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INTRODUCTION

The Laboratory of Aerospace Biology has made substantial progress towards the goal of describing a mechanism of inert gas narcosis during the period covered by this report. Contained in this report are two articles detailing _in vivo_ experiments currently under way and two manuscripts prepared since the last status report which will be submitted for publication.

One very interesting discovery reported herein is that a strain of mice, genetically altered to increase susceptibility to botulin poisoning, appears to _increase_ metabolic rate while breathing argon in contrast to the decrease which we have observed in several animal species. This phenomenon may be of great significance in the search for a mechanism of gas narcosis since botulin toxin is known to work at the synapse. Therefore, it can be inferred that these mice have a genetically altered synaptic response to both botulin toxin and narcotic gases, suggesting/that gas narcosis may also involve the synapse. We intend to pursue such a hypothesis using this strain of mice, which were made available through a cooperative effort with the Army Research Office, as a model.

A second and on-going study is designed to ascertain whether or not the metabolic depression we have observed in animals breathing heavier diluent gases is a function of the degree of narcosis. Using

human subjects breathing either air or a 30% mixture of nitrous oxide while at rest or during exercise, we are measuring the occurance of oxygen debt and recovery therefrom as a gauge of metabolic response to narcosis during a controlled state of body activity. Our preliminary findings are that nitrous oxide narcosis does not appear to cause pronounced metabolic depression either at rest or during exercise.

A third keystone finding of the laboratory is detailed in a manuscript by Dr. Christopher Schatte, which is included in the appendix of this report. He found that altering the fatty acid composition of phospholipids in the brain and muscle of mice increased their resistance to the metabolically depressant effects of argon but had no effect on pronounced nitrous oxide narcosis. Potentially, these results suggest that metabolic narcosis is achieved in a manner different from that of pronounced sensory narcosis.

The second manuscript contained in the appendix is a result of work done in the Department of Physiology and Biophysics, but not under the auspices of NASA funds, to which Dr. Schatte was a consultant and author of the manuscript.

In addition to the projects described in this document, David Clarkson, a pre-doctoral student in the laboratory, is pursuing his study of mitochondrial function in the presence of various narcotic gases which was outlined in the 31 October 1972 Status Report.

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BOTULIN SUSCEPTIBILITY AND RESISTANCE TO NARCOSIS:

A PRELIMINARY REPORT

The phenomenon of profound sensory narcosis primarily entails disruption of central nervous system (CNS) function and, perhaps, peripheral nerve conduction (1). However, narcotic gases also effect other cells directly since bacteria (2), fungi (3), and tissue slices (4) are depressed metabolically in vitro, apart from any neurohumoral or electrical influence. This laboratory has amply demonstrated that the metabolic depression seen in vitro occurs also in vivo at atmospheric pressure and in the absence of any visible CNS aberrations (5,6,7). But we do not know whether or not the metabolic effects in vivo are centrally mediated or a direct, non-specific influence of inert gases on all cells in general.

Our search for a mechanism of gas narcosis is currently centered on the function of various subcellular components of both neural and somatic tissues. Examples of these endeavors include: a) the effects of altered membrane fatty acids on the response to narcotic gases, the results of which are contained in a manuscript in this status report; b) the testing of liver mitochondria exposed to anesthetic gases as described elsewhere in this report; c) the response of isolated synaptosomes to nitrogen and argon, reported in the 30 April 1972 status report.

Because botulin toxin is known to act only on the CNS, probably

at the synapse, we were recently intrigued by a report of Dr. Carl

Lamanna of the U.S. Army Office of the Chief of Research and Develop-

ment showing that germ-free mice were significantly more susceptible

to botulin poisoning than normal mice (8). Since it has been postulated

that narcotic gases also act at the synapse, we decided to see whether

or not these mice would respond differently to a narcotic environment.

Methods:

Through the assistance of Dr. Lamanna, we obtained mice from a

genetic strain developed at Fort Dietrick and known to be botulin-

susceptible. The mice were shipped and housed in sanitary but non-

germ-free facilities prior to testing. As a measure of narcotic sus-

ceptibility, we measured the relative change in oxygen consumption

while breathing argon at ambient pressure (630 mm Hg) as compared to

air. The Fort Dietrick mice were tested simultaneously against a group

of Charles Rivers mice raised normally and of similar sex, age and

weight. The chamber system has been described in detail in the 31

October 1972 status report. Twenty-four animals from each group were

acclimatized to chamber conditions for 14 days in air, then exposed

for three days each in air, argon-oxygen (4:1) and air. Chamber

temperatures were maintained between 27 and 29° C, the thermal neutral

range of mice for both nitrogen and argon. Parameters measured were

oxygen and food consumption, weight change and the specific activity of expired $^{14}CO_2$ after a total injection of 1.2 ml of a 15 μCi/ml concentration of sodium acetate-1-^{14}C on the final day of each exposure period.

Results:

The results are shown in Table 1. The value k represents the slope of a least squares regression analysis of expired $^{14}CO_2$ specific activity as described in the 30 April 1969 status report. The greater the -k value, the steeper is the slope of the curve. When ln $(C_0$-P) is plotted vs time after injection of the sodium acetate-1-^{14}C where C_0 is the initial amount of radioactivity in the injected acetate and P is the amount expired as $^{14}CO_2$. The slope of the curve (-k) is directly proportional to the rate of catabolism of the injected acetate to $^{14}CO_2$ and approximates a direct relationship to metabolic rate (9). The curve in this instance was a plot of μCi/mmole $^{14}CO_2$ expired/μCi injected versus time (120 minutes). A second but related parameter is the curve area which represents the integration of the area under the curve. Both parameters are interpreted on a relative basis and can show changes either in rate or metabolic pathway.

It can be seen that argon predictably depressed metabolism in the control animals (Charles Rivers strain). The 5.7% decrease in $\dot{V}O_2$ was somewhat less than the 10-15% depression we normally find, but the fact that $\dot{V}O_2$ rose during recovery indicates that it was a valid change.

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Table 1. Mean oxygen consumption ($\dot{V}O_2$) in ml per gram body weight per day; weight change (wt.) in mg per gain body weight per day; food consumption (food) in mg per gram body weight per day; and the values for k and curve derived from the specific activity of expired $^{14}CO_2$ as described in the text. The Charles Rivers mice are controls and the Fort Dietrick mice are the botulin-susceptible strain. Oxygen, food and weight measurements were made on 24 animals in each group; $^{14}CO_2$ specific activity data was taken from 12 mice in each group.

Strain	Gas	$\dot{V}O_2$	Wt.	Food	$-k(x10^3)$	Area
Charles Rivers	Air	3.58	8.7	146	14.1	88.6
	Ar/O_2	3.38	-2.1	114	11.5	68.6
	Air	3.49	1.6	118	13.6	81.8
Ft. Dietrick	Air	3.93	-7.6	*	11.5	84.5
	Ar/O_2	4.33	4.7	*	12.9	102.1
	Air	3.84	3.0	112	12.6	89.3

* data unavailable

A similar pattern of reduction in argon and partial recovery in air was observed for all the other parameters. The lack of total recovery to pre-exposure values reflects the inadequate time period allowed to re-adapt to nitrogen.

In contrast to the control animals, the botulin-susceptible mice increased metabolism in argon relative to air. Oxygen consumption increased 10.1% and dropped below pre-exposure levels during recovery. These animals were losing weight prior to argon exposure, then gained weight while breathing argon. When re-exposed to air, the rate of weight gain decreased. Unfortunately, food consumption data on the Ft. Dietrick mice for two of the measurement periods were lost due to technical problems. However, the $^{14}CO_2$ specific activity parameters supported the oxygen and weight data by showing an increased metabolic rate in argon with partial return to pre-exposure values during recovery.

Using a smaller chamber system capable of more accurate oxygen measurements, we obtained an 8.3% increase in $\dot{V}O_2$ during exposure of the Ft. Dietrick mice to the argon-oxygen mixture, thus confirming that argon does increase metabolic rate.

Discussion:

The exact mechanism of botulin poisoning has not been elucidated but it is known that neurotransmission at the synapse is affected, perhaps as the primary locus of activity (8). It appears that this factor also alters the response to heavy diluent gases. The fact

that the response to exposure to an argon-oxygen mixture in the Ft.
Dietrick mice was exactly opposite to the response normally seen
argues that the receptor organ of the genetic change also plays a
crucial role in the mechanism of gas narcosis.

The results present two major implications relative to the search
for a mechanism of action of "inert" gas narcosis. First, the pos-
sibility that a genetic alteration can influence the organism's
response to rare gases, suggests that the mechanism could involve a
specific biochemical reaction rather than a non-specific physical
effect on membranes. This implies that the synapse may indeed be the
primary focus of gas narcosis. Second, it suggests that the influence
of a heavy rare gas such as argon on metabolic rate could be largely
a function of CNS activity rather than a general, non-specific effect
on all tissues. If metabolic depression in argon is CNS-mediated,
it would support the view that metabolic narcosis at atmospheric
pressure is simply a subtle manifestation of the profound sensory
narcosis seen at higher partial pressures.

Because the Fort Dietrick mice offer a potential solution to
several problems in this area, we will continue to use them as a
model system for comparative differences with normal mice. First,
we will perform a more extensive study similar to this one in order
to challenge/confirm the data reported here. Second, to see if their
apparent resistance to narcosis can be extended to profound sensory

narcotic states, the anesthetic endpoint in N_2O as indicated by loss of the righting reflex will be determined. If protection exists under such conditions, the concept of a common mechanism for both metabolic and sensory narcosis will be supported. Third, we will ascertain whether or not metabolic depression in argon is centrally mediated. This can be done using hypophysectomized and normal mice injected with α - or β - adrenergic blocking agents. If neurohumors or catecholamines play a major role in narcotic depression of metabolism, the responses should be altered accordingly by the lack of these hormones. An extension of this work would involve altering body acetylcholine levels, the main synaptic transmitter, by the use of antiacetylcholinesterase or reserpine, which depletes acetylcholine stores.

Finally, it is possible that the genetic alteration of the Fort Dietrick mice is a generalized tissue change. By comparing the metabolism in vitro of brain and liver homogenates from these and normal animals in the presence of heavy diluent (or narcotizing) gases, the relative magnitude of a direct tissue effect by rare gases can be studied.

It is likely that no one factor is completely responsible for gas narcosis, rather that each of those mentioned above plays a part to one degree or another. We think that we will be able to quantify the relative importance of each and thereby identify the most pertinent aspects from which to develop a method of artificial control of metabolism using diluent gases of the rare gas family.

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Acknowledgement:

OXYGEN UTILIZATION IN THE NARCOTIC STATE

We have reported that rare gases can alter metabolic rate in vivo
(5,6,7) under conditions in which the subjects appear to be in a
"normal" state. Although these gases may cause their effects via a
direct, non-specific effect on all body tissues, it is possible that
metabolic depression in argon, for instance, at a pressure of one
atmosphere is due to central nervous system (CNS) which, in turn,
slows activity and energy requirements. Thus, metabolic depression in
the absence of visible CNS depression may be due to a subtle manifes-
tation of the profound effects such as mental aberrations observable
during sensory narcosis at elevated partial pressures of these gases.

Schatte et al (6) have reported evidence suggesting that inert
gases such as helium and argon can alter the availability or utilization
of oxygen in the cell at atmospheric pressure. The preponderance of
in vivo work, however, suggests that oxygen utilization in the narcotic
state is not reduced other than as a function of body activity (1).
But a recent report by Choteau et al (10) strongly implied that oxygen
availability to the cell was a limiting factor during exposure to nitro-
gen or helium at high pressure. And, Bradley and Dickson (11) observed
a slight but significant decrease in oxygen consumption of men breath-
ing 15 or 30% nitrous oxide both at rest and during exercise. The
sedative effect of nitrous oxide narcosis at rest would be expected to

reduce oxygen needs at rest when compared to air. But the reduced uptake during exercise, during which the body must be in a similar state of activity as in air, implies that a direct effect of narcotic gases may be exerted on oxygen utilization independent of activity considerations.

Because the data represented possible confirmation of Schatte's findings and a potential source of information concerning the mechanism of narcosis, we have begun to make similar tests with the intent of using relative oxygen debt kinetics as an assay for changes in oxygen flux while breathing a narcotic gas.

Methods:

The testing entails performance of exercise, incurrence of an oxygen debt and post-exercise recovery while breathing a narcotic versus a non-narcotic gas. If oxygen availability or utilization is impaired during narcosis, it is presumed that a greater oxygen debt will be required during exercise and a longer period of recovery necessary to repay it. By measuring oxygen consumption and blood lactate, the two classic indicators of oxygen debt, a relative measure of aerobic function both at rest and in a controlled state of activity can be used to compare the effects of narcosis on oxygen kinetics in vivo.

Six males and two females, between the ages of 21 and 29 are the subjects. All are non-smokers and untrained, but in comparatively good athletic fitness. Each are being tested twice within the same week

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to insure uniformity of fitness and at the same time of day to preclude possible diurnal effects. Air is used as the non-narcotic gas and a mixture of 30% N_2O: 49% N_2: 21% O_2 as the narcotic one. This percentage of nitrous oxide gives a mild narcosis which causes some visible mental effects but allows full motor coordination to perform exercise. The subjects receive the gases in random order to eliminate pattern effects.

A typical experimental protocol is as follows. The subject begins at 1000 hours after having refrained from breakfast, weighed and an oral temperature taken. The test gas is breathed in the supine position for 25 minutes to insure adequate equilibration in the body and to allow sufficient time for a true resting condition to be attained. Throughout the experiment, noise protectors with music via an earphone isolate the subject from extraneous noise and blinders reduce photic stimuli. Air is breathed from the surroundings and the mixture delivered from a Douglas bag. The subject breathes through a modified Otis-McKerrou valve (Warren E. Collins, Co.) into an American standard wet gas meter. Mixed expired gas is sampled on the exhaust side of the gas meter, analyses made for oxygen (Servomex paramagnetic) and CO_2 (Beckman LB-1 infrared), and recorded on strip chart recorders. The analyzers are calibrated with standard mixtures containing all components of the expired gas. Respiratory rate is taken from a pressure switch in the mouthpiece and tallied electromechanically. Heart rate is derived from a standard EKG recording. Data are recorded during every five minutes of rest, then every minute for the final 5 minutes

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of rest, 5 minutes of exercise, and the first 10 minutes of recovery, after which at five minute intervals until return to pre-exercise values.

Prior to the start of exercise, a 5 ml blood sample is withdrawn from a superficial vein in the forearm and immediately deproteinized in cold perchloric acid. Exercise is performed in the supine position on a Godart constant load bicycle ergometer. Work load is 175 watts for the males and 100 watts for the females at a rate of 60 rpm. After 5 minutes of exercise, another blood sample is drawn and recovery begun. Additional blood samples are taken at 5, 10, and 15 minutes of recovery.

Blood lactate is determined enzymatically using the Sigma Lactic Acid Test. Oxygen consumption, CO_2 production and minute ventilation are calculated and converted to STPD conditions and corrected for body weight. Additional parameters include heart rate, respiratory rate, and respiratory quotient.

Results:

To date, four subjects have been tested. Figures 1 and 2 show the mean values during rest, exercise, and recovery for oxygen consumption and lactate metabolism. These two parameters are the key determinants in this study of oxygen utilization. Their absolute values and the levels at different time periods throughout recovery will be used as an indication of oxygen utilization and general metabolic efficiency. The remaining respiratory and cardiovascular data have not

Figure 1. Mean oxygen consumption ($\dot{V}O_2$) of four subjects during rest, exercise and recovery while breathing air or 30% nitrous oxide. The values represent the oxygen consumption per kilogram body weight for successive five minute measuring periods.
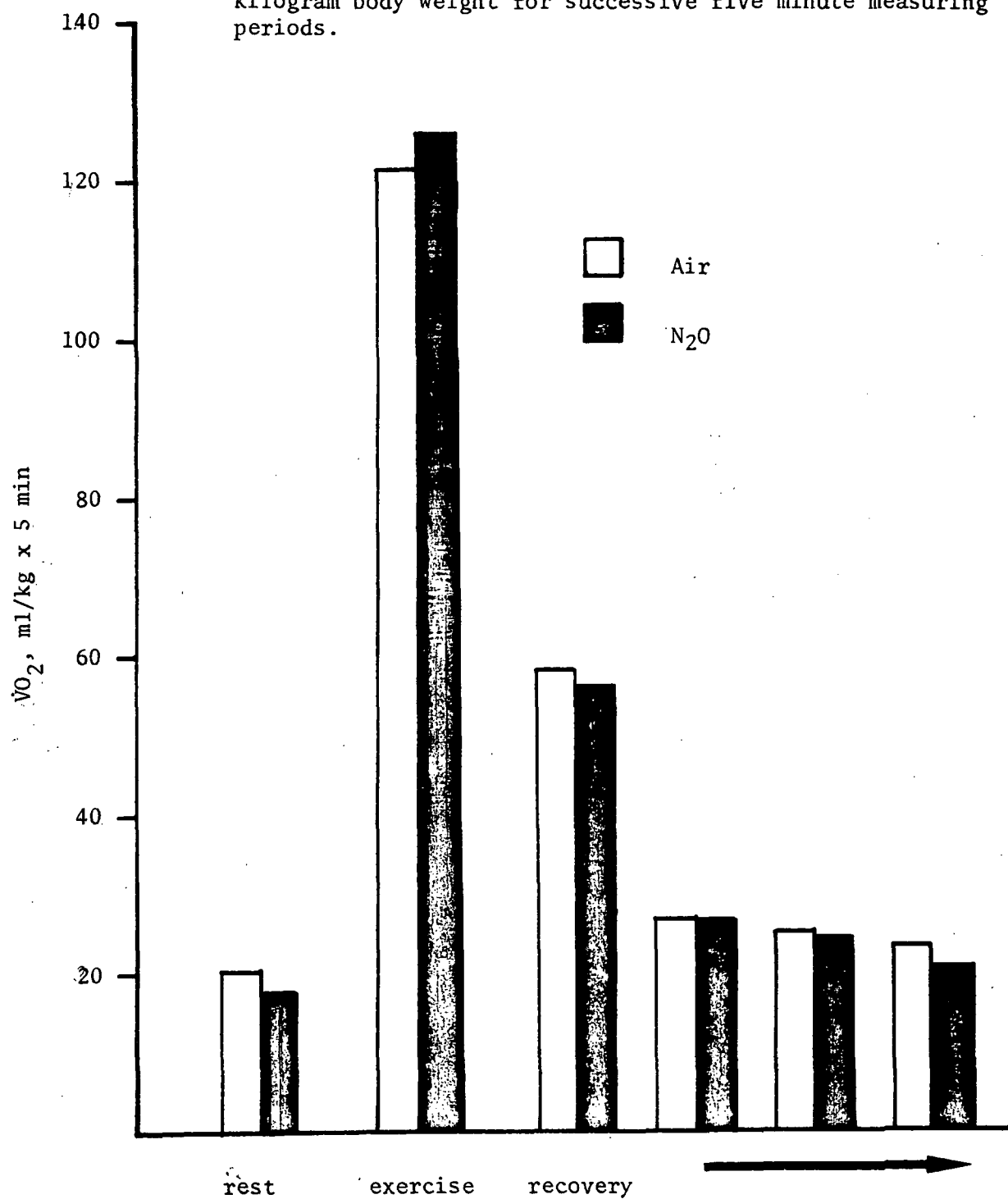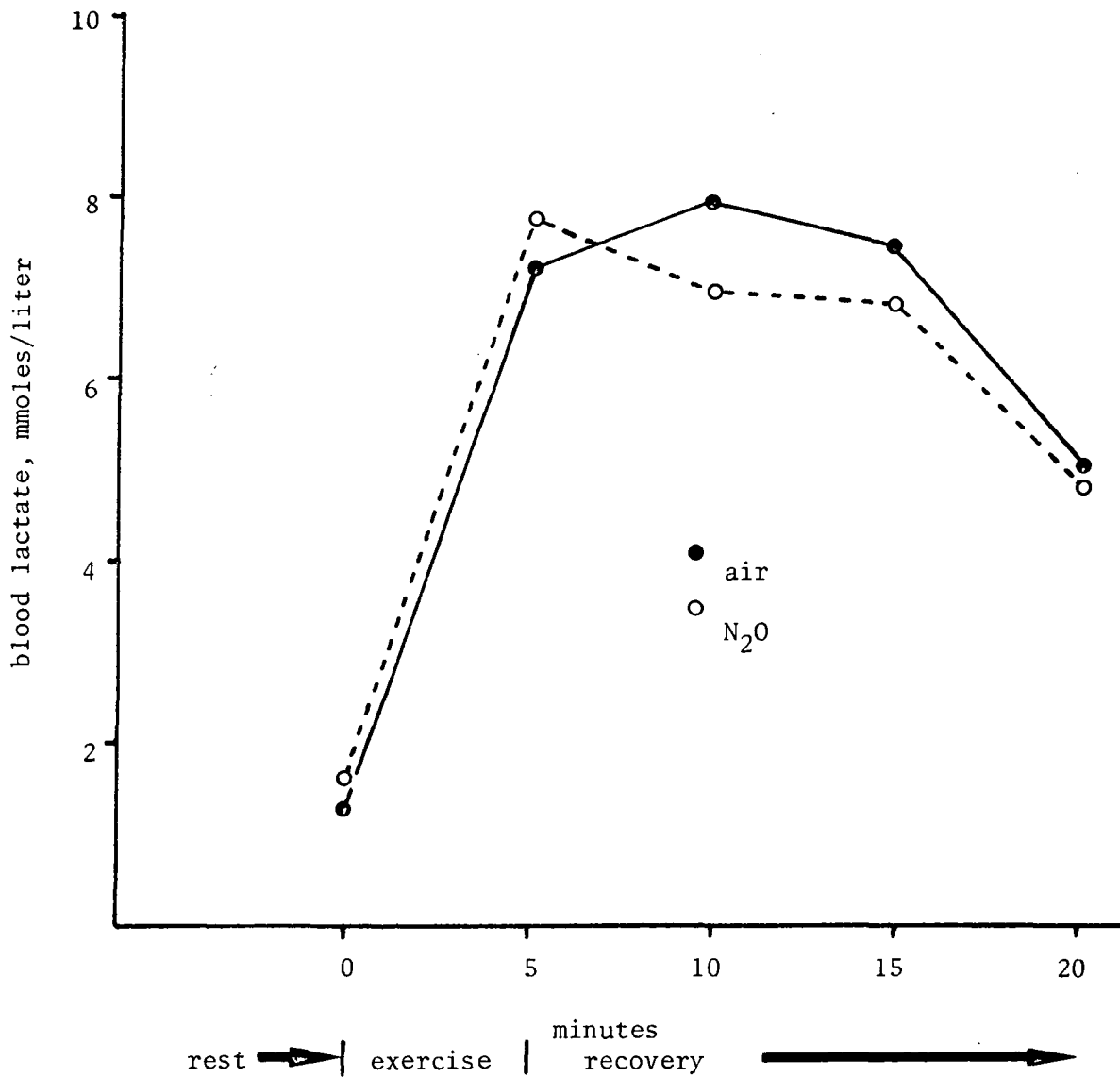
Table 2. Mean blood lactate levels immediately prior to exercise and during recovery while breathing air or a 30% mixture of nitrous oxide. Each mean represents four subjects.

y-axis: blood lactate, mmoles/liter

- air
- N_2O

x-axis: minutes

rest → | exercise | recovery →

been analyzed yet but is expected to reflect a pattern similar to that of oxygen consumption.

Except during exercise, N_2O depresses mean oxygen consumption relative to air although the differences are less than 5%. Despite the general mean depression of $\dot{V}O_2$, the four subjects tested to date have consumed 6.16% more oxygen during exercise and recovery in N_2O than in air.

Mean lactate levels, considered to be an indicator of anerobic energy conversion during exercise, indicate that lactate production is comparatively high during rest and after exercise but declines more rapidly during recovery than in air. An interpretation of such a change cannot be made with the present data but it may be pertinent that the subjects appear to be more narcotic after exercise than before. This is probably due to greater blood flow and, hence, distribution of N_2O during exercise. If this is the case, any effect of narcosis on the enzymes involved in lactate metabolism might be more pronounced during recovery and result in a change in the relationship of recovery levels to those during pre-exercise rest period when being compared to air.

The slight difference between mean oxygen and lactate values in air and N_2O, a great deal of subject variability and the small number of subjects tested to date preclude any statistical significance between the results in air and N_2O.

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Comment:

This project is designed to show whether or not the metabolic depression we have documented in several species breathing heavy diluent gases at ambient pressure is intensified by a more pronounced degree of narcosis. In a previous report from this laboratroy (12), in which the metabolic response of goats to 7 atmospheres of nitrogen was assessed, the data failed to show any significant depression of metabolic rate although changes in the relative importance of various pathways may have occurred. Other reports measuring _in vivo_ cerebral metabolism indicate no decrease and even an increase in oxygen consumption in the presence of 70% N_2O (13,14). In one exercise study similar in design to this, Weber et al (15) also showed an increased oxygen consumption during exercise although the accuracy of the analytical measurements are questionable. Bradley and Dickson (11) worked human subjects whilst breathing 30% N_2O and found a slight, consistent and occasionally significant decrease of $\dot{V}O_2$ in N_2O.

Although the present results are preliminary, the data suggest that N_2O may reduce $\dot{V}O_2$ and lactate values slightly but not significantly although it should be remembered that total oxygen consumption is actually higher in N_2O. It would be premature to attempt an interpretation of the results but it seems likely that the increased level of narcosis is not accompanied by an increased degree of metabolic alteration relative to the situation in argon at ambient pressure.

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If the use of 30% N_2O does not show a significant change in metabolism, a 60% mixture of N_2O will be tested as the maximum degree of narcosis compatible with the work performance. If narcosis does not depress metabolism independent of body activity, we expect to see little difference between the results in 0, 30, and 60% N_2O.

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A P P E N D I X   A

The following paper entitled "Influence of Membrane Composition on Susceptibility to Narcosis and Oxygen Toxicity" by C.L. Schatte, J.E. Loader, T.K. Akers and J.P. Jordan will be submitted for publication in Aerospace Medicine. The work was funded by NASA Grant 06-002-075 at Colorado State University.

Influence of Membrane Composition on Susceptibility

To Narcosis and Oxygen Toxicity

by

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INFLUENCE OF MEMBRANE COMPOSITION ON SUSCEPTIBILITY

TO NARCOSIS AND OXYGEN TOXICITY

Abstract:

The relative susceptibilities to narcosis and oxygen poisoning
were tested as a function of dietary and membrane fatty acid (MFA)
composition. Mouse pups were fed chow, safflower oil or beef tallow
diets in order to raise or lower MFA unsaturation of brain and muscle.
The degree of unsaturation was substantially lowered in the tallow-
fed mice but unaffected in those fed safflower oil. The depression
of $\dot{V}O_2$ in argon relative to nitrogen was less in mice fed high-fat
diets irrespective of composition but membrane levels of myristate
varied with response to argon. The partial pressure of N_2O required
for loss of righting reflex was slightly but significantly higher
in oil-fed animals but MFA composition was probably not the cause.
Tallow-fed mice were dramatically protected against and oil-fed
animals susceptible to respiratory distress, convulsion and death
during exposure to hyperoxia. Dietary and MFA influence on narcotic
indices was minimal while diet profoundly affected the response to OHP.
Possible effects of MFA composition on narcosis and oxygen poisoning
are discussed.

Introduction:

The narcosis resulting from exposure to certain rare gases and the toxicity of oxygen at high pressure (OHP) entail ionic disturbances and abnormal CNS function as a primary symptom (3,18). In addition to CNS effects, both OHP and narcotic gases influence the metabolism of other tissues in vitro, suggesting a general effect on all cells. In each case, the evidence suggests that cell and mitochondrial membranes are major loci at which narcotic gases and oxygen influence physiological processes (5,6).

Nearly all the theories proposed to explain inert gas narcosis are based on an interaction of the gas with the non-polar or lipoidal portion of membranes. Indeed, the best substantiated hypotheses for narcosis deal with the lipid solubility of the various gases and their ability to alter membrane function. Such alterations may include expansion of the membrane lipid bilayer (29), a reduction in membrane surface tension (9), an increased permeability to cations (2), and reduction of fatty acid chain motional freedom in relation to surrounding molecules (31).

In the case of oxygen poisoning, the reported susceptibility of membrane fatty acids (MFA) to peroxidation (19), the inhibition of

membrane-associated metabolic pathways required for energy supply to the cell (27) and the physical disintegration of membranes (22) are documented facts implicating cellular membranes as a central component in the toxicity of oxygen at high pressure.

Therefore, it is interesting to know whether or not the composition of membranes is a factor determining their role in the response to toxic gases and if changes in composition might alter those responses. This study was undertaken to test the hypothesis that membrane fatty acid composition, to the extent that it can be influenced by diet, represents one component controlling the in vivo response to narcotic gases and OHP.

<u>Methods and Materials</u>:

Pregnant Sprague-Dawley mice (Horton Laboratories, Oakland, Cal.) were obtained, taken to term, and the pups immediately placed on one of three combinations of diet and temperature acclimatization:

A.   fed Lab Chow (Ralston Purina) at $18 \pm 1°C$

B.   fed a safflower oil diet at 5-10°C

C.   fed a beef tallow diet at 34-37°C

The compositions of the high fat diets are listed in (Table 1). Caging and lighting conditions were the same for all groups.

The mothers and pups were placed on the diets immediately after parturition to insure that the pups would be exposed to the high fat diets during the maturation of the CNS. The temperatures were used primarily in conjunction with another experiment but presented the following considerations to this one. First, acclimatization temperature is known to alter MFA composition in poikilotherms (20) and at least that of depot fat in hibernators (11). Mice have been reported to be poor temperature regulators during the first few weeks of life and thus may be somewhat poikilothermic during that time (16). If ambient temperature did influence membrane compositon in this work, we anticipated that cold exposure of mice fed diets high in unsaturated fatty acids would enhance the intended incorporation of those acids into cellular membranes and vice versa. Second, the

Table 1.   Composition of treatment diets:

A.   control, Purina Lab Chow

B.   experimental, high in unsaturated fat

43%   safflower oil

34%   lactalbumin

 9%   alphacellulose

William Briggs salt mix*

vitamins

C.   experimental, high in saturated fat

46%   beef tallow

38%   lactalbumin

1.5% safflower oil

William Briggs salt mix*

vitamins

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*supplemented with 4 mg/kg Cr++ and 2 mg/kg Se++

liquid consistency of the safflower oil diet at room temperature
necessitated a lower ambient temperature to solidify and apparently
make it more palatable. Food consumption and growth on the safflower
oil diet was improved at 5°C over that at the 18°C temperature used
in a preliminary experiment.

The pups, that approximated 2:1 female to male in each group
when born, were raised under these conditions for about four months
during which they were tested as follows.

Depression of $\dot{V}O_2$ by Argon:

Previous work from this laboratory has shown that substitution
of argon for nitrogen at an ambient pressure of one atmosphere or less
reduces $\dot{V}O_2$ 10-15% relative to air (8,28). This criterion of relative
$\dot{V}O_2$ depression by argon was used as one measure of susceptibility to
a mildly narcotic environment.

After three months' exposure to the diets, the mice from each
treatment group were exposed in groups of four to air and 80% argon:
20% oxygen at ambient pressure (635 mm Hg.). The chamber system
used has been previously described (8). The sole modification
was the installation of a thermoswitch under the floor which, via
a relay and solenoid valve in the coolant line, maintained chamber
temperature at 20 ± 0.5°C.

All experiments were performed at the same time of day with
the exposure to air or the argon mixture being done on separate days.

Exposure period was for four hours; oxygen consumption readings were taken every five minutes for the final two hours and averaged.

The data for six groups of four mice each within the three treatment groups were analyzed using a paired t-test comparison between the $\dot{V}O_2$ in air and argon and interpreted on the basis of a single-tailed probability.

Loss of Righting Reflex in Nitrous Oxide:

The relative susceptibility to profound narcosis as indicated by loss of righting relfex was determined by exposure to nitrous oxide (N_2O) at an elevated partial pressure. Testing was done under the auspices of the Man-in-the-Sea program at the University of North Dakota. The mice were exposed in pairs inside a glass jar placed in a hyperbaric chamber (volume = 10 liters). The animals had been brought to ambient temperature (22°C) for 48 hours prior to testing. The chamber was flushed with 100% oxygen then compressed at 30 psi per minute with N_2O. During compression, the chamber was rocked so that the mice had to actively maintain balance inside the rolling jar. Loss of righting reflex was taken as the time in minutes at which the animals could no longer regain a four-point stance. Including decompression at 30 psi per minute, the entire sequence was complete in 4-5 minutes. Ambient temperature in all cases rose from about 26°C to about 31°C during compression. Occasional tremor and convulsion were observed during decompression.

The endpoint in psi was recorded for each animal of all three

groups and analyzed using a one-way analysis of variance and a two-

tailed t-test comparison between the controls and the two experimental

groups.

Time to Respiratory Distress, Convulsion and Death in OHP:

The relative susceptibility to acute oxygen poisoning was tested

during a 300 minute exposure to 100% oxygen at 60 psi. The mice were

four months old at this time and were brought to ambient temperature

(22°C) 48 hours prior to testing. Eight mice from each treatment

group were simultaneously exposed in a hyperbaric chamber (volume =

1000 liters) fitted with a 24-compartment wood and wirecloth cage

which allowed full view of each individual animal. Compression rate

was 15 psi per minute after which the chamber was flushed at a rate

sufficient to maintain a 0.2% difference between affluent and effluent

oxygen analyses (Servomex paramagnetic oxygen analyzer). Temperature

rose from 21-26°C during the exposure period. Sixteen animals from

each group were tested during two separate exposures.

The number and time in minutes of mice from each group which

incurred respiratory distress, convulsion and death was recorded.

Respiratory distress was regarded as when obvious labored breathing

occurred, presumably due to pulmonary edema and atelectasis. Time

to convulsion was recorded upon full clonic seizure entailing the

mouse's loss of an upright stance. Time to death was taken as the

last visible respiratory movement.

The data for respiratory distress and convulsion time were analyzed using a one-way analysis of variance and a two-tailed t-analysis between the controls and the two experimental groups; only a t-test was applied to the results for death time.

Tissue Lipid Analysis:

The eight mice in each group not used in the OHP experiments were sacrificed and Folch extracts (12) prepared from the brains and a segment of abdominal skeletal muscle.

A portion of the total lipid extract from each tissue was analyzed using silicic acid thin-layer chromatography. Spots were developed to a plate height of 4 cm. in chloroform:methanol:water:acetic acid:65:25:4:1 then to a height of 16 cm. with petroleum ether:diethyl ether:water:85:20:3. The plates were dried, sprayed with saturated potassium dichromate in 75% H_2SO_4, and charred at 180°C for 45 minutes. Spots were quantitated using a Joyce-Loebl Chromoscan Densitometer.

The remainder of the total lipid extract was applied to silicic acid columns and the polar (phospholipid) component eluted with methanol following elution with chloroform and acetone (26). The methanol eluate was then hydrolyzed and the fatty acids converted to their methyl esters (23). Fatty acid methyl esters were analyzed on a Perkin Elmer gas chromatograph Model 880 equipped with flame

ionization detector using the following conditions:

column - 6' x 2mm stainless steels with SP-222-PS
          (Supelco, Inc., Bellafonte, Penn.)

oven   - 200°C

detector - 240°C

injector - 215°C

nitrogen gas flow - 32 ml/minute

Samples were applied in CS_2 and peaks identified by comparison
of relative retention times with NIH-D, GLC-60 and RM-3 standard
mixtures (Supelco, Inc.,) and calculation of "carbon number" (36).

The index of unsaturation (IU) was calculated according to
the formula:

$$IU = \sum_{k=22}^{a=12} \text{(weight percent of fatty acid)} \text{(no. double bonds of fatty acid)}$$

where a and k are the minimum and maximum chain lengths considered,
respectively.

Results:

References to the three treatment combinations will be as follows:

A.   control   - fed Lab Chow at 18°C

B.   experimental - fed diet of safflower oil at 5-10°C

C.   experimental - fed diet of beef tallow at 34-37°C

Depression of $\dot{V}O_2$ by Argon:

Mean oxygen consumption while breathing argon was significantly reduced relative to air for all treatment combinations (Table 2). Based on the relative percentage reduction of $\dot{V}O_2$ in argon, the experimental treatments appeared to increase resistance to argon, particularly in the case of treatment C.

It is not clear what effect the temperature component of the experimental treatments had on the response to argon. The cold-acclimated mice showed an expected higher $\dot{V}O_2$ than the other two groups. Our previous experience with rats and hamsters has shown that reduction of $\dot{V}O_2$ in argon is greater in animals with higher metabolic rates, particularly when accelerated by low temperature. Since this was not the case in the present study, it seems likely that the dietary component of the treatments played the more dominant role in determining the response to argon. Further, the lesser percentage reduction of $\dot{V}O_2$ in argon for both the experimental groups suggests that a high fat diet, irrespective of degree of unsaturation, modifies the metabolic response of mice to a mildly narcotic agent.

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Table 2.   Mean ± s.d. weight in grams and $\dot{V}O_2$ observed during exposure
to air or argon:oxygen. Each value is the mean of 6 (x 4 mice
each) replications. Depression of $\dot{V}O_2$ in argon is expressed
as a percent of the value in air. The values for t and P are
based on a paired, single-tail analysis.

$\dot{V}O_2$ (ml/min. kg^{-1})

Treatment	Weight	Air	Ar-O$_2$	%Depression	t	P
A	36.38 ± 1.74	52.66 ± 4.93	46.82 ± 6.73	11.09	1.673	<.025
B	32.16 ± 2.40	68.44 ± 8.09	63.42 ± 5.75	7.33	2.719	<.005
C	33.06 ± 2.46	53.29 ± 4.98	50.71 ± 4.60	4.84	1.943	<.025

Loss of Righting Reflex in Nitrous Oxide:

The partial pressure of N_2O required to produce narcosis was increased somewhat by treatment B but unaffected by treatment C (Table 3). While numerically small, the significant 5.7% increase in partial pressure of N_2O required to anesthetize the B group suggests that a diet high in unsaturated fatty acids offers some protection against narcotic gases.

However, the lack of any influence by treatment C casts some doubt on the involvement of the diet in protection. It may be pertinent that the B-treated mice had a higher metabolic rate, as measured by oxygen consumption, than either the controls or group C, which had similar metabolic rates. Since hypothermia is known to reduce the amount of anesthetic required, it seems reasonable to suppose that a hypermetabolic animal requires a higher dose of an anesthetic agent to attain a given level of narcosis. In view of the fact that the B mice, with an increased metabolic rate, required more N_2O than the A and C groups, which had the same metabolic rate and loss of righting reflex endpoint, we feel that the dietary treatment probably was not responsible for the observed effects.

Susceptibility to Acute Oxygen Poisoning:

The graphical depiction (Figure 1) and statistical analyses (Table 4) of the results for time to respiratory distress, convulsion and death during exposure to OHP indicate a clear enhancement of

Table 3.   Mean ± s.d. pressure of nitrous oxide in pounds per
            square inch gauge required to produce loss of righting
            reflex. The t analysis was two-tailed. The P value
            listed for treatment A refers to the level of signifi-
            cance of the F test while those for treatments B and
            C represent the significance of the t analysis between
            these groups and the controls.

Treatment	n	PSIG	F	t	P
A	23	38.32 ± 2.17	8.22		<.001
B	24	40.52 ± 2.05		3.55	<.001
C	24	38.31 ± 2.25		.021	NS

Figure 1.   Mean ± s.d. time in minutes to respiratory distress,

convulsion and death during exposure to 100% oxygen at

60 psia.   The number in parentheses represents the number

of animals out of 16 showing effects within 300 minutes.

There were no deaths in group C.

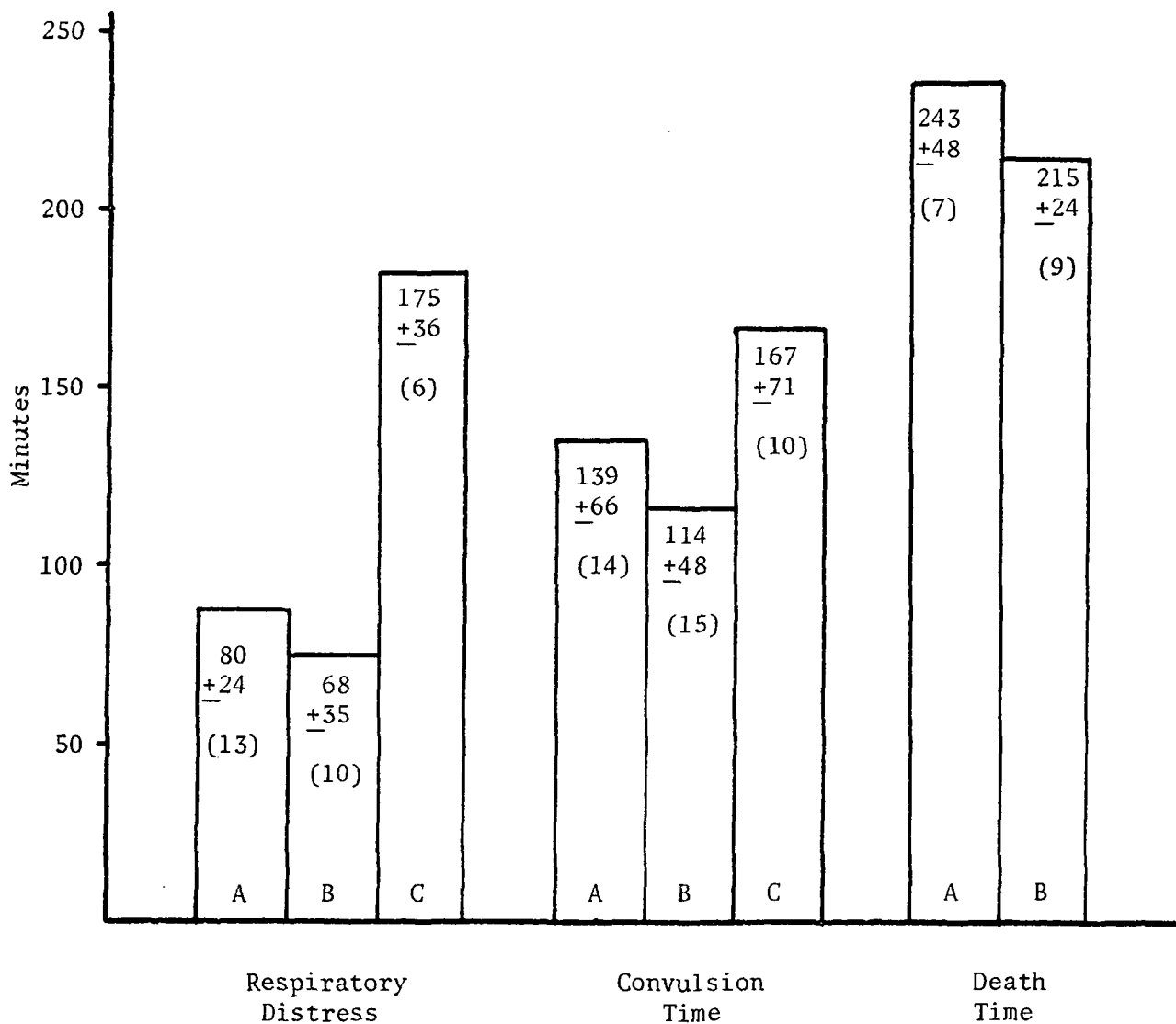Respiratory Distress   Convulsion Time   Death Time

Table 4. Statistical analyses of parameter responses to OHP.

All t values represent two-tailed tests between control (A) and experimental (B,C) treatment combinations.

Treatment	No.	Weight	Respiratory Distress				Convulsions				Death		
			No.	F	t	P	No.	F	t	P	No.	t	P
A	16	34.5+3.4	13	25.66		.005	14	2.487		NS	7		
B	16	33.5+3.1	10		0.940	NS	15		1.181	NS	9	1.456	NS
C	16	24.6+3.4	6		6.873	.001	10		0.974	NS	0		

susceptibility by treatment B and protection by treatment C to acute oxygen toxicity. In every case except respiratory distress, there were more B and fewer C mice experiencing the three stages of oxygen poisoning than controls. In the case of all three parameters, the B-treated mice had lesser mean times to onset of these symptoms while the C group had longer times to onset than did controls. Of particular note was the absence of any deaths in the C mice during the exposure period.

In addition to these three criteria, it was the opinion of the observers that both the intensity and duration of convulsions after the initial episode was greater in the B group and negligible in the C mice when compared to controls. Thus, the B-treated animals seemed to convulse violently and almost continually between the onset of convulsions and death. Conversely, the C group showed a comparatively mild seizure at the onset of convulsions followed by a subdued appearance; convulsions after the initial episode were rarely observed in this group.

Paradoxically, the C mice exhibited a curious phenomenon at the beginning of exposure to OHP. Almost immediately upon initiation of chamber flush with 100% oxygen at ambient pressure, a few of the mice were seen to undergo a pre-convulsive behavior characterized by tremors and arching of the back. Only C-treated mice were observed to undergo these tremors. After a few seconds, this behavior

disappeared and the mice either became apparently normal again or were slightly more subdued compared to the others.

An interesting finding of these experiments were significant (P<.05), negative coefficients of regression and correlation between body and weight and convulsion time. Previous studies (7,15) have shown no dependence of oxygen poisoning on body weight. Such a finding here was due to a significantly lower (P<.005) mean weight of the C mice, coupled with a longer mean convulsion time than controls, of group B. The difference in weights was a result of a substantial loss in the C animals during the few weeks prior to testing, probably as a result of a less palatable diet. Since appreciable parametric differences were noted between the controls and group B, despite no appreciable difference in weight, it seems likely that weight was not the determining factor of the response to OHP. It is the authors' opinion that factors other than weight produced the results and that the significant interaction between weight and convulsion time was peculiar to these particular circumstances rather than a general phenomenon.

One possible combination of factors other than diet which could have produced these results should be noted. If oxygen poisoning is partially dependent on metabolic rate, as has been suggested (27), then the B mice, with an elevated oxygen consumption, would be expected to show signs of poisoning sooner than controls, as was the

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case. Further, starvation is known to protect against OHP symptoms (13). The loss of weight in the C mice during the weeks prior to OHP exposure implies that they had a reduced food intake; it is possible that their resistance to oxygen toxicity may have been a result of this reduced dietary intake.

Tissue Lipid Analyses:

Tables 5 and 6 show the percentage composition of the major lipid classes and phospholipid fatty acids for brain and muscle in mice from each of the treatment groups. The phospholipid fatty acid composition was considered to reflect MFA composition since phospholipids form the bulk of membrane lipid and do not exist in large amounts elsewhere in the cell (1). Thin-layer chromatographic analysis of the methanol eluate, containing primarily the polar lipid fraction, indicated that phospholipids comprised most of this fraction with slight contamination by cholesterol esters.

Based on the analysis of major lipid classes, the high-fat diets had no major effect on brain lipid distribution but did increase the relative amounts of triglycerides in muscle. This influence appeared to be independent of diet fatty acid composition.

Dietary composition did have a major influence on phospholipid fatty acids but the effects were not consistent. As in the case of major lipid classes, muscle was altered to a greater extent than was brain. Both high-fat diets increased the percentage of myristic

Table 5. Percentage composition of major lipid classes in brain

and muscle from mice in each treatment group. The

analyses represent the pooled tissue from four animals.

Lipid Class	Brain A	Brain B	C	Muscle A	Muscle B	C
Cholesterol Ester-Triglyceride*	12.21	10.63	13.79	86.39	96.13	96.04
Fatty Acids	3.03	4.32	3.30	- - -	- - -	- - -
Cholesterol	32.21	36.40	31.07	- - -	- - -	- - -
Monoglycerides	14.51	14.59	16.12	- - -	- - -	- - -
Phospholipids	35.25	32.07	33.01	12.16	3.87	3.96
Origin	2.87	1.98	2.72	1.45	- - -	- - -
Total	99.99	99.99	99.99	100.00	100.00	100.00

*, incomplete separation of these two classes

-, undetected or trace amounts

Table 6. Percentage composition of fatty acid methyl esters obtained
from the phospholipids of brain and muscle for each of the
treatment groups. The analyses represent the pooled tissues
of four animals. The percentage unsaturation, the index
of unsaturation and the ratio of saturated to unsaturated fatty
acids were calculated only from positively identified acids
of up to chain length C-22.

| | Brain | | | Muscle | | |
Methyl Ester	A	B	C	A	B	C
12:0	- - -	.85	- - -	- - -	1.50	.22
14:0	1.40	23.316	51.96	4.40	24.66	55.86
15:0	trace	2.54	.92	- - -	- - -	1.47
16:0	21.31	20.19	15.58	17.97	12.20	12.48
16:1	1.18	1.90	.30	- - -	1.30	.55
17:0	2.36	- - -	1.06	- - -	1.50	1.34
18:0	14.23	12.68	9.74	5.81	8.04	6.51
18:1	13.27	11.52	8.45	5.81	5.89	7.49
18:2	.88	1.69	1.02	5.02	13.11	1.87
18:3	trace	- - -	.40	- - -	1.37	trace
20:1	2.06	1.48	1.45	5.81	1.81	1.07
unknown	- - -	.42	- - -	trace	- - -	trace
20:4	trace	trace	trace	6.34	1.25	.49
22:0	7.30	7.93	3.41	4.76	1.00	3.42
unknown	6.86	2.11	1.75	19.21	7.42	1.60
unknown	2.58	- - -	1.12	10.57	5.77	- - -
unknown	- - -	3.17	1.30	- - -	1.86	.71
24:0	- - -	- - -	- - -	trace	1.22	.36
unknown	26.55	10.15	1.56	14.27	10.10	4.56
TOTAL	99.98	99.99	100.02	99.97	100.00	100.00
Total > C-22	35.99	15.43	5.73	44.05	26.37	7.23
percent unsaturation	17.39	16.59	11.22	22.98	24.73	11.47
index of unsaturation	18.27	18.70	12.24	47.02	44.33	14.81
saturated/unsaturated	2.67	4.07	7.40	1.43	1.97	7.08

acid (14:0) considerably with the greatest increase produced by the beef tallow regimen. As expected, this highly saturated diet decreased the percentage of unsaturated fatty acids, the index of unsaturation and increased the ratio of saturated to unsaturated fatty acids in both brain and muscle.

Surprisingly, the indices showed no appreciable difference in unsaturation of brain or muscle from the B mice relative to control. With the exception of percent unsaturated fatty acids in muscle, the B mice apparently had a somewhat greater degree of saturation of both brain and muscle MFA. Particularly intriguing is the fact that these animals did not show pronounced increases in C-18 unsaturated acids despite the fact that safflower oil contains 75% linoleic (18:2) acid (17). Rather, it appears that the dietary acids were largely hydrogenated and oxidized to myristate.

Another trend of the high-fat diets was to reduce the relative percentages of fatty acids with greater retention times than behenate (22:0) or branched chain acids. Using the sum percentage of all fatty acids beyond C-22, it can be seen that both high-fat diets decreased the relative amounts of these higher weight acids compared to control. Since, the relative percentages of these acids as a group varied inversely with the percentage of myristate (14:0) for all tissues, it is interesting to speculate that myristic acid may have been preferentially incorporated into phospholipids at the expense of the long chain, branched chain and polyunsaturated fatty acids.

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Discussion:

It has been shown that narcosis and oxygen poisoning involve an apparent increase in memebrane permeability to cations and that the CNS symptoms produced by both phenomena can be obviated by pre-treatment with the same cationic detergents or anti-inflammatory agents (4,5). The fact that a single agent can attenuate the effects of both narcotic gases and OHP implies that at least one aspect of their etiologies is common to both. The present data suggest that this common aspect does not involve membrane fatty acids since changes in MFA had relatively little influence on narcotic indices in contrast to a dramatic effect on susceptibility to oxygen poisoning.

Membrane Fatty Acids and Narcosis:

Based on the proposition that narcosis results from a transient increase in membrane permeability, factors contributing to increased membrane permeability might predispose cells to the influence of narcotic gases. Studies with electron spin labels have shown that anesthetic gases cause a reduction in vitro of the motional freedom of fatty acids in phospholipid suspensions (31). Such gases in vivo cause a loss of K+ and an increased uptake of Na+ by cells (4,24) which can be interpreted as an increased permeability to these cations. The liver mitochondria of rats fed a diet deficient in or supplemented with essential fatty acids are reported to exhibit decreased fatty acid chain motional freedom with increasing unsaturation (32). Additionally,

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the frequency of oscillation (swelling), largely dependent on the membrane, is slowed. Permeability to cations was not appreciably altered, although an increase in the permeability of liposomes to cations has been reported as a function of increasing degree of unsaturation and shorter fatty acid chain length (10). If narcosis does depend on an increase in cation permeability and such an increase is facilitated by a high index of unsaturation (IU), animals with highly unsaturated MFA should be more susceptible to narcosis and vice versa.

Using metabolic rate as an index of mild narcosis at ambient pressure, our data show that the C mice, with a low IU, were protected somewhat against the depressant effects of argon. The 5% depression of $\dot{V}O_2$ by argon in this group is of biological significance since our past experience with several species has shown a minimum 10% depression of $\dot{V}O_2$ by argon as typified by the 11% decrease seen in the present controls (A group). However, the fact that $\dot{V}O_2$ was also depressed to a lesser degree than control in the B-treated mice (7%) suggests that MFA unsaturation may not be a primary causative factor of the apparent lessened susceptibility to metabolic narcosis.

We cannot be absolutely sure that the lesser depression of $\dot{V}O_2$ by argon in mice fed high-fat diets was caused by a change in MFA composition. But it is interesting, however, that the most pronounced change in MFA composition in both experimental groups was a marked increase in the percentage of myristic acid (14:0) relative to controls.

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Further, the C mice, with the highest levels of myristate showed the greatest tolerance to the depressant effects of argon, Accordingly, the B mice had intermediate levels of this fatty acid and showed an intermediate degree of $\dot{V}O_2$ depression in argon. The control animals, with low levels of myristate had the greatest decrease in metabolic rate while breathing argon. While there is no evidence to suggest that myristate levels and $\dot{V}O_2$ were related, it may be significant that short chain fatty acids, including myristate, have been reported to facilitate the metabolic function in vitro of synthetic liposomes (10).

The profound narcosis induced by N_2O was not consistently dependent on diet composition, either. The small but significantly increased amount of N_2O required to narcotize the B mice, coupled with no change in narcotic susceptibility of the C mice, suggested that changes in MFA composition were not a critical factor. This is supported by the fact that the C mice, which showed a substantial change in MFA composition were equally susceptible to profound narcosis as were controls. Conversely, the B mice had an MFA composition similar to that of controls but showed an increased tolerance to N_2O narcosis. The authors are thus inclined to discount the apparent protective effect of a highly unsaturated diet on N_2O narcosis as recorded in this study. If such an effect truly exists, it appears to be relatively small even though statistically significant.

Dietary composition appeared to have some effect on metabolic

narcosis at ambient pressure but not on profound narcosis at elevated pressures. The effects observed in argon may have been a result of changes in MFA composition, primarily the increased levels of myristic acid, but the relative degree of unsaturation does not appear to be crucial to membrane function during narcosis.

We conclude that the changes in MFA composition which occurred in this study do not profoundly alter the response to breathing a narcotic gas. It is possible that certain fatty acids, the percentages of which were not altered by their diets, may play an important role in the mechanism of narcosis. Or, perhaps cholesterol levels and lipid-protein interactions are more important in membrane function during narcosis. But it appears that non-specific changes in MFA composition or degree of unsaturation will not consistantly alter membrane function in the presence of a narcotic gas.

Fatty Acid Composition and Oxygen Poisoning:

The results suggest a dramatic influence of dietary composition on relative susceptibility to acute oxygen poisoning. Mice fed a saturated diet had a lower incidence and severity of respiratory distress, convulsion and death; those animals which showed these symptoms had longer mean times to their onset. Conversely, the mice fed a highly unsaturated diet showed a greater incidence than controls of convulsion and death with shorter mean times to their onset. The fact that the response to OHP was completely opposite in the two

experimental groups relative to controls implies that the divergent

compositions of their diets probably was a major causative agent.

The data do not allow identification of the mechanism by which

dietary composition alters the response to OHP but three possibilities

are pertinent. Wood has presented strong evidence that a block in the

metabolism of the inhibitory neurotransmitter gamma-aminobutyric acid

(GABA) is the primary lesion in oxygen toxicity (35). It had also been

shown that reduced ATP availability is responsible, at least in part,

for the convulsions associated with OHP (27). Since GABA and ATP

metabolism are largely membrane-associated, changes in membrane composition

might differentially influence the response to OHP via a conformational

change in membrane structure. Related to this, the second possibility

centers on the fact that the most OHP-susceptible also had a higher

metabolic rate as evidenced by $\dot{V}O_2$ in nitrogen and argon. Their

increased susceptibility to OHP would have been due to faster depletion

of GABA and ATP because of their high metabolic rate. But the fact

that the C mice, with a metabolic rate similar to control values,

showed a marked difference in OHP susceptibility relative to controls

suggests that some other factor was dominant in the response.

The most plausible explanation is based on the lipid peroxide

theory. Cellular peroxides have been shown in vitro to increase

as a function of MFA unsaturation (19), oxidize sulfhydryl-containing

amino acids (25), and inhibit certain enzymes (33). However, in vivo

evidence correlating brain peroxide levels with severity of oxygen poisoning is conflicting (34,37). While our results do not prove that peroxide levels were different among the three treatment groups, it is tempting to reason that the OHP-resistant C mice might have had lower peroxide levels as a result of a lower degree of MFA unsaturation. But the converse was apparently not true since the OHP-susceptible B mice had a degree of MFA unsaturation similar to that of the controls indicating that the response to OHP was not based on quantitative amounts of unsaturated MFAs. It is possible that one or more individual fatty acids are not quantitatively as important as those located in other lipid fractions or existing freely in the cell.

The only other comparable work, by Hill and Begin-Heick (14), indicated that mice fed high-fat diets, regardless of composition, were more susceptible to OHP toxicity than controls but dietary treatment was not begun until five weeks of age. Perhaps the discrepency between our results and theirs was due to the influence of diet in our mice during the first five weeks of life.

The fact that α-tochopherol can protect against lipid peroxidation in vitro and against the effects of OHP in vivo (30) suggests an important role of peroxides in the pathology of oxygen poisoning. Whether these peroxides are dependent on quantitative amounts of unsaturated fatty acids, certain key unsaturated acids located within the cell at critical points, or can be mitigated by the presence of other acids (i.e., myristate?) cannot be determined from our data.

An additional alternative is that MFA composition has no effect at all on OHP susceptibility. As noted previously, the response to OHP could have been caused by a potentiating effect of higher metabolic rate in the B mice and protection by a presumed state of reduced food intake by the C group. While the authors do not consider these factors to have been completely responsible for the observed effects, they may have enhanced the influence of diet and MFA composition on OHP suscepti- bility. A more detailed investigation of the qualitative and quantita- tive effects of MFA composition is in progress to delineate their role and that of peroxides in the etiology of oxygen poisoning.

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A P P E N D I X B

The following paper entitled "The Susceptibility of Altitude-
Acclimatized Mice to Acute Oxygen Toxicity" by Peter Hall, Christopher
L. Schatte and John W. Fitch will be submitted for publication in the
Journal of Applied Physiology. This paper is included because C. Schatte
is an author and consultant although the work was supported by funds
from the Department of Physiology and Biophysics at Colorado State
University.

The Susceptibility of Altitude-Acclimatized Mice to Acute

Oxygen Toxicity

by

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The Susceptibility of Altitude-Acclimatized Mice to Acute Oxygen Toxicity

Abstract:

The influence of hypoxic acclimatization at altitudes of 0, 5000 and 15,000 feet on acute oxygen poisoning was determined for 300 adult female mice. After acclimatization periods of 1, 2, 4 and 8 weeks, the mice were exposed to oxygen at high pressures (OHP) of 4, 6, or 9 ATA and the times to convulsion and death recorded. Statistically distinguishable differences between parameter means were found only at an OHP level of 4 ATA. Pre-exposure to hypoxia reduced mean convulsion and death times although this was consistently significant only at 15,000 feet. Duration of acclimatization tended to increase convulsion time, had a mixed effect on death time, and tended to decrease time between convulsion and death. Body weight did not significantly influence any parameter. A common effect of hypoxia and hyperoxia on gamma-aminobutyric acid metabolism was postulated as a possible explanation of the results. It was concluded that altitude effects on oxygen toxicity are practically significant at altitudes above 5,000 feet, at OHP levels of 4 ATA or less, and at any duration of acclimatization studied.

Introduction:

In addition to the traditional professions of caisson and under-
water work, there has been increasing exposure of humans to oxygen at
high pressure (OHP) during the treatment of various diseases, radio-
therapy and sport diving. A substantial number of these people live
at an altitude above sea-level and therefore are acclimatized to a
reduced inspired oxygen tension.

If hypoxic acclimatization prior to a hyperoxic episode alters the
individual's susceptibility to the symptoms of oxygen toxicity, it is
necessary to properly adjust exposure times and OHP levels to maintain
safe operating conditions. Brauer et al (1) have reported that rats
acclimatized to an altitude of 17,400 feet (382 mm Hg) survived the
pulmonary damage attendant with an OHP level of 1.08 ATA more than
three times longer than sea-level controls. But, at seven ATA, the
altitude rats convulsed in half the time of controls.

A further possible interaction between hypoxia and OHP toxicity
is implied by the work of Wood and associates who have demonstrated
that the CNS neuroinhibitory transmitter, gamma-aminobutyric acid (GABA),
is altered by both hypoxia and hyperoxia (3,4).

In both conditions, brain GABA is significantly reduced below
normal and the reduction is reversible, like the effects of hypoxia and
hyperoxia, upon return to air at sea-level. Animals convulsing during
OHP exposure had GABA reductions commensurate with convulsion severity;
prior administration of GABA or its metabolic precursors protected

against both convulsions and pulmonary damage (2).

In view of the apparent interaction between hypoxia and OHP, the present study was undertaken to verify Brauer et al's results at seven ATA and further investigate any relationship between acclimatization to altitude, duration of acclimatization (DA) and acute toxicity of oxygen.

Methods and Materials:

A group of 300 adult female mice (CFW strain, Carworth Farms)
with a mean ± s.d. weight of 19.34 ± 1.71 grams were raised at sea-
level, randomly divided into three groups and placed in three chambers
at altitudes of 0 feet (760 mm Hg), 5000 feet (635 mm Hg) and 15,000
feet (437 mm Hg) in air. All were housed under similar conditions of
caging, light cycle, and food (Purina Lab Chow). Once each day, the
chambers were brought to ambient pressure (635 mm Hg) briefly for
servicing. The ranges of chamber temperatures (26-29°C), relative
humidities (19-56%), and CO_2 levels (.06-.19%) were regulated by ad-
justment of gas flow through the chambers.

At intervals of one, two, four and eight weeks, a randomly selected
group of 24 mice was removed from each chamber, weighed, and randomly
divided into three groups of eight animals. These groups were exposed
to OHP at four, six, or nine ATA (60,90,or 135 psia) in a hyperbaric
chamber (volume = 1000 liters) fitted with a 24 compartment wood and
wirecloth cage, which allowed an unobstructed view of all subjects.
All oxygen exposures had eight mice from each of the three altitude
groups, thereby insuring a valid comparison between groups for every
OHP test.

Compression rate was 0.66 ATA per minute and a flow was maintained
through the chamber such that oxygen analyses of the gas entering and
leaving the chamber differed by no more than 0.2% (Servomex paramagnetic
analyzer).

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The time to convulsion was recorded as the time in minutes between reaching pressure and the onset of full clonic spasm. Time of death was taken as the time in minutes between reaching pressure and the last visible respiratory movement.

Each of the parameters was statistically evaluated using a one way analysis of variance, a pooled correlation design, and a multiple range test. Additionally, a factorial analysis of variance was used to determine possible interactions between treatment factors. All references to statistical significance in this paper represent chance probabilities of 5% or less $(P \leq .05)$.

Results:

Since the Duncan's multiple range test used to analyze differences between means is somewhat conservative, a least significant difference evaluation was also made. Consistent with its more liberal tolerances, the least significant difference method revealed a few more significant differences than the multiple range test; but since these additional analytical results reinforced the trend indicated with the multiple range test, we have reported mean differences based on the latter.

Weight:

Significant mean body weight differences as a function of altitude occurred in one group of eight animals at 1, 2, and 4 weeks; in each case, one group of sea level mice weighed more than those at either altitude. These differences reflected the significant interaction between body weight and altitude found for all treatment groups. The relationship can be described as primarily linear but with a sizeable quadratic component.

However, there was no clear relationship between body weight and duration of acclimatization and, of major concern, no significant correlation with convulsion, death, or convulsion to death times for any treatment combination. This latter observation suggests that acute

OHP toxicity is independent of body weight and that altitude-related effects can be predicted accordingly.

Convulsion Time:

Only at an OHP level of four ATA were any significant differences in convulsion time observed; at six and nine ATA, convulsion time was statistically indistinguishable for all treatment combinations. After two, four and eight weeks, mice living at 15,000 feet showed a significantly reduced convulsion time relative to those at 5,000 feet and sea-level. Except for a pronounced decrease after two weeks at 15,000 feet, there was a gradual but non-significant improvement in convulsion time with duration of acclimatization at all altitudes although the least improvement occurred at 15,000 feet. As might be expected, there was significant interaction between the OHP level and convulsion time, a function which was largely linear with a small quadratic component.

The data indicate that convulsion time is dependent primarily on OHP level and, at four ATA, varies significantly as a function of altitude. While an increasing acclimatization period appears to prolong convulsion time, there was no significant influence of acclimatization time on convulsion time.

Death Time:

As with convulsion time, significant differences in mean death time were observed only at an OHP of four ATA. Acclimatization to 15,000 feet

significantly reduced death time at two, four, and eight weeks relative
to the other two altitudes. In contrast to CT, there was a significant
decrease in death time after two weeks as compared with one, four, and
eight weeks for altitudes of 0 and 5000 feet; no significant effect
of duration was seen at 15,000 feet.

Although not significant, there was a clear, primarily linear,
interaction between death time and OHP level. Since death time sig-
nificantly correlated with convulsion time at OHP levels of four and
six ATA, it is likely that death time can be predicted as a function of
convulsion time for a given combination of altitude, acclimatization
period and OHP level.

Time Between Convulsion and Death:

This parameter indicates relative susceptibility or resistance to
death following a convulsive episode. As with its determinant para-
meters, convulsion and death times, significant variation in convulsion
to death time occurred only at an OHP level of four ATA. There was,
however, a conflicting pattern of this parameter vs. altitude. At two
weeks, mice raised at 15,000 feet had significantly longer times than
those at lower elevations. But after four weeks, sea-level mice were
significantly more resistant to death after convulsion than those at 5000
or 15,000 feet.

At all three altitudes, an acclimatization period of two weeks
resulted in a significantly longer convulsion to death time than at

one, four or eight weeks. Significant correlations between convulsion to death times, convulsion times, and death times were found at OHP levels of 60 and 135 psia but only between convulsion to death and death times at six ATA. There was a strong but non-significant, totally linear, interaction between decreasing convulsion to death time and increasing altitude for all treatment combinations. It thus appears that this parameter is largely a function of convulsion time and death time but that a predictable relationship between it and altitude might be made.

Discussion:

The data suggest that acclimatization to low oxygen tensions prior to OHP exposure can enhance susceptibility of mice to convulsions and death depending on the OHP level to which the animal is exposed (Figure 1). Of the three levels tested in this study, the effects of altitude acclimatization were discernible only at four ATA (Figure 2). The statistically insignificant results at six and nine ATA were probably due to at least two factors. First, the comparatively small time intervals to convulsion and death made accurate observations under the test conditions more difficult. Both observers expressed lesser confidence in their results at six and nine ATA, particularly the latter than at four ATA. Thus, lack of any significant differences at the two higher OHP levels may have been due, at least in part, to lesser accuracy and precision.

More likely, though, is the second factor of a possible reduced range of biological variation at higher levels of OHP. If one views the symptoms of oxygen poisoning as a combination of a short-term physical component and a longer-term biochemical one, it could be postulated that the relative contribution of these two factors might vary as a function of OHP level. If the level is relatively low ($\leq$ four ATA), convulsions may result from the development of biochemical lesions causing a reduction of certain CNS metabolites and pulmonary damage. Such biochemical lesions might be the principle focus of any influence exerted by altitude acclimatization. Conversely, if the OHP level is high enough ($\leq$ six ATA) that the physical cause of convulsions,

Figure 1.   Graph of mean convulsion (CT), death (DT) and convulsion

to death (CDT) times of mice exposed to 60 psia oxygen

after acclimatization for eight weeks at sea level, 5000
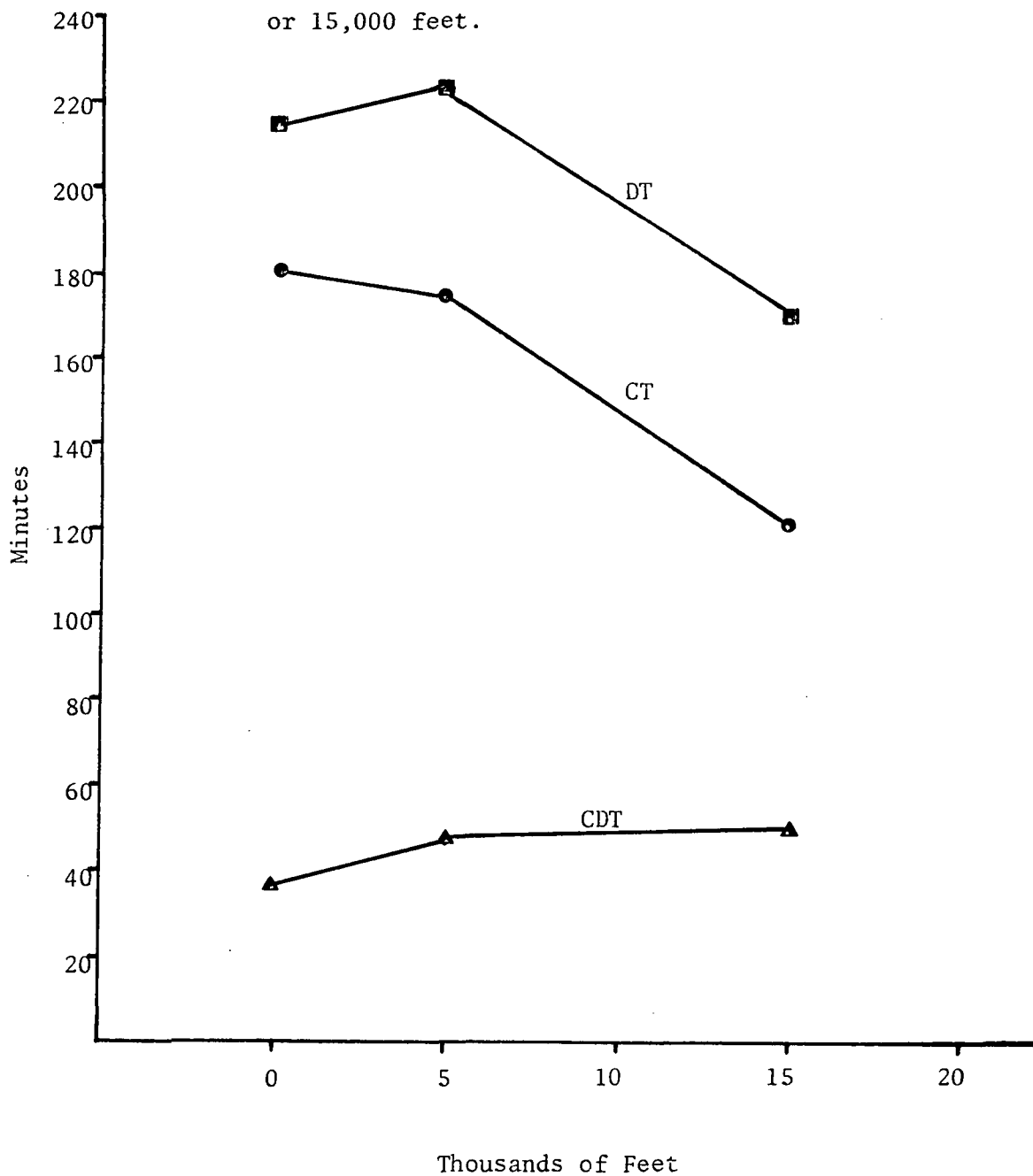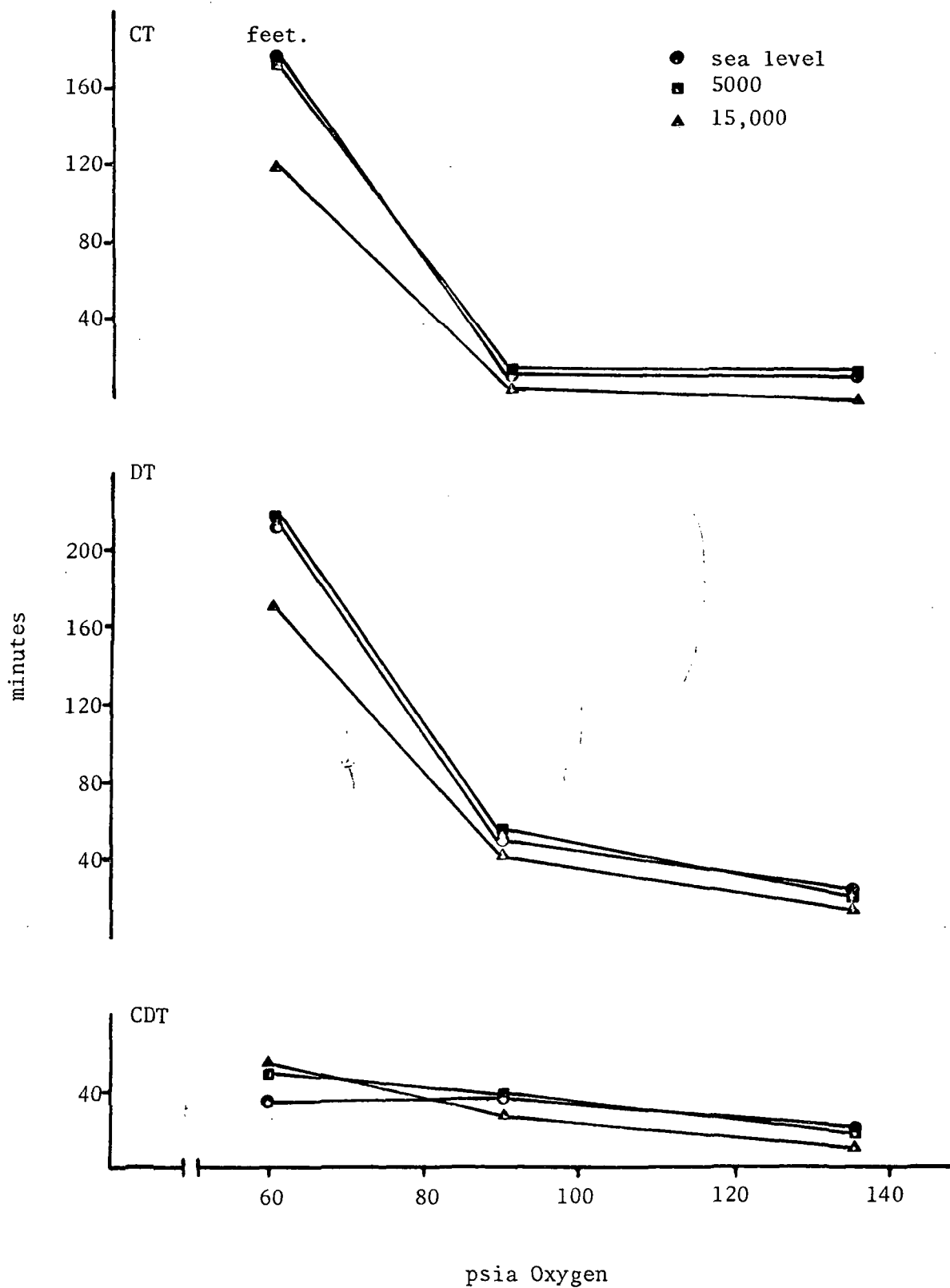
or 15,000 feet.

Figure 2. Graph of mean convulsion (CT), death (DT) and convulsion to death (CDT) times as a function of oxygen pressure (psia) for mice acclimatized eight weeks at sea level, 5000, or 15,000 feet.

perhaps an abnormal ionic distribution across the neuronal membrane,
were effected without appreciable biochemical involvement, altitude
acclimatization or other such treatment probably would have little ef-
fect. It is the authors' opinion that this is a possible explanation
for the present results and that the effects of altitude acclimatization
are of practical significance at four ATA or less.

It is pertinent to note that Brauer et al (1) did observe a
statistically significant reduction in the convulsion time of rats
acclimatized to 17,400 feet for eight weeks and exposed to an OHP level
of seven ATA. Their results are not consistent with ours at a similar
OHP level but might be attributable to a greater influence of hypoxia
on rats than on mice. These investigators also reported that hypoxic
acclimatization did protect against the pulmonary lesions caused by
OHP at 1.08 ATA oxygen in nitrogen as compared to sea-level controls.
This is a reversal of the influence of hypoxia observed by them and us
at higher OHP levels but is consistent with the presumed locus of hypoxic
influence being primarily biochemical in nature.

Considering our results for duration of acclimatization, there
was a general tendency for convulsion time to increase, no clear pattern
for death time and a decrease in convulsion to death time with time
duration of acclimatization (Figure 3). A marked decrease in convulsion
time after two weeks at 15,000 feet coupled with little change in death
time produced a high convulsion to death time. At 5000 feet altitude,
all three parameters were notably higher after two weeks than at one or

Figure 3. Graphs of mean convulsion (CT), death (DT) and convulsion to death (CDT) times of mice acclimatized to sea level, 5000 or 15,000 feet for one, two, four or eight weeks. Values are results from exposure to 60 psia oxygen.

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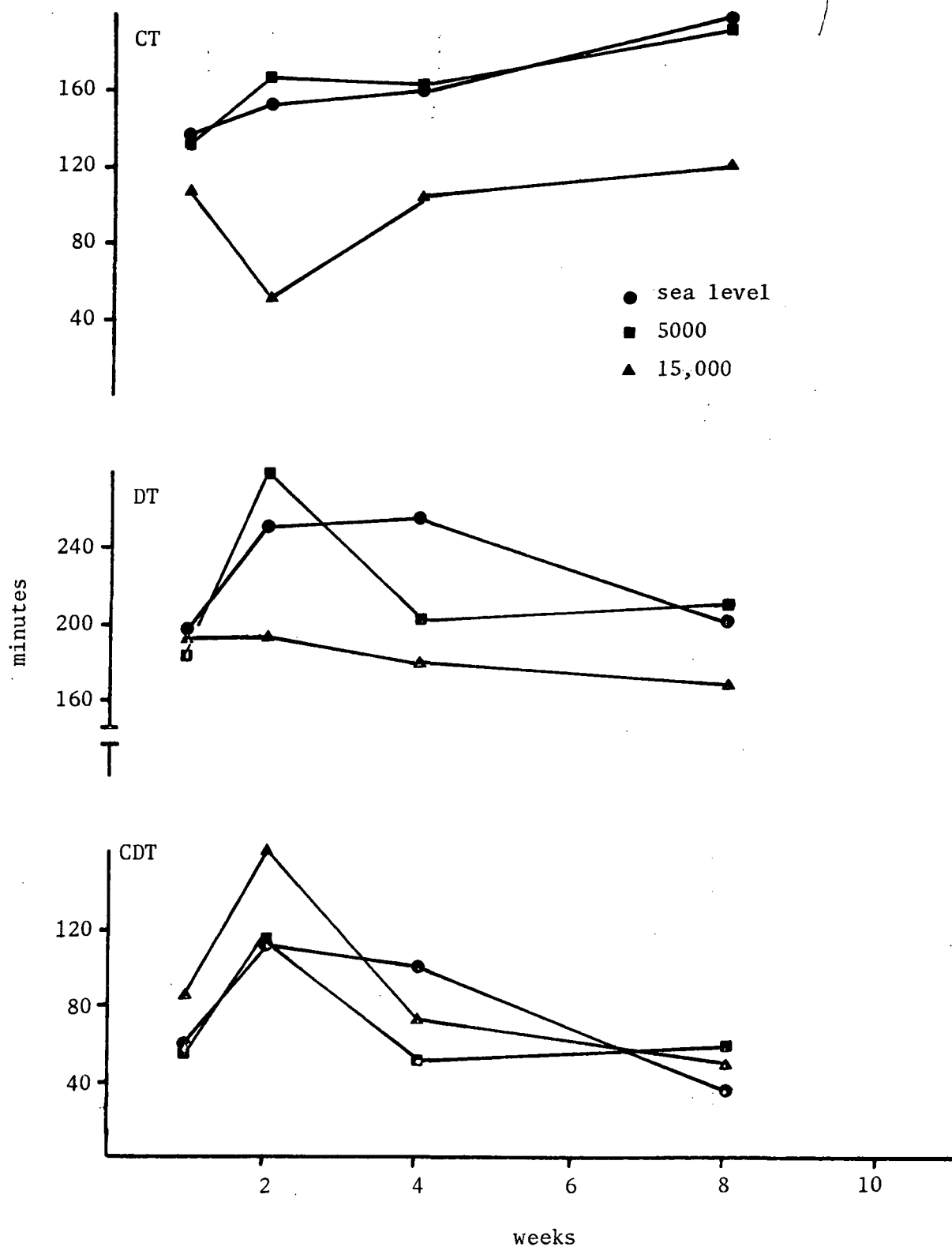
four weeks duration. The sea-level mice showed a similar peak at the second and fourth weeks for death time and convulsion to death time while convulsion time increased almost linearly throughout the acclimatization period. The tendency towards a pronounced change in these parameters after about two week's exposure to a given altitude may result from the occurrence of a critical phase in hypoxic adaptation at that time. However, since sea-level control values underwent similar fluctuations, these peculiar results may have been artefacts. It is possible, too, that acclimation to factors other than hypoxia, which are inherent to a closed environment, may have produced the peculiar results after two weeks. In any case, it is our opinion that the data do not offer a clear relationship between duration of acclimatization, convulsion time, and death time upon which accurate predictions for a given treatment combination could be made.

Neither does the data reflect a mechanism by which acclimatization to hypoxia might increase susceptibility to OHP-induced convulsions and death. Nevertheless, the concept of impaired GABA metabolism is consistent with the results. Hypoxia has been shown to lower brain GABA levels (4) as does exposure to OHP both prior to and following convulsions (2,3). Although the reduction of CNS GABA levels in vivo has not been proven as the primary biochemical event in oxygen poisoning, such a suggestion is supported by the fact that treatment with GABA prior to OHP exposure prevents convulsions and pulmonary damage (2). It is reasonable, then, to hypothesize that a reduction of GABA by

hypoxic acclimatization might increase susceptibility to oxygen toxicity by eliminating some of the chemical protection against OHP; the further reduction by OHP of GABA to the convulsion threshold level could thus be accomplished more quickly.

The present data suggest that the hypoxic effect is not consistently significant at an altitude of 5000 feet but becomes so on or before reaching 15,000 feet. At higher elevations, the pre-disposition toward OHP-induced convulsions and death is significantly apparent in both mice and rats (1) when compared to sea-level. The duration of hypoxic exposure does not seem to be as important as the degree in regard to severity of OHP symptoms; nevertheless, increasing duration of exposure did appear to decrease the effects of altitude on convulsions but not death in the present study.

These findings have practical implications for hyperoxic therapy in disease, underwater diving and any circumstances in which a resident at altitude becomes exposed to oxygen at elevated partial pressures. The altitude factor may be even more important if other conditions which predispose an individual to oxygen poisoning (exercise, hypercapnia, high temperature, drugs) are present. In such a set of circumstances, it might be necessary to reduce the maximum allowable OHP level and exposure duration to it on the premise that the predisposing factors are synergistic in their effect.

Finally, this study and the work of Brauer et al(1) show that the influence of hypoxic acclimatization offers protection against

lung damage at an OHP of about one atmosphere but predisposes an animal to convulsions at four ATA and above. Further work is required to delineate the nature of these two mechanisms and the optimum level of OHP at which hypoxic acclimatization ceases to be protective and becomes detrimental during OHP exposure.

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