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Studies On The Effects Of Centrally Acting Drugs At Increased Pressures Of Air

G D. Fields

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STUDIES ON THE EFFECTS OF CENTRALLY ACTING DRUGS AT INCREASED PRESSURES OF AIR

by

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amphetamine. Similarly, hyperbaric air enhanced the locomotor stimulation produced by morphine sulphate (15 and 30 mg/kg). In regard to the depressant drugs, exposure to 7 but not 4 ata air significantly prolonged the sleep time induced by pentobarbital 45 mg/kg. No significant prolongation was observed at 35 mg/kg the other dose tested. In contrast, hyperbaric air acted to reduce the DPH (10 and 20 mg/kg), induced depression of spontaneous activity. Similarly, the combination of alcohol (1.75 and 2.75 g/kg) and hyperbaric air generally did not depress spontaneous activity. Such an effect was only observed at the high dose at 7 ata, and was transitory. In the other cases, the combination tended to increase spontaneous activity. Studies with helium/oxygen confirmed that these effects were due to N_2.

In humans, hyperbaric air impaired memory but not neuromuscular coordination. The drugs alone did not impair performance, and in general did not significantly worsen performance at pressure. In study-I, alcohol (0.25 g/kg) did combine with hyperbaric air to impair neuromuscular coordination. This was not observed at the higher dose in study-II, in more practiced subjects.

The results indicate that N_2 at pressures in the "air diving range" can modify the effects of centrally acting drugs. The modification of drug effects, however, is more complex than would be predicted by the hypothesis that N_2 produces a generalized depression of CNS activity. In particular the results in mice suggest that both stimulation (disinhibition) and depression of neuronal function occur. Current concepts of anesthetic action, and data on inert gas effects on CNS activity also question the hypothesis that N_2 produces a generalized

depression of CNS activity. It is proposed that a hypothesis of N_2's effects on CNS function based on current concepts of anesthesia offer a better means of explaining the results.

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List of Abbreviations

ARAS	Ascending Reticular Activating System
ARS	Ascending Reticular System
CSF	Cerebrospinal fluid
DA	Dopamine
HPNS	High Pressure Nervous Syndrome
LVFA	Low voltage fast activity (in the EEG)
MRF	Mesencephalic Reticular Formation
NAc	Nucleus Accumbens
PCPA	Para-chlorophenylalanine
pCO_2	Blood carbon dioxide levels
PHe	Partial pressure of Helium
PO_2	Partial pressure of oxygen
PN_2	Partial pressure of Nitrogen
RF	Reticular formation
5 HT	5-hydroxytrptamine
6-OHDA	6-hydroxydopamine

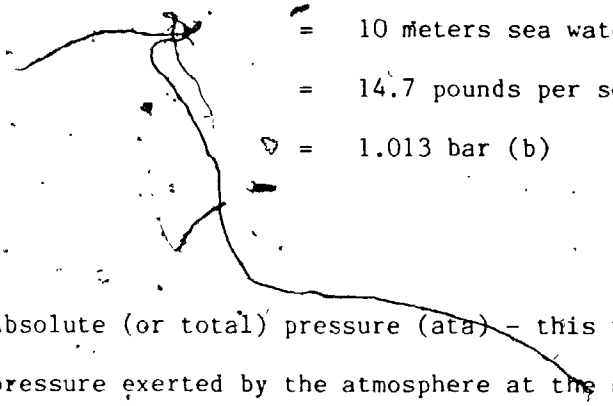
PRESSURE CONVERSION CHART

- 1 atmosphere (atm) = 33 feet sea water (fsw)

= 10 meters sea water (msw)

= 14.7 pounds per square inch (psi)

= 1.013 bar (b)



- Absolute (or total) pressure (ata) - this takes into account the pressure exerted by the atmosphere at the surface. Therefore for example, at a depth of 33 fsw, the gauge pressure is 1 atm, but the absolute pressure is 2 ata.

CHAPTER 1

GENERAL INTRODUCTION AND PURPOSE

At present there is an urgent need for information regarding the ways in which responses to centrally acting drugs may be modified during exposure to increased pressures of air. In recent years there has been a virtual explosion in all forms of diving activity, the largest increases occurring in the recreational and commercial areas. As an example, in 1978 it was estimated that the population of active recreational divers in the United States was 2 million, with a predicted increase of 135,000 new divers/year (McAniff, 1981). Estimates put the number of recreational divers in Canada at around 60,000, and it is likely that there are another 2 million scattered throughout the rest of the world.

The rapid increase in the amount of diving activity, coupled with the fact that drugs are now an integral part of modern life, has greatly increased the likelihood that drugs will be used during, or closely preceeding exposure to a hyperbaric air environment. For example, drugs such as analgesics, antihistamines, nasal decongestants, and antimotion sickness preparations, may be taken by the recreational diver for treatment of minor ailments. Also alcohol, and more recently drugs of abuse such as marihuana, are being taken prior to diving.

The variety of drugs that may be used in commercial diving is even greater (Cox, 1980 ; McIver, 1980). In commercial diving, in order to prevent having to decompress divers every day following work at depth, saturation exposures are usually employed. In this system, divers are maintained in a pressure chamber, at or near a pressure equivalent to the depth at which they are working. After their work is finished, they are then slowly decompressed to 1 ata (see Conversion Chart). Thus, in addition to the medication for the treatment of minor ailments referred to above, the extremely long time required for decompression from saturation exposures raises the possibility that divers may require medical treatment for trauma or infection while at pressure. Furthermore, as McIver (1980) notes, working efficiency and safety demand that divers get some sleep in noisy surroundings, and therefore sleep inducing medication is sometimes administered.

At present however, very little is known about how the response of such drugs may be altered by exposure to hyperbaric air. There is concern however, that the effects of drugs may be altered at increased pressures of air, possibly with tragic consequences. Exposure to hyperbaric air is associated with increases in the partial pressures of nitrogen (N_2), oxygen (O_2), as well as increases in hydrostatic pressure and perhaps pCO_2 levels, all of which are capable of producing changes in central nervous system (CNS) function (see Chapter 2). The behavioral changes seen at pressures of air in excess of 4ata, and characterized by stimulation, ammnesia and impaired neuromuscular co-ordination (Bennett, 1966 ; 1975 ; see also Chapter 2), are an indication of the marked alterations in CNS function that can occur. Thus in a hyperbaric air environment, a drug is no longer acting on a

"normal" individual, and consequently drug effects might be expected to differ from those under normal conditions. For example, the effects of a drug normally tolerated at 1 ata, might in combination with those produced by hyperbaric air compromise CNS function to such an extent that the ability of the diver to react appropriately to a real or perceived danger is impaired. Alternatively, the changes in the physiological state of the diver might alter the normal pharmacological or toxic profile of a drug. Such interactions in the diving environment given the ever present dangers of decompression sickness (the bends) and drowning, could have tragic consequences.

There are a few scattered clinical reports in the literature of adverse effects of drugs at increased pressures of air. Nemiroff (1977), reported the case of a diver who had ingested 2-4 ounces of alcohol and "snorted" an unknown amount of phencyclidine before diving. On surfacing, he was combative, disoriented, and showed marked hyperreflexia and coarse nystagmus to the left. On recompression, he gradually returned to normal and was diagnosed as having mild residual organic brain syndrome. McIver (1980), cites the case of a diver who took two aspirins and ephedrine nasal spray before diving to 110 feet (4.3 ata) for 60 minutes. Towards the end of the dive he felt "narked" (suffering from narcosis) and was uncoordinated on the ascent and aggressive once in the chamber. After a series of skirmishes, he was left to sleep it off, having been sedated with an antihistamine. At the time, hypoglycemia was diagnosed. Reports of hallucinations in a diver given distalgesic (a mixture of dextropropoxyphene and acetaminophen). at approximately 110 feet (4.3 ata) , and vertigo and unconsciousness from overuse of nasal spray containing oxymetazocine hydrochloride at

136 feet (approx. 5 ata), are noted by Cox (1980) in his report on drug use at pressure in commercial diving operations. He also cited the case of a diver who dived while taking hyoscine butylbromide for an undisclosed ailment, and who developed dizziness and vomiting and died. The depth at which this occurred was not given. In the above cases, it would be hasty to attribute all of these effects to the interactions of the drugs with those of hyperbaric air, as other factors could also be responsible. For example, in the case of the diver who had taken ephedrine, the effects of hypoglycemia could have been responsible. However, in the case of distalgesic and oxymetazocine hydrochloride, the pressures of air at which these effects were observed should normally prove no hazard to the experienced diver.

It could be argued that given the large number of divers, these represent very few interactions. One reason for this however, may be the absence of an adequate reporting and collection network. For example, the majority of the cases cited above are from commercial diving operations, where the drugs taken by divers and any of their adverse effects can be readily monitored. Even so, it appears that it is only within the past few years that this has become fairly standard practice. As McIver (1980) states in regard to diving accidents, "there are really no accurate figures with which to assess the relative safety of diving operations. A fatality will make the news, but rarely is there a centre to analyze fatal diving accidents, and relate them to causative factors or to the associated number of divers or dives performed. In general, 'near-misses' and non-fatal accidents pass unrecorded." He goes on to say that the figures relating to sports diving accidents are even less reliable. These sentiments have been

echoed in later reports on underwater accidents, and diving safety (Elliott and Davis, 1982; McAniff, 1981). The situation is improving however, as evidenced by the establishment of a National Underwater Accident Data Center in the United States. Even though this is a step in the right direction, enough attention is not being placed on the possible role of drugs in diving accidents. For example, in their "Underwater Accident Research Form", questions pertaining to drugs taken prior to diving, (with the exception of alcohol), are not included.

At the time of starting this thesis, there was very little information on the effects of drugs at increased pressures of air. Thus the aim of this thesis was to examine the ways in which the effects of a number of specially selected centrally acting drugs might be modified by hyperbaric air, at pressures that would be normally encountered by sport and commercial divers. A limit of 120 ft sea water (4.75 ata) is generally accepted as the depth limit for sports divers, and then only for the highly experienced ones. The information obtained from these studies would add to the pool of data on this topic, and act as a starting point for further studies to probe the nature of such effects. Experiments were restricted to the 1-7 ata range. This was done partly for practical reasons (existing facilities do not permit studies at higher pressure), but also because this range adequately covers the air diving range.

Work commenced with studies in mice. During the course of these studies however, results of drug - hyperbaric air interactions in humans were published which were at variance with the results of the studies in mice, as well as with the main body of animal data. These conflicting

results questioned the relevance of the data from animal studies to the human situation, and highlighted the need for more experiments in humans to clarify these conflicting findings. Therefore, two studies using human subjects were also performed.

Finally based on new concepts of anesthetic action, and a re-evaluation of the effects of N_2 on CNS function, a hypothesis of N_2's action on CNS function was proposed, and its implications for the modification of drug effects by hyperbaric air discussed.

In the following chapter, the effects of increases in PN_2, PO_2, pCO_2 and hydrostatic pressure on CNS function will be described. This will provide an indication of how drug effects might be expected to differ in a hyperbaric air environment. Those factors most relevant to interactions with, or modification of CNS drug effects at the pressures of air normally encountered in sports diving will be emphasized. Also the possible effects of exposure to hyperbaric air on drug pharmacokinetics will be discussed.

CHAPTER 2

FACTORS IN THE HYPERBARIC AIR ENVIRONMENT AND THEIR IMPORTANCE WITH REGARD TO MODIFICATION OF DRUG EFFECTS

2.1 NITROGEN

At the pressures encountered in the air diving range, the increased PN_2 is probably the most important factor with regard to modification of centrally acting drugs. Increasing the pressure of air correspondingly increases the PN_2, and in accordance with Henry's law (that the solubility of a gas in a fluid is directly proportional to its pressure) more N_2 dissolves in the membranes of nerves and other tissues. It is this increase of N_2 in neurons in the CNS which is thought to be responsible for the behavioral changes produced by hyperbaric air, and hence this syndrome is termed N_2 narcosis (Bennett, 1966; 1975). The term "inert gas narcosis" is also used since the changes are also seen with other inert gases such as Argon (Ar), and Xenon (Xe), although these gases are more potent than N_2. The primary role of N_2 in producing these changes was not always accepted however. Therefore, following a brief review of the behavioral changes produced by hyperbaric air, the evidence that they are due to N_2 will be presented. Finally, the mechanism by which N_2 was thought to produce its effects will be discussed. The reader is directed to other reviews by Bennett (1966,1975).

2.1.1 Behavioral Changes Produced by Hyperbaric Air

The behavioral changes commonly associated with exposure to hyperbaric air are excitement, euphoria, stimulation, a slowing of cognitive function, ammnesia for events at depth and impaired neuromuscular co-ordination. It should be noted that these are not the only changes that occur, but are the ones that are most easily observable. Other changes such as slowed reaction time (Kiessling and Maag, 1962) and decreases in standing steadiness (Jones et al., 1979; Adolfson et al., 1972) have been shown by specific tests.

The changes produced by hyperbaric air are usually manifest at pressures in excess of 4ata, and increasing the pressure of air increases the severity of the narcosis (Bennett, 1969; Adolfson, 1964, 1965). In fact, it is thought that at sufficient pressure, hyperbaric air could act as an anesthetic. The effects at lower pressures (4 ata) have been likened to the effects of alcohol, hypoxia, and the earlier stages of anesthesia (Behnke et al., 1935; Schilling and Willgrube, 1937; Bennett, 1975). At higher pressures (13 ata) however, the effects are more typical of those due to psychedelic drugs such as LSD, rather than alcohol, (Adolfson, 1965; Bennett, 1969). Tolerance to these effects can develop from frequent exposure to hyperbaric air (Bennett, 1966), and mental function appears to be more affected than motor co-ordination (Behnke et al., 1935; Case and Haldane, 1941; Kiessling and Maag, 1962; Bennett, 1966).

2.1.2 Theories To Explain The Behavioral Effects Produced By Hyperbaric Air

2.1.2.1 Early Reports and Theories

Reports began to surface in the early 19th century regarding the ability of hyperbaric air to produce behavioral changes in man. However it was not until 1935, some 100 years after the first published report of such changes, that N_2 was proposed as the causative agent.

The first report is usually attributed to Junod, who, in 1835, noted that when breathing hyperbaric air, "the functions of the brain are activated, imagination is lively, thoughts have a peculiar charm, and in some cases, symptoms of intoxication are present." Later Green (1861) noted that breathing compressed air while diving produced a "feeling of sleepiness". At 160 feet (5.8 ata) he also noted hallucinations and impairment of judgement, and suggested that the signs and symptoms were of sufficient severity to merit an immediate return to atmospheric pressure (Bennett, 1966). Paul Bert, well known for his work on oxygen toxicity and decompression sickness, in his book La Pression Barometrique (1878), also alluded briefly to the "narcotic" condition of divers at great depths (Bennett, 1966).

Despite these reports however, it appears that no serious investigations into the behavioral effects of hyperbaric air were made until the studies by Hill and co-workers in the early 1930's on the problems of deep diving and submarine escape (Hill and Phillips, 1932; Hill et al., 1933). This lack of interest was probably due to the fact that the main use of hyperbaric air in the latter half of the 19th

century and early 20th century appeared to be in caissons to keep back the water encountered in the driving of shafts and tunnels (Walder, 1982). In this work, the pressures used are quite low (< 5 ata) and at these pressures, the major problem was not from the narcosis produced by hyperbaric air, but rather from decompression sickness (the bends). There is some evidence that "narcotic" effects were observed however. For example, Moxon in 1881 states that "the workmen feel more lively and elastic and go up their ladders with ease ", and Hill and Greenwood in 1906, make mention of "the subjective effects of increased pressure ". These effects however were considered to be due either to the increased pressure forcing blood into the brain (Moxon, 1881),or "psychical conditions" such as anxiety or excitement (Hill and Greenwood,1906).

Hill and co-workers (Hill and Phillips, 1932) provided quite detailed accounts of the behavioral changes observed in divers at depths in the range of 270 to 300 feet (approx. 10-11 ata). These included many of the changes now associated with N_2 narcosis, such as ammnesia for events at depth, a tendency to fixation of ideas, increased difficulty in assimilating facts and making quick decisions, and loss of consciousness. These changes were assumed to be an extreme case of the subtle change in character and behavior which was observed at lower pressures. N_2 however, was not considered to be the cause of these behavioral changes. Instead, these workers speculated that the effects of hyperbaric air were due to the high PO_2, or to impurities in the air breathed. Flushing the diving helmet with air appeared to offer some relief, and it was suggested that CO_2 might also be a factor in these changes. It is reported that subsequent observations caused Hill to rule out O_2 and CO_2 as the cause of these effects (Damant,1930). The

evidence which led Hill to conclude this is not presented however. Eventually, based on the finding that the divers most affected seemed to be of "the nervous type", Hill concluded that the psychological make up of the diver was the main cause of the mental effects of deep diving, and suggested that potential divers be interviewed to detect those with mental instability, which in his opinion was contraindicated in deep diving (Hill and Phillips, 1932).

2.1.2.2 Nitrogen Theory

The first proposal that the increased PN_2 was the cause of the changes produced by hyperbaric air was made by Behnke and co-workers (Behnke et al., 1935). In an experiment to rule out abnormal factors in the diving environment such as darkness, cold, claustrophobia, as the cause of the narcosis, trained laboratory personel were exposed to hyperbaric air in a large well lit pressure chamber, and their ability to perform routine laboratory tasks monitored. Behavioral changes were observed as the pressure reached (4 ata) and increased in severity with increasing pressure. The changes were grouped into three categories: (i) emotional:- stimulation, alertness, and a feeling of well-being, (ii) impairment of the higher mental processes:- response to stimuli was slowed, tendency to fixation of ideas, and increased difficulty in recollection and concentration, and (iii) impairment of neuromuscular control:- manifested by increased difficulty in the manipulation of burettes and pipettes, and increased breakage of glassware. In one experiment, the group of subjects was exposed to 10 ata, and at this

pressure the symptoms were reported to be more severe, however, no further detailed information is given. The authors only report that an extreme numbness was felt by one subject which they likened to "partial stupefaction", and stated that the performance of a simple task, such as palpation of the pulse, was performed with great difficulty. As PO_2's in excess of those encountered in this study had previously been shown not to produce similar behavioral changes (Behnke et al., 1934), O_2 was ruled out as the cause of these effects. It was concluded that the increased PN_2 (including the small percentage of Ar normally found in air) was responsible. They also proposed, based on the Meyer-Overton Hypothesis (Meyer, 1899; Overton, 1901), that the high lipid solubility of N_2 allowed it to act as a "narcotic", and predicted that replacement of N_2 in the breathing mixture by a gas with a low partition co-efficient, would minimize the "psychic" effects caused by breathing air at pressure.

Following this, further support for N_2 as the causative agent was obtained in experiments by Behnke and Yarbrough (1938), and Case and Haldane (1941), as well as from the reports of dives made breathing He/O_2(80/20) mixtures instead of air (End, 1937, 1938; Behnke and Willmon, 1939), all of which demonstrated the lack of behavioral changes when breathing He/O_2 instead of air at pressure.

In addition, such factors as the small quantities of CO_2 and Ar normally present in air were ruled out as the cause of the effects. For example, Case and Haldane (1941) found in their experiments that whereas breathing CO_2 at concentrations up to 6% produced distress in their subjects, it produced very little of the impairment seen at 11 ata air.

As this concentration of CO_2 was greater than the 4% calculated to be present in the air at 11 ata, they suggested that CO_2 was not responsible for the effects seen. Using a similar approach Behnke and Yarbrough (1939) ruled out the small % of Ar in the air as the cause of the narcosis.

It should be pointed out that in most of the above reports dealing with the evidence for N_2 as the causative agents, the experimental details are sketchy,and in some cases the results are based on only a few subjects. The differential effects of air compared to He/O_2 on behavior, subjective feelings, and performance appear large enough however, to justify the conclusions drawn.

Although Behnke and co-workers (Behnke and Yarbrough, 1938 ; 1939) and Case and Haldane (1941) agreed on N_2 as the causative agent, they disagreed as to the mechanism of action. As stated earlier, Behnke and co-workers (Behnke et al., 1935; Behnke and Yarbrough, 1938, 1939) considered that the lipid solubility of N_2 was the most important factor with regard to its mechanism of action. Thus their findings that He with its low oil/water solubility was inactive (Behnke and Yarbrough, 1938) seemed to support their theory. The greater "narcotic" potency of Ar compared to N_2 despite their similar oil/water solubility ratios did not fit with the theory however. This inconsistency was explained by postulating that the difference was due to Ar being twice as soluble as N_2 in lipid (Behnke and Yarbrough, 1939). They also suggested however, that the different molecular weights of the gases might be responsible.

Case and Haldane (1941) however, disagreed with the lipid solubility theory. Their disagreement appeared to stem from the

discrepancy between the lipid solubility of H_2 and its "narcotic" potency. They reasoned that if the lipid solubility theory was correct, then 10.8 ata H_2 should have a "narcotic" potency aproaching that of 9.7 ata air (i.e. 7.8 ata N_2). This was not supported by their experimental evidence however (Case and Haldane,1941). As an alternative, they proposed that the differences in adsorption of these gases to charcoal at 0^oC more closely reflected their differences in potency, and suggested that adsorption on surfaces within or on the cell may be as important as lipid solubility in determining the "narcotic" properties of these gases. It should be noted however, the adsorption data would suggest N_2 to be more potent than Ar since the volume of N_2 adsorping is greater than that of Ar. (Adsorption data : H2, He, Ar, N_2/ 4, 2, 12, and 15 volumes respectively).

The primacy role of N_2 was not accepted by all workers at the time however. Shilling and Willgrube (1937) the authors of the other major study at that time, disagreed with the proposal that N_2 was the sole cause of the narcosis. In their experiment, which examined the effect of hyperbaric air on mental function and reaction time, they observed that the greatest decrement in performance was noticeable immediately upon reaching depth, and if anything lessened as the subjects became adjusted. They considered this to negate the lipid solubility theory, since if the theory were correct, then the effects should increase not decrease with time spent at pressure. They suggested instead, that the cause of the narcosis might be due to a combination of all the previously considered factors, i.e. high PO_2, N_2, and psychological factors,or could be entirely due to the pressure itself.

Thus, by the early 1940's there was strong evidence in favor of N_2 as the cause of the narcosis although there was disagreement as to the mechanism of action, and its primary role was not universally accepted. Confirmation of N_2 as the cause of the narcosis was to take a long time however, as in subsequent years alternative theories received strong support. These opposing theories will now be discussed.

2.1.2.3 Opposition to Nitrogen As The Cause Of The Narcosis

Carbon Dioxide Theory

In a series of experiments on anesthetized dogs, Bean (1947,1950) found that rapid compression to 70-100 psi (approx. 5.6-7.1 ata) produced an increase of 0.15 to 0.2 units in the acidity of the blood (Bean, 1947) and an increase in alveolar CO_2 tension (Bean, 1950). He suggested that this increase in alveolar CO_2 was due to blockade of expiration by the inflow of air during compression. Furthermore, he proposed that it would diminish or reverse the normal CO_2 diffusion gradient, thereby driving CO_2 back into the blood and resulting in a hyperoxic - hypercapnia. Based on these findings and the already etablished "narcotic" effect of CO_2, he proposed that an increase in CO_2 in the blood and tissues produced by compression was more likely than N_2 to be the cause of the effects produced by increased pressures of air.

To further support his proposal, he cited the arguments raised against the lipid solubility theory by other investigators (Schilling and Willgrube, 1937), namely that it could not explain the rapidity of

symptom onset, nor the finding that the maximum effect was experienced immediately on reaching pressure and did not increase in severity with time, but rather tended to decrease during the continued exposure to pressure. He also stated that the apparent alleviation of the symptoms by substitution of He for N_2 in the breathing mixture did not invalidate CO_2 as the cause of the narcosis, because, (i) substitution of He for N_2 very largely but not entirely alleviated the symptoms, and (ii) that due to their different physical properties He and N_2 would be expected to have quite different effects on CO_2 retention.

Further support for the CO_2 theory came from Buhlman (1961,1963), Seusing and Drube (1960), and Seusing (1961), see Bennett (1966) for a review of their arguments. These researchers also considered CO_2 retention to be the cause of the narcosis. They differed from Bean (1950) however, in that they considered this to be due to hypoventilation brought about by the increased breathing resistance at depth. They also predicted that this CO_2 retention would be further aggravated by the high PO_2 which in itself would produce hypoventilation (Bennett, 1966). Thus, like Bean (1950), they predicted a state of hyperoxic-hypercapnia. As a corollary of their theory it was suggested that narcosis could be alleviated by using a less dense gas such as He, or by using auxilliary breathing equipment to ensure satisfactory alveolar ventilation. Bennett (1966) states that Buhlman used both assisted ventilation, and He to overcome narcosis. No details are given however.

Hypoxia - Vail's hypothesis

Vail in 1971, contended that "N_2-narcosis" was a myth, and stated that there was no experimental facts to show than an inert gas in itself, produced narcosis through a biochemical action in humans. Instead he proposed that all undersea narcosis was due to hypoxia, complicated by CO_2 retention. These effects could result from decreased ventilation due to the increased density of the breathing mixture at pressure. This proposal was based on the fact that the maximum expiratory flow, an important determinant of alveolar ventilation is independent of voluntary respiratory effort, but instead is determined by the physical properties of the airway and the density of the breathing mixture (Pride et al., 1967; Mead et al., 1967). Since flow is inversely related to density, he argued that at pressure the increased gas density would reduce the maximum expiratory flow leading to hypoventilation. Furthermore as the maximum expiratory flow is effort independant, any attempt to increase ventilation through increased expiratory effort would be ineffective. In fact, he suggested that such effort might lead to collapse of the airway and further reduce expiratory flow. The hypoventilation coupled with the increased work of breathing would lead to decreased alveolar gas exchange resulting in hypoxia and CO_2 retention. Vail proposed that this collapse would occcur at the level of the subsegmental bronchi, and analysis of previous experimental data (Lord et al., 1966), seemed to support this.

In choosing hypoxia as the main cause of the narcosis, he suggested that CO_2 retention could not explain all of the symptoms produced by hyperbaric air, but that the symptoms such as stimulation, excitement,

and euphoria were classic symptoms of hypoxia. In resting subjects at pressure he considered it possible for a diver to receive adequate oxygenation since the PO_2 would be elevated above normal values. Once the diver began to work however, the increased expiratory effort in response to the work would lead to airway collapse producing the signs and symptoms of narcosis.

The greater potency of Ar vs. N_2, and the ability of Xe to produce anesthesia at normal atmospheric pressure, was also explained using this theory. Vail proposed that because of their greater density compared to N_2, airway collapse and the resultant behavioral effects would occur at lower pressures than seen with N_2.

2.1.2.4 Confirmation of Nitrogen as the Cause of the Narcosis

Evidence Against the Carbon Dioxide Theory

Shortly after the resurgence of interest in CO_2 as the cause of the narcosis produced by hyperbaric air (Bean, 1950), Rashbass (1955) provided evidence which tended to reject this. Studies were conducted on the effect of exposure to 8.6 ata air on the ability of subjects to perform an arithmetic task while breathing normally, and also after hyperventilating to reduce alveolar CO_2. Exposure to hyperbaric air produced a slight increase in mean alveolar CO_2, from a value of 5.1% at the surface to 5.4 % at pressure, and reduced the number of problems correct from a mean value of 24.1 at the surface to 16.8. Hyperventilating reduced the mean alveolar CO_2 from 5.4 to 3.9%, but did

not improve arithmetic performance.

Direct measurements of cortical CO_2 and O_2 levels during exposure to increased pressures of inert gases, also argued against increases in CO_2 tissue levels as the cause of narcosis (Bennett, 1963, 1965, 1966). In studies on (chloralose) anesthetized cats, Bennett determined cortical levels of CO_2 and O_2 during exposure to raised pressures of N_2, Ar and He and attempted to correlate the changes in these parameters with the level of narcosis. Both normoxic and hyperoxic mixtures (2.34 ata O_2) were studied, the partial pressures of the inert gases in these cases being 10 and 8.67 ata respectively. Depression of auditory evoked potentials was used as the indicator of narcosis. Critical evaluation of the results of this study is made difficult by the method of presentation of the results and the fact that the data for each treatment condition is based on a very small number of animals (2 in most cases). This is particularly so in Bennett (1965). In this study, the changes in CO_2 are presented as arithmetic rather than % change, and it is not clear as to the point in time at which the changes reported occurred. The data is presented somewhat more satisfactorly in Bennett (1963, 1966) where the mean % change in CO_2 as a function of time is presented.

Bearing the above comments in mind, the results in general showed little correlation between cortical CO_2 levels and narcosis. For example, both the normoxic and hyperoxic N_2 mixtures produced narcosis. In the former, however, the cortical CO_2 was not different from that at 1 ata air, thus ruling out CO_2 as the cause. In regard to the hyperoxic mixture, Bennett reported that there was a slight increase in CO_2 levels

above that seen at 1 ata air. This increase in CO_2 did not seem to be responsible for the "narcotic" effect however, since a similar increase in CO_2 levels was seen breathing pure O_2 at 2.34 ata, the PO_2 equivalent in the hyperoxic mixture, without any signs of narcosis. The results however, did seem to support the contention that increases in PO_2 and gas density would tend to promote CO_2 retention (Buhlman, 1961, 1963; Seusing and Drube, 1960; Seusing, 1961). Thus, for a particular inert gas the tissue levels of CO_2 were higher with the hyperoxic mixtures, and Ar, the most dense gas, produced the greatest increase in CO_2 levels. Helium, the least dense of the gases, apparently did not produce any increase in cortical CO_2 levels.

Later studies in man (Hesser et al., 1971, 1978; Bennett and Blenkarn, 1974) also provided evidence against the CO_2 theory. To assess the relative roles of N_2 and CO_2 in the narcosis produced by hyperbaric air, Hesser et al., (1971) evaluated the performance of 10 subjects at 6 ata air in the presence of 0, 2 , 4, and 6% CO_2, on a perceptual motor task, and the Stroop test. End tidal CO_2 values were continuously recorded throughout the experiment. In order to control for possible effects of the increased PO_2 in the air at 6 ata, experiments were also carried out at 1.3 ata O_2, and the results of these experiments compared.

Exposure to 6 ata air alone produced a sight elevation in end tidal CO_2 levels (32.9 vs 30.6 mm Hg) over that seen at 1.3 ata O_2, and significantly decreased performance on the perceptual motor test. The decrement in performance did not appear to be due to the slight increase in end tidal CO_2, since in the experiments at 1.32 ata O_2 end tidal

concentrations in excess of 40 mm Hg were required to disrupt performance on this test. Increases in the end tidal CO_2 did however, disrupt performance on the Stroop Test in a concentration dependent manner. The authors concluded however, that it was not clear to what extent this effect of CO_2 represented a "narcotic" effect, or difficulty in vocalizing the words due to hypercapnic hypernea. Increases in both PN_2 and end tidal CO_2 produced greater decrements on both tests than observed with either agent alone, supporting the earlier findings (Case and Haldane, 1941) that CO_2 could enhance the decrement in CNS function produced by hyperbaric air. Based on their findings the authors concluded that the CO_2 component in narcosis was negligible below a P_aCO_2 (alveolar CO_2) of 40 mm Hg. A similar study conducted later by Hesser et al., (1978), confirmed these findings.

In their experiment, Bennett and Blenkarn (1974) showed that despite similar pH, pCO_2 and pO_2 values in subjects exposed to air or He/O_2 at 6.7 and 9.6 ata, narcosis, as evidenced by decrements in performance on an arithmetic and digit symbol test, was only observed when air was breathed. It should be noted that in contrast to the hypercapnia observed in other studies at depth, eg. Hesser et al., (1971), these subjects were hypocapnic, alkalotic, and demonstrated a small positive base excess (mean 4.1 + 1.1 m eq/litre). These studies were performed during decompression stops from a saturation dive to 27.2 ata He/O_2, and the authors suggested that the hypocapnia and other acid/base changes were due to a chronic metabolic adaptation to hypercapnia occurring during the saturation exposure. These changes do not however, invalidate the results of the study.

The experiments reviewed in this section, provide strong evidence against CO_2 as the cause of the narcosis. Bennett (1966) states that Buhlman in a personal communication indicated that he interpreted the term narcosis as a loss of consciousness. If this is so for other supporters of the CO_2 theory, then this may explain some of the differences between their views and the supporters of the N_2 theory. It would also explain Buhlman's assertion that assisted ventilation can ameliorate the narcosis produced by hyperbaric air. Improved ventilation would reduce CO_2 levels and prevent the interaction between its effects and those of N_2.

Evidence Against Hypoxia As The Cause of Narcosis: Vail's Theory

As stated earlier, Vail (1971) considered that the narcosis was due to the increased gas density at pressure which resulted in hypoxia and CO_2 retention. There is both experimental and theoretical evidence however to reject this proposal.

Experimentally, the studies by Bennett and Blenkarn (1974) in humans, and Bennett (1963, 1965) in cats discussed previously, clearly show that adequate oxygenation occurs under conditions in which narcosis is present. Also, the finding that during Xe anesthesia, pO_2 and pCO_2 levels are within the normal range (Morris et al., 1955) argues against hypoxia resulting from hypoventilation being the cause of narcosis.

On theoretical grounds, Vail's hypothesis fails in that it does not predict the occurrence of narcosis during a resting dive. During a resting dive Vail predicted that despite reduced ventilation, a diver would be able to obtain adequate oxygenation due to the increased PO_2,

and therefore the symptoms of narcosis would not occur. Studies have in fact shown that the first part of his prediction is correct with adequate ventilation being present not only for resting divers but also for divers doing light work (Lanphier, 1963; Fagraeus and Linnarson, 1975). Narcosis however, has been repeatedly demonstrated during resting dives (Ackles and Fowler, 1971; Bennett et al., 1969; Bennett and Blenkarn, 1974; Hesser et al., 1971), therefore refuting Vail's hypothesis.

There is also ample evidence to reject the increased density of air at pressure as the causative factor. For example, Bennett, Dossett and Kidd (1960) showed that elevation of the electroshock threshold (their indicator of narcosis) in rats was related to the PN_2 and not to the density of the breathing mixture. Also the fact that a Xe/O_2 mixture at 1ata is anesthetic (Morris et al., 1955) whereas air at 10 ata which has twice the density (288 vs.114), is not, tends to refute the molecular density hypothesis (Bennett, 1966). Similarly, a mixture of N_2O/O_2 can produce narcosis without a significant change in density from that of air (Bradley and Dickson, 1975) Thus, from the above examples the hypothesis that the molecular density of the breathing mixture is causative factor in the production of narcosis would appear to be adequately disproved.

In summary, therefore, the evidence would indicate that neither elevated CO_2 tissue levels, nor hypoxia are responsible for the narcosis produced by hyperbaric air. Instead the evidence suggests that it is due to the increased PN_2. CO_2 however appears capable of increasing the CNS effects of N_2 (Case and Haldane, 1941; Hesser et al., 1971; 1978).

2.1.3 Proposed Mechanism of Nitrogen's Effect on CNS Function

Classically, N_2 and the other the inert gases (Ar,Xe), were thought to act like other anesthetics (French et al., 1953), producing a general depression of CNS activity, due to blockade of nerve transmission in the reticular formation (RF), (Bennett, 1966). A review of the information on the effects of the inert gases on CNS function tended to support the proposal that they produced a depression of CNS function. There were questions however, regarding the role of depression of the RF in the production of these effects. In the following the evidence that N_2 produced a depression of CNS function will be reviewed. See Bennett (1966, 1969, 1975) for other reviews.

Early studies in frogs (Marshall and Fenn, 1950; Marshall, 1951), and mice (Carpenter, 1954), provided evidence which suggested that the inert gases acted as CNS depressants. Exposure to increased PN_2 and PAr was shown to abolish the EEG of the frog brain, and also to protect mice against electroshock induced convulsions. [NB The ability to protect against electroshock induced convulsions was considered as evidence of central depressant activity (Carpenter, 1953)]. Helium at similar pressures was ineffective indicating that these effects were due to an anesthetic action of these gases, and not to the effects of increased hydrostatic pressure.

These experiments also demonstrated the similarity of the action of the inert gases to those of traditional anesthetics. Like traditional anesthetics, transmission in peripheral nerve was much more resistant than CNS activity to the effects of the inert gases. For example, in the experiments on frogs, 54 ata N_2 abolished the EEG, whereas a

pressure of 96 ata N_2 had no effect on conduction in sciatic nerve (Marshall, 1951). Similarly, 18 ata N_2, and 12 ata Ar protected mice against electroshock, whereas 310-340 ata Ar was ineffective in blocking sciatic nerve conduction (Carpenter, 1954). The pressure of N_2 required to block axonal conduction was not determined. Also in comparison to nerve conduction, reflex activity in the frog spinal cord was extremely sensitive to the effects of the inert gases, being blocked by 17 ata N_2 and 10 ata Ar (Marshall, 1951). This differential sensitivity of spinal synaptic transmission was similar to that seen with traditional anesthetics (Carpenter, 1954), and suggested that like these agents, the inert gases produced their depressant effects by blockade of central synaptic transmission rather than axonal conduction. The finding by Carpenter (1954), that a good correlation existed between anesthetic potency and lipid solubility for the inert gases as well as for the other more common anesthetic gases, was taken as evidence that all of these agents had the same mechanism of action.

Later studies on reflex activity in the spinal cord of decerebrate cats, (Chun, 1959), and on synaptic potentials and nerve conduction in lightly anaesthetized rats (Bennett, 1963), provided direct in vivo evidence that the inert gases blocked transmission at mammalian central synapses. The latter study also confirmed the differential sensitivity of synaptic transmission, compared to axonal conduction, to the effects of inert gases. Furthermore, the sensitivty of the various components of the synaptic potentials to Ar was similar to that of other anaesthetics, providing more evidence of their apparent similarity of action.

The above experiments therefore provided fairly good evidence that the inert gases acted at central synapses to produce their effects. The Particular synapses in the CNS involved however, were not known. Studies by French et al., (1953), had shown the ability of anesthetics to depress evoked potentials in the RF and had led to their theory that anesthetics acted by producing a reversible depression of this system. Subsequent studies by Arduini and Arduini (1954) had confirmed these findings, and had also indicated the ability of ether and pentobarbital to block the EEG desynchronization produced by natural stimuli or stimulation of the RF.

Indirect evidence that the inert gases also impaired transmission through the RF was obtained shortly after by Bennett and coworkers. In experiments by Bennett and Glass (1961) it was observed that exposure to hyperbaric air prevented the EEG desynchronization (i.e the blockade of the normal alpha rhythm) normally seen in subjects at 1 ata air while working on an arithmetic problem. When He/O_2 was breathed instead of air the the EEG desynchronization returned, indicating that the increased PN_2 was responsible for its abolition. In addition, there was some suggestion of a correlation between alpha blocking and deterioration in mental performance, however, this could not be proven conclusively.

The onset of this effect appeared to vary inversely with pressure. Thus, whereas the effect could be observed at pressures as low as 2.5 ata, long times (e.g. 50 min.) were required. At 7 ata similar effects could be observed within 3 min. In fact from the data obtained, it was calculated that the time to abolition of the EEG

desynchronization was inversely proportional to the square of the pressure. Based on this it was concluded that the blockade of EEG desynchronization occurred when a critical concentration of N_2 was exceeded at synapses in the CNS. Earlier studies by Moruzzi and Magoun (1949) had indicated that the ascending reticular activating system (ARAS) was responsible for producing EEG desynchronization. Thus the ability of N_2 to block the EEG desynchronization suggested that it acted similarly to other anesthetics (French et al., 1953 ; Arduini and Arduini, 1954) in producing a depression of the ARAS.

These experiments also provided an indication that the cortex might be affected by increased PN_2, as a diminution in the amplitude of the alpha waves was observed at pressures in excess of 7 ata. Based on this, it was suggested that at these pressures, N_2, in addition to its effect on the reticular formation, might also exert a depressant action on cortical synapses.

Further evidence that the inert gases affected the RF was obtained in experiments on " flicker fusion frequency " (FFF), i.e. the frequency at which a flickering light is perceived to become steady. Exposure to hyperbaric air produced a decrease in the FFF. Like the abolition of the EEG desynchronization, the time required for this to occur was inversely proportional to the square of the pressure. Furthermore in the same individual the blockade of EEG desynchronization and the decrease of FFF occurred simultaneously (Bennett, 1958). This was taken by Bennett as further evidence that a fundamental change in CNS function occurred when a critical concentration of N_2 was exceeded at certain synapses in the CNS. Previous studies had shown that the

reticular activating system affected the FFF (Lindsley, 1958), once again suggesting the role of the RF in the changes produced by N_2 and the inert gases.

Direct evidence that the inert gases exerted depressant effects on reticular formation and at the cortex was provided by the finding that the inert gases could depress auditory evoked potentials in cats (Bennett, 1964) and in humans (Bennett et al., 1969). In the former, anesthetized cats were exposed to 12.3 ata of N_2/O_2, Ar/O_2 or He/O_2 mixtures (PO_2 = 2.3 ata), and their effects on evoked potentials recorded at the brain stem reticular formation, and cortex determined.

Both the cortical and brain stem potentials were diphasic. The cortical potential consisted of a small fast initial component followed by a high voltage slow response. The brain stem potential was more varied however, with sometimes only a single component being observed, whereas at other times a triphasic response was seen. Depression of these potentials only occurred with the N_2 and Ar mixtures. At the brain stem only the initial component of the potential was affected. Surprisingly Ar and N_2 appeared equipotent in their effects, each producing decrements of 60-70%. At the cortex, both components of the potential were affected. In contrast to the effects seen at the brain stem however, the initial component was first enhanced 10-25% by N_2 and Ar. This was, followed about 10 min. later by a progressive decline to about 35% of the control value. No initial augmentation was seen with the secondary component of the cortical potential, it being decreased in a similar manner to the initial component of the brain stem. This potential was more sensitive to the effects of Ar compared to N_2, being

depressed 80-85 % by Ar and 60-70 % by N_2. N_2 at a lower partial pressure, 5ata, exerted similar but reduced effects on these potentials.

In the human study, the effects of increased PN_2 and PHe were determined on auditory evoked potentials recorded at the vertex. In addition the effects on evoked potentials were compared with effects on arithmetic performance. Exposure to 10 ata air depressed auditory evoked potentials (by 55%), and also impaired arithmetic performance. A similar depression of the evoked potential was produced by a mixture of $N_2/H_2/O_2$ at 10 ata, in which the PN_2 was the same as that in air at 10 ata but the PO_2 remained at normoxic levels. This confirmed that the effect with air was due to N_2, and not to an increase in PO_2. Exposure to 10 ata He/O_2 (80/20) also produced a decrement in the evoked response. The magnitude of the decrement in this case was much smaller however, only 28-29%, and in addition, no decline in arithmetic performance was observed. A similar decrement was seen when pure O_2 was breathed at a pressure equivalent to that in the He/O_2 mixture, suggesting that the decrement was due to O_2.

Additionally, in the experiments with air or the N_2 mixture, there was an apparent correlation between the decline in arithmetic performance and the magnitude of the depression of the evoked potentials. This correlation appeared to provide a direct link between impairment of nerve transmission in the reticular formation and the behavioral effects of N_2. This finding strengthened the proposal that N_2 and the inert gases acted by the depression of synapses in the reticular formation, and possibly in the cortex as well.

Subsequent work by Bevan (1971) confirmed the ability of hyperbaric air to depress transmission through the RF, but questioned the ability of N_2 to exert similar effects at the cortex. In this study performed at 10 ata, hyperbaric air was found to depress the auditory evoked response, whereas the Contingent Negative Variation, supposedly an indicator of cortical transmission, was not affected.

In contrast to the evidence reviewed above which indicated that N_2 and the inert gases produced CNS depression, there was evidence to suggest however, that they were also capable of producing CNS excitation. For example, Jullien et al., (1953) reported signs of neural excitability in cats under light Dial anesthesia, exposed to 5 ata air. They reported that, "Neurons were more apt to receive impulses, and the rhythmic repetition of strychnine potentials was facilitated. Also responses at the cortex evoked by light stimuli showed an initial dimunition in the absolute refractory phase, which was later followed by an increase". Similarly, Roger et al (1955) in studies on human subjects at 10 ata air, found changes in the EEG indicative of increased excitation in the CNS. The frequency of the EEG was augmented, however the amplitude was diminished. In addition evoked potentials produced by intermittent photic stimulation were augmented, and unexpected sensory stimuli which had no effect at 1 ata tended to elicit spikes in the EEG. They stated that these changes were accompanied by "an enhancement of perceptive activities and of the global activity of the subjects, together with emotional lability, with a tendency to euphoria". The authors concluded that this state of cortical excitation might account for the syndrome of N_2 narcosis.

There were two schools of thought regarding the cause of this excitation; (i) that it was due to the increased PN_2, but was only transitory (Bennett, 1964; 1966), or (ii) that the effects were not due to N_2, but rather to the increased PO_2 (Albano et al., 1962 ; Albano and Crisuoli, 1962 ; Crisuoli and Albano, 1971). This suggestion by the latter authors was based on the different effects of 12 ata air compared to a N_2/O_2 mixture in which the PN_2 was equivalent to that in air, but the PO_2 was only 0.4 ata. Exposure to the N_2/O_2 mixture produced an impairment of memory, and clouding of the senses, along with other decrements. Air on the other hand, produced euphoria, and emotional instability in addition to other decrements. (Albano et al., 1962; Albano and Crisuoli, 1962 ; Crisuoli and Albano, 1971). In addition, the EEG at 12 ata air showed changes which were between the slowing seen with the N_2/O_2 mixture, and the signs of cortical hyperexcitability produced by O_2 at 2.5 ata. These findings therefore led these workers to conclude that O_2 was the cause of both the EEG and behavioral excitability.

Bennett (1966) however, considered that this excitation was similar to that seen e.g. during the onset of Xe anesthesia (Morris et al., 1955) or to the short-lived augmentation of the initial component of the cortical potential, observed in his experiments on cats (Bennett, 1964). He suggested that this effect could either be the signs of asphyxial depolarization, given the apparent similarity of the effects of inert gases to hypoxia (Bennett, 1963), or alternatively to disinhibition resulting from faster blockade of inhibitory synapses compared to excitatory ones, shown to occur by Chun (1959).

Subsequently, however, Bennett and Hayward (1967) suggested that hyperexcitability might be a natural function of inert gas narcosis. This proposal was based on their studies in cats which they suggested that exposure to increased PN_2, and PAr, but not PHe produced an increase in neuronal intracellular Na^+ and Cl^-. The experimental evidence in support of this is weak and as it stands, does not justify the conclusions made by the authors. This will be discussed in greater detail in the section dealing with the cellular mechanism of action of N_2 and the inert gases.

The controversy as to the cause of the excitation was not resolved however. There was some support for Bennett's claim of the transitory nature of the excitation. For example Roger et al., (1955), used the term "transitory" in their report to describe the excitation observed. Furthermore, in their report Crisuoli and Albano (1971) state that a short-lived (4-7 min.) desynchronization of the EEG occurred following compression with both the air and N_2/O_2 mixture. They attributed this to excitation of the ARAS due to hypercapnia occurring during compression. No evidence was given to support this however. This statement however, helps to clear up the controversy, since it would appear that Crisuoli and Albano were not trying to explain the transient excitation produced by hyperbaric air described by Roger et al., (1955), but rather the long lasting hyperexcitability changes.

In addition to this evidence of increased CNS excitation following exposure to hyperbaric air, the role of depression of the RF in the production of narcosis was not as clear as it initially seemed. Experiments by Ackles and Fowler (1971) contradicted the earlier finding

by Bennett et al., (1969) of a correlation between depression of evoked potentials and a decline in arithmetic performance. These workers found that whereas an Ar/O_2 mixture (80/20), was much more potent than air at equivalent pressures, in disrupting arithmetic performance, no such difference in potency was found in their effects on visual or auditory evoked responses. Furthermore as would be expected from the above statement, analysis of the data showed no correlation between the depression of the evoked response and arithmetic performance. They also claimed that the correlation obtained by Bennett et al., (1969) was erroneous since it was calculated using mean values for the depression of evoked potentials and arithmetic performance, rather than the individual values. They also stated that a reanalysis of the data using the individual values for each subject revealed no significant correlation. The finding that the visual evoked response was similarly reduced by hyperbaric air, did confirm however, that the effect on the auditory potential was indeed due to an effect on the CNS, and not to peripheral attenuation of the auditory stimulus.

Other experiments (Schreiner, 1971) were cited by Bennett (1975) to support the correlation between depression of evoked potentials and behavioral correlates of narcosis. The data presented does not allow a critical evaluation of this however. Furthermore it appears that this correlation would be based on < 3 subjects, therefore its validity is questionable. Thus although the evidence suggested that depression of evoked potentials was due to an "anesthetic" effect since similar depressions were not seen with He (Bennett, 1969), the significance of this effect was unclear.

In summary therefore, the evidence seemed to indicate that increased PN_2 exerted primarily a depressant effect on CNS function, although the mechanism by which this occurred was unclear. The evidence of CNS excitation was difficult to reconcile with the apparently strong evidence of CNS depression, namely the abolition of the frog EEG, the anticonvulsant effect in mice, and the depression of evoked potentials. This excitation seemed best explained by Bennett's proposal that it represented the transient excitatory phase normally seen during the onset of anesthesia.

Cellular Mechanism Of Action

The mechanism by which N_2 exerts it's effects at the cellular or membrane level is poorly understood. Over the years many theories have been proposed. These included a histotoxic hypoxia, depression of metabolism, cell membrane stabilization causing a block in ion permeability, a transient increase in permeability to cation, among others, see Bennett (1966, 1969, 1975) for a review of these and other theories. Many of these theories have not been experimentally tested, and are no longer seriously considered (Bennett, 1975). Studies however, have clearly shown that the inert gases do not depress the oxidative metabolism of nerve tissue (Pittinger et al., 1951; Levy and Featherstone, 1954; Carpenter, 1956), thereby ruling out this as the mechanism by which N_2 produces it's effects. Thus, it would appear that N_2 and the inert gases act similarly to the more common anesthetics by disrupting some aspect of membrane function (Seeman, 1972), although the

actual mechanism remains elusive.

At the time of commencing this thesis, it was proposed that N_2 and the inert gases acted by producing a transient increase in permeability to cation (Bennett, 1975). The evidence in favor of this was not very strong however. This theory was based on the results of studies in cats which showed that exposure to Ar/O_2 or N_2/O_2 mixtures produced a significant depression of the cortical auditory evoked response, used as the indicator of narcosis, as well as a decrease in the concentration of Na^+ and Cl^- ions in the cerebral spinal fluid (CSF) (Bennett and Hayward, 1967). Similar exposure to the "non-narcotic" He/O_2 however, did not decrease the auditory evoked response or reduce the concentration of ions in the CSF. The existence of similar permeability changes following exposure to hyperbaric air or inert gases, in blood (Barthelemy, 1963), urine (Radomski and Bennett, 1971) and in transport across frog skin (Gottleib et al., 1968), as well as the increase in cation permeability of model systems produced by anesthetics (Bangham et al., 1965), were cited in support of this (Bennett, 1975). The ability of the cationic detergents stearylamine and cetyltrimethyl ammonium bromide, to prevent the occurrence of inert gas narcosis (measured by depression of evoked potentials) in rats (Bennett and Dossett, 1970), also seemed to confirm this theory. These drugs were also capable of protecting against carbon tetrachloride induced liver necrosis (Bangham et al.,1962), and were thought to act by reducing membrane permeability to cation. The anionic detergents, sodium hexadecyl sulphate and sodium duodecyl sulphate, which did not prevent liver necrosis also did not prevent the depression of the evoked potentials by the inert gases.

In the initial study in cats however, there was no correlation between the degree of narcosis and the magnitude of the permeability change (Bennett and Hayward, 1967). For example the Ar mixture decreased the potentials by 44.7 % compared to 18.6 % for the N_2 mixture. With the N_2 mixture however, the decline in the Na^+ and Cl^- concentrations was 14.2 and 9.6 M.eq. respectively, compared to a fall of 11.2 and 6.0 for the Ar mixture. Thus despite the fact that the mean depression of the potentials by Ar was twice that of N_2, the mean changes in permeability were greater with N_2. Furthermore in studies on liquid crystals, Bennett et al., (1967) could not reliably obtain permeability changes following exposure to the "narcotic" inert gases.

The evidence provided in support of this theory is also weak. It is not clear the extent to which one can extrapolate changes in the ionic composition of blood, and urine, to neurons in the CNS. Furthermore the permeability changes produced by the n- alcohols in the study by Bangham et al., (1965) do not correlate very well with their synaptic blocking potencies. The ability of the cationic drugs to prevent the depression of evoked potentials is interesting (Bennett and Dossett, 1970). If these drugs do act by reducing permeability to cation, this would provide strong support for the increase in permeability theory, despite the lack of other hard evidence. It is therefore unfortunate that in this experiment, the effectiveness of these drugs on permeability changes were not concurrently measured. This would have provided evidence to accept or reject this theory.

2.2 Effects of Changes in the Other Environmental Factors on CNS Function

2.2.1 Oxygen

It is not clear to what extent the changes in PO_2 occurring in the air diving range are important with regard to modification of drug effects. Oxygen at pressures in excess of 2-3 ata have been shown to produce toxic effects in both animals and man (Donald, 1947a,b; Lambertsen, 1978; Clark, 1982). The primary manifestations of this are CNS excitation (convulsions), and pulmonary damage. At the pressures of air used in the experiments in this thesis, and at those in the sport diving range, the maximum PO_2's attained are < 1.5 ata. These values are below those required for O_2 toxicity (Clark and Lambertsen, 1971), therefore, the CNS excitation and other changes associated with O_2 toxicity would not be expected to occur. There is evidence from both human and animal studies however, to suggest that small increases in PO_2 (< 2 ata), can produce changes in CNS function and therefore may be important with regard to modification of drug effects.

For example in man, PO_2's of 1.6 and 1.7 ata respectively, have been shown to decrease standing steadiness (Adolfson et al., 1972), and impair arithmetic performance (Hesser et al., 1978). In addition, studies in man (Frankenhauser et al., 1963), rats (Thomas, 1974; Thomas et al., 1976) and insects (Fenn, 1965) have demonstrated that increased PO_2 is capable of potentiating the effects of N_2.

Frankenhauser et al., (1963) compared the effect of exposure to a fixed PN_2 (approx. 3.9 ata) while varying the PO_2, on the performance of 12 subjects on psychomotor tests. The tests used were simple and choice reaction time, and a mirror drawing test. The PO_2's studied were 0.2, 1.03 and 2.60 ata. Exposure to the 2.60 ata PO_2 mixture produced a significant increase in simple reaction time compared to 1 ata air. No significant increase was seen with the other mixtures however. In addition the increase in reaction time was also significantly different from the normoxic mixture. The authors also reported a synergistic effect between the effects of N_2 and O_2 on the mirror drawing test. This apparently is based on the finding that increasing the PO_2 produced an increase in the speed at which the test was performed. This is misleading however, as it does not take into account the number of mistakes made on the test. When this is done, it is seen that at the lowest PO_2 (0.2 ata) the subjects had the same error rate as at the 2.60 ata mixture even though they took longer to do the test. This suggests that in fact the greatest disruption of performance occurred with the lowest PO_2 mixture. Whether this finding indicates that the potentiation of N_2 by O_2 will not occur on all aspects of CNS function is not known.

In animal studies, the interaction between N_2 and O_2 has been studied over a wide range of PN_2, in rats performing on differential reinforcement of low rate (DRL), and fixed interval (FI), schedules (Thomas, 1974; Thomas et al., 1976). Increasing the PN_2 resulted in an increased rate of responding on the DRL task (Thomas, 1974). At the higher PN_2 (7.2 and 8.8 ata) tested increasing the PO_2 above 0.8 ata resulted in further increases in the rate of responding. No such effect

was seen at the lower PN_2's (4.0 and 5.6 ata) however, even at PO_2's as high as 1.4 ata. In the study on FI responding, the PN_2 was varied from 0.8 to 10.4 ata in 2.4 ata steps, and the PO_2 from 0.2 to 2.2 ata in 1.0 ata steps. As in the DRL task, increasing the PN_2 alone resulted in increased response rates and also decreased the "index of curvature" (a measure of the temporal distribution of responding). No interaction between O_2 and N_2 was seen at a PO_2 of 1.2 ata, even at the maximum PN_2 of 10.6 ata. However, when the PO_2 was raised to 2.02 ata, an enhancement of N_2's effect on response rate and "index of curvature" was observed at a PN_2 as low as 3.2 ata. The greatest disruption of responding at this PO_2 occurred at the highest PN_2. These results of these experiments appear to suggest therefore, that the interaction between N_2 and O_2 is greater the higher the PN_2, and that the value of the PO_2 may determine the minimum PN_2 at which an interaction is seen.

Finally, the experiments on Drosophila (Fenn, 1965) indicated the marked lethality to these insects of a combination of elevated PN_2 and PO_2. Thus whereas exposure to a normoxic mixture of 30 ata N_2 resulted in a survival time in excess of 2 days, increasing the PO_2 to 1.0 ata reduced survival to 5 hr. This was not due to an effect of the increased PO_2 alone since the survival time in pure O_2 at 1 ata was 1 week. Similar effects were seen with Ar but not with He, confirming that the effect was not a pressure effect.

At present, the mechanism by which O_2 produces these effects is not known. Some workers suggest that the synergistic effect of increased PO_2 is in fact due to an increase in pCO_2 caused by impaired removal of CO_2 from the tissues (Frankenhaeuser et al., 1963; Thomas et al.,

1976). Studies by Smith and Payton (1976) however, have demonstrated the ability of increased O_2 to produce anesthesia in mice, provided the convulsions are blocked by pretreatment with an anticonvulsant. This finding, coupled with the effects in Drosophila (Fenn, 1965), would suggest that the effects of O_2 may actually be direct effects independent of any increase in CO_2.

2.2.2 Carbon Dioxide

As discussed previously, increases in pCO_2 enhance the narcosis produced by N_2 (Case and Haldane; 1941; Hesser et al., 1973; Hesser et al., 1978). Therefore, an increase in pCO_2 in the CNS during exposure to hyperbaric air may be important in regard to modification of drug effects. In addition, it is conceivable that a sustained increase in pCO_2 could alter the pH of blood and CSF, and thereby alter the effect of a drug by changing its degree of ionization. The importance of this effect, however, in sport diving or in the experiments reported in this thesis is not clear. In these cases the exposures are of short duration, and the changes in pCO_2 would not be expected to be great enough to significantly alter blood or CSF pH for any sustained length of time. In contrast to this, however, large increases in pCO_2 due to hypoventilation have been observed in some divers under these conditions (Morrison and Reimers, 1982).

The extent to which CO_2 retention occurs during hyperbaric air is primarily a function of the ambient pressure (Vail, 1971; Lamphier, 1975). However, the activity level of the subject is also important. Studies in man have shown that at rest or during light exercise no

significant CO_2 retention occurs, but quickly becomes a problem at heavy work loads (Lanphier, 1963; Lanphier and Busby, 1962; Fagraeus and Linnarsson, 1973).

To my knowledge, there is no information on pCO_2 changes in animals during short exposure to hyperbaric air. Some data are available in anesthetized animals (Bean, 1950; Bennett, 1965), however the applicability of these findings to unanesthetized animals is questionable. By extrapolation from man however, one could also assume that the activity level of an animal would determine the amount of CO_2 retention occurring at a particular pressure. Whether an increase in activity will have as great an effect on CO_2 retention in animals as it does in man however is not known.

Thus, in summary, increased pCO_2, because it enhances N_2 narcosis would be expected to worsen the interaction between a drug and hyperbaric air. This effect would (i) depend on the activity level of the subject and (ii) be more important at higher pressure.

2.2.3 Increased Hydrostatic Pressure

There is ample evidence demonstrating the effects of increased hydrostatic pressure on CNS function, (Brauer, 1975 ; Hunter and Bennett, 1974). For example, exposure to high (He) hydrostatic pressures has been shown to produce behavioral changes in both animals and man (Brauer, 1975 ; Hunter and Bennett, 1974) and also to affect a wide variety of cellular processes (MacDonald, 1975). The behavioral syndrome in man and animals has been termed the High Pressure Nervous Syndrome (HPNS), and consists of tremor, convulsions, and impaired

motor- coordination, among other things (Brauer, 1975; Hunter and Bennett, 1974). The pressures encountered in air diving range are below the values (10-25 ata), required to produce the above effects (Brauer, 1975 ; Brauer et al., 1982 ; Hunter and Bennett, 1974). Exposure to He/O_2 in the 7 ata range have been shown to produce very minor disruption of performance in both humans (Behnke and Yarbrough, 1939), and rats (Thomas, 1973a). It has not been clearly estsblished however, that these changes are due to hydrostatic pressure, rather than stress, or the effect of the increased PO_2. In any case they are minimal compared to the effects produced by similar pressures of N_2. It is not expected therefore that the increase in nydrostatic pressure would be a significant factor in the interaction of drugs with hyperbaric air.

2.2.4 Alterations in the Pharmacokinetics of Drugs

At the time of commencing this thesis, there was no information on the pharmacokinetics of drugs at increased pressures of air. Some work had been done with He/O_2 in which it was shown that exposure to 11 ata He/O_2 increased the metabolism of morphine in mice (Tofano and DeBoer, 1976). In this same study 11 ata He/O_2 was also reported to produce diuresis in rats. There were no reports of experiments at lower pressures of He/O_2.

2.3 Summary

In summary therefore, the evidence suggested that the increased PN_2 was the most important factor in the hyperbaric air environment in regard to modification of the effects of centrally acting drugs. In this pressure range, the effects of the increased PO_2 and hydrostatic pressure were considered to be minor compared to those produced by N_2. In addition, there was no evidence to indicate whether drug pharmacokinetics would be altered by exposure to hyperbaric air in this pressure range. Finally as N_2 appeared to produce a generalized depression of CNS activity, this suggested that exposure to hyperbaric air would tend to enhance the effects of depressants and reduce those of stimulants.

CHAPTER 3

ANIMAL STUDIES

3.1 Introduction

As discussed in the previous chapter, the evidence suggested that hyperbaric air produced a depression of CNS activity. Based on this therefore, it would be predicted that the effects of CNS depressants would be enhanced by exposure to hyperbaric air, and those of stimulants reduced. At the time work on this thesis began, there was very little data to verify or refute this prediction, with only a few studies on this topic having been performed. In fact these studies provided both conflicting and supporting results.

Thomas (1973b), examined the effects of chlordiazepoxide, and amphetamine, at 1 and 8.6 ata on the performance of two rats on differential reinforcement of low rate, (DRL), and fixed ratio (FR) schedules. Doses used were 1.0 to 20 mg/kg for chlordiazepoxide, and 0.5 to 2.5 mg/kg for amphetamine. At the surface both drugs disrupted performance, the general effect observed being a tendency to increased rates of responding at lower doses of the drugs, followed by a decline from these elevated values at the higher doses. Exposure to hyperbaric air did modify the effects of the drugs in that their dose effect

functions were different from those at 1 ata. The results were very difficult to interpret however, given the small number of animals, the biphasic effect of the drugs on response rate, and the fact that hyperbaric air alone affected responding. The higher doses of the drugs at pressure however, were associated with marked decreases in responding, suggesting a synergistic effect between the drugs at these doses, and hyperbaric air on performance. In subsequent studies however, which provided clearer data, exposure to hyperbaric air was shown to enhance not reduce the effect of amphetamine on schedule controlled responding (Walsh, 1974 ; Thomas, 1976). Thus on performance on a DRL schedule, it was found that the combination of amphetamine and 7.1 ata air, produced a greater disruption of performance than that seen with either agent alone (Walsh, 1974). Similarly, on performance on a progressive ratio schedule, exposure to 7.1 ata appeared to shift the dose effect function to the left. At pressure, the maximum number of reinforcements were obtained at lower doses than at the surface, and doses which produced optimal performance at 1 ata were associated with a decline in reinforcements at depth. The results of these experiments were therefore at odds with the classical theory of inert gas narcosis, which would have predicted an antagonism between the effects of amphetamine and hyperbaric air on performance.

In the other studies on the effects of drugs at high pressure (Hart, 1974a,b), a different approach to that in the studies reported above was used. In these studies, the ability of hyperbaric air to modify drug-induced behaviors or physiological effects was examined. Hart (1974a), showed that exposure to hyperbaric air enhanced the anesthetic effect of pentobarbital in mice, awakening times following

administration of pentobarbital (50 and 75 mg/kg) being significantly increased at 2, 4, 6 and 8 ata, compared to 1 ata. No enhancement was observed at 100 mg/kg the other dose tested. In contrast, a similar enhancement was not seen in rats, cats or guinea pigs. In the same study, exposure to 6 ata air was shown to significantly increase the LD_{50} for picrotoxin but not caffeine, in mice. This protective effect seemed to be due to the increased PO_2 and not the PN_2 however, for if the PO_2 was maintained at normoxic levels, no protective effect was seen even though the PN_2 was increasd. Hart (1974b), also examined the effect of hyperbaric air on the antipyretic effect of salicylate in rats. No change in the antipyretic effect was observed at pressures up to 9 ata.

In contrast to the results from the operant studies, the prolongation of the effect of pentobarbital in mice was in keeping with the theory that increased PN_2 produced a general depression of CNS activity. There were some inconsistencies however. For example, the failure to detect this effect in rats, guinea pigs, or cats. Furthermore, in the experiments with mice, the effect did not show a consistent dose response relationship with regard to the PN_2, as in a few cases lower PN_2's produced greater prolongation of awakening times than higher values. This finding was inconsistent with the view that the central depressant effect of N_2 increased with increasing pressure (Bennett, 1975).

Three other studies were reported prior to 1977 in which drugs had been used at pressure (Bennett, 1962; 1963a,b). The focus of these experiments was different however, as they were designed to find drugs

which would be capable of preventing inert gas narcosis. One of these studies however, (Bennett, 1962), still provided some information regarding the interaction of drugs with hyperbaric air. In this study the ability of a wide variety of drugs to antagonize the N_2 induced elevation of the electroshock threshold in rats was determined. Surprisingly, stimulants such as methamphetamine and metrazol were found to enhance not reduce this effect of N_2. This was a somewhat similar finding to those of Thomas (1973b , 1976) and Walsh (1974) with amphetamine. The relevance of the findings on electroshock to N_2 narcosis and the interaction of drugs with hyperbaric air was questioned however, by the fact that carbachol, a drug which should not cross the blood brain barrier, was extremely effective in preventing the elevation of the threshold produced by N_2.

Thus the studies on the effects of drugs at increased pressures of air prior to 1977 did indicate that exposure to hyperbaric air could modify the effects of centrally acting drugs. These studies provided very little to go on however, due to their small number, and the apparent incosistencies, particularly in the studies with amphetamine, which were unexplained. Therefore, given the lack of information on the effects of drugs at increased pressures of air, it was decided that as the first step the effects of a number of different drugs would be determined at increased pressures of air to provide a pool of data on this topic. The information obtained from these studies could then be used to formulate more specific experiments to aid in elucidating the nature of the changes observed.

The approach taken to the study of drugs at pressure was the same as that used by Hart(1974a), i.e. to examine the ability of hyperbaric air to modify drug-induced behavior. This approach was chosen over examining the effects of drugs at pressure on performance of a learned task, since it was thought that this approach would simplify the interpretation of the results. With performance tests such as operant responding for example, it might be difficult to determine if the decrements in performance are due to a deficit in the learned response, or to an interaction of hyperbaric air with other behavioral effects of the drug (Rapp and Robbins, 1976). In addition, the mechanisms by which drugs exerted their behavioral effects were generally more widely studied. Therefore potentially more information was available to allow speculation on the mechanism of any changes in drug effects at pressure.

Drugs Studied

Selection of drugs was made so as to cover a wide variety of drugs which would be of practical interest, (i.e. with respect to use by divers), as well as of theoretical interest. Initial studies were performed with pentobarbital and amphetamine, drugs of theoretical interest as prototype depressants and stimulants respectively, of CNS activity (Harvey, 1975 ; Innes and Nickerson, 1975). Amphetamine in particular was chosen because it is a drug which has been widely studied and a great deal is known about the mechanism by which it produces some of its behavioral changes, such as locomotor activity and stereotyped behavior (Kelly, 1977). Thus a study of the effect of hyperbaric air on these behaviors could act as a point of departure for more detailed studies to elucidate some of the changes occurring in the CNS during

exposure to hyperbaric air. Amphetamine was also of practical interest because it is an indirect acting sympathomimetic, similar to drugs which may be used in nasal decongestants, and anti-motion/sickness compounds (Wood and Graybiel, 1972). Subsequent studies examined the effects of morphine, diphenhydramine (DPH), and alcohol, which are of practical relevance to the diving situation.

Morphine was chosen because it is widely used as an analgesic and it is possible that it could conceivably be used under hyperbaric condtions to ease the pain resulting from trauma or severe Type I, (pain only) decompression sickness. Theoretically it is of interest because morphine produces its effects through an effect on opiate receptors (Jaffe and Martin, 1975), and at the time there was some evidence suggesting a role of endogenous opiates in the production of anesthesia (Fink et al., 1977).

DPH was chosen as a repesentative of the antihistamine class of compounds which are frequently used in cough and cold remedies, and antimotion sickness preparations. DPH in particular was chosen because of its strong sedative (CNS depressant) properties (Douglas, 1975).

Alcohol was chosen because it enjoys wide social use and is known to produce significant CNS dysfunction (Ritchie, 1975).

The parameters chosen for study for the drugs were as follows: (i) pentobarbital:- sleep time; (ii) amphetamine:- convulsive activity, stereotyped behavior, locomotor activity; (iii) morphine, DPH, alcohol:- locomotor activity.

With the exception of amphetamine, two doses of each drug were studied, one having minimal, and the other marked effects on the parameter studied. Amphetamine because of its multiple effects on activity was studied in greater detail.

3.2 General Methodology

Subjects

Experimental animals were male, Swiss mice, 25-30 g. They were housed individually in cages and maintained on a light/ dark schedule (12/12) which generally coincided with normal day/night. The mice used in the studies with DPH were maintained on a reversed light/dark schedule (10/14) to permit more efficient scheduling of experiments. Food and water was provided ad libitum. At least one week was allowed for the mice to acclimatize to their new environment. Two or three days prior to entering an experiment, the mice were myringotomized under ether anesthesia using a fine but blunt probe. The total proceedure from exposure to anesthesia to myringotomy lasted less than 4 min. Destruction of the tympani permits rapid equalization of pressure within the inner ear and sinuses and reduces the ear discomfort that normally occurs during compression, which could conceivably have influenced the results. Mice which displayed post-operative problems such as excessive weight loss, discharge of fluid from the ear, or disturbances in gait were excluded from the experiments. The occurrence of this was extremely rare, with only 4 mice in the entire series of experiments being so discarded.

Experimental Design

The experiments were performed at 1, 4, and 7 ata air to determine the dose response relationship to N_2. To determine if an effect seen with hyperbaric air was in fact due to the increased PN_2, rather than to the concurrent increases in PO_2, or hydrostatic pressure, experiments were performed at the appropriate pressure with a He/O_2 (80/20) mixture. In this case the PO_2 and hydrostatic pressure are the same as at the equivalent pressure of air, the only difference being that the "narcotic" gas N_2 is replaced by the "non-narcotic" gas He. Therefore if a similar effect was not seen, this would suggest that the effect seen with air was due to N_2. In some cases, experiments were also performed with a N_2/O_2 mixture, (95/5) to further clarify this. This does two things. Firstly it allows a test of the effect at higher PN_2's than are possible with air, and therefore provides a means of extending the dose response relationship. Secondly, it provides a test of the importance of O_2 in the effect seen since it allows the PN_2 to be raised while maintaining the PO_2 at near normoxic levels. Thus for example, the PO_2 with this mixture at 7ata would be only 0.35 ata, compared to a value approaching 1.5 ata for air. The experiments with air and N_2/O_2 were conducted at $24 \pm 1^{\circ}$ C. Those with He/O_2 were performed at $31 \pm 1^{\circ}$ C to counteract the hypothermic effect resulting from its high thermal conductivity (Brauer, et al., 1977).

The effects of exposure to hyperbaric air on the different doses of a drug were tested in separate experiments. A mixed randomized block, factorial design was used, with each new batch of mice constituting a block (Goldstein, 1964). The order of exposure to the various

pressure/gas mixture combinations was counterbalanced as far as possible across the batches of mice tested. Assignment of mice into groups for testing was done using a table of random numbers (Goldstein, 1964).

Statistical Analysis

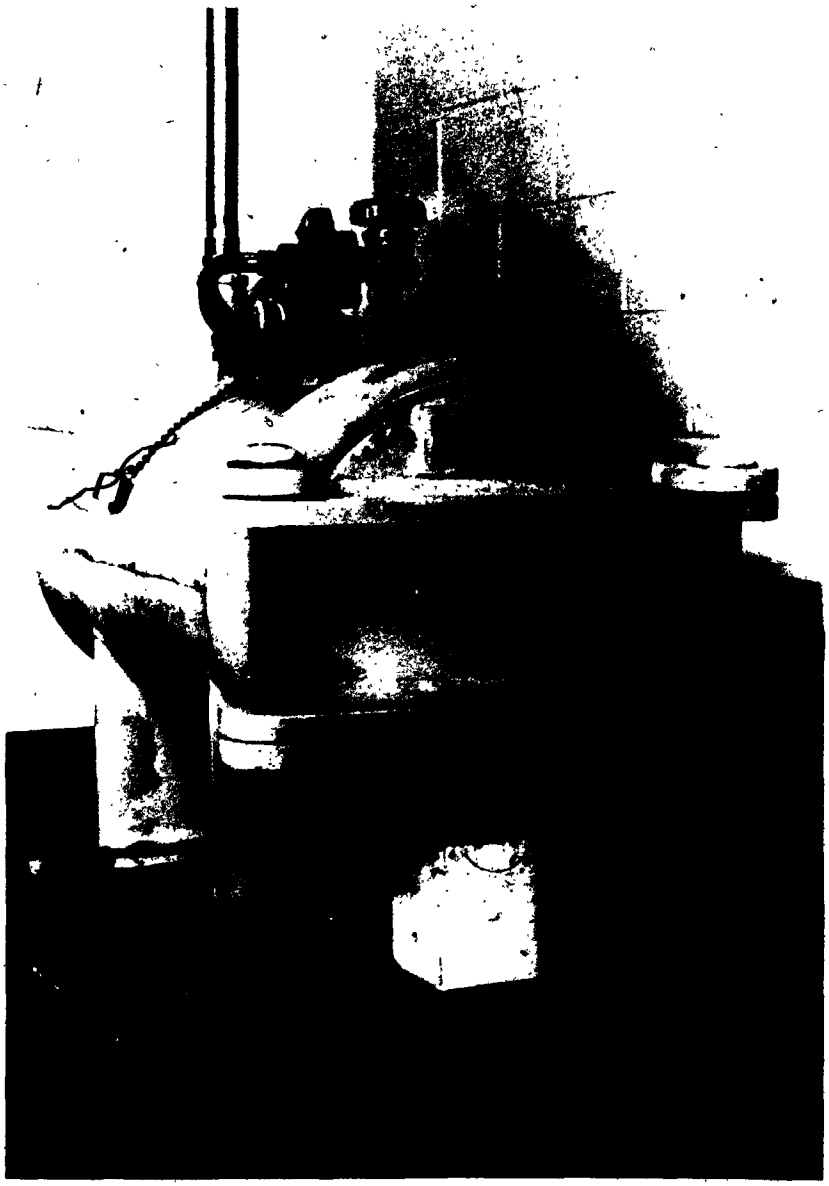
Unless otherwise specified, analysis of variance (Winer, 1971), was used to determine statistical significance. If a statistically significant effect was obtained, this was followed by the Student Newman-Keuls test (Winer, 1971), to determine the differences between individual treatments. Statistical significance was set at the 5% level.

Hyperbaric Chamber

Experiments were conducted in a small animal hyperbaric chamber (Dominion Welding Engineering Ltd., Montreal) measuring 75 cm in length and 34 cm internal diameter. The chamber was outfitted with a glass viewing-port, and outlets for electrical connections, Figure (1). The temperature inside the chamber was controlled using an external system of copper coils which encircled the chamber, and through which hot water or coolant could be circulated. This type of system does not permit rapid temperature control, however, once the desired temperature is reached, very little fluctuation in temperature occurs due to the large mass of the chamber. A YSI temperature probe (No: 15-176-28) and telethermometer (Model 42SC) were used to monitor the temperature in the chamber. During each experiment, trays containing Baralyme, and Drie-rite were placed in the chamber to absorb CO_2 and water vapour

Figure 1

Photograph of hyperbaric chamber.



respectively. The chamber was also vented continuously throughout each experiment. The gases used in the experiments were supplied from commercial gas cylinders. Illumination of the chamber was provided by an external light source positioned at the viewing port.

Compression Procedure

Compression was conducted at the rate of 1 ata/min., with stops at approximately 1.5 ata (2 min.), and also at 5 ata (1 min.) for those experiments performed at 7 ata. These stops were made to further facilitate equalization of pressure within the inner ear and sinuses.

Compression was always associated with a short-lived rise in temperature. The extent of this rise could be controlled to about 4 to 5° C however, by a rapid venting of the chamber at appropriate times during compression. Also, by maintaining the temperature inside the chamber about 2° C below the mean experimental temperature prior to starting an experiment, the temperature rise was effectively reduced to only 2 to 3° C. After commencing an experiment at 1 ata, the chamber was repeatedly pressurized to approximately 1.5 ata then vented, over a 5min. period. This was done to simulate the noise produced by pressurization of the chamber in the experiments at 4 and 7 ata in an attempt to control for any effects that this might have on the behaviour of the mice.

Measurement of Activity and Behavioural Observations

The activity of the mice was measured using specially designed boxes, and a sensor developed in our laboratory, see Appendix I . In addition, in order to provide more information as to the type of activity the mice were engaged in, and also to provide a check on the accuracy of the sensing device, behavioural observations were made at selected times throughout each experiment using a time sampling behavioural categorization technique (Bindra and Spinner 1958; Wincour et al., 1969; . Taylor et al., 1973). Briefly, the procedure is as follows. At the time of observation, each mouse is observed for a specified period of time, e.g. 1 min, and at fixed intervals during that period a record of its behaviour made using the appropriate selection from a list of preset categories. The categories used in these experiments are shown in Table (1). In these experiments, a record of behavior was made at 2 sec intervals during the observation period.

The categories are designed so that they are mutually exclusive, which means that an activity which involves behaviours from more than one category has to be recorded as belonging to only one of the categories. In such cases, the category to which the activity is assigned is the one which is higher in the hierarchy. For example, if a mouse is observed to be rearing and sniffing at the same time, the activity will be recorded as rearing. Similarly, if a mouse is observed to be walking and sniffing, walking is recorded. The justification for this hierarchy stems from the fact that sniffing is frequently associated with rearing and walking (Bindra and Spinner, 1958). For

TABLE 1

CATEGORY	DESCRIPTION	SUBDIVISION	RECORD
Gnawing	Biting wall or floor of cage	Wall Floor	BW Bf
Rearing	Front limbs off floor with hind limbs extended	Free At a Wall	R RW
Walking	Movements involving four limbs	None	W
Sniffing	Head movements with rear limbs immobile, twitching of vibrissae	Floor Wall Air	Sf Sw Sa
Grooming	Scratching, biting or licking of coat	With Head With Forelegs With Hindlegs	M FL HL
Immobile	Freezing, total inactivity	None	I
Miscellaneous	Eating feces and other infrequent behaviour	None	X

NB: Some of the categories have been subdivided so that a more accurate description of the activity can be obtained, enabling one to make a more detailed analysis of a compound's effect on a particular category, if necessary.

each observation period the totals for each category are calculated, thereby providing information on the different activities that the animal was engaged in, and their frequency. This technique has the advantage over other forms of recording behavior such as rating procedures in that it provides not only qualitative but also quantitative information about the behaviour of an animal (Taylor et al., 1974). The latter facilitates statistical analysis of the data. The categories used in these experiments were further subdivided to provide more detailed information on the behaviour of the mice in the event that this might prove useful in analyzing the results.

In the studies reported in this thesis, the data from the behavioral observations was not statistically analyzed. Data from the walking and rearing categories are presented however, to support the activity data obtained from the sensor.

3.3.1 STUDIES ON AMPHETAMINE

3.3.1.1 Introduction

The effects of hyperbaric air on amphetamine induced "convulsive activity", stereotyped behavior, and locomotor activity were studied. These are characteristic, well defined, dose-dependent effects, with stimulation of locomotor activity occuring at low doses (1-5 mg/kg) (Maickel, et al., 1974 ; Villareal et al., 1973), followed by a switch over to stereotyped behavior at moderate doses (> 5-6 mg/kg), (Randrup and Munkvad, 1967) and high doses (> 15mg/kg) triggering convulsions and death (Gardocki et al., 1966 ; George and Wolf, 1966). A study of these behaviors therefore, would allow the effects of hyperbaric air on the CNS stimulation produced by amphetamine over a wide dose range. Also as stated earlier, there was strong evidence to suggest that the locomotor activity and stereotyped behavior produced by amphetamine, were critically dependent on different sites in the CNS for their production, the nucleus accumbens (NAc) for locomotor activity, and the striatum for stereotyped behavior (Kelly et al., 1975). Thus it was thought that in addition, a study of these behaviors would provide some information as to how different areas in the CNS might be affected by hyperbaric air.

3.1.1.2 METHODS

General

D-amphetamine sulphate (Dexedrine, Smith, Kline and French) was used. It was dissolved in saline and injected (i.p.) in a volume of 0.1 ml/10g body weight. Experiments were conducted 9 hr (±30 min) into the light cycle.

Convulsive Activity

Mice were tested in kinetographs (K020, Canadian Scientific). The kinetograph Fig.(2) consists of a plexiglass box (11.5 x 5 X 6 cm) attached to a flexible metal blade on which a movement sensitive crystal is mounted. Movement of the mouse in the box is transferred via the metal blade to the crystal, resulting in a voltage output. The output from the kinetograph was fed into a Grass Model 7 Polygraph to obtain a record of activity. The sensitivity of the Grass recorder was adjusted and the vibration of the box dampened so that the recording produced by violent movement of the mouse in the box, such as that occurring during "convulsive activity", was distinguishable from that produced by other movements such as sniffing, grooming or gnawing.

Two mice were tested at one time. Following a 30 min acclimatization period in the chamber, the mice were injected with amphetamine 30 mg/kg and exposed to the appropriate pressure/gas mixture combination. Recording from the kinetographs commenced 5 min after injection and was continued for a period of 3 hr. Periods of convulsive

Figure 2

Photograph of kinetograph.

activity were noted on the chart, as well as any deaths that occurred. In addition a brief record of the behavior of the mice was made at 15 and 20 min following injection, and thereafter at 10 min intervals for the first 90 min. Following this, observations were made every 15 min. At the end of the experiment, surviving mice were placed in individual cages, with food and water and kept for a further 24 hr. The number of mice dying during this period was also recorded.

Experiments were conducted at 1, 4 and 7 ata air, and at 7 ata He/O_2. Six runs each were performed at 1 and 7 ata air, and four each at 4ata air and 7ata He/O_2, giving a total of 12 mice at each of the former pressures, and 8 mice at each of the latter pressures.

Locomotor Activity

Studies were performed at 3, 4 and 5 mg/kg. The initial studies were performed at 3 mg/kg as this was found to produce a significant increase in activity, but was far enough removed from the dose (approx. 6 mg/kg), at which stereotyped behavior started to occur. Based on the finding that exposure to hyperbaric air enhanced the effect of this dose of amphetamine, additional experiments were conducted at 4 and 5 mg/kg to examine this effect in more detail. In particular, given the fact that the locomotor activity and stereotyped behavior appeared to be mediated through different sites in the CNS (Kelly et al., 1975), it was of interest to determine if exposure to hyperbaric air would precipitate the switch over from locomotor activity to stereotyped behavior at a lower dose.

Prior to testing, the mice were acclimatized to the test boxes and experimental procedure. The acclimatization period was as follows. On the day prior to testing, the mice were placed in the test boxes for 1.5 hr in the laboratory, followed by a saline injection and a further 1 hr acclimatization period in the chamber. At the commencement of the period in the chamber the chamber was repeatedly pressurized and vented over a 5 min period to simulate the noise during compression. On the test day the same procedure was followed. However, upon completion of the acclimatization period in the chamber, the mice were injected with the appropriate dose of amphetamine, and the experiment started.

Activity counts were recorded at 5 min intervals commencing 10 min after injection. Behavioral observations were made at 15 and 20 min after injection, and thereafter at 10 min intervals for the remainder of the experiment. The length of an experiment was adjusted to the duration of action of the dose given. Experiments at 3 mg/kg were of 90 min duration and those at 4 and 5 mg/kg of 120 min duration. Experiments were conducted at 1, 4, and 7 ata air. Three mice were tested at one time and three runs were performed at each pressure thereby giving a total of 9 animals per treatment group. In the initial experiments, i.e. at the 3 mg/kg dose, experiments were also conducted with He/O_2 to determine if the effect produced by hyperbaric air was due to N_2. Two runs with He/O_2 were performed giving a total of 6 mice at this pressure.

Stereotyped behavior

The stereotyped behavior produced by amphetamine in mice and rats consists of a syndrome of sniffing, licking, gnawing and biting (Randrup and Munkvad, 1967 ; Kelly, 1977). At lower doses sniffing predominates, however as the dose of amphetamine is increased licking, gnawing and biting are more frequent and at higher doses these behaviors completely dominate behavior (Randrup and Munkvad, 1967 ; Costall et al., 1977). The initial experiments were performed using 10 mg/kg as this produced a rapid onset, well defined stereotyped response manifested by continuous licking/gnawing at the sides of the cage. Experiments were also performed at 8 mg/kg which produced a less intense response to look for a dose response effect.

The procedure followed was identical to that used in the experiments on locomotor activity, with the exception that locomotor activity was not monitored, and a new category licking/gnawing/biting (L), was added to the existing list. Licking, biting and gnawing behaviors appear to be manifestations of the same effect of amphetamine, and in the analysis of stereotyped behavior no distinction is made between them (Kelly et al., 1975 ; Costall et al., 1977).

Experiments were conducted at 1, 4 and 7 ata air. Three mice were tested at one time, and three runs were performed at each pressure giving a total of 9 mice per treatment group. Experiments were of 2 hr duration. For the 8 mg/kg dose, a separate series of experiments comparing the effects of air and He/O_2 at 7 ata on the stereotyped response was performed, to determine if the reduction in licking/biting produced by hyperbaric air was due to N_2.

3.3.1.3 RESULTS

Convulsive Activity

At 1 ata air, injection of amphetamine 30 mg/kg produced hyperactivity in the mice manifested primarily by vigorous biting of the neck, arching of the head and tremor, interspersed with outbursts of explosive uncontrolled motor activity which resulted in the mouse being thrown against the sides of the cage. These outbursts of uncontrolled motor activity, were generally of short duration (0.5 to 2 sec) and resembled the uncontrolled activity seen in convulsions. These were usually preceeded by a short period in which the mouse was motionless, with its legs tensed against the sides of the cage. In some cases convulsions, in which the episodes of uncontrolled motor activity were of longer duration and accompanied by loss of balance, occurred. The biting arching of head, tremor and other manifestations of hyperactivity were very rapid in onset commencing 10-15 min after injection. These lasted for approximately 50 min, following which the predominant behaviour observed was a less vigorous licking or gnawing at the sides of the box. The inital episode of violent motor activity was usually observed within 10- 30 min after injection. The time of peak effect varied between mice but generally occurred in the 30-80 min period following injection. This dose of amphetamine was lethal to 4 mice, one of these dying within the 3 hr experimental period.

For the purpose of analysis the violent motor episodes and convulsions were collectively described as "convulsive activity" and for each pressure the number of mice displaying at least one episode of such

activity, as well as the time spent in such episodes determined. These data are shown in Table (2) along with the 24 hr lethality at each pressure.

Exposure to 4 and 7 ata air significantly reduced the number of mice displaying convulsive activity, Table (2), see also Fig.(3). [Binomial test (Goldstein, 1964); $x^* = 2.59$, 2.58, respectively, $p<0.05$.]. The time spent in episodes of convulsive activity was also reduced. The other indicators of hyperactivity such as biting, and arching of the head, were still evident however. In addition at these pressures amphetamine was not lethal to any of the mice. The percentage of mice displaying convulsive activity following exposure to 7 ata He/O_2 was not significantly different from that at 1 ata, and the mean time spent in such episodes was also similar. This pressure of He/O_2 however, did protect the mice from the lethality of this dose of amphetamine Table (2).

Locomotor Activity

Graphs of 5 min activity counts are shown in Figs.(4a,b,c). To determine the effect of exposure to the various pressures on the locomotor activity produced by amphetamine, the cumulative activity totals over the entire experimental period, Table (3), were analyzed. The 60 min cumulative activity totals of saline-treated mice are also presented in Table (3) for comparison. At 1 ata increasing the dose of amphetamine produced a dose dependent increase in activity Table (3). At 3 mg/kg, the major pattern of behavior observed was periods of

TABLE 2

RESULTS OF EXPOSURE TO HYPERBARIC CONDITIONS ON AMPHETAMINE-INDUCED CONVULSIVE ACTIVITY, AND LETHALITY

GAS	PRESS. (ATA)	# MICE DISPLAYING CONVULSIVE ACTIVITY	TIME SPENT IN CONVULSIVE ACTIVITY(s)*	% LETHALITY
AIR	1	10/12	30.8 ± 11.6	33.3
	4	2/8**	6.5 ± 0.7	0
	7	3/12**	1.3 ± 0.4	0
$He-O_2$	7	6/8	29.2 ± 12.2	0

*values are Mean ± SEM

**sig vs 1 ata

Figure 3

Photograph of recording of the output from a kinetograph illustrating the difference in the tracings produced by convulsive activity vs. other behaviors. The top tracing of each figure represents the integrated recording. The large spikes in the tracing at 1 ata (A), indicates the type of recording seen during convulsive activity. The tracing at 7 ata (B), is representative of the type of recording seen at this pressure.

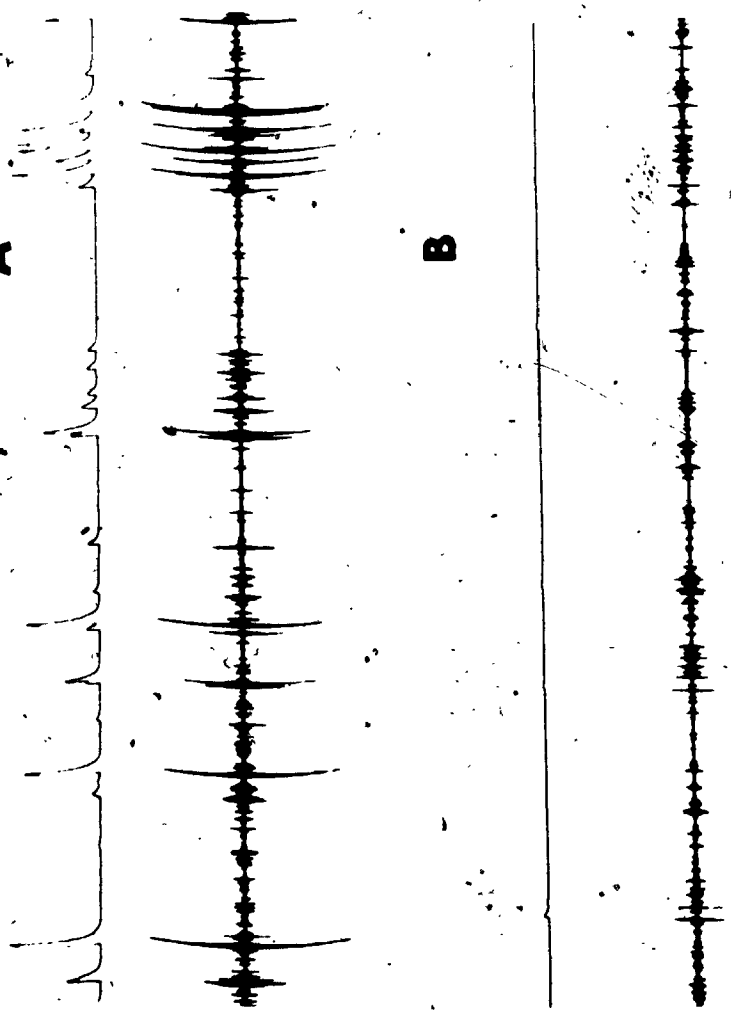

A
B

Figure 4a

Graph of 5 min. activity totals of mice given amphetamine 3.0 mg/kg at 1 (●), 4 (▲) and 7 (■) ata air, and 7 ata He/O_2 (□). Vertical bars = 1 SEM. (n=9) for experiments with air. (n=6) for experiments with He/O_2.

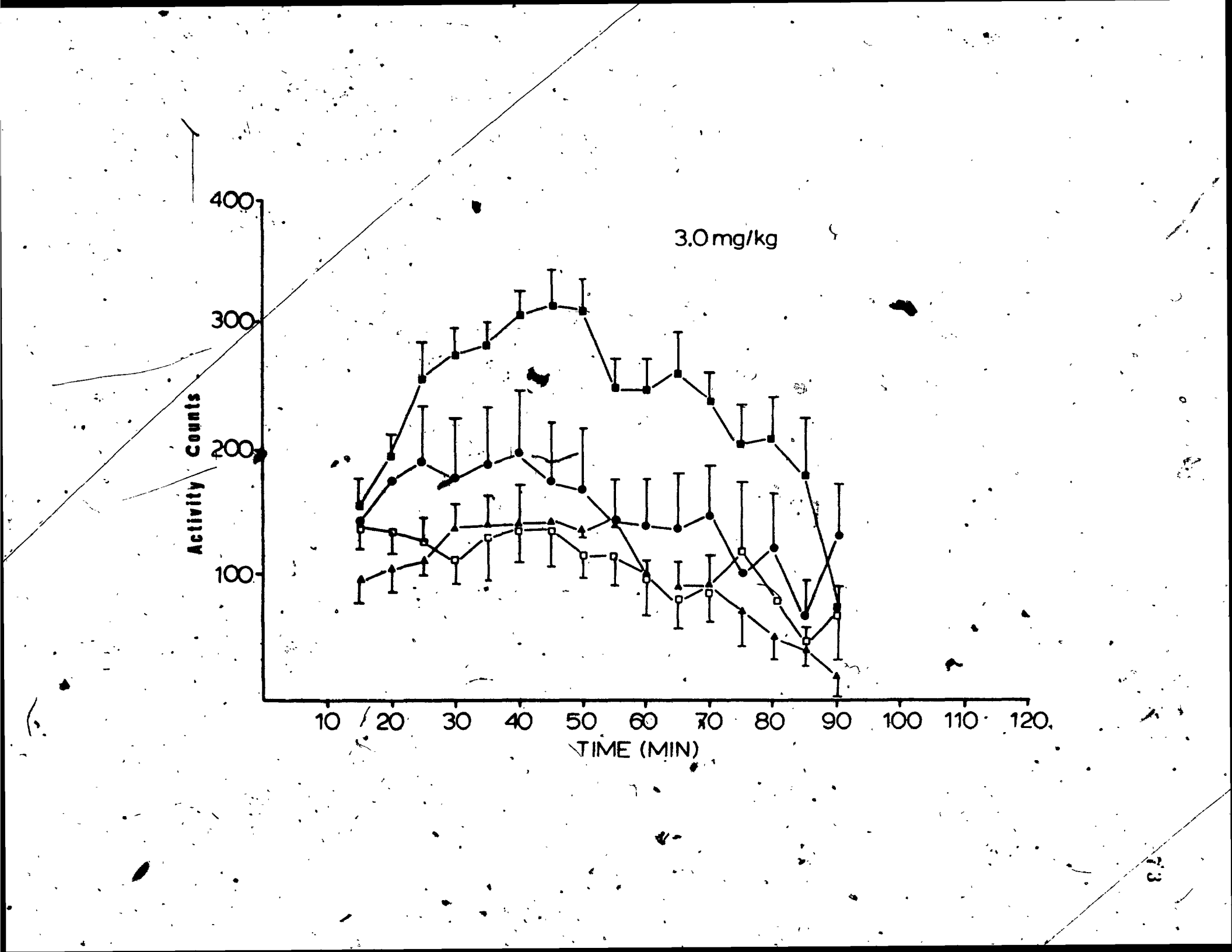

3.0 mg/kg
Activity Counts
400
300
200
100
10
20
30
40
50
60
70
80
90
100
110
120
TIME (MIN)

Figure 4b

Graph of 5 min. activity totals of mice given amphetamine 4.0 mg/kg at 1 (●), 4 (▲) and 7 (■) ata air. Vertical bars = 1 SEM. (n=9).

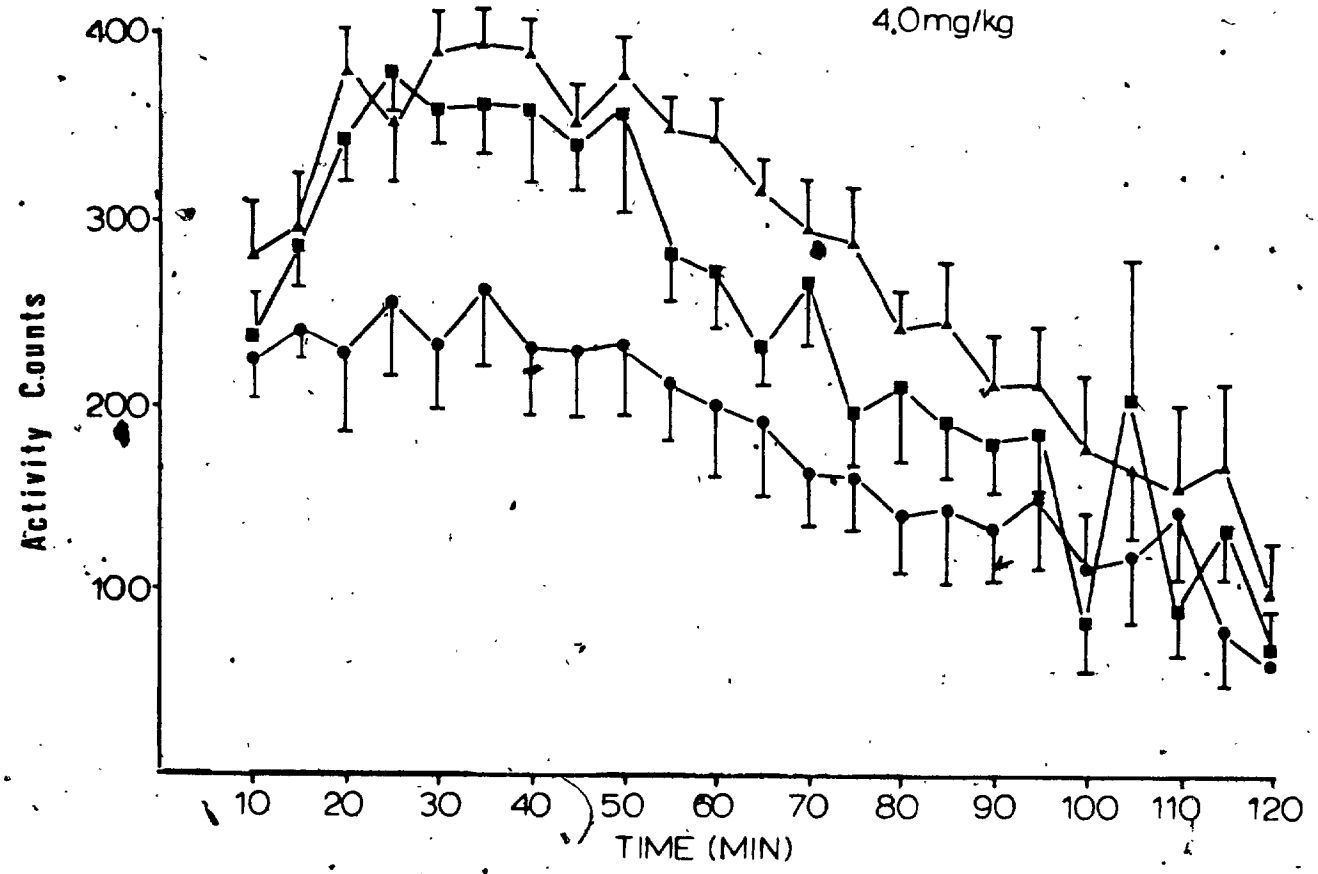

4.0mg/kg
Activity Counts
400
300
200
100
10
20
30
40
50
60
70
80
90
100
110
120
TIME (MIN)

Figure 4c

Graph of 5 min. activity totals of mice given amphetamine 5.0 mg/kg at 1 (●), 4 (▲) and 7 (■) ata air. Vertical bars = 1 SEM. (n=9).

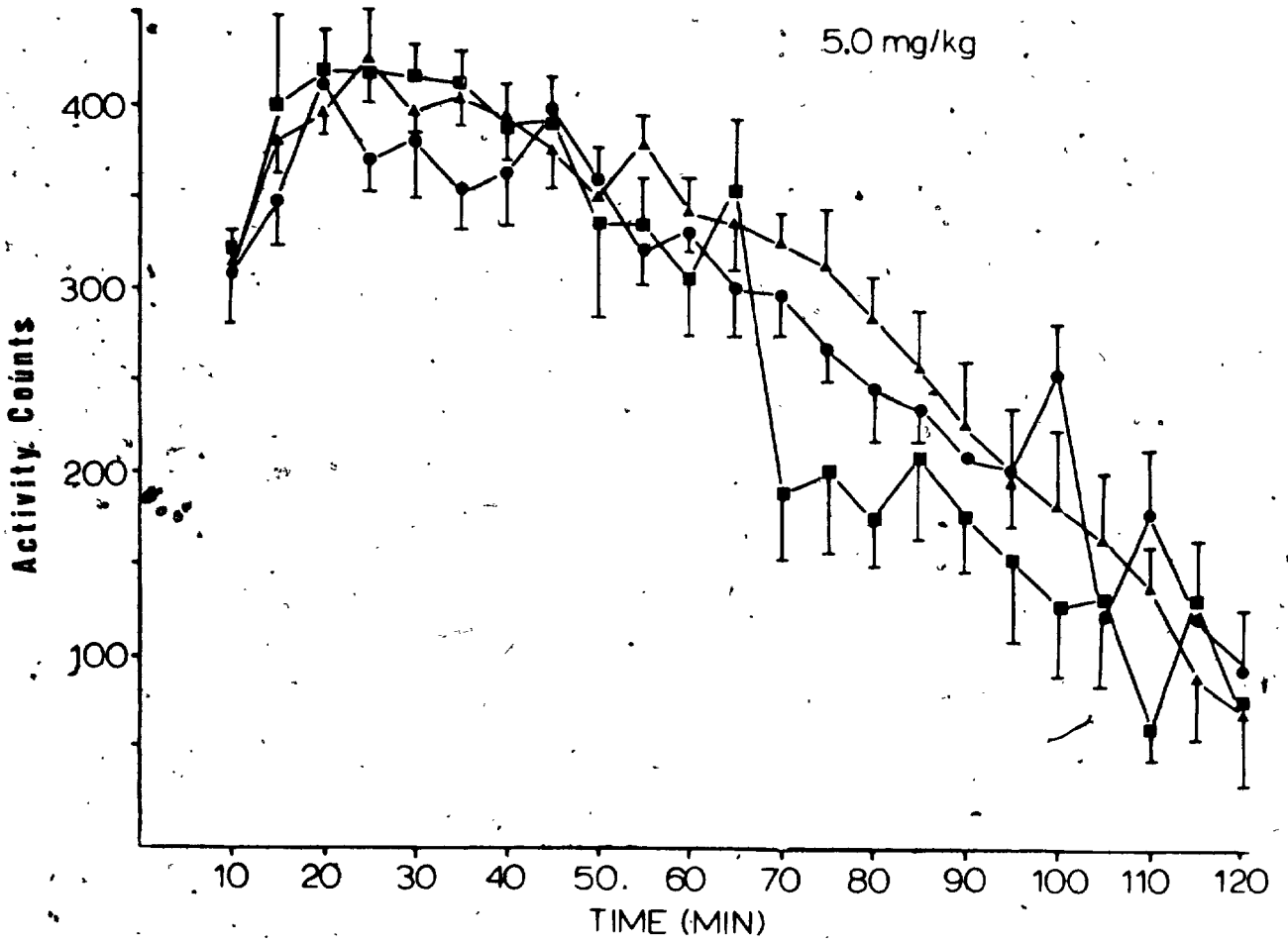

5.0 mg/kg
Activity Counts
400
300
200
100
10
20
30
40
50.
60
70
80
90
100
110
120
TIME (MIN)

TABLE 3

CUMULATIVE ACTIVITY OF AMPHETAMINE AND SALINE-TREATED MICE

PRESSURE

DOSE (mg/kg)	1 ATA AIR	4 ATA AIR	7 ATA AIR	7 ATA He-O_2
3	2396.96 ± 583.1	1618.0 ± 311.4	3740.8 ± 211.8*	1778.7 ± 300.2
4	4267.3 ± 363.2	6261.9 ± 386.5**	5416.7 ± 409.9**	
5	6122.3 ± 284.8	6386.0 ± 308.4	5443.0 ± 349.5	
Saline controls†	489.7 ± 100.1		270.5 ± 51.4	301.7 ± 76.5

Values (Mean ± SEM)

* sig vs 3.0 mg/kg at 1; 4 ATA air, 7 ATA He-O_2

** sig vs 4.0 mg/kg at 1 ATA air

† saline treated mice tended to fall asleep approximately 30 min after being replaced in the chamber, accounting for the low counts.

walking interspersed with periods of inactivity and sniffing. At 4 and 5 mg/kg there was a reduction in the periods of inactivity and an increase in walking, running and rearing Fig.(5).

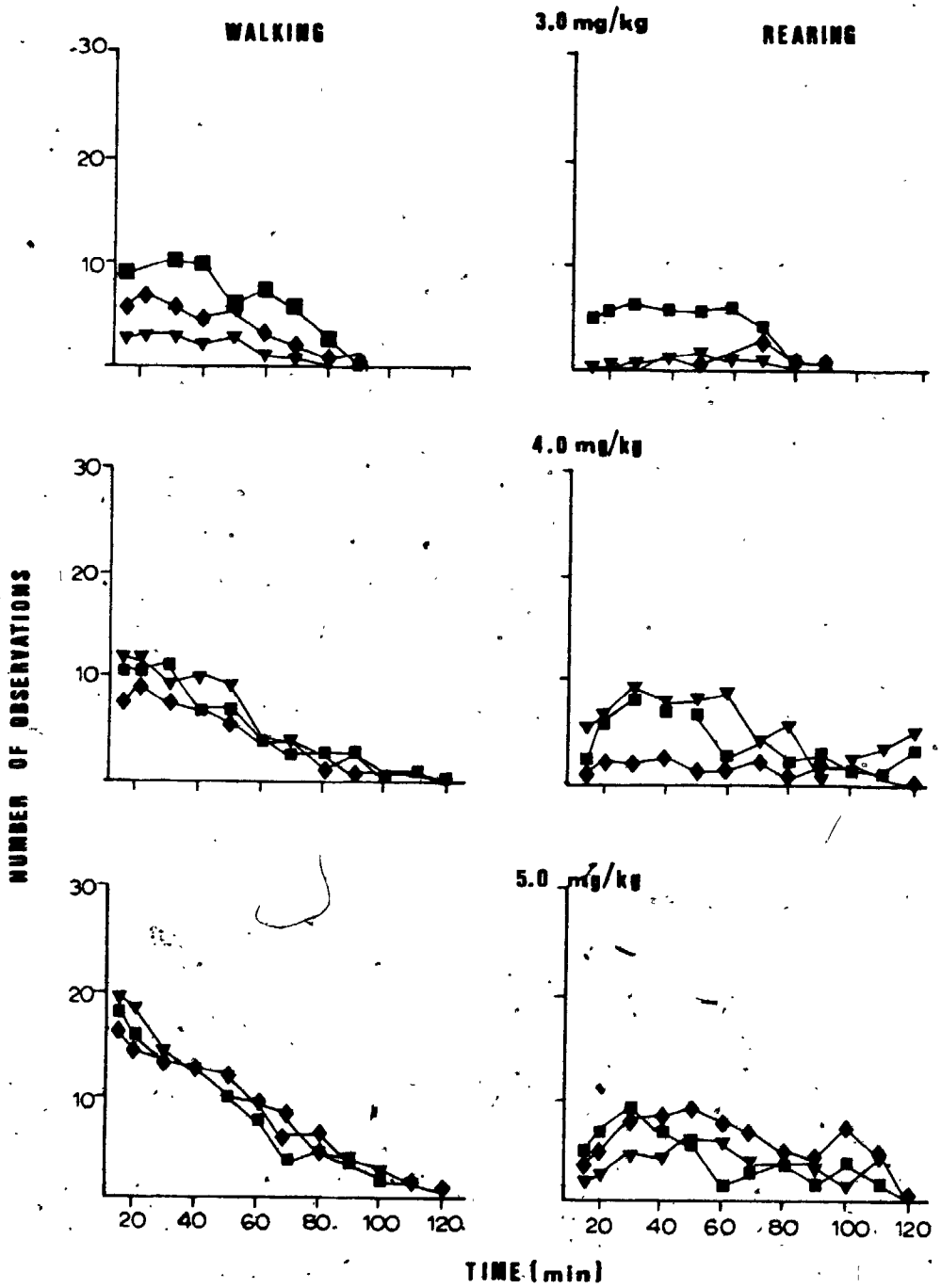
At the 3 mg/kg dose, exposure to 7 ata air resulted in higher levels of activity compared to that at 1 ata. This appeared to be due to an increase in the peak level of activity rather than a prolongation of the effect. At 4 ata air and 7 ata He/O_2 however, the mean activity totals were less than at 1 ata. Analysis of the cumulative activity totals revealed a significant effect between pressures [$F(2, 4) = 48.20$ $p < 0.01$, air only, $F(3,3) = 25.5$ $p < 0.05$, with He/O_2]. Further tests revealed that this was due to the activity at 7 ata air being significantly higher than at all the other pressures tested. No other statistically significant differences were observed.

At the 4 mg/kg dose, exposure to both 4 and 7 ata air resulted in increases in activity compared to 1 ata, with the greatest increase in activity occurring at 4 ata. Once again this appeared to be due to an effect on peak activity rather than on duration. Analysis of cumulative totals showed a significant effect of pressure of air [$F(2,18) = 6.83$, $p < 0.05$.], and tests between means showed that activity totals at 4 and 7 ata were significantly higher than at 1 ata, but were not significantly different from each other.

No significant differences in the cumulative activity totals were observed at the 5 mg/kg dose. Activity was lowest at 7 ata air, however this did not appear to be due to a decline in peak activity but rather to a sharp decline in the activity of the mice at 7 ata approximately 65 min after injection, Fig.(4c). This decrease in activity was associated

Figure 5

Incidence of walking and rearing for mice given amphetamine 3, 4 and 5 mg/kg at 1 (◆), 4 (▼) and 7 (■) ata air. (n=9)


WALKING
REARING
3.0 mg/kg
4.0 mg/kg
5.0 mg/kg
NUMBER OF OBSERVATIONS
TIME (min)
30
20
10
20
40
60
80
100
120

with an increase in grooming. This is the behavior normally seen as the effect of a particular dose wears off. This trend to rapid decline from the peak response at 7 ata is in fact evident at all of the doses. It is more marked at the 5 mg/kg dose however.

In the experiments with the saline-treated mice, no statistically significant differences were seen between the 60 min activity totals at 1 and 7 ata air, or 7 ata He/O_2. This indicates that the enhancement was not due to an increase in activity produced by hyperbaric air.

Stereotyped Behavior

The total number of observations in the licking/biting category at each observation period were calculated for each mouse, and following a square root transformation to normalize the variances, (Winer, 1971), analyzed to determine the effect of the various hyperbaric exposures on this response. Graphs of the incidence of stereotyped licking at the various pressures as a function of time, are presented in Fig.(6). [An alternative method of analysis in which the effects of the hyperbaric environments on the whole curve are examined is presented in Appendix III].

Injection of amphetamine 10 mg/kg produced an intense stereotyped licking/ biting response peaking approximately 40-50 min after injection, Fig.(6a). Exposure to 4 or 7 ata air did not significantly affect the onset or peak level of this response. In fact exposure to both 4 and 7 ata appeared to slightly increase the rate of onset, as mean values at 15 min tended to be higher at these pressures compared to

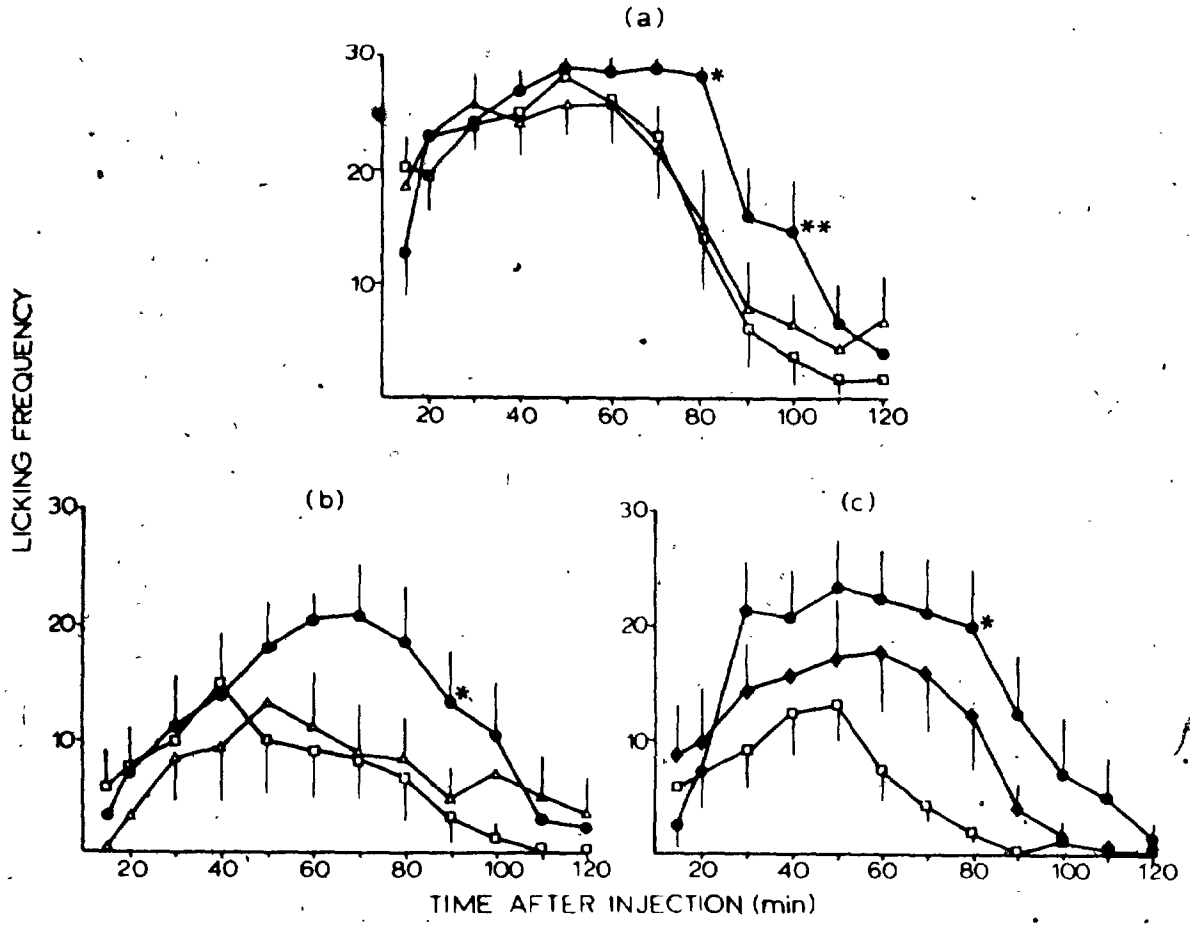
Figure 6

a) Incidence of stereotyped licking/biting for mice given amphetamine 10 mg/kg at 1 (●), 4 (△) and 7 (□) ata air. * sig. vs. 4,7 ata air. ** sig. vs. 7 ata air.

b) Incidence of stereotyped licking/biting for mice given amphetamine 8 mg/kg at 1 (●), 4 (△) and 7 (□) ata air. * sig. vs. 7 ata air.

c) Incidence of stereotyped licking/biting for mice given amphetamine 8 mg/kg, at 1 (●) and 7 (□) ata air , and 7 ata He/O_2 (◆). * sig. vs. 7 ata air.

Vertical Bars = 1 SEM. (n=9) in all cases.


(a)
(b)
(c)
LICKING FREQUENCY
TIME AFTER INJECTION (min)
30
20
10
20
40
60
80
100
120
*
**

1/ata air, Fig.(6a). This effect was not significant however. Exposure to hyperbaric air did significantly increase the rate of decline of the response, with statistically significant differences in the incidence of the licking/biting response at pressure being observed at 80 and 100 min following injection, [$F(2,18) = 5.69$, 3.08 respectively, $p < 0.05$], Fig.(6a).

At 1 ata, amphetamine 8 mg/kg produced a less intense stereotyped response with both the rate of onset and peak level being reduced compared to that at 10 mg/kg, Fig.(6a,b). In contrast to the 10 mg/kg dose, exposure to 4 and 7 ata did tend to reduce the peak response, with mean values at 4 and 7 ata from 50 min onwards being lower than at 1 ata, Fig.(6b). Analysis of variance revealed a statistically significant difference in the incidence of licking/biting between pressures at 90 min, the incidence at 7 ata being significantly reduced compared to 1 ata [$F(2,18) = 3.71$, $p<0.05$]. Trends to significant differences ($p<0.1$) were also observed at 60,70 and 100 min following injection. To more clearly show the effect of hyperbaric air on the response, the cumulative response over the 60-90 min period (i.e. the sum of the responses over this period) was analyzed. The analysis showed that both 4 and 7 ata significantly reduced the incidence of licking/biting over this period, [$F(2,18) = 4.23$, $p < 0.05$].

In the experiments with He/O_2, exposure to 7 ata He/O2 also resulted in lower mean peak levels compared to 1 ata, however this effect was not as marked as at 7 ata air, Fig.(6c). A statistically significant difference in licking/biting between the various conditions was obtained 80 min after injection, [$F(2,18) = 4.81$, $p<0.05$], with the

incidence at 7 ata air being significantly reduced compared to 1 ata air. At this time period the incidence of licking at 7 ata He/O_2 was not significantly different from 1 ata air. Trends to significant differences ($p<0.1$), were also observed at 60 and 70 min following injection. An analysis of the cumulative response over the 60 - 90 min revealed that the response was significantly reduced by exposure to 7 ata air, but not 7 ata He/O_2, [$F(2,18) = 4.44$, $p<0.05$.].

3.3.1.4 DISCUSSION

The results show that hyperbaric air in the range of 4-7 ata can modify the behavioral effects of amphetamine in mice. Thus exposure to hyperbaric air reduced the convulsive activity and abolished the lethality produced by the 30 mg/kg dose. Also, hyperbaric air acted to reduce the stereotyped licking/biting/gnawing response. This effect of hyperbaric air was more marked at the lower dose, with the peak response being reduced. The more intense response produced by the higher dose was less affected, with the only effect seen being a faster decline in the response at pressure compared to 1 ata. The effects on locomotor activity were more complex with the effects appearing to depend on the dose of amphetamine as well as the pressure of air. At the lowest dose (3 mg/kg), 7 but not 4 ata air enhanced activity. At the 4 mg/kg dose both pressures significantly increased activity compared to 1 ata air, the maximum activity however, occurred at 4 instead of 7 ata. No significant differences in activity were observed at 5 mg/kg. As was the case for the 4 mg/kg dose however, the greatest mean activity occurred at 4 ata air. In fact for this dose, mean activity at 7 ata was lower than at 1 ata.

In general, the effects of hyperbaric air on the amphetamine induced behaviors would appear to be due primarily to the increased PN_2 rather than to concurrent increases in PO_2 or hydrostatic pressure. This is illustrated by the failure of 7 ata He/O_2 to significantly modify amphetamine induced convulsive activity, locomotor activity or stereotyped behavior. In regard to locomotor activity experiments with

He/O_2 were not performed at the 4.0 mg/kg dose, and therefore it could be argued that it has not been clearly established that the enhancement seen at this dose is due to N_2. However, given the findings at the 3.0 mg/kg dose it is not unreasonable to assume that N_2 was the cause. Also, with regard to stereotyped behavior, exposure to 7 ata He/O_2 did produce a non significant decrease in the response to 8 mg/kg. This suggests therefore, that the increased PO_2 or hydrostatic pressure might also be capable of affecting this behavior, and therefore could have contributed to the effect produced by hyperbaric air. As will be discussed later however, this effect of He/O_2 may be related to a tendency for He to reduce drug effects in mice.

The factors in the hyperbaric air environment responsible for the protection against the amphetamine induced lethality are not as clear cut since exposure to 7 ata He/O_2 also protected against the lethality. Thus it is not possible to resolve whether the effect was due to N_2 or the increased PO_2 or hydrostatic pressure. Possible explanations for this effect of He/O_2 will be discussed later.

Although this was not tested, it is unlikely that the modifications observed were primarily due to an alteration in the pharmacokinetics or distribution of amphetamine at pressure. For example it is not possible to explain the enhancement of locomotor activity, as well as the reduction of convulsive activity, and stereotyped behavior at the 8 mg/kg dose, on the basis of an alteration in pharmacokinetics. Also, since the peak response to 10 mg/kg was unaffected by exposure to hyperbaric air, it is unlikely that the decreased response to the 8 mg/kg dose was due to a decline in the amount of amphetamine entering

the CNS. This possibility however, cannot be ruled out completely as an explanation for the effect on convulsive activity. The fact that the other manifestations of hyperactivity , biting, arching of head, tremor seen at 1 ata were present at pressure, suggests however, that significant amounts of amphetamine were getting into the CNS. Furthermore, there is no evidence to suggest that exposure to hyperbaric air reduces the amount of drugs entering the CNS. In fact in the only study to my knowledge which has looked at this is that performed by Johnston (1983) in this laboratory. In this experiment, exposure to hyperbaric air did not significantly alter the brain levels of apomorphine in mice given a 50 mg/kg dose, despite significantly decreasing the rearing response to the drug.

It would appear therefore, that the modifications of the behavioral effects observed are primarily due to an interaction with the central effects of N_2. The results however, are not all explainable in terms of the classical theory that N_2 produces a generalized slowing of CNS activity (Bennett, 1966 ; Bennett, 1975). The effects on convulsive activity and stereotyped behavior are compatible with this view. The results on locomotor activity in general are not, since in many cases an enhancement of activity was observed. The results on locomotor activity are similar to those obtained in the operant studies with amphetamine, in which hyperbaric air enhanced the disruptive effect of amphetamine on FR (Thomas, 1976), and DRL schedules (Walsh, 1974). These results suggest that hyperbaric air produces some increase in CNS excitation. This is at first surprising in view of the fact that N_2 was supposed to produce a generalized depression of CNS activity. Anesthetics in low doses however, do produce some CNS excitation (disinhibition) at low

doses, one of the manifestations of this in animals being an increase in locomotor activity (Hynes and Berkowitz, 1979 ; Waters and Walczak, 1980). Also, low doses of anesthetics have been shown to enhance the locomotor stimulation produced by a number of drugs (Waldeck, 1974 ; Waters and Walczak, 1980 ; Rushton and Steinberg, 1963). Of particular interest to this study is the fact that low doses of the barbiturate amobarbital and amphetamine in combination act synergistically to increase the activity of rats (Rushton and Steinberg, 1963). The stimulation, excitement and euphoria seen in humans at low pressures of air (Behnke et al., 1935), suggests that N_2 may also produce some CNS excitation at low pressures. Thus the enhancement of locomotor activity may represent an interaction with the disinhibitory effects produced by N_2. This will be discussed in more detail in chapter 5.

It is interesting that the enhancement of the behavioral effects of amphetamine was limited to locomotor activity. The difference in the effects of hyperbaric air on locomotor activity and stereotyped behavior may be due to the fact that they appear to be mediated through different areas in the CNS. Both of these effects would appear to be the result of release of DA by amphetamine (Kelly, 1977). The evidence suggests however, that mesolimbic systems such as those projecting to the nucleus accumbens (NAc) may be involved in the locomotor response (Kelly et al., 1975; Teitelbaum et al., 1979 ; Pijenburg et al., 1975), whereas extrapyramidal DA systems are responsible for the stereotyped behavior (Kelly, 1977 ; Costall et al., 1977).

It should be pointed out that the site or sites at which amphetamine acts to induce stereotyped licking/biting are not as clear

cut as originally thought. Although the study by kelly et al., (1975) appeared to implicate the striatum as the site of prime importance, other evidence suggests that it is not that simple. For example local application of amphetamine to the striatum does not produce the licking/biting response (Costall et al., 1972). In addition, whereas 6-OHDA lesions of the striatum attenuate the biting response to amphetamine (Kelly et al., 1975) extensive electrolesions of the striatum fail to modify amphetamine-induced stereotypy (Divac, 1972; Costall and Naylor, 1974). There is some evidence that the globus pallidus is a key area (Costall et al. 1977). For example amphetamine is more effective in producing stereotyped behavior when applied to the globus pallidus than to the striatum (Costall et al., 1972). Furthermore, 6-OHDA lesions of the globus pallidus has been shown to modify amphetamine stereotypy (Costall et al., 1977). Thus it may be that the stereotyped behavior produced by amphetamine may be the net result of its effects at several areas in the basal ganglia. Possible mechanisms for the modifications of the amphetamine induced behaviors by N_2 will be discussed in chapter 5.

The effect of He/O_2 to protect against amphetamine induced lethality was surprising particularly in view of the fact that He/O_2 would not be expected to have a depressant effect on CNS function (Bennett, 1966 ; 1975). Furthermore it did not protect against the convulsive activity Table (2). It is not clear however, whether the lethality to this dose of amphetamine is in fact due to a central effect. In 3 out of the 4 mice that died, death occurred after the 3 hr experimental period rather than during "convulsive activity". Thus, it is likely that the lethality was due to the stresses which would be

produced by the marked peripheral and central effects of this dose of amphetamine (Estler, 1975). If this is so then there are a number of ways in which He/O_2 could have reduced the stress on the mice. For example the increased PO_2 could have been effective either through its mild anesthetic effect (Smith and Payton, 1976), or by providing better oxygenation of the tissues, offset some of the stress. Recall, the increased PO_2 was shown to be the cause of the protection afforded by hyperbaric air against picrotoxin induced lethality in mice (Hart, 1974a). In this case it was proposed that the increased PO_2 acted by preventing the lethal hypoxia which occurred during convulsions. Also, He due to its high thermal conductivity (Brauer et al., 1977), could be expected to increase the heat loss from the mice, and reduce the hyperthermia which would be expected to occur with this dose (Bizzi, et al., 1970). Hyperthermia, has been suggested as one of the major causes of the lethality to amphetamine in aggregated mice (Askew, 1962), and it is possible that it could be a contributing factor in isolated mice as well.

There is another possibility however. It is becoming increasingly apparent that He, apart from any pressure effects is capable of modifying the effects of drugs in mice. For example, exposure to 1 ata He/O_2 (80/20) has been shown to significantly reduce the sleep time to alcohol (Alkana and Malcolm, 1981). Similarly studies in our laboratory have shown that the phenylquinone writhing response, (Siegmund et al., 1957) was reduced by exposure to 1 ata He/O_2, [Mean $\pm$ sem $=23.8 \pm 7.8$, 14.9 ± 8.8, $(n=11)$], (unpublished observations). Thus He/O_2 would appear to have a tendency to reduce the effect of some drugs in mice. In fact, if one considers the effect of 7 ata He/O_2 on all of the

amphetamine induced behaviors it is apparent that there is a tendency to a decreased response in all of them, see Tables (2,3), and Figs.(4a,6c). This occurred even in the case of locomotor activity at the 3 mg/kg dose where 7 ata air enhanced activity. Thus it may be that the reduction in lethality seen with He/O_2 at 7 ata is just a manifestation of this general effect of He.

The mechanism by which He produces the effects referred to above is not known. Exposure of rats to 1 ata He/O_2 has been shown to markedly increase their O_2 consumption (Leon and Cook, 1960), this apparently due to a need to offset the heat loss resulting from the high thermal conductivity of He. Similar findings have been observed in mice (Cook et al., 1951). In addition, exposure to 11 ata He/O_2 has been shown to produce diuresis in rats (Tofano and DeBoer, 1976). Whether these effects are involved in producing the decreased response to He/O_2 is not known.

In summary, exposure to hyperbaric air in the range 1-7 ata is capable of modifying the behavioral effects of amphetamine. The alterations of behavior however, are more complex than would be predicted based on the classical theory of inert gas narcosis (Bennett, 1966 ; 1975). In particular the results suggest that there are both disinhibitory and depressant changes produced in the CNS by exposure to hyperbaric air. This will be disussed in more detail in chapter 5.

3.3.2 STUDIES ON MORPHINE

3.3.2.1 Introduction

General

Morphine sulphate at doses above 10 mg/kg produces a marked stimulation of locomotor activity in mice (Villareal et al., 1973 ; Carroll and Sharp, 1972 ; Eidelberg and Erspamer, 1975). Based on a review of the activity data in isolated mice (Villareal et al., 1973 ; Eidelberg and Erspamer, 1975), a dose of 15 mg/kg was selected for study. This produced an acceptable stimulation of locomotor activity, and was chosen as the low dose. A dose of 30 mg/kg which produced a much more marked stimulation of locomotor activity was chosen as the high dose.

3.3.2.2 Methods

Morphine sulphate (BDH), was dissolved in saline and administered (i.p.), in a volume of 0.1 ml/10 g body weight.

As in the experiments with amphetamine the mice were acclimatized to the activity boxes prior to testing. Experiments were conducted at 1, 4 and 7 ata. Also at the 15 mg/kg dose, experiments at 7 ata He/O_2 were performed to determine if the effects were due to N_2. The experiments were of 2 hr duration, with activity counts and behavioural observations being recorded as described in the general methods. Three

mice were tested at one time and three runs were performed at each pressure thereby giving a total of 9 animals at each dose/pressure combination. The order of exposure to the pressures of air was randomized using a Latin Square design.

3.3.2.3 Results

Graphs of the mean 5 min activity counts are presented in Figs.(7,8), To determine the effect of hyperbaric air on the locomotor activity produced by morphine, the cumulative activity totals for the 5-60, 60-120, and 5-120 min periods following injection Table (4A,B), were analyzed.

Injection of morphine sulphate at 1 ata produced a dose dependent increase in the activity of the mice compared to the activity of similarly acclimatized mice injected with saline; [For activity of saline treated mice see amphetamine results section, Table (3)]. The increase in activity consisted mainly of intermittent walking or running interspersed with periods of immobility, or sniffing. At the higher dose the episodes of walking and running were increased and those of immobility reduced. In contrast to the effects seen with amphetamine however, rearing was rarely seen Fig.(9). At the 15 mg/kg dose there was an approximately 15-20 min delay before the stimulatory effect of morphine on activity was apparent, Fig.(7). During this period the mice lay quietly in one corner of the cage with periodic sniffing activity being observed. This lag period was not seen at the higher dose, Fig.(8).

For both doses, exposure to hyperbaric air resulted in higher mean cumulative activity totals over the experimental period compared to similarly treated mice at 1 ata Table (4A,B). For the two doses however, the pressure at which the maximum activity occurred varied. For the 15 mg/kg dose the greatest activity occurred at 7 ata, whereas at the 30 mg/kg dose the highest activity occurred at 4 ata air.

For the 15 mg/kg dose, analysis of variance revealed a significant difference in the 5-60 and 5-120 min cumulative activity totals at the various pressures of air [$F(2,18) = 3.82$, and 3.88, $p<0.05$, respectively], Table (4A). For the 5-60 min totals this was due to activity at 7 ata being significantly higher than at 1 ata. For the 5-120 min totals, activity at both 4 and 7 ata was higher than at 1 ata. No significant difference between activity at 4 and 7 ata was observed at any of the time intervals.

At the 30 mg/kg dose, a significant difference in cumulative activity totals between pressures was only observed for the 5-120 min totals, [$F(2,18) = 3.73$, $p<0.05$.]. Further tests showed that this was due to the activity totals at 4 ata being significantly higher than at 1 ata. No other differences were statistically significant.

Exposure to 7 ata He/O_2 did not enhance the activity produced by morphine sulphate (15 mg/kg), (Table 4C).

Figure 7

Graph of 5 min. activity totals for mice given morphine sulphate 15 mg/kg at 1 (◆), 4 (▼) and 7 (■) ata air. (n=9). Standard error bars have been omitted for the sake of clarity.

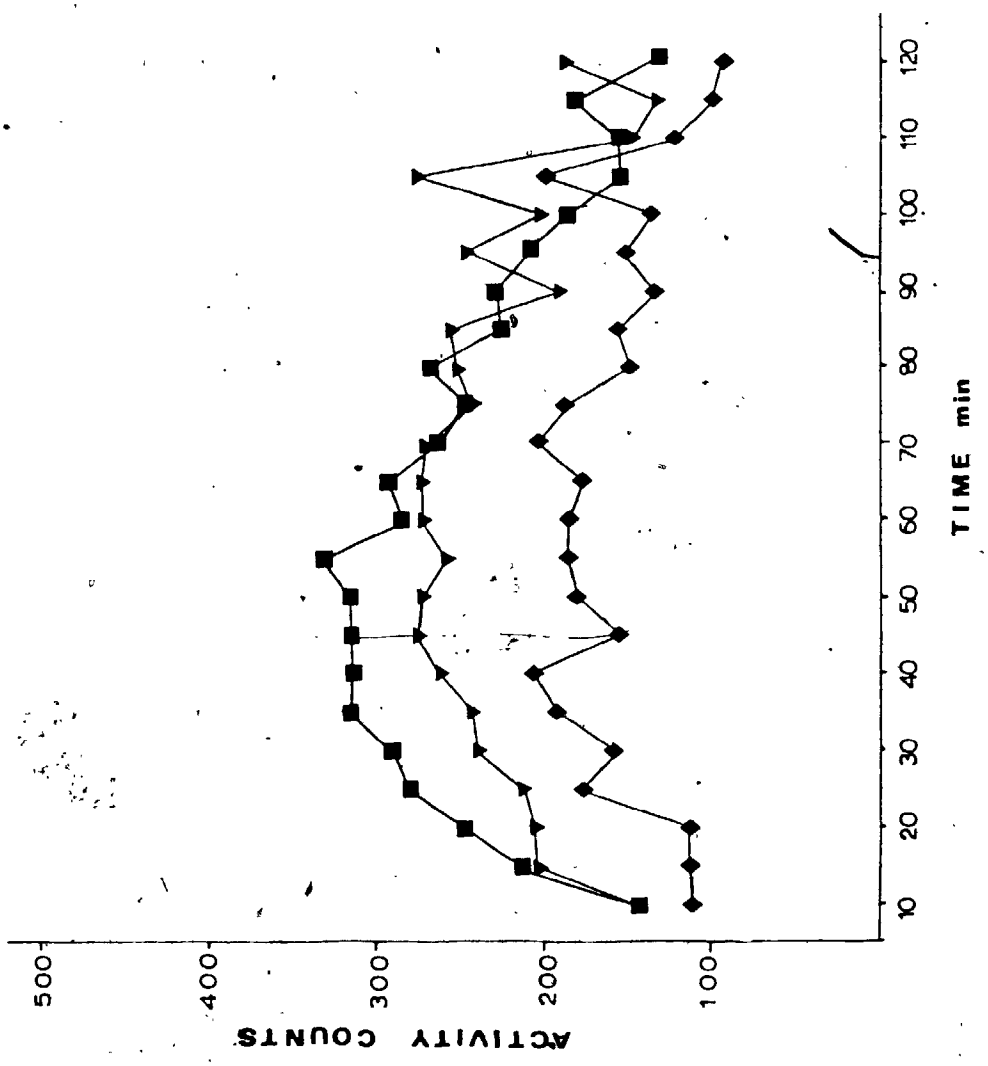

ACTIVITY COUNTS
500
400
300
200
100
10
20
30
40
50
60
70
80
90
100
110
120
TIME min

Figure 8

Graph of 5 min. activity totals for mice given morphine sulphate 30 mg/kg at 1 (◆), 4 (▼) and 7 (■) ata air. (n=9). Standard error bars have been omitted for the sake of clarity.

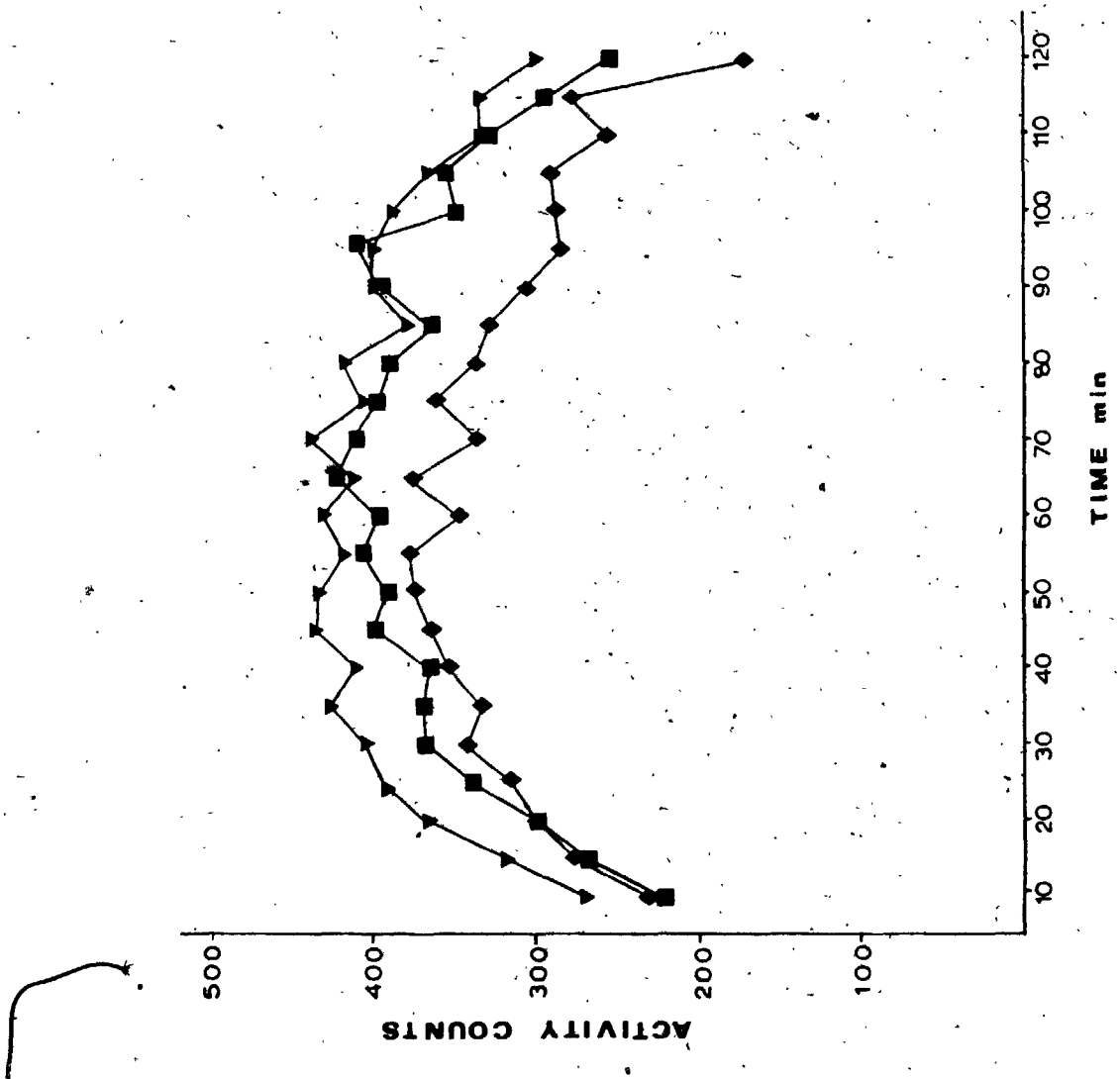

ACTIVITY COUNTS
500
400
300
200
100
10
20
30
40
50
60
70
80
90
100
110
120
TIME min

Figure 9

Incidence of walking and rearing at 1 (◆), 4 (▼) and 7 (■) ata air for mice given morphine sulphate 15 and 30 mg/kg. (n=9).

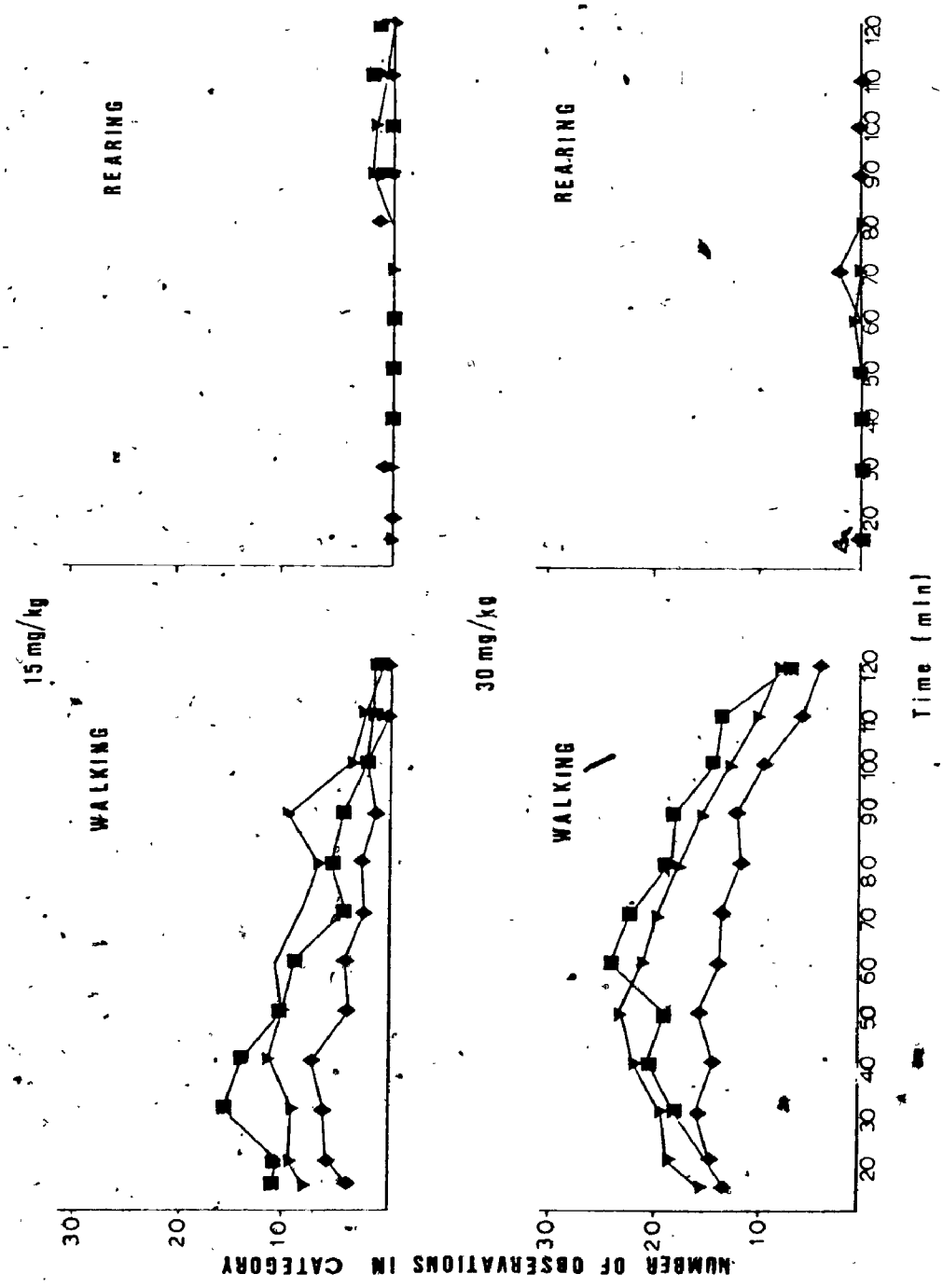

REARING
REARING
15 mg/kg
30 mg/kg
WALKING
WALKING
NUMBER OF OBSERVATIONS IN CATEGORY
Time (min)

TABLE 4

'A'

Morphine Sulphate 15 mg/kg. Cumulative Activity Totals

(Mean ± SEM) at 1, 4 and 7 ata air.

Time Interval (min)	Pressure of Air (ata) 1	4	7
5- 60	1771.7 ± 300.0	2588.4 ± 392.4	3051.4 ± 281.3*
60-120	1699.8 ± 283.5	2561.2 ± 225.8	2422.9 ± 294.6
5-120	3471.4 ± 540.6	5260.8 ± 655.4*	5585.4 ± 565.9*

*sig vs 1 ata, $p<0.05$

'B'

Morphine Sulphate 30 mg/kg. Cumulative Activity Totals

(Mean ± SEM) at 1, 4 and 7 ata air.

Time Interval (min)	Pressure of Air 1	4	7
5- 60	3612.7 ± 409.9	4336.2 ± 305.9	3813.4 ± 291.3
60-120	3596.7 ± 410.1	4537.7 ± 316.3	4364.1 ± 235.3
5-120	7220.4 ± 767.2	8873.9 ± 580.2*	8174.2 ± 418.2

*sig vs 1 ata, $p<0.05$

TABLE 4

"C'

Morphine Sulphate 15 mg/kg.Cumulative Activity Totals

(Mean + SEM) at 1, 7 ata air, 7 ata He/O_2

Time Interval (min)	Pressure 1 Air	4 Air	7 He/O_2
5-60	1021.7 +209.8	2151.7 +444.1	1230.8 +286.5
60 -120	1152.7 +227.4	1598.0 +279.6	1172.5 +203.1
5--120	2174.3 + 425.3	3744.7 +616.9	2399.7 +470.1

* $F(2,2)=39.1,31.3$; p 0.05 vs 1 ata air, 7 ata He/O_2

5-60 , 5- 120 min respectively.

3.3.2.4 DISCUSSION

The results of these experiments show that hyperbaric air in the range of 4-7 ata can significantly modify the locomotor activity induced by 15 and 30 mg/kg morphine sulphate. In contrast to what would be predicted by the classical theory of inert gas narcosis however, (Bennett, 1966,1975), exposure to hyperbaric air tended to enhance not decrease the locomotor stimulation produced by morphine. At the 15 mg/kg dose, significant increases in activity occured at both 4 and 7 ata, with the maximum activity being obtained at 7 ata. At the 30 mg/kg dose however a significant increase in activity was only observed at 4 ata. As with amphetamine the increase in activity was due to an increase in peak activity levels rather than in the duration of the response.

The effect of hyperbaric air on the morphine induced activity is similar to that seen in the experiments on amphetamine-induced locomotor activity, in that as the dose of drug is increased, and higher activity levels are obtained at 1 ata, the pressure of air at which the maximum activity occurs switches from 7 to 4 ata. There are some differences however. As discussed previously, in the case of amphetamine the peak levels did not appear to be affected. Instead the lower activity was due to a more rapid decline from peak values at 7 ata compared to 4 ata, figs.(4b,c). For morphine the cause of the reduction at the higher dose appeared to be due to a tendency to a decline in the peak values at 7 ata compared to 4 ata, Fig.(8). At present the reason for the decline in activity is not clear. Increasing the dose of morphine at 1 ata increases activity up to a certain point, beyond which further increases

in dose produce less activity. Thus this effect may just reflect a shift in the dose response curve to the left produced by hyperbaric air. In the present experiments a full dose response curve for the effect of morphine at 1 ata was not performed, thus it is not possible to determine the magnitude of the shift. An attempt was made to do this by using dose response curves in the literature. The dose at which the peak effect of morphine occurred varied quite considerably between studies however. In a number of studies it was the order of 100 mg/kg and above (Villareal et al., 1973 ; Carroll and Sharp, 1972 ; Ayhan et al., 1979). There is one study however, in which the effect peaked at 30 mg/kg (Hynes and Berkowitz, 1979). Thus given this variation, and the differences in experimental design between the present study and others in the literature, it was not possible to come up with an estimate from the literature.

The rapid onset of the changes in activity and their complex nature, make it unlikely that they are due to an alteration of in morphine pharmacokinetics at pressure.

As is the case for amphetamine, the NAc also appears to be implicated in the locomotor stimulation produced by morphine. Thus lesions of the NAc also reduce the locomotor response to morphine in mice although the reduction is not as complete as for amphetamine (Teitelbaum et al., 1979). It is possible therefore, that as suggested for amphetamine, the enhancement of morphine-induced hyperactivity may also be due to an interaction with the disinhibition produced by N_2 at these pressures. Possible mechanisms for these changes at pressure will be discussed in the general discussion in chapter 5.

3.3.3 EFFECT OF HYPERBARIC ENVIRONMENTS ON SPONTANEOUS ACTIVITY

3.3.3.1 INTRODUCTION

Experiments were conducted to determine if increased pressures of air, or the other gas mixtures N_2/O_2, and He/O_2, that might be used in the experiments with DPH and alcohol, affected spontaneous activity. Two series of experiments were performed. In the first, the activity of saline treated mice was determined at 1, 4, and 7 ata air, and in the second at 1, and 7 ata air, and 7 ata of He/O_2, and N_2/O_2.

3.3.3.2 METHODS

The experimental design was as described in the general methods. Mice from three litters were used in the first experiment, and from two in the second. Three mice were tested at one time thus giving a total of 9 mice per treatment group in the first experiment and a total of 6 in the second experiment. After injection with saline (0.1 ml/10g body weight), the mice were transferred to the chamber and exposed to the appropriate pressure/gas mixture combination. A red light was used for handling, and observation of the mice. Activity counts were recorded at 5 min intervals commencing 5 min after injection. Also, behavioral observations were made at 15, 20 and 30 min after injection, and thereafter at 15 min intervals for the remainder of the experiment. The procedure followed in this, and the later alcohol study, was slightly different from that described in the general methods however. In these studies, the observation period was reduced from 1 min to 30 sec. Also,

due to the dim lighting conditions, it was only possible to make detailed observations on two of the mice. These, and the subsequent experiments with DPH and alcohol, were conducted 4 hr ± 30 min into the dark cycle, and were of 2 hr duration.

3.3.3.3 RESULTS

In analyzing the results of this and the subsequent studies with DPH and alcohol, a different method to that used in the studies on amphetamine and morphine was employed. In comparison to the well defined, uniform effects on locomotor activity produced by amphetamine and morphine, the effects of these drugs particularly alcohol were more complex, and in some cases short lived. Thus an analysis of cumulative activity over the 5-60, or 60-120 min periods following injection, would in some cases cause significant effects on activity to be missed. Therefore to determine the effect of a particular treatment on activity, it was decided as a standard procedure to analyze the 15 min activity totals, as well as the 5-60 and 60-120 min cumulative totals. This it was felt would allow for adequate detection of drug effects.

The cumulative activity totals for the experiments with air are presented in Table (5). At 1 ata saline treated mice displayed high levels of activity which declined only slightly throughout the 2 hr experimental period. Exposure to 4 or 7 ata did not produce any marked changes in activity compared to that at 1 ata, and no significant differences were found between the cumulative activity totals, Table (5). Similarly, no difference was observed between the 15 min activity

TABLE 5

Cumulative Activity Totals for Saline Treated Mice

at 1, 4 and 7 ata air

Time Interval (min)	Pressure		
	1 ata	4 ata	7 ata
5 - 60	3527.9 ± 206.8	3176.2 ± 285.2	3466.9 ± 218.5
60 - 120	3156.4 ± 207.1	3191.1 ± 372.1	3566.4 ± 266.0
5 - 120	6584.3 ± 307.8	6367.3 ± 588.6	7033.3 ± 419.2

Values are mean ± SEM (n=9)

totals (data not shown). The predominant types of activity displayed by the mice were walking or running, rearing at the walls of the boxes, and biting the rods of the cage.

In the second experiment, similarly none of the hyperbaric environments produced any significant alteration in activity, see Fig (10), Table (6). There was a tendency however, to increasing activity levels with increasing PN_2 Table (6).

3.3.3.4 DISCUSSION

From these results it can be concluded that the hyperbaric environments tested have no appreciable effect on the spontaneous activity of mice. In particular no depressant effect is observed . If anything, exposure to increased pressures of N_2 in this range would appear to have a tendency to enhance activity. Thus the mean 5-120 min cumulative activity levels for all the hyperbaric exposures were higher than at 1 ata, this effect being most marked for 7 ata N_2O_2

TABLE 6

Cumulative Activity Totals for Saline Treated Mice

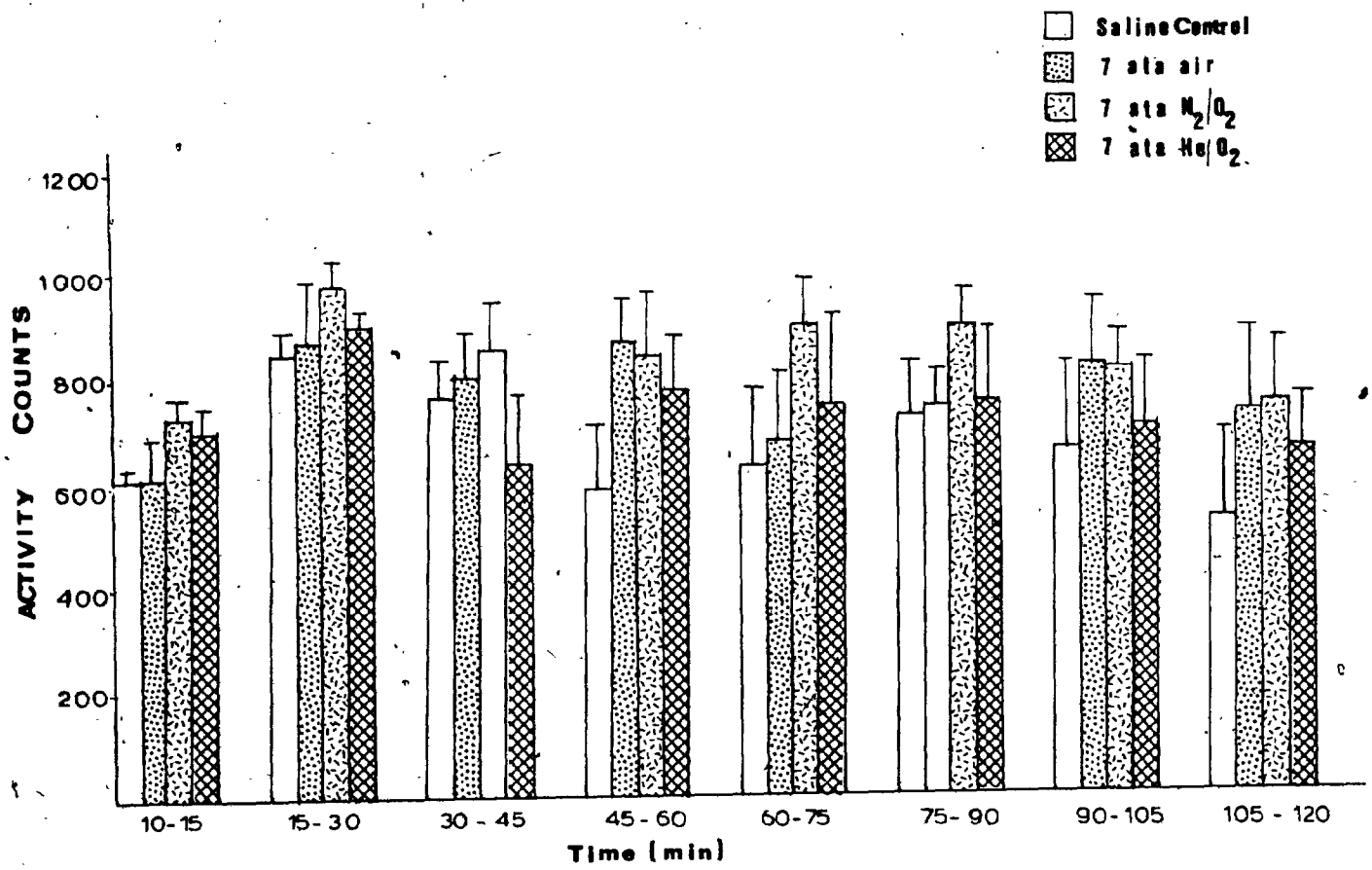
at 1, 7 ata Air, and 7 ata He/O_2 and N_2/O_2

Time Interval (min)	Breathing Mixture			
	1 ata air	7 ata air	7 ata N_2/O_2	7 ata He/O_2
5 - 60	2796.5 ± 218.2	3114.2 ± 284.8	3394.8 ± 274.7	3064.0 ± 217.5
60 - 120	2585.8 ± 503.6	3007.8 ± 426.3	3356.8 ± 316.1	2871.7 ± 513.6
5 - 120	5382.3 ± 712.3	6122.0 ± 499.2	6751.7 ± 584.8	5935.7 ± 696.1

Values are (mean ± SEM) (n=6)

Figure 10

Graph of 15 min. totals for saline treated mice at 1, and 7 ata air, 7 ata N_2/O_2, and 7 ata He/O_2. Vertical Bars = 1 SEM. (n=6).


Saline Control
7 ata air
7 ata N_2/O_2
7 ata He/O_2
ACTIVITY COUNTS
1200
1000
800
600
400
200
10-15
15-30
30-45
45-60
60-75
75-90
90-105
105-120
Time (min)

3.3.4 STUDIES ON DIPHENHYDRAMINE

3.3.4.1 INTRODUCTION AND METHODS

DPH is known to produce sedation in humans (Douglas, 1975). Very litte information is available however, in regard to its effects, or those of other antihistamines, on locomotor activity in animals such as mice and rats. In fact only two studies in which information regarding the effect of DPH on locomotor activity was presented were found. In one study, DPH 40 mg/kg i.p., was reported to produce excitation in mice, the mice being observed to run or jump intermittently with slight attacks of clonic seizures (Chen and Bohner, 1958). In the other study, DPH 50 mg/kg (p.o.), was reported not to significantly affect the activity of mice (Bastian, 1961). In both of these studies the reporting of the experimental details, and results on locomotor activity were very sketchy.

In the present experiments the effect of DPH, 10 and 20 mg/kg s.c., (free base), were studied. These doses were found in preliminary experiments to produce minimal and marked depression of spontaneous activity respectively. DPH was administered as the hydrochloride salt (Parke/Davis), in a volume of 0.05 ml saline/10g body weight.

In all experiments three mice were tested at one time. For the 10 mg/kg dose experiments were conducted at 1, 4 and 7 ata air, with saline controls at 1 ata also being performed. Three runs were performed for each treatment, giving a total of 9 mice/treatment group.

For the 20 mg/kg dose, three series of experiments were performed. In the first series the effect of DPH was tested at 1, 4 and 7 ata air. Two runs were performed at each pressure giving a total of 6 mice/treatment group. In the second series, experiments with N_2/O_2 (95/5) were conducted to determine the effect of increasing the PN_2's to values greater than those in the 1-7 ata air range, on the response to DPH. Experiments were performed at 1 and 7 air, and 7 ata N_2/O_2. Three runs were performed at each pressure giving a total of 9 mice/treatment group. Saline controls were included in two of these runs, giving a total of 6 mice in this group. In the third series, the effect of 4 ata He/O_2 on the response to DPH was tested. A pressure of 4 ata was used, since in the experiments with air this pressure was more effective than 7 ata in counteracting the DPH-induced depression of spontaneous activity. In this series, experiments were conducted at 1 and 4 He/O_2, with saline controls at 1 ata He/O_2 also being performed. Two runs were performed for each treatment, giving a total of 6 mice/treatment group.

At the time these studies were performed, the decision to institute detailed behavioral observations using the behavioral categorization technique had not been made. Brief observations of the mice were made at 10 min intervals throught the experiment however.

3.3.4.2 RESULTS

At 1 ata, injection of DPH 10 mg/kg produced a short-lived depression of activity over the 15-45 min period following injection, Fig.(11). Exposure to 4, and 7 ata air appeared to counteract the effect of DPH to decrease activity, as the decline in activity over the same period at pressure was minimal Fig.(11).

Analysis of the 15 min totals Fig.(12), revealed a statistically significant difference between treatments only at the 30-45 min period following injection [$F(3,6) = 6.47$, $p < 0.05$]. Further tests revealed that over this period the activity of DPH treated mice at 1 ata was significantly lower than that of saline controls. In contrast there was no statistically significant difference between the activity of DPH treated mice at 4 and 7 ata air and saline controls, confirming the ability of hyperbaric air to counteract the depression of activity produced by this dose of DPH. A trend to statistical significance was observed for the 105-120 min period following injection, [$F(3,6) = 4.30$, $p < 0.1$]. This was due to the tendency for higher activity levels over this period in DPH treated mice at 7 ata air, compared to the other treatments, Fig.(12). Analysis of the 5-60, and 60-120 min cumulative totals Table (7), revealed no statistically significant differences between the various treatments.

DPH at 20 mg/kg produced a much greater depression of activity compared to the 10 mg/kg dose, with activity being markedly depressed over the 15-60 min period following injection, Figs.(13,15). As was seen at the 10 mg/kg dose, exposure to 4 and 7 ata air, tended to antagonize the effect of DPH on activity. Four ata air

Figure 11

Graph of 5 min. activity counts for mice given DPH 10 mg/kg at 1 (◇), 4 (▽) and 7 (□) ata air, and saline controls (◆) at 1 ata air. (n=9). Standard error bars have been omitted for clarity.

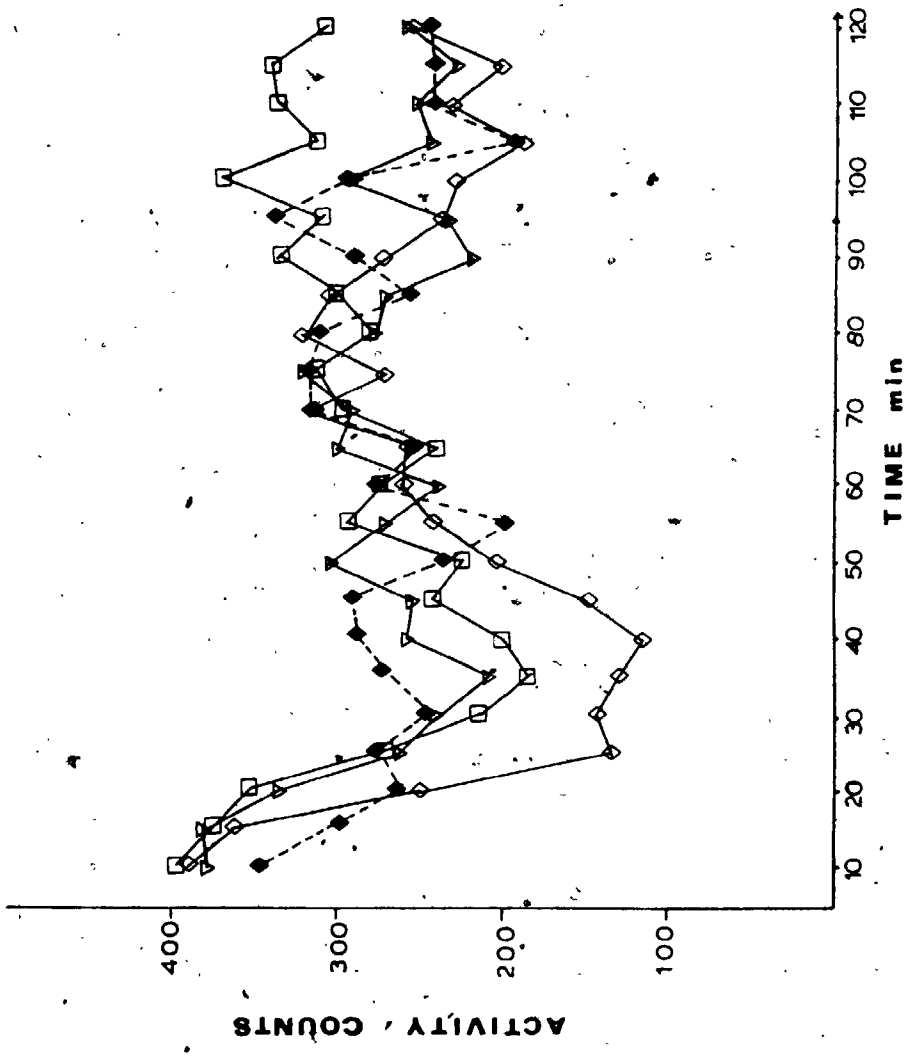

ACTIVITY / COUNTS
400
300
200
100
10
20
30
40
50
60
70
80
90
100
110
120
TIME min

Figure 12

Graph of 15 min. activity totals for mice given DPH 10 mg/kg at 1, 4, and 7 ata air , and saline controls at 1 ata air. (n=9). Vertical bars = 1 SEM. * sig. vs saline controls at 1 ata.

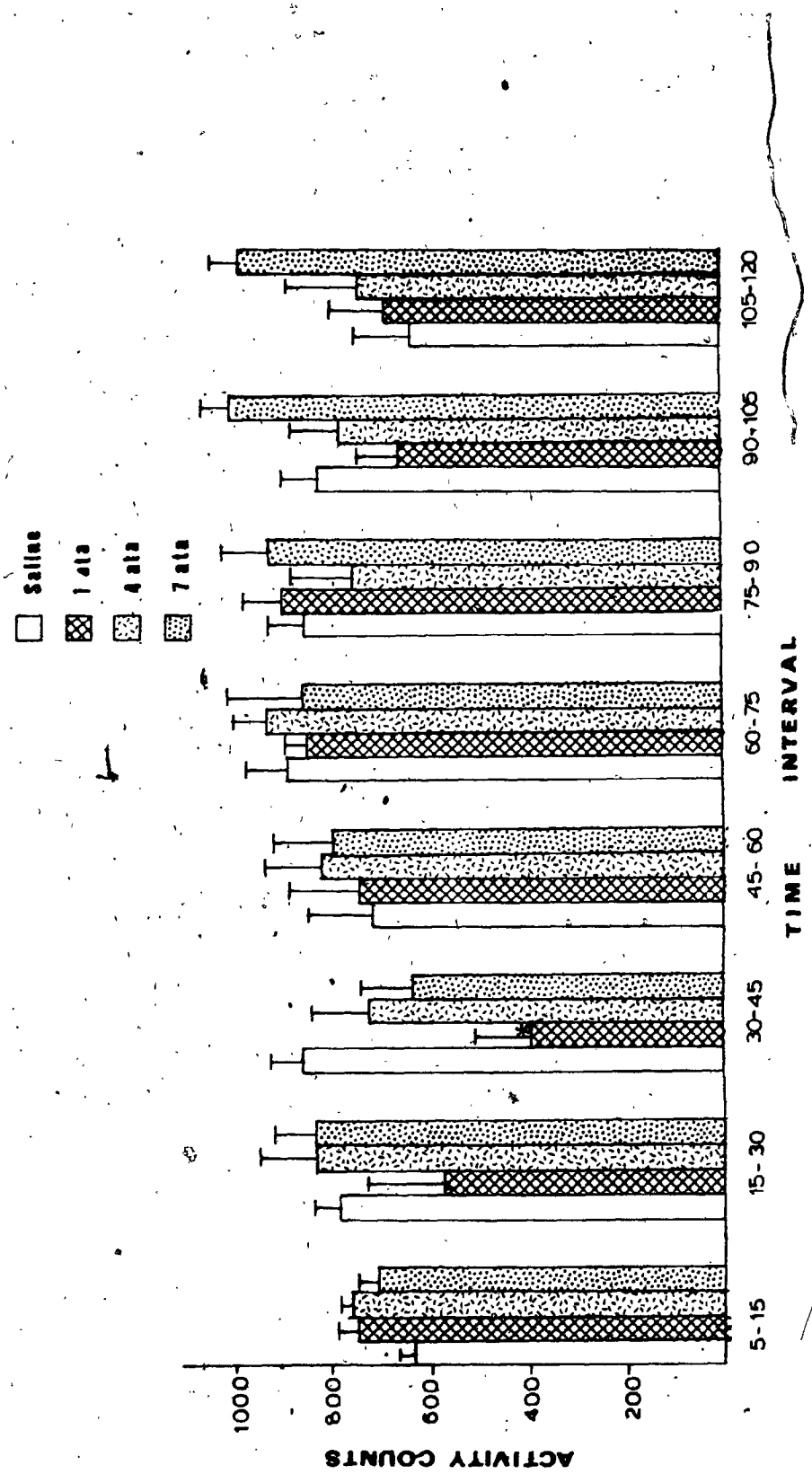

Saline
1 ata
4 ata
7 ata
ACTIVITY COUNTS
1000
800
600
400
200
5-15
15-30
30-45
45-60
60-75
75-90
90-105
105-120
TIME INTERVAL

TABLE 7

Cumulative Activity Totals for DPH (10 mg/kg) Treated Mice at 1, 4, 7 ata Air, and Saline Controls at 1 ata Air

Time Interval (min)	Saline	DPH		
	1 ata	1 ata	4 ata	7 ata
5 - 60	3002.1 ± 299.6	2472.2 ± 316.9	3138.9 ± 305.2	3041.1 ± 389.7
60 - 120	3116.8 ± 327.3	3079.4 ± 270.7	3213.0 ± 368.7	3747.8 ± 323.9

Values are (mean ± SEM) (n=9)

appeared more effective than 7 ata air in this regard, Fig.(13). During the second 60 min period, the activity levels of DPH treated mice at 1 and 4 ata air tended to return to saline control levels. For mice at 7 ata however, the recovery from the depression produced by DPH was not as complete as at the other pressures, and activity tended to remain below control levels, Fig.(13).

Analysis of the 15 min totals revealed statistically significant differences between treatments over the 30-45 and 45-60 min time periods, [$F(3,3) = 19.3$, 18.5 respectively, $p < 0.05$], the activity of DPH treated mice at all pressures being significantly reduced compared to that of the saline controls, Fig.(14). No significant differences were observed between the activity of DPH treated mice at 1, 4 or 7 ata air however. A statistically significant difference between treatments was also observed for the 5-60 min cumulative activity totals, [$F(3,3) = 11.8$, $p < 0.05$], Table (8A). Over this period the activity of DPH treated mice at 1 and 7, but not 4 ata air was significantly reduced compared to that of the saline controls. This confirmed the greater ability of air at 4, compared to 7 ata, to offset the depression of activity produced by this dose of DPH. No statistically significant differences in activity were observed over the 60-120 min period following injection.

In the experiments with N_2/O_2 the effects of DPH in mice exposed to 1 and 7 ata air were similar to those seen at these pressures in the first series of experiments with air, Fig.(15). Increasing the PN_2 through exposure to 7 ata N_2/O_2, still did not enhance the depression of activity produced by DPH. Instead, like 7 ata air, 7 ata N_2/O_2 acted to

Figure 13

Graph of 5 min. activity counts for mice given DPH at 20 mg/kg at 1 (◇), 4 (▽) and 7 (□) ata air, and saline controls (◆) at 1 ata air. (n_i = 6). Standard error bars have been omitted for clarity.

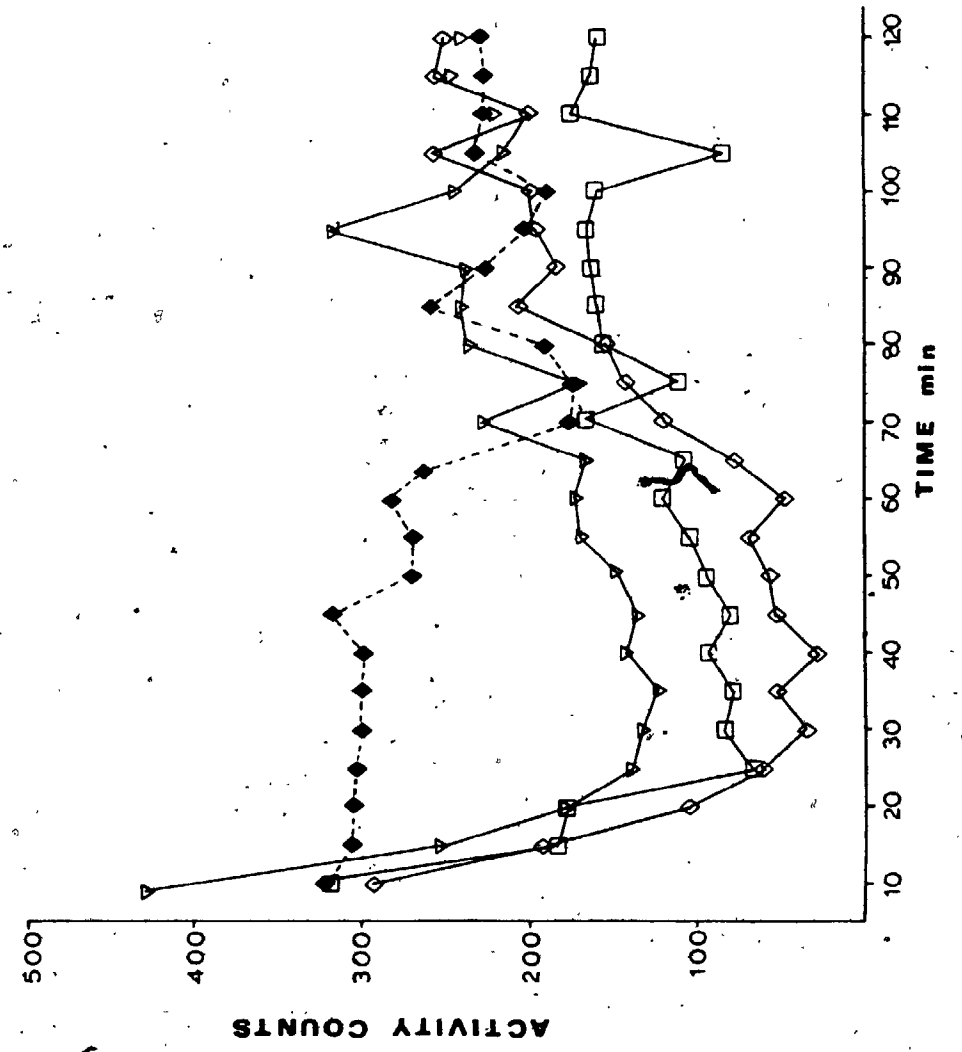
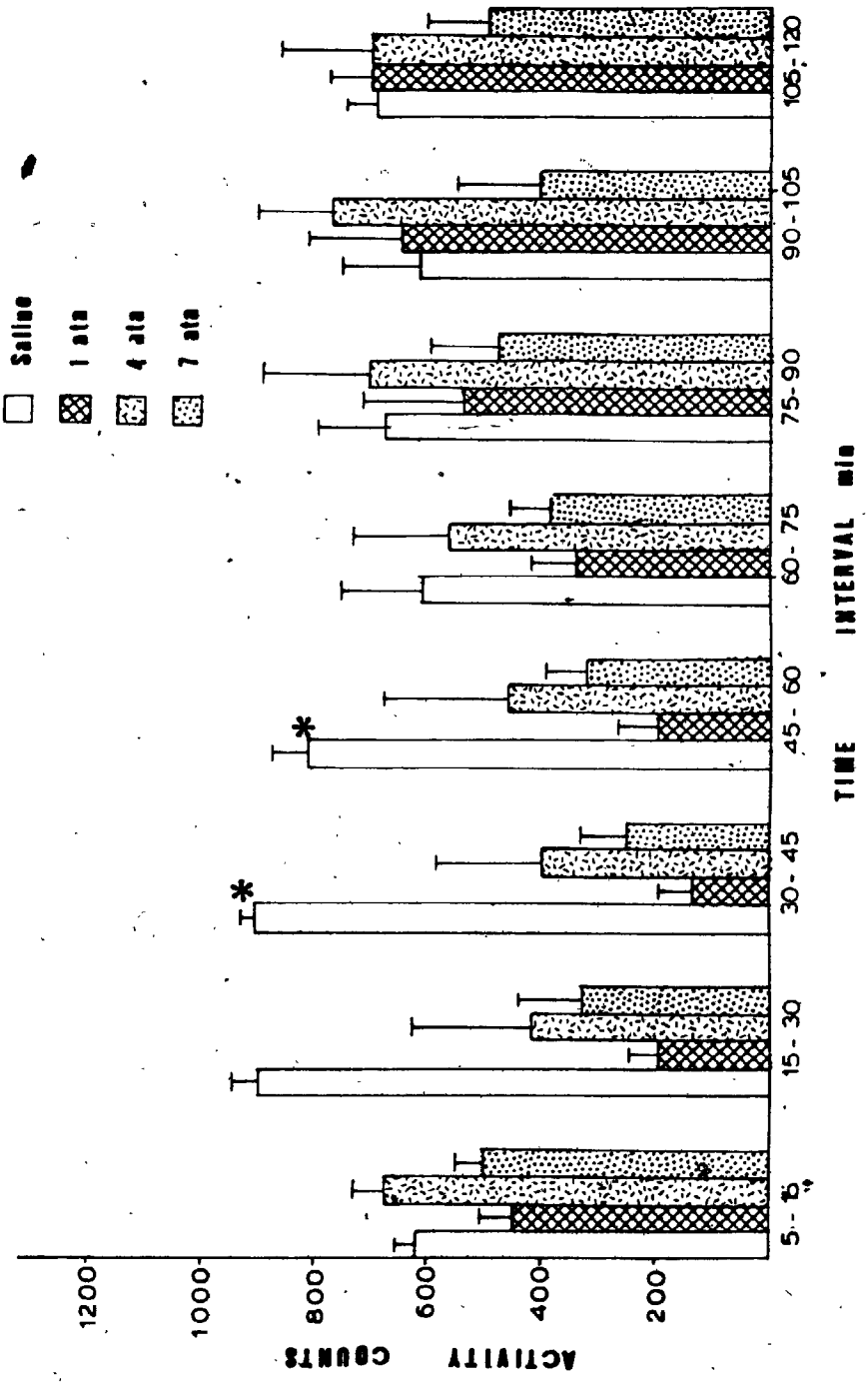

ACTIVITY COUNTS
500
400
300
200
100
10
20
30
40
50
60
70
80
90
100
110
120
TIME min

Figure 14

15 min. activity totals for mice given DPH 20 mg/kg at 1, 4 and 7 ata air, and saline controls at 1 ata air. (n = 6). Vertical bars = 1 SEM. * sig. p, 0.05 vs. DPH treated mice at 1, 4 and 7 ata air.


Saline
1 ata
4 ata
7 ata
ACTIVITY COUNTS
1200
1000
800
600
400
200
5-15
15-30
30-45
45-60
60-75
75-90
90-105
105-120
TIME INTERVAL min
*
*

reduce the depressant effect of DPH on activity Fig.(15). Their effects over the second hr of the experiment differed however. Whereas activity at 7 ata N_2/O_2 returned to control levels during the second 60 min period, as was the case in the first series of experiments with air, activity at 7 ata air again tended to remain below control levels, Fig.(15).

Analysis of the 15 min activity totals for the two series of experiments in which saline controls were included, revealed significant differences in activity over the 15-30 and 30-45 min periods after injection [$F(3,3) = 43.3$, 123.5 respectively, $p < 0.01$]. At both of these time periods the activity of saline treated mice was significantly higher than that of DPH treated mice at all pressures, Fig.(16). Over the 5-60 min period, activity of DPH treated mice at all pressures was also significantly depressed compared to saline controls [$F(3,3) = 31.8$, $p < 0.01$], Table (8B). Analysis of the data for DPH alone, indicated that over the 30-45 min period following injection, the activity of DPH treated mice at 7 ata air, and 7 N_2/O_2 was significantly higher than that at 1 ata, [$F(2,4) = 10.0$, $p < 0.05$], Fig.(16). This is an indication of the trend for these mixtures to reduce not enhance the DPH induced depression of spontaneous activity. No statistically significant differences between the effects of 7 ata air and 7 ata N_2/O_2 were observed at any time. Also no statistically significant differences between treatments were observed over the 60-120 min period.

In contrast to the effects produced by hyperbaric air, exposure to 4 ata He/O_2 did not reduce the effect of DPH 20 mg/kg on activity, Table (9).

Figure 15

Graph of 5 min activity totals for mice given DPH 20 mg/kg at 1 (◇), and 7 (□) ata air, 7 ata N_2/O_2 (■), and saline controls at 1 ata (◆). (n=9) for DPH treated mice. (n=6) for saline controls. Standard error bars have been omitted for clarity.

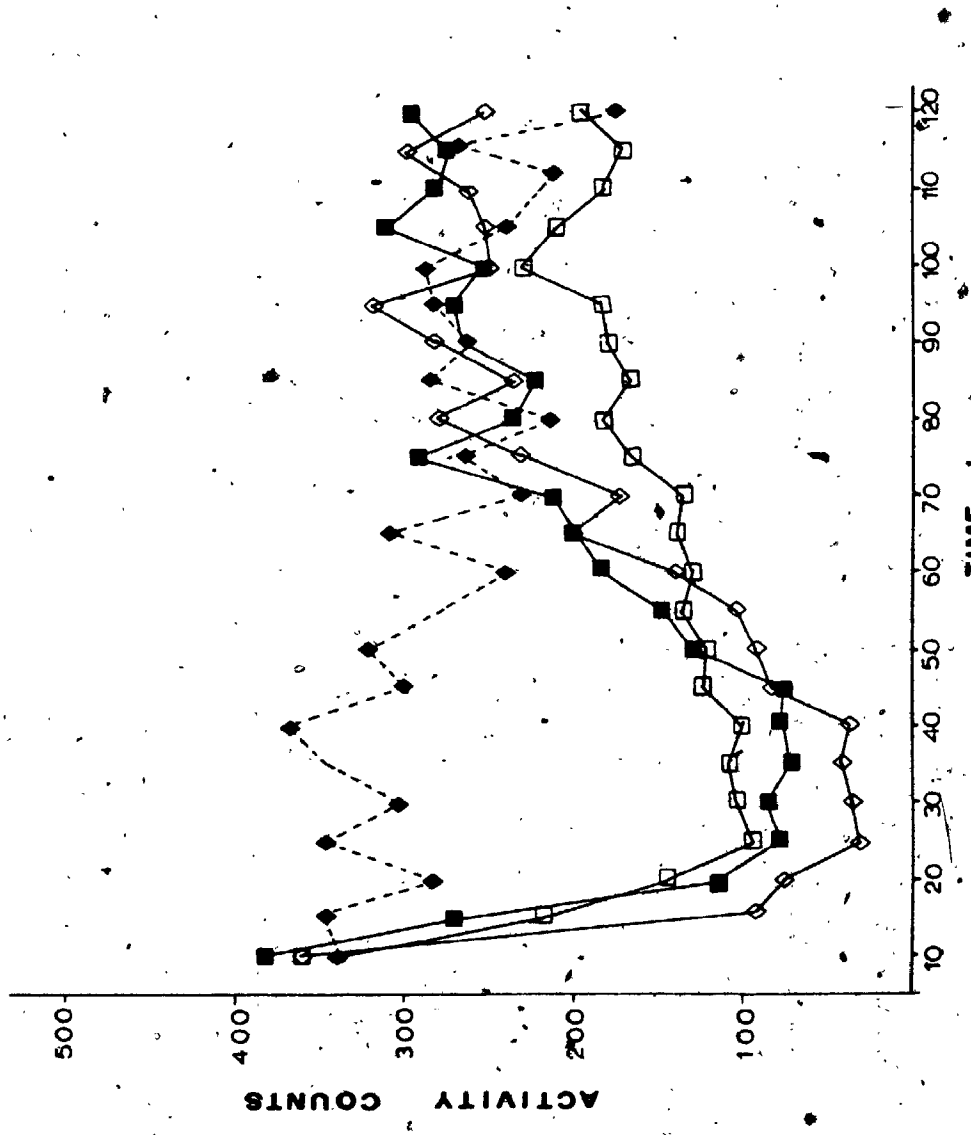

ACTIVITY COUNTS
500
400
300
200
100
10
20
30
40
50
60
70
80
90
100
110
120
TIME min

Figure 16

15 min activity totals for mice given DPH 20 mg/kg at 1 and 7 ata air 7 ata N_2/O_2, and saline controls at 1 ata air. (n=9) for DPH treated mice. (n=6) for saline controls. Vertical bars = 1 SEM.

* sig. $p < 0.05$ vs. DPH treated mice

** sig. $p < 0.05$ vs DPH treated mice at 1 ata air.

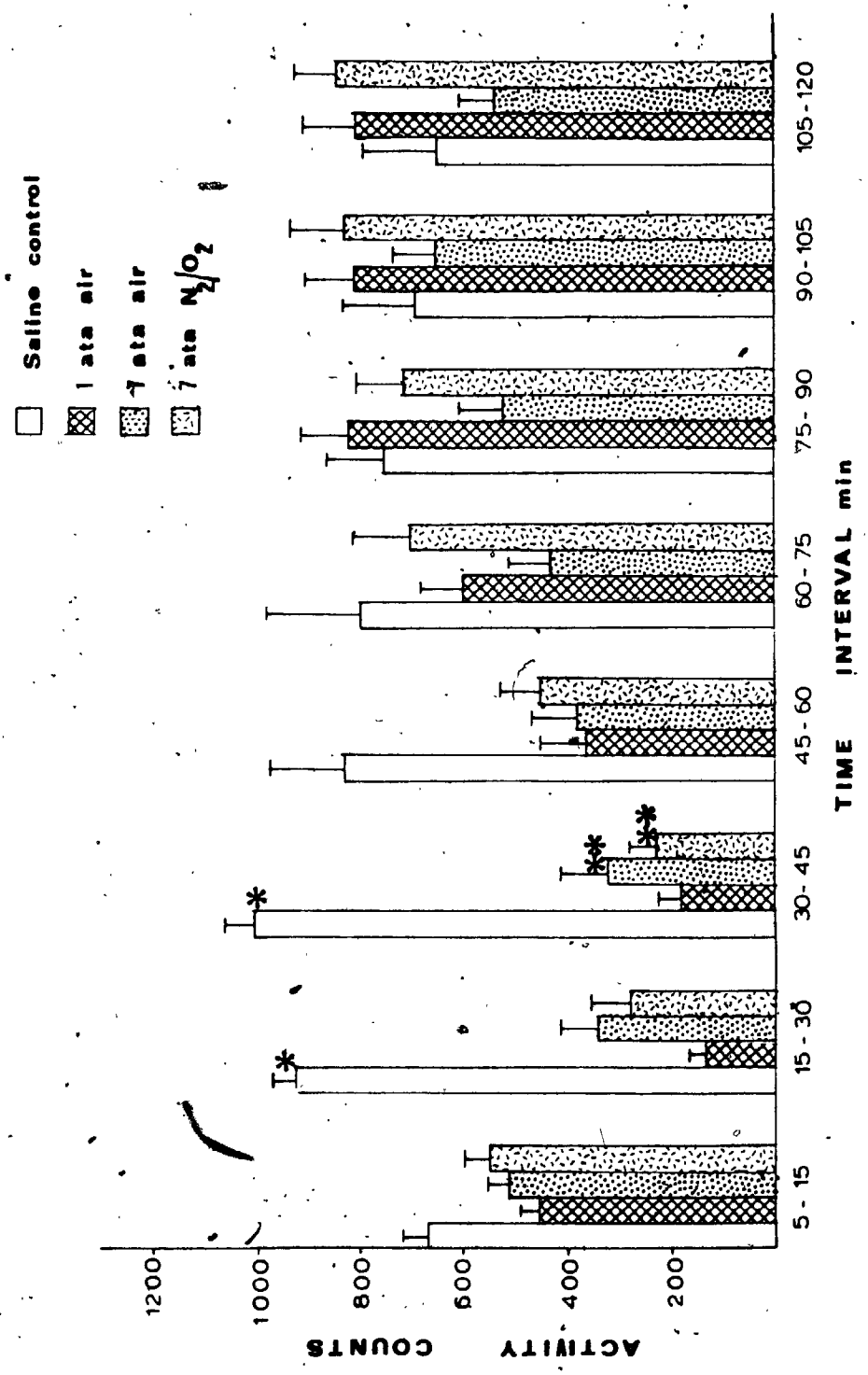

Saline control
1 ata air
4 ata air
4 ata N_2/O_2
ACTIVITY COUNTS
1200
1000
800
600
400
200
5 - 15
15 - 30
30 - 45
45 - 60
60 - 75
75 - 90
90 - 105
105 - 120
TIME INTERVAL min

TABLE 8A

Cumulative Activity Totals for DPH (20 mg/kg) Treated Mice at 1, 4, 7 ata Air, and Saline Controls at 1 ata Air

	Saline	DPH		
Time Period (min)	1 ata	1 ata	4 ata	7 ata
5 - 60	3296.2 ± 157.3*	990.8 ± 194.4	2027.5 ± 681.9	1421.8 ± 292.5
60 - 120	2672.3 ± 355.2	2263.5 ± 455.3	2778.8 ± 628.0	1787.2 ± 347.4

Values are (mean ± SEM) n=6
*sig vs 1, 7 ata p 0.05

TABLE 8B

Cumulative Activity Totals for DPH (20 mg/kg) Treated Mice at 1, 7 ata Air, and 7 ata N_2/O_2, and Saline Controls at 1 ata

	Saline	DPH		
Time Period (min)	1 ata Air	1 ata Air	7 ata Air	7 ata N_2/O_2
5 - 60	3436.5 ± 146.6*	998.7 ± 165.7	1543.5 ± 391.5	1579.0 ± 288.2
60 - 120	2911.7 ± 330.7	2751.0 ± 416.7	2361.0 ± 173.6	3473.3 ± 267.6

Values are mean ± SEM DPH (n=9) Saline (n=6)
*sig vs 1, 7 ata air, 7 ata N_2/O_2.

TABLE 9

Cumulative Activity Totals for DPH (20 mg/kg) Treated Mice at 1 and 4 ata He/O_2, and Saline Controls at 1 ata He/O_2

Time Interval (min)	Saline	DPH	
	1 ata	1 ata	4 ata
5 - 60	2561.0 ± 194.2	996.7 ± 369.2*	1035.0 ± 245.7*
60 - 120	2372.7 ± 230.5	1940.3 ± 154.5	1915.2 ± 217.5

Values are mean ± SEM (n=6)

* sig. vs saline controls [$F(2,2) = 39.68$, $p < 0.05$]

3.3.4.3 DISCUSSION

DPH produced a dose dependent depression of the activity of mice, the peak effect occurring over the 15-45 min period following injection. Exposure to hyperbaric air did not enhance this depression of activity produced by DPH, but instead tended to reduce it. This was evident at both doses of DPH, but was more marked at the lower dose. Surprisingly, 4 ata air was more effective than 7 ata air in in counteracting the DPH induced depression of activity, Table (8A). Increasing the PN_2 by using the N_2/O_2 mixture, was also ineffective in enhancing the depression produced by DPH. This gas mixture also acted to reduce the DPH induced depression of activity.

This effect of hyperbaric air would appear to be due to the effect of the increased PN_2, rather than the PO_2 or hydrostatic pressure. This is shown by the fact that 4 ata He/O_2, unlike 4 ata air, had no effect on the depression of activity produced by DPH Table (9). In addition the tendency of 7 ata N_2/O_2 (in which the PO_2 is only 0.35 ata), to counteract the effect of DPH, confirms the unimportance of the increase in PO_2 for the effects seen.

The rapidity of the effect, and the fact that 4 ata was more effective than 7 ata, suggests that these effects are not due to an alteration in the pharmacokinetics, or reduction in brain levels of DPH at pressure. Also, the reduction in the effect of DPH would not appear to be due to the trend for activity of mice to be increased with increasing PN_2, (see Table (6), spontaneous activity studies). If this were so, then 7 ata should have been more effective than 4 ata air in counteracting the depression of activity produced by DPH. This was not

the case however. Thus the modification of DPH's effect on activity would appear to be due to an interaction between the central effects of the drug and those of N_2.

Similarly to the results with amphetamine and morphine, these results do not support the classical view that N_2 produces a generalized depression of CNS activity (Bennett, 1966 ; 1975). In fact these results suggest that excitatory effects are produced in the CNS by increased PN_2 which tend to counteract the depression produced by DPH. The fact that air at 4 ata was more effective than at 7ata in reducing the effect of DPH was at first surprising. This can be explained however, by postulating that N_2 produces both excitatory and depressant effects on CNS activity. Thus whereas the excitatory changes produced by N_2 would tend to offset the depressant effect produced by DPH, the depressant effects of N_2 would tend to enhance it. The net result on activity therefore, would depend on the relative strength of these two effects. At 7 ata therefore, it is possible that the greater depressant effect of N_2 at this pressure adds to the CNS depression produced by DPH and thereby tends to offset the antagonism produced by the excitatory effects. At 4 ata however, the depressant effect at this pressure may not be great enough to significantly add to the CNS depression produced by DPH. Therefore the antagonism of the depressant effect of DPH by the excitatory changes at this pressure, may not be offset to any great extent by a corresponding increase in CNS depression. Consequently, a greater antagonism of the DPH induced depression of spontaneous activity will be observed at this pressure. Further support for the proposal that N_2 produces both excitatory and depressant effects on CNS function will be presented in chapter 5.

The fact that in contrast to the other groups, the activity of DPH treated mice at 7 ata air never fully returned to control levels, Figs.(11,13), is interesting. This effect would appear to be related to an interaction between N_2 and the increased PO_2, since it did not occur with the N_2/O mixture at the same pressure. In the latter mixture, the PO_2 was only 0.35 ata, compared to approx. 1.5 ata with air. This effect would appear to be reproducible since it was observed in the series of experiments with air, as well as in those with N_2/O_2. At present the reason for this effect is not known. As was described in chapter 2 (section 2.2.1), it was considered that an increase in PO_2 could enhance the effect of N_2 in two ways. For example the anesthetic effect of O_2 (Smith and Payton, 1976) could add to that of N_2 to increase its depressant effect. Alternatively, it was proposed that CO_2 retention produced by breathing elevated PO_2's, was responsible (Frankenhauser, et al., 1963). Whether these or other mechanisms are involved is not known.

In summary therefore, the experiments indicate that exposure to hyperbaric air can modify the effects of DPH on spontaneous activity. Although the significant modifications appeared to be primarily due to N_2, the changes observed were complex and suggestive of the fact that N_2 in this range produces both excitatory and depressant effects.

3.3.5 STUDIES ON ALCOHOL

3.3.5.1 INTRODUCTION AND METHODS

Alcohol is known to produce biphasic effects on locomotor activity in mice, with excitation being observed at low doses and depression at high doses (Matchett and Erickson, 1977 ; Deimling and Schnell, 1980). The doses used in the present experiments were 1.75 and 2.75 g/kg. These were found to produce significant alterations in activity, and were below doses producing loss of righting reflex.

Alcohol was administered i.p. as a 10% (w/v) solution in saline. The use of this solution necessitated the use of fairly large volumes, 0.44 - 0.53 ml for the low dose, and 0.69 - 0.825 ml for the high dose. These volumes, although large are still within the acceptable limits for i.p. injection in mice (McLeod et al., 1970). The 10% solution was used in preference to the 20% (w/v) solution frequently cited in the literature and which allows smaller volumes to be used, because in initial experiments the higher concentration appeared to produce gastrointestinal (GI) discomfort (writhing) in mice. Furthermore a trial experiment revealed that activity over the 5 - 60 min period following injection of alcohol 1.75 g/kg, was significantly lower for mice given the 20 % solution compared to the 10% solution which produced no writhing [Mean Activity 1563.2 + 317.5 vs. 2414.2 + 258.8, n = 6 in each group]. Thus to avoid non-specific effects on activity due to GI discomfort, the more dilute solution was used.

The experimental design, activity measurements, and behavioral observations were as described for the studies on spontaneous activity (Section 3.3.3.2). Experiments were performed at 1, 4 and 7 ata air, and a saline control at 1 ata was performed at each dose. The volume of saline injected was adjusted to be commensurate with the larger volumes administered in these experiments. Experiments were also carried out with He/O_2 to determine that the effect seen with air was due to N_2.

3.3.5.2 RESULTS

Injection of alcohol 1.75 g/kg at 1 ata produced a rapid onset excitation (1.5 - 2 min following injection), manifested by running, and rearing at the sides of the test boxes. Ataxia was also observed. This initial excitation persisted with gradually diminishing intensity, until 15 min after injection, and was followed by a steady decrease in activity over the remainder of the experiment, Figs.(17,18). Injection of 2.75 g/kg at 1 ata also produced a rapid onset excitation and ataxia. The initial excitatory phase was of shorter duration however, being replaced by a quiescent phase in which some activity was observed, but mice were frequently observed lying in their cages or grooming themselves. Following this, activity levels tended to increase until approximately 60 min post injection, after which a gradual decline in activity occurred Figs.(19,20).

Exposure to hyperbaric air affected the response to alcohol in a complex manner, with the effect observed depending on the dose and pressure of air. At the lower dose, exposure to both 4 and 7 ata air acted to enhance the initial excitation in that higher mean activity levels were observed at these pressures over this period Figs.(17,18). Analysis of the 15 min totals over this period, or the 5-60 min cumulative totals Table(10A), did not reveal any significant differences between treatments however. Thus this effect of hyperbaric air was not significant. In addition, exposure to 7 but not 4 ata air, appeared to offset the gradual decline in activity produced by this dose at 1 ata air. For example, the rate of decline in activity tended to be slower at 7 ata compared to 4 ata, and unlike at 4 ata, appeared to plateau

Figure 17

Graph of 5 min. activity counts for mice given alcohol 1.75 g/kg at 1 (◇), 4 (▽) and 7 (□) ata air, and saline controls (◆) at 1 ata air. Standard error bars have been omitted for clarity.

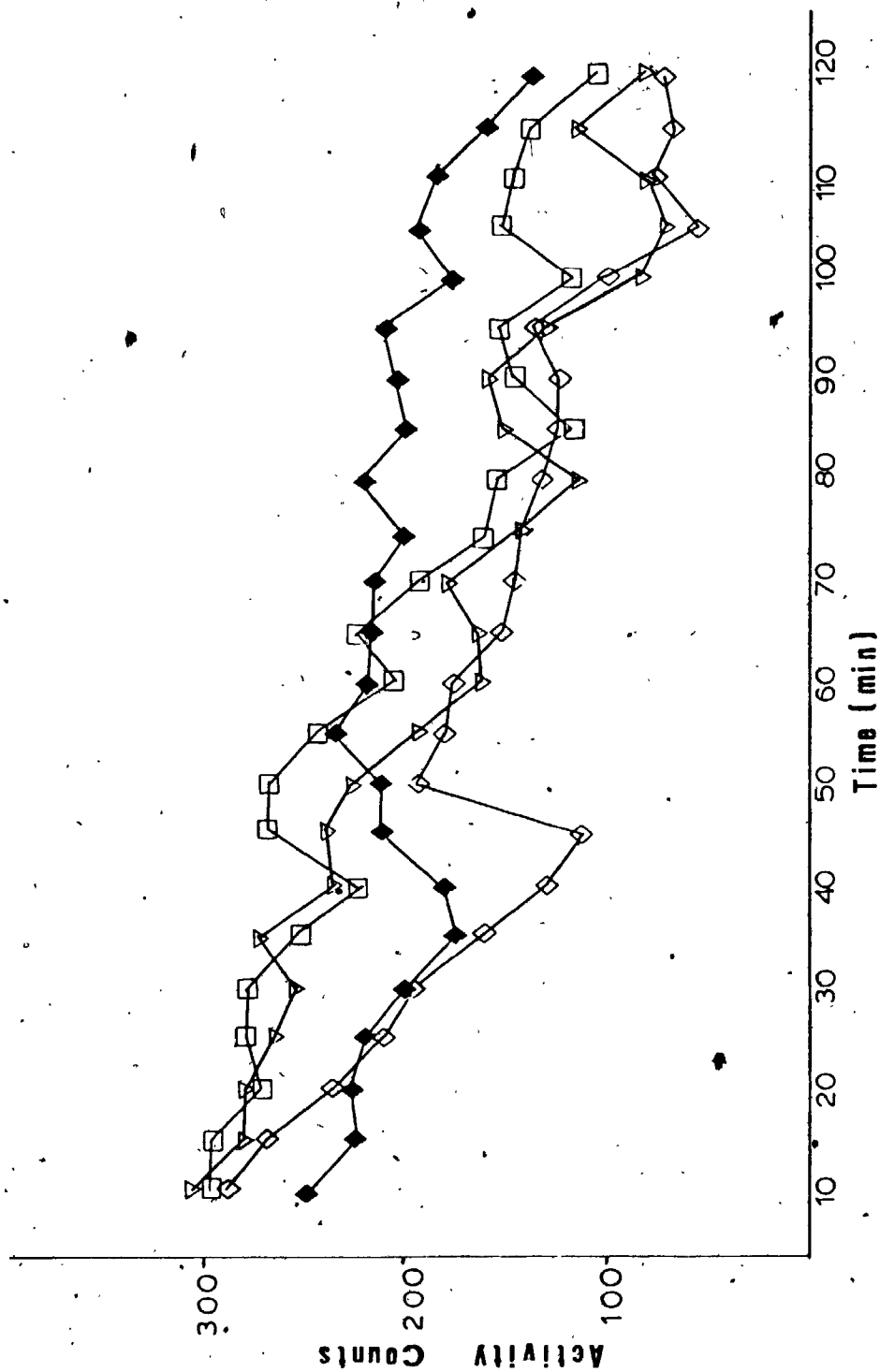
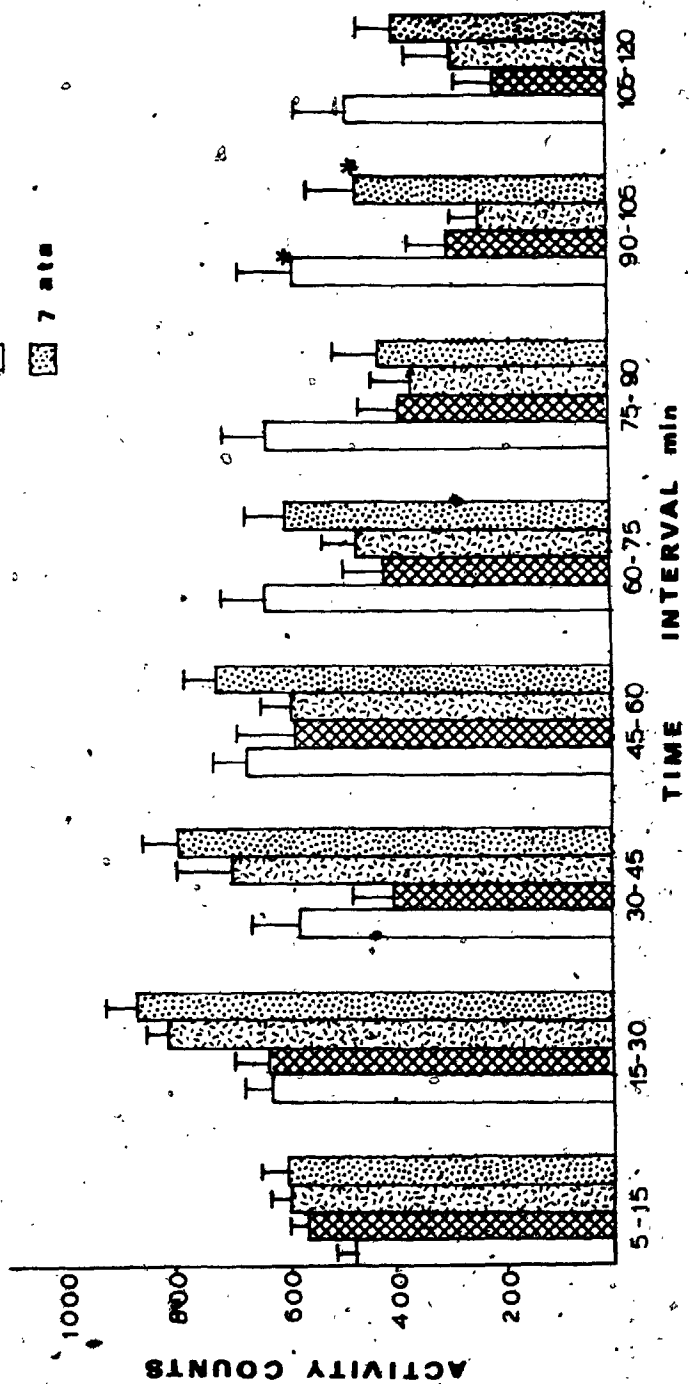

Activity Counts
300
200
100
10
20
30
40
50
60
70
80
90
100
110
120
Time (min)

Figure 18

Graph of 15 min activity totals for mice given alcohol 1.75 g/kg at 1, 4 and 7 ata air, and saline controls at 1 ata air. Vertical bars = 1 SEM.

* sig. vs. alcohol treated mice at 1 and 4 ata air.


Saline
1 ata
4 ata
7 ata
ACTIVITY COUNTS
1000
800
600
400
200
5-15
15-30
30-45
45-60
60-75
75-90
90-105
105-120
TIME INTERVAL min

approximately 90 min post injection, Figs.(17,18). Analysis of the 15 min activity totals over the second hr revealed a statistically significant difference between treatments only for the 90-105 min post injection period. This was due to activity of alcohol treated mice at 1 and 4 ata being significantly reduced compared to that of saline controls, and alcohol treated mice at 7 ata air, [$F(3,6) = 12.3$, $p < 0.05$], Fig.(18). Also, an analysis of the cumulative activity totals revealed a statistically significant diference between treatments over the 60-120 min period following injection [$F(3,6) = 6.78$, $p < 0.05$], this being due to a significant reduction in the activity of alcohol treated mice at 1 and 4 ata compared to saline controls. These findings indicate therefore that the effect of 7 ata air to offset the gradual depression of activity was statistically significant. Exposure to hyperbaric air also tended to enhance the motor incoordination produced by this dose of alcohol. For example the mice had difficulty in maintaining their balance when rearing or trying to groom themselves. The mice appeared to regain their co-ordination 35-45 min post injection.

In contrast to their qualitatively similar effects at the lower dose, 4 and 7 ata air produced different effects on the response to the 2.75 mg/kg dose. As at the lower dose, exposure of alcohol treated mice to 4 ata air resulted in elevated mean activity levels compared to similar treated mice and saline controls at 1 ata. At this dose however, the effect of 4 ata was more marked and persisted for a longer period of time Figs.(19,20). During this period which lasted some 45-60 min after injection, the mice displayed aimless running, and rearing at the sides of the activity box Fig.(21). This effect of 4 ata air,

Figure 19

Graph of 5 min. activity counts for mice given alcohol 2.75 g/kg at 1 (◇), 4 (▽) and 7 (□) ata air, and saline controls at 1 ata air (◆). (n=9). Standard error bars omitted for clarity.

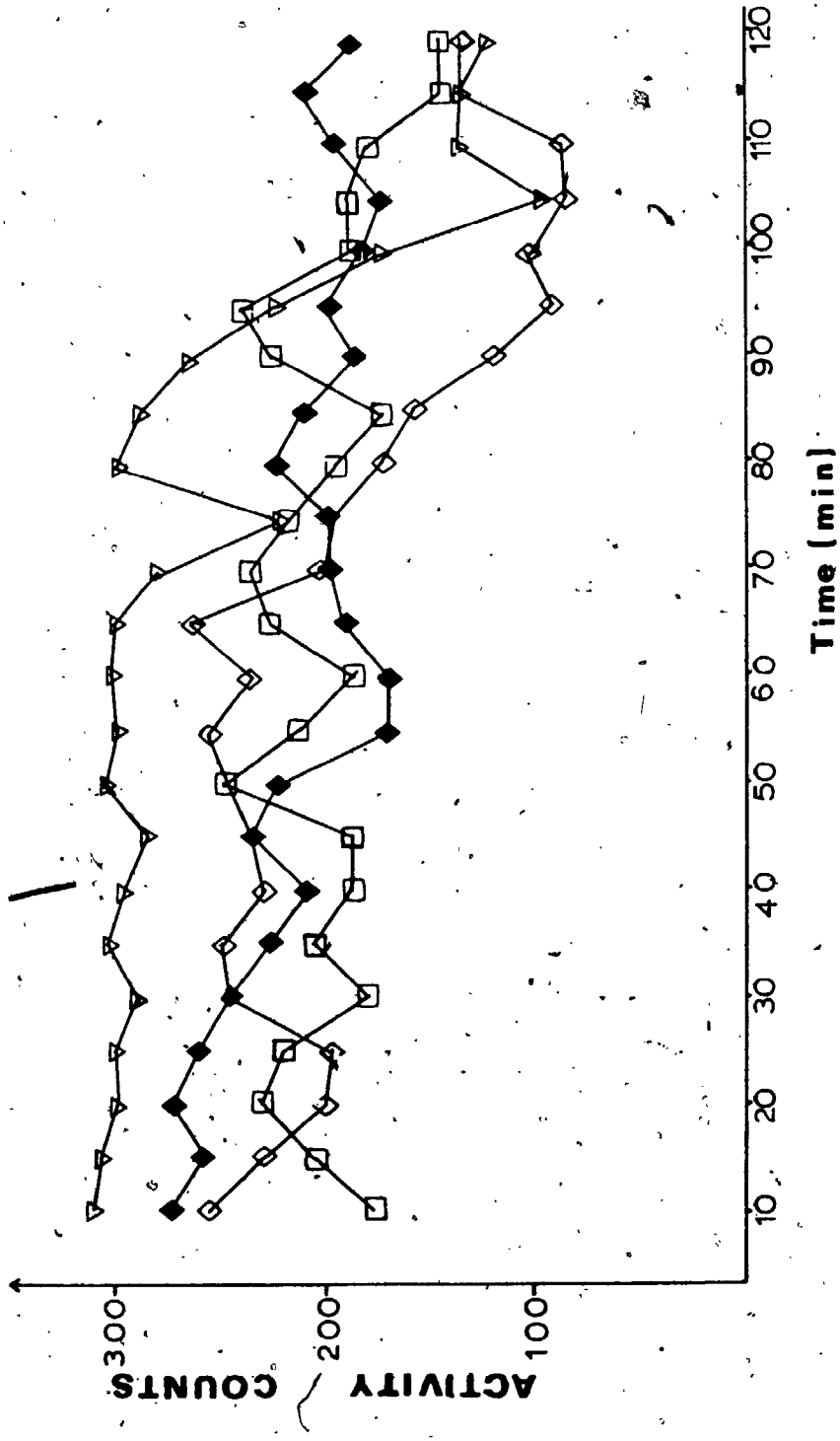

ACTIVITY / COUNTS
100
200
300
Time (min)
10
20
30
40
50
60
70
80
90
100
110
120

Figure 20

15 min. activity totals for mice given alcohol 2.75 g/kg at 1, 4 and 7 ata air , and saline controls at 1 ata air. Vertical bars = 1 SEM. (n=9) in all cases.

* sig. vs. saline controls, and alcohol treated mice at 4 ata air.

** sig. vs. alcohol treated mice at 1 and 7 ata, and saline controls.

*** sig. vs. saline controls.

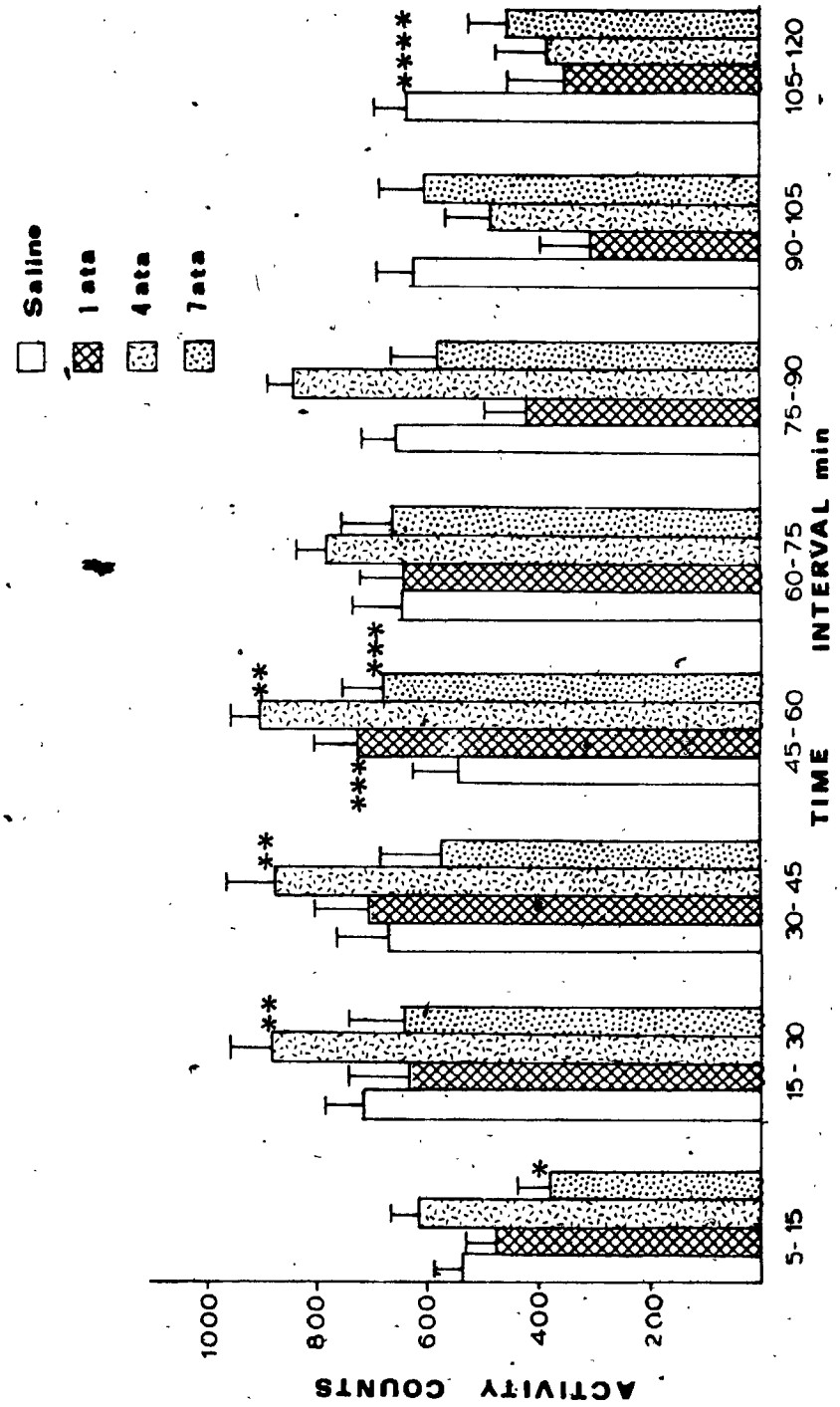

Saline
1 ata
4 ata
7 ata
ACTIVITY COUNTS
1000
800
600
400
200
TIME INTERVAL min
5-15
15-30
30-45
45-60
60-75
75-90
90-105
105-120
*
**
**
***
***
****

Figure 21

Incidence of walking and rearing for mice given alcohol 1.75, and 2.75 g/kg, at 1 (◆), 4 (▼) and 7 (■) ata air.

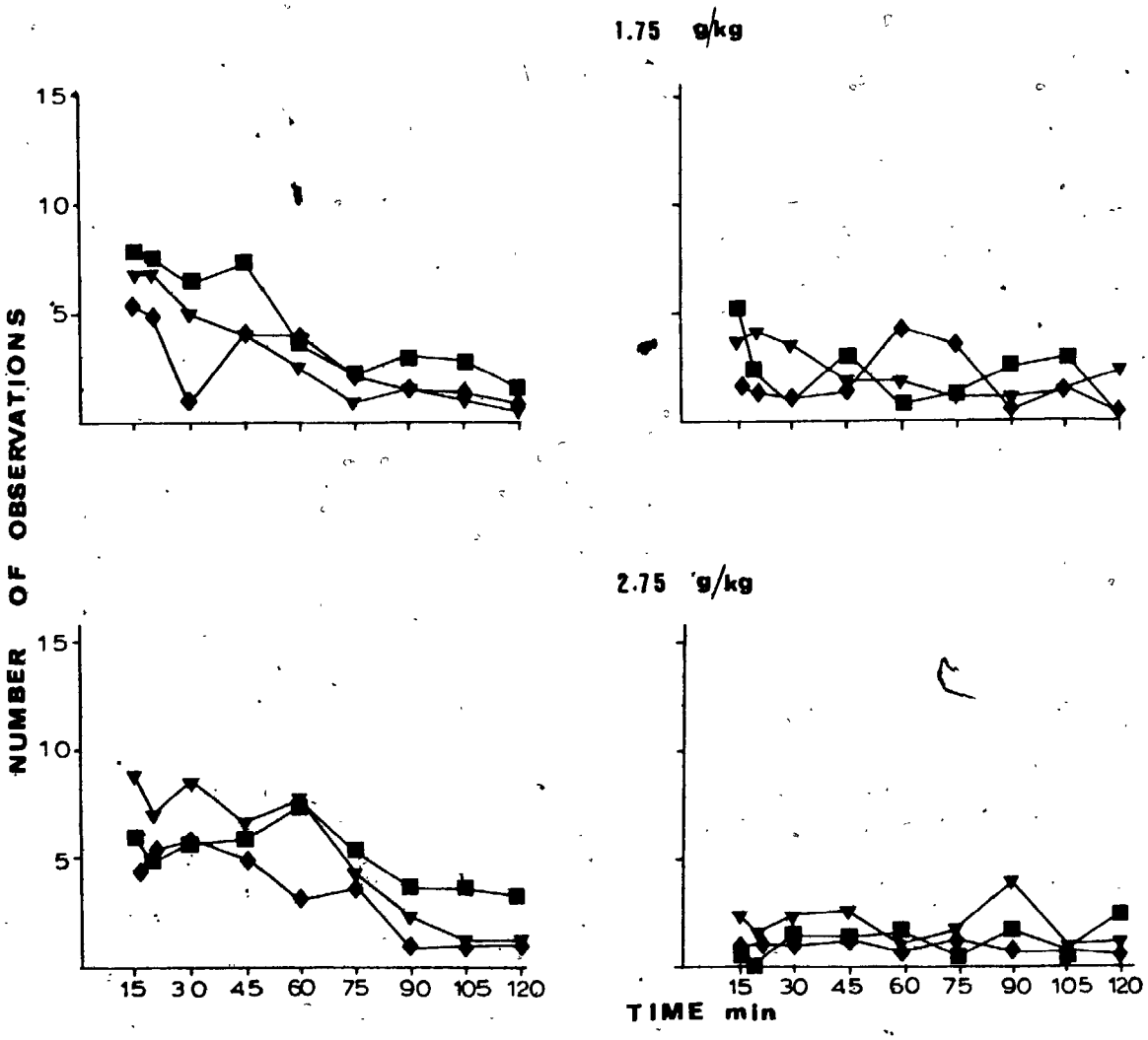

1.75 g/kg
2.75 g/kg
NUMBER OF OBSERVATIONS
15
10
5
15 30 45 60 75 90 105 120
TIME min

TABLE 10 A

Alcohol 1.75 g/kg 5 - 60, and 60 - 120 min Activity Totals
(mean ± SEM) (n=9/group)

Time	Saline Controls	1 ata	4 ata	7 ata
5 - 60	2359.4 ± 186.9	2073.0 ± 243.8	2742.4 ± 145.9	2965.0 ± 210.3
60 - 120	2319.8 ± 307.6*	1300.9 ± 180.7	1371.2 ± 136.6	1843.9 ± 241.9

*sig vs 1 and 4 ata

TABLE 10 B

Alcohol 2.75 g/kg 5 - 60 and 60 - 120 min Activity Totals
(mean ± SEM) (n=9/group)

Time	Saline Controls	1 ata	4 ata	7 ata
5 - 60	2461.1 ± 240.7	2547.3 ± 319.0	3281.6 ± 240.7*	2268.4 ± 312.3
60 - 120	2568.4 ± 213.4	1720.6 ± 211.8	2492.2 ± 156.5	2232.0 ± 218.4

*sig vs saline controls and 1 and 7 ata

resulted in the activity levels of these mice being significantly elevated compared to alcohol treated mice at 1 and 7 ata, and saline controls, over the 5-60 min post injection period, [$F(3,6) = 6.78$, $p < 0.05$], Table (10B). This was also reflected in statistically significant differences in activity over the 15-30, 30-45, and 45-60 min periods following injection Figs.(19,20). During the second hr however, as was the case at 1 ata, activity levels continually declined eventually falling below those of saline controls between 90-105 min after injection. In contrast, exposure to 7 ata reduced activity during the early post injection period, activity levels during the 5-15 min post injection period being significantly lower compared to saline controls, and ethanol treated mice at 4 ata, [$F(3,6) = 7.47$, $p < 0.05$], Fig.(20). During this period, it appeared as if some of the mice might lose their righting reflex. Similarly to 1 ata, following this initial depression, activity levels gradually increased with time over the first hr. They never approached those seen at 4 ata however, Figs.(19,20). As was seen at the lower dose, 7 ata air appeared to act to offset the decline in activity over the second hr, as the rate of decline was slower than at 1 or 4 ata Figs.(19,20). Analysis of the data over the 60-120 min period following injection, revealed a significant effect only over the 105-120 min period. Over this time interval the activity of alcohol treated mice at all pressures was significantly reduced compared to saline controls [$F(3,6) = 6.15$, $p < 0.05$]. Thus at this dose, the effect of 7 ata air to offset the gradual depression of activity was not statistically significant.

In contrast to the effects produced by air, exposure to 4 ata He/O_2 did not enhance the activity produced by alcohol at this dose.

Surprisingly, exposure to 1 ata He/O_2 did increase the activity of alcohol treated mice over saline controls at 1 ata air, Table (11).

TABLE 11

Cumulative Activity Totals For Alcohol (2.75 g/kg) Treated Mice

At 1, and 4 ata He/O_2, and 4 ata Air, and Saline Controls at 1 ata Air

	Saline	Alcohol		
Time Interval (min)	1 ata Air	1 ata He/O_2	4 ata He/O_2	4 ata Air
5 - 60	2373.6 ± 126.8	3592.2 ± 301.3*	1831.2 ± 126.7	3145.8 ± 240.3*
60 - 120	2250.0 ± 72.1	2240.4 ± 153.5	2207.8 ± 266.2	2413.9 ± 207.6

Values are mean ± SEM (n=9)
* sig vs 4 ata He/O_2, and 1 ata Air, [$F(3,6) = 8.96$, $p < 0.05$]

3.3.5.3 DISCUSSION

As for the previous drugs studied, exposure to hyperbaric air altered the response to alcohol in a complex manner, with the effect seen depending to some extent on the dose of drug and the pressure of air. At the 1.75 g/kg dose, both 4 and 7 ata air tended to enhance the initial stimulation produced by alcohol. In addition 7 ata tended to offset the subsequent decline in activity seen over the second hr of the experiment. At the higher dose 4 ata air again acted to increase the activity of the mice, the effect in this case being more marked than at the lower dose. In contrast, at this dose, exposure to 7 ata air produced a short lived, but significant depression of activity over the 5-15 min period following injection. At this dose, 7 ata air was not as effective in preventing the decline in activity seen over the latter stages of the experiment.

The significant modifications of the response to alcohol would appear to be due to the increased PN_2. For example, exposure to 4 ata He/O_2, did not produce a similar increase in activity as was observed following exposure to 4 ata air. This tends to argue against the effect of air being due to the increased hydrostatic pressure, or the increase in PO_2. With regard to the transient depression of activity seen at the 2.75 mg/kg dose, although it cannot be stated conclusively that this effect was due to N_2 since studies with 7 ata He/O_2 were not performed, evidence from other studies would suggest that this is the case. For example, exposure to increased pressure is known to reduce rather than enhance anesthetic induced loss of righting reflex (Lever, et al., 1971). This effect occurs at very high pressures however. Furthermore,

it has been clearly demonstrated that at pressures encompassing those used in the present study, exposure to He/O2 (Alkana and Malcolm, 1981 ; 1982) unlike hyperbaric air (Alkana and Malcolm, 1982), reduces rather than enhances the sleep time produced by alcohol. This has been erroneously considered as evidence of pressure reversal of anesthesia (Alkana and Malcolm, 1981). In coming to this conclusion the authors fail to take into consideration that just the act of switching the breathing mixture from air to He/O_2 without any increase in pressure, produces a marked reduction in sleep time. Furthermore in a number of cases the reduction in sleep produced by increasing the pressure of He/O_2 is minimal compared to that produced by switching the breathing mixture. These studies indicate however, that He/O_2 acts to reduce rather than enhance the depressant effects of alcohol. Therefore, it is unlikely that in the present study, that either the increased PO_2, or hydrostatic pressure is responsible for the transient depression of activity observed. It would appear that this effect of hyperbaric air is also probably due to the increased PN_2.

As discussed for amphetamine and DPH, given the rapidity of the modifications and their complex nature, it is unlikely that they are due to an alteration in the pharmacokinetics of alcohol at pressure. In support of this, studies in humans (Jones et al., 1979), have shown that exposure to 4 or 6 ata air did not affect the rate of metabolism of alcohol. It would appear therefore, that these effects are the result of an interaction with changes in the CNS produced by N_2.

The results obtained are not compatible with the theory however, that N_2 in this pressure range produces a generalized depression of CNS

activity. In this study, one of the main effects of increasing the dose of alcohol from 1.75 to 2.75 g/kg was to shorten the initial excitatory phase, and to produce a more quiescent phase over this period. Thus whereas for the 1.75 mg/kg dose activity in the 5-15 min period following injection tended to be elevated (although not significantly) above that of saline controls, at the 2.75 g/kg dose activity tended to be depressed below that of their corresponding saline controls Figs.(17,19). Furthermore a dose of 3.25 g/kg produces a transient loss of righting reflex in mice (data not shown). Thus if N_2 did produce a generalized depression of activity, one would have predicted that exposure to hyperbaric air would have resulted in lower activity levels in the 5-15 min period following injection, or perhaps in a loss of righting reflex. Such an effect was apparent only at the high dose of alcohol at 7 ata air, where the combination of alcohol and N_2 acted to transiently decrease spontaneous activity Figs.(19,20). At the lower dose at 7 ata, and at 4 ata for both doses, the trend was for the combination of alcohol and N_2 to increase activity in the initial period following alcohol administration Figs.(17-20).

The transient depression of activity produced by the combination of 7 ata air and 2.75 mg/kg alcohol, together with the observation that hyperbaric air appeared to enhance the ataxia produced by alcohol, clearly indicates that the effects of N_2 can add to those of alcohol. This is also supported by studies in the literature. For example in humans, exposure to 4 and 6 ata has been shown to potentiate the decrease in standing steadiness produced by alcohol (Jones et al., 1979). Furthermore, in the study by Alkana and Malcolm (1982) referred to earlier, exposure to hyperbaric air in the range of 8-10 ata has been

shown to prolong the sleep time in mice injected with ethanol. Interestingly, in this study, no significant prolongation of sleep time was observed at 6 ata air, a pressure within the range used in the present studies. "Wake up" concentrations at this pressure however, as at the higher ones were significantly lower than at 1 ata. This suggests therefore that the effect of N_2 at this pressure was enhancing somewhat the effects of alcohol. Thus the reason for the failure of N_2 in the present study to combine with alcohol to produce a prolonged depression of activity, may be due to the fact that at these pressures, as at 6 ata in the study referred to above, the depressant effects of N_2 are not strong enough.

The above nowever, does not explain the action of N_2 to combine with alcohol to enhance activity. It is suggested that these results can be explained by proposing, as was done in the case of DPH, that N_2 produces excitatory as well as depressant effects on CNS activity. As for alcohol (Ritchie, 1975), and other anesthetics (Rosner and Clark, 1973), the excitatory effects would occur first, and be more important at lower concentrations. The depressant effects however, would be minimal at the lower concentrations, but would become progressively more important as the concentration, i.e. pressure of N_2 is increased. Thus the net effect of the combination of a given dose of alcohol and pressure of air, would depend on the outcome of the interaction of the excitatory and depressant effects of alcohol with those of N_2. At the low dose of alcohol at both pressures, and also at the high dose at 4 ata, where the combination of alcohol and N_2 acted to stimulate activity, it is likely that their combined depressant effects were not great enough to depress activity. Thus the combination of the

excitatory effects predominated leading to the observed action to stimulate activity. At the higher dose however, the greater depressant effect exerted by this dose may be such that in combination with that of N_2 it can depress activity. The transient nature of the depression is probably due to the fact that as the concentration of alcohol in the CNS declines with time it falls below the critical value needed to interact with N_2 to depress activity. The failure to see a similar effect at 4 ata at this dose is probably due to the fact that the depressant effect of N_2 at 4 ata air is still to weak to add to that of alcohol. Thus what is observed is an enhancement of the excitatory effects without any corresponding increase in the depressant effect. This gives rise to the significant enhancement of activity seen. Possible mechanisms for the excitatory and depressant effects of alcohol will be presented in chapter 5.

As stated earlier, at the lower dose of alcohol exposure to hyperbaric air tended to offset the gradual decline in activity which occurred over the latter part of the experiment. The cause of this secondary decline in activity is not known. It is different from the initial depression of activity following high doses of alcohol where ataxia and loss of righting reflex are observed. This effect was maximal towards the end of the experiment when concentrations of alcohol in the CNS would be low and ataxia and loss of righting reflex were no longer present. This effect would therefore appear to be due to changes produced in the CNS by the acute effects of alcohol. Thus one explanation for exposure to 7 ata air to counteract this effect, may be due to the fact that the excitatory effect of N_2 at this pressure counteracts this depression. At this time there is no large

concentration of alcohol present to add to the depressant effect of N_2, and thus the excitatory effect of N_2 will predominate. It should be noted that a similar effect also tended to occur at the higher dose of alcohol Figs.(19,20). At this dose however, 7 ata was not as effective in counteracting this effect. Thus it may be at this higher dose the secondary depressant effect was too strong for the effect of N_2 to counteract it. Also, the failure of 4 ata to have the same effect may be due to the fact that the excitatory effect of N_2 at this pressure is to weak to counteract this secondary depressant effect. Support for the fact that N_2 produces excitatory and depressant effects, and further discussion of the effects of alcohol, will be presented in chapter 5.

Finally the mechanism by which 1 ata He/O_2 enhanced the activity produced by 2.75 mg/kg is not known. As discussed earlier for amphetamine (section 3.3.1.4), He/O_2 would appear to be capable of significantly affecting the effects of drugs in mice. Furthermore in the studies by Alkana and Malcolm (1981 ; 1982) referred to earlier, it is apparent that He/O_2 at 1 ata acts to reduce the sleep time to alcohol in mice. Thus it may be that this effect of 1 ata He/O_2 in this study is a manifestation of this poorly understood effect of He.

3.3.6 STUDIES ON PENTOBARBITAL

3.3.6.1 INTRODUCTION AND METHODS

The effect of exposure to hyperbaric air on the sleep time induced by sodium pentobarbital (Abbott) was determined. The doses used were 35 and 45 mg/kg, these being administered i.v. in a volume of 0.05 ml/10g body weight. Following injection, the mice were placed in separate plexiglass boxes (12.5 X 8.5 X 13 cm), and transferred to the chamber. Sleep time was calculated as the time between loss and regaining of the righting reflex. Experiments were conducted at 1, 4 and 7 ata air. In addition at the 45 mg/kg dose, experiments at 7 ata He/O_2 were also performed. Three mice were tested at one time.

3.3.6.2 RESULTS

Injection of pentobarbital produced a rapid loss of righting reflex in mice, this normally occurring within 1 min post injection. In a few cases the injection was not completed successfully. Data from such mice were not included in the results. During compression there was a slight tendency for some mice to paw at their ears and to try to right themselves. The intensity of this behavior was markedly reduced from that seen in mice which had not been myringotomized.

At the low dose, exposure to hyperbaric air did not significantly affect the sleep time induced by pentobarbital. At 4, but not 7 ata air, the mean sleep time was increased above that at 1 ata, Table (12).

TABLE 12

Sleep times following administration of pentobarbital

	Sleep Time (min)			
	Air			He/O_2
Dose	1 ata	4 ata	7 ata	7 ata
35 (mg/kg)	16.6 ± 1.67 (7)	26.2 ± 6.0 (8)	16.9 ± 1.4 (8)	-
45 (mg/kg)	36.8 ± 2.3 (9)	48.2 ± 4.6 (9)	54.6 ± 5.4* (8)	40.7 ± 6.5 (7)

Values are (mean ± SEM); numbers in parentheses are number of animals tested.

*sig vs 1 ata air.

This however, was not significant. At the higher dose the mean sleep times at both 4 and 7 ata air were increased over that at 1 ata, Table (12). A one way analysis of variance revealed a statistically significant difference in sleep times between pressures, [$F(3,30) = 3.26$, $p < 0.05$]. Further tests revealed that this was due to the sleep time at 7 ata being significantly prolonged compared to 1 ata. No significant differences were observed between the sleep time at 4 and 7 ata air. Also in contrast to air, 7 ata He/O_2 did not significantly prolong sleep time.

3.3.6.3 DISCUSSION

The results indicate that exposure to hyperbaric air is capable of prolonging the sleep time induced by pentobarbital in mice. This effect was only significant however, at the longer sleep times produced by the higher dose of pentobarbital, and at 7 ata air. As a similar prolongation of sleep time was not produced by exposure to 7 ata He/O_2, this implies that the effect seen with air was due to the increased PN_2.

It has been demonstrated that N_2 can produce anesthesia in mice (Miller et al., 1967). Thus it is likely that the prolongation of sleep time seen in this study is due to an interaction of the anesthetic effects of N_2 and pentobarbital. There are some other possibilities that need to be considered however. Pentobarbital is known to produce hypothermia in mice (Hart, 1974a). In addition, hypothermia is known to enhance the anesthetic effect of pentobarbital (Fuhrman, 1947). This raises the possibility that the prolongation of the sleep time was due

to exposure to hyperbaric air enhancing the hypothermia produced by pentobarbital. As rectal temperatures of the mice were not taken this cannot be refuted completely. The earlier study by Hart (1974a), in which prolongation of sleep time was observed without any significant increase in hypothermia, would tend to argue against an increase in hypothermia being the cause. Also it could be possible that N_2 could act to inhibit the metabolism of pentobarbital and give rise to the effect seen. This however, would not be able to explain the effect at the lower dose where the mean sleep time at 4 ata tended to be longer than at 7 ata. Thus it would appear that this effect represents an interaction of the effects of pentobarbital with the CNS effects of N_2.

The results at the higher dose would appear to support the classical theory that N_2 produces CNS depression. The finding at the lower dose where a correlation between sleep time and PN_2 was not apparent, is in conflict with this. At present the reason for this is not known. Some possibilities are discussed in chapter 5 however.

3.4 SUMMARY OF ANIMAL STUDIES

The results indicate that hyperbaric air in the range 4 to 7 ata is capable of modifying the behavioral effects of the drugs tested, even though air at these pressures produced no discernible effects on the behavior of control (saline treated) mice. At these doses and pressures however, no lethal or overtly debilitating interactions were observed. In general the modifications of behavior appeared to be due to the increased PN_2 rather than to changes in PO_2 or hydrostatic pressure since, in practically all of the cases tested, equivalent pressures of He/O_2 (80/20) did not produce significant modifications of the drug effects. The only exception to this was in the case of amphetamine-induced lethality in which, as with hyperbaric air, no deaths were observed following exposure to 7 ata He/O_2. In some experiments exposure to He/O_2 did tend to produce slight differences in behavioral effects of some drugs, e.g. the stereotyped behavior produced by amphetamine 8 mg/kg, Fig.(6c). In addition exposure to 1 ata He/O_2 enhanced the activity of mice given alcohol 2,75 g/kg, Table (12). It was suggested however, that the effects were probably related to the high thermal conductivity of He.

Although this was not confirmed experimentally, the rapid time course and the complex nature of the modifications of drug effects suggest that alterations in the pharmacokinetics of the drugs were not the primary cause of the changes produced. Thus the results would appear to be due to the central effects of N_2. The results however are not compatible with the view that N_2, at least in this pressure range, produces a generalized slowing (depression) of CNS activity. Indeed

several of the modifications observed suggest that in this pressure range exposure to hyperbaric air was associated with some slight increase in CNS excitation (disinhibition). Examples of this would be the ability of hyperbaric air to enhance the locomotor stimulant properties of amphetamine at 3 and 4 mg/kg, morphine at 15 mg/kg, and to significantly increase the locomotor activity of mice given alcohol, 2.75 g/kg. In all, there were only a few instances where N_2 appeared to act in accordance with the predictions of the classical theory. This was seen for example in the ability of 4 and 7 ata to antagonize amphetamine induced convulsive activity Table (2), the brief but significant enhancement by exposure to 7 ata air of the depression of activity produced by alcohol 2.75 g/kg Fig.(20), and the prolongation of pentobarbital sleep time Table (12). Exposure to 7 ata air also reduced the stereotyped behavior in mice produced by amphetamine 8 mg/kg. However, as will be discussed later it is not clear whether this is in fact due to a depressant effect of N_2.

Another important feature of the modifications of drug effect by hyperbaric air is the fact that in the experiments on motor activity, the changes produced appeared to be influenced by both the dose of the drug and the pressure of air. This effect was observed for all the drugs.

In general therefore, the results of these studies would suggest that at these pressures N_2 produces both excitatory (disinhibitory) and depressant effects in the CNS which are capable of modifying the effects of centrally acting drugs. Studies by other investigators on the interaction of centrally acting drugs with hyperbaric air, also tend to

support this view. For example support for the fact that increased PN_2 produces excitatory changes in the CNS comes from operant studies with drugs such as amphetamine (Walsh, 1974 ; Thomas, 1976 ; Thomas and Walsh, 1978), delta-9 THC (Walsh and Burch, 1977a), and alcohol (Thomas and Walsh, 1978). In the above mentioned studies exposure to hyperbaric air tended to enhance the action of stimulants such as amphetamine, and to reduce the effects of depressants delta-9 THC, and alcohol. Support for the depressant action is suggested by the findings that hyperbaric air can prolong the sleep time of mice given alcohol (Alkana and Malcolm, 1982) and reduce the dose of diazepam required to induce sleep in guinea pigs (Nicodemus et al, 1980). As stated earlier, further discussion of the modifications of the drug effects wil be given in chapter 5.

CHAPTER 4

HUMAN STUDIES

4.1 INTRODUCTION

The early work on drug effects at pressure was performed in animals and it was not until Jones et al., (1979) that the first full report dealing with such studies in humans was published. In this study , the combined effects of alcohol and hyperbaric air (4 and 6 ata) on body sway were investigated. Both alcohol, and hyperbaric air, individually increased body sway, and a marked synergism was observed between their effects. Later in the same year, Walsh and Burch (1979) presented a preliminary report on the effects of commonly used drugs, in combination with hyperbaric air, on the ability of divers to learn and perform a complex task. Five drugs were tested, aspirin, acetaminophen, caffeine, dramamine, and diphenhydramine (DPH), and presssures up to 6 ata were employed. All of the drugs were reported to produce decrements in the learning of the task. The analgesics and caffeine produced minimal effects, whereas DPH produced the largest decrement with error rates being three times baseline. Dramamine's effects appeared to depend on individual susceptibility. Interestingly, however, performance of a well learned task was not affected by the combination of drugs and hyperbaric air.

The enhancement of the effects of the central depressants alcohol, and diphenhydramine, by hyperbaric air in these studies, provided support for the theory that hyperbaric air acted as a central depressant (Bennett, 1966). The results were somewhat surprising however, in the light of earlier studies with these drugs in animals. Studies in rats had indicated that the disruption of scheduled controlled behavior by alcohol was reduced, not enhanced by exposure to 7 ata air (Thomas and Walsh, 1978). Also, using similar techniques, Walsh and Burch (1977b) failed to find any decrement in performance with DPH at therapeutic doses even at pressures up to 7 ata. Significant interactions were reported to occur only at 5-10 times the customary therapeutic doses. Furthermore, in the animal studies reported in this thesis (see Chapter 3) exposure to 4 or 7 ata air did not enhance the depressant effects of DPH on spontaneous activity.

The results of the human studies were also at odds with other experiments in animals with drugs such as amphetamine (Walsh, 1974; Thomas, 1976; Thomas and Walsh, 1978) and delta-9 tetrahydrocannibinol (delta-9 THC) (Walsh and Burch, 1977a). In these studies the evidence suggested that hyperbaric air produced CNS excitation rather than depression, since the effects of amphetamine were enhanced and those of delta-9 THC reduced.

The reasons for these conflicting results in the human vs. animal studies were not clear as there was no evidence to suggest that hyperbaric air produced different effects in animals vs. humans. In fact a great deal of work leading to the proposal that hyperbaric air acted as a central depressant came from animal studies (Bennett, 1963;

1964). These conflicting findings however, raised doubts regarding the applicability of animal data on drug-hyperbaric air interactions to man, and highlighted the need for more studies in humans to provide information against which the animal data could be compared. Therefore, studies were performed in humans with the central depressants DPH and alcohol, the two drugs common to the animal and human studies, and widely used by divers.

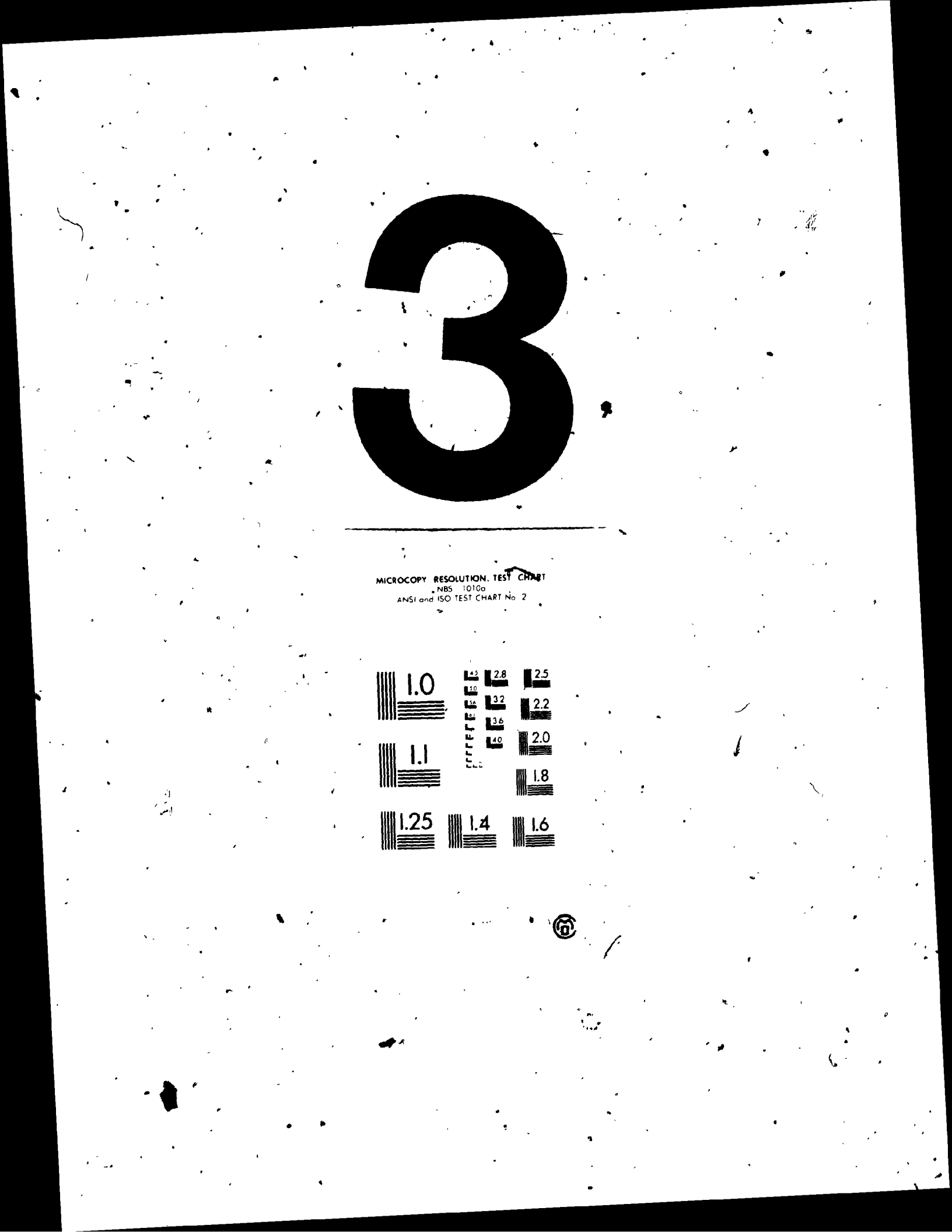
It was not possible to make a strict experimental comparison between the human and animal studies performed in this thesis however, since in the latter, spontaneous activity was the behavioral measure used. In addition it was becoming apparent that the modification of drugs by hyperbaric air was not as simple as predicted by the classical theory. In particular, it appeared that the choice of test or behavior might influence the type of modification observed. Therefore, the tests used in this study were chosen to explore this possibility, as well as to provide information which would be of practical relevance to the diving situation. The basic hypothesis remains the same however, namely that if exposure to hyperbaric air produces CNS depression, the combination of these depressant drugs and hyperbaric air should result in a greater decrement in performance than that seen with either drug or hyperbaric air alone.

Memory and neuromuscular co-ordination (as measured by the ball bearing test) were the parameters chosen to investigate the effects of these drugs at pressure. Deficits in these parameters have been repeatedly observed following exposure to hyperbaric air (Fowler, 1973; Fowler and Ackles, 1975; Bennett, 1966; 1975) these effects being due

to N_2. In addition they are important aspects of diver function, deficits in which could conceivably limit the usefulness and decrease the safety of divers at depth. Furthermore, they presumably depend on quite different central processes for their execution, (cognitive and motor respectively) with very little, if any, overlap, and therefore, should provide some indication of any differences in the combined effects of the drugs and hyperbaric air on those areas of the brain important for cognitive and motor skills.

Memory was chosen in preference to other tests reflecting cognitive ability such as arithmetic tests which are also affected by hyperbaric air (Bennett, 1966; 1975), as it has been widely studied, and there are a number of tests which can be used to probe in detail any deficits produced. For example tests can be performed to determine whether long term memory (LTM) or short term memory (STM) are affected, and whether any deficit in LTM is due to a failure of input or retrieval. The choice of memory therefore, might allow detection of differences between the effects of alcohol, DPH, and hyperbaric air, or their combinations on CNS function which might not be possible with tests such as the arithmetic test.

These studies were performed at 5 ata air. Previous studies at 10 ata had indicated that hyperbaric air affected LTM but not STM, and that input into rather than retrieval from memory was affected (Fowler, 1973; Fowler and Ackles, 1975). Thus tests were chosen to determine if similar effects would occur at 5 ata, as well as to provide a detailed account of the way in which the presence of the drugs might alter any deficit observed. It should be noted that LTM and STM are not


3
MICROCOPY RESOLUTION TEST CHART
NBS 1010a
ANSI and ISO TEST CHART No 2
1.0
1.1
1.25
1.4
1.6
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.8

necessarily defined in terms of the time between presentation of material and recall, but rather represent two memory stores with different functional characteristics (Atkinson and Shiffrin, 1968).

An immediate recall test (Glanzer, 1971) was used to determine effects on LTM and STM. In addition tests of delayed recall, and recognition, were administered to provide information on the nature of any deficit observed. These latter tests both provide information on LTM. The recognition test however, provides a truer picture of the memory store since it bypasses retrieval mechanisms which can limit recall (McCormack, 1972). Thus by comparing performance on delayed recall vs. recognition, one can draw inferences as to the cause of the deficit.

4.2 METHODS

General

Two studies were performed. Both were conducted at the Canadian Underwater Training Centre (C.U.T.C.) , a commercial diving school, located in Toronto, Ontario, Canada.

All testing was performed in a compression chamber 4.5m x 1.3m equipped with a double lock, viewing ports, exterior lighting and communications system. The dive consisted of a 35 min. exposure to a pressure equivalent of 39 msw (5 ata).Pressurization was completed within 3-5 min.,and the subjects were decompressed according to Royal Navy Table II. Testing at pressure commenced approximately 2 min. after reaching depth.

4.2.1 STUDY I

Subjects

Eighteen volunteer male divers, mean age and body weight 23.3 yrs (range 18-33) and 79.6 kg. (range 64-102) respectively, students at the C.U.T.C. served as subjects. By nature of their training and prior experience, they were familiar with the effects of hyperbaric air. Prior to these experimental dives, they had been diving regularly to similar depths and therefore could be considered at their maximum tolerance to the effects of narcosis encountered. Divers with prior experience of an adverse reaction to DPH or extremely susceptible to its soporific effects, and non-drinkers, did not take part in the study.

They were instructed to fast from 2200 hrs. on the night prior to the test day and to obtain a good night's rest. Aspirin was the only medication permitted for 48 hrs. prior to and during the study. A consent form was read and signed by all subjects before entering the study.

Tests of Memory

(a) Immediate Recall: At each test session, subjects were shown seven lists of words (fifteen words/list, 2 sec./word) and following presentation of the last word in a list were allowed one min. to write down in any order as many words as they could remember. Thirty sec. were allowed between the end of recall from one list and presentation of the next list. During this interval, subjects were instructed not to rehearse or discuss words previously presented. The words were taken from Thorndike and Lorge (1944) [see Roberts (1972) for a detailed description of the characteristics of the words], and were printed in black (letter size 3cm X 2cm) on white flash cards (10cm X 28cm). Due to the size of the chamber, words were presented simultaneously at two viewing ports, three subjects per port. Three groups of lists were used in the study, their order of use being counterbalanced across days, test sessions, and viewing ports.

(b) Delayed Recall: Approximately 2 min. after completion of the immediate recall test, subjects were given 5 min. to write down as many words as they could from all of the lists presented in the test session.

(c) Recognition Test: Subjects were given a recognition sheet (see Appendix (II), Fig (25) on which were listed, in random order, the

words from the first two lists presented during that test session (old words) together with an equal number of distractor words, ie. words not used in any of the test or practice lists. The distractor words were also drawn from Thorndike and Lorge (1944), and had the same characteristics as those in the test lists. The subjects were told to indicate which words they thought had been previously presented during the test session, as well as their confidence in their decision by marking, 1 (word not presented), 2 (fairly sure word presented), or 3 (positive word presented) beside each word on the sheet. To reduce guessing or concentration by the subjects on words from the first two lists, they were not told the number of 'old' words on the sheet or the lists from which they were taken. They were instructed to work as rapidly as possible and to make a decision on every word. Two min. (i.e. about 2 sec./word) were allowed for completion of the test. This rate was chosen to reduce as much as possible the use of complex retrieval strategies.

Ball Bearing Test

Subjects were required to pick up ball bearings (11mm in diameter) with a forceps (25cm long) and place them in a vertically mounted tube 12mm in diameter. The number placed in the tube in 45 sec. was recorded.

Design and Procedure

The subjects were randomly assigned to three treatment groups. One group received diphenhydramine (DPH) 50 mg, another alcohol 0.25 g/kg (60 ml of 40% alcohol/70 kg body weight), and the third, the control group, received placebo vehicle (orange juice). Performance of the subjects on the tests was determined on three occasions, once before receiving their appropriate treatment (pre treatment session) to provide baseline data, and twice after, once at the surface (post treatment session) followed immediately by an evaluation at pressure (dive). The subjects were tested in groups of six comprised of two members from each treatment group, and one such group was tested per day. The order of administration of test sessions and drugs are shown in Table (13). This arrangement was necessary to allow for the much slower rate of absorption of DPH compared to alcohol (Carruthers et al., 1978; Spector et al., 1980; Lin et al., 1976). The subjects were not told the content of their drink, and at the times of drug administration, those subjects not receiving any drug always received the placebo vehicle. Additionally, to conceal the absence of alcohol, 5 ml of it were gently layered onto the surface of the orange juice to provide the taste and smell. A light breakfast consisting of two slices of toast and jam, and one cup of decaffeinated instant coffee (98% caffeine-free) was given approximately 20 min. before alcohol administration.

In order to prevent rehearsal of words between the tests on memory, during these periods the subjects either performed the ball bearing test or alternatively a number classification test. In the latter, subjects were asked to classify fifteen two digit numbers between 0 and 60 as to

TABLE 13

'A'

Schedule of Test-Sessions and Drug Administration

Elapsed Time (min)	Item
0	Pre-treatment Test
30	DPH Administration
130	Breakfast
165	Alcohol Administration
190	Blood Sampling
195	Post-treatment Test
225	*Dive and Test at Depth
255	Decompression

whether they were High (H) >30, Low (L) <30. and odd (O) or even (E) by writing the appropiate letters from the set HLOE beside each number. Thus, the order of tests within each session was as follows:

(i) immediate recall

(ii) ball bearing test/number classification

(iii) delayed recall

(iv) ball bearing test/number classification

(v) recognition test

Three of the subjects performed the ball bearing test between the immediate and delayed recall test, and the others between the delayed recall and recognition test.

All of the material necessary for the tests on memory and the number classification test was placed on clipboards which were given to the subjects on entering the chamber. These clipboards were equipped with sides to prevent the subjects from seeing each others responses. Immediately upon completion of recall from a list the subjects placed their responses face down in the plastic bag provided.

Practice sessions were conducted on the immediate recall, ball bearing, and number classification tests on the day prior to the test day. Three practice lists were given for the immediate recall test, and one practice run was allowed on the number classification test. The ball bearing test was practiced until three successive scores were within two of each other.

Blood Alcohol Determination

A capilliary blood sample (100 ul) was collected from a fingertip, anticoagulated with sodium citrate (3.8%) and centrifuged to obtain a plasma sample. Following deproteinization with perchloric acid and subsequent centrifugation, the supernatant was removed and stored in a tightly capped Eppendorf tube at -20° C until assayed. Alcohol concentration was determined by an enzymatic method (Sigma Tech. Bulletin, No. 332-UV) based on the method of Bucher and Redetzki (1951).

Statistical Analysis

Results were analyzed using a two factor analysis of variance with repeated measures on one factor (Winer, 1971). In addition, the following tests were used to determine the source of significant effects. The t-test (unpaired) was used to test for differences between treatment groups when main effects were significant and the interaction was not. In the latter case orthogonal comparisons were used. Differences between test sessions were analyzed using the paired t-test.

4.2.2 STUDY II

Design and Procedure

The procedure used in this study was basically the same as that in Study I, however, the following changes were made:

(1) The dose of DPH was increased from 50 mg to 75 mg, and the dose of alcohol from 0.25 to 0.375 g/kg.

2) Eighteen different divers mean age and body weight 26.5 years (range 21-37) and 79.6 kg (range 69-93) respectively, were used. They were randomly assigned to two treatment groups, DPH or alcohol, and a crossover design was used in which each subject was tested on two days, on one day receiving the drug assigned to him and on the other, the placebo vehicle. As in Study I the subjects were tested on three occasions on each test day, pre treatment, post treatment at the surface, and post treatment at pressure. Six divers, three from each drug group were tested at one time, with two test groups being run each day. Drug administration was arranged to balance, as far as possible, drug conditions between test groups and across test days. The interval between dives for each subject was 36 hours.

(3) Three additional groups of test lists, and additional practice lists were prepared using words similar to those used in Study I. As in Study I, the order of presentation of the groups of lists was counterbalanced across days and viewing ports.

(4) The amount of practice on these tests was increased. Three practice lists were given on the immediate recall test, followed by a

delayed recall test, and two recognition tests using words from the first two lists. After practising the ball bearing test until scoring consistently within two, subjects were rested for 15 min. and then taken to the chamber where a practice session on all tests was conducted. In addition, a practice run was allowed on the ball bearing test prior to the pre treatment test on each day. In a trial in our laboratory this level of practice was found to be adequate to eliminate practice effects.

(5) Two scores were obtained for each subject on the ball bearing test compared to only one in the first study, once at the end of the immediate recall test and again after the delayed recall test. The mean of these scores was used in the analysis.

(6) At the end of the study, a questionnaire was administered to the subjects to learn (i) how effective were the attempts at making the study single-blind, and (ii) information on any side effects experienced by the divers. They were asked to identify (i) which drug they had received as well as the day on which they thought it was administered, (ii) if they could detect a difference in the colour, smell, or taste of the drink containing the drug, (iii) any side effects experienced (side effects listed on the questionnaire were, drowsiness, dizziness, intoxication, difficulty in thinking and nausea), and (iv) if they thought that, as a result of the drug, their performance during the dive was worse, better, or did not change.

Statistical Analysis:

The scores for the pre treatment tests on both days and the placebo post treatment test were compared using a one way analysis of variance. If no significant difference was obtained, these scores were pooled and the mean taken to represent control performance. A three factor analysis of variance with repeated measures on two factors was used to analyze the data (Winer, 1971).

4.3 RESULTS

4.3.1 STUDY I

General:

None of the subjects spontaneously reported any ill effects from the combination of drugs and hyperbaric air. The mean (±SEM) blood alcohol level 25 min. after administration was 0.013% (±0.002). As a check on the sensitivity of the assay, four replicate analyses were performed on a solution containing alcohol 0.015% w/v. The results of these analyses gave a mean value (±SEM) of 0.0149% (±0.0001).

Tests on memory

For three subjects, the group of lists used in the pre treatment tests was inadvertently reused on their post treatment test at the surface, thereby invalidating the results for this test session. The data for these subjects were excluded from the analysis, and therefore the results on memory are based on five subjects per group.

Immediate Recall:

Serial position curves which reflect the % recall as a function of position in the list were plotted and are shown in Figs. (22,a,b,c). The curves show the characteristic U shape indicating that recall is better for words at the beginning (primacy region) and end (recency region) of the list compared to the middle. The primacy and middle regions are thought to represent recall from LTM, and the recency region

recall from STM (Glanzer, 1971). From observation of these curves, positions 1-3, 4-10, and 11-15 were designated the primacy middle and recency regions respectively. The % recall within each region was calculated Table (14), and after an arcsine transformation to normalize the variances, analyzed to determine the effects of the various treatments on recall from these regions.

Analysis of the data revealed statistically significant group, and test session effects in the primacy region [$F(2,12) = 7.0$ $p<0.01$, and $F(2,24) = 18.2$ $p<0.001$, respectively], and a significant test session effect in the middle region [$F(2,24) = 5.4$ $p<0.025$]. No significant effects occurred in the recency region.

Further analysis showed that the significant between group difference in the primacy region was due to better recall in this region in the alcohol group compared to the control ($p<0.01$) and DPH groups ($p<0.001$) over all test sessions.

In the primacy region, analysis of the differences in recall between test sessions indicated that for all groups, recall at pressure was significantly lower than that in both the pre treatment test ($p<0.05$), and post treatment test at the surface ($p<0.001$). In the middle region, however, recall at pressure was only significantly decreased compared to that for the surface post treatment test ($p<0.005$). In addition, in both the primacy and middle regions, recall was significantly higher in the post treatment test at the surface compared to pre treatment levels ($p<0.01$, $p<0.05$, respectively) possibly reflecting a practice effect.

Figure 22b

Serial position curves for subjects in the alcohol group-Study I. Note the decline in recall in the primacy region at depth. This is not as marked as in the control or DPH groups however. Also there is a decline in the late middle early recency regions which is not seen in the other groups.

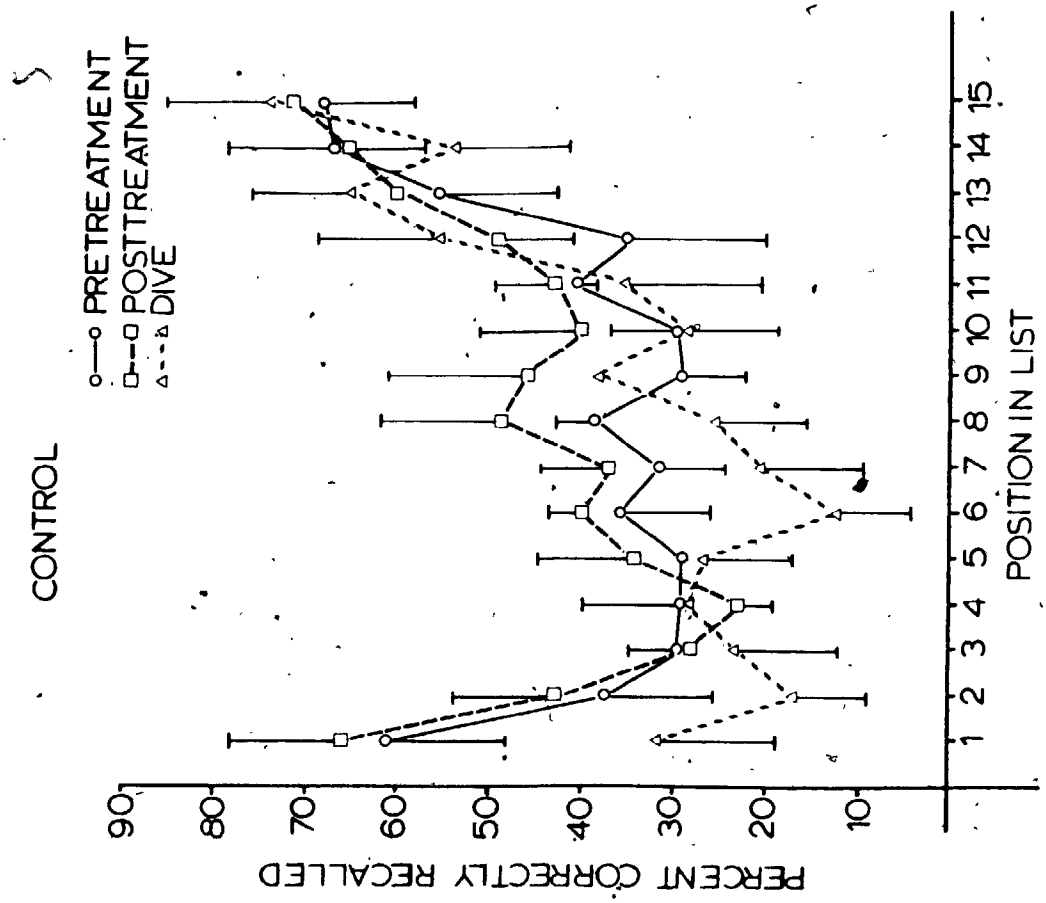

CONTROL
PRETREATMENT
POSTTREATMENT
DIVE
PERCENT CORRECTLY RECALLED
90
80
70
60
50
40
30
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
POSITION IN LIST

Figure 22a

Serial position curves for subjects in the control group-Study I. Note the decline in recall in the primacy and middle regions at depth.

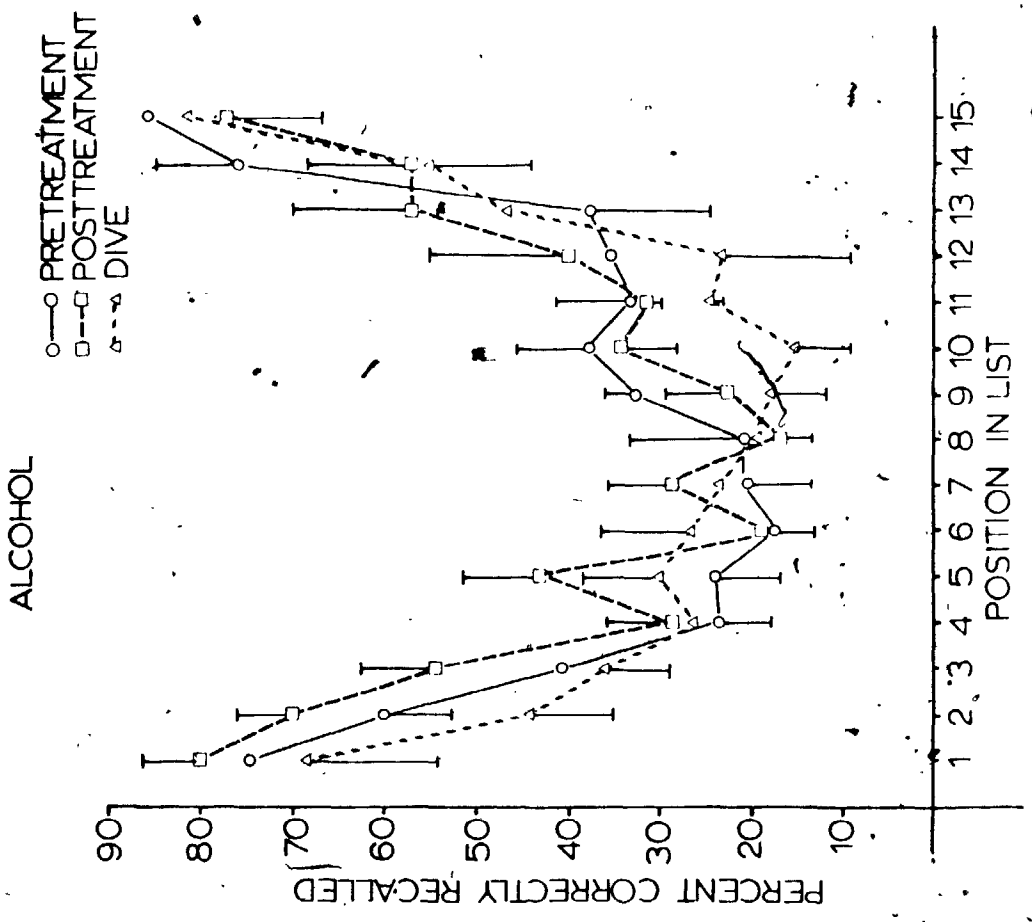

ALCOHOL
PRETREATMENT
POSTTREATMENT
DIVE
PERCENT CORRECTLY RECALLED
90
80
70
60
50
40
30
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
POSITION IN LIST

Figure 22c

Serial position curves for subjects in the DPH group-Study I. Note the decline in recall in the primacy and middle regions at depth.

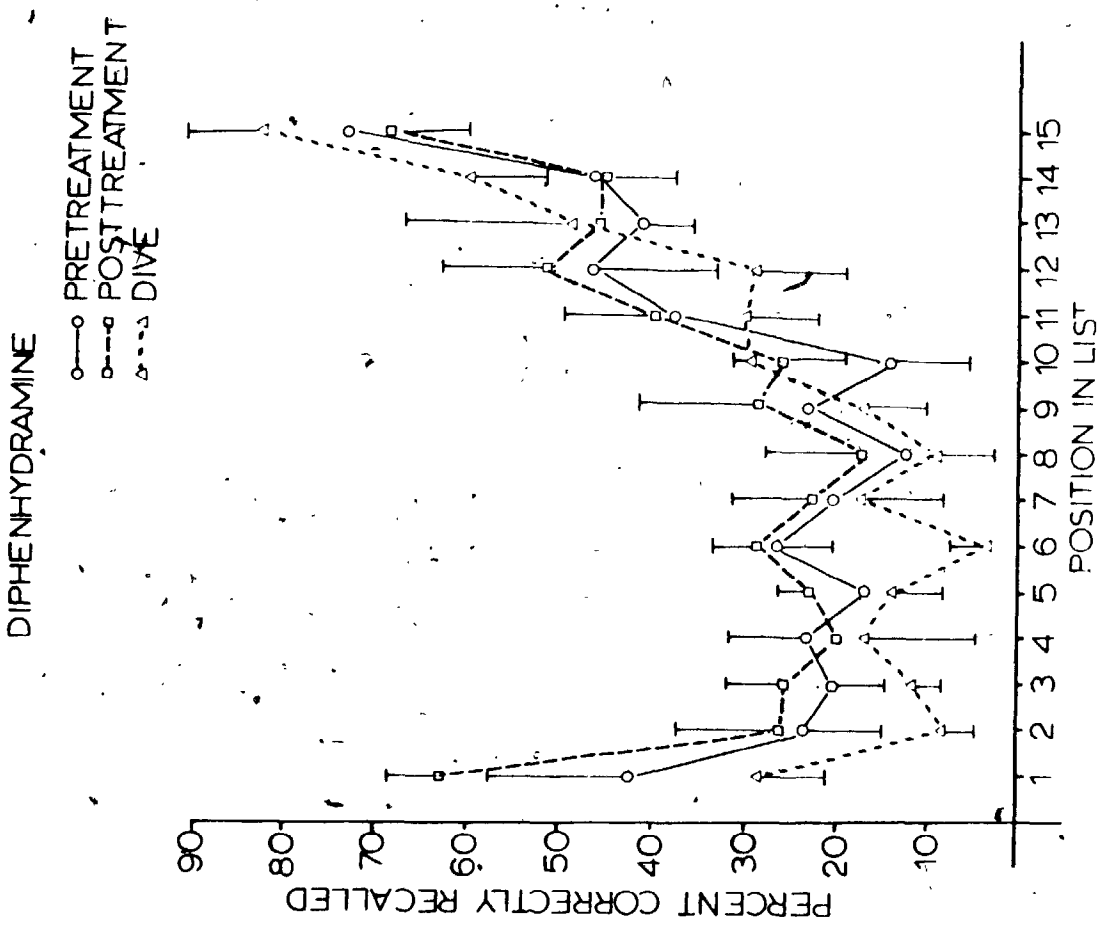

DIPHENHYDRAMINE
PRETREATMENT
POST TREATMENT
DIVE
PERCENT CORRECTLY RECALLED
90
80
70
60
50
40
30
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
POSITION IN LIST

TABLE 14

Percentage of Words Recalled in Primacy, Middle and Recency Regions at Each Test Session (Pooled Data)

		TEST SESSION		
	Group	Pre Treatment	Post Treatment	Dive
PRIMACY REGION	Control	42.8 ± 7.3	46.0 ± 5.9	24.1 ± 10.0
	Alcohol	49.4 ± 4.5	64.8 ± 6.0	51.3 ± 9.8
	DPH	26.7 ± 4.6	38.1 ± 5.8	18.2 ± 5.2
MIDDLE REGION	Control	31.8 ± 7.7	38.4 ± 7.6	25.8 ± 7.2
	Alcohol	24.9 ± 1.3	27.3 ± 2.6	25.4 ± 3.3
	DPH	19.6 ± 4.3	20.8 ± 4.5	14.6 ± 3.6
RECENCY REGION	Control	52.5 ± 4.4	58.5 ± 1.9	56.8 ± 5.8
	Alcohol	56.0 ± 7.6	52.6 ± 6.7	46.1 ± 7.9
	DPH	49.0 ± 5.8	50.8 ± 6.0	53.3 ± 5.7

(Values are Mean ± SEM)

In neither the primacy or middle regions was the groups X test session interaction significant, indicating that the drugs alone did not affect recall nor did they significantly alter the effect of hyperbaric air.

Delayed Recall:

The results are presented in Table (15) . Analysis of the data revealed that the test session effect alone was significant [$F(2,24) = 29.22$ $p<0.001$]. Further analysis showed that this was due to recall at pressure being significantly decreased ($p<0.001$) in all groups compared to the tests at the surface. The drugs alone did not affect performance on this test nor did they enhance the effect of hyperbaric air as evidenced by the non - significant group X test session interaction [$F(4,24) = 1.52$ $p>0.1$].

Recognition Test:

In the type of recognition test used in these studies, a subject responds either yes or no to each word and therefore has a 50% chance of correctly identifying an 'old' word. Thus, the proportion of 'old' words correctly identified (hit rate) by itself is not an accurate measure of memory retention since this will be affected by the response bias of the subject, i.e. his willingness to guess. For example, hit rates can be artifically inflated if a subject simply responds yes to most words. This approach however, would lead to a large number of distractor words being incorrectly identified as 'old' i.e a large false

TABLE 15

STUDY 1

Number of Words Recalled in Delayed Recall Test at Each Test Session

Group	TEST SESSION		
	Pre-Treatment	Post-Treatment	Dive
Control	16.2 ± 3.7	17.4 ± 2.3	8.4 ± 3.7*
Alcohol	14.4 ± 1.9	13.8 ± 1.6	7.6 ± 3.8*
DPH	12.6 ± 1.3	12.2 ± 1.1	8.6 ± 1.1*

Values (Mean ± SEM)

*Sig vs Pre and Post Treatment $p < 0.05$

alarm rate. On the other hand, a subject can be very conservative in his responding and respond no to most items giving rise to a low hit rate, but at the same time keeping his false alarm rate near zero. To overcome this problem imposed by response bias, the data were therefore analyzed using Signal Detection Theory (SDT) which allows one to obtain a measure of memory retention while accounting for the effects of response bias (Banks, 1966). Hit and false alarm rates were determined for each rating category and from these the nonparametric indices of sensitivity (retention) A' (Norman and Galanter, 1964) and response bias B'' (Grier, 1971) were calculated. A value of A' equal to 0.5 represents chance level of performance and the the larger of A', the better the recognition performance. B" varies between 1 and -1 with larger values indicating less willingness to guess.

The A' scores are shown in Table (16). Mean A' scores were greater than 0.5 indicating that recognition performance was better than chance. Analysis of the A' scores indicated no effect of any of the experimental conditions on recognition performance. The data for response bias are presented in Appendix II.

Ball Bearing Test:

The results of the ball bearing test are presented in Table (17). No significant group [$F(2,15) = 0.52$, $p>0.1$] or test session [$F(4,30) = 0.37$, $p>0.1$] effects were observed. The group X test session interaction however, was significant [$F(4,30) = 3.11$, $p<0.05$]. Further analysis revealed that whereas no significant difference in performance was observed between the groups at the pre and post treatment tests, a

TABLE 16

STUDY I

Recognition Data A' Scores

Group	TEST SESSION		
	Pre-Treatment	Post-Treatment	Dive
Control	0.71 ± 0.04	0.70 ± 0.04	0.66 ± 0.05
Alcohol	0.68 ± 0.05	0.77 ± 0.04	0.66 ± 0.05
DPH	0.67 ± 0.05	0.63 ± 0.02	0.67 ± 0.03

Values (Mean ± SEM)

TABLE 17

STUDY I

Number of Ball Bearings Transferred At Each Test Session

	TEST SESSION		
Group	Pre-Treatment	Post-Treatment	Dive
Control	9.7 ± 1.3	10.83 ± 4.7	10.83 ± 4.7
Alcohol	9.5 ± 1.6	10.5 ± 1.6	8.00 ± 2.2
DPH	10.3 ± 2.3	9.3 ± 2.8	12.3 ± 2.4

Values (Mean ± SEM)

significant difference was observed between alcohol and DPH groups at pressure [$F(1,30) = 4.38$, $p<0.05$]. This apparently was due to decreased performance ($p<0.05$), in the alcohol group, and a tendency to increased performance in the DPH group, compared to post treatment levels. Neither the drugs nor hyperbaric air alone affected performance on this test.

4.3.2 STUDY II

General

Blood alcohol levels 20-25 min. after administration were 0.019 (± 0.003)%. For all tests, the pre treatment scores for Day I and Day II were compared by the paired t test to determine if performance had been affected by learning or by the experimental conditions. No significant differences were found indicating that performance had stabilized, and that carry over effects were not a problem. On all of the tests, with the exception of the recognition test, there was no significant difference between the pre treatment tests on Days I and Day II, or the placebo post treatment test at the surface in either the alcohol or DPH group. Thus these values were pooled as described in the methods to give baseline performance. The analysis of the recognition data is described in its section.

Tests on memory

Immediate Recall: The serial position curves are shown in Fig (23), and the percentage of words correctly recalled in the primacy, middle and recency regions is given in Table (18). Analysis of the pooled data revealed no significant effects of any treatment conditions in either the primacy, middle, or recency regions. Some trends were observed in the primacy and middle regions however.. In the primacy region the effect of hyperbaric air tended to significance [$F(1,16)= 4.45$, $p<0.1$] this being due to a tendency for decreased recall in this region at pressure. In the middle region a trend to a significant group X drug condition interaction [$F(1,16)= 4.21$, $p<0.1$] was observed. This was due to a tendency for DPH to decrease recall in the middle region. None of the other effects tended to significance indicating no interaction between the drugs and hyperbaric air.

Delayed Recall: The results are shown in Table (19). Exposure to hyperbaric air alone significantly decreased recall compared to that at the surface [$F(1,16)= 27.89$, $p<0.01$]. The drug condition X pressure interaction was not significant however [$F(1,16)= 2.27$, $p<0.1$] indicating that the drugs did not potentiate the effect of hyperbaric air. There was a tendency however, in both groups for recall to be decreased in the drug vs. placebo condition both at the surface and at pressure, [$F(1,16)=3.63$, $p<0.1$].

Figure 23

Serial position curves for subjects in alcohol and DPH groups - Study II. Standard error bars omitted for clarity. (○——○), (□--□), (▽- -▽), (▲- -▲), denotes: no drug control, post drug, pressure, pressure + drug, respectively. These show the virtual lack of effect of the various treatments on recall. The only effects are a tendency to decreased recall in the primacy regions at pressure, which is more marked in the DPH group, and a tendency for DPH to decrease recall in the middle region.

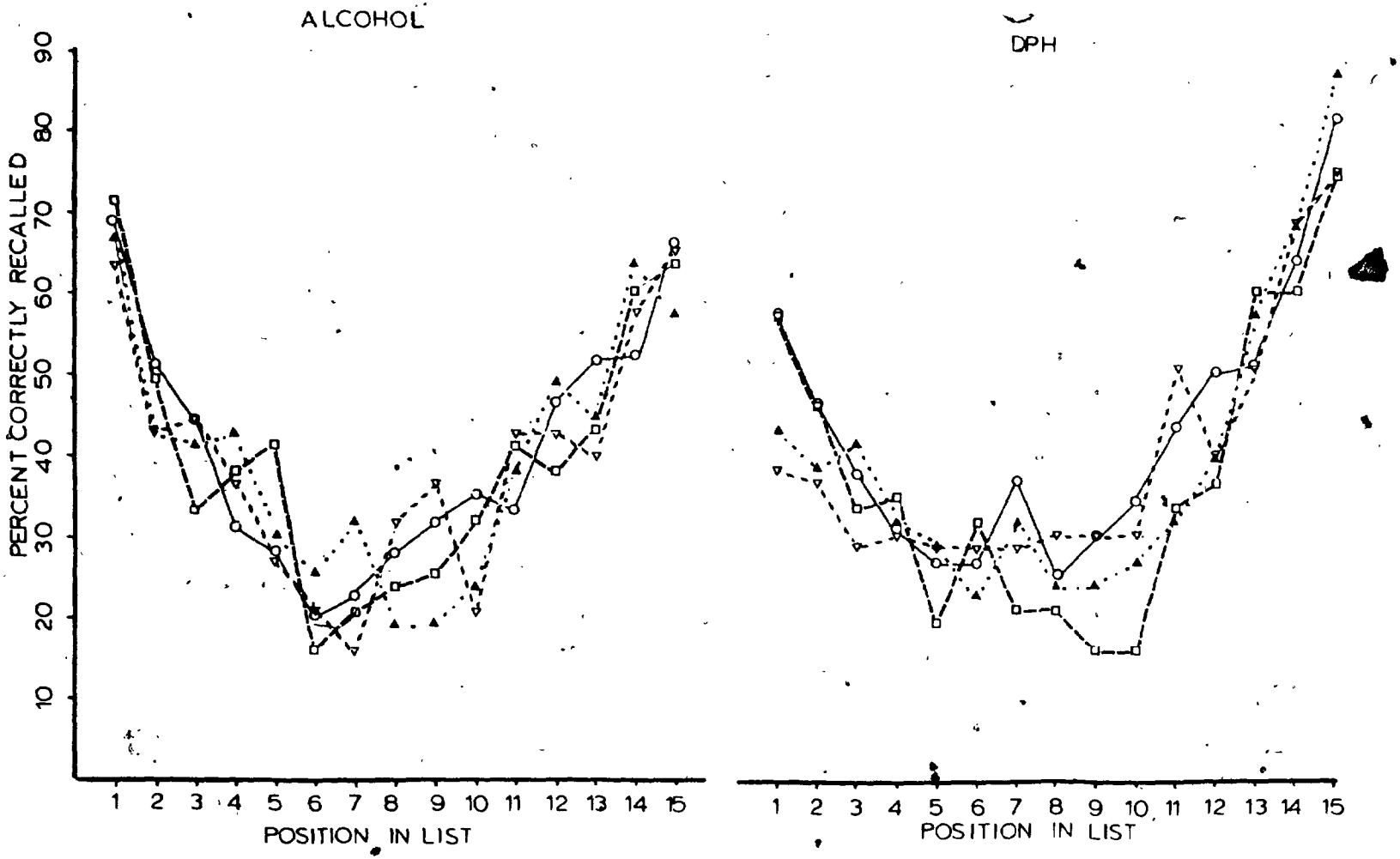

ALCOHOL
DPH
PERCENT CORRECTLY RECALLED
10
20
30
40
50
60
70
80
90
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
POSITION IN LIST
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
POSITION IN LIST

TABLE 18

STUDY II

Percentage of Words Recalled in Primacy, Middle, and Recency Region at Each Test Session (Pooled Data)

	Group	Treatment	TEST SESSION		
			Pre Treatment	Post Treatment	Dive
PRIMACY REGION	Alcohol	Placebo	56.6 ± 6.4	52.4 ± 5.0	50.3 ± 6.3
		Drug	56.1 ± 7.1	51.3 ± 7.5	50.8 ± 5.0
	DPH	Placebo	49.7 ± 9.0	40.7 ± 8.1	33.9 ± 7.2
		Drug	49.2 ± 9.3	45.5 ± 8.5	40.7 ± 8.8
MIDDLE REGION	Alcohol	Placebo	27.0 ± 5.5	27.2 ± 3.3	27.0 ± 3.9
		Drug	30.1 ± 8.3	28.1 ± 4.4	27.4 ± 3.4
	DPH	Placebo	30.8 ± 6.8	28.1 ± 5.2	29.5 ± 6.6
		Drug	30.8 ± 5.3	22.7 ± 5.0	27.6 ± 6.2
RECENCY REGION	Alcohol	Placebo	51.7 ± 7.3	50.2 ± 6.6	49.8 ± 7.9
		Drug	51.7 ± 7.2	48.2 ± 8.8	50.1 ± 7.5
	DPH	Placebo	60.3 ± 8.0	57.5 ± 6.3	56.8 ± 6.1
		Drug	56.2 ± 7.3	53.0 ± 5.8	56.8 ± 7.7

Values are mean ± SEM

TABLE 19

STUDY II

Number of Words Recalled in Delayed Recall Test At Each Test Session on Placebo and Drug Days

Group	Treatment	TEST SESSION Pre Treatment	Post Treatment	Dive
Alcohol	Placebo	13.4 ± 2.2	11.6 ± 2.3	6.4 ± 1.2*
	Drug	13.3 ± 3.1	8.5 ± 1.0	4.5 ± 1.0*
DPH	Placebo	14.6 ± 3.4	11.1 ± 2.0	6.2 ± 2.5*
	Drug	12.3 ± 2.3	9.9 ± 2.1	6.9 ± 2.2*

Values are mean ± SEM

*Sig vs Pre and Post Treatment, $p < 0.05$

Recognition test:

The mean A' scores are presented in Table (20.A). As in study I mean A' scores were above 0.5 in all cases indicating that recognition performance was better than chance. In the alcohol group however, performance on the placebo post treatment test was significantly lower compared to that on the pre teatment tests on both days. This precluded pooling of these scores to obtain baseline performance. Therefore for each subject the mean % change from the pre treatment score on the drug and placebo days were computed and used in the analysis. These data are presented in Table (20.B). Analysis of variance revealed no significant effect of any of the experimental conditions.

The data for response bias are presented in Appendix II.

Ball Bearing Test

The results are presented in Table (21). Analysis of variance revealed no significant effect of any of the treatment conditions.

Results of Questionaire:

All subjects correctly identified the drug they received and all but one (in the DPH group) correctly identified the day on which it was given (he indicated both days). All of the subjects in the alcohol group identified it by taste and/or smell, and five out of nine subjects receiving DPH detected a difference in taste. All of the subjects in the DPH group reported feelings of drowsiness, two of them, feelings of dizziness and six difficulty in thinking. In contrast in the alcohol

TABLE 20

STUDY II

A.

Recognition Data. A' Scores

Group	Treatment	TEST SESSION Pre Treatment	Post Treatment	Dive
Alcohol	Placebo	0.66 ± 0.03	0.59 ± 0.03	0.60 ± 0.04
	Drug	0.72 ± 0.03	0.63 ± 0.02	0.58 ± 0.03
DPH	Placebo	0.68 ± 0.03	0.67 ± 0.03	0.62 ± 0.02
	Drug	0.63 ± 0.04	0.60 ± 0.02	0.62 ± 0.04

B.

A' Scores. % Change from Pre Treatment Value

Group	Treatment	TEST SESSION Post Treatment vs Pre Treatment	Dive vs Pre Treatment
Alcohol	Placebo	- 9.1 ± 3.2	- 7.8 ± 7.7
	Drug	-12.8 ± 4.1	-20.0 ± 5.7
DPH	Placebo	- 0.6 ± 4.7	- 6.9 ± 6.7
	Drug	- 3.1 ± 5.8	- 0.4 ± 9.0

Values are mean ± SEM

TABLE 21

STUDY II

Number of Ball Bearings Transferred at Each Test Session

Group	Treatment	TEST SESSION		
		Pre Treatment	Post Treatment	Dive
Alcohol	Placebo	11.8 ± 0.6	11.8 ± 0.6	13.8 ± 0.9
	Drug	12.3 ± 0.6	12.3 ± 0.7	13.0 ± 1.1
DPH	Placebo	12.3 ± 1.2	12.1 ± 0.7	13.1 ± 1.0
	Drug	11.8 ± 0.7	11.2 ± 1.5	11.3 ± 1.1

Values are mean ± SEM

group no feelings of drowsiness or dizziness were reported. Four subjects reported difficulty in thinking, and four feelings of intoxication. No nausea was reported by any of the subjects. Five subjects in the alcohol group thought that their performance was worse at pressure as a result of the drug, one that his performance was better, and the others detected no change. In the DPH group five thought that their performance was worse, one that his performance was better, and two detected no change.

4.4 DISCUSSION

The results from these studies confirm the ability of hyperbaric air to produce decrements in memory and in addition show that this can occur at pressures as low as 5 ata. In contrast, no significant decrement in neuromuscular co-ordination (as measured by performance on the ball bearing test) was observed. Of all the tests on memory, delayed recall was the most sensitive to the effects of hyperbaric air with highly significant decrements being observed in both studies. Immediate recall also showed some sensitivity to the effects of hyperbaric air with recall from the primacy and middle regions of the list being significantly decreased in Study I. In Study II, no significant decrements were observed although a trend to decreased recall in the primacy region was observed. The failure of hyperbaric air to significantly decrease immediate recall in Study II may have been due to the greater level of practice allowed on this test in this study compared to Study I. As a result of this, performance on the test may have been more established and therefore less sensitive to the

disruptive effects of hyperbaric air. Performance on the recognition test was not affected by hyperbaric air in either study. The implications of these results for the nature of memory deficit produced by hyperbaric air will be discussed later.

In contrast to the effects of hyperbaric air on memory, the drugs themselves did not significantly affect memory, nor did they worsen or alter the effect of hyperbaric air. In Study II, with the higher doses of the drugs, there was a tendency for the drugs alone to decrease recall in the delayed recall test. In this study recall at pressure in the drug condition tended to be lower than in the no-drug condition Table (19), indicating possibly a trend towards an additive effect between the drugs and hyperbaric air. This, however, was not significant. Similarly, the drugs alone did not affect neuromuscular co-ordination nor did they consistently produce a significant impairment at pressure. In Study I, however, a significant decrement was observed in the alcohol group at pressure. This was not observed, however, in Study II at the higher dose of alcohol. The failure of the drugs in Study II to significantly affect performance suggests that even though attempts to make the study single-blind failed, this did not appear to materially affect the results.

If hyperbaric air produces its effects through a general slowing of CNS activity, then the failure of these drugs to consistently worsen performance at pressure, especially on the memory tests, is somewhat surprising. With regard to alcohol, the doses used were quite low and the blood levels are, in general, below those (>0.035%) reported to produce significant performance impairment on mental and motor function

(Forney and Harger, 1965). In addition they are below those (0.07%), which potentiated the effect of hyperbaric air on standing steadiness (Jones et al., 1979). However, blood levels in the range of 0.02% have been reported to produce detectable CNS dysfunction. For example, decrements in the flicker fusion test (Goldberg, 1943) have been shown to occur in this range. Therefore, given the significant decrement in performance at pressure on the ball bearing test in Study I, it is surprising that the higher levels in Study II, in the presence of the additional disruptive effect of N_2 did not produce significantly greater impairment of performance on this test. Again, this maybe due to the increased level of practice in Study II so that performance on the tests was more established and less vulnerable to disruption. In support of this, performance on the ball bearing test in Study II was generally better than in Study I Tables (17,21). Additionally, the mean pretreatment score for subjects in Study II on their first day of testing was significantly higher than the comparative scores in Study I (12.4 ± 0.49 vs. 9.8 ± 0.44, $t = 4.02$, $p<0.001$). Other studies have indicated that the level of practice of a subject on a task may influence the extent to which performance is disrupted by alcohol (Eggleton, 1955). Thus it may be that in these experienced divers these doses of alcohol are too low to significantly add to the disruptive effects of hyperbaric air , provided that the subjects are well practiced on the tests. It is possible however that at higher doses of alcohol significant interactions would be observed even in well practiced subjects. For memory, this is suggested by the tendency to additive effects between alcohol and hyperbaric air on delayed recall, Table (19).

The failure of DPH to significantly worsen performance at depth is harder to explain in terms of the doses being too low. These doses have been reported to produce marked sedation, a sign of CNS depression, in humans (Carruthers, et al 1978; Spector et al., 1980). Furthermore from the observation of the subjects in both studies, and the results of the questionaire in Study II, it was apparent that significant sedation occurred in those subjects receiving DPH. This lack of effect is also surprising in view of the results of Walsh and Burch (1979) who found that commonly used doses of DPH at pressure significantly impaired the ability of divers to learn a complex task. The report of this study is very sketchy however. The doses of DPH used are not stated nor is it clear at what pressure the significant increase in error rates occurred. Thus, a strict comparison between their study and the present studies is not feasible. Also, it is conceivable that the learning of a task may require coordination of a number of different aspects of CNS function. Thus, it may be that this type of test is more sensitive than memory to the disruptive effects of DPH and hyperbaric air.

The contrast between the effects of DPH and hyperbaric air on memory and sedation is striking however, and may, offer some clues to explain the lack of effect of DPH. For example, whereas DPH produced marked sedation (generally taken to be a sign of CNS depression) it did not have any significant effect on memory. The opposite however, was true for hyperbaric air. This would suggest therefore, that DPH at these doses exerts a very strong effect on certain areas of the brain important for the production of sedation, but has very little effect on those areas of the brain involved in memory, whereas the converse would be true of hyperbaric air. Thus, the memory impairment produced by

hyperbaric air would appear to be a primary effect on areas of the CNS important for memory rather than a secondary effect mediated through a generalized CNS depression. If this is the case, then it is not that surprising that the CNS depression produced by DPH did not worsen the memory impairment.

The failure of these drug doses to significantly impair performance at depth should not be taken as an indication, however, that they do not pose an additional hazard in a hyperbaric air environment. The subjects used in the studies were experienced divers, and in addition, the test used may not be adequate predictors of the ability of divers to react appropriately to emergency situations. Emergencies, by their very nature, tend to be unfamiliar and quite often complex situations. Therefore a diver in reacting to an emergency may be, unlike the subjects in our study who were familiar with the tasks, in a learning rather than a performance mode. The results of Study I with alcohol, in which the less well practiced subjects showed a decrement in neuromuscular co-ordination at pressure, and those of Walsh and Burch (1979), on the differential effects of DPH plus hyperbaric air on the ability of divers to learn or perform a complex task, if they can be substantiated, suggest that in such situations the responses of divers may be more sensitive to the combination of drugs and hyperbaric air.

Finally, the results in the control group in Study I, and those from the no-drug tests in Study II provide some insight into the nature of the memory deficit produced by hyperbaric air. Classically memory has been considered to consist of two separate storage systems STM and LTM with each of these systems having different characteristics

(Atkinson and Shiffrin, 1968; Lewis, 1979). STM is usually considered to be of limited capacity, and the route of entry to LTM for new memories, its contents being temporary and fragile, either decaying rapidly unless rehearsed, or transferring rapidly to LTM. LTM is considered to store memories permanently, and to have almost unlimited capacity. Based on this model, most forgetting in memory is considered to be due to either input failure due to breakdown in transference of information from STM to LTM, or retrieval failure in which the item is in memory but cannot be retrieved.

The highly significant decrement at pressure in delayed recall in both studies, and the decrement in immediate recall from the primacy and middle regions of the curve, in Study I suggests that hyperbaric air affects recall from LTM. The failure of hyperbaric air to similarly affect immediate recall in the Study II, suggests however that in well practiced subjects a significant decrement in recall from LTM may not occur when recall is tested immediately after presentation. This finding would suggest therefore that the deficit seen in delayed recall is due to retrieval rather than input failure. This is confirmed by the differential effects of hyperbaric air on the delayed recall vs. recognition test. As stated earlier, recognition tests bypass the retrieval process and provide direct access to the memory store. Thus the lack of a significant difference on recognition performance contrasted with the decrement in recall, would suggest that retrieval rather than input failure is the cause of the deficit. In addition the results of the immediate recall test suggest that STM is not affected by hyperbaric air, since in neither study were significant decrements observed in the recency region which is thought to represent recall from

STM (Glanzer, 1971).

Although the effect of hyperbaric air on memory has been discussed in terms of the classical concepts of memory, it should be noted that the validity of such a two storage system is disputed by several workers, see Lewis (1979) for a review of the arguments. In brief, these workers argue against the need for STM, and consider forgetting to stem primarily from retrieval failure. No attempt will be made to discuss the pros and cons of these different views. Suffice it to say that the nature of the memory deficit reported here is compatible with both views.

These results, in general, support those of Fowler and co-workers, which suggest that hyperbaric air affects LTM but not STM (Fowler, 1973; Fowler and Ackles, 1975). In addition, the effect of hyperbaric air on immediate recall in Study I is similar to that reported by Fowler et al., (1980) for N_2O, lending support to their proposal that N_2O might be useful as a more practical and easier means of studying some of the effects produced by hyperbaric air. There is disagreement however, between the data reported here and that of Fowler and co-workers regarding the nature of the deficit produced by hyperbaric air. In contrast to the results reported here which suggest that the deficit is due to retrieval failure, their work suggests that input into rather than retrieval from LTM is impaired by both hyperbaric air, and N_2O. The reason for this discrepancy is not clear. However, some possibilities suggest themselves. Firstly, their studies with air were performed at 10 ata, and therefore it is possible that the greater "narcotic" effect at this pressure might produce a deficit in input. A

similar case can be made for the experiments with N_2O as 35% N_2O, the concentration used in their studies, may be more narcotic than 5 ata air (Biersner et al., 1977). Secondly, different retrieval aids were used in their studies, ie. cued recall compared to recognition in the studies reported here, and it is possible that hyperbaric air, and N_2O might disrupt the association between the cue and its target word. If this occurs, this would decrease the effectiveness of the cue, and give rise to an apparent input failure. There is some evidence that this may occur for hyperbaric air (see Fowler and Ackles,1975, Experiment III). In this experiment , exposure to 10 ata air prevented the facilitatory effect of cueing on recall of materials learned at the surface. Further studies at 10 ata air, or with 35% N_2O, using a recognition test instead of cued recall would resolve this.

In summary, the results show that significant decrements in memory similar to those previously seen at 10 ata (Fowler,1973; Fowler and Ackles,1975), can occur at pressures as low as 5 ata air. DPH at the doses used, despite producing marked sedation, did not worsen the deficit in memory produced by hyperbaric air, or combine with hyperbaric air to affect motor co-ordination. Prior consumption of alcohol also did not consistently worsen performance at depth, although a signifcant decrement in performance in the ball bearing test was observed at the low dose of alcohol in study I. It is suggested that the failure of alcohol to consistently impair performance at depth may have been due to the doses being too low to produce any decrements in these tests in well practiced, experienced divers. Furthermore the contrasting effects of DPH and hyperbaric air on memory and sedation would suggest that the effect of hyperbaric air on memory, is not due to a general slowing

(depression) of CNS function, but instead may be due to effects on specific areas of the brain. One possibile area might be the hippocampus as this is often suggested to play an important role in memory (Drachman and Arbit, 1966). The implications of these results for the classical theory of N_2 narcosis, and the modification of centrally acting drugs by hyperbaric air will be discussed in Chapter 5.

CHAPTER 5

DISCUSSION

5.1 General

The experiments reported in this thesis clearly indicate that hyperbaric air at pressures in the air diving range is capable of modifying the effects of centrally acting drugs. In general the modifications appear to be due to the increased PN_2 rather than to increases in PO_2 or hydrostatic pressure, and are quite complex. Furthermore, the rapidity with which the modifications occurred and their complex nature, make it unlikely that they were due to alterations in the pharmacokinetics of the drugs at pressure. Thus they would appear to be due to the CNS effects of N_2.

Crucial to an understanding of the modification of the effects of centrally acting drugs by hyperbaric air, is the way in which N_2 is thought to affect CNS function, and thus would alter the changes produced by the drugs. It should be noted that drug-induced changes in CNS function may consist of two actions, these being the initial action of the drug at its defined target site or sites (e.g. specific receptors for most drugs), and the secondary changes in areas not directly affected by the drug. Thus the effects of N_2 on both the initial and secondary (or indirect) actions of the drug must be

considered.

As described earlier (Chapter 2), historically N_2 has been considered to produce a generalized depression of CNS function, due to its apparent ability to depress the hypothesized ascending reticular activating system (ARAS).[a] As you may recall the ARS was considered to be the "energizer" of the brain, being responsible for transferring to the cortex the tonic activating sensory inflow necessary to maintain the awake state (Moruzzi and Magoun, 1949). Progressive reduction of this flow by anesthetics was thought to deprive the cortex and other diencephalic structures of their activating input, resulting in a progressive slowing of CNS activity, and at some point, loss of consciousness (French et al., 1953). Presumably therefore, depression of the ARS by N_2 at pressures below those required to cause loss of consciousness, could slow CNS activity sufficiently to result in effects associated with N_2 narcosis, such as impairment of cognitive function, neuromuscular co-ordination and memory.

Evidence indicating the ability of N_2 to produce increased activity in some areas of the CNS (Roger et al., 1955 ; Jullien et al., 1955) was not seriously considered. Similarly to the more conventional anesthetics, this was thought to be transitory, due possibly to an initial disinhibition by N_2 (Bennett, 1966). The result of subsequent experiments in cats (Bennett and Hayward, 1967) which suggested that N_2

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a)Moruzzi (1972) subsequently revised the earlier concept of the ARAS, in particular the notion of a non-specific "activating" system. In keeping with this, the ARAS will be referred to hereafter as the ascending reticular system (ARS).

produced an increase in neuronal CL^+ and Na^+, led Bennett to reconsider and suggest that increased excitability might indeed be a component of inert gas narcosis. In his later reviews, however, (Bennett, 1969 ; 1975 ; 1981) this theory was never developed.

Of importance with regard to the way in which this hypothesis would consider N_2 to modify drug effects, was the fact that the ARS appeared to be the only site in the CNS directly affected by N_2 in the 1-7 ata air range. Similarly therefore to the findings with anesthetics (French et al., 1953), the ARS appeared to be extremely sensitive to depression by N_2. Thus the ARS was depressed at pressures at which other areas in the CNS such as spinal synapses, and more importantly the cortex were unaffected (Bennett, 1963a ; 1963b ; Bennett and Glass, 1961 ; Bevan, 1971). For example experiments on "alpha blocking" (Bennett and Glass, 1961) suggested that in humans, the ARS could be depressed at pressures as low as 3-4 ata, although quite long times, 30-50 min were required. In contrast the results of the study by Bevan (1971) in which the Contingent Negative Variation was used as the indicator of cortical function, suggested that N_2 did not directly affect the cortex even at pressures of 10 ata air.

In summary therefore, this hypothesis would suggest that there are only two effects of N_2 of importance regarding the modification of drug effects by hyperbaric air, (i) the depressant effect on the ARS, and (ii) the secondary depression produced in other areas of the CNS due to the depression of the ARS. Direct effects of N_2 on other areas of the CNS are not considered as important. In fact even if there were taken into consideration they would not alter the predictions regarding the

modification of drug effects by hyperbaric air, since they too would be expected to produce depression of neuronal activity.

This view of N_2's effect on CNS activity and the predictions it makes regarding the modifications of centrally acting drugs by hyperbaric air, is not adequate, however, to explain all of the results obtained in this thesis. As discussed earlier, based on this view one would predict that exposure to hyperbaric air would enhance the depression produced by CNS depressants, and reduce the excitatory effects of stimulants. The results obtained in the present experiments are more complex, however. For example the antagonism by N_2 of amphetamine- induced convulsive activity (Table 2) and stereotyped licking (Fig 6), the prolongation of pentobarbital sleep time (Table 12), and the transient enhancement of the depressant effect of the high dose of alcohol on locomotor activity (Fig 20) are at first glance, compatible with the view that N_2 produces a depression of CNS activity. The enhancement of the locomotor activity induced by amphetamine (Table 3), and morphine (Table 4), the increased activity seen at the high dose of alcohol at 4 ata air (Table 10B), and the reduction of the DPH-induced depresssion of activity (Tables 7,8), do not fit in with this view, however. Furthermore in the human studies, the ability of hyperbaric air to impair memory without producing sedation (Tables 15,19), whereas DPH which produced marked sedation failed to impair memory or enhance that produced by hyperbaric air, also questions the view that N_2 at these pressures produces a generalized depression of CNS activity. The results obtained from the animal experiments in this thesis appear to suggest that both increases as well as decreases in neuronal activity are occurring in the CNS at these pressures. Reports

in the literature on the effects of centrally acting drugs at increased pressures of air tend to substantiate this (Walsh, 1974 ; Walsh and Burch, 1977a ; Thomas and Walsh, 1978 ; Jones et al., 1979).

The hypothesis that increased PN_2 produces a generalized depression of CNS activity was not based on a detailed systematic study of the effects of N_2 on the CNS. Rather it was formulated based on data obtained in the early 1950's and mid 1960's, which suggested that it and the other inert gases acted similarly to the conventional anesthetics (Bennett, 1966). As such this hypothesis was heavily influenced by concepts of anesthetic action at the time, namely (i) that anesthetics produced a generalized depression of CNS activity (Geudel, 1937 ; Himwhich, 1951), (ii) the apparent exquisite sensitivity of the ARS to depression by anesthetics and the implications that this would have on CNS activity (French et al., 1953) and (iii) the consideration that the stimulation (disinhibition) seen at low concentrations of anesthetics was only transitory (Geudel, 1937 ; Himwhich, 1951). Since the early work to elucidate the mechanism of action of N_2, very little detailed studies have been done to confirm the original hypothesis or to enhance our knowledge regarding the effects of N_2. Significant advances have been made, however, in the understanding of the mechanism of action of anesthetics and their effects on CNS activity, the newer concepts differing quite markedly from those existing at the time the mechanism of N_2's action was formulated. It is proposed that an alternative hypothesis of N_2's effects on CNS function based on current concepts of anesthesia, offers a better means of explaining the present results, and predicting the possible outcome of the effects of centrally acting drugs at pressure. A review of the recent literature, however, indicates that

there has been very little attempt to incorporate new information regarding anesthetic action into discussions of the effects of N_2 on CNS activity, or the modification of centrally acting drugs by hyperbaric air.

In the following, current concepts of anesthesia will be briefly reviewed, and evidence will be presented to show that N_2 and other inert gases adhere to these. Then using this information, an alternative hypothesis for the effects of N_2 on CNS activity over the air diving range will be proposed, and contrasted with the earlier view. In addition their different implications regarding the modification of drugs by hyperbaric air will be highlighted. Finally, an attempt will be made as far as possible to postulate mechanisms by which N_2 could produce the results obtained in the experiments in this thesis.

5.2 Current Concepts of Anesthetic Action

5.2.1 Effects on CNS Activity

The concepts of anesthetic action existing at the time the early work on the CNS effects of N_2 was performed, were formulated primarily from studies with ether and the barbiturates (French et al., 1953), anesthetics which at concentrations above those producing loss of consciousness do tend to produce a generalized depression of CNS activity (Winters, 1976 ; Rosner and Clark, 1973). Data accumulated in the ensuing years from more detailed studies on these and other anesthetics suggest another view, however. Before reviewing the data, it should be noted that in general such studies have been neither

systematic nor exhaustive, thus there are many gaps in the information regarding the effects of many anesthetics on various areas of the CNS. This is particularly so in regard to the low concentration ranges important for the present experiments. Thus although detailed maps of the effects of anesthetics on CNS activity cannot be constructed, the data does allow one to identify a number of characteristics common to all anesthetics, and in particular to refute the notion that anesthetics necessarily produce a generalized depression of CNS function.

The available data clearly indicate that the concept that anesthetics produce a generalized depression of CNS activity is an oversimplification of the changes produced by anesthetics in CNS activity, and in fact is totally incorrect for some. Rather complex changes are observed with both increases and decreases in activity being possible. Furthermore it is clear that anesthetics do not all act alike, but instead display some degree of specificity in their CNS effects. Thus different types of anesthetics produce their own characteristic sequence of stimulation and depression of CNS activity, the areas of the brain affected and the type of effect observed depending upon the kind of anesthetic and the dose (Mori et al., 1967 , 1968 ; Rosner and Clark, 1973 ; Winters, 1976). For example whereas the barbiturates, halothane and ether at concentrations above those producing loss of consciousness do tend to produce generalized CNS depression, enflurane and ketamine do not. They produce increasing excitation in certain areas of the CNS, and in the case of enflurane, seizures may result (Winters, 1976).

At concentrations below those causing loss of consciousness, i.e. those most important for effects of N_2 in the air diving range, studies have confirmed the ability of anesthetics to increase activity in certain areas of the CNS (Mori et al., 1968; Winters, 1976). Also as will be seen later, stimulation of activity is not necessarily transitory as origionally thought, but will persist if pre anesthetic concentrations are maintained.. The stimulation of activity at these concentrations is not thought to be due to a direct stimulant effect of the anesthetic. Rather it is thought to be due to disinhibition due to preferential depression of sensitive inhibitory neurons (Ritchie, 1975).

Of great importance in regard to the validity of the early view that N_2 produced its effects through depression of the ARS, is the fact that the ARS is activated, not depressed, at these concentrations. Activation of the ARS is thought to be responsible for the EEG desynchronization, i.e the low voltage, fast activity (LVFA) of the alert state, commonly observed at these concentrations.[a] Thus in cats, mesencephalic transections which interrupt the connection between the mesencephalic reticular formation (MRF), the major site of the ARS, and the cortex, prevented the LVFA normally induced by ether, N_2O or cyclopropane (Rossi and Zirrondoli, 1955). In contrast transection at the pontine level, leaving the connection between the MRF and cortex intact, did not block the response. Also, the dominant frequency and scalp distribution of the EEG desynchronization produced

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a[Cyclopropane may be an exception to this (Mori et al., 1972). LVFA does occur, however, following cyclopropane administration in animals with pre trigeminal rostropontine transections (Rossi and Zirondoli, 1955), leading Rosner and Clark (1973) to speculate that cyclopropane may also excite brain stem regions below the level of the trigeminal root, which may somehow block the appearance of LVFA.]

by anesthetics was similar to that seen in normal arousal (Rossi and Zirondoli, 1955 ; Rosner and Clark, 1973), suggesting that it was produced by the same neurons responsible for the response during normal arousal. Further evidence is provided by the fact that in experiments with ether in cats, the appearance and subsequent disappearance of LVFA appeared to be correlated with changes in the firing rate of some neurons in the MRF (Schlag and Brand, 1958). Thus it would appear that the ARS responsible for mediating electrical and behavioral arousal is initially activated by anesthetics before it is depressed. Furthermore studies with ether and barbiturates indicate that if pre anesthetic concentrations are maintained, this activation of the ARS is not transitory, but may persist until the point at which consciousness is lost (Bellville and Artusio, 1955 ; Artusio, 1954 ; Finesinger et al., 1947).

The extent to which increases in activity occur in different areas of the CNS is not known. Also, since anesthetics display some degree of specificity in their CNS actions, the areas exhibiting increased activity may vary for different types of anesthetics. Based on their review of studies with barbiturates, Rosner and Clark (1973) suggested that the initial rapid activity generated in the ARS by anesthetics, is propagated to other areas of the CNS such as the medial thalamus (Okuma et al., 1957), basal ganglia (Hinko et al., 1970), and the hippocampus (Brazier, 1969). From an examination of the references cited this appears to be true for the hippocampus. In this study, pre anesthetic doses were used and the LVFA was persistent making it easy to verify the statement. In the other studies anesthetic doses were used and as a result the LVFA was only transitory. From the data presented in these

studies, it is difficult to tell if a spread of excitation did occur. At present therefore, it is difficult to completely verify their suggestion. It should be noted, however, that increased activity is not generalized. For example depressant effects can be observed in certain parts of the CNS, e.g. the RF, with all anesthetics tested (Mori et al., 1967 ; Winters, 1976).

The ability of anesthetics to exert some degree of specificity in their CNS actions is also observed at concentrations below those causing loss of consciousness. Thus for example, differences are observed in the ability of ether and barbiturates to depress the transcallosal response, and transmission through the RF (Darbinjan et al., 1971). Indirect evidence of the ability of anesthetics to exert some degree of specificity in their CNS actions at these low concentrations is also provided by their physiological effects. Thus for example analgesia is present in the very early stages of ether anesthesia (Artusio, 1954), whereas barbiturates tend to be hyperalgesic (Harvey, 1975). It is appreciated that these are only a few examples. Detailed information on anesthetic effects at these concentrations is limited, however.

5.2.2 Cellular Mechanism

The ability of anesthetics to display some degree of specificity in their actions has also led to changes in the way anesthetics are thought to exert their effects on neural function. As you may recall, classically, anesthetics were thought to act alike and alter neural function through a nonspecific effect on membrane lipids (Richards, 1978 ; Roth, 1979). Lipid theories of anesthesia still receive much support, more recent theories suggesting that anesthetics disrupt

membrane function by expanding the membrane (Roth and Seeman, 1972), altering lipid fluidity (Lawerence and Gill, 1975), or changing the phase transition temperature of the lipids (Trudell, 1977a). Specificity of action is incompatible, however, with proposals that anesthetics act through a nonspecific effect on membrane lipids, and this, together with the fact that the validity of the recent lipid theories are in doubt (Richards et al., 1978 ; Trudell, 1977b ; Franks and Lieb, 1979), has led to strong support for theories which propose that anesthetics act through direct effects on membrane proteins (Richards, 1978 ; Richards et al., 1978 ; Labella, 1980). Hydrophobic pockets in proteins have been suggested to have the necessary structure to allow specificity of anesthetic action (Richards et al., 1978 ; Labella, 1980), specificity being explained by proposing that the various functional proteins at different synapses in the CNS have differential sensitivity to different anesthetics. Indeed, specificity of action has been observed at the synaptic level with for example differences being observed in the effects of different types of anesthetics on glutamate (Richards and Smaje, 1976), and acetylcholine responses (Smaje, 1976). The site of anesthetic action, however, whether lipid, protein or both, is as yet unresolved.

5.2.3 Summary

In summary therefore, current data on anesthetic action suggests that at concentrations below those producing loss of consciousness, i.e. the range of interest for the present experiments, anesthetics produce neither generalized depression nor stimulation of CNS activity. Rather a mixture of stimulatory (disinhibitory) and depressant effects are

observed. Furthermore the types of effects and the areas affected depend upon the type of anesthetic and its dose. Also, it is clear that anesthetic-induced changes in CNS activity at these concentrations do not stem from a depressant effect of the anesthetic on the ARS. In fact at these concentrations anesthetics appear to disinhibit rather than depress the ARS. The differing effects produced by different types of anesthetics suggest instead that anesthetics have multiple sites of action, an effect on the ARS being only one of them.

5.3 Review of Effects of N_2 and Inert Gases on CNS Activity

Although detailed studies of the effects of N_2 and the other inert gases on CNS activity have not been performed, there is evidence from both human and animal studies to indicate that they, like other anesthetics, do not produce a generalized depression of CNS activity at concentrations below those causing loss of consciousness. Rather, they also appear to produce some CNS stimulation and exhibit some degree of specificity in their CNS actions.

5.3.1 Stimulatory Effects

In humans, evidence of CNS stimulation is provided by the behavioral stimulation seen at low pressures of air, manifested by excitement and loquacity (Behnke et al., 1935 ; Bennett, 1975). Additional evidence is provided by the fact that the EEG desynchronization (LVFA), produced by anesthetics at low concentrations, and thought to represent activation of the ARS (Rosner and Clark, 1973), also appears to occur with hyperbaric air (Roger et al., 1955;

Cruscioli and Albano, 1971; Z'altsman, 1968), N_2/O_2 mixtures (Cruscioli and Albano, 1971; Townsend et al., 1971), and Xe (Morris et al., 1955). These studies were reviewed earlier (Section 2.1.3). Finally, the EEG changes, and LSD like effects seen at 13-14 ata air, are similar to those observed in Stage II anesthesia (Z'altsman, 1968 ; Adolfson, 1965 ; Winters, 1976). This stage of anesthesia is considered to be associated with CNS stimulation not depression (Winters, 1976), suggesting that CNS stimulation occurs at pressures of air well beyond the sport diving range.

In animals there is less direct evidence of stimulation. Bennett and Dossett (1973) reported an increase in frequency of the alpha and beta 1 and 2 bands in rats, following compression to 4 ata with air or N_2 (PO_2 fixed at 2 ata). At pressures beyond 4 ata, however, the increase in frequency in these bands declined. This decline in fast activity at these low pressures is surprising in view of the fact that a short-lived LVFA is still seen at pressures of 10 and 12 ata in humans (Roger et al., 1955 ; Cruscioli and Albano, 1971). Humans are generally thought to be more sensitive to the effects of the inert gases compared to animals. For example whereas it is believed that at pressures not much greater than 13 ata air (PN_2 = approx. 10 ata) would produce anesthesia in humans, the ED_{50} for the loss of righting reflex in mice (Miller et al., 1967), and rats (Bennett et al., 1980), are approximately 34, and 18 ata N_2 respectively. Therefore, given the greater sensitivity of humans to the effects of the inert gases it would have been predicted that the whole sequence of changes produced by the inert gases, namely the appearance and disappearance of LVFA and onset of anesthesia, would occur at lower pressures in humans compared to

animals. The rate of compression in the study by Bennett and Dossett (1973) was very fast however, approximately 4 ata/min. Thus both the initial excitatory effect and the subsequent decline they reported, might have been influenced by effects due to CO_2 build up. An additional problem with these results is that straight lines were used to illustrate changes in the EEG between the pressures at which measurements were made. This may be inappropriate as it implies a linear relationship between the changes in the EEG and pressure where one may not exist. Other evidence that the inert gases can produce an increase in CNS excitation in animals comes from the work of Jullien et al., (1955). These authors reported signs of neuronal hyperexcitability in cats under light dial anesthesia at 6 ata air. Responses at the cortex evoked by light stimuli showed a dimunition in the absolute refractory phase, this effect lasting for 35-40 min.

The evidence reviewed above, although indirect in the majority of cases, would suggest that exposure to increased PN_2 at pressures below those causing loss of consciousness does produce some increase in CNS activity. Of importance for the modification of the effects of centrally acting drugs by hyperbaric air, however, are the pressures at which increases in CNS activity would be present, and whether they are only transitory, and therefore not of much concern. Presently, there is not a great deal of information to thoroughly answer these questions. Some speculations can be made, however, based on the information available.

As reviewed earlier, the increases in CNS activity produced by ether and pentobarbital at low concentrations are not transitory

(Artusio, 1954 : Finesinger et al., 1947). As you may recall, this stimulation of CNS activity was thought to be due to a preferential depression of highly sensitive inhibitory neurons (Ritchie, 1975). Based on this, there is no a priori reason not to assume that at low pressures N_2 will also preferentially depress these neurons, and give rise to a sustained CNS stimulation.

With regard to the pressures of air at which CNS excitation would be observed, Bennett and Dossett (1973) have reported that based on experiments in animals and man, Z'altsman (1968) related the LVFA stage of anesthesia to air pressures of 5-7 ata, with the stage of reduction in activity occurring at 9 ata. This sequence of changes would appear to be compatible with the results of other human studies. For example the behavioral stimulation seen with air normally commences around pressures of 4 ata (Behnke et al., 1935). Similarly, the claim that the stage of reduction in activity occurs at pressures around 9 ata, is compatible with the data of Roger et al., (1955) and Cruscioli and Albano (1971). As you may recall in these studies performed at 10 and 12 ata air respectively, although LVFA was observed it was only transitory, presumably disappearing as the concentration of N_2 in the CNS increased beyond a certain point. Finally, as discussed earlier in this section, the similarity of the effects of air at 13-14 ata to stage II anesthesia (Adolfson, 1965 ; Z'altsman, 1968 ; Winters, 1976), suggests that even at these pressures, stimulation is present in certain areas of the CNS.

In animals, the only experimental data available is that from the study in rats by Bennett and Dossett (1973), referred to earlier. Their

results suggested that in rats, increased CNS activity, manifested by LVFA, would be limited to pressures of 4 ata and below. However, as discussed previously (Section 5.3.1), this is not compatible with the relative sensitivities of animals compared to humans to the effects of N_2, and could have been influenced by CO_2 effects. Some predictions as to the situation in mice may be inferred from the experiments on spontaneous activity reported in this thesis. In these experiments, there was a tendency for exposure to 7 ata N_2/O_2 to stimulate the activity of naive, saline treated mice (Table 6). In fact the results suggested that significant stimulation of activity would have occurred at slightly higher pressures, since activity was increasing with increasing PN_2. Exposure to a 35% N_2O mixture stimulates locomotor activity in mice (Hynes and Berkowitz, 1979). Thus based on a comparison of the relative anesthetic potencies of N_2 and N_2O (Miller et al., 1967), significant increases in the locomotor activity of mice might be predicted to occur around pressures of 9-10 ata air. Thus although from the present data conclusions cannot be drawn as to the PN_2 at which disinhibitory effects would commence, the inference that significant stimulation of activity would occur at 9-10 ata, suggests that disinhibition would still be prominent at these pressures.

In summary, the evidence suggests that in humans increased activity would occur in certain areas of the CNS, commencing at 4-5 ata and possibly persisting up to pressures of 13-14 ata. In mice the pressures at which excitatory changes would commence is not known. It is predicted however, that they would still be prominent at pressures of 9-10 ata air.

5.3.2 Specificity of Action

Evidence that N_2 and the other inert gases exhibit some degree of specificity in their CNS actions is provided for example by the different effects of N_2 compared to pentobarbital and halothane on visual evoked potentials in the guinea pig (Hempel et al., 1979). Thus, whereas anesthetic doses of barbiturates and halothane were required to depress the electroretinogram, this was reduced by 16 ata N_2, which did not produce anesthesia in the guinea pig (Hempel et al., 1979). Also the effect of N_2 on the visual cortex potential was quite different from that produced by halothane, or pentobarbital (Hempel et al., 1979). The latter anesthetics produced a restructuring of this potential, the biphasic early component being transferred into a single large positive wave. No such effect was seen with N_2. Based on their results these authors concluded that the effects of pentobarbital and halothane are so unlike those produced by high pressures of N_2 that postulation of a common mechanism for their effects is disallowed.

Additional evidence for some degree of specificity is provided by the fact that during Xe anesthesia, the EEG pattern observed is quite different to that produced by ether or cyclopropane, (Morris et al., 1955; Pittinger et al., 1955). For example, burst- suppression and total suppression, effects seen with the other anesthetics were not observed with Xe in studies in man (Morris et al., 1955), nor in monkeys, even at a pressure of 3 ata (Pittinger et al., 1955). In both of the studies, profound anesthesia was still present, despite the absence of these changes in the EEG. Also, Xe does not produce the slow single rhythmic pattern produced by ether or cyclopropane (Morris et

al., 1955).

Specificity of action is also evident from the differences between the physiological effects of Xe compared to other anesthetics. For example, Xe produces sufficient analgesia for surgical operations at concentrations which do not significantly affect important systems such as respiration. Barbiturates on the other hand are hyperalgesic at low doses, and only produce analgesia at very high levels, which also depress respiration (Harvey, 1975). Also, as Pittinger et al., (1955) report, the order of disappearance of the reflexes when Xe is administered is quite different from the order when ether is used.

From this review of inert gas effects in the CNS it seems reasonable to conclude that N_2 and the other inert gases like the more common anesthetics, produce at low concentrations (pressures) increased activity in certain areas of the CNS, such as the ARS, and exhibit some degree of specificity in their CNS actions.

The evidence reviewed earlier (Section 5.3.1) which suggests that N_2 at these pressures activates the ARS, is contrary to the conclusions of the early studies on "alpha blocking" (Bennett and Glass, 1961), and evoked potentials (Bennett, 1964), which instead suggested that N_2 depressed it. This apparent contradiction is resolved in the following section.

5.3.3 Review of the Role of the RF in the CNS Effects of N_2

Present knowledge suggests that the conclusions of ARS depression by N_2 drawn from the studies on "alpha blocking" and evoked potentials are erroneous. Thus as will be shown below, both the sensory evoked response in the RF used by Bennett, (1964), as well as the vertex potentials used by Bevan (1971) and later Bartus and Kinney (1975), would appear to be invalid as measures of the activity of the ARS. In fact only "alpha blocking", the parameter used by Bennett and Glass (1961), would appear to be valid as a measure of the functional state of the ARS. The conclusions of their study have been questioned however (Towensend et al., 1971).

The sensory evoked response has been widely used in anesthesia research as a measure of the activity of the ARS (French et al., 1953 ; Arduini and Arduini, 1954 ; Bennett, 1964), being considered to represent the excitation of ARS neurons transferring an arousing or activating stimulus to the cortex (Moruzzi and Magoun, 1949 ; French et al., 1952). This conclusion stemmed from studies which showed that the areas in the RF at which sensory evoked potentials could be recorded were co-existent with those which upon electrical stimulation produced EEG and behavioral arousal (Moruzzi and Magoun, 1949 ; French et al., 1952). This, plus the similarity of the EEG and behavioral arousal produced by sensory, or electrical stimulation of this area, led to the link being formed between the sensory evoked response and activation of the ARS. Sensory impulses from collaterals of the specific sensory pathways were thought to merge, losing specificity in the RF, and produce an ascending activating influence on the cortex (Moruzzi and

Magoun, 1949 ; French et al., 1952).

Present data, however, question this. For example, studies have shown that depression or abolition of the sensory evoked response is not necessarily correlated with depression of the ARS. Thus the sensory evoked response can be reduced or abolished at a stage when the ARS is displaying increased activity (Mori et al., 1968), and electrical stimulation can still produce EEG arousal (Darbinjan et al., 1971). Furthermore, single unit studies on the sensory input into the RF show a level of organization which is not commensurate with a non specific activating role. Thus although convergence of sensory impulses does exist, in that a number of cells are multimodal, responding to somatic, auditory and visual stimuli (Schiebel 1980 ; 1981), this convergence is not unlimited, nor non specific as originally thought. Multimodal cells instead show a considerable degree of congruence (Scheibel, 1980 ; 1981). Thus the convergence of different sensory stimuli onto a particular cell appears to be determined by the spatial position of the stimulus in regard to the animal. For example, an auditory sensitive unit which also responds to hind limb stimulation is likely to respond to auditory stimuli presented well to the rear of the organism. Scheibel (1980 ; 1981) also suggests that this sensory map is linked with a motor map, which would act to centre the fovea on the spatial point around the animal which is represented multimodally at the stimulation site . He concludes that the usefulness of this kind of sensory motor organization in the conjoint control of eye and head movement is apparent.

In fact, the case for the involvement of the sensory evoked response in the control of muscle activity has been argued quite convincingly by Siegel (1979). On the basis of a review of studies on evoked activity in pontomedullary RF neurons, Siegel concluded that the sensory evoked response in RF neurons represents a discharge related to control of muscle activity, rather than an arousing stimulus. For example cells responding to sensory input still show phasic discharges which are correlated with motor activity even though the sensory input into these neurons was eliminated. Thus elimination of the sensory input into a cell discharging in relation to auditory or visual input by blocking the ear with wax, or by placing the animal in a light tight box, did not abolish its activity. Phasic discharges correlated with muscle activity in the EMG were still observed (Siegel and McGinty, 1977). Data from invertebrates such as the dogfish (Restieaux and Satchell, 1958), in which the RF is the major site of sensory motor organization, also tend to support this hypothesis (Siegel, 1979). Sensory evoked responses in the reticular cells of such species show convergence and congruence similar to that seen in mammals (Scheibel, 1980 ; 1981). In these fish it is proposed that the reticular cells function to orient the animal to meaningful stimuli in the environment (Restieaux and Satchell, 1958). Siegel (1979) also argues that the long latency and tendency to habituate does not fit in with their role as tonically activating stimuli. He suggests instead that the EEG activation produced by sensory stimulation of the ARS could be carried by smaller cells which might be missed in electrophysiological studies due to their small size.

It should be noted that the cells whose motor correlates were studied were located in the pontomedullary region of the RF (Siegel and McGinty, 1977), and not the MRF, the site most commonly recorded from in studies on anesthesia (Bennett, 1964 ; French et al., 1953). The cells responding to sensory input in the MRF would appear to be similar to those in the pontomedullary RF showing properties such as convergence and habituation (Scheibel, 1979 ; 1980). Thus there is no reason to suppose that the function of the sensory evoked response in the MRF would be any different. The evoked response in mammals therefore, may, as in the dogfish, represent neural activity mediating the orientation response to meaningful stimuli rather than an activating stimulus.

Similarly to the sensory evoked response, present data question the use of the vertex potential as a measure of ARS activity. Thus although the neural generators of the vertex potentials have not been clearly established, Hillyard and Picton (1979) in a recent review suggest that the most reasonable conclusion is that they are generated in widespread cortical areas under the control of the primary cortex. This conclusion is based partly on the fact that transmission of the sensory impulse to its primary cortical receiving area appears to be essential for the generation of the vertex potential (Williamson et al., 1970 ; Domino et al., 1965). Sensory impulses transmitted through the RF are not conducted to their primary cortical receiving areas (French et al., 1952). The findings by Williamson et al., (1970) and Domino et al., (1965) militate therefore, against the RF being involved in the generation of these potentials, and their validity as measures of the functional state of the ARS.

As stated earlier, of the three measures used as indicators of ARS function only the inhibition of EEG desynchronization (alpha blocking) was valid. "Alpha blocking" refers to the replacement by LVFA of the "alpha waves" normally seen in the EEG of a resting subject upon presentation of an alerting stimulus. Since the appearance of LVFA is thought to be due to activation of the ARS (Moruzzi and Magoun, 1949), the failure of an alerting stimulus to produce this is suggestive of the fact that the ARS is impaired. The results of the study by Bennett and Glass (1961) suggesting that "alpha blocking" is inhibited at pressures as low as 4-7 ata air, have been contradicted, however, by a subsequent study (Townsend et al., 1971). In the latter, no evidence of inhibition of "alpha blocking" was observed at PN_2's up to 7.5 ata. A great part of the discrepancy between the results of the two studies centers around the definition of "alpha blocking". In the study by Townsend et al., (1971) " alpha blocking " was considered to have occurred when the stimulus (a flash of light) resulted in a 50% reduction in the amplitude of the ongoing 8-13 Hz activity for 0.5 sec. In the study by Bennett and Glass (1961), however, the stimulus was an arithmetic problem, and the degree of alpha blocking was calculated over the entire time the subject was working on the problem. As far as can be ascertained from the example given this time would appear to be normally well in excess of 2 sec. Based on this, Townsend et al., (1971) argued that the measure used by Bennett and Glass (1961) was more related to the ability to maintain a certain level of activity over a period of time rather than to the classical definition of "alpha blocking", i.e. the perception or attention to a stimulus. The data of Townsend et al., (1971) does show that the ARS is still functional in

[redacted]t it can respond to an alerting stimulus. Furthermore the data of Bennett and Glass (1961) is hard to reconcile with the data reviewed earlier suggesting activation of the ARS at pressures in the air diving range. Thus the arguments raised against the study of Bennett and Glass (1961) are probably valid, and thus their data does not provide evidence that the function of the ARS is depressed at these pressures.

5.4 Alternative Hypothesis for N_2's Effects on CNS Function

In summary, current concepts of anesthetic action and a review of inert gas effects on CNS activity reject the hypothesis that N_2 produces its effects through depression of the ARS leading to a generalized depression of CNS activity. Rather the evidence suggests that the effects of N_2 on CNS activity and behavior, stem from actions of N_2 at different areas in the CNS, these displaying different sensitivities to its effects. Furthermore the alterations in neuronal function at a particular site in the CNS would be the result of the direct and indirect effects on that area. Also, like the other anesthetics, both increased and decreased neuronal function would be expected, the effect observed depending on the area and the PN_2. Thus a plot of the changes in activity in different areas of the CNS as a function of PN_2 would not produce curves indicating only depression as initially proposed. Rather a more complex pattern would be expected with both increases and decreases being observed. As with the other anesthetics (Richards, 1978 ; Roth, 1979), the direct effects of N_2 would be predicted to result from actions such as reduced tramsitter release, potentiation of

inhibitory mechanisms, alteration of receptor function, among others, these leading to alteration of synaptic function. Furthermore the ability of N_2 to display some degree of specificity in its actions, suggests that the relative sensitivity of various synapses in the CNS to its effects would differ from other types of anesthetics. This in turn would lead to some differences between the alterations in CNS activity and behavioral changes produced by N_2, compared to those of other anesthetics. At the pressures in this thesis it would be predicted that disinhibition, as well as depressant effects would be present. N_2 also appears to be capable of activating the ARS (see Section 5.3.1), and this may be the source of some of the disinhibitory effects produced by N_2.

This alternative hypothesis for the way in which N_2 would act to modify CNS function has important implications for the modification of drug effects by hyperbaric air. Since this rejects the view that N_2 at these pressures produces a generalized depression of CNS activity, one can no longer simply consider whether the depression of CNS activity produced by N_2 would enhance the depression produced by depressants, or reduce the excitation produced by stimulants. Several different possibilities now exist. For example one has to consider how the direct actions of N_2 might alter the effects of the drug. Also the ability of N_2 to produce disinhibition, creates the possibility of the enhancement of the effects of stimulants. Furthermore, this hypothesis also suggests that the modification of a drug's effect by hyperbaric air could be influenced by all of the following factors (i) the behavior or task chosen for study, (ii) the drug and the dose used, (iii) the pressure at which the experiment was conducted , and (iv) the species

chosen for study. The behavior chosen for study is important since it would determine those areas of the CNS which are involved in the performance of the behavior or task; the other factors are important since they would determine the effects (direct or indirect) of the drugs and N_2 on these areas of the CNS.

This hypothesis for interpreting the modification of drugs by hyperbaric air, can be used to explain the complex and and apparently contradictory results obtained in the present experiments. As discussed earlier (Sections 3.3.1.4, 3.3.2.4, 3.3.5.3), the disinhibitory effects of N_2 could explain the ability of hyperbaric air to enhance the stimulatory effects of amphetamine, morphine and alcohol on activity. This is strengthened by the fact that anesthetic induced stimulation of activity appears to have some similarities to that produced by amphetamine and morphine. Thus, like that of the latter drugs (Kelly et al., 1975 ; Teitelbaum et al., 1979), anesthetic induced stimulation of activity is markedly reduced by haloperidol (Waters and Walczak, 1980 ; Liljequist et al., 1981). This suggests that DA systems in the NAc are also involved in stimulation of activity produced by anesthetics. This could be tested by determining the effect of direct administration of haloperidol into the NAc on anesthetic induced stimulation of locomotor activity.

In regard to the human studies, the proposal that the effects of N_2 at these pressures, including the impairment of memory, do not stem from a generalized depression of CNS activity, makes it easier to understand the inability of the drugs, in particular DPH which produced marked sedation, to significantly worsen performance at depth. Thus instead of

assuming that the CNS depression produced by DPH would automatically enhance that produced by N_2 to worsen performance, one has to consider how the actions of these drugs would alter those effects through which N_2 impairs memory or neuromuscular co-ordination. It has been suggested that the sedation produced by DPH and thought to be due to a blockade of H-1 receptors (Uzan, 1979 ; Quach et al., 1980), may be mediated through an effect on the ARS. Thus if as suggested earlier (Section 4.4) the effects of DPH on performance are secondary to its sedative effects, it is possible that the activation of the ARS suggested to occur at these pressures (Zaltsman, 1968), or other disinhibitory effects of N_2 would offset the sedation and performance decrements produced by DPH. In fact such a trend was observed in the first study on the ball bearing test where perfomance at pressure tended to be elevated above the post drug test at the surface (Table 17). A similar effect was not observed at the higher dose of DPH, however. It could be that the stimulation produced by N_2 might not have been capable of counteracting the greater depressant effect produced by this dose. Alternatively, it may be that an interaction might be taking place between the membrane effects of N_2 and those of DPH which would be more prominent at the higher dose (Douglas, 1975).

Finally the seemingly paradoxical action of a given PN_2 to stimulate amphetamine induced activity but yet reduce convulsive activity and stereotyped behavior, and prolong pentobarbital-induced sleep time, can be explained by proposing that different behavioral effects are mediated through different areas in the CNS. Thus the fact that anesthetics including N_2 may affect different CNS areas differently, could lead to the differntial alterations observed.

5.5 Possible Neurochemical Explanations for N_2's Effects

Presently there is virtually no information regarding the neurochemical changes produced by N_2. Furthermore given the numerous possibilities it is unwise to make specific proposals. Despite this, however, some possibilities will be discussed.

Early studies suggested that 5HT transmission might be altered by N_2. This was primarily based on the similarity between some of the effects of lysergic acid diethylamide (LSD) and those of hyperbaric air, e.g. the hallucinations and dysphoria occurring at high pressures (Adolfson, 1967 ; Bennett, 1969). Support for this view was provided by the fact that frenquel, a drug capable of counteracting the effect of LSD on the EEG (Rinaldi and Himwhich, 1955), appeared also to be effective in preventing the effects of the inert gases. Thus frenquel prevented the increase in the electroshock threshold produced by N_2 and argon in rats (Bennett, 1963), and also appeared to offer some protection against performance decrements in man (Bennett, 1963a). It must be pointed out, however, that the data on frenquel although suggestive, was rather weak. For example the specificity of frenquel's effect on electroshock was questioned by the fact that several other drugs such as carbachol, phenacetin, and aspirin were also capable of preventing the increase in electroshock threshold produced by N_2 (Bennett, 1962). Also the data from the human studies were only preliminary, the author himself concluding that little reliability could be placed on the few experiments performed (see Bennett, 1963).

Although the experimental evidence for the involvement of 5HT in the changes produced by N_2 on CNS activity is weak, a LSD-like effect of

N_2 is an attractive possibility. LSD is thought to act as an agonist on 5HT autoreceptors located on the soma and presynaptic terminals of 5HT neurons (Haigler and Aghajanian, 1977 ; Ennis and Cox, 1982), thereby acting to inhibit their firing, as well as to decrease the amount of 5 HT released from the terminal. An action of N_2 to simulate the effect of LSD, either through a reduction in the amount of transmitter released, or disruption of postsynaptic receptor function, could explain a number of the effects observed. Since 5 HT appears to be an inhibitory transmitter (Haigler and Aghajanian, 1977), such an action would disinhibit those areas in the brain innervated by 5 HT neurons, and consequently might produce some of the disinhibition observed at low pressures of N_2. In this regard, it is interesting to note that LSD produces LVFA (Rinaldi and Himwhich, 1955), an effect also seen with N_2 and other anesthetics at low concentrations (Z'altsman, 1968 ; Rosner and Clark, 1973). Also a decrease in 5HT transmission could explain the differential effects of hyperbaric air on amphetamine induced locomotor activity and stereotyped behavior. For example, treatment with parachlorophenylalanine (PCPA) which depletes brain 5HT through inhibiting its synthesis (Koe and Weisman, 1966), has been shown to result in an enhanced locomotor but decreased stereotyped response to amphetamine (Jacobs et al., 1975 ; Segal, 1976). Finally, LSD has also been reported to impair memory (Jarvik et al., 1955). Unlike the effects of N_2 in the present experiments, both recall and recognition were affected. The tests of memory used in that study were quite different from those employed in the present experiments, however.

An effect on cholinergic systems may also be a possibility. Thus an effect to decrease cholinergic transmission has been proposed by

Waters and Walczak (1980) as the primary mechanism by which anesthetics stimulate locomotor activity. The evidence on which this was based was indirect, however, and centered on the additivity of the stimulatory effects of phenobarbital and scopolamine on locomotor activity in their experiments, and on the ability of barbiturates to depress transmitter release (Weakly, 1969). This needs to be tested in more detail before any conclusions about its validity can be drawn.

Interestingly, an effect to impair cholinergic transmission would also be compatible with the impairment of memory produced by N_2. For example anticholinergic agents such as scopolamine also impair recall from LTM but not STM (Ghoneim and Mewaldt, 1977 ; Petersen, 1977). Scopolamine, however, unlike N_2 at the pressure used in the present studies, also impairs recognition performance, suggesting that it affects input into memory. This difference does not necessarily indicate a different mechanism of action, however. For example it is possible that this discrepancy is due to a difference in the magnitude of their effects rather than to a basic difference in their effects on memory. As you may recall, the study by Fowler (1973) indicates that at higher pressures (10 ata air) N_2 may also impair input into memory. One has to be cautious, however, about drawing conclusions about the involvement of ACh in N_2's effect on memory based solely on its similarity to the deficit produced by scopolamine. For example the memory deficit produced by diazepam is similar to that produced by scopolamine, however, it is not reversed by physostigmine as is the case for scopolamine (Ghoneim and Mewaldt, 1977). It would be interesting, however, to determine the effect of a drug such as physostigmine on the memory deficit produced by N_2.

Finally, it is possible that some of the depressant effects of N_2 may be mediated through alteration of GABA systems. Studies by Martz et al., (1983) and Liljequist and Engel (1982), have shown the apparent involvement of GABA in the motor incoordination and loss of righting reflex produced by alcohol. Studies on in vitro preparations have shown the ability of alcohol (Nestoros, 1980 ; Davidoff, 1973) and other anesthetics (Barker and Ransom, 1978 ; Barker et al., 1980) to enhance the action of GABA. It is possible therefore that N_2 also exerts an action on GABAergic systems, and that this may be responsible for its ability for example to prolong pentobarbital sleep time in mice. It is unlikely that a reduction in GABA activity is responsible for the disinhibitory effects of N_2, however, since the ability of haloperidol to block the locomotor activity produced by anesthetics (Waters and Walczak, 1980 ; Liljequist et al., 1981), would tend to argue against this. As stated earlier the primary effect of haloperidol to block the locomotor stimulation produced by anesthetics is thought to be its effect on DA receptors in the NAc. The output system of the NAc would appear to be GABAergic (Mogenson et al., 1983 ; Mogenson, 1984). Thus the ability of haloperidol to block the increased activity would suggest that like amphetamine and morphine, anesthetics act prior to the GABAergic neurons.

In summary, the limited knowledge of the neurochemical actions of anesthetics at low concentrations permitted only a cursory and selective examination of the potential mechanisms for the effects of N_2 observed in the present experiments. Presently no firm conclusions can be drawn as to the validity of these proposals. The lack of knowledge regarding the effects of N_2 on the CNS is unsatisfactory. More work needs to be

done to provide such information as a clear understanding of these effects is essential to a full understanding of how drug effects might be modified by hyperbaric air.

5.6 Summary

Although the results have shown the ability of hyperbaric air to modify the effect of centrally acting drugs, the applicability of these results to the human situation is fraught with difficulty. It is difficult to make generalizations from the animal data to the human situation primarily because different tests and doses of drugs were used, and also some of the behaviors chosen have no, or little relevance to behaviors carried out by humans. The need for caution is also underscored by the fact that the animal studies indicate that the behavior chosen for study may influence the effect that is observed. Also there is the as yet unanswered question as to the similarity of the effects of N_2 in humans versus animals at a particular pressure.

Despite these difficulties, however, there are some encouraging points. For example in the case of DPH, it is interesting to note that in both the animal and human studies exposure to hyperbaric air did not enhance the depression produced by this drug. In the animal studies the effect of DPH was actually reversed, and it is interesting that in the first human study a tendency for hyperbaric air to reduce the effect of DPH was also seen in the ball bearing test. In addition, the animal studies strongly suggest that there is a marked excitatory component in N_2's effect, and that this is is capable of enhancing the effect of stimulants. This has not been taken into consideration before and some thought should be given to this effect of N_2 and its potential for harm. Finally, the observation in the animal studies that the type of behavior chosen for study could influence the modification observed, suggests that in testing the safety of a drug at pressure a wide variety of tasks

should be monitored to account for this possibility. Whether this will be seen in humans as it was in these animal studies is not known. The possibility cannot be excluded, however.

SUMMARY AND CONCLUSIONS

1. The results show that exposure to hyperbaric air in the 4-7 ata range can significantly modify the effects of a number of centrally acting drugs in mice. These modifications occurred without any discernible effects of hyperbaric air on the behavior of the mice. At the doses of the drugs used, no debilitating or toxic interactions were observed.

2. Thus, exposure to hyperbaric air significantly reduced the "convulsive activity" produced by d-amphetamine sulphate (30 mg/kg), and also protected against the lethality induced by this dose. Similarly, it acted to reduce the stereotyped licking/biting/gnawing response produced by amphetamine (8 and 10 mg/kg,), this effect being more marked at the lower dose. The modification of the locomotor activity induced by amphetamine (3, 4 and 5 mg/kg) was more complex, however, with the effect appearing to depend on the dose of drug as well as the pressure of air. Thus, at the 30 mg/kg dose 7 but not 4 ata air enhanced activity. At the 4 mg/kg dose both pressures enhanced activity but the greatest effect was observed at 4 ata. No significant differences in activity were observed at 5 mg/kg. As was the case for the 4 mg/kg dose, the greatest mean activity occurred at 4 ata air.

3. Exposure to hyperbaric air enhanced the locomotor activity induced by morphine sulphate (15 and 30 mg/kg). As for amphetamine the effect seen was influenced by the dose of the drug as well as the pressure of air. Thus, at the lower dose, 7 ata air produced the greatest enhancement of activity. At the high dose however, the greatest

enhancement was seen at 4 ata.

4. Exposure to hyperbaric air acted to reduce not enhance the depression of spontaneous activity produced by DPH (10 and 20 mg/kg s.c.). The reduction of the depressant effect of DPH was greater at the lower dose, and in general 4 ata was more effective than 7 ata air.

5. Exposure to hyperbaric air resulted in a complex modification of the effects produced by alcohol. At the low dose (1.75 g/kg) both 4 and 7 ata tended to enhance the initial stimulation of activity produced by alcohol. In addition, 7 but not 4 ata tended to offset the alcohol induced decline in activity over the 60-120 min period following injection. At the higher dose (2.75 g/kg), however, 4 and 7 ata exerted different effects on activity. Similarly to its effect at the low dose, 4 ata enhanced activity compared to 1 ata, the enhancement being more marked at this dose. At 7 ata however, no enhancement of activity was observed. In fact, during the 10-15 min period following injection, a significant depression of activity was observed.

6. Exposure to hyperbaric air significantly prolonnged the sleep time induced by pentobarbital 45 mg/kg, but not that induced by 35 mg/kg.

7. In general, these effects of hyperbaric air appeared to be due to an effect of N_2, rather than to the increased pO_2 or hydrostatic pressure. Furthermore, the rapidity with which the modifications were observed, and their complex nature, make it unlikely that there were due to an alteration in the pharmacokinetics of the drugs at pressure. Thus the modification of drug effects would appear to be due primarily to the actions of N_2 on the CNS.

8. In the human studies, exposure to 5 ata produced a significant decrement in memory , but had no effect on neuromuscular co-ordination as measured by the ball bearing test. The memory impairment seemed to be due to a retrieval rather than an input failure. Neither DPH (50 and 75 mg) nor alcohol (0.25 or 0.375 g/kg) significantly affected memory or neuromuscular co-ordination, and in general, did not combine with hyperbaric air to worsen performance at pressure. In the first study, however, at the low dose of alcohol, alcohol and hyperbaric air did combine to impair neuromuscular co-ordination. A similar effect was not seen in the second study at the higher dose in well practiced subjects.

9. The results of these experiments do not support the hypothesis that increased PN_2 at least in the 1-7 ata air range, produces a generalized depression of CNS activity. In particular the results in mice suggest that both increases and decreases in neuronal activity are produced by N_2.

10. Current concepts of anesthetic action and a review of inert gas effects on CNS activity also do not support the concept that N_2 at these pressures produces a generalized depression of CNS activity. It is proposed that a hypothesis based on current concepts of anesthesia, which postulates that N_2 acts at multiple sites in the CNS and produces both stimulation (disinhibition) as well as depression of neuronal activity at these pressures, offers a better means of explaining the results observed.

11. This hypothesis suggests that the modification of drug effects by hyperbaric air should not be considered simply from the point of view as to whether the depression produced by N_2 would enhance that produced by

a depressant, or reduce the excitation produced by a stimulant. Rather one has to examine the ways in which the effects of N_2 might modify those of the drug. Furthermore the fact that N_2 may affect different areas of the CNS differently, and that the effects may vary with pressure, suggests that different effects of a drug might be modified differently by hyperbaric air, and that the modification observed might be influenced by the pressure of air.

12. Presently very little is known about the effects of N_2 on the CNS at these pressures in terms of the areas affected and the neurochemical changes produced. Such knowledge is needed to aid in the understanding of the modification of drugs by hyperbaric air.

SUGGESTIONS FOR FUTURE WORK

It is clear that we are still very far away from making predictions regarding the safety of centrally acting drugs when used in a hyperbaric air environment. A lot more information is required regarding the changes produced by hyperbaric air in the CNS in the air diving range, and their interaction with centrally acting drugs. There is also an immediate need for information on the combined effects of these drugs and hyperbaric air on human performance, to provide data of practical relevance to the diving community. Thus the following studies are proposed.

(i) Detailed electrophysiological studies should be performed in animals mapping the changes that occur in various areas of the CNS over the 1-10 ata air range. These experiments should be conducted at 2 or 3 ata intervals to allow conclusions to be drawn about the sequential changes that occur in CNS activity. This information would facilitate explanation of the modification of drug effects by hyperbaric air.

(ii)Studies on the *in vitro* preparations normally utilized in the study of the effects of conventional anesthetics should be instituted to provide information on the actions on N_2 at the neural level. Experiments could be performed to determine its effects on the electrical properties of neural tissue, as well as on the responses to various transmitters and putative transmitters.

(iii) Experiments could be performed in rats to determine the neural substrates of anesthetic induced stimulation of activity as this

might shed some light on the mechanism of the disinhibition produced N_2. These would involve injection of various neurotransmitter agonists and antagonists into various areas of the brain, e.g. the NAc, in an attempt to modify the stimulation of activity.

(iv) The concept of performing studies with low concentrations of anesthetics such as N_2O as a substitute for studies at high pressures of air should be pursued. The greater experimental control, and safety which would be afforded by the ability to perform the experiments at normal pressure, would allow more, and greater detailed studies to be performed.

"APPENDIX I

APPARATUS FOR MEASUREMENT OF ACTIVITY

The apparatus consists of two main parts (i) an activity box and (ii) the sensors and counters.

Activity Box

The activity box (Fig.24) is constructed from plexiglass (18 X 11.5 X 14 cm) and has a floor consisting of stainless steel rods (0.16 cm diam.) spaced 4 mm apart. The rods are wired so that they form a pattern of pairs of rods of different polarity, separated by a neutral rod. These rods are insulated with a clear plastic. In addition, the "live" rods are also insulated starting from the points at which they enter the sides of the box, and extending for a further 5 mm away from the sides of the box. The purpose of the neutral rods, and the insulation of the ends of the rods is to prevent feces and urine respectively from bridging the gap between the rods of different polarity and interferring with the measurement of activity.

Principle of Operation

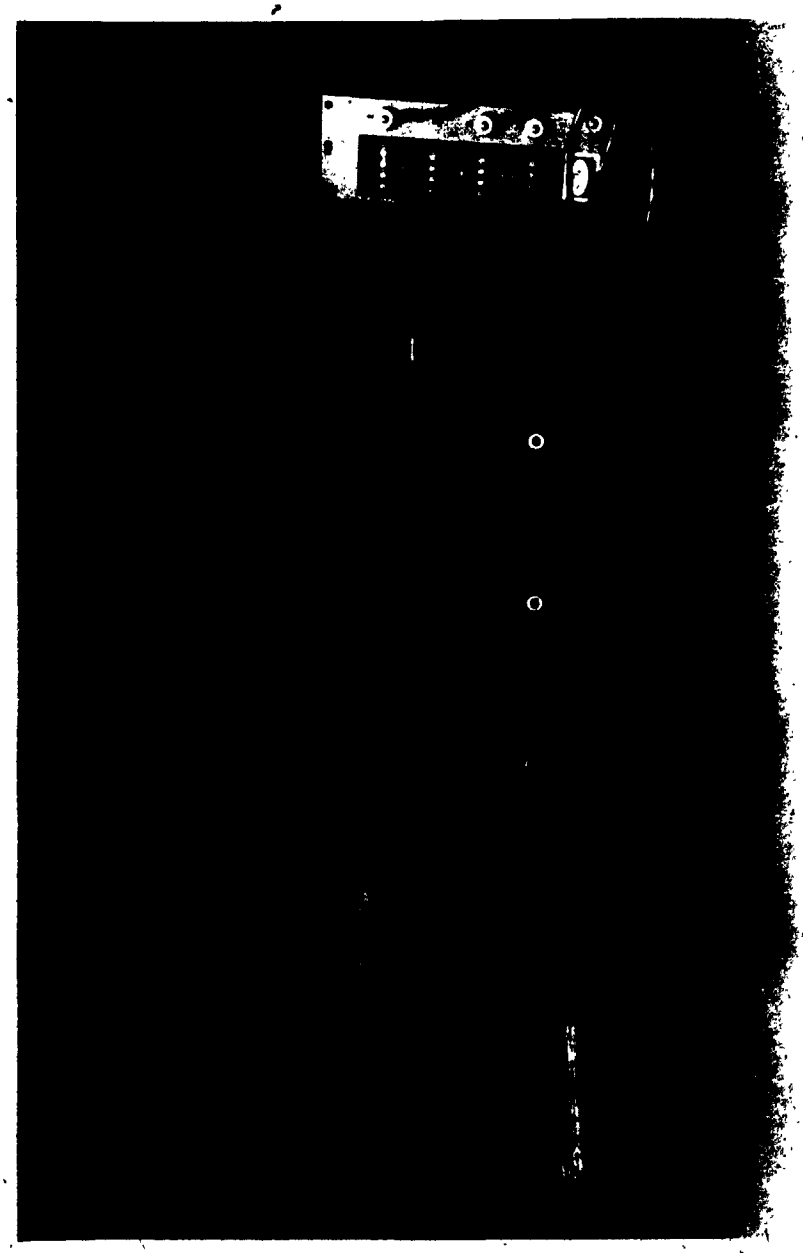
The measurement of activity is based on the principle that as the mouse moves across the floor of the cage and makes and breaks contact with rods of different polarity, the resistance of the box (i.e. the resistance between rods of different polarity) changes. In this apparatus, a voltage is applied across the rods of the cage, and the current flowing between the rods over successive preset time intervals is continuously compared by the sensor. Shifts in the position of the mouse in the box produce changes in the current flow across the rods.

If the change between successive time intervals is greater than a preset value, a signal is sent to the counter and a count is registered. The current passing across the mouse was always less than 3 uA. This apparently did not significantly affect the behavior of the mice, since naive animals continued to show high levels of exploratory activity when placed in the box. The gross sensitivity of the sensor is set internally. For fine adjustment of sensitivity, however, the sensitivity switch seen on the front panel (Fig. 24) is used.

With the present sensor, sampling times ranging from 0.0167 to 0.25 sec, in steps of 0.0167 sec can be obtained. In trials of the sensor, a sampling time of 0.0334 sec was found to give the best correlation of activity counts with observed behavior. This short sampling time posed a problem however, in that changes in resistance occurring after a movement had been executed, due e.g. to the mouse making firmer contact with the rods, were also capable of triggering a count. These double counts were avoided by incorporating a feature into the circuit which allows one to set up a refractory period of of adjustable duration after each count, during which no further counts can be registered. The length of the refractory period and consequently the maximum number of counts possible per sec is controlled by the bandwidth knob on the front panel (Fig. 24). In these studies, the maximum number of counts per sec was set at 4.

Figure 24

Front view of activity sensor panel, and test box.



Types of Activity Measured by the Sensor

Essentially the sensor will record any movement of the mouse which produces a change in resistance greater than the pre-set value. Therefore activities such as walking, rearing, shifting position in the box (e.g. turning, movement of legs), biting or licking rods will produce counts. For this reason, it is advisable to observe the mice periodically to determine the type of behaviors that they are involved in. Grooming produces very few activity counts however.

The ability of several different movements besides walking and running, to trigger counts is a potential weakness of the apparatus, particularly if one wants to use the activity counts as an index of locomotor or exploratory activity. In the case of "shifting position in the box", this does not occur with very great frequency. Furthermore, over an identical time interval, shifting position does not generate as many counts as walking, running and rearing, the behaviours normally observed during exploratory activity, and stimulation of locomotor activity. Consequently, the counts generated by this type of movement do not mask the effects of drugs on activity. The counts produced by the mouse licking or gnawing the rods are more of a problem since if done repeatedly this produce a large number of counts. In experiments using acclimatized mice this is not of great concern since this behavior does not occur very frequently. Naive mice however, in trying to get out, frequently gnaw at the rods of the box, in particular the last rod at each end of the box. This behavior appears to be an integral part of the behavior spectrum displayed by such mice, and can be considered to be contributing to the overall activity of the mouse.

After treatment with depressent drugs in doses that reduce activity, this gnawing at the rods is reduced concomitantly with decreases in walking and rearing and therefore they do not mask the drug's effect. For depressants therefore, the counts produced by gnawing at the bars do not constitute a problem. In the case of drugs which increase activity however, gnawing in control mice might tend to mask an increase in locomotor activity produced by a drug. Over identical time periods however, a mouse walking or running in the cage will display higher activity counts than one biting at the rods. Thus, if the drug produces greater amounts of walking and running, higher activity counts will be obtained. Additionally since the majority of the gnawing is done at the ends of the box, the contribution of this behavior to the total activity, was reduced by insulating the end rods as well.

In general the system worked well, and the insulation prevented urine and feces from short circuiting the system. Furthermore a comparison of the activity counts with the behavioral observations made for the various drugs (see their respective results sections), indicates that there is a good correlation of the incidence of walking and rearing with activity counts recorded by the sensor.

APPENDIX II

Figure 25

Sample recognition sheet.

RECOGNITION SHEET A

TUMBLE	LEMON	COMPOUND	BUTLER
FOOTBALL	PARLOR	FORTUNE	PONY
JAZZ	COLUMN	BEGGAR	LETTER
CLOTHING	CONGRESS	PILOT	SURVEY
ARROW	PRODUCT	PATTERN	PROGRAM
WAGON	EAGLE	DOCTRINE	MISTRESS
DISGUISE	CONFLICT	BEDROOM	BASIN
MERIT	FELLOW	HABIT	SENTENCE
VOYAGE	ABSENCE	IDEAL	COSTUME
PUBLIC	STANZA	NATIVE	LUMBER
RESEARCH	COLLAR	POCKET	BIBLE
HERO	PAYMENT	CULTURE	SHERIFF
KITCHEN	TEMPER	CARPET	WEAKNESS
PEPPER	MOTOR	SHELTER	BEAUTY
SHEPHERD	FLAVOUR	MOVIE	VALUE

TABLE 22

STUDY I

'A'

Criterion Value (B_s") For Each Test Session

Group	TEST SESSION		
	Pre Treatment	Post Treatment	Dive
Control	0.38 ± .08	0.35 ± .07	0.35 ± .12
Alcohol	0.45 ± .18	0.56 ± .19	0.40 ± .10
DPH	0.34 ± .17	0.16 ± .13	0.30 ± .14

'B'

Criterion Value (B_s") For Each Test Session

Group	TEST SESSION		
	Pre Treatment	Post Treatment	Dive
Control	0.05 ± .11	0.00 ± .14	-0.02 ± .20
Alcohol	0.05 ± .07	-0.13 ± .33	0.07 ± .17
DPH	0.09 ± .20	0.02 ± .10	-0.05 ± .16

TABLE 23

STUDY II

'A'

Criterion Value: B_3"

Group	Treatment	Pre Treatment	Post Treatment	Dive
		TEST SESSION		
Alcohol	Placebo	0.36 ± 0.12	0.34 ± 0.13	0.11 ± 0.14
	Drug	0.41 ± 0.11	0.33 ± 0.08	0.30 ± 0.14
DPH	Placebo	0.30 ± 0.09	0.43 ± 0.08	0.23 ± 0.09
	Drug	0.51 ± 0.10	0.22 ± 0.05	0.35 ± 0.11

Values are Mean ± SEM

'B'

Criterion Value: B_2"

Group	Treatment	Pre Treatment	Post Treatment	Dive
		TEST SESSION		
Alcohol	Placebo	0.09 ± 0.13	0.01 ± 0.06	0.01 ± 0.12
	Drug	-0.08 ± 0.14	0.00 ± 0.03	0.00 ± 0.16
DPH	Placebo	0.00 ± 0.14	0.08 ± 0.12	0.12 ± 0.12
	Drug	0.06 ± 0.08	0.03 ± 0.03	0.09 ± 0.12

Values are Mean ± SEM

APPENDIX III

APPENDIX III

Regression Analysis of Stereotyped Behavior

As an alternative means of analysis, the data was used to generate multiple regression equations describing the time course of the stereotyped response under the various dose/pressure conditions. These equations are of the form $y = a + bx + cx^2$, where "y" represents the response and "x" time. Where appropriate, the slopes of the curves obtained under the different conditions were analyzed to determine effects on stereotyped behavior.

RESULTS

The values for the curve parameters are presented in Tables 24 A,B,C. The data for the 10 mg/kg dose fitted this type of equation the best as is evident by the higher R-Square values at this dose. In the light of the poor fit of the curve at the 8 mg/kg dose, only the slopes for the 10 mg/kg dose were analyzed. In order to determine whether the slopes obtained under thw different conditions were significantly different the 95 % confidence limits (Goldstein, 1964) for each of the parameters were calculatedand analyzed to determine if an overlap occurred. A difference in the slope "b", was observed for those curves at 4 and 7 ata, compared to the ones at 1 ata. No differences were found in the slope "c".

Table 24 A

Parameters of regression curve 10 mg/kg

Parameter	Pressure of Air 1 ata	4 ata	7 ata
a	6.14	19.9	16.9
b	.80 ± .11	.22 ± .14	.35 ± .110
c	-.0007 ± .00081	-.0032 ± .0011	=.0044 ± .008
R	.71	.57	.74
R-Square	.51	.33	.55

Table 24 B

Parameters of regression curve 8 mg/kg

Pressure of Air

Parameter	1 ata	4 ata	7 ata
a	-7.71	- 2.7	8.1
b	.83 ±.14	.41±.13	.23±.13
c	-.0064 ± .001	-.0031±.001	-.0027 ±.0009
R	.53	.28	.48
R-Square	.28	.079	.23

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DISCUSSION

The slope "b" can be conceptualized as the rate of rise of the response from the initial starting point (15 min in this case). The fact that the slope at 1 ata is larger than at 4 or 7 ata indicates that the rate of rise of the response is greater than that observed following exposure to hyperbaric air. This suggests that since at all pressures the initial response is almost the same, that the effect of N_2 is to dampen the increase in the response. In regard to the lack of effect on "c", this could be used to provide some data on the rate of decline of the response at the three pressures. This slope is a lot smaller than "b" and therefore only becomes important as X, and consequently X^2 becomes large, which occurs towards the end of the curve where the response is declining. The fact therefore that no significant differences were observed in this slope suggests that the rate of decline in the response at the three pressures is similar. This suggests therefore that the significant decreases at the later time points when each point is analyzed separately (see Fig 6a) is probably not due to a faster rate of decline at pressure, but instead due to the smaller overall response obtained at pressure.

In conclusion, this method of analysis in general supports the analysis carried out in the thesis.

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