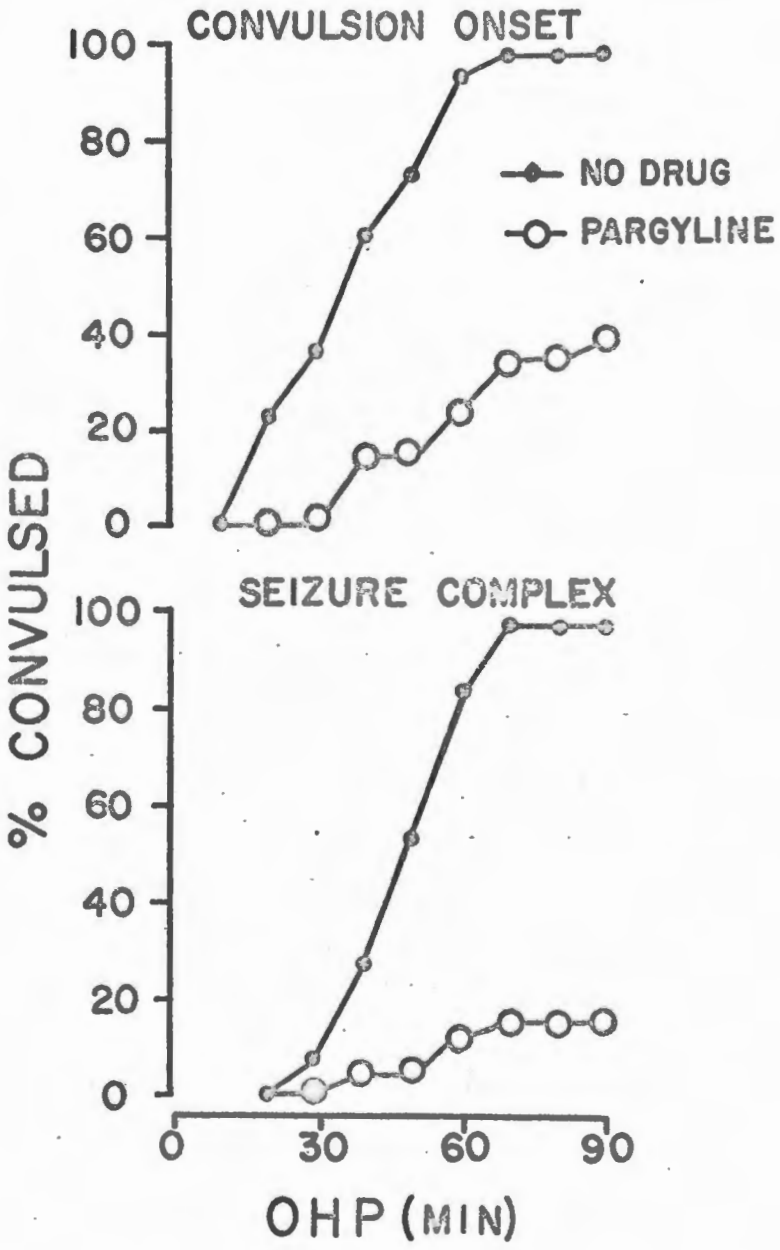


Figure 3. EFFECT OF PARGYLINE ON HYPERBARIC OXYGEN CONVULSIONS. ○- pargyline (100 mg/kg), ● - saline. Open symbols denote significant difference from corresponding saline value at the 0.05 level using chi-square test in groups of 26-30 mice.

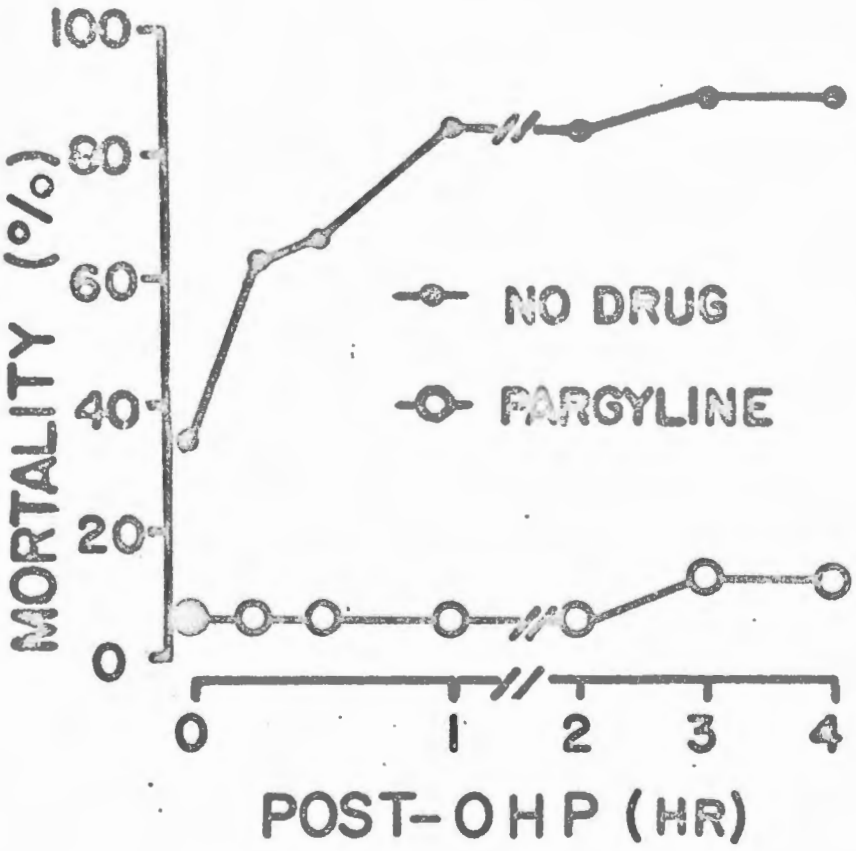


Figure 4. EFFECT OF PARGYLINE ON LETHALITY FOLLOWING HYPERBARIC OXYGEN EXPOSURE. ○- pargyline (100 mg/kg), ● - saline. Open symbols denote significant difference from corresponding saline value at the 0.05 level using the chi-square test in groups of 26-30 mice.

OHP significantly increased lung weight and lung water content (Table 1). Pargyline decreased these changes approximately to control levels (Table 1). There was also significantly less pulmonary hemorrhage in pargyline-treated mice as evidenced by the fact that OHP exposure elevated lung hemoglobin content by 98% in vehicle-treated mice as opposed to only the 15% increase seen in pargyline-treated mice (Table 2).

Brain GABA levels were significantly decreased (35%) after 90 min of OHP exposure (Figure 5). Pargyline significantly elevated brain GABA (42%) in room air-exposed mice and prevented the OHP-induced decrease in brain GABA levels (Figure 5). Since GABA levels were highest 120 min after pargyline injection (Figure 5), this time period was used for the dose-response curve (Figure 7). Actual means, standard errors, levels of significance and percent changes in brain GABA are shown in Table 2 of the appendix. There was a significant correlation between brain GABA levels and the susceptibility of the individual mice to OHP convulsions in that the vehicle-treated mice had lower GABA levels and shorter latencies to seizures than did the pargyline-treated mice (Figure 6). The correlation coefficients were 0.71 and 0.80 for latency to convulsion onset and latency to seizure complex, respectively (Figure 6). There was also a significant correlation ($r = 0.88$, $p < 0.0005$) between the dose of pargyline administered and the elevation in brain GABA levels (Figure 7). The increase in GABA was near its maximum at 100 mg/kg since increasing the dose of pargyline to 200 mg/kg produced only a further seven percent elevation in brain GABA (Figure 7). Actual means, standard errors, levels of significance and percent changes in brain GABA are shown in Table 3 of the appendix.

Table 1. EFFECT OF PARGYLINE ON HYPERBARIC OXYGEN-INDUCED ALTERATIONS IN LUNG WEIGHT AND LUNG WATER CONTENT

| Treatment[c] | Time (min) | Lung as % Body Weight (Mean ± S.E.)[d] | % Change | % Water Content (Mean ± S.E.)[d] | % Change[e] |
|---|---|---|---|---|---|
| Saline | 120 | 0.46 ± 0.014 | --- | 77 ± 0.4 | -- |
| Saline + OHP (60 psig) | 120<br>90 | 0.72 ± 0.065[a] | +57 | 81 ± 0.3[a] | +5 |
| Pargyline | 120 | 0.47 ± 0.016[b] | +2 | 76 ± 0.3[b] | -1 |
| Pargyline + OHP | 120<br>90 | 0.50 ± 0.011[a,b] | +9 | 79 ± 0.4[a,b] | +3 |

[a]Significantly different from saline controls at the 0.05 level using the Student "t" test.

[b]Significantly different from saline + OHP at the 0.05 level using the Student "t" test.

[c]Pargyline (100 mg/kg) or saline (10 ml/kg body weight) was injected intraperitoneally.

[d]Mean of six pairs of lungs.

[e]Percent change from saline controls. Plus represents increase and minus, decrease.

Table 2. EFFECT OF PARGYLINE ON HYPERBARIC OXYGEN-INDUCED ALTERATIONS IN LUNG HEMOGLOBIN CONTENT

| Treatment[b] | Time (min) | mg Hemoglobin/lung[a] (Mean ± S.E.) | % Change[e] |
|---|---|---|---|
| Saline | 120 | 8.55 ± 0.50 | -- |
| Saline + OHP (60 psig)[c] | 120<br>90 | 16.97 ± 2.41 | +98.5 |
| Pargyline (100 mg/kg) | 120 | 8.39 ± 0.40[d] | -1.8 |
| Pargyline + OHP | 120<br>90 | 9.85 ± 0.25[c,d] | +15.3 |

[a]Each value represents the mean of five pairs of lungs.

[b]Saline or pargyline (10 ml/kg body weight) was injected intraperitoneally. Dose is given in parentheses.

[c]Significantly different from saline controls at 0.05 level (Student "t" test).

[d]Significantly different from saline + OHP at the 0.05 level (Student "t" test).

[e]Plus indicates increase and minus, decrease.

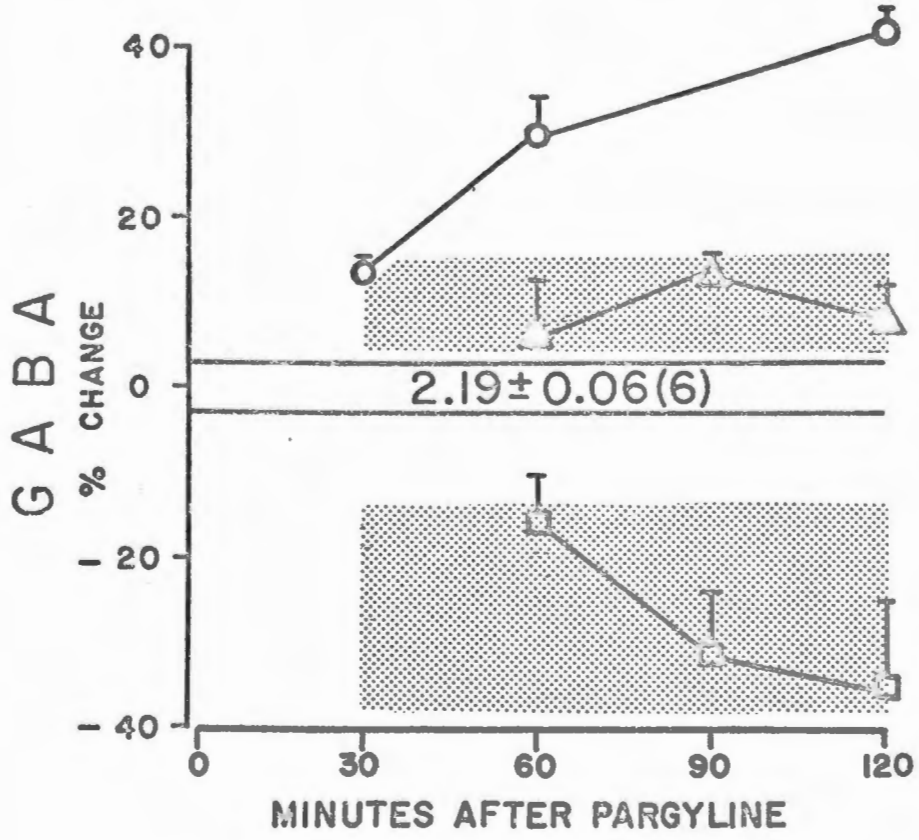


Figure 5. ALTERATION OF MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID BY PARGYLINE AND HYPERBARIC OXYGEN. Percent change is represented as mean ± S.E. Open symbols denote values significantly different from controls at 0.05 level using Student "t" test. GABA concentration of control animals is given in μmoles/g brain as mean ± S.E. ( ) represents number of mice used. ○- pargyline (100 mg/kg), □ - saline + OHP (60 psig). △ - pargyline + OHP.

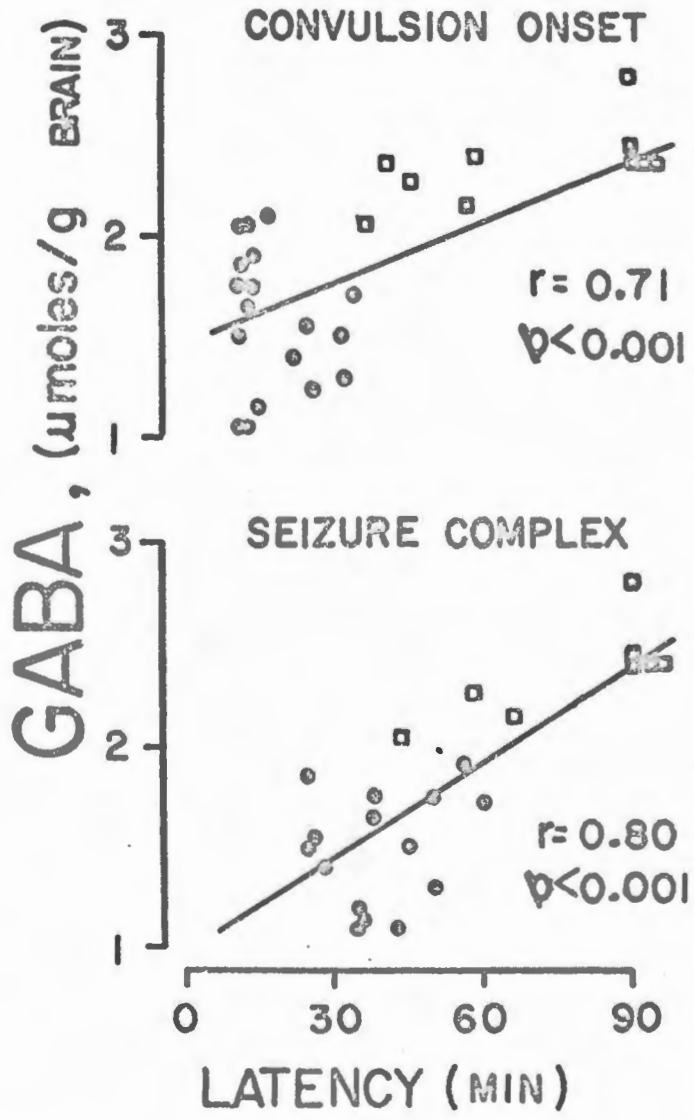


Figure 6. CORRELATION BETWEEN BRAIN GAMMA-AMINOBUTYRIC ACID AND SUSCEPTIBILITY TO HYPERBARIC OXYGEN SEIZURES IN PARGYLINE-TREATED MICE. ● - OHP, □ - pargyline. The animals not convulsing within 90 min were assigned an arbitrary convulsion latency of 90 min. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

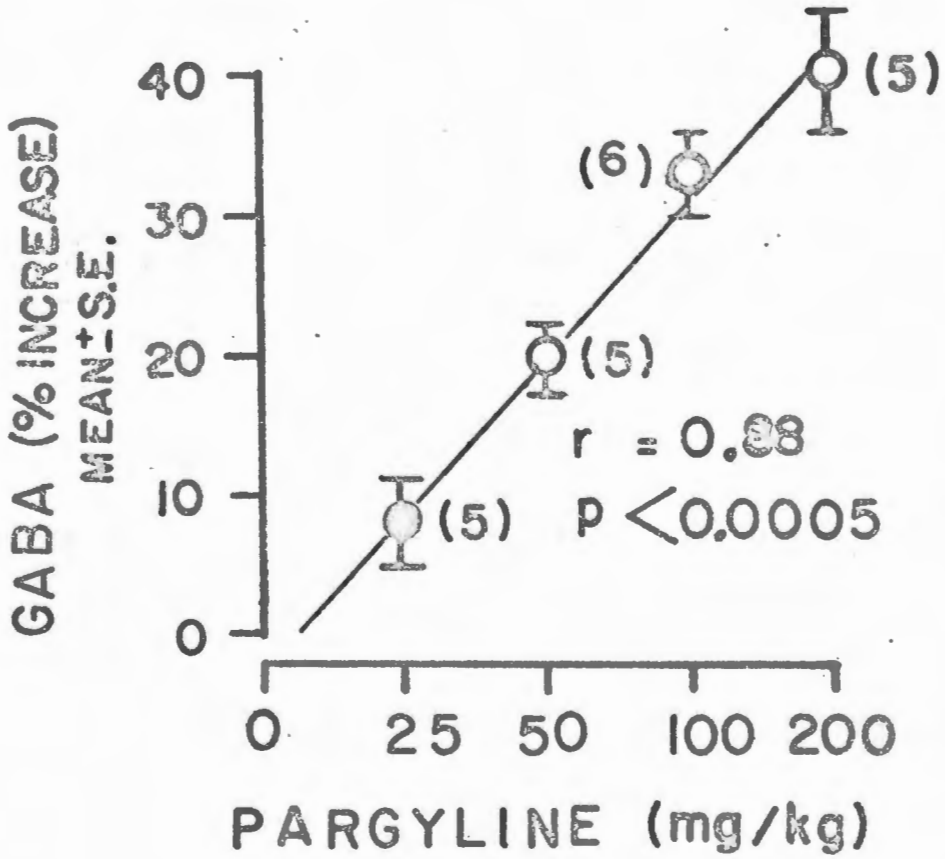


Figure 7. EFFECT OF VARIOUS DOSES OF PARGYLINE ON MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID LEVELS. Pargyline was administered 120 min prior to sacrifice. Each point represents the mean percent increase ± S.E. of duplicate samples. Number in parentheses represents number of animals used. Open symbols denote significant difference from controls at the 0.05 level using the Student "t" test. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

The activity of the GABA degrading enzyme, GABA-T, in mouse brain was not altered by OHP exposure (Tables 3, 8 and 13). Pargyline decreased GABA-T activity in the brains of mice exposed to room air at normal atmospheric pressure and also in those of mice exposed to OHP, although the degree of inhibition was less in the pargyline-treated, OHP-exposed mice (Table 3). The inhibition of GABA-T in pargyline-treated, room air-exposed mice was not significantly different from that observed in pargyline-treated mice exposed to OHP.

OHP exposure (90 min) decreased the activity of the GABA synthesizing enzyme, GAD, by about 20% (Tables 4, 9 and 14), and pargyline treatment did not significantly alter this OHP-induced decrease in enzyme activity (Table 4). Pargyline treatment had no effect on brain GAD activity in mice exposed to room air (Table 4).

OHP exposure elevated brain ammonia levels and decreased brain glutamine levels (Tables 5, 10 and 15). The maximal elevation in ammonia (173%) occurred after 30 min of OHP exposure (Table 5) which was the time at which most saline-treated mice began to demonstrate severe convulsive activity (Figure 3). The decreased glutamine levels, on the other hand, were greatest after 60 min (37%) and 90 min (36%) of OHP exposure (Tables 5, 10 and 15). Pargyline had no effect on brain ammonia or glutamine levels in mice exposed to room air (Table 5). Pargyline treatment significantly lessened the elevation in brain ammonia produced by OHP exposure and partially prevented the OHP-induced decrease in brain glutamine (Table 5).

There was a significant correlation between brain ammonia levels and the susceptibility of the individual mice to OHP seizures after pargyline treatment (Figure 8). The correlation coefficients were -0.73 and

Table 3. ALTERATION OF MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID TRANSAMINASE ACTIVITY BY PARGYLINE AND HYPERBARIC OXYGEN

| Treatment | | | | | |
|---|---|---|---|---|---|
| Drug[b]-time[a] | OHP[c]-time[a] | µmoles[e]/g brain/h (Mean ± S.E.)[f] | P[d] | % Change[g] |
| Saline | -120 | 0 | 229 ± 9.3 | -- | -- |
| Saline | -60 | 30 | 227 ± 3.0 | >0.05 | -3 |
| Saline | -90 | 60 | 225 ± 2.5 | >0.05 | -2 |
| Saline | -120 | 90 | 223 ± 3.2 | >0.05 | -1 |
| Pargyline | -30 | 0 | 190 ± 6.1 | <0.005 | -17 |
| Pargyline | -60 | 0 | 154 ± 9.5 | <0.0005 | -33 |
| Pargyline | -120 | 0 | 171 ± 5.2 | <0.0005 | -25 |
| Pargyline | -60 | 30 | 196 ± 4.5 | <0.01 | -15 |
| Pargyline | -90 | 60 | 186 ± 4.0 | <0.0025 | -19 |
| Pargyline | -120 | 90 | 201 ± 4.6 | <0.01 | -13 |

[a] Minutes.

[b] Saline (10 ml/kg body weight) or pargyline (100 mg/kg) was injected intraperitoneally.

[c] OHP exposure at 60 psig.

[d] Student "t" test.

[e] µmoles succinic semialdehyde.

[f] Each value represents mean of five mice.

[g] Minus represents decrease.

Table 4. ALTERATION IN MOUSE BRAIN GLUTAMIC ACID DECARBOXYLASE ACTIVITY BY PARGYLINE AND HYPERBARIC OXYGEN

| Treatment | | | μmoles $CO_2$/g brain/h | | |
|---|---|---|---|---|---|
| Drug[b]-time[a] | | OHP[c]-time[a] | (Mean ± S.E.)[e] | P[d] | % Change[f] |
| Saline | -120 | 0 | 16.3 ± 0.92 | -- | -- |
| Saline | -60 | 30 | 13.7 ± 0.70 | <0.05 | -16 |
| Saline | -90 | 60 | 12.9 ± 0.47 | <0.005 | -21 |
| Saline | -120 | 90 | 12.8 ± 0.81 | <0.01 | -22 |
| Pargyline | -30 | 0 | 16.0 ± 0.88 | >0.05 | -2 |
| Pargyline | -60 | 0 | 15.7 ± 0.46 | >0.05 | -4 |
| Pargyline | -120 | 0 | 16.0 ± 0.76 | >0.05 | -2 |
| Pargyline | -60 | 30 | 14.1 ± 1.10 | >0.05 | -13 |
| Pargyline | -90 | 60 | 13.2 ± 0.71 | <0.0125 | -19 |
| Pargyline | -120 | 90 | 12.8 ± 0.85 | <0.01 | -21 |

[a]Minutes.

[b]Saline (10 ml/kg body weight) or pargyline (100 mg/kg) was injected intraperitoneally.

[c]OHP exposure at 60 psig.

[d]Student "t" test.

[e]Each value represents mean of five mice.

[f]Minus represents decrease.

Table 5. EFFECT OF PARGYLINE ON HYPERBARIC OXYGEN-INDUCED ALTERATIONS IN MOUSE BRAIN AMMONIA AND GLUTAMINE

| Treatment | | | | | | |
|---|---|---|---|---|---|---|
| Drug[c]-time[a] | | OHP[d]-time[a] | Ammonia[b] | % Change[e] | Glutamine[b] | % Change[e] |
| Saline | -60 | 0 | 0.318 ± 0.09 | -- | 4.63 ± 0.12 | -- |
| Saline | -60 | 30 | 0.868 ± 0.03[f] | +173 | 3.74 ± 0.43[f] | -19 |
| Pargyline | -60 | 0 | 0.289 ± 0.05[g] | -9 | 4.72 ± 0.27[g] | +2 |
| Pargyline | -60 | 30 | 0.472 ± 0.07[g] | +48 | 4.86 ± 0.25[g] | +5 |
| Saline | -90 | 0 | 0.338 ± 0.05 | -- | 4.14 ± 0.25 | -- |
| Saline | -90 | 60 | 0.810 ± 0.14[f] | +140 | 2.60 ± 0.32[f] | -37 |
| Pargyline | -90 | 0 | 0.309 ± 0.04[g] | -9 | 3.81 ± 0.15[g] | -8 |
| Pargyline | -90 | 60 | 0.492 ± 0.05[g] | +46 | 3.95 ± 0.15[g] | -5 |
| Saline | -120 | 0 | 0.320 ± 0.09 | -- | 4.68 ± 0.20 | -- |
| Saline | -120 | 90 | 0.677 ± 0.06[f] | +112 | 3.01 ± 0.30[f] | -36 |
| Pargyline | -120 | 0 | 0.343 ± 0.03[g] | +7 | 4.82 ± 0.17[g] | +3 |
| Pargyline | -120 | 90 | 0.484 ± 0.94[g] | +51 | 3.68 ± 0.32[f,g] | -21 |

[a] Minutes.

[b] µmoles/g brain ± S.E. Mean of five mice.

[c] Saline (10 ml/kg body weight) or pargyline (100 mg/kg) was injected intraperitoneally.

[d] OHP exposure at 60 psig.

[e] Percent change compared to saline controls. Plus represents increase and minus, decrease.

[f] Significantly different from saline controls at the 0.05 level (Student "t" test).

[g] Significantly different from saline + OHP at the 0.05 level (Student "t" test).

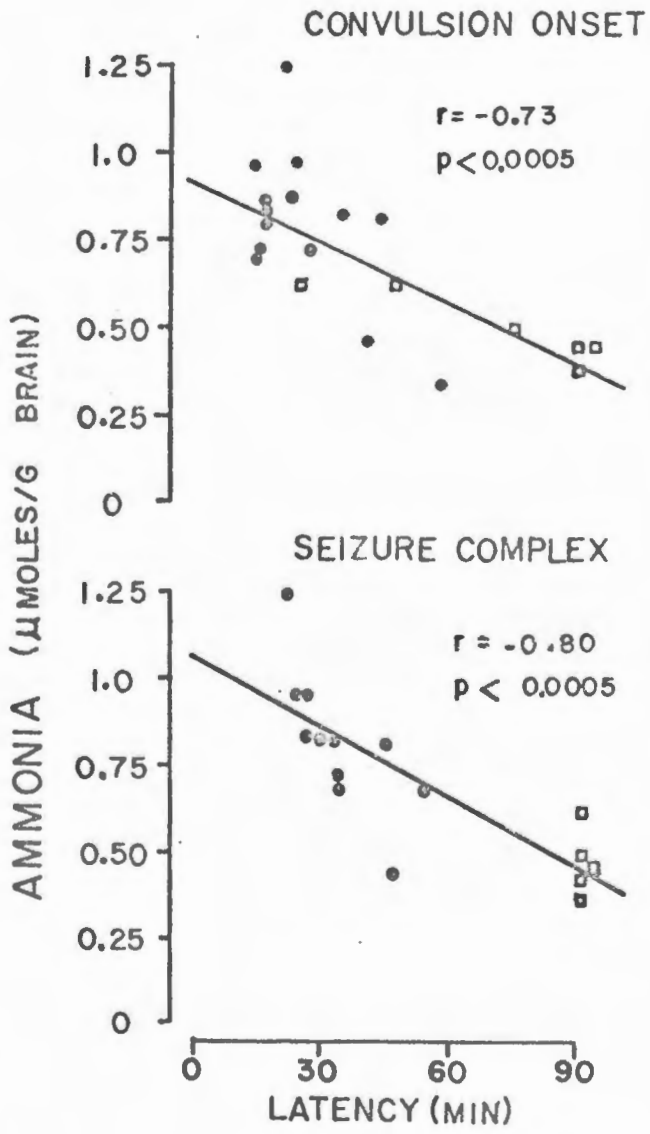


Figure 8. CORRELATION BETWEEN BRAIN AMMONIA AND SUSCEPTIBILITY TO HYPERBARIC OXYGEN SEIZURES IN PARGYLINE-TREATED MICE. ● - OHP, □ - pargyline. The animals not convulsing within 90 min were assigned an arbitrary convulsion latency of 90 min. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

-0.80 for convulsion onset and seizure complex, respectively (Figure 8). The correlation between decreased levels of glutamine and OHP seizure susceptibility ($r = 0.21$) was not significant in the case of latency to convulsion onset. There was, however, a significant correlation ($r = 0.65$, $p < 0.01$) between decreased glutamine levels and seizure susceptibility in the case of seizure complex (Figure 9).

SUCCINIC ACID

Preliminary studies showed that the intraperitoneal injection of 12 mmoles/kg (1.39 g/kg) succinic acid 60 min prior to OHP exposure decreased the incidence of OHP convulsions more than did other doses given at other time intervals (appendix Table 4). Both the latency to convulsion onset and the latency to seizure complex were prolonged by prior succinic acid treatment (Figure 10). The percentage of animals that had convulsed at the end of the 90 min OHP exposure was approximately 60% and 40% for convulsion onset and seizure complex, respectively, in succinic acid-treated mice, whereas all saline-treated mice had convulsed prior to the end of the OHP test period (Figure 10). As in the case of pargyline, the decreased incidence of OHP seizures after succinic acid treatment was not due to the pH of the drug solution (pH 7.9) since a succinic acid solution adjusted to pH 6.5 was as effective as the more basic solution in decreasing the incidence of OHP seizures (appendix Table 4).

Succinic acid significantly decreased post-OHP mortality (Figure 11). After OHP exposure, 85% of the saline-treated mice died within 4 h as opposed to only 20% in the case of succinic acid-treated mice (Figure 11).

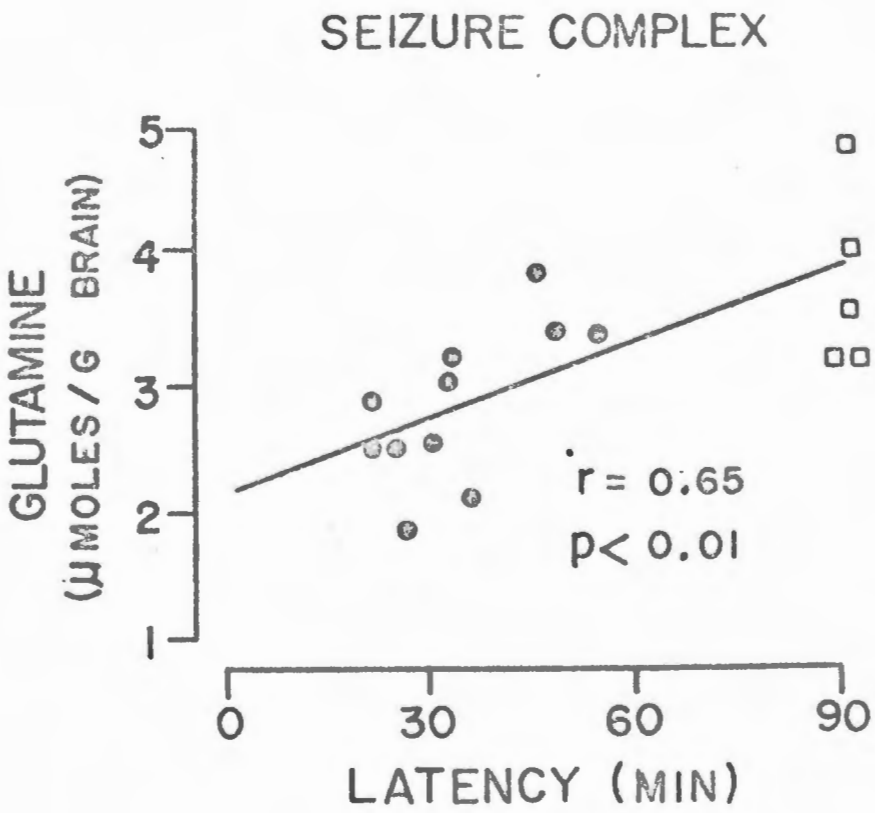


Figure 9. CORRELATION BETWEEN BRAIN GLUTAMINE AND SUSCEPTIBILITY TO HYPERBARIC OXYGEN SEIZURES IN PARGYLINE-TREATED MICE. ●- OHP (60 psig), □- pargyline (100 mg/kg). The animals not convulsing within 90 min were assigned an arbitrary convulsion latency of 90 min. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

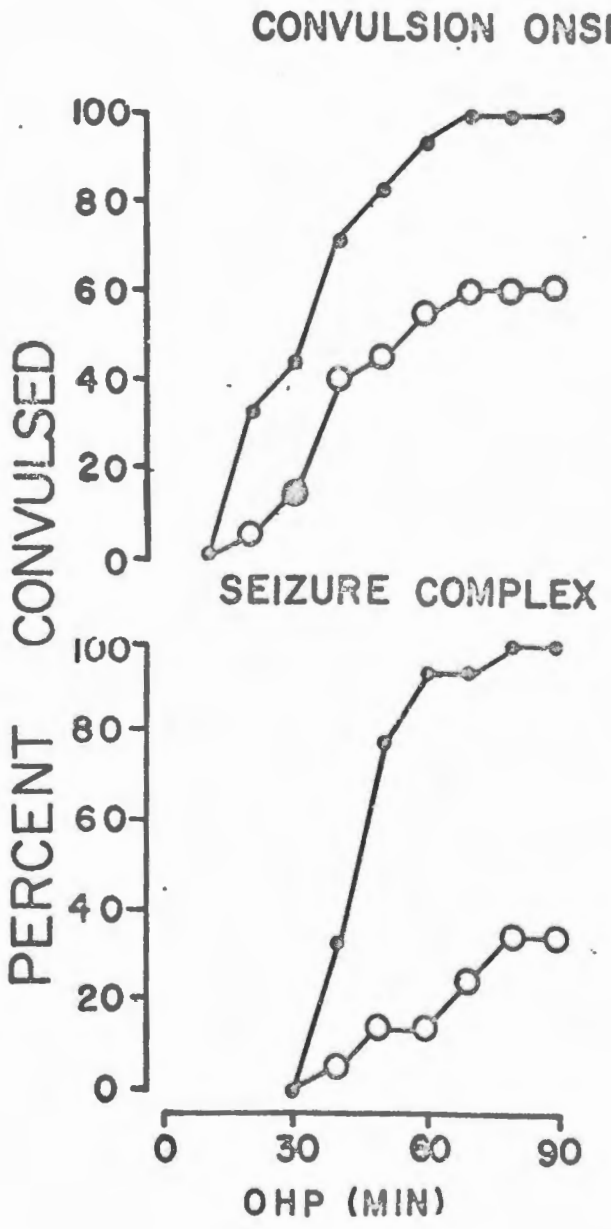


Figure 10. EFFECT OF SUCCINIC ACID ON HYPERBARIC OXYGEN CONVULSIONS. ○- succinic acid (12 mmoles/kg), ● - saline. Open symbols denote significant difference from corresponding saline value at the 0.05 level using chi-square test in groups of 18-20 mice.

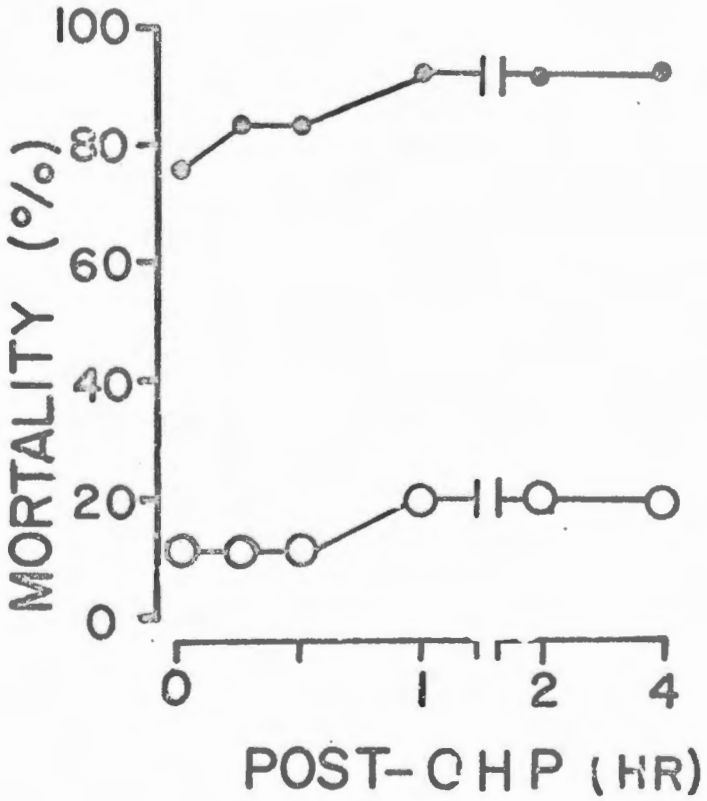


Figure 11. EFFECT OF SUCCINIC ACID ON LETHALITY FOLLOWING HYPERBARIC OXYGEN EXPOSURE. ○- succinic acid (12 mmoles/kg), ● - saline. Open symbols denote significant difference from corresponding saline value at the 0.05 level using chi-square test in groups of 18-20 mice.

Table 6. EFFECT OF SUCCINIC ACID ON HYPERBARIC OXYGEN-INDUCED ALTERATIONS IN LUNG WEIGHT AND LUNG WATER CONTENT

| Treatment[c] | Time (min) | Lung as % Body Weight (Mean ± S.E.)[d] | % Change[e] | % Water Content (Mean ± S.E.)[d] | % Change[e] |
|---|---|---|---|---|---|
| Saline | 150 | 0.52 ± 0.014 | --- | 74 ± 0.6 | -- |
| Saline + OHP (60 psig) | 150<br>90 | 0.81 ± 0.047[a] | +56 | 78 ± 1.0[a] | +6 |
| Succinic acid | 150 | 0.49 ± 0.008[b] | -6 | 74 ± 0.2[b] | 0 |
| Succinic acid + OHP | 150<br>90 | 0.61 ± 0.047[b] | +17 | 74 ± 0.7[b] | 0 |

[a]Significantly different from saline controls at the 0.05 level using the Student "t" test.

[b]Significantly different from saline + OHP at the 0.05 level using the Student "t" test.

[c]Succinic acid (12 mmoles/kg) or saline (10 ml/kg body weight) was injected intraperitoneally.

[d]Mean of five pairs of lungs.

[e]Percent change from saline controls. Plus indicates increase.

Succinic acid treatment significantly decreased the OHP-induced increase in lung weight and lung water content (Table 6). There was also significantly less pulmonary hemorrhage in succinic acid-treated mice as evidenced by the fact that OHP exposure elevated lung hemoglobin content by 98% as opposed to the 15% increase seen in the succinic acid-treated mice (Table 7).

Brain GABA levels were significantly decreased (35%) at the end of the OHP test period (Figures 12 and 19). GABA levels were elevated by about 20% 150 min after succinic acid injection (Figure 12). The maximal elevation in GABA levels was seen at 90 min after succinic acid treatment (Figure 12), and this time period was used in the dose-response curve (Figure 14). After 30 min of OHP exposure, GABA levels were still elevated by 20% in the succinic acid-treated mice but this level decreased to that of saline-treated, room air-exposed mice at the end of 90 min of OHP exposure (Figure 12). Actual means, standard errors, levels of significance and percent changes in GABA levels are shown in Table 5 of the appendix. There was a significant correlation between brain GABA levels and the susceptibility of the individual mice to OHP convulsions in succinic acid-treated mice (Figure 13). The correlation coefficients were 0.56 and 0.77 for latency to convulsion onset and latency to seizure complex, respectively (Figure 13). There was also a significant correlation between the dose of succinic acid administered and the degree to which brain GABA was elevated ($r = 0.86$, $p < 0.0005$) (Figure 14). Although the highest dose of succinic acid (24 mmoles/kg) elevated GABA levels by 60%, one of the five mice died before sacrifice and the remaining four mice appeared sick (Figure 14).

Table 7. EFFECT OF SUCCINIC ACID ON HYPERBARIC OXYGEN-INDUCED ALTERATIONS IN LUNG HEMOGLOBIN CONTENT

| Treatment | Time (min) | mg Hemoglobin/lung[a] (Mean ± S.E.) | % Change[e] |
|---|---|---|---|
| Saline | 150 | 8.55 ± 0.50 | --- |
| Saline + OHP (60 psig) | 150<br>90 | 16.97 ± 2.41[c] | +98.5 |
| Succinic acid (12 mmoles/kg) | 150 | 8.17 ± 0.44[d] | -4.5 |
| Succinic acid + OHP | 150<br>90 | 9.87 ± 0.56 | +15.4 |

[a]Each value represents the mean of five pairs of lungs.

[b]Saline or succinic acid (10 ml/kg body weight) was injected intraperitoneally. Dose is given in parentheses.

[c]Significantly different from saline controls at the 0.05 level (Student "t" test).

[d]Significantly different from saline + OHP at the 0.05 level (Student "t" test).

[e]Plus represents increase and minus, decrease.

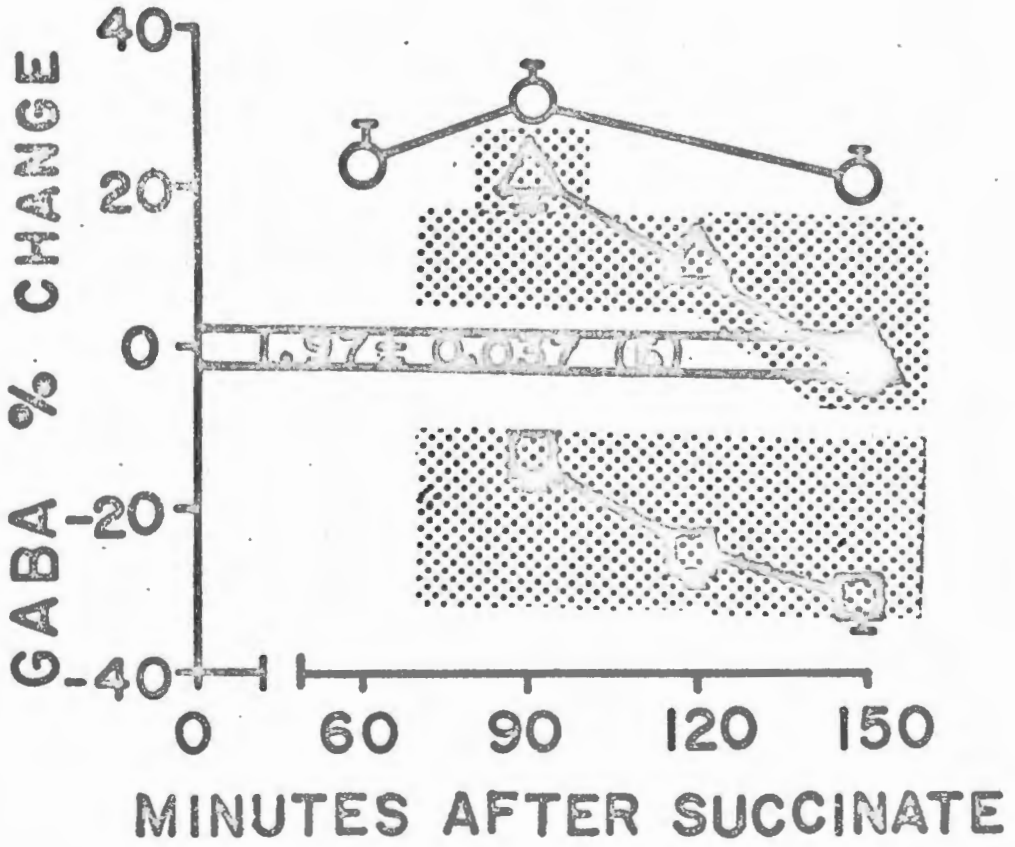


Figure 12. ALTERATION OF MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID BY SUCCINIC ACID AND HYPERBARIC OXYGEN. Percent change is represented as mean ± S.E. Open symbols denote values significantly different from controls at 0.05 level using Student "t" test. GABA concentration of control animals is given in μmoles/g brain as mean ± S.E. Shaded area denotes OHP exposure. ( ) represents number of mice used. ○ - succinic acid (12 mmoles/kg), □ - saline + OHP (60 psig), △ - succinic acid + OHP.

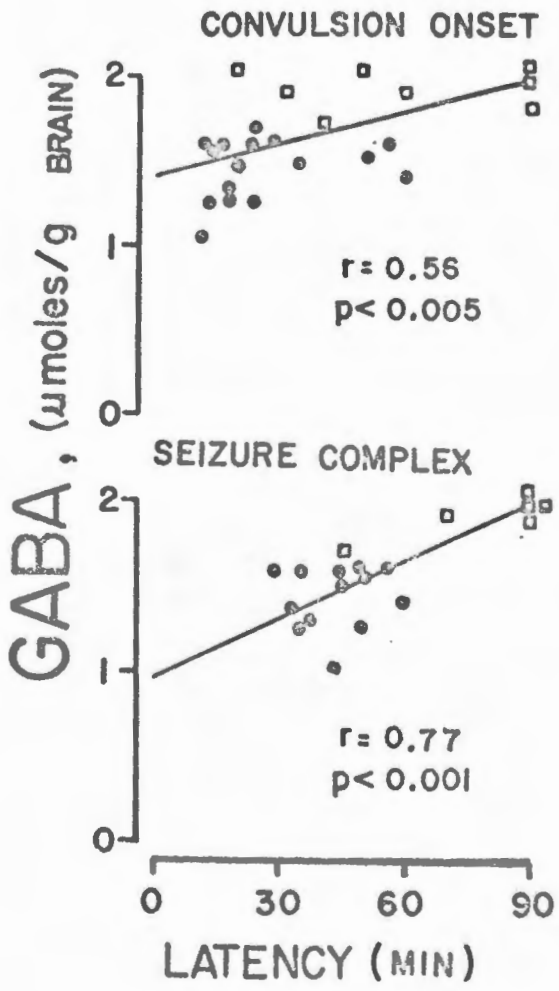


Figure 13. CORRELATION BETWEEN BRAIN GAMMA-AMINOBUTYRIC ACID AND SUSCEPTIBILITY TO HYPERBARIC OXYGEN SEIZURES IN SUCCINIC ACID-TREATED MICE. ● - OHP, □ - succinic acid. The animals not convulsing within 90 min were assigned an arbitrary convulsion latency of 90 min. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

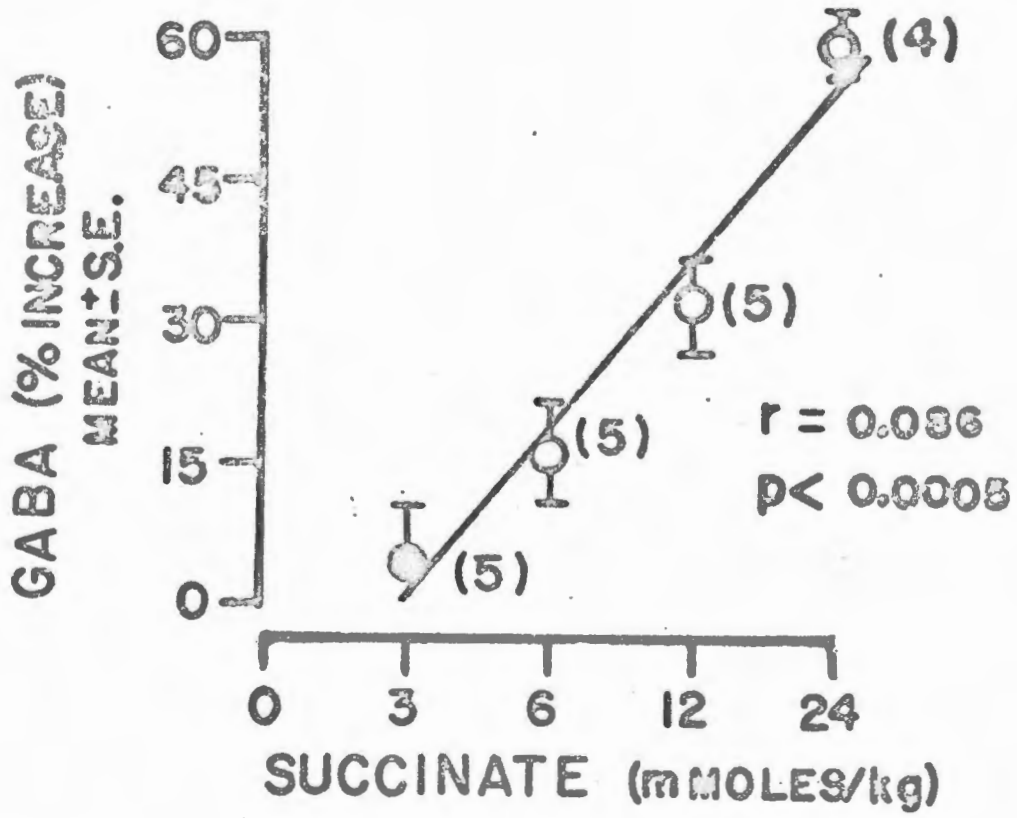


Figure 14. CORRELATION BETWEEN DOSE OF SUCCINIC ACID AND BRAIN GAMMA-AMINOBUTYRIC ACID LEVEL. Succinic acid was administered 90 min prior to sacrifice. Open symbols denote significant difference from saline controls at the 0.05 level (Student "t" test). P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero. At the highest dose, 1/5 mice died and the remaining mice appeared sick.

Actual means, standard errors, levels of significance and percent changes in GABA levels are shown in Table 6 of the appendix.

GABA-T activity was significantly increased (15%) 150 min after succinic acid treatment; however, the GABA-T activity of succinic acid-treated mice exposed to OHP was not significantly different from that of saline-treated, room air-exposed animals (Table 8).

GAD activity was also increased (45%) 150 min after succinic acid treatment (Table 9). There was no OHP-induced decrease in GAD activity in succinic acid-treated mice (Table 9); in fact, GAD activity was significantly increased (23%) in succinic acid-treated mice after 30 min of OHP exposure. This increase in GAD activity was reduced to about 10% after 90 min of OHP exposure (Table 9).

The OHP-induced increase in brain ammonia levels was significantly less in succinic acid-treated mice than in saline-treated mice, and succinic acid-treated mice did not exhibit significantly greater ammonia levels than did saline-treated, room air-exposed animals (Table 10). The OHP-induced decrease in brain glutamine levels was also significantly less in succinic acid-treated mice (Table 10). Glutamine levels were not significantly decreased in succinic acid-treated, OHP-exposed mice when compared to saline-treated, room air-exposed controls (Table 10). As in the case of pargyline, there was a significant correlation between elevation in brain ammonia levels and the susceptibility of the individual mice to OHP seizures in succinic acid-treated mice (Figure 15). The correlation coefficients were -0.76 and -0.81 for latency to convulsion onset and latency to seizure complex, respectively (Figure 15). The correlation between decreased glutamine levels and OHP seizure susceptibility ($r = 0.27$) was not significant in the case of convulsion

Table 8. ALTERATION OF MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID TRANSAMINASE ACTIVITY BY SUCCINIC ACID AND HYPERBARIC OXYGEN

| Treatment | | | | | |
|---|---|---|---|---|---|
| Drug[b]-time[a] | | OHP[c]-time[a] | µmoles[e]/g brain/h (Mean ± S.E.)[f] | p[d] | % Change[g] |
| Saline | -150 | 0 | 224 ± 3.6 | -- | --- |
| Saline | -90 | 30 | 227 ± 3.0 | >0.05 | +1 |
| Saline | -120 | 60 | 225 ± 2.5 | >0.05 | 0 |
| Saline | -150 | 90 | 223 ± 3.3 | >0.05 | 0 |
| Succinic acid | -60 | 0 | 247 ± 5.3 | <0.01 | +10 |
| Succinic acid | -90 | 0 | 262 ± 7.6 | <0.005 | +17 |
| Succinic acid | -150 | 0 | 258 ± 7.6 | <0.005 | +15 |
| Succinic acid | -90 | 30 | 220 ± 4.4 | >0.05 | 0 |
| Succinic acid | -120 | 60 | 224 ± 3.0 | >0.05 | 0 |
| Succinic acid | -150 | 90 | 223 ± 3.3 | >0.05 | 0 |

[a] Minutes.

[b] Saline (10 ml/kg body weight) or succinic acid (12 mmoles/kg) was injected intraperitoneally.

[c] OHP exposure at 60 psig.

[d] Student "t" test.

[e] µmoles succinic semialdehyde.

[f] Each value represents mean of five mice.

[g] Plus represents increase.

Table 9. ALTERATION OF MOUSE BRAIN GLUTAMIC ACID DECARBOXYLASE ACTIVITY BY SUCCINIC ACID AND HYPERBARIC OXYGEN

| Treatment | | | μmoles $CO_2$/g brain/h | | |
|---|---|---|---|---|---|
| Drug[b]-time[a] | | OHP[c]-time[a] | (Mean ± S.E.)[e] | P[d] | % Change[f] |
| Saline | -150 | 0 | 15.5 ± 0.56 | -- | --- |
| Saline | -90 | 30 | 13.7 ± 0.70 | <0.05 | -12 |
| Saline | -120 | 60 | 12.9 ± 0.47 | <0.0025 | -17 |
| Saline | -150 | 90 | 12.8 ± 0.81 | <0.0125 | -18 |
| Succinic acid | -60 | 0 | 18.2 ± 0.84 | <0.01 | +17 |
| Succinic acid | -90 | 0 | 21.2 ± 0.88 | <0.0005 | +37 |
| Succinic acid | -150 | 0 | 22.5 ± 0.03 | <0.0005 | +45 |
| Succinic acid | -90 | 30 | 19.1 ± 0.82 | <0.0025 | +23 |
| Succinic acid | -120 | 60 | 17.0 ± 0.57 | <0.05 | +9 |
| Succinic acid | -150 | 90 | 16.9 ± 0.80 | >0.05 | +9 |

[a] Minutes.

[b] Saline (10 ml/kg body weight) or succinic acid (12 mmoles/kg) was injected intraperitoneally.

[c] OHP exposure at 60 psig.

[d] Student "t" test.

[e] Each value represents mean of five mice.

[f] Plus represents increase and minus, decrease.

Table 10. EFFECT OF SUCCINIC ACID ON HYPERBARIC OXYGEN-INDUCED ALTERATIONS IN MOUSE BRAIN AMMONIA AND GLUTAMINE

| Treatment | | | | | | |
|---|---|---|---|---|---|---|
| Drug-time[c] [a] | | OHP-time[d] [a] | Ammonia[b] | % Change[e] | Glutamine[b] | % Change[e] |
| Saline | -90 | 0 | 0.32 ± 0.09 | --- | 4.63 ± 0.12 | --- |
| Saline | -90 | 30 | 0.87 ± 0.03[f] | +173 | 3.74 ± 0.43[f] | -19 |
| Succinic acid | -90 | 0 | 0.31 ± 0.06[g] | -3 | 4.55 ± 0.15 | -2 |
| Succinic acid | -90 | 30 | 0.44 ± 0.07[g] | +37 | 4.74 ± 0.15[g] | +2 |
| Saline | -120 | 0 | 0.34 ± 0.05 | --- | 4.14 ± 0.25 | --- |
| Saline | -120 | 60 | 0.81 ± 0.14[f] | +140 | 2.60 ± 0.32[f] | -37 |
| Succinic acid | -120 | 0 | 0.34 ± 0.03[g] | 0 | 3.88 ± 0.15[g] | -6 |
| Succinic acid | -120 | 60 | 0.50 ± 0.07[g] | +48 | 3.88 ± 0.22[g] | -6 |
| Saline | -150 | 0 | 0.32 ± 0.09 | --- | 4.68 ± 0.20 | --- |
| Saline | -150 | 90 | 0.68 ± 0.06[f] | +112 | 3.01 ± 0.17[f] | -36 |
| Succinic acid | -150 | 0 | 0.40 ± 0.07[g] | +25 | 4.69 ± 0.24[g] | 0 |
| Succinic acid | -150 | 90 | 0.42 ± 0.04[g] | +31 | 3.74 ± 0.24[g] | -20 |

[a] Minutes.

[b] μmoles/g brain ± S.E. Mean of five mice.

[c] Saline (10 ml/kg body weight) or succinic acid (12 mmoles/kg) was injected intraperitoneally.

[d] OHP exposure was at 60 psig.

[e] Percent change compared to saline controls. Plus represents increase and minus, decrease.

[f] Significantly different from saline controls at the 0.05 level (Student "t" test).

[g] Significantly different from saline + OHP at the 0.05 level (Student "t" test).

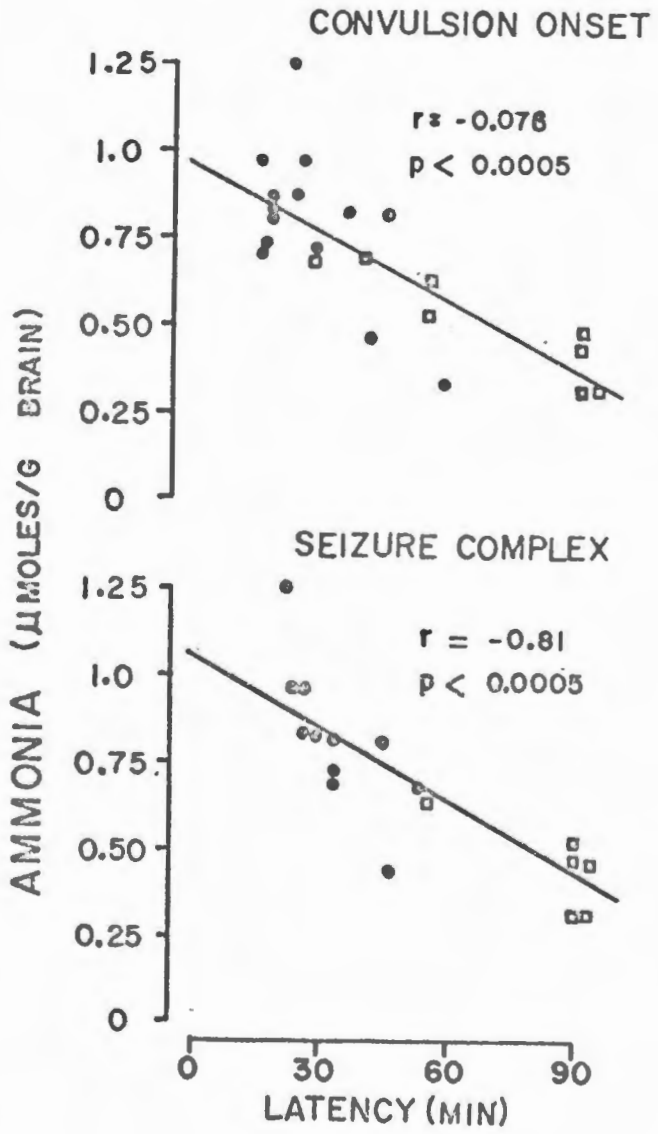


Figure 15. CORRELATION BETWEEN BRAIN AMMONIA AND SUSCEPTIBILITY TO HYPERBARIC OXYGEN SEIZURES IN SUCCINIC ACID-TREATED MICE. o - OHP (60 psig), - succinic acid (12 mmoles/kg, IP). The animals not convulsing within 90 min were assigned an arbitrary convulsion latency of 90 min. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

onset. There was a significant correlation between glutamine levels and seizure susceptibility ($r = 0.66$, $p < 0.005$) in the case of the seizure complex (Figure 16).

L-ASCORBIC ACID

Preliminary studies (appendix Table 7) showed that the intraperitoneal injection of 12 mmoles/kg (2.11 g/kg) of L-ascorbic acid 60 min prior to OHP exposure decreased the incidence of OHP convulsions more than did other doses given at other time intervals (appendix Table 7). L-ascorbic acid significantly decreased the incidence of OHP convulsions and significantly increased the latency to OHP seizures when compared to saline-treated mice (Figure 17). In the case of convulsion onset and seizure complex, approximately 20% of the ascorbic acid-treated mice had convulsed at the end of the OHP test period as opposed to 100% of the saline-treated animals (Figure 17). The decreased incidence of OHP seizures after ascorbic acid treatment was not due to the pH of the drug solution (pH 2.5) since an ascorbic acid solution adjusted to pH 6.5 was as effective as the more acidic solution in decreasing the incidence of OHP convulsions (appendix Table 7).

Ascorbic acid-treated mice demonstrated significantly less post-OHP mortality than did saline-treated mice for up to 12 h after OHP exposure (Figure 18). At the end of 72 h, however, post-OHP mortality was about 90% in both groups of mice (Figure 18).

Ascorbic acid treatment significantly decreased the OHP-induced increase in lung weight when compared to saline-treated mice (Table 11). In spite of ascorbic acid treatment, however, lung weights were significantly higher than those of saline-treated or ascorbic acid-treated,

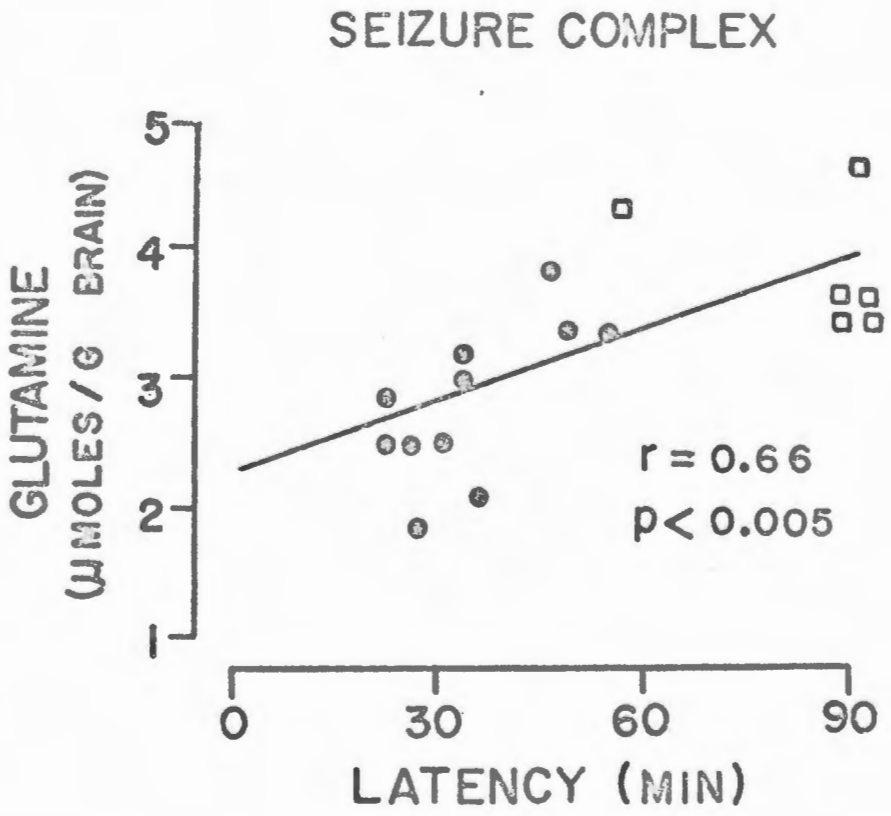


Figure 16. CORRELATION BETWEEN BRAIN GLUTAMINE AND SUSCEPTIBILITY TO HYPERBARIC OXYGEN SEIZURES IN SUCCINIC ACID-TREATED MICE. ● - OHP (60 psig), □- succinic acid (12 mmoles/kg). The animals not convulsing within 90 min were assigned an arbitrary convulsion latency of 90 min. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

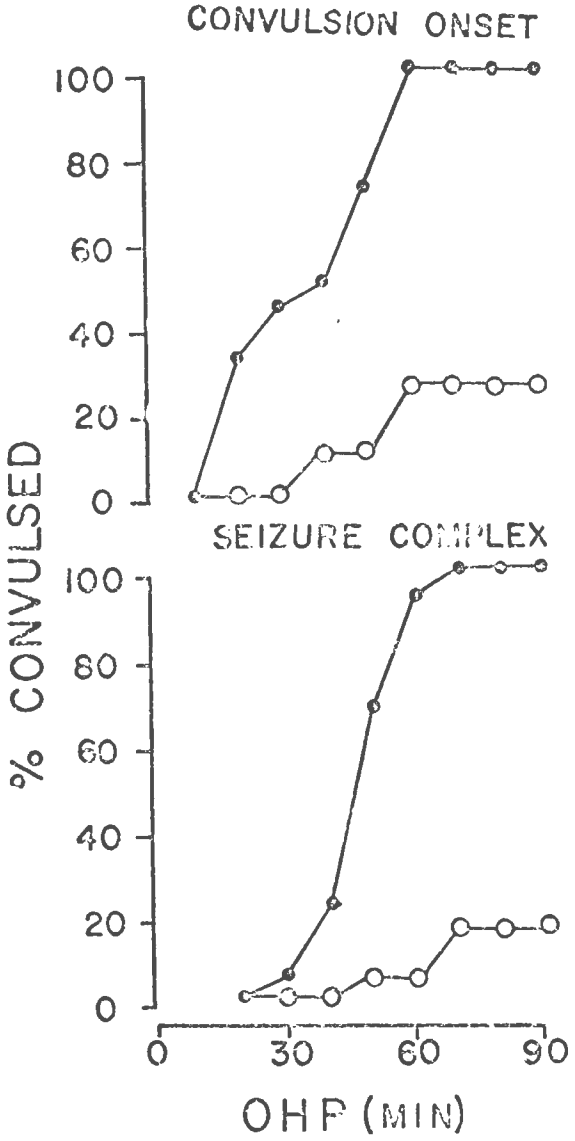


Figure 17. EFFECT OF L-ASCORBIC ACID ON HYPERBARIC OXYGEN CONVULSIONS. ○- ascorbic acid (12 mmoles/kg), ○ - saline. Open symbols denote significant difference from corresponding saline value at the 0.05 level using chi-square test in groups of 18-19 mice.

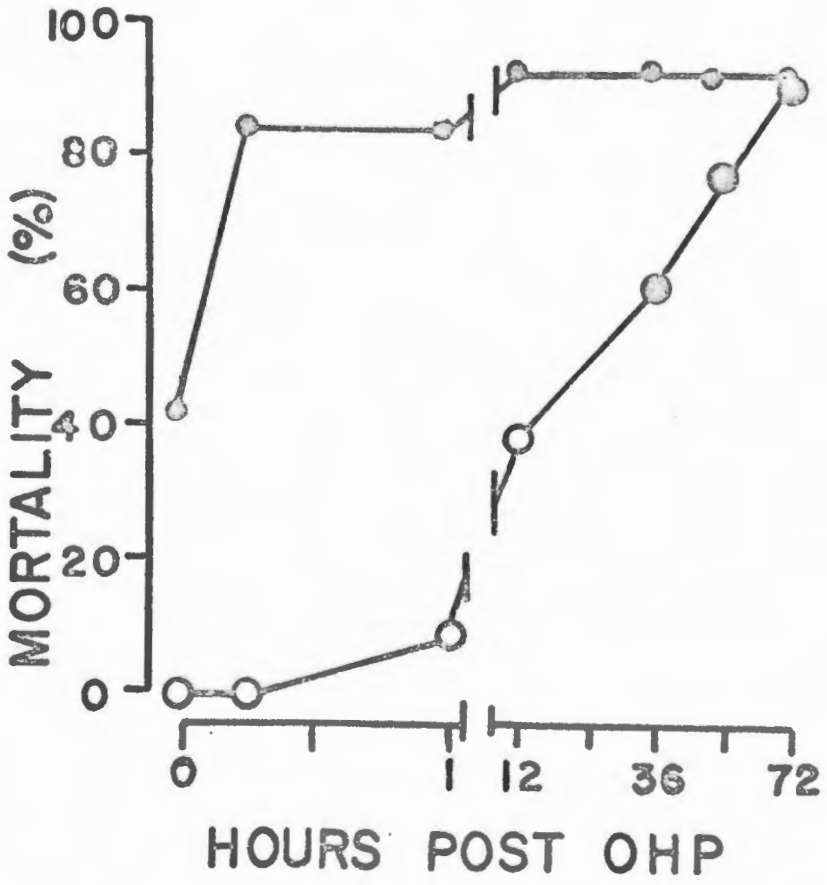


Figure 18. EFFECT OF L-ASCORBIC ACID ON LETHALITY FOLLOWING HYPERBARIC OXYGEN EXPOSURE. ○ - ascorbic acid (12 mmoles/kg), ● - saline. Open symbols denote significant difference from corresponding saline value at the 0.05 level using chi-square test in groups of 18-19 mice.

Table 11. EFFECT OF L-ASCORBIC ACID ON HYPERBARIC OXYGEN-INDUCED ALTERATIONS IN LUNG WEIGHT AND LUNG WATER CONTENT

| Treatment[c] | Time (min) | Lung as % Body Weight (Mean ± S.E.)[d] | % Change[e] | % Water Content (Mean ± S.E.)[d] | % Change[e] |
|---|---|---|---|---|---|
| Saline | 150 | 0.52 ± 0.014 | --- | 74 ± 0.5 | --- |
| Saline + OHP (60 psig) | 150<br>90 | 0.81 ± 0.047[a] | +56 | 78 ± 1.0[a] | +6 |
| Ascorbic acid | 150 | 0.53 ± 0.022[b] | +2 | 71 ± 0.5[b] | -4 |
| Ascorbic acid + OHP | 150<br>90 | 0.67 ± 0.044[a,b] | +29 | 78 ± 1.9[a] | +6 |

[a]Significantly different from saline controls at the 0.05 level using the Student "t" test.

[b]Significantly different from saline + OHP at the 0.05 level using the Student "t" test.

[c]L-ascorbic acid (12 mmoles/kg) or saline (10 ml/kg body weight) was injected intraperitoneally.

[d]Mean of five pairs of lungs.

[e]Percent change from saline controls. Plus represents increase and minus, decrease.

room air-exposed mice (Table 11). Ascorbic acid treatment had no significant effect on the OHP-induced increase in lung water content (Table 11). Ascorbic acid-treated mice exhibited less pulmonary hemorrhage after OHP exposure when compared to saline-treated mice (Table 12). There was still, however, a 34% increase in lung hemoglobin in the ascorbic acid-treated mice (Table 12) as opposed to only a 15% increase in lung hemoglobin seen after pargyline (Table 2) or succinic acid treatment (Table 7).

Ascorbic acid treatment had no effect on brain GABA levels in room air-exposed mice nor did it prevent the OHP-induced decrease in brain GABA levels (Figure 19). Actual means, standard errors, levels of significance and percent changes in brain GABA levels are shown in Table 8 of the appendix. Further, there was no significant correlation between brain GABA levels and individual susceptibility of the mice to OHP seizures. The correlation coefficients for latency to convulsion onset and latency to seizure complex were 0.23 and 0.27, respectively.

Ascorbic acid treatment had no effect on GABA-T activity in OHP- or room air-exposed mice (Table 13). Also, ascorbic acid treatment had no effect on GAD activity in room air-exposed mice nor did it prevent the OHP-induced decrease in GAD activity (Table 14).

The OHP-induced increase in brain ammonia levels was significantly less in ascorbic acid-treated mice, and ascorbic acid-treated mice demonstrated no significant increase (35%) in brain ammonia levels when compared to saline-treated, room air-exposed animals (Table 15). The OHP-induced decrease in brain glutamine was also significantly less in ascorbic acid-treated mice (Table 15). There was no significant decrease in brain glutamine in ascorbic acid-treated mice after 30 or 60 min of

Table 12. EFFECT OF L-ASCORBIC ACID ON HYPERBARIC OXYGEN-INDUCED ALTERATIONS IN LUNG HEMOGLOBIN CONTENT

| Treatment[b] | Time (min) | mg Hemoglobin/lung[a] (Mean ± S.E.) | % Change[e] |
|---|---|---|---|
| Saline | 150 | 8.55 ± 0.45 | --- |
| Saline + OHP (60 psig) | 150<br>90 | 16.95 ± 2.41[c] | +98.5 |
| Ascorbic acid (12 mmoles/kg) | 150 | 8.93 ± 0.25[d] | +4.4 |
| Ascorbic acid + OHP | 150<br>90 | 11.49 ± 0.45[c] | +34.4 |

[a] Each value represents mean of five pairs of lungs.

[b] Saline or ascorbic acid (10 ml/kg body weight) was injected intraperitoneally. Dose is given in parentheses.

[c] Significantly different from saline controls at the 0.05 level (Student "t" test).

[d] Significantly different from saline controls at the 0.05 level (Student "t" test).

[e] Plus represents increase.

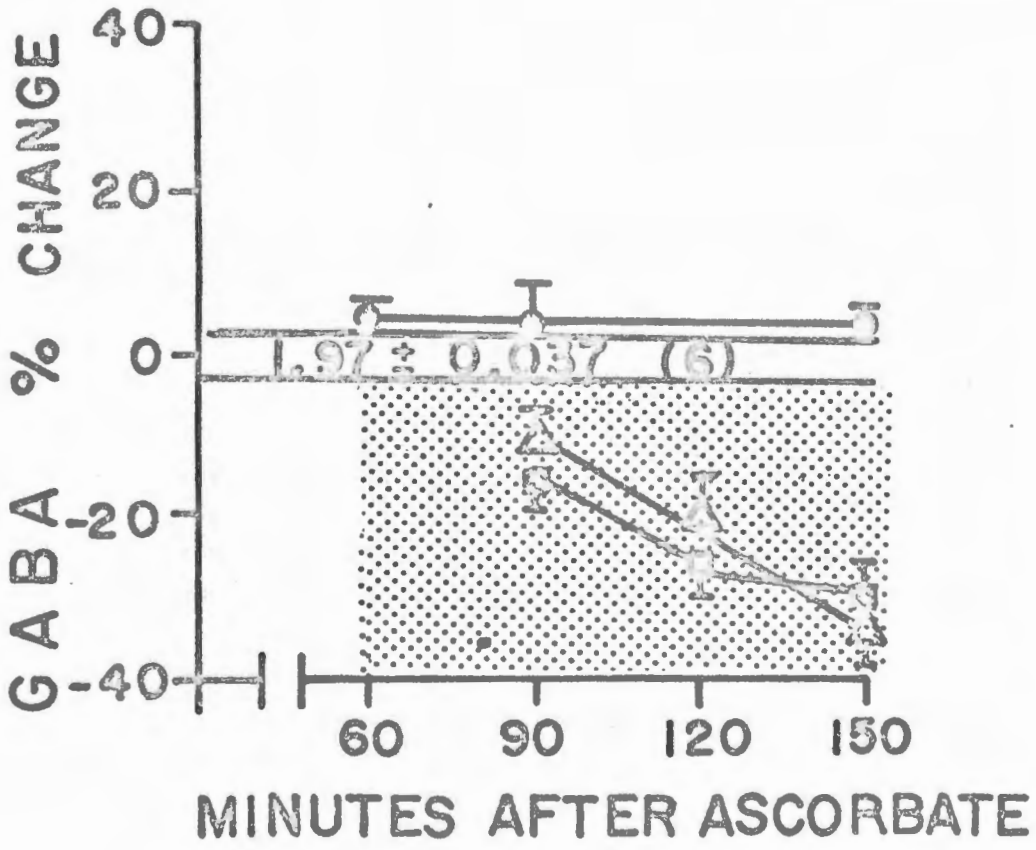


Figure 19. ALTERATION OF MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID BY L-ASCORBIC ACID AND HYPERBARIC OXYGEN. Percent change is represented as mean ± S.E. Open symbols denote values significantly different from controls at 0.05 level using Student "t" test. GABA concentration of control animals is given in μmoles/g brain as mean ± S.E. ( ) represents number of mice used. ○ - ascorbic acid (12 mmoles/kg), □ - saline + OHP (60 psig), △ - ascorbic acid + OHP. Shaded area represents OHP exposure.

Table 13. ALTERATION OF MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID TRANSAMINASE ACTIVITY BY L-ASCORBIC ACID AND HYPERBARIC OXYGEN

| Treatment | | | µmoles[e]/g brain/h | | |
|---|---|---|---|---|---|
| Drug[b]-time[a] | OHP[c]-time[a] | | (Mean ± S.E.)[f] | p[d] | % Change[g] |
| Saline | -150 | 0 | 224 ± 3.6 | -- | -- |
| Saline | -90 | 30 | 227 ± 3.0 | >0.05 | +1 |
| Saline | -120 | 60 | 225 ± 2.5 | >0.05 | 0 |
| Saline | -150 | 90 | 223 ± 3.3 | >0.05 | 0 |
| Ascorbic acid | -60 | 0 | 226 ± 4.3 | >0.05 | +1 |
| Ascorbic acid | -90 | 0 | 223 ± 1.3 | >0.05 | -1 |
| Ascorbic acid | -150 | 0 | 226 ± 3.7 | >0.05 | +1 |
| Ascorbic acid | -90 | 30 | 223 ± 3.5 | >0.05 | -1 |
| Ascorbic acid | -120 | 60 | 223 ± 2.8 | >0.05 | -1 |
| Ascorbic acid | -150 | 90 | 222 ± 3.4 | >0.05 | -1 |

[a] Minutes.

[b] Saline (10 ml/kg body weight) or ascorbic acid (12 mmoles/kg) was injected intraperitoneally.

[c] OHP exposure at 60 psig.

[d] Student "t" test.

[e] µmoles succinic semialdehyde.

[f] Each value represents mean of five mice.

[g] Plus represents increase and minus, decrease.

Table 14. ALTERATION OF MOUSE BRAIN GLUTAMIC ACID DECARBOXYLASE ACTIVITY BY L-ASCORBIC ACID AND HYPERBARIC OXYGEN

| Treatment | | | | | |
|---|---|---|---|---|---|
| Drug[b]-time[a] | | OHP[c]-time[a] | μmoles $CO_2$/g brain/h (Mean ± S.E.)[e] | p[d] | % Change[f] |
| Saline | -120 | 0 | 15.6 ± 0.80 | -- | --- |
| Saline | -90 | 30 | 13.7 ± 0.70 | >0.05 | -12 |
| Saline | -120 | 60 | 12.9 ± 0.47 | <0.01 | -18 |
| Saline | -150 | 90 | 12.8 ± 0.81 | <0.025 | -18 |
| Ascorbic acid | -60 | 0 | 15.9 ± 0.66 | >0.05 | +2 |
| Ascorbic acid | -90 | 0 | 15.4 ± 0.53 | >0.05 | -1 |
| Ascorbic acid | -150 | 0 | 15.9 ± 0.36 | >0.05 | +1 |
| Ascorbic acid | -90 | 30 | 13.7 ± 1.10 | >0.05 | -12 |
| Ascorbic acid | -120 | 60 | 12.1 ± 0.91 | <0.0125 | -23 |
| Ascorbic acid | -150 | 90 | 12.8 ± 0.87 | <0.025 | -18 |

[a] Minutes.

[b] Saline (10 ml/kg body weight) or L-ascorbic acid (12 mmoles/kg) was injected intraperitoneally.

[c] OHP exposure at 60 psig.

[d] Student "t" test.

[e] Each value represents mean of five mice.

[f] Plus represents increase and minus, decrease.

Table 15. EFFECT OF L-ASCORBIC ACID ON HYPERBARIC OXYGEN-INDUCED ALTERATIONS IN MOUSE BRAIN AMMONIA AND GLUTAMINE

| Treatment | | | | | | |
|---|---|---|---|---|---|---|
| Drug[c]-time[a] | OHP[d]-time[a] | Ammonia[b] | % Change | Glutamine[b] | % Change[e] |
| Saline | -90 | 0 | 0.32 ± 0.09 | --- | 4.63 ± 0.12 | --- |
| Saline | -90 | 30 | 0.87 ± 0.03[f] | +173 | 3.74 ± 0.43[f] | -19 |
| Ascorbic acid | -90 | 0 | 0.38 ± 0.06[g] | +18 | 4.72 ± 0.27[g] | +2 |
| Ascorbic acid | -90 | 30 | 0.43 ± 0.04[g] | +36 | 4.86 ± 0.25[g] | +5 |
| Saline | -120 | 0 | 0.34 ± 0.05 | --- | 4.14 ± 0.25 | --- |
| Saline | -120 | 60 | 0.81 ± 0.14[f] | +140 | 2.60 ± 0.32[f] | -37 |
| Ascorbic acid | -120 | 0 | 0.38 ± 0.05[g] | +11 | 4.16 ± 0.29[g] | 0 |
| Ascorbic acid | -120 | 60 | 0.47 ± 0.07[g] | +38 | 4.17 ± 0.20[g] | 0 |
| Saline | -150 | 0 | 0.32 ± 0.09 | --- | 4.68 ± 0.20 | --- |
| Saline | -150 | 90 | 0.68 ± 0.06[f] | +112 | 3.01 ± 0.30[f] | -36 |
| Ascorbic acid | -150 | 0 | 0.33 ± 0.02[g] | +4 | 4.81 ± 0.27[g] | +43 |
| Ascorbic acid | -150 | 90 | 0.43 ± 0.01[g] | +34 | 3.82 ± 0.18[g] | -18 |

[a] Minutes.

[b] µmoles/g brain ± S.E. Mean of five mice.

[c] Saline (10 ml/kg body weight) or ascorbic acid (12 mmoles/kg) was injected intraperitoneally.

[d] OHP exposure at 60 psig.

[e] Percent change compared to saline controls. Plus represents increase and minus, decrease.

[f] Significantly different from saline controls at the 0.05 level (Student "t" test).

[g] Significantly different from saline + OHP at the 0.05 level (Student "t" test).

OHP exposure when compared to saline-treated, room air-exposed animals, but there was a significant decrease of 18% after 90 min of OHP exposure (Table 15). There was a significant correlation between brain ammonia levels and the susceptibility of the individual mice to OHP seizures in ascorbic acid-treated animals (Figure 20). The correlation coefficients were -0.76 and -0.86 in the case of latency to convulsion onset and latency to seizure complex, respectively (Figure 20). There was no significant correlation between glutamine levels and seizure susceptibility ($r = 0.35$) in the case of latency to convulsion onset. The correlation between glutamine levels and seizure susceptibility ($r = 0.75$, $p < 0.001$) was, however, significant in the case of latency to seizure complex (Figure 21).

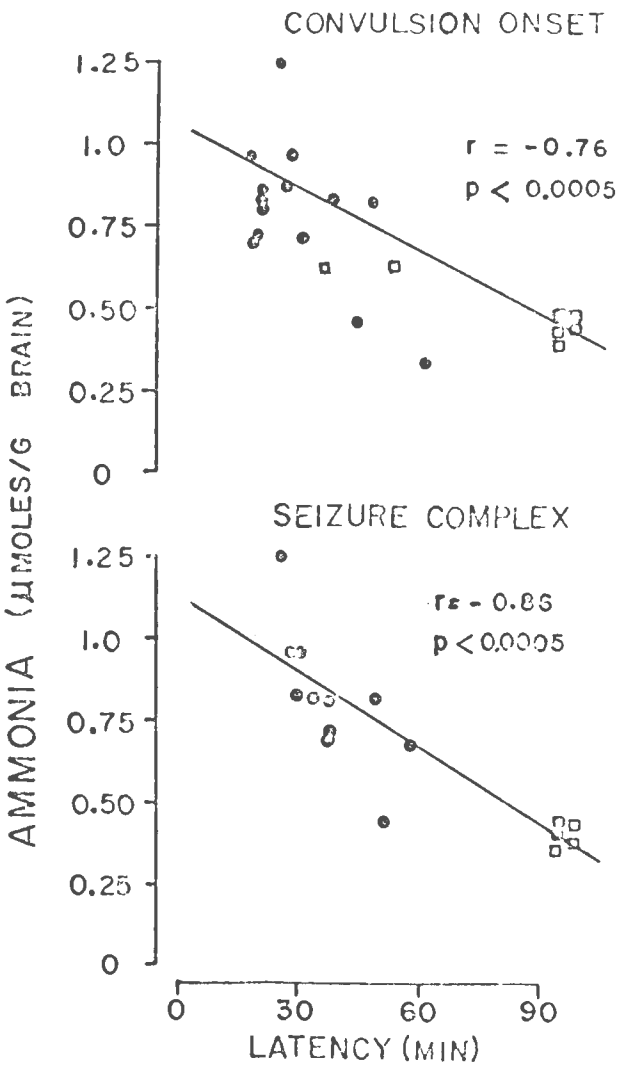


Figure 20. CORRELATION BETWEEN BRAIN AMMONIA AND SUSCEPTIBILITY TO HYPERBARIC OXYGEN SEIZURES IN ASCORBIC ACID-TREATED MICE. ● - OHP (60 psig), □ - ascorbic acid (12 mmoles/kg, IP). The animals not convulsing within 90 min were assigned an arbitrary convulsion latency of 90 min. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

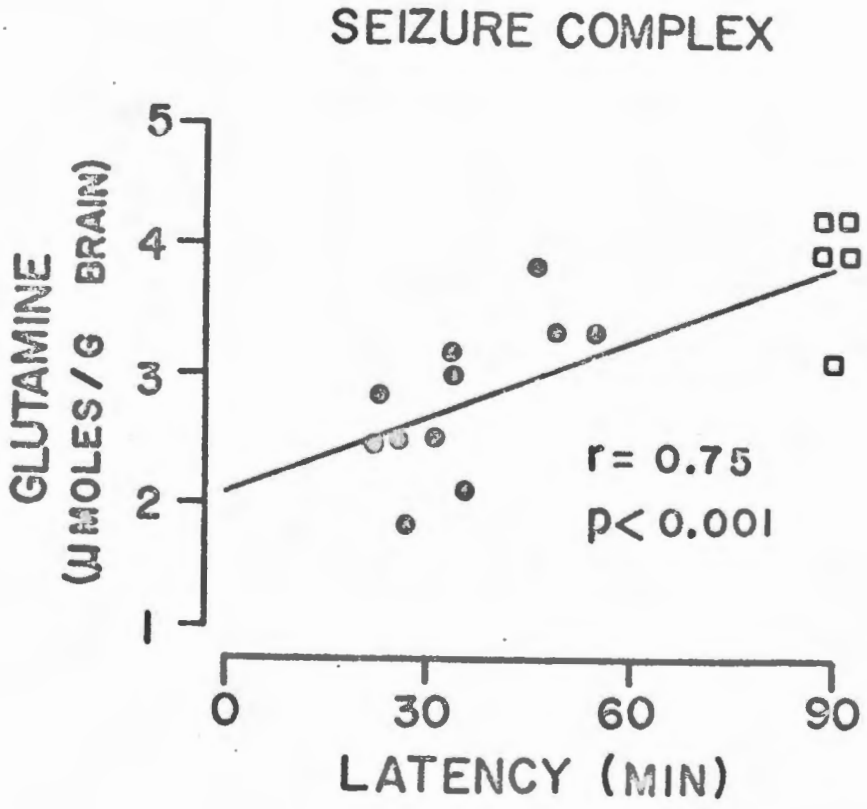


Figure 21. CORRELATION BETWEEN BRAIN GLUTAMINE AND SUSCEPTIBILITY TO HYPERBARIC OXYGEN SEIZURES IN ASCORBIC ACID-TREATED MICE. ● - OHP (60 psig), □- ascorbic acid (12 mmoles/kg, IP). The animals not convulsing within 90 min were assigned an arbitrary convulsion latency of 90 min. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

# V. DISCUSSION

## HYPERBARIC OXYGEN CONVULSIONS

That pargyline protected against OHP-induced convulsions (Figure 3) is in agreement with the findings of previous investigators (Blenkarn et al., 1969; Faiman et al., 1971). Further, the pretreatment time and dose of pargyline that provided the greatest degree of protection against OHP seizures in mice was the same as that employed by Faiman et al. (1971).

The pretreatment time and dose of succinic acid that provided maximal protection against OHP-induced convulsions (Figure 10) was the same as that employed by Woodhall et al. (1971). Blenkarn et al. (1969) reported that succinic acid was a more effective protective agent against OHP seizures than was pargyline; however, we did not find this to be true. Although equimolar doses of pargyline (0.5 mmoles/kg) and succinic acid (12 mmoles/kg) were not used in our study or in that of Blenkarn et al. (1969), comparison of the doses of agent that were most effective in preventing OHP convulsions showed that pargyline (Figure 3) was more effective in increasing the latency to convulsion onset than was succinic acid (Figure 10). Both agents in the doses used were, however, approximately as effective in their ability to increase the latency to seizure complex. This discrepancy may be due to the fact that we did not use fasted animals as did other investigators (Woodhall et al., 1971) since we were able to demonstrate significant protection against OHP

convulsions after succinic acid treatment in non-fasted mice (Figure 10). DeFeudis and Elliott (1967) have reported that the injection of large volumes of hypertonic solutions of diverse substances will also increase tolerance of animals to OHP convulsions and thus, obscure the basic actions of the protective agents being tested. Although we concur that our succinic acid solution is indeed hypertonic, we do not consider the dosage volume injected (10 ml/kg) to be a large quantity for use in mice since it is the volume usually employed in studies conducted in most laboratories. Further, injection of hypertonic solutions of NaCl produced the same degree of brain dehydration as did hypertonic succinic acid solutions, but the protective effect of NaCl against OHP seizures was inconsistent compared to the protective effect of succinic acid (DeFeudis and Elliott, 1967). Thus, we feel that possible artifacts due to fasting or injection of large volumes of hypertonic solutions are not responsible for the protective effects against OHP convulsions in the case of any of the three agents that we have tested.

Our results also showed that L-ascorbic acid protected against OHP convulsions (Figure 17). Jamieson and Van Den Brenk (1964), however, reported a slight decrease in the latency to OHP convulsions after ascorbic acid treatment. This discrepancy may be due to two factors. First, the dose of ascorbic acid (1.74 g/kg) used by Jamieson and Van Den Brenk (1964) was lower than the dose of ascorbic acid used in our studies (2.11 g/kg). Second, Jamieson and Van Den Brenk (1964) used a 10 min pretreatment time as opposed to the 60 min pretreatment time used in our studies. Further, our preliminary results (appendix Table 7) showed that a 30 min pretreatment time was not as effective in reducing the incidence of OHP seizures compared to the 60 min pretreatment time.

In a recent paper, Serrill et al. (1971) reported that ascorbic acid decreased the latency to OHP convulsions in mice. Again, the dosage employed (0.5 g/kg) was lower than that used in our studies. Also, the ascorbic acid was given in combination with an equal dose of acetylsalicylic acid (Serrill et al., 1971). We feel that these differences are due to either the low dosage of ascorbic acid employed or to the fact that both drugs were given in combination since higher doses of ascorbic acid (Figure 17) or acetylsalicylic acid (Puglia et al., 1970) given individually protected against OHP convulsions.

Comparison of the protective effects of pargyline, succinic acid and ascorbic acid were based on the doses of the agents that were most effective in decreasing the incidence of OHP convulsions. That the doses of ascorbic acid and succinic acid employed in the study were equimolar is coincidental.

Ascorbic acid increased the latency to convulsion onset (Figure 17) more than did pargyline (Figure 3) or succinic acid (Figure 10), but all three agents were approximately equal in their effectiveness in increasing the latency to the OHP seizure complex.

Since two of the protective agents used in our studies were acidic when in solution, we undertook to determine if protection against OHP convulsions was related to a pH change caused by injection of the acidic solutions into the peritoneal cavity. Our results showed that solutions of pargyline (appendix Table 1), succinic acid (appendix Table 4), or ascorbic acid (appendix Table 7) adjusted to pH 6.5 with 2N NaOH or 2N HCl did not decrease the effectiveness of these drugs as protective agents against OHP convulsions. We, therefore, suggest that the protective effect of these agents against OHP convulsions is not due

to any pH change produced by intraperitoneal injection of an acidic substance or basic substance.

Since OHP exposure decreased body temperature and pargyline enhanced this decrease (Puglia et al., 1970), the protective effect of pargyline against OHP convulsions may have been due to hypothermia. Since our hyperbaric chamber was not equipped to monitor body temperature, we were not able to measure body temperature in our studies. It is known, however, that phenacetin, like pargyline, enhanced the decrease in rectal temperature produced by OHP, but phenacetin, unlike pargyline, did not protect against OHP convulsions (Puglia et al., 1970). Further evidence against the protective effect of pargyline, succinic acid or ascorbic acid as being due to their ability to enhance OHP-induced hypothermia is provided by the finding that acetylsalicylic acid, which protected against OHP convulsions, partially prevented the OHP-induced hypothermia (Puglia et al., 1970).

LETHALITY FOLLOWING HYPERBARIC OXYGEN EXPOSURE

That pargyline increased the number of animals surviving after OHP exposure (Figure 4) is in agreement with the results of previous investigators (Blenkarn et al., 1969). We have not seen any investigations concerning the effect of succinic acid on post-OHP mortality, but our studies have shown that succinic acid, like pargyline, increased the number of animals surviving after OHP exposure (Figure 11). Jamieson and Van Den Brenk (1964) reported that ascorbic acid treatment did not alter survival times of mice exposed to OHP; however, these animals were left in the hyperbaric chamber until death, and hence, the results are not a measure of post-OHP mortality. We have found that ascorbic acid

decreased post-OHP mortality for about 12 h, but at the end of 72 h the number of ascorbic acid-treated mice surviving OHP exposure was the same as that of saline controls (Figure 18). The possible reasons for this lack of protection against post-OHP mortality after ascorbic acid treatment will be discussed later in relation to the effect of ascorbic acid on OHP-induced alterations in brain GABA and ammonia levels.

HYPERBARIC OXYGEN-INDUCED PULMONARY DAMAGE

To our knowledge, no investigations have been undertaken to study the effects of pargyline or succinic acid on OHP-induced pulmonary damage.

Since adrenergic blocking agents decreased OHP-induced pulmonary toxicity (Gerschman et al., 1955; Johnson and Bean, 1957) and since MAO inhibitors can reduce peripheral sympathetic activity via production of "false neurotransmitters" (Kopin et al., 1965), we had at first thought that pargyline protection against OHP pulmonary toxicity (Tables 1 and 2) may have been due to a similar inhibitory effect on peripheral and/or central sympathetic activity. The time required for the production of "false neurotransmitters" is, however, much longer (Kopin et al., 1965) than that required for pargyline to exert its protective effect against OHP-induced pulmonary damage (Tables 1 and 2). Further, it has already been established that no apparent correlation existed between absolute levels or turnover rates of brain NE, DA or 5-HT and susceptibility to OHP convulsions (Blenkarn et al., 1969; Faiman et al., 1971), and possibly, this is also true in the case of pulmonary damage. As mentioned in the literature review, it is not firmly established whether

pulmonary damage is of central origin and a result of seizure activity or is due to a direct toxic effect of OHP on the lung.

Both pargyline (Tables 1 and 2) and succinic acid (Tables 6 and 7) were equally effective in preventing the OHP-induced increase in lung weight, lung water content and lung hemoglobin content.

The fact that ascorbic acid prevented the OHP-induced increase in lung weight (Table 11) is in agreement with previous findings (Jamieson and Van Den Brenk, 1964). Comparison of the effect of ascorbic acid on OHP-induced pulmonary damage (Tables 11 and 12) to the effects of succinic acid (Tables 6 and 7) or pargyline (Tables 1 and 2) shows that ascorbic acid was not as effective as the other two agents in preventing the OHP-induced increase in lung weight, lung water content and lung hemoglobin content. Possible reasons for this difference will be discussed later in regard to the effect of ascorbic acid on OHP-induced alterations in brain GABA and ammonia levels.

## GAMMA-AMINOBUTYRIC ACID, GLUTAMIC ACID DECARBOXYLASE AND GAMMA-AMINOBUTYRIC ACID TRANSAMINASE

The OHP-induced decrease in brain GABA shown in Figures 5, 12 and 19 is in agreement with previous findings (Wood *et al.*, 1963; Wood *et al.*, 1966).

To our knowledge, the elevation in brain GABA levels after pargyline treatment (Figure 5) has never been reported before. This effect was due to inhibition of GABA degradation by GABA-T (Table 3). Pargyline had no effect on the GABA synthesizing enzyme, GAD (Table 4). These data are in agreement with Popov and Mattheis (1969) who found that other MAO inhibitors elevated brain GABA by inhibiting GABA-T, and these MAO inhibitors in the doses used had no effect on GAD activity.

Succinic acid, on the other hand, elevated GABA levels (Figure 12) by increasing GABA synthesis (Table 9). Although the activity of both GAD (Table 9) and GABA-T (Table 10) were increased by succinic acid treatment, GAD was stimulated to a greater degree than was GABA-T. We established a dose-response relationship between the dose of pargyline or succinic acid administered and the degree of elevation in brain GABA (Figures 5 and 12) although by different mechanisms. The ability of pargyline or succinic acid to prevent the OHP-induced decrease in brain GABA (Figures 5 and 12) is responsible, at least in part, for the protective effects of these agents against OHP toxicity. There was also a significant correlation between OHP seizure susceptibility and brain GABA levels in pargyline- (Figure 6) or succinic acid-treated mice (Figure 13). Further support for decreased levels of GABA being involved in OHP toxicity is provided by the findings that GABA administration decreased OHP convulsions (Wood et al., 1963) and pulmonary damage (Wood et al., 1965).

Results obtained using succinic acid may be explained in relation to ATP availability. Since ATP is decreased during OHP exposure (Woodhall et al., 1971) and both GAD and GABA-T are Pyr P requiring enzymes (Baxter, 1970), OHP may inhibit pyridoxal kinase thus decreasing the availability of the Pyr P cofactor. That OHP inhibits GAD preferentially may be explained by the fact that the cofactor is only loosely bound to GAD whereas Pyr P has a greater affinity to GABA-T (Baxter, 1970) and, therefore, decreased Pyr P availability might be expected to have a greater effect on the activity of GAD than on that of GABA-T. Succinic acid, by means of increasing ATP levels, could increase Pyr P availability and consequently, elevate GAD and GABA-T activity.

Succinic acid could also maintain normal ATP levels during OHP exposure and thus, prevent the OHP-induced decrease in GAD activity.

Pargyline, like other MAO inhibitors (Tapia and Pasantes, 1971), may inhibit GABA-T by preventing combination of the Pyr P cofactor with the enzyme thus decreasing GABA-T activity. That pargyline preferentially inhibited GABA-T and was without effect on GAD may be explained in light of the fact that GAD and GABA-T have a different subcellular localization (Baxter, 1970). For this reason, pargyline may not be allowed access to the subcellular compartment containing GAD and cannot, therefore, inhibit GAD.

Although our experiments were all _in vivo_ studies and may not accurately reflect cellular events, they do enable us to gain some insight as to possible biochemical events and enable us to make the preceding speculations. Substantiation of these speculations would require extensive research on the _in vitro_ aspects of OHP toxicity and the effects of pargyline, succinic acid and ascorbic acid thereon.

The ability of pargyline to elevate brain GABA may at first seem paradoxical since pargyline is used as an antidepressant, whereas GABA has neuronal inhibitory properties. It may be that GABA is not involved in mood, and pargyline's effect on GABA is, therefore, not related to pargyline's ability to combat depression. Also, our study involved the administration of only one dose of pargyline and is, therefore, short-term in nature, whereas chronic administration of pargyline is required for this drug to exhibit its antidepressant effect.

Other factors are involved in the production of OHP toxicity as indicated by the fact that ascorbic acid protected against OHP toxicity (Figures 17 and 18; Tables 11 and 12) but did not increase GABA levels

or prevent the OHP-induced decrease in brain GABA levels (Figure 19). Further, there was no correlation between GABA levels and OHP seizure susceptibility in ascorbic acid-treated mice.

AMMONIA AND GLUTAMINE

The elevation in brain ammonia levels during OHP exposure (Gershenovich and Krichenskoya, 1954; Szam, 1969) is thought to be due to inhibition of glutamine synthetase (Gershenovich et al., 1963). Glutamine synthetase catalyzes the combination of glutamic acid and ammonia to form glutamine (Figure 1) and this reaction is, therefore, a means of ammonia detoxication in CNS. This is believed to be the main pathway for ammonia detoxication in brain (see Weil-Malherbe, 1962) especially since the CNS does not have all the enzymes of the urea cycle as does the liver (Katanuma et al., 1965).

The fact that the increase in brain ammonia levels during OHP exposure (Tables 5, 10 and 15) was greatest at the time of maximal seizure activity (Figures 3, 10 and 17) and that administration of ammonium salts produced convulsions (Salvatore and Bocchini, 1961; Ugarte et al, 1968) and pulmonary damage (König and König, 1949) indicates that elevated brain ammonia levels may be involved in the etiology of OHP toxicity. Support for this hypothesis also lies in the ability of pargyline (Table 5), succinic acid (Table 10) or ascorbic acid (Table 15) to lessen the OHP-induced elevation in brain ammonia and to protect against OHP toxicity. Further, there was a significant correlation between OHP seizure susceptibility and brain ammonia levels in pargyline-, succinic acid- or ascorbic acid-treated mice (Figures 8, 15 and 20). The decrease in brain glutamine levels during OHP exposure (Table 5, 10

and 15) also correlated well with OHP seizure susceptibility in pargyline-, succinic acid- or ascorbic acid-treated mice (Figures 9, 16 and 21). This decrease is thought to be due to inhibition of glutamine synthetase (Gershenovich et al., 1963); hence, the ability of the brain to detoxify ammonia was impaired.

The source of this ammonia could be, in part, due to increased MAO activity in the presence of elevated oxygen tension (Novick, 1966). This would increase various deamination reactions resulting in the observed elevation in brain ammonia levels and decrease in brain DA, NE and 5-HT during OHP exposure (Faiman et al., 1971). Pargyline offset this OHP-induced ammonia elevation (Table 5) possibly by preventing the OHP-induced increase in MAO activity.

Succinic acid prevented the OHP-induced decrease in brain ATP levels (Woodhall et al., 1971) and may, therefore, prevent OHP-induced inhibition of glutamine synthetase if the enzyme inhibition is due to decreased ATP availability.

Ascorbic acid, like succinic acid, can supply reducing equivalents to the mitochondrial respiratory chain (Mapson, 1967) and could, therefore, also prevent the OHP-induced decrease in brain ATP levels. Both succinic acid and ascorbic acid may in this manner allow the brain's ammonia detoxication pathway to function normally during OHP exposure and consequently, prevent the OHP-induced buildup in brain ammonia and decrease in brain glutamine levels.

## RELATIONSHIP BETWEEN BRAIN AMMONIA AND GAMMA-AMINOBUTYRIC ACID

As can be seen in Figure 1, both ammonia and GABA are intimately related in that they have the common precursor glutamic acid. Although

pargyline, succinic acid or ascorbic acid all protected against OHP convulsions, ascorbic acid was not as effective against the total OHP toxicity syndrome. This, and the fact that all three agents lessened the OHP-induced elevation in brain ammonia, but ascorbic acid, unlike pargyline and succinic acid, did not prevent the OHP-induced decrease in brain GABA, may point to a relationship between ammonia and GABA in the etiology of OHP toxicity. We feel that decreased brain GABA levels and increased ammonia levels are primarily responsible for the OHP toxicity syndrome.

There is another common link between these protective agents in that they all have an effect on the mitochondria whose function is impaired early in the course of OHP toxicity (Haugaard, 1968). Mitochondrial dysfunction could, therefore, account for the reported decrease in brain ATP levels (Woodhall et al., 1971) and consequent inhibition of glutamine synthetase (Gershenovich et al., 1963) and pyridoxal kinase (Baxter, 1970). This inhibition could, therefore, account for the altered brain GABA and ammonia metabolism observed in our studies and by others (Wood et al., 1966; Wood et al., 1969; Gershenovich and Krichenskoya, 1954; Gershenovich et al., 1963).

If increased MAO activity contributes to mitochondrial dysfunction, then pargyline could prevent this effect. Also, MAO is associated with the outer leaf of the mitochondrial membrane (Sottocasa, 1967; Schnaitman et al., 1967) and pargyline could maintain mitochondrial integrity by binding to this membrane-associated enzyme possibly stabilizing the mitochondrial membrane. In either case, stabilization of mitochondrial function could be the result, and consequently, normal ATP levels could be maintained.

Normal ATP levels are maintained after succinic acid treatment in OHP-exposed animals (Sanders et al., 1966), and this may also be true in the case of ascorbic acid since both agents can supply reducing equivalents to the mitochondrial respiratory chain for ATP production. The decreased protective effect of ascorbic acid compared to succinic acid could be due to the fact that ascorbic acid enters the respiratory chain at a later step than does succinic acid (Mapson, 1967) and consequently, would not provide for as much ATP production as would succinic acid.

Therefore, although decreased brain GABA and increased ammonia levels may be responsible for the actual OHP toxic syndrome, the common underlying cause of these biochemical changes in GABA, ammonia and glutamine and GABA enzymes could be due to decreased ATP production or availability, and pargyline, succinic acid or ascorbic acid could exert their protective effect by maintaining normal ATP levels during OHP exposure.

# VI. SUMMARY AND CONCLUSIONS

1. Hyperbaric oxygen produced convulsions and post-OHP mortality and increased weight, water content and hemoglobin content of the lungs.
2. Pargyline, succinic acid or ascorbic acid protected against all of the above aspects of OHP toxicity to varying degrees. The doses of pargyline and succinic acid that were maximally effective in protecting against OHP convulsions were approximately equal in their ability to reduce the severity of the rest of the OHP toxicity syndrome. The dose of ascorbic acid providing maximal protection against OHP convulsions was as effective as was pargyline or succinic acid in decreasing OHP convulsions but was less effective in reducing the severity of the other aspects of oxygen poisoning.
3. OHP exposure decreased brain GABA levels and brain GAD activity but had no effect on GABA-T activity. Both pargyline and succinic acid increased brain GABA levels and prevented the OHP-induced decrease in brain GABA. The ability of these agents to elevate brain GABA correlated well with the dose of the agent administered. There was also a correlation between GABA levels and OHP seizure susceptibility in pargyline- or succinic acid-treated mice.
4. Pargyline elevated brain GABA and prevented the OHP-induced decrease in GABA via inhibition of GABA-T activity in mice exposed to room air or OHP. Pargyline had no effect on GAD activity nor did it prevent the OHP-induced decrease in GAD activity.

5. Succinic acid increased GABA-T and GAD activity in room air-exposed mice.
6. Succinic acid increased brain GABA levels by increasing the activity of GAD. The increase in GABA-T activity after succinic acid was less than that of GAD, and GABA-T activity returned to control levels during OHP exposure while GAD activity remained slightly elevated.
7. Ascorbic acid had no effect on brain GABA levels nor did it prevent the OHP-induced decrease in brain GABA. Ascorbic acid had no effect on the GABA enzymes nor did it prevent the OHP-induced decrease in brain GAD activity. There was no correlation between GABA levels and OHP seizure susceptibility in ascorbic acid-treated mice.
8. Hyperbaric oxygen exposure increased brain ammonia and decreased brain glutamine levels.
9. Pargyline, succinic acid or ascorbic acid had no effect on brain ammonia or glutamine levels in room air-exposed mice.
10. Pargyline, succinic acid and ascorbic acid markedly reduced the OHP-induced increase in brain ammonia levels and also reduced the degree to which brain glutamine levels were decreased by OHP exposure.
11. There was a correlation between brain ammonia levels and OHP seizure susceptibility in pargyline-, succinic acid- or ascorbic acid-treated mice. This was also true for glutamine levels and OHP seizure susceptibility.
12. The effectiveness of pargyline, succinic acid and ascorbic acid as protective agents against OHP toxicity is related to their ability to alter the deleterious effects of OHP exposure on brain ammonia and GABA metabolism.

REFERENCES

Balzer, H., Holtz, P. and Palm, D.: Reserpin und γ-aminobuttersäuergehalt des gehirns. Experentia 17: 38-40, 1960.

Baxter, C. F.: The nature of γ-aminobutyric acid. In Handbook of Neurochemistry, ed. A. Lajtha, vol. 3, pp. 289-353, Plenum Press, New York, 1970.

Bean, J. W.: Effects of oxygen at increased pressure. Physiol. Rev. 25: 1-147, 1945.

Bean, J. W. and Johnson, P. C.: Epinephrine and neurogenic factors in the pulmonary edema and CNS reactions induced by $O_2$ at high pressure. Am. J. Physiol. 180: 438-444, 1955.

Bean, J. W., Zee, D. and Thom, B.: Pulmonary changes with convulsions induced by drugs and oxygen at high pressure. J. Appl. Physiol. 21: 865-872, 1966.

Behnke, A. R., Johnson, F. S., Poppen, J. R. and Motley, E. P.: The effect of oxygen on man at pressures from 1 to 4 atmospheres. Am. J. Physiol 110: 565-572, 1934-5.

Benitez, D., Pscheidt, G. R. and Stone, W. E.: Formation of ammonium ion in the cerebrum in fluoracetate poisoning. Am. J. Physiol. 176: 488-492, 1954.

Bernhard, W. F., Norman, J. C., Ishizuka, R. and Carr, J. G.: Evaluation of hyperbaric surgery in infants with congenital cardiac defects. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp. 345-361, National Academy of Sciences and National Research Council, Washington, D. C., 1966.

Bert, P.: La Pression Barometrique. (1878). English translation by M. A. Hitchcock and F. A. Hitchcock, College Book Co., Columbus, Ohio, 1943.

Blenkarn, G. D., Schanberg, S. M. and Saltzman, H. A.: Cerebral amines and acute hyperbaric oxygen toxicity. J. Pharmacol. Exp. Ther. 166: 346-353, 1969.

Brummelkamp, W. H.: Treatment of anaerobic infections with hyperbaric oxygen. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp. 492-500, National Academy of Sciences and National Research Council, Washington, D. C., 1966.

Buttery, P. J. and Roswell, E. V.: Enzymic assays for ammonia and L-glutamine in tissue extracts. Analyt. Biochem. 39: 297-310, 1971.

Churchill-Davidson, I.: Radiation therapy with hyperbaric oxygen. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp. 643-647, National Academy of Sciences and National Research Council, Washington, D. C., 1966.

Clark, J. M. and Lambertsen, C. J.: Pulmonary oxygen toxicity: A review. Pharmacol. Rev. 23: 37-133, 1971.

DeFeudis, F. V. and Elliott, K.A.C.: Delay or inhibition of convulsions by intraperitoneal injections of diverse substances. Can. J. Physiol. Pharmacol. 45: 857-865, 1967.

Diaz, P. A., Ngai, S. H. and Costa, E.: Effect of oxygen on brain serotonin metabolism in rats. Am. J. Physiol. 214: 591-594, 1966.

Del Guercio, L.R.M., Cohn, J. D., Greenspan, M., Feins, N.R. and Kornitzer, G.: Pulmonary and systemic arteriovenous shunting in clinical septic shock. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp. 337-342, National Academy of Sciences and National Research Council, Washington, D. C., 1966.

Dickens, F.: The toxic effect of oxygen on nervous tissue. In Neurochemistry, ed. K.A.C. Elliott, I. H. Page and J. H. Quastel, pp. 851-867, Charles C. Thomas Publishers, Springfield, Illinois, 1962.

Dixon, W. J. and Massey, F. J.: Introduction to Statistical Analysis, 3rd edition, McGraw-Hill, New York, 1969.

Faiman, M. D. and Noble, A. R.: The effect of hyperbaric oxygenation on cerebral amines. Life Sci. 5: 2225-2234, 1966.

Faiman, M. D., Mehl, R. G. and Meyers, M. B.: Brain norepinephrine and serotonin in central oxygen toxicity. Life Sci. 10: 21-34, 1971.

Folbergrova, J.: Free glutamine levels in the rat brain in vivo after methionine sulfoximine administration. Physiol. Bohemoslov. 13: 21-27, 1964.

Gerschman, R. and Fenn, W. O.: Ascorbic acid content of adrenal glands of rat in oxygen poisoning. Am. J. Physiol. 176: 6-8, 1954.

Gerschman, R., Gilbert, D. L., Nye, S. W., Price, W. E. and Fenn, W. O.: Effects of autonomic drugs and of adrenal glands on oxygen poisoning. Proc. Soc. Exp. Biol. Med. 88: 617-621, 1955.

Gershenovich, Z. S. and Krichevskoya, A. A.: Ammonia and glutamine in the brain at increased oxygen pressure. Doklady Akad. S.S.S.R. 95: 837-840, 1954.

Gershenovich, Z. S., Krichevskoya, A. A. and Kolousek, A.: The effect of raised oxygen pressure and of methionine sulphoximine on the glutamine synthetase activity of brain. J. Neurochem. 10: 79-82, 1963.

Gesell, R.: On the chemical regulation of respiration: Regulation of respiration with special reference to the metabolism of the respiratory centre and the coordination of the dual function of hemoglobin. Am. J. Physiol. 66: 5-49, 1923.

Gottleib, S. F. and Cymerman, A.: The independent effects of restraint and ammonium salts on susceptibility to oxygen toxicity. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp. 97-105, National Academy of Sciences and National Research Council, Washington, D. C., 1966.

Graham, L. T. and Aprison, M. H.: Fluorometric determination of aspartate, glutamate and γ-aminobutyrate in nerve tissue using enzymatic methods. Analyt. Biochem. 15: 487-497, 1966.

Haggendal, J.: The effect of high pressure air or oxygen with and without carbon dioxide added on the catecholamine levels of rat brain. Acta. Physiol. Scand. 69: 147-152, 1967.

Haggendal, J.: Effect of hyperbaric oxygen on monoamine metabolism in central and peripheral tissue of the rat. Eur. J. Pharmacol. 2: 323-325, 1968.

Hainline, A. Jr.: Standard Methods of Clinical Chemistry, vol. 2, ed. D. Seligson, pp. 49, Academic Press, New York, 1958.

Haugaard, N.: Cellular mechanisms of oxygen toxicity. Physiol. Rev. 48: 311-373, 1968.

Hitchcock, C. R., Haglin, J. J. and Telander, R. L.: Treatment of clostridial infections with hyperbaric oxygen. Surgery 62: 759-769, 1967.

Jamieson, D. and Van den Brenk, H.A.S.: Pulmonary damage due to high pressure oxygen breathing in rats. (2) Changes in dehydrogenase activity of rat lung. Aust. J. Exp. Biol. Med. Sci. 40: 51-56, 1962.

Jamieson, D. and Van den Brenk, H.A.S.: The effects of antioxidants on high pressure oxygen toxicity. Biochem. Pharmacol. 13: 159-164, 1964.

Jones, R. C.: Tetanus and gas gangrene; present attitudes. Postgrad. Med. 41: 641-648, 1967.

Johnson, P. C. and Bean, J. W.: Effect of sympathetic blocking agents on the toxic action of oxygen at high pressure. Am. J. Physiol. 188: 593-598, 1957.

Karsner, H. T.: The pathological effects of atmospheres rich in oxygen. J. Exp. Med. 23: 149-170, 1916.

Karsner, H. T. and Ash, J. E.: A further study of the pathological effects of atmospheres rich in oxygen. J. Lab. Clin. Med. 2: 254-255, 1917.

Katunuma, N., Okada, M. and Nishi, Y.: Regulation of the urea cycle and TCA cycle by ammonia. Adv. Enz. Reg. 4: 317-334, 1965.

König, H. and König, R.: Production of acute pulmonary edema by ammonium salts. Proc. Soc. Exp. Biol. Med. 70: 375-380, 1949.

Kopin, I. J., Fischer, J. E., Musacchio, J. M., Horst, W. D. and Weise, V. K.: "False neurochemical transmitters" and the mechanism of sympathetic blockade by monoamine oxidase inhibitors. J. Pharmacol. Exp. Therap. 147: 186-193, 1965.

Kramer, B. S., Sanders, A. P., Lesage, A. M. and Woodhall, B.: The effect of profound hypothermia on preservation of cerebral ATP content during circulatory arrest. J. Thorac. Cardiovasc. Surg. 56: 699-709, 1968.

Kravitz, E. A.: Acecylcholine, gamma-aminobutyric acid and glutamic acid: physiological and chemical studies related to their roles as neurotransmitter agents. In The Neurosciences, ed. G. C. Quarton, T. Melenchuk and F. O. Schmitt, pp. 433-444, Rockefeller University Press, New York, 1967.

Lambertsen, C. J.: Effects of oxygen at high partial pressure. In Handbook of Physiology, vol. 2, ed. W. O. Fenn and H. Rahn, pp. 1027-1046, Williams and Wilkins Co., Baltimore, 1965.

Lehman, A.: Audiogenic seizures. Data in mice supporting new theories of biogenic amine mechanisms in the central nervous system. Life Sci. 6: 1423-1431, 1967.

Mapson, L. W.: Ascorbic acid. IX Biochemical systems. In The Vitamins, ed. W. H. Sebrell and R. S. Harris, vol. 1, pp. 386-398, Academic Press, New York, 1967.

McIlvaine, T. C.: A buffer solution for colorimetric comparison. J. Biol. Chem. 49: 183-186, 1921.

Mejne, N. G.: Hyperbaric oxygen in cardiac surgery. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp. 353-362, National Academy of Sciences and National Research Council, Washington, D. C., 1966.

Moor, G. F., Fuson, R. L., Margolis, G., Brown, J. W. and Smith, W. W.: Protective effect of hyperbaric oxygenation on the central nervous system during circulatory arrest. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp.

363-371, National Academy of Sciences and National Research Council, Washington, D. C., 1966.

Myles, W. S. and Wood, J. D.: The effect of hyperbaric oxygen on the GABA shunt pathway in brain homogenates. Can. J. Physiol. Pharmacol. 46: 669-671, 1968.

Nathanson, L., Brown, B., Maddock, C. and Hall, T. C.: Effects of antitumor agents and hyperbaric oxygen in normal and tumor-bearing rodents. Cancer 19: 1019-1025, 1966.

Neff, N. H. and Costa, E.: The effect of oxygen on the turnover of biogenic amines in vivo. I. Catecholamine. (Abstract) Fed. Proc. 26: 463, 1967.

Nelson, B. S., Stansell, G. B. and Kraemer, J. G.: Hyperbaric oxygen in experimental burn. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp. 604-605, National Academy of Sciences and National Research Council, Washington, D. C., 1966.

Norman, J. N., MacIntyre, J., Shearer, J. R. and Smith, G.: Use of a one-man, mobile pressure chamber in the treatment of carbon monoxide poisoning. Brit. Med. J. 2: 333-334, 1970.

Novick, W. J.: Effect of oxygen tension on monoamine oxidase activity. Biochem. Pharmacol. 15: 1009-1012, 1966.

Oliver, J. H., Little, J. M. and Pirch, J. H.: Effect of reserpine and other drugs on the CNS and lethal effects of hyperbaric oxygen in mice. Arch. Int. Pharmacodyn. 183: 215-223, 1970.

Paine, J. R., Keys, A. and Lynn, D.: Manifestation of oxygen poisoning in dogs confined in atmospheres of 80 and 100% oxygen. Am. J. Physiol. 133: 406-407, 1941.

Peter, R. H., Rau, R. W., Whalen, R. W., Entman, M. L. and McIntosh, H. D.: Effect of hyperbaric oxygenation on coronary artery occlusion in pigs. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp. 395-399, National Academy of Sciences and National Research Council, Washington, D. C., 1966.

Pletscher, A. L., Gey, K. F. and Zeller, P.: Monoamine-oxydaschemmer. In Progress in Drug Research, ed. E. Jucker, vol. 2, pp. 417-591, Birkhäuser Publishers, Stuttgart, Germany, 1960.

Plotinkoff, N., Juang, J. and Havens, P.: Effect of monoamine oxidase inhibitors on audiogenic seizures. J. Pharm. Sci. 52: 172-173, 1963.

Popov, N. and Matthies, H.: Some effects of monoamine oxidase inhibitors on the metabolism of gamma-aminobutyric acid in rat brain. J. Neurochem. 16: 899-907, 1969.

Prescott, B. A. and Waelsch, H.: A microdetermination of glutamic acid and its application to protein analysis. J. Biol. Chem. 164: 331-343, 1946.

Prockop, D. J., Parkhurst, A. S. and Brodie, B. B.: Anticonvulsant properties of monoamine oxidase inhibitors. Ann. N. Y. Acad. Sci. 80: 643-651, 1959.

Puglia, C. D., Glauser, E. M. and Glauser, S. C.: Effect of hyperbaric $O_2$ on rat rectal temperature. (Abstract) Fed. Proc. 29: 743, 1970.

Riechert, W.: Beitrag zum cardiazol-lungenodem. Arch. Exp. Path. Pharmakol. 197: 620-624, 1941.

Roberts, E.: γ-aminobutyric acid. In Neurochemistry, ed. K.A.C. Elliott, I. H. Page and J. H. Quastel, pp. 636-655, Charles C. Thomas Publishers, Springfield, Illinois, 1962.

Roberts, E. and Simonsen, D. G.: Some properties of L-glutamic acid decarboxylase in mouse brain. Biochem. Pharmacol. 12: 113-134, 1963.

Salvatore, G. D. and Bocchini, V.: Enhancement of urea biosynthesis by some amino acids as a model of ammonia detoxication. Biochimica Applicata 8: 228-234, 1961.

Sanders, A. P., Hall, I. H., Cavanaugh, P. J. and Woodhall, B.: Effects of hyperbaric oxygenation on metabolism. I. ATP concentration in rat brain, liver and kidney. Proc. Soc. Exp. Biol. Med. 121: 32-34, 1966.

Sayers, G. and Sayers, M. A.: The pituitary-adrenal system. Ann. N. Y. Acad. Sci. 50: 522-539, 1949.

Schlesinger, K., Boggan, W. and Freedman, D. X.: Genetics of audiogenic seizures. I. Relation to brain serotonin and norepinephrine in mice. Life Sci. 4: 2345-2351, 1965.

Schlesinger, K., Boggan, W. and Freedman, D. X.: Genetics of audiogenic seizures. II. Effects of pharmacological manipulation of brain serotonin, norepinephrine and gamma-aminobutyric acid. Life Sci. 7: 437-447, 1968.

Schnaitman, C., Erwin, V. G. and Greenwalt, J. W.: The submitochondrial localization of monoamine oxidase. J. Cell. Biol. 32: 719-735, 1967.

Serrill, S., Jefferson, D., Quick, J. and Mengel, C. E.: Effect of acetylsalicylic acid and ascorbic acid on oxygen toxicity. Aerospace Med. 42: 436-438, 1971.

Shene, W. G., Norman, J. N. and Smith, G.: Effect of hyperbaric oxygen in cyanide poisoning. In Proceedings of the Third International Conference on Hyperbaric Medicine, ed. J. W. Brown and B. G. Cox, pp. 705-710, National Academy of Sciences and National Research Council, Washington, D.C., 1966.

Shilling, C. W. and Adams, B. H.: A study of convulsive seizures caused by breathing oxygen at high pressure. U. S. Naval Med. Bull. 31: 112-121, 1933.

Smith, F.J.C., Bennett, G. A., Heim, J. W., Thomson, R. M. and Drinker, C. K.: Morphological changes in the lungs of rats living under compressed air conditions. J. Exp. Med. 56: 79-89, 1932.

Smith, J. L.: The pathological effects due to increase of oxygen tension in the air breathed. J. Physiol. 24: 19-35, 1899.

Sottocasa, G. L.: Biochemical properties of inner and outer mitochondrial membranes of liver. Biochem. J. 105: 1-2P, 1967.

Stadie, W. C., Riggs, B. C. and Haugaard, N.: Oxygen poisoning. Am. J. Med. Sci. 207: 84-114, 1944.

Stromme, J. H.: Metabolism of disulfuram and diethyldithiocarbamate in rats with demonstration of an in vivo ethanol-induced inhibition of the glucuronic acid conjugation of the thiol. Biochem. Pharmacol. 14: 393-410, 1965.

Szam, I.: Beitrag zur pathogenese der hyperbaren sauerstoffvergiftung. Der Anaesthesist 18: 39-43, 1969.

Tabor, C. G.: Hyperbaric oxygenation of burns of less than forty percent. Korean J. Intern. Med. 10: 53-61, 1967.

Tapia, R. and Pasantes, H.: Relationship between pyridoxal phosphate availability, activity of vitamin $B_6$-dependent enzymes and convulsions. Brain Res. 29: 111-122, 1971.

Tennekoon, G. E.: Pulmonary edema due to thiosemicarbazide. J. Path. Bact. 67: 341-347, 1954.

Ugarte, G., Pereda, T. and Vallega, A.: Influencia de la intoxicacion y del dano hepatico agudo sobre el acido gama-aminobutirico cerebral en ratas. Arch. Biol. Med. Exp. 5: 1-3, 1968.

Van den Brenk, H.A.S. and Jamieson, D.: Pulmonary damage due to high pressure oxygen breathing in rats. (1) lung weight, histological and radiological studies. Aust. J. Exp. Biol. Med. Sci. 40: 37-50, 1962.

VanTassel, P. V.: A hyperbaric chamber for small animals. J. Appl. Physiol. 20: 342-345, 1965.

Warren, K. S. and Schenker, S.: Effect of an inhibitor of glutamine synthesis (methionine sulfoximine) on ammonia toxicity and metabolism. J. Lab. Clin. Med. 64: 442-449, 1964.

Weil-Malherbe, H.: Ammonia metabolism in the brain. In Neurochemistry, ed. K.A.C. Elliott, I. H. Page and J. H. Quastel, pp. 321-327, Charles C. Thomas Publishers, Springfield, Illinois, 1962.

Wolfe, L. S. and Elliott, K.A.C.: Chemical studies in relation to convulsive disorders. In Neurochemistry, ed. K.A.C. Elliott, I. H. Page and J. H. Quastel, pp. 694-727, Charles C. Thomas Publishers, Springfield, Illinois, 1962.

Wood, J. D.: Seizures induced by hyperbaric oxygen and cerebral gamma-aminobutyric acid in chicks during development. J. Neurochem. 17: 573-579, 1970.

Wood, J. D., Stacey, N. E. and Watson, W. J.: Pulmonary and central nervous system damage in rats exposed to hyperbaric oxygen and protection therefrom by gamma-aminobutyric acid. Can. J. Physiol. Pharmacol. 43: 405-410, 1965.

Wood, J. D. and Watson, W. J.: Gamma-aminobutyric acid levels in the brain of rats exposed to oxygen at high pressures. Can. J. Biochem. Physiol. 41: 1907-1913, 1963.

Wood, J. D. and Watson, W. J.: The effect of amino-oxyacetic acid and hydroxylamine on rats breathing oxygen at high pressure. J. Neurochem. 12: 663-669, 1965.

Wood, J. D. and Watson, W. J.: The effect of intraperitoneal injections of hyperosmotic solutions on convulsions induced by drugs and hyperbaric oxygen. Can. J. Physiol. Pharmacol. 46: 649-652, 1968.

Wood, J. D., Watson, W. J. and Clydesdale, F. M.: Gamma-aminobutyric acid and oxygen poisoning. J. Neurochem. 10: 625-633, 1963.

Wood, J. D., Watson, W. J. and Ducker, A. J.: Oxygen poisoning in various mammalian species and the possible role of gamma-aminobutyric acid metabolism. J. Neurochem. 14: 1067-1074, 1967.

Wood, J. D., Watson, W. J. and Murray, G. W.: Correlation between decreases in brain γ-aminobutyric acid levels and susceptibility to convulsions induced by hyperbaric oxygen. J. Neurochem. 16: 281-287, 1969.

Wood, J. D., Watson, W. J. and Stacey, N. E.: A comparative study of hyperbaric oxygen-induced convulsions with particular reference to γ-aminobutyric acid metabolism. J. Neurochem. 13: 361-370, 1966.

Woodhall, B., Kramer, R. S., Currie, W. D. and Sanders, A. P.: Brain energetics and neurosurgery. A review of recent studies done at Duke University. J. Neurosurg. 34: 3-14, 1971.

# VITA

Robert Alfred Schatz was born on January 3, 1943 in Fitchburg, Massachusetts, where he obtained his elementary and high school education. Mr. Schatz enrolled at the Massachusetts College of Pharmacy in 1961 and received his Bachelor of Science degree in pharmacy in June, 1966. He then entered the Graduate School at Northeastern University, Boston, in September, 1966, where Mr. Schatz completed the requirements for the Master of Science degree of Pharmacology in June, 1968. Mr. Schatz entered the Graduate School of the University of Rhode Island in September, 1968 and completed the requirements for the Doctor of Philosophy degree in pharmacology in June, 1972. He is a member of the American Association for the Advancement of Science, Rho Chi and Sigma XI.

Mr. Schatz is married to the former Anne Elizabeth Sawyer of South Portland, Maine. Their two children are Jonathan Robert, age 5, and Heidi Anne, age 1.

Mr. Schatz has accepted a postdoctoral research position in neurochemistry at the Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan.

# APPENDIX

Table 1. EFFECT OF PRETREATMENT TIME AND DOSE OF PARGYLINE ON HYPERBARIC OXYGEN CONVULSIONS

| Dose[a] (mg/kg) | Pretreatment time (min) | N | Minutes of OHP Exposure 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % Convulsed | | | | | | | | |
| | | | CONVULSION ONSET | | | | | | | | |
| 0 | 30 | 30 | 0 | 23 | 37 | 60 | 70 | 93 | 100 | 100 | 100 |
| 50 | 30 | 7 | 0 | 13 | 13 | 43 | 57 | 86 | 86 | 86 | 100 |
| 100 | 30 | 26 | 0 | 0 | 0 | 19 | 19 | 23 | 35 | 35 | 39 |
| 200 | 30 | 13 | 0 | 0 | 15 | 15 | 23 | 23 | 46 | 60 | 60 |
| 100 | 60 | 7 | 0 | 0 | 15 | 13 | 29 | 43 | 43 | 57 | 71 |
| 100 | 120 | 10 | 0 | 0 | 10 | 20 | 20 | 40 | 60 | 70 | 70 |
| 100 | 240 | 7 | 0 | 0 | 13 | 29 | 29 | 57 | 71 | 71 | 86 |
| 100[b] | 30 | 7 | 0 | 0 | 0 | 13 | 13 | 29 | 29 | 29 | 43 |
| | | | SEIZURE COMPLEX | | | | | | | | |
| 0 | 30 | 30 | 0 | 0 | 7 | 23 | 51 | 81 | 100 | 100 | 100 |
| 50 | 30 | 7 | 0 | 0 | 0 | 13 | 29 | 29 | 57 | 71 | 71 |
| 100 | 30 | 26 | 0 | 0 | 0 | 4 | 4 | 8 | 12 | 12 | 12 |
| 200 | 30 | 13 | 0 | 0 | 0 | 8 | 15 | 23 | 38 | 54 | 54 |
| 100 | 60 | 7 | 0 | 0 | 0 | 0 | 13 | 29 | 43 | 57 | 57 |
| 100 | 120 | 10 | 0 | 0 | 0 | 10 | 10 | 20 | 20 | 20 | 40 |
| 100 | 240 | 7 | 0 | 0 | 0 | 13 | 13 | 29 | 29 | 57 | 57 |
| 100[b] | 30 | 7 | 0 | 0 | 0 | 13 | 13 | 13 | 13 | 29 | 29 |

[a] Pargyline or saline (10 ml/kg body weight) was injected intraperitoneally.

[b] Pargyline solution was adjusted to pH 6.5 with 2N NaOH.

Table 2. ALTERATION OF MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID BY PARGYLINE AND HYPERBARIC OXYGEN

| Treatment | | | | | |
|---|---|---|---|---|---|
| Drug[a]-time[b] | | OHP[c]-time[b] | µmoles GABA/g brain (Mean ± S.E.)[e] | p[d] | % Change[f] |
| Saline | -120 | 0 | 2.19 ± 0.065 | -- | --- |
| Saline | -60 | 30 | 1.85 ± 0.108 | <0.05 | -16 |
| Saline | -90 | 60 | 1.43 ± 0.124 | <0.005 | -32 |
| Saline | -120 | 90 | 1.43 ± 0.142 | <0.005 | -35 |
| Pargyline | -30 | 0 | 2.47 ± 0.057 | <0.01 | +13 |
| Pargyline | -60 | 0 | 2.83 ± 0.142 | <0.005 | +30 |
| Pargyline | -120 | 0 | 3.12 ± 0.081 | <0.001 | +42 |
| Pargyline | -60 | 30 | 2.33 ± 0.064 | >0.05 | +6 |
| Pargyline | -90 | 60 | 2.46 ± 0.077 | >0.05 | +13 |
| Pargyline | -120 | 90 | 2.35 ± 0.091 | >0.05 | +7 |

[a] Saline (10 ml/kg body weight) or pargyline (100 mg/kg) was injected intraperitoneally.

[b] Minutes.

[c] OHP exposure was at 60 psig.

[d] Significantly different from saline controls at the 0.05 level (Student "t" test).

[e] Each value represents mean of six mice.

[f] Plus represents increase and minus, decrease.

Table 3. EFFECT OF VARIOUS DOSES OF PARGYLINE ON MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID

| Treatment[a] | µmoles GABA/g brain, Mean ± S.E. (N)[b] | P[c] | % Change[d] |
|---|---|---|---|
| Saline | 2.08 ± 0.079 (5) | -- | --- |
| Pargyline (25 mg/kg) | 2.24 ± 0.089 (5) | >0.05 | +8 |
| Pargyline (50 mg/kg) | 2.48 ± 0.094 (5) | <0.02 | +19 |
| Pargyline (100 mg/kg) | 2.76 ± 0.089 (6) | <0.001 | +33 |
| Pargyline (200 mg/kg) | 2.91 ± 0.107 (5) | <0.001 | +40 |

[a] Saline or pargyline (10 ml/kg body weight) was injected intraperitoneally 120 min prior to sacrifice. Dose is given in parentheses.

[b] Values were obtained from duplicate samples.

[c] Significantly different from saline controls at the 0.05 level (Student "t" test).

[d] Plus represents increase.

Table 4. EFFECT OF PRETREATMENT TIME AND DOSE OF SUCCINIC ACID ON HYPERBARIC OXYGEN CONVULSIONS

| Dose[a] (mmoles/kg) | Pretreatment time (min) | N | Minutes of OHP Exposure 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % Convulsed | | | | | | | | |
| CONVULSION ONSET | | | | | | | | | | | |
| 0 | 60 | 20 | 0 | 30 | 40 | 70 | 80 | 90 | 100 | 100 | 100 |
| 6 | 60 | 7 | 0 | 0 | 13 | 13 | 43 | 43 | 57 | 71 | 71 |
| 12 | 60 | 18 | 0 | 6 | 11 | 39 | 44 | 50 | 57 | 57 | 57 |
| 15 | 60 | 7 | 0 | 13 | 13 | 29 | 43 | 43 | 57 | 57 | 57 |
| 12 | 30 | 7 | 0 | 13 | 13 | 13 | 29 | 57 | 71 | 71 | 71 |
| 12 | 120 | 7 | 0 | 0 | 13 | 29 | 29 | 29 | 43 | 57 | 57 |
| 12 | 240 | 7 | 0 | 13 | 29 | 29 | 57 | 57 | 71 | 86 | 86 |
| 12[b] | 60 | 7 | 0 | 0 | 0 | 13 | 29 | 43 | 43 | 43 | 57 |
| SEIZURE COMPLEX | | | | | | | | | | | |
| 0 | 60 | 20 | 0 | 0 | 0 | 30 | 80 | 90 | 90 | 100 | 100 |
| 6 | 60 | 7 | 0 | 0 | 0 | 13 | 29 | 29 | 43 | 43 | 57 |
| 12 | 60 | 18 | 0 | 0 | 0 | 6 | 11 | 11 | 22 | 33 | 33 |
| 15 | 60 | 7 | 0 | 0 | 0 | 0 | 0 | 13 | 43 | 43 | 43 |
| 12 | 30 | 7 | 0 | 0 | 0 | 13 | 13 | 57 | 71 | 71 | 71 |
| 12 | 120 | 7 | 0 | 0 | 0 | 13 | 13 | 13 | 29 | 29 | 43 |
| 12 | 240 | 7 | 0 | 0 | 0 | 29 | 29 | 43 | 57 | 57 | 71 |
| 12[b] | 60 | 7 | 0 | 0 | 0 | 13 | 13 | 13 | 29 | 29 | 29 |

[a]Succinic acid or saline (10 ml/kg body weight) was injected intraperitoneally.

[b]Succinic acid solution was adjusted to pH 6.5 with 2N HCl.

Table 5. ALTERATION OF MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID BY SUCCINIC ACID AND HYPERBARIC OXYGEN

| Treatment | | | | | |
|---|---|---|---|---|---|
| Drug[a]-time[b] | | OHP[c]-time[b] | µmoles GABA/g brain (Mean ± S.E.)[e] | p[d] | % Change[f] |
| Saline | -150 | 0 | 1.97 ± 0.037 | -- | --- |
| Saline | -90 | 30 | 1.72 ± 0.072 | <0.005 | -13 |
| Saline | -120 | 60 | 1.48 ± 0.067 | <0.001 | -25 |
| Saline | -150 | 90 | 1.33 ± 0.080 | <0.001 | -33 |
| Succinic acid | -60 | 0 | 2.40 ± 0.097 | <0.005 | +22 |
| Succinic acid | -90 | 0 | 2.58 ± 0.128 | <0.005 | +31 |
| Succinic acid | -150 | 0 | 2.36 ± 0.061 | <0.001 | +20 |
| Succinic acid | -90 | 30 | 2.42 ± 0.158 | <0.02 | +23 |
| Succinic acid | -120 | 60 | 2.23 ± 0.068 | <0.01 | +13 |
| Succinic acid | -150 | 90 | 1.91 ± 0.049 | >0.05 | -3 |

[a] Saline (10 ml/kg body weight) or succinic acid (12 mmoles/kg) was injected intraperitoneally.

[b] Minutes.

[c] OHP exposure was at 60 psig.

[d] Significantly different from saline controls at the 0.05 level (Student "t" test).

[e] Each value represents mean of six mice.

[f] Plus represents increase and minus, decrease.

Table 6. EFFECT OF VARIOUS DOSES OF SUCCINIC ACID ON MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID

| Treatment[a] | μmoles GABA/g brain, mean ± S.E. (N) | P[c] | % Change[d] |
|---|---|---|---|
| Saline | 2.03 ± 0.063 (5) | -- | --- |
| Succinic acid (3 mmoles/kg) | 2.12 ± 0.103 (5) | >0.05 | +4 |
| Succinic acid (6 mmoles/kg) | 2.37 ± 0.098 (5) | <0.01 | +17 |
| Succinic acid (12 mmoles/kg) | 2.63 ± 0.135 (5) | <0.005 | +30 |
| Succinic acid (24 mmoles/kg) | 3.25 ± 0.098 (4)[b] | <0.0005 | +60 |

[a]Saline or succinic acid (20 ml/kg body weight) was injected intraperitoneally 90 min prior to sacrifice. Dose is given in parentheses.

[b]One out of five mice was dead prior to sacrifice and the remaining four appeared sick.

[c]Significantly different from saline controls at the 0.05 level (Student "t" test).

[d]Plus represents increase.

Table 7. EFFECT OF PRETREATMENT TIME AND DOSE OF L-ASCORBIC ACID ON HYPERBARIC OXYGEN CONVULSIONS

| Dose[a] (mmoles/kg) | Pretreatment time (min) | N | Minutes of OHP Exposure 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % Convulsed | | | | | | | | |
| CONVULSION ONSET | | | | | | | | | | | |
| 0 | 60 | 19 | 0 | 42 | 47 | 53 | 74 | 100 | 100 | 100 | 100 |
| 6 | 60 | 7 | 0 | 13 | 43 | 71 | 71 | 71 | 71 | 71 | 71 |
| 12 | 60 | 18 | 0 | 0 | 0 | 17 | 17 | 28 | 28 | 28 | 28 |
| 15 | 60 | 7 | 0 | 0 | 0 | 13 | 29 | 29 | 29 | 43 | 43 |
| 12 | 30 | 7 | 0 | 43 | 43 | 43 | 57 | 57 | 57 | 57 | 57 |
| 12 | 120 | 7 | 0 | 0 | 0 | 13 | 13 | 29 | 29 | 29 | 43 |
| 12 | 240 | 7 | 0 | 0 | 13 | 13 | 29 | 29 | 43 | 43 | 43 |
| 12[b] | 60 | 7 | 0 | 0 | 0 | 13 | 13 | 13 | 13 | 29 | 29 |
| SEIZURE COMPLEX | | | | | | | | | | | |
| 0 | 60 | 19 | 0 | 0 | 5 | 26 | 74 | 95 | 100 | 100 | 100 |
| 6 | 60 | 7 | 0 | 0 | 13 | 29 | 29 | 71 | 71 | 71 | 71 |
| 12 | 60 | 18 | 0 | 0 | 0 | 0 | 6 | 6 | 17 | 17 | 17 |
| 15 | 60 | 7 | 0 | 0 | 0 | 0 | 13 | 13 | 13 | 29 | 29 |
| 12 | 30 | 7 | 0 | 0 | 13 | 13 | 29 | 29 | 29 | 43 | 43 |
| 12 | 120 | 7 | 0 | 0 | 0 | 13 | 13 | 13 | 29 | 29 | 29 |
| 12 | 240 | 7 | 0 | 0 | 13 | 13 | 13 | 29 | 29 | 43 | 43 |
| 12[b] | 60 | 7 | 0 | 0 | 0 | 0 | 13 | 13 | 13 | 13 | 29 |

[a]Ascorbic acid or saline (10 ml/kg body weight) was injected intraperitoneally.

[b]Ascorbic acid solution was adjusted to pH 6.5 with 2N NaOH.

Table 8. ALTERATION OF MOUSE BRAIN GAMMA-AMINOBUTYRIC ACID BY ASCORBIC ACID AND HYPERBARIC OXYGEN

| Treatment | | | µmoles GABA/g brain (Mean ± S.E.)[e] | P[d] | % Change[f] |
|---|---|---|---|---|---|
| Drug[a]-time[b] | | OHP[c]-time[b] | | | |
| Saline | -150 | 0 | 1.97 ± 0.037 | -- | --- |
| Saline | -90 | 30 | 1.72 ± 0.072 | <0.005 | -13 |
| Saline | -120 | 60 | 1.48 ± 0.067 | <0.001 | -25 |
| Saline | -150 | 90 | 1.33 ± 0.080 | <0.001 | -33 |
| Ascorbic acid | -60 | 0 | 2.02 ± 0.060 | >0.05 | +3 |
| Ascorbic acid | -90 | 0 | 2.03 ± 0.067 | >0.05 | +3 |
| Ascorbic acid | -150 | 0 | 2.01 ± 0.094 | >0.05 | +2 |
| Ascorbic acid | -90 | 30 | 1.78 ± 0.072 | <0.05 | -10 |
| Ascorbic acid | -120 | 60 | 1.75 ± 0.185 | <0.05 | -11 |
| Ascorbic acid | -150 | 90 | 1.31 ± 0.099 | <0.001 | -34 |

[a] Saline (10 ml/kg body weight) or L-ascorbic acid (12 mmoles/kg) was injected intraperitoneally.

[b] Minutes.

[c] OHP exposure was at 60 psig.

[d] Significantly different from saline controls at the 0.05 level (Student "t" test).

[e] Each value represents mean of six mice.

[f] Plus represents increase and minus, decrease.