

THE BIOLOGICAL EFFECTS OF GASES AT PRESSURE

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by

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(44)

Less than one hundred years ago Paul Bert discovered that oxygen at raised pressures was injurious to a wide range of organisms and, in a number of experiments conducted over several years, he investigated this and other problems which form the basis for what has subsequently become high-pressure physiology. It seems however that the large amount of research which has been done in an attempt to understand the nature of oxygen poisoning has tended to confuse the original simple view of oxygen as a universal poison. A very large number of reactions which depend upon enzymes are adversely affected by raised pressures of oxygen, sufficient indeed to make it likely that the 'cause' of oxygen poisoning will not be found, in the sense that no single critical reaction will be identified as giving rise to any one of the various toxic manifestations. Instead the emphasis must be upon studying the way in which cellular metabolism is modified and finally disrupted by raised pressures of oxygen; and in particular by trying to identify the rate-limiting step within the complex of reactions.

The present work has been upon one aspect of oxygen toxicity, namely the development of convulsions in mice which have been exposed to raised pressures of oxygen. These experiments have been based upon two hypotheses; the first of these was that the dose-response relationship was related to the dynamics of the underlying biochemical processes and particularly, that the rate at which toxic signs developed would be determined by whichever reaction was rate-limiting. Attempts to correlate biochemical and physiological information have not so far

been possible since there have been no estimates available of the rate at which toxicity develops. The first task was therefore to define as accurately as possible the dose-response relationship between the ambient oxygen pressure and the time taken for convulsions to appear, and then, by dividing each exposure into two parts, to attempt to measure the rate at which toxicity develops.

The second hypothesis was that the time to convulsions was not uniquely determined by the partial pressure of oxygen inspired and hence that the convulsion time would be modified by the presence of other respirable gases. Since it was thought that the nature of such modifications would give further information about the process which leads to convulsions, the interactions between oxygen and other gases, namely, Carbon Dioxide, Nitrous Oxide, Nitrogen and Helium therefore form the second part of this work.

The first notable feature of oxygen convulsions in mice was that at least two types of convulsion were distinguishable. The first type, referred to as minor convulsions, tended to occur early, was of short duration and was minor in intensity, while the second tended to occur later, was more prolonged and more violent and was called a major convulsion. A few animals showed chronic rolling movements after relatively long exposures.

The second point of importance was that, for any particular oxygen pressure, convulsions in mice were found to be distributed in a skew manner. This could however be converted to a normal distribution by a logarithmic transformation, which also had the desirable feature that the

standard deviation of the transformed results was independent of the mean, unlike the untransformed data.

Consideration of the dose-response curve lead to an approximate relationship in which the product of the ambient oxygen pressure and the mean logarithmic convulsion time for that pressure was found to be constant. A similar relationship was also found to describe the occurrence of minor convulsions and the relationship between minor and major convulsions expressed as the ratio between the slopes of the respective dose-response lines.

When an initial exposure, short of convulsions, was immediately followed by a second exposure leading to convulsions, such divided exposures were found to be additive; that is, the parts were capable of being described by the same mathematical relationship as the whole. These divided exposures also lead to the definition of a provoked threshold which for mice was at the pressure of about 3 atm. of oxygen. Exposure to pressures above this threshold shortened subsequent exposures, while exposures below the threshold had no detectable effect upon subsequent exposures.

Other experiments using divided exposures, in which the time of the first exposure was varied, were interpreted as showing that the process leading to convulsions was, as a first approximation, exponential in form and that its rate was proportional to the ambient oxygen pressure. Estimates for the half-time of this process at 3 atm. and 4 atm. were 35 mins. and 22 mins. respectively.

The first interaction to be investigated was that between oxygen

and carbon dioxide and the results obtained were found to be in agreement with the findings of Marshall and Lambertsen (1961), since carbon dioxide at low levels hastened the onset of convulsions, although at high levels no significant effect could be demonstrated. This would seem to be consistent with the view that high carbon dioxide levels protect against convulsions and would point to a cross-over point at about 7 atm. of oxygen for carbon dioxide levels of 0.03-0.07 atm.

Similar results were obtained for nitrogen, these two gases, carbon dioxide and nitrogen both changed the slope and the intercept of the dose-response line as is shown diagrammatically in Figure 17. While the mechanism of action is not known it is speculated that both may lead to changes in intracellular pH. at lower concentrations, while at higher concentrations the anaesthetic properties of these gases may preponderate.

The evidence obtained from the interaction with nitrous oxide seemed to show that more than one type of interaction was possible, since this gas altered the slope of the dose-response line, 0.54 atm. of N_2O gave a marked protection against convulsions, but did not appreciably alter the intercept.

Helium was found to produce a marked acceleration in the onset of oxygen convulsions, but no evidence was found for any convulsant action of helium itself, since supra-liminal oxygen pressures were necessary in order to show the effects of helium. The magnitude of the effect, judged by the change in slope of the dose-response time, was proportional to the helium partial pressure; hence the possibility has been considered that this effect was due not to any physiological action of helium, but rather to the effects of pressure 'per se'.

The significance of the threshold, i.e. the minimum effective pressure to produce convulsions and the minimum response time, has been discussed. Two possible mechanisms to account for the threshold were considered. The first of these depends upon the fact that the tissue oxygen consumption tends to keep the tissue oxygen tension within normal limits until the total quantity of oxygen supplied exceeds the quantity being consumed. The second possibility which was considered was that of a competing recovery process which removed some product of the convulsion-producing reaction at a rate different from that at which it was produced. While on balance the first explanation was thought more likely, recovery undoubtedly does occur, and both may therefore be involved.

Regarding the minimum response time, this was thought to be a simple consequence of the rate of the process to convulsions reaching a maximum value; although the reason for this is not obvious, it was suggested that it might be due to the progressive inactivation of enzymes by oxygen at pressure.

Consideration of the possible sites of action of oxygen in producing convulsions may perhaps be premature, since it is uncertain whether the convulsions represent a generalised reaction of brain tissue or whether there are particularly sensitive areas on the basal ganglia from which the convulsions originate. It was thought however that there was sufficient evidence to show that oxygen acts by disturbing cellular oxidations to make the choice of the mitochondrion, as the site of action, not unlikely. Using the previous assumption and available data, an estimate

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was made of the toxic level of oxygen in the brain of man as being of the order of 40-65 min. Hg. at the mitochondria, or 70-100 min. Hg. in the venous blood.

It was shown that the dose-response curve might be interpreted as resulting from a process leading to convulsions. It was further suggested that this hypothetical process to convulsions represented the rate-limiting step of a cyclic or chain reaction involved in tissue oxidations and that the kinetics of this process might help to identify the site or sites at which oxygen acts to produce convulsions.

The existence of interactions between oxygen and other gases has been confirmed and it has been suggested that these interactions may represent interference with tissue oxidations either at the same sites as those involved in oxygen toxicity or at adjacent sites. The evidence suggested that there were two principal modes of interaction, the first represented by the anaesthetic gases and the second by the action of helium, although alterations in intra-cellular pH. may also be of some importance.

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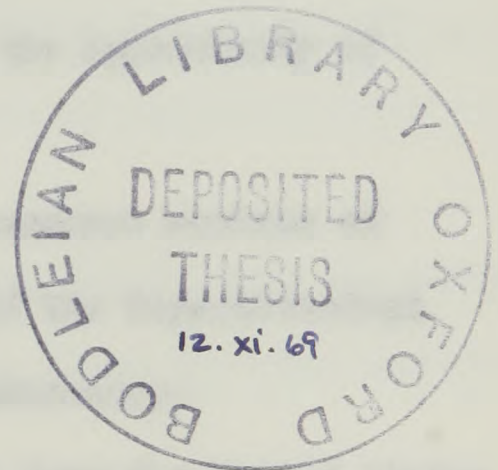
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GENERAL INTRODUCTION

The discovery of the basic nature of certain of the processes of the human mind was made by Paul Hertz and published in his book "The Psychology of Knowledge" in 1970. The experiments which this book described showed that not only birds and dogs but other mammals, insects, many invertebrates and plants and 'ferrets' were all in some way susceptible to this new principle.

GENERAL CONCLUSIONS

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CHAPTER 1. INTRODUCTION

The discovery of the toxic nature of oxygen at pressures greater than normal was made by Paul Bert and published in his book "La Pression Barometrique" in 1878. The extensive studies which this book contains showed that not only birds and dogs but other mammals, frogs, lizards, many invertebrates and plants and 'ferments' were all in some way susceptible to this new poison.

OXYGEN CONVULSIONS

Perhaps the most remarkable of Bert's findings was the way in which oxygen at raised pressures produced convulsions in his experimental animals, convulsions which he characterised as being similar to those produced by phenol or strychnine. As a result of his observations he summarised his views in a Report to the Academy of Sciences which he gave in February 1873.

- "1. Oxygen acts like a poison which is rapidly fatal, when its quantity in the animal (arterial) blood rises to about 35 cubic centimetres/100 cubic centimetres of liquid.
2. The poisoning is characterised by convulsions which according to the intensity of the symptoms represent the different types of tetanus, strychnine, phenol, epilepsy, etc.
3. These symptoms, which are quieted by chloroform, are due to an exaggeration of the excite-motor power of the spinal cord.
4. They are accompanied by a considerable and constant drop of the body temperature."

This summary, which might almost be taken to apply to more recent 'in vivo' experiments in a number of species, does not include the broader view which Bert expressed as to the relationship between oxygen convulsions and the more general effects of oxygen toxicity. Starting from the premise that 'ordinary atmospheric pressure' represented the optimum condition for healthy animals Bert expected pressures above this to be generally harmful and therefore interpreted the convulsions seen in higher animals as a special phenomenon, which masked the presumed general effects.

P. 842 "we conclude from these data that the death of higher animals in compressed oxygen, although its immediate cause is the super-excitation of the central nervous system, as we have demonstrated, is really due to a general effect of the oxygen upon the whole organism. But the nervous elements, which are more susceptible react first, disturb the vital mechanisms, so that death occurs before the other elements are noticeably affected."

In summary, Bert's achievement was considerable.

1. He showed that a very wide range of organisms was affected by raised oxygen pressures.
2. He recognised the dependance of the toxic effects of oxygen upon both pressure and time and defined a limit in terms of arterial oxygen levels and ambient pressures below which these effects did not appear.
3. He showed that aether and chloroform protected against convulsions as did section of the relevant motor nerve for the muscles supplied by it.

4. He commended the anaesthetic properties of carbon dioxide to the attention of surgeons, but seems wrongly to have discounted the effects of the high levels of carbon dioxide in his experiments, an aspect discussed by Bean (1945).
5. As a result of comparing the effects of exposures to oxygen and to pressures of air with an equivalent oxygen pressure, Bert was of the opinion that the only factor involved in producing the toxic effects seen was the pressure of the oxygen.

EFFECTS UPON THE LUNGS

As a result of a series of experiments upon the attenuation of microbes by oxygen at high pressure, J. Lorrain Smith (1899) observed that oxygen at a tension of more than 1 atm. produced pneumonia in normal animals. He confirmed that a range of animals including pigeons, rats, mice and guinea-pigs responded as had those in the studies reported by Bert. However, his interest in the respiratory system led him to believe that the lungs would be affected by oxygen, since oxygen had to pass through the lungs in order to reach the nervous system. Post-mortem studies indeed showed an inflammatory reaction of the lungs. Smith's interpretation of this seems to have been coloured by the view which he and J. S. Haldane at that time held, that respiration in the lung involved the active physiological process of absorption of oxygen. He describes inflammation as "in a sense directly continuous with the normal process of respiration" and offered the following explanation for the toxic effects of oxygen.

" ... oxygen which at the tension of the atmosphere stimulates the lung cells to active absorption at a higher tension acts as an irritant, or pathological stimulant and produces inflammation."

Both the convulsive effect of oxygen and its effect upon the lungs of experimental animals have been amply confirmed by a large number of workers, as can be seen from the extensive review of the effects of oxygen at increased pressure published by Bean (1945).

THE EFFECTS OF OXYGEN IN MAN

Convulsions

The second world war acted as a considerable stimulus to research into oxygen toxicity largely as a result of the offensive use of divers breathing oxygen from closed-circuit breathing apparatus. The most extensive series of human exposures to raised pressures of oxygen was undertaken by volunteers from the Royal Navy, the results being published after the war by Donald (1947).

This study showed that men also developed epileptiform convulsions, associated with electro-encephalographic changes, which seemed very similar to those seen in animals. However, there were aspects of considerable practical importance for which there seemed no obvious explanation, firstly, exposures made in a diving-suit in water were more dangerous than similar exposures made in a dry chamber. Secondly, the toxic effects of oxygen were potentiated by work and by alterations in the ambient temperature. The most quoted finding however was the extreme variability of the results, the range between the most susceptible and the most resistant individual was extremely wide and the day-to-day variation in suscepti-

bility in a single individual considerable. The unpredictability of the effects in man of raised oxygen pressures was thought to be such as to make diving on pure oxygen below 25 ft. ($PO_2 = 1.7 \text{ atm.}$) "a hazardous gamble."

Pulmonary oxygen tolerance

There is a significant effect of oxygen upon the lungs, although this is usually less severe than that observed in animals. This effect has recently been studied by Clark and Lambertsen (1966) who observed seven normal men exposed to 2 atm. oxygen pressure for from 8 to 12 hours. After about 8 hours the subjects showed progressively severe pulmonary irritation and coughing consistent with the development of a tracheo-bronchitis. It has been suggested that one of the simplest and most practicable ways of detecting early pulmonary damage is by looking for alterations in vital capacity, since such alterations occur in conjunction with the development of symptoms Clark and Lambertsen (1967).

Retinal changes

One of the principal effects of oxygen is upon the cerebral vasculature where it produces a marked vaso-constriction. Bean (1945) (1961). The retinae of premature infants seem particularly liable to injury leading to the condition of retrolental fibroplasia, Patz (1953).

ENZYME INHIBITION

Concurrent with the work of Donald experiments by Dickens (1946), Mann and Quastel (1946) and by Stadie Riggs and Haugaard (1945) showed that several of the enzyme systems which they studied were sensitive to the action of oxygen, particularly those which contained sulphhydryl groups.

Previous work in this field was reviewed by Stadie et al (1944) and has recently been brought up to date by Haugaard (1968).

Dickens (1962) has pointed out that the time-course of the development of convulsions is normally measured in minutes; whereas the fall in the respiration rate of rat brain slices or liver homogenates, used as a measure of oxygen toxicity, may continue for hours. Reactions have been studied however which are sufficiently rapid to be involved in the genesis of convulsions. Jamieson and Chance (1966) showed that oxygen at 5 atm. and at 12 atm. produced oxidation of cytochrome c and cytochrome a and a₃ in rat liver mitochondria. This reaction was strongly potentiated by lowering the pH and was accompanied by a fall in the respiratory rate of the mitochondria. At 12 atm. the oxidation of cytochrome c appeared more or less complete in about 30 minutes. Using rat liver and pigeon heart mitochondria Chance Jamieson and Coles (1965) have shown that pressures of 2-3 atm. gave rise to readily detectable changes in the steady state level of reduced pyridine nucleotide towards a state of greater oxidation. These changes were reversible in pigeon heart mitochondria but only partly so in rat liver mitochondria. As an indication of the rapidity of some of these changes, experiments with sub-mitochondrial particles at 23°C showed approximately 50% inhibition of the rate of reduction of pyridine nucleotide by an exposure of 30 seconds at 12 atm. pressure. These authors concluded that under hyperbaric conditions there was a change in the steady state of reduced pyridine nucleotide which was followed by convulsive activity and suggest that the interval of time between these two events may be due to an alteration in the level of some intermediate metabolite, possibly related to the metabolism of gamma-

amino-butyric acid (G.A.B.A.).

Wood and Watson (1962) injected rats intra-peritoneally with GABA thirty minutes before exposure to 75 p.s.i. of oxygen ($PO_2 = 6.1$ atm.). They were able to show a significant reduction, both in the number of deaths and in the number and severity of convulsions in those animals which had received GABA compared with the control animals injected with saline. Wood and Watson (1963) also found a fall in GABA levels in the brain in rats which had been convulsed with oxygen. They suggested that intra-peritoneal GABA acted by raising the level of GABA in the brain and restoring some specific but conjectural function. A similar suggestion has been put forward by Paton (1967) that convulsions may occur as a result of disturbing the balance between glutamic acid, which appears to be excitant to central neurones, and gamma-amino-butyric acid which is depressant. Convulsions might thus be expected to occur when the level of GABA has fallen sufficiently for the nervous system to respond to the excitatory effect of glutamic acid.

AGENTS WHICH MODIFY THE TOXIC EFFECTS OF OXYGEN

Carbon Dioxide

A number of studies support the view that carbon dioxide accelerates the onset of convulsions, for example, Marshall and Lambertsen (1961) found in mice that while this was so for lower partial pressures of carbon dioxide, at higher pressures there was some protective action.

An indication that the inter-action of carbon dioxide with the effects of oxygen may not be simple is the finding of Hempleman (1956) that prior exposure of rats to 15% oxygen in the presence of 6% carbon dioxide or

exposure to 6% carbon dioxide with normal oxygen levels, produced a marked prolongation of convulsion times upon subsequent exposure to pure oxygen at pressure. These findings are supported by those of Walker (1961) who showed that mice adapted to living in 10-20% carbon dioxide convulsed later than control animals when subsequently tested at 6 atm. with pure oxygen.

The shortening of convulsion time when CO₂ is added to oxygen at pressure is at least partly due to the vascular effects of carbon dioxide which were studied by Lambertsen, Kough, Cooper, Emmel, Loeschcke and Schmidt (1953) and again by Lambertsen, Ewing, Kough, Gould and Stroud (1955) in men. These studies showed a 15% decrease in cerebral blood flow when oxygen breathing at 1 atm. and a change from normal levels to about 1,000 mm. Hg. in the average cerebral venous PO₂ upon breathing 2% carbon dioxide in oxygen at 3.5 atmospheres.

That the effect of carbon dioxide is not perhaps entirely due to its action upon blood vessels is indicated by the experiments of Williams and Beecher (1944) who found that carbon dioxide also enhances the action of oxygen upon *Drosophila*.

A protective effect of tris (hydroxy methyl) amino methane (THAM) was demonstrated in mice by Sanger Nahas Goldberg and Allesio (1961) and the possible mode of action of THAM discussed by Nahas (1965). While the administration of THAM produced a significant fall in oxygen uptake in mice, Nahas interpreted the effects of THAM as being primarily a function of "its antacid, basic property", which suggests that the effect of carbon dioxide upon oxygen convulsions may be to some extent due to alterations in intracellular pH.

Anaesthetics

From the observations of Bert onwards there is considerable evidence for the protective effect of anaesthetics against oxygen convulsions. This is perhaps a misnomer since what is meant is a delay in or abolition of convulsions due to the presence of an anaesthetic agent. That this effect can scarcely be thought of as protective is suggested by the observations of Van Den Brenk and Jamieson (1962) (1964) that the residual brain damage seen in rats after exposure to oxygen was increased by a variety of anaesthetics including Nitrous Oxide and pentobarbital. Pre-treatment of mice exposed to raised pressures of oxygen with a barbiturate resulted in residual brain damage, while no such damage was seen in the absence of barbiturate.

The mechanism by which anaesthetics reduce or abolish oxygen convulsions is not known but studies by Bean and Zee (1965) using sodium pentobarbital and α -chloralose in propylene glycol showed a marked effect both against convulsions and pulmonary damage.

A similar protective effect was seen after administration of dinitrophenol or L-thyronine. The authors concluded that the protective action seen was not due to a general depression of metabolism. They also suggested that the association of convulsions and pulmonary damage justified the inference that these effects were related and that "central and neurogenic factors" may be involved in the production of pulmonary damage. The implicit assumption here seems to be that anaesthetics only act centrally.

However, in a later paper Bean Zee and Thom (1966) reported that

pulmonary changes, similar to those seen after exposure to oxygen, could be induced in rats by convulsants such as metrazol and picrotoxin. Anaesthetics which prevented the convulsion also protected against lung damage, while curare did not prevent lung damage but abolished convulsions and 'sympatholytic or anti-epinephrine' drugs prevented the lung damage but not the convulsions. This evidence strengthens the view that autonomic or endocrine factors may be partly responsible for the pulmonary damage seen during exposure to raised pressures of oxygen.

Hormones

The general nutritional and endocrine state of an animal determines to some extent its reaction to oxygen at pressure. Campbell (1937) showed that there was a fall in the body temperature of rats exposed to oxygen at ambient temperatures below 33°C. If this fall was prevented by maintaining the animal at 33°C then upon exposure to 6 atm. of oxygen for 30 minutes, followed by decompression in 20 minutes, there were rarely any survivors; while exposure at 24°C rarely led to any deaths. Campbell also tried the effects of a number of substances - including thyroxin, dinitrophenol, posterior lobe pituitary extract and insulin, with negative results. A definite protective effect was, however, obtained as a result of thyroidectomy.

The converse, that is the effects of hyperthyroid states, was studied by Grossman and Penrod (1949) who found that the survival rate of rats at 5.5 atm. was less in animals receiving thyroid powder than in untreated animals or those treated with propyl-thiouracil.

Bean and Johnson (1952) showed that hypophysectomy in rats conferred

a significant degree of protection against oxygen at pressure; the role of "the combined hypophyseal-adreno-cortical and the sympatho-adreno-medullary 'defense' mechanisms", in the reaction to oxygen at increased pressure has been discussed by Bean (1955) (1965).

Non-toxic gases

There are a number of non-toxic gases which produce anaesthesia, but which have been considered either chemically or physiologically inert. Since it is possible to demonstrate their effects upon biological systems it seems preferable not to refer to them as inert gases, nor to their effects as inert-gas narcosis.

These non-toxic gases include Nitrous Oxide, Nitrogen, the noble gases (Helium, Neon, Argon, Krypton and Xenon) various hydro-carbons and halogenated hydro-carbons, sulphur hexafluoride and Hydrogen. The anaesthetic pressures for a large number of these gases have been tabulated by Miller Paton and Smith (1965) (1967). With regard to Hydrogen, Helium and Neon there is some doubt as to whether these gases show anaesthetic effects. Experiments by Miller Paton Streete and Smith (1967) showed that the Italian Newt *Triturus* became unresponsive at between 165-245 atm. whether the pressure was achieved with Neon, Helium or hydrostatic pressure. Brauer, Johnsen, Pesotti and Redding (1966) observed convulsions in Rhesus monkeys exposed to some 50 atm. at an oxygen partial pressure of about 1 atm., the remainder being either Hydrogen and Helium. Clear Electro-encephalographic evidence of anaesthesia was obtained with Nitrogen at 28 atm. but not with either Hydrogen or Helium at pressures as high as 110 atm.

A number of interesting interactions between oxygen and other gases have been reported by Fenn (1965). In studying the survival time of fruit-flies (*Drosophila*), it was found that the addition of some 500 p.s.i. of Nitrogen ($\text{PN}_2 = 34 \text{ atm.}$) reduced the survival time for all oxygen pressures below about 5.5 atm. Similar results were also obtained with Argon and a few experiments with Helium suggested that this might also interact with oxygen.

The growth rate of *Neurospora crassa* was studied by Schreiner Gregoire and Lawrie (1962) in the presence of 0.95 atm. of various non-toxic gases - and 0.05 atm. of oxygen. The growth rate was found to be markedly reduced by all the gases studied and in an extension of this work Buchheit Schreiner and Doebbler (1966) gave the partial pressures of non-toxic gases required for 50% inhibition of growth in *Neurospora* as Xe (0.8 atm.) Kr (1.6 atm.) Ar (3.8 atm.) Ne (35 atm.) and Helium (ca 300 atm.). It is interesting to note that all these gases depressed the growth rate more than Nitrogen, that is to say compared with their anaesthetic effects there is a reversal of ranking between Nitrogen and Neon and between Nitrogen and Helium, although within the Helium group of gases the effect was found to be proportional to the logarithms of the polarizabilities and ionization potentials of the gases.

CHAPTER 2. APPARATUS AND GENERAL METHODS

(Any departure from the general methods described below has been outlined in the relevant section under the heading of Method).

PRESSURE CHAMBERS

(a) Low-pressure chamber (4.7 litres internal volume).

This chamber consisted of a vertical cylinder of 'perspex' 14 cms. internal diameter and 31 cms. long designed for a maximum working pressure of four atmospheres. The ends were of stainless-steel seating by O-rings and held in place by tie-rods and wing-nuts, the upper end-plate bearing the inlet, exhaust and safety valves and electrical connections.

(b) Medium-pressure chamber (13.8 litres internal volume).

A horizontal mild-steel cylindrical chamber designed for a maximum working pressure of 10 atmospheres, of internal dimensions 18.4 cms. internal diameter and 51.3 cms. long. The chamber was fitted with a small inward-opening door at one end and a circular 'perspex' window at the other with a long 'perspex' window on the top. Other fittings were inlet, exhaust and safety valves and a terminal block for electrical connections.

(c) High-pressure chamber (2.6 litres internal volume)

A horizontal mild-steel chamber designed for a maximum working pressure of 136 atmospheres, which was 25.4 cms. long and 11.4 cms. internal diameter. Both ends were threaded internally, each taking a heavy plug which sealed upon an O-ring; one was used as a door and the other contained a very small 'perspex' plug which was used for lighting the inside of the chamber. The upper surface had two similar plugs

which could be used as windows with a rather limited field of view. Other facilities were similar to the chambers previously described.

ANCILLARY EQUIPMENT

Gauges

A variety of Bourdon type pressure gauges was used. These gauges were calibrated by Mr. W. B. Butt of the Royal Naval Physiological Laboratory using an air-balance type of dead-load tester. After calibration all gauges were accurate within 1% of the indicated pressure, re-calibration was only considered necessary if a gauge was dropped or otherwise damaged, but occasional checks were made against a mercury column for pressures up to about 4.5 atm., or of one gauge against another for higher pressures. In the majority of experiments at least two gauges were used for pressure measurements, one for the lower pressures and another which covered the whole pressure-range for which the chamber was designed.

Cages

In order to confine the animals to a position from which they could be seen throughout the exposure and also to provide as far as possible a uniform environment, small metal cages were used. These consisted of circular metal boxes 8.5 cms. in diameter and 3 cms. deep. A wall of perforated zinc sheet extended upwards for 2.5 cms. and was topped by a 'perspex' lid which was held in place by three rubber-bands. The total internal volume of each cage was 0.3 litres and the 6 mm. diameter holes in the perforated zinc represented approximately 10% of the total surface area of the cage.

Temperature control

Experiments were at first carried out at ambient temperature until it became obvious that the animals were frequently too cold, since their tails appeared blue and they occasionally showed shivering. As a result it was decided to heat the chambers with hot-water. Each chamber was wrapped round with a rubber tube which was then lagged with cotton-wool on the outside. The tube was connected to a reservoir of water containing a heater and thermostat, the heated water was circulated through the system by a pump. The ambient temperature selected, a wall temperature of 30°C was intended to provide an environment in the region of the neutral temperature zone for mice, Paton and Speden (1965); the need for such control of the ambient temperature was emphasised by the finding of Fertwee (personal communication) that oxygen pressures above 3 atmospheres produced hypothermia in mice.

Gases

Oxygen of industrial grade that is not less than 99.5% oxygen, was supplied in 1.5 cu. ft. cylinders charged to 2,500 p.s.i. This grade of gas is quite satisfactory for such experiments differing from medical or diving grades mainly in its moisture content.

Oxygen and carbon dioxide mixtures were supplied in cylinders containing 120 cu. ft. by British Oxygen Company special gases department to the specification $0.5\% \pm 0.13$ carbon dioxide in oxygen and $1.0\% \pm 0.13$ carbon dioxide in oxygen.

Helium. Pure mineral Helium to the specifications of the U.S. Bureau of Mines i.e. 99.995% pure of which the principal impurities were

Neon and Nitrogen, was supplied in 1.5 cu. ft. cylinders charged to 2,500 p.s.i.

Nitrogen. So-called 'white-spot' oxygen-free Nitrogen was supplied in 1.5 cu. ft. cylinders charged to 2,500 p.s.i.

Nitrous oxide to the British Pharmacopoeia standard of purity was supplied in cylinders containing 400 gallons.

All gases were supplied by the British Oxygen Company.

EXPERIMENTAL ANIMALS

Six-week old male white mice of the Tuck No. 1. strain weighing between 25 and 35 grammes were used for all animal exposures. Animals arrived by rail, in batches of 50, early in the week, and were not used until the following day. Where possible each group of treatments in an experiment was carried out using the same batch of mice, however, a complete Latin square of treatments might need three or four different batches.

The number of animals used to produce any result was gradually increased as a result of experience. Early exposures were made with groups of ten, however, it was soon found that for significant differences between groups to be demonstrated a minimum of twelve, but preferably fifteen to eighteen results were needed.

At the end of each experiment the animals were killed either by stunning and breaking the neck, or by an overdose of aether.

EXPERIMENTAL TECHNIQUES

Chamber atmosphere

The first step in producing the desired atmospheric composition is to flush out the air from the chamber. It is usual to remove this air

by passing a current of gas through the chamber between the inlet and exhaust valves. The design of many chambers is, however, such that a large proportion of the gas within the chamber may be left unstirred, leading to considerable contamination of the atmosphere with the air originally present. In order to avoid this a method was devised which involved the expansion of any sequestered gas which it was hoped would improve the mixing of gases and hence the efficiency of the flushing procedure.

Serial dilution

The air present in the pressure chamber at the beginning of each exposure was removed by diluting it with oxygen. The oxygen was added until a pre-determined flushing pressure was reached and the mixture then exhausted to the atmosphere. This process was repeated in a serial manner which, on the assumption that mixing was complete between the gas added and the gas already in the chamber, should give an exponential approach to 100% oxygen according to the following expression:

$$\text{Percentage oxygen} = 100 \left(1 - \frac{0.8}{(P)^n} \right)$$

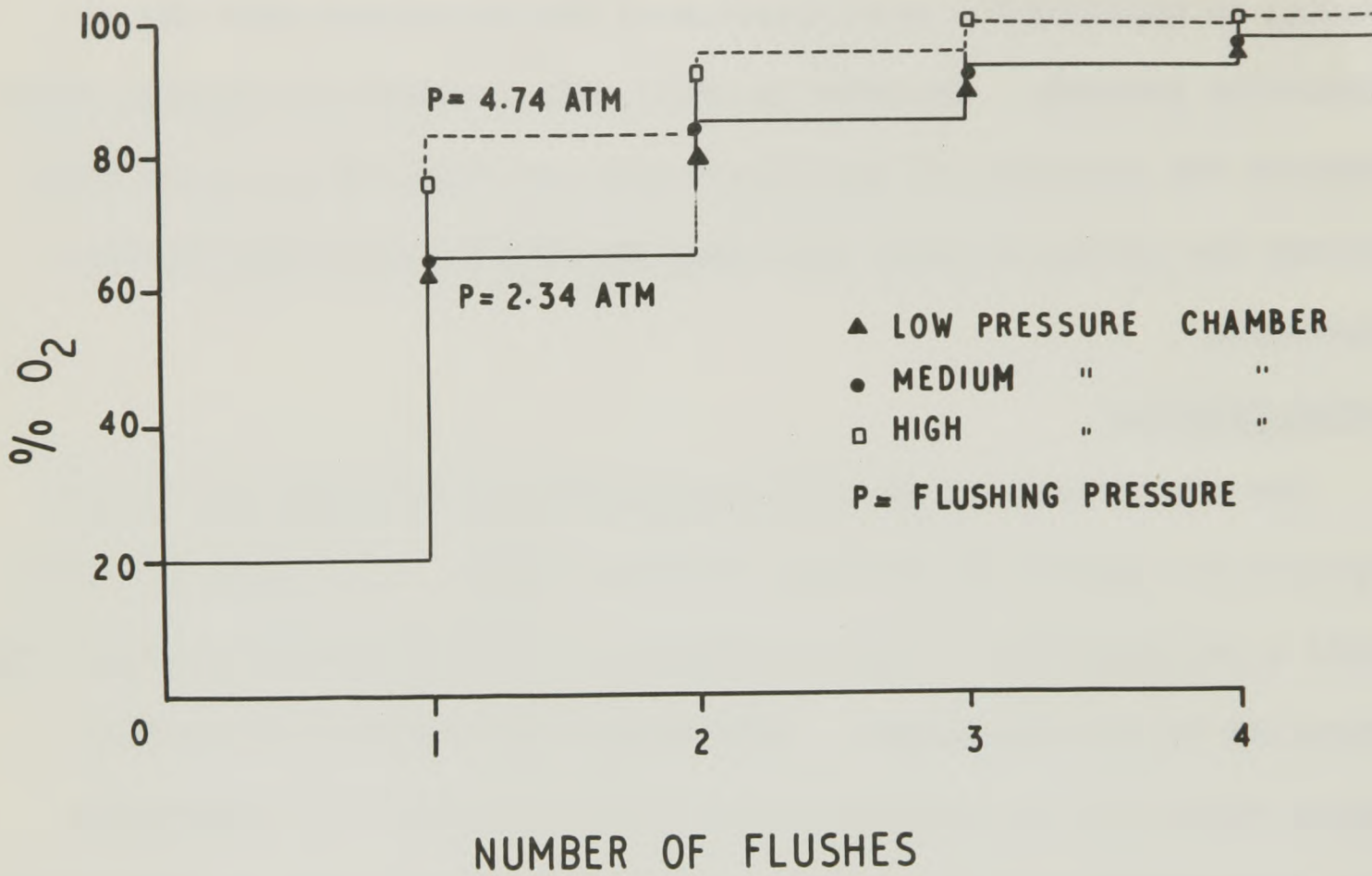
Where P = flushing pressure (atmospheres absolute)

n = the number of complete flushes

To check the validity of this method of flushing, measurements were made using a Beckman D.2 paramagnetic oxygen analyser. The analyser was first calibrated with pure Nitrogen, air and pure oxygen, using the scale marked in mm. of mercury, and a Fortin barometer.

The PO_2 of the exhaust gas was measured after expansion to 1

FIGURE 1



Serial dilution: A comparison between the observed and the predicted oxygen levels. The measured levels are indicated by symbols superimposed upon the theoretical values, indicated by lines.

atmosphere, following each dilution, and three sets of measurements obtained for each chamber. The mean values for the first and subsequent dilutions have been plotted against the corresponding theoretical values in Fig. 1 which shows satisfactory agreement between the observed and the calculated values, most of the difference between them was probably due to imperfect mixing.

The time taken for flushing was variable, but was between one and two minutes.

Carbon Dioxide

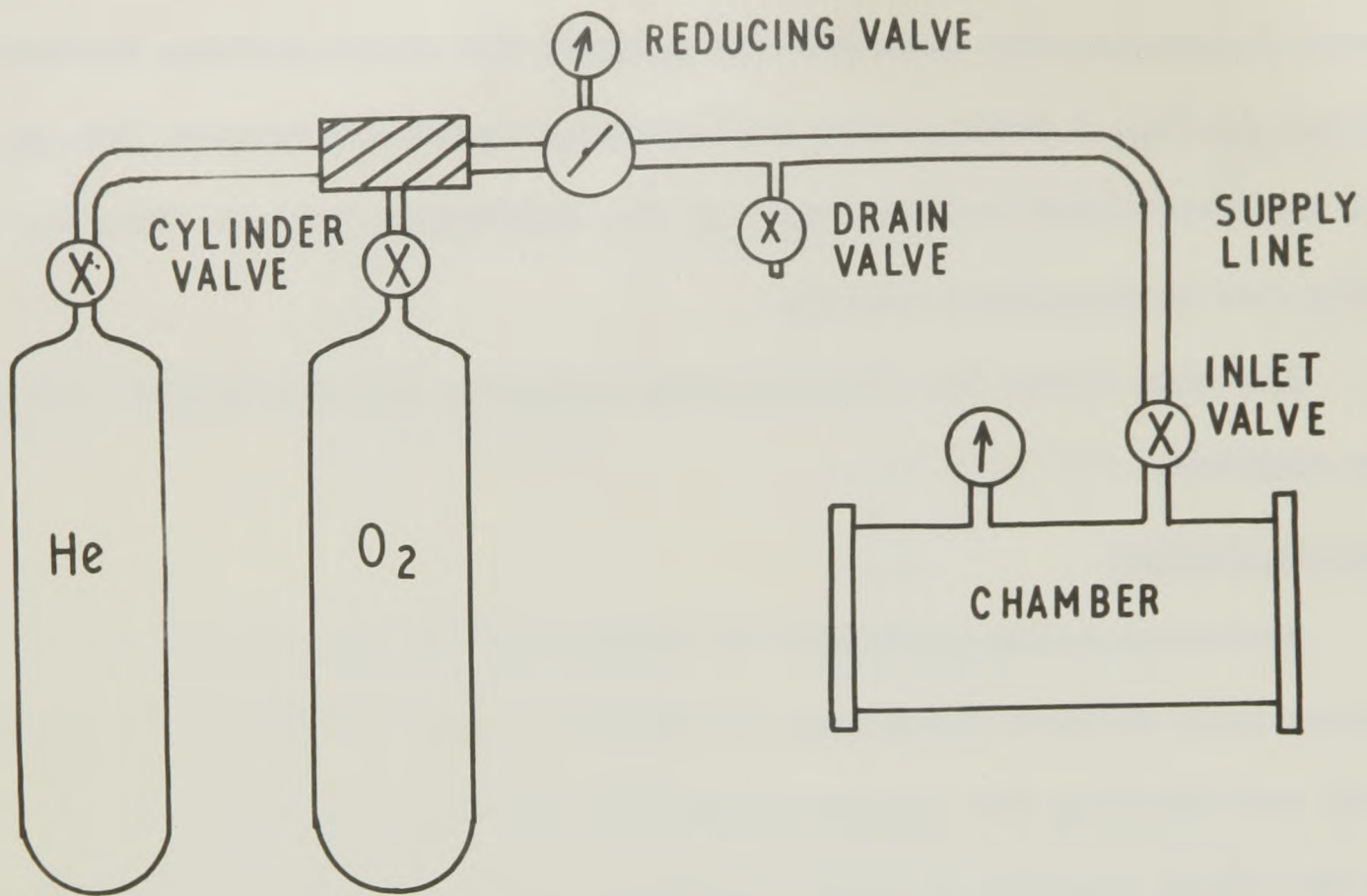
Exposures using a mixture of carbon dioxide and oxygen were of course made without absorbent, in this case the pre-mixed gas was used both for flushing the chamber initially and also for compression; while at the final pressure a small continuous flow was passed in at one end of the chamber and out at the other through a 'Rotameter' flow-meter.

Carbon Dioxide absorption

Absorption of the carbon dioxide produced by the animals was by self-indicating granular soda-lime contained in open trays beneath the cages. In the high pressure chamber the absorbent was placed in a rectangular tray 21 x 2.8 x 1.5 cms. which held about 30 gms. of absorbent.

Measurements of the carbon dioxide in the atmosphere, in the presence of absorbent, were made following exposures of up to 30 mins. at 22 atm., in order to check the efficiency of the absorbent. Samples were removed through the exhaust valve and allowed to expand to atmospheric pressure whence they were passed through a Grubb-Parsons infra-red carbon dioxide detector. Preliminary experiments put the resolving power of this

FIGURE 2



Arrangements for mixing Gases within the chamber.

instrument at about 0.05% of 1 atm. at maximum sensitivity. No carbon dioxide was detected from the pressure chamber at 22 atm. and it is therefore considered that the carbon dioxide partial pressure was less than 0.01 atm.

As a result of some observations by M. J. Lever (personal communication) it was thought that considerable inhomogeneity might exist in the chamber atmosphere, to counter this a small fan driven by a low-voltage synchronous motor was installed in one end of the chamber for some of the later experiments at high pressures.

Gas mixing within the chamber

When only oxygen was to be used for an exposure this was added as soon as flushing was complete.

However, when oxygen was used with some other gas, the detailed procedure depended upon the relative proportions of the gases in the final mixture. For example, in exposures using oxygen and helium where the greater part was to be helium, the following procedure was used:

1. Flush chamber with oxygen.
2. Add O_2 to the required pressure.
3. Shut off O_2 and drain supply lines to atmosphere (see Fig. 2).
4. Open Helium cylinder and add gas until required pressure reached.

When oxygen was the predominant gas in the mixture, for example, when used with nitrous oxide, a similar procedure was used, viz.

1. Flush chamber with oxygen.
2. Shut off O_2 and drain supply line.
3. Add N_2O to required pressure.

4. Shut off the N_2O cylinder and drain the supply line.
5. Open O_2 cylinder and add gas until required final pressure.

This method allowed the pressure of the gas which formed the lesser proportion in the mixture to be measured on the more accurate low pressure gauge.

Compression

Having obtained an atmosphere consisting mainly of oxygen by the flushing procedure outlined above, the chamber was then filled to the required pressure with oxygen or whatever gas mixture was being tested. The rate at which this compression was achieved was variable since it was thought more convenient to use a constant compression time, usually of the order of one minute. However, some slower compressions of five minutes were also used.

Timing

All times were taken from the start of compression, that is, after flushing; they were measured by stop-watch and recorded to the nearest five seconds.

Units

There are no generally accepted units in pressure physiology, pounds per square inch, kilogrammes per square centimetre, millimetres of Mercury and feet of sea water being used with both cubic feet and litres. As far as possible all pressures have been expressed in atmospheres absolute (atm.) where one atmosphere represents the mean atmospheric pressure at sea-level and the standard acceleration of gravity.

The following equivalents have been used.

760 Torr
1 Atmosphere (atm.) = 14.7 lbs/ins² (p.s.i.)
33 feet of sea-water

For mixtures of gases the partial pressure of each gas has been expressed in atmospheres absolute (atm.).

of the cage, as explained and average partial which may last for some time
minutes. The animals were seen to breathe freely, usually sitting still but
checking occasional climbing movements. Before a procedure there is
usually a stage of excitement which is shown by increased respiration,
that is regular movements within the cage. Increased frequency of gas
working and for-cleaning movements and sometimes swimming for
studies.

First procedure

The first procedure was in reality a test, both to determine
severity and to determine what is a slight change of the first procedure
with repeated movements of the animal. The above stated procedure
initially lasts only about five to ten minutes. It was found that such
signs may be associated with some general disturbance of central nervous
system to determine effects in some. In these cases the animal may still
behave and usually breathe for a brief period before appearing very
rest and appearing abnormally normal. A few animals were kept
straight from quiescence or still hypoxemia by a water procedure
without any preliminary signs.

Second procedure

These are of course exact but may be recorded by recording several the

CHAPTER 3. OXYGEN CONVULSIONS IN MICE

GENERAL FEATURES

When exposed to potentially convulsive pressures of oxygen the behaviour of mice is at first little changed. The initial reaction is a rapid exploration of the available space and then gnawing of the sides of the cage, an explore and escape pattern which may last for some five minutes. The animals then seem to settle down, mainly sitting still but showing occasional cleaning movements. Before a convulsion there is usually a stage of excitement which is shown by increased restlessness, that is random movements within the cage, increased frequency of paw-washing and fur-cleaning movements and sometimes spasmodic running in circles.

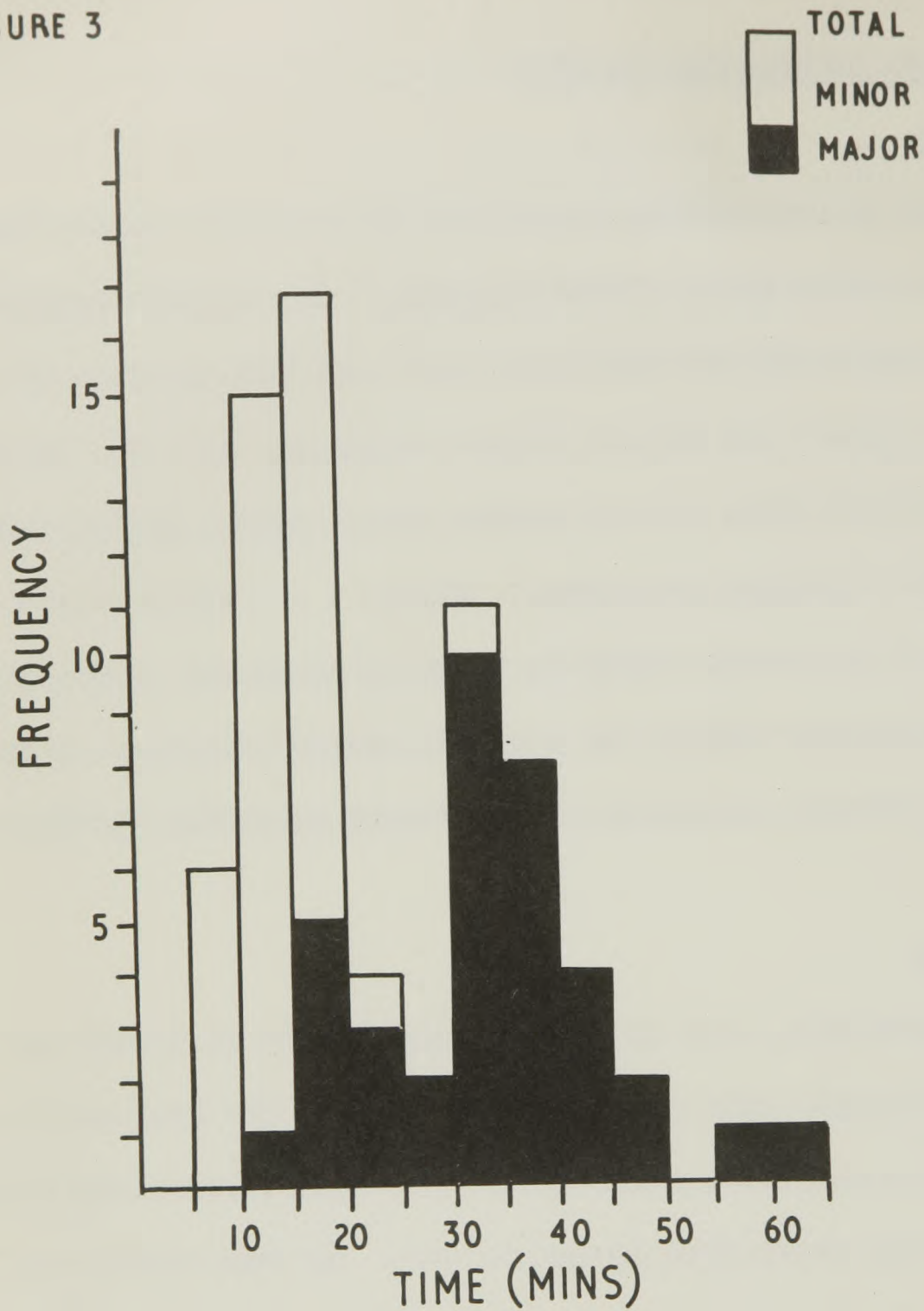
Minor convulsions

The first convulsion seen is usually minor, both in extent and severity and is commonly seen as a rapid nodding of the head associated with repeated movements of one fore-limb; the whole attack characteristically lasts only about five to ten seconds. In some cases such focal signs may be associated with more general radiation of motor spasms, similar to Jacksonian attacks in man; in these cases the animal may fall backwards and remain immobile for a brief period before regaining its feet and appearing substantially normal. A few animals seem to go straight from quiescence or mild hyperactivity to a major convulsion without any premonitory signs.

Major convulsions

These are of sudden onset but may be preceded by running around the

FIGURE 3



The distribution of convulsions in time.
The frequencies of all convulsions seen at 5.4 atm.
are shown plotted against their time of occurrence.

cage; the convulsions involve all muscle groups, so that the animal spins violently within the cage. The convulsion is usually associated with incontinence of urine and there may also be ejaculation of semen. The duration of this violent phase of the convulsion is usually from 30 to 60 seconds, after which it is followed by a tonic spasm in which the animal lies upon its side, eyes closed, mouth gaping, fore-paws flexed and its hind-limbs extended. This latter phase usually lasts for between 15 and 30 seconds, the animal may then get up and move about the cage in a normal manner until a further convulsion ensues. These convulsions differ from epileptic attacks in man in that they are usually terminated by a tonic phase, whereas in man the tonic generally precedes the clonic phase.

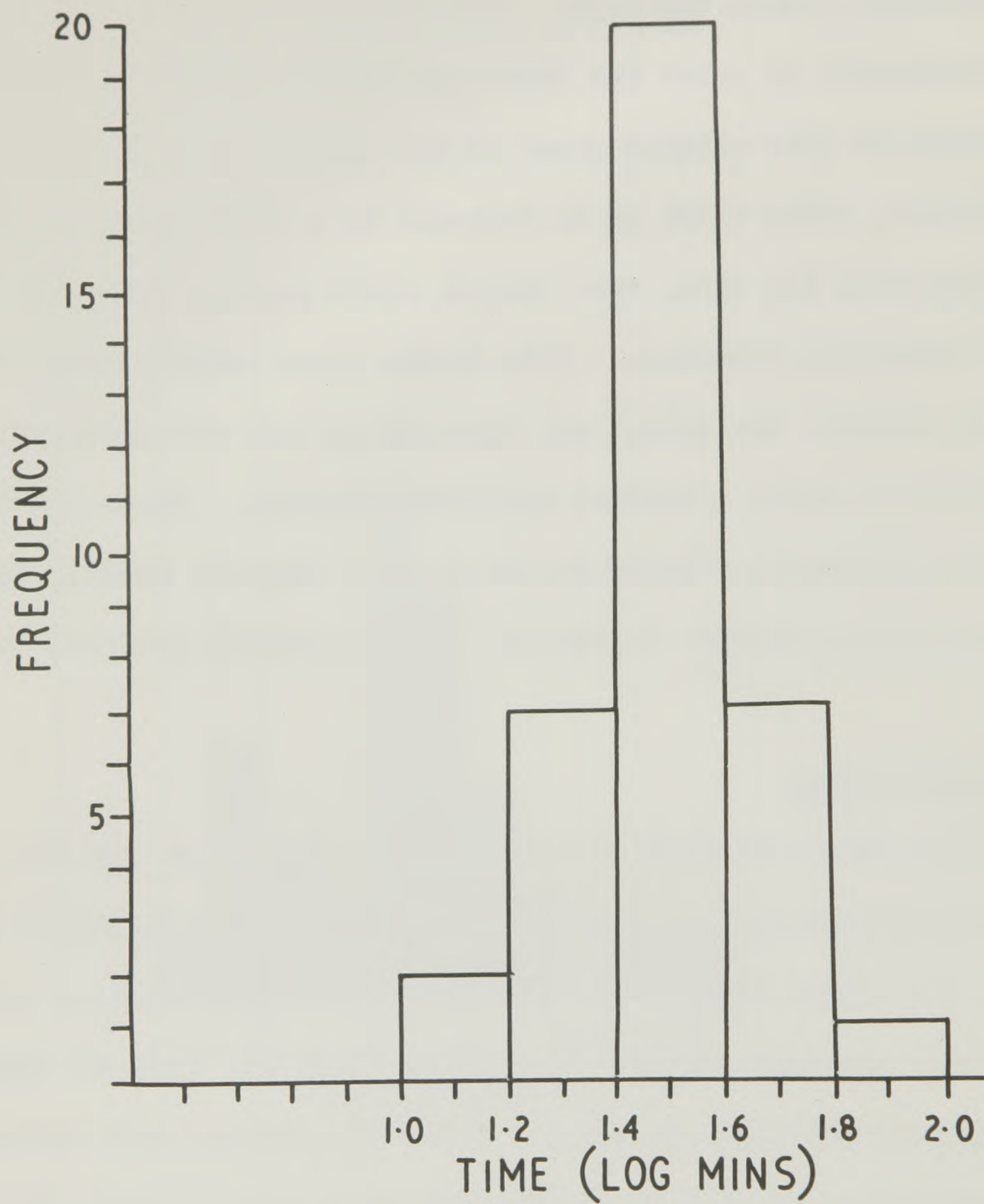
Chronic convulsions

Animals which are exposed to high oxygen pressures may not convulse in the typical manner described, but instead may seem to become ill, lying in the bottom of the cage with only occasional bouts of activity. Such animals commonly develop convulsions which are slow and which include characteristic rolling movements. These convulsions also differ from major convulsions in that their duration tends to be longer, two minutes or more, and the animal may progress to death with intermittent chronic convulsions and increasing respiratory embarrassment as shown by slow irregular gaping or gasping respiratory movements.

Time Relationships

If one considers the distribution in time of the three types of convulsion described, Minor, Major and Chronic, then relative to the median time for Major convulsions, Minor convulsions occur earlier and

FIGURE 4



The Logarithmic Transformation of Convulsion time.
The convulsion times obtained at 5.4 atm. have been transformed
and the resulting frequency distribution shown above.

Chronic convulsions occur later. The quantitative relationship between Major and Minor convulsions is discussed later.

EXPOSURE, DOSE AND RESPONSE.

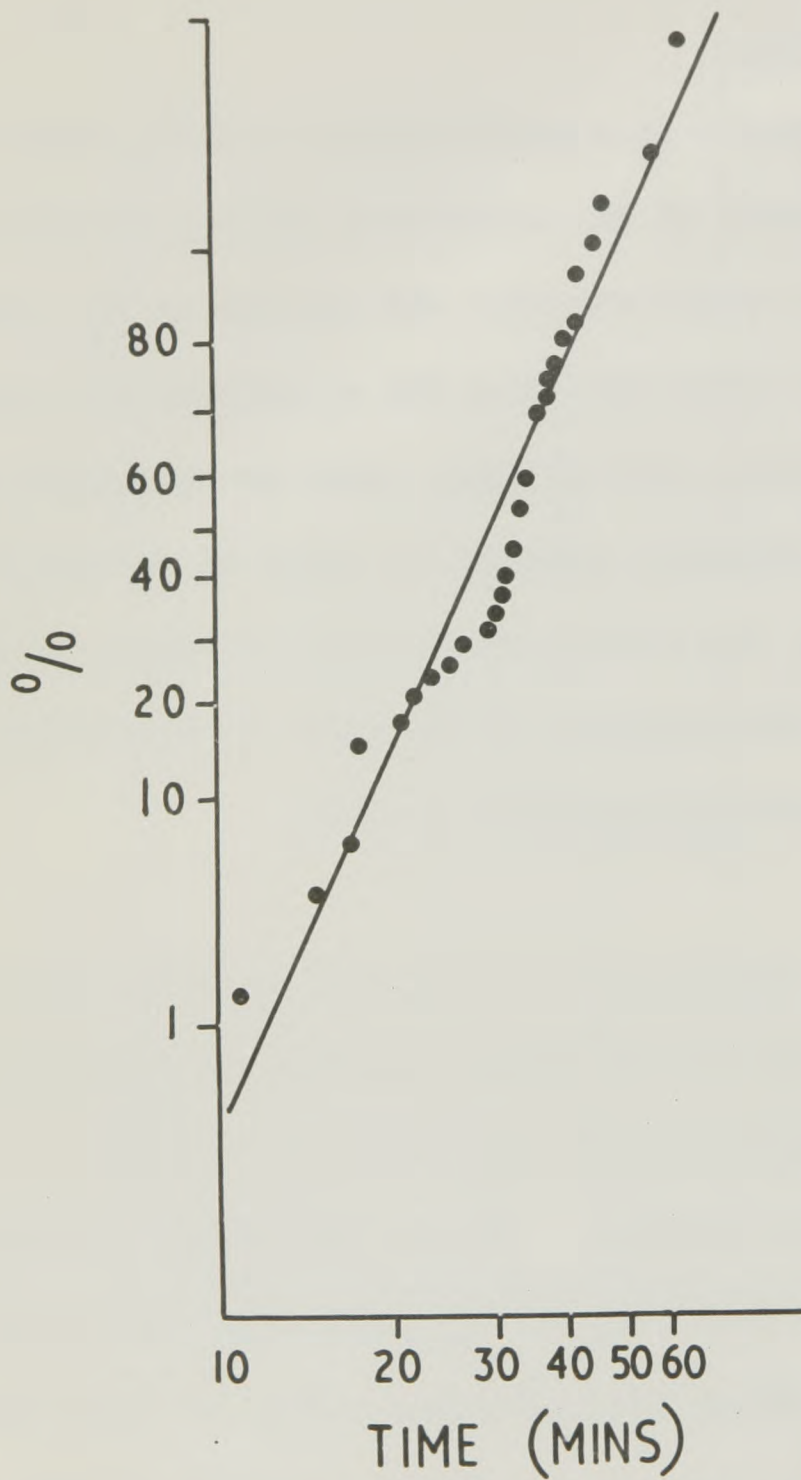
When an animal is exposed to a sufficiently high PO_2 there is a latent period before the development of the convulsion which marks the end-point. This time may be regarded as the response and treated as the dependent variable. However, it is also true that the occurrence of a convulsion is a function both of pressure and of time, hence an aspect of the dose is the time spent at a particular pressure in order to produce a convulsion. Since, however, the relationship which is being studied is that between the PO_2 and the response, it is usual to treat the ambient pressure only as the independent variable or dose.

The Response

If one considers the responses to an ambient pressure sufficient to cause convulsions in most of the animals exposed they are found to be distributed in the manner shown in Fig. 3, where it will be seen that Minor and Major convulsions overlap. It was originally intended to use as an end-point the first convulsion of any kind, that this would have been unsatisfactory is shown by the following table, in which the number of animals convulsing at 5.4 atm. is tabulated according to the type of convulsion seen.

Table 1. Convulsions at 5.4 atm.				
MINOR ONLY	MINOR AND MAJOR	MAJOR ONLY	NO CONVULSION (OR CHRONIC)	TOTAL
4	30	7	1	42

FIGURE 5



The Probit Transformation: The convulsion times obtained at 5.4 atm. have been used to calculate the empirical probits, upon which have been superimposed the fitted probit line.

Had the first convulsion been used as an end-point nearly one-fifth of the animals (8) would have convulsed at some 30 minutes, while the remainder (34) would have convulsed at about 13 minutes; thus the distribution of the first convulsion is clearly bimodal.

The distribution of responses about the mean for one type of convulsion seems at first sight to be normal, since it is roughly symmetrical and the mean, median and mode are quite close together. Clearly, however, negative convulsion times have no meaning, and in fact convulsions are rarely seen in less than five minutes, this means that one tail of the distribution is incomplete.

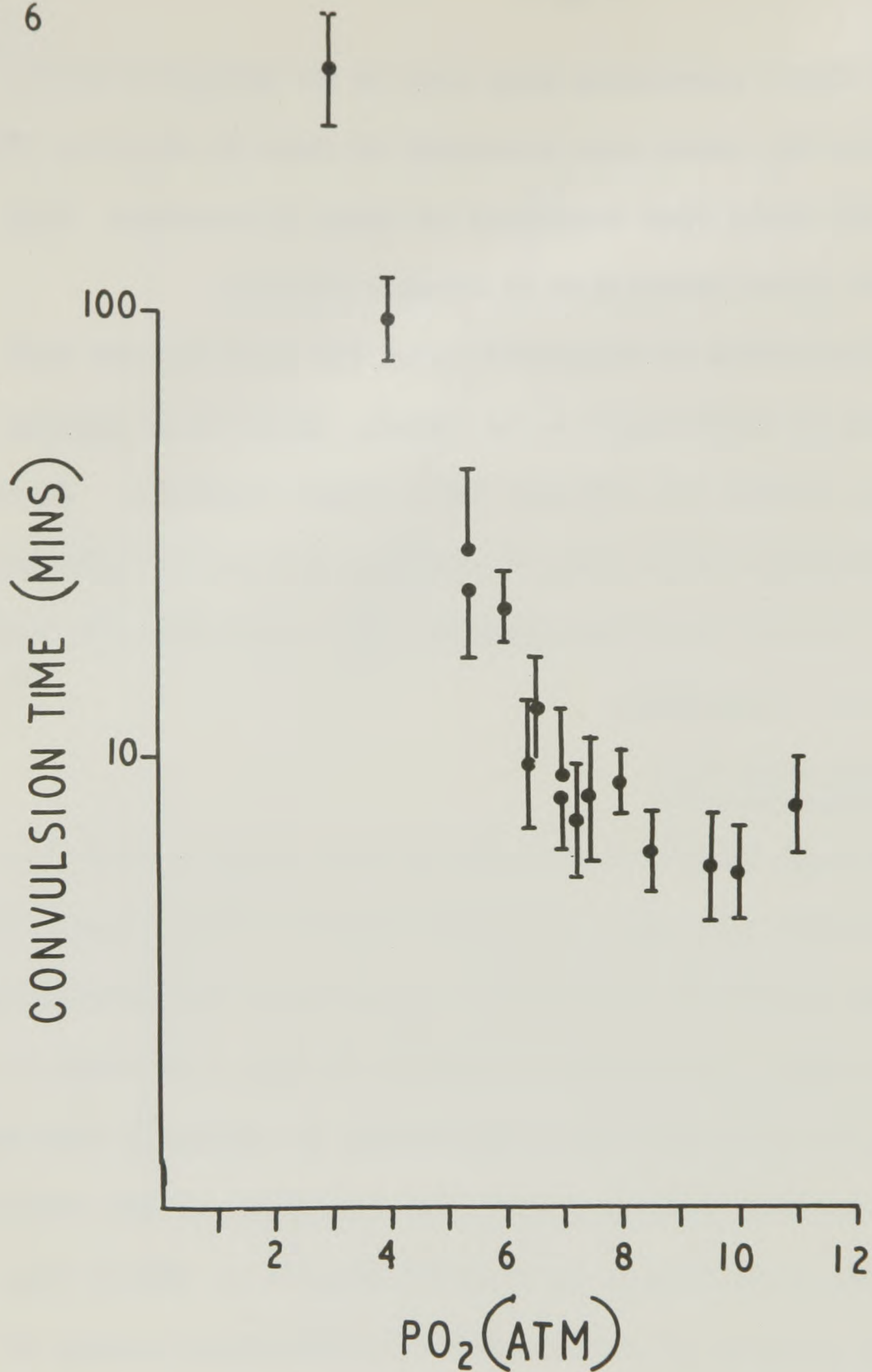
The convulsion time ' t_c '

Marks (1944) showed that a plot of the probit against the logarithm of the convulsion time gave a straight line for each oxygen pressure tested. The result of performing a logarithmic transformation upon the data for major convulsions contained in Fig. 3 is shown in Fig. 4 where it can be seen that the distribution is obviously more symmetrical. A plot of empirical probits against the logarithm of the convulsion time shows that the distribution is slightly negatively skewed Fig. 5, but the mean and standard deviation give a satisfactory measure of the distribution, since there is no significant difference between the empirical probits and the fitted probit line.

Thus, in order to calculate the convulsion time t_c , all times were converted to logarithms and the mean log. time calculated. This is equivalent to the geometric mean of the observed time in minutes.

The effect of this logarithmic transformation is to convert a

FIGURE 6



Oxygen Dose-response Curve: The sample mean convulsion times t_c and their standard deviations σ have been plotted against the respective ambient oxygen pressures.

positively skewed distribution in which the error is proportional to the mean, into an approximately normal distribution for which the standard deviation is independent of the mean.

The Dose-response curve

The dose-response curve for mice exposed to ambient oxygen pressures from three to eleven atmospheres is shown in Fig. 6.

The three important features of the dose-response curve are:

- (a) the shape of the curve
- (b) the threshold pressure: defined as that PO_2 to which the curve appears asymptotic upon the pressure axis
- (c) the minimum response time: defined as the convulsion time to which the curve appears asymptotic upon the time axis.

The shape of the curve

From the appearance of the dose-response curve, one of the simplest and most obvious relationships to try was that for a rectangular hyperbola of the general form

$$(x - b)(y - a) = c$$

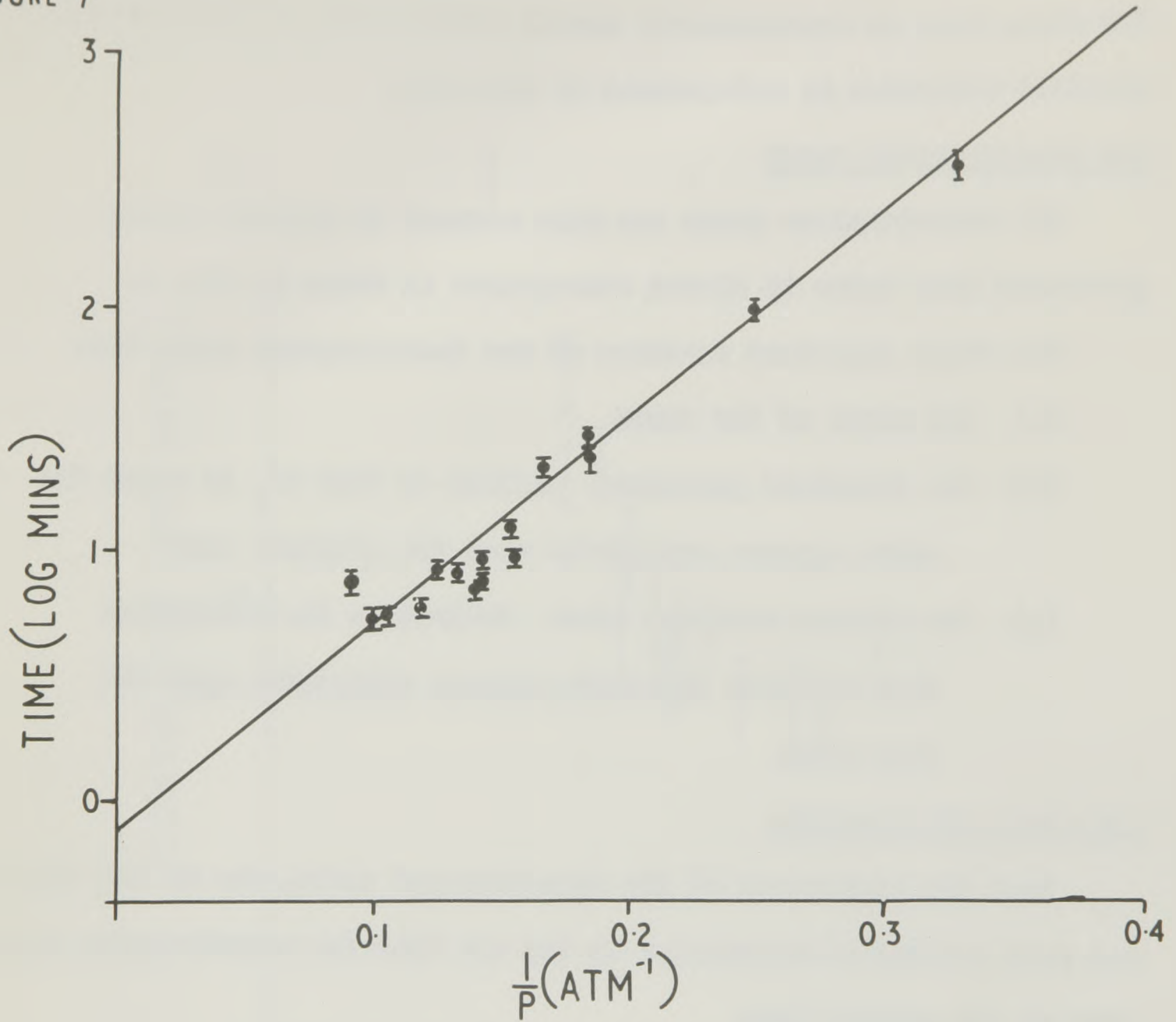
where a and b are the asymptotes parallel to the x and y axes respectively.

Re-arranged in the form $y = a + c \left(\frac{1}{x - b} \right)$ it is in the form of the equation for a straight line.

The desired equation for the present case, after logarithmic transformation would therefore become

$$\log_{10} t_c = a + c \left(\frac{1}{P - b} \right)$$

FIGURE 7



Empirical relationship for Major Convulsions: The Mean Convulsion time t_c for each sample and its standard error σ_n has been plotted against the reciprocal of the ambient oxygen pressure. Superimposed is the regression line obtained by the method of least squares.

where $\log_{10} t_c$ = the logarithm of the major convulsion time.

a = the minimum response time.

c = the proportionality constant.

P = the ambient PO_2 .

b = the threshold pressure.

When the asymptote is not known it may be estimated in the manner described by Riggs (1963). This method involves fitting of the best straight line by trial and error, which may be done graphically. It was considered more satisfactory however to fit a regression line to the data by the method of 'least-squares' and to select the line which gave the highest co-efficient of linear correlation.

The mean logarithmic convulsion times, their standard errors σ_n and the empirical equation selected viz.

$$\log t_c = -0.1 + 8.1 \left(\frac{1}{P}\right)$$

are shown in Fig. 7. The highest co-efficient of linear correlation $r = 0.98$ for values of b between 0 and 3 atm. was achieved for $b = 0$.

Goodness of fit

Although the fit between the observed values and the derived empirical equation is extremely good, there are nevertheless two inconsistencies in this analysis; the first of these concerns the value for the minimum response time. As the PO_2 increases it is found that the response time becomes shorter, lying between about five and eight minutes for exposure above 7 atm. It was not found possible to explore this limit of the curve further since at pressures above 11 atm. convulsions appear to be suppressed. Exposure to 16 atm. and 20 atm. produced collapse within about three minutes and lead to death within ten minutes

without any sign of convulsions. This had previously been observed by Paton (1967) who considered that the absence of convulsions was consistent with the view that oxygen at such pressures acts as an anaesthetic.

Whatever the reasons for the absence of convulsions at high oxygen pressures, this finding makes it impossible to confirm by direct experimentation the extremely short minimum response time, of about 50 seconds, predicted by this equation.

The second inconsistency concerns the threshold pressure which, like the minimum response time, is also extremely difficult to verify directly, since at pressures in the region of 3 atm. of oxygen the animals become ill and major convulsions are less common, chronic convulsions or respiratory distress apparently taking their place. In this connection Smith (1899) considered that a degree of protection against convulsions was conferred by the lung-damage which resulted from prolonged exposure to oxygen. In the present work, those animals which were examined post-mortem at 3, 4 and 4.5 atm. usually showed quite gross lung-damage as evidenced by dense dark haemorrhagic lungs and frequently also by a sanguineous pleural effusion. As will be discussed however in a later section it is unlikely that lung damage is the major factor in producing the apparent threshold, which for mice is about 3.0 atmospheres of oxygen.

Minor convulsions (Figure 8)

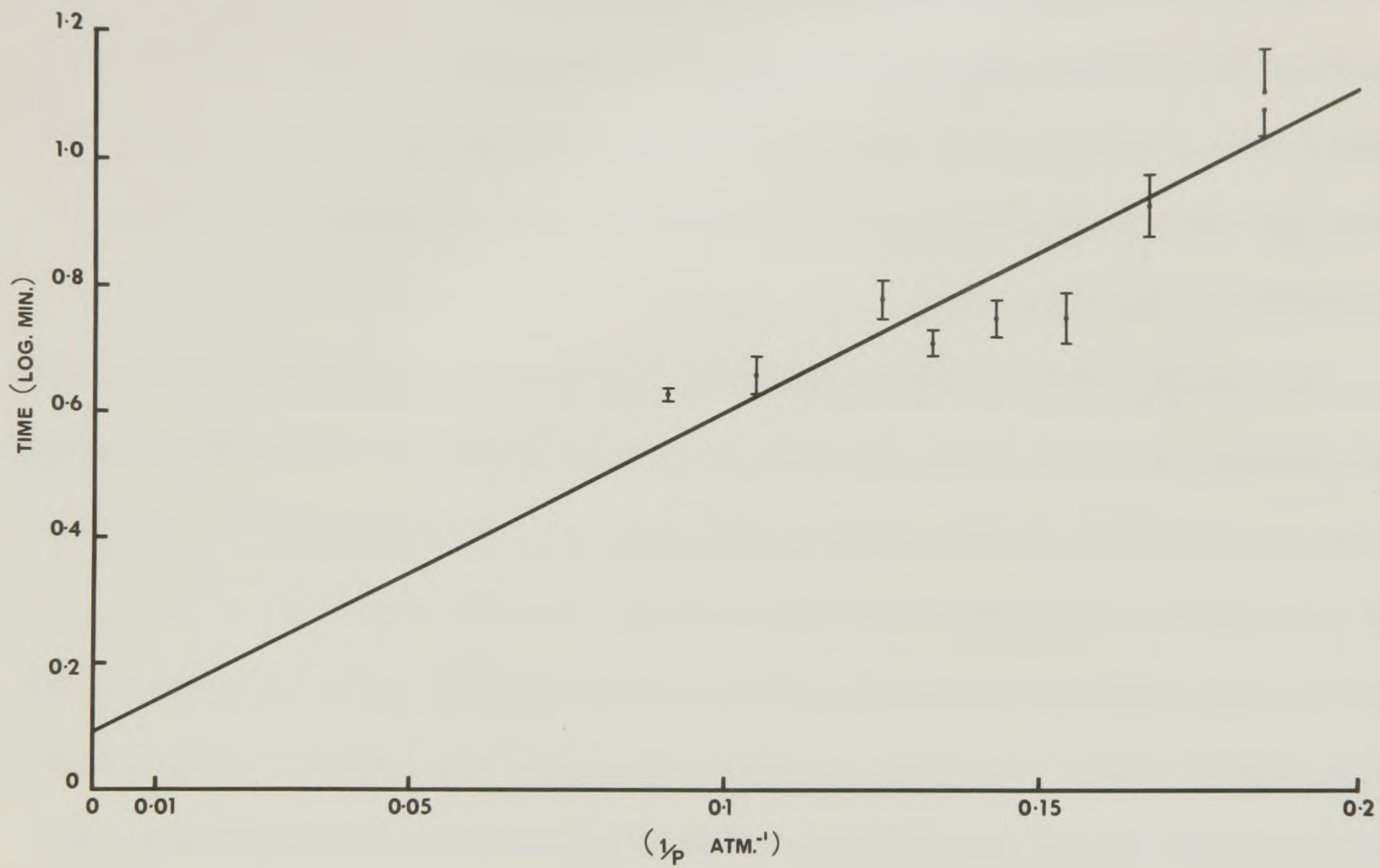
Analysis of the dose-response curve for minor convulsions in exactly the same way as for major convulsions leads to the empirical equation

$$\log t_m = + 0.1 + 5.1 \left(\frac{1}{P}\right)$$

where $\log t_m$ = the logarithm of the time to minor convulsions

$\left(\frac{1}{P}\right)$ = the reciprocal of the ambient PO_2 .

FIG. 8



Empirical relationship for Minor Convulsions:
 The Mean Convulsion time t for each sample and its standard error σ_n has been^m plotted against the reciprocal of the ambient oxygen pressure. Superimposed is the regression line obtained by the method of least squares.

The co-efficient of linear correlation given by the data is
 $r = 0.93$.

Thus, not only are minor convulsions distinct from major convulsions in their appearance, but the slope of the regression line is also different.

The slope-ratio for major and minor convulsions may be obtained from the equations given, whence:

$$\text{slope ratio} = \frac{8.1}{5.1} = 1.6$$

ADDENDUM Page 29.

Only animals which showed both a definite Minor convulsion, followed in the same exposure by a definite Major convulsion, were used to calculate the values of t_m .

While Major convulsions were usually repeated if the exposure was extended, Minor convulsions were rarely repeated and were never seen to follow a Major convulsion.

The approximate Time-relationship between Major and Minor convulsions was found to be, that Minor convulsions developed in about half the time taken for Major convulsions.

CHAPTER 4. DIVIDED EXPOSURES

The onset of convulsions in warm-blooded animals is characteristically preceded by a latent period during which the animal appears normal, the nature of the changes which must be taking place in the central nervous system during this period and which lead to convulsions, is not known. However, a large number of in vitro studies, Davies and Davies (1965) Haugaard (1968) have shown that oxygen inhibits many of the enzymes essential to cellular metabolism, but as has been remarked by Dickens (1962) this inhibition usually proceeds at a far slower rate than the rate at which convulsions develop. In order to study the nature of the process which leads to convulsions a series of experiments was devised. This involved dividing the exposure into two parts, each part at a different pressure. The first experiments were designed to see whether it would be possible to detect the effects of a sub-liminal exposure by subsequent titration to convulsions at some supra-liminal pressure.

METHOD

Animals were used in pairs, one control and one experimental animal.

THE EXPERIMENTAL ANIMAL

This was placed in the medium pressure chamber, in the presence of soda-lime absorbent, and exposed to oxygen for a time t_1 minutes at a pressure of P_1 atm. The pressure was then increased to P_2 atm. and the time t_2 minutes taken for the animal to convulse was recorded.

THE CONTROL ANIMAL

(a) For exposures at an initial pressure P_1 of one atmosphere the

control animal was placed in the low pressure chamber, in the presence of soda-lime absorbent, breathing air; it was then transferred to the chamber holding the experimental animal. The chamber was then flushed with oxygen and both animals compressed to the pressure P_2 .

(b) When the pressure P_1 was greater than 1 atm. the control animal was exposed separately in order to avoid the complication of decompressing both animals. The control exposure in this case was made at the selected pressure P_2 for a time t_c necessary to produce convulsions, but without any prior exposure.

Selection of the control animal and the site occupied by each animal was by the toss of a coin.

SUB-LIMINAL EXPOSURES

The first series of exposures was made using 15 minutes at 1 atm. breathing either air or oxygen followed by exposure at 5.4 atm. of oxygen; both this and the subsequent series of 1 hour at 1 atm. followed by 5.4 atm. were entirely negative. Table 2.

In a further attempt to find a level which would provide a change in the convulsion time, exposures were made at 4 atm. for 30 minutes, followed by exposure to 7 atm. of oxygen. Table 3. These experiments showed a significant shortening of the convulsion time when compared with the control value at 7.0 atm. Reverting downwards the next exposures were made at 2 atm. for 30 minutes and the convulsion time compared with that for the control group at 7.0 atm. These results showed no significant difference. Table 2.

Finally exposures of 30 minutes at 3 atm. gave a highly significant

Table 2
The effects of sub-liminal exposures

P_1 (atm.)	t_1 (mins.)	P_2 (atm.)	t_2 (mins.)	$\log t_2$	σ_n
1 O ₂	15	5.4 O ₂	29.4	1.47	0.07
1 AIR	15	"	29.3	1.47	0.05
1 O ₂	60	"	36.6	1.56	0.03
1 AIR	60	"	29.0	1.46	0.06
2 O ₂	30	7.0 O ₂	10.3	1.01	0.01
CONTROL		"	9.2	0.97	0.02

Table 3
The shortening of convulsion times by supra-liminal exposures

P_1 (atm.)	t_1 (mins.)	P_2 (atm.)	t_2 (mins.)	$\log t_2$	σ_n
4 O ₂	30	7.0 O ₂	6.3	0.80 [‡]	0.04
CONTROL		"	9.2	0.97	0.02
3 O ₂	30	6.0 O ₂	11.9	1.08*	0.05
CONTROL		"	21.4	1.33	0.02
3 O ₂	30	7.5 O ₂	5.6	0.75*	0.01
CONTROL		"	8.1	0.91	0.04

[‡] Significant at the 1% level
 * Significant at the 0.1% level

shortening of the convulsion time tested at both 6.0 atm. and 7.5 atm.

Table 3.

It seems, therefore, that one may talk of a threshold to provocation which, for mice, lies between 2 atm. and 3 atm. Prior exposure to pressures below this provoked threshold does not lead to any detectable alteration in the convulsion time at some higher pressure; while exposure to pressures above this provoked threshold produces a marked shortening of the time to convulsions observed at some other supra-liminal pressure. While there is no evidence which would identify this provoked threshold with the threshold recognised from simple exposures, the two pressures seem sufficiently similar to make identity quite probable.

THE CONVULSIVE PROCESS

While no evidence can be offered that any process leading to convulsions exists below the threshold pressure, it is clear that for exposures above the threshold pressure one may regard the latent period as representing the time taken for the development of convulsions by some process which is assumed to begin on first exposing an animal to a raised oxygen pressure and to end with the convulsion. The mathematical form which such a process might take has not previously been investigated except in a recent paper by Fern Henning and Philpott (1967) in which they suggest that the course of oxygen poisoning in 'Drosophila' might be linear rather than exponential.

FRACTIONAL EXPOSURES

When an animal is exposed at a supra-liminal pressure P_1 for a time t_1 short of the time t_{c1} needed to produce convulsions, then upon changing

the pressure to some other supra-liminal pressure P_2 , there will be a time t_2 after which the animal will convulse. If the fractional exposure is defined as the ratio of the exposure time to the convulsion time for that pressure, then providing that the process being considered is a linear function with time, the two parts should combine in the following manner:

$$\frac{t_1}{t_{c1}} + \frac{t_2}{t_{c2}} = 1$$

which may be re-arranged to give:

$$t_2 = t_{c2} \left(1 - \frac{t_1}{t_{c1}} \right)$$

Such an expression should be able to be used to predict the time to convulsions in a divided exposure. In order to avoid confusion between the observed and the predicted values the symbol t_a has been used for the predicted time at pressure P_2 . The results of a series of exposures designed to test this relationship are shown in Table 4. Superficially the agreement appears to be satisfactory, however, half the results were obtained with an initial fractional exposure of about one-third; while these show agreement which is remarkably close, both smaller and larger fractional exposure ratios give results which are inconsistent with the proposed relationship and suggest that the process to convulsions is non-linear.

PARTIAL EXPOSURES

A further attempt to find a way of adding the two parts of a divided exposure was made in the following way:

1. It was assumed that the relationship previously derived for

Table 4
 Fractional Exposures
 $t_a = t_{c2} (1 - \frac{t_1}{t_{c1}})$

Pressure P_1 (atm.)	Exposure time t_1 (mins.)	Convulsion time t_{c1} (mins.)	Fractional exposure t_1/t_{c1}	Pressure P_2 (atm.)	Convulsion time t_{c2} (mins.)	Predicted time t_a (mins.)	Observed time t_2 (mins.)
3	30	346	0.09	6	21.4	19.5	11.9
3	30	346	0.09	7.5	8.1	7.4	5.6
4	30	94	0.32	5.4	29	19.7	18.4
4	30	94	0.32	6.0	21.4	14.6	8.6
4	30	94	0.32	7.0	9.2	6.3	6.3
4	30	94	0.32	7.5	8.1	5.5	5.1
4	30	94	0.32	11.0	7.7	5.2	4.3
5.4	10	29	0.35	6.0	21.4	14.1	14.1
6.0	5	21.4	0.23	5.4	29	22.2	13.0
6.0	10	21.4	0.47	5.4	29	15.4	8.4

convulsive exposures could also be used to describe partial exposures, and also

2. That the approximate relationship $P \log t = K$ could be substituted for the empirical equation

$$\log t_c = -0.1 + 8.1 \left(\frac{1}{P}\right)$$

3. The partial exposures were defined as follows

(a) The exposure at P_1 may be considered to continue for a total time of $t_1 + t_2$ minutes giving rise to a partial exposure $y_1 = P_1 \log (t_1 + t_2)$

(b) The exposure at P_2 may be considered as being added to that at P_1 such that the effective pressure is $(P_2 - P_1)$. This pressure acting for a time t_2 gives rise to a partial exposure

$$y_2 = (P_2 - P_1) \log t_2$$

(c) Since the total exposure gives rise to a convulsion

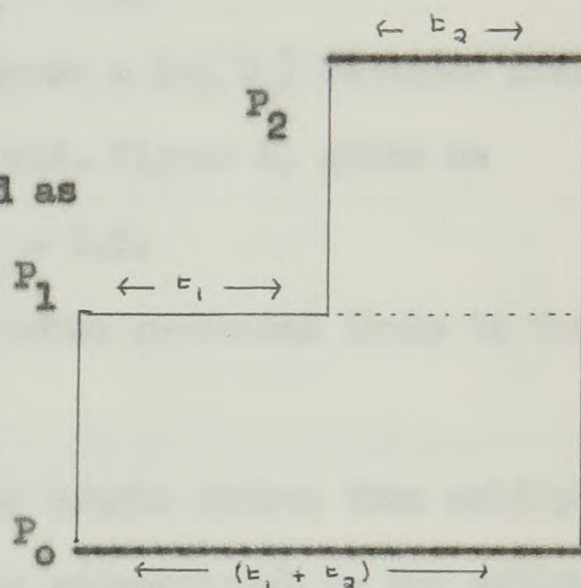
$$y_1 + y_2 = K \text{ or } P_1 \log (t_1 + t_2) + (P_2 - P_1) \log t_2 = K$$

whence K will be the slope of the dose-response curve.

Method

Groups of three mice were exposed for three or more different times at pressure P_1 (e.g. 5, 10 and 20 minutes) and then exposed to a higher pressure P_2 until major convulsions were seen. The pressure P_1 selected was above the provoked threshold, either at 3.0 or 4.0 atm. of oxygen, while P_2 was either 5.4 atm. or 6.0 atm.

The times for the initial exposure were selected in a random order



and the experiments arranged as four Latin squares. The results are presented in Table 5 and Table 6. The latter Table shows that 15 independent estimates of K are in satisfactory agreement, giving a mean value for K = 7.5 with a standard error of $\sigma_n = 0.1$.

By comparison the mean product of (Pressure x log t_c) obtained from the values shown in the dose-response curve vid. Figure 6, gives an estimate for K = 7.3 with a standard error $\sigma_n = 0.2$.

It is therefore considered that the evidence presented leads to the following conclusions:

1. The convulsive process appears to be single rather than multiple.
2. The rate of this process is directly proportional to the ambient oxygen pressure.
3. As a first approximation its form may be said to be exponential (vid. CHAP. 9).

Table 5
Partial exposures

$P_1 = 3.0 \text{ atm. } P_2 = 5.4 \text{ atm.}$

First exposure t_1 (mins.)	Second exposure t_2 (mins.)	Mean log Convulsion time $\log t_2$	Standard deviation σ	Number in sample n
7.5	23.0	1.36	0.13	12
15	18.4	1.26	0.11	12
30	15.2	1.18	0.27	12
60	5.7	0.76	0.28	12

$P_1 = 4.0 \text{ atm. } P_2 = 6.0 \text{ atm.}$

t_1	t_2	$\log t_2$	σ	n
5	11.1	1.05	0.09	12
10	10.2	1.01	0.18	12
20	11.8	1.07	0.18	13

$P_1 = 4.0 \text{ atm. } P_2 = 5.4 \text{ atm.}$

t_1	t_2	$\log t_2$	σ	n
7.5	16.7	1.22	0.15	18
15	15.3	1.18	0.17	18
30	9.2	0.97	0.18	16

$P_1 = 3.0 \text{ atm. } P_2 = 6.0 \text{ atm.}$

t_1	t_2	$\log t_2$	σ	n
5	11.8	1.07	0.15	13
10	13.6	1.13	0.10	13
20	11.1	1.05	0.12	12
40	6.3	0.80	0.17	15
80	4.6	0.67	0.28	15

Table 6
 The evaluation of the relationship
 $P_1 \log (t_1 + t_2) + (P_2 - P_1) \log t_2 = K$
 or $y_1 + y_2 = K$

$P_1 = 3.0 \text{ atm.}$ $P_2 = 5.4 \text{ atm.}$

t_1 (mins.)	t_2 (mins.)	y_1	y_2	k
7.5	23.0	4.45	3.27	7.7
15	18.4	4.57	3.03	7.6
30	15.2	4.97	2.84	7.8
60	5.7	5.45	1.82	7.3

$P_1 = 4.0 \text{ atm.}$ $P_2 = 6.0 \text{ atm.}$

t_1 (mins.)	t_2 (mins.)	y_1	y_2	k
5	11.1	4.83	2.09	6.9
10	10.2	5.22	2.02	7.2
20	11.8	6.01	2.14	8.2

$P_1 = 4.0 \text{ atm.}$ $P_2 = 5.4 \text{ atm.}$

t_1 (mins.)	t_2 (mins.)	y_1	y_2	k
7.5	16.7	5.53	1.71	7.2
15	15.3	5.92	1.66	7.6
30	9.2	6.36	1.35	7.7

$P_1 = 3.0 \text{ atm.}$ $P_2 = 6.0 \text{ atm.}$

t_1 (mins.)	t_2 (mins.)	y_1	y_2	k
5	11.8	3.68	3.22	6.9
10	13.6	4.12	3.40	7.5
20	11.1	4.48	3.14	7.6
40	6.3	5.00	2.40	7.4
80	4.6	5.77	1.99	7.8

CHAPTER 5. CARBON DIOXIDE

Numerous workers have shown that carbon dioxide has a marked effect upon oxygen induced convulsions Bean (1945) Haugaard (1968). In a series of experiments using mice as subjects Marshall and Lambertsen (1961) found that at lower pressures of carbon dioxide, oxygen convulsions were potentiated, while at higher pressures of carbon dioxide seizures became less violent and the end-point less precise, suggesting that there was some protective effect.

As a first step therefore in studying the interactions of other gases with the phenomenon of oxygen toxicity, the following experiments were made in an attempt to find a quantitative relationship which would describe this interaction.

METHOD

Groups of three mice were exposed in the medium pressure chamber either to a mixture of oxygen with carbon dioxide or to oxygen alone: six different types of exposure were arranged in a 6 x 6 Latin square as follows:-

Treatment	FCO_2	FO_2	P TOTAL	Gas mixture used
A	0.07	6.93	7.0	1% CO_2 in O_2
B	0.04	6.96	7.0	0.5% CO_2 in O_2
C	--	7.0	7.0	Oxygen
D	--	5.4	5.4	Oxygen
E	0.05	5.35	5.4	1% CO_2 in O_2
F	0.03	5.37	5.4	0.5% CO_2 in O_2

One 6 x 6 square was chosen at random from those tabulated by Fisher and Yates (1948).

When mixtures of oxygen and carbon dioxide were used, the chamber was first flushed in the usual way using the mixture, compression followed and a steady current of mixture was allowed to vent from the chamber, at a rate of 2 - 2.5 litres/minute, through a 'Rotometer'; the inflow was adjusted so that the chamber pressure remained constant.

The control exposures to pure oxygen were carried out using soda-lime absorbent and without a flow of gas.

EXPERIMENTAL DESIGN

The design of the experiment was based upon the following considerations.

The use of a Latin square was intended to allow the segregation of two possible causes of variation: first, any diurnal variation in susceptibility which might be expected to appear as a difference between columns; secondly, any variation in sensitivity between batches, or between successive samples from the same batch, such as might be produced by ageing. Such effects should be recognised as a difference between rows.

In order, as far as possible, to take account of the first of these sources of variation, it was necessary to carry out a complete group of six exposures in one day. The lowest convenient pressure which would allow this was 5.4 atm. at which the mean convulsion time was about 30 minutes.

In choosing the higher pressure level, reference to Marshall and Lambertsen (1961) suggested that a PO_2 of about 7 atm. with a PCO_2 of

0.03 to 0.07 atm. (ca. 20 - 55 mm. Hg.) would be likely to give the optimum effect.

If carbon dioxide was to show a significant protective effect at 7 atm. total pressure, then this might be expected to show itself to some extent as an absence of convulsions. Such an absence of the end-point might be qualitatively convincing but would make analysis of the results more difficult if several missing values had to be computed. Groups of three mice were therefore used, the mean convulsion time for each group being computed, ignoring any missing values.

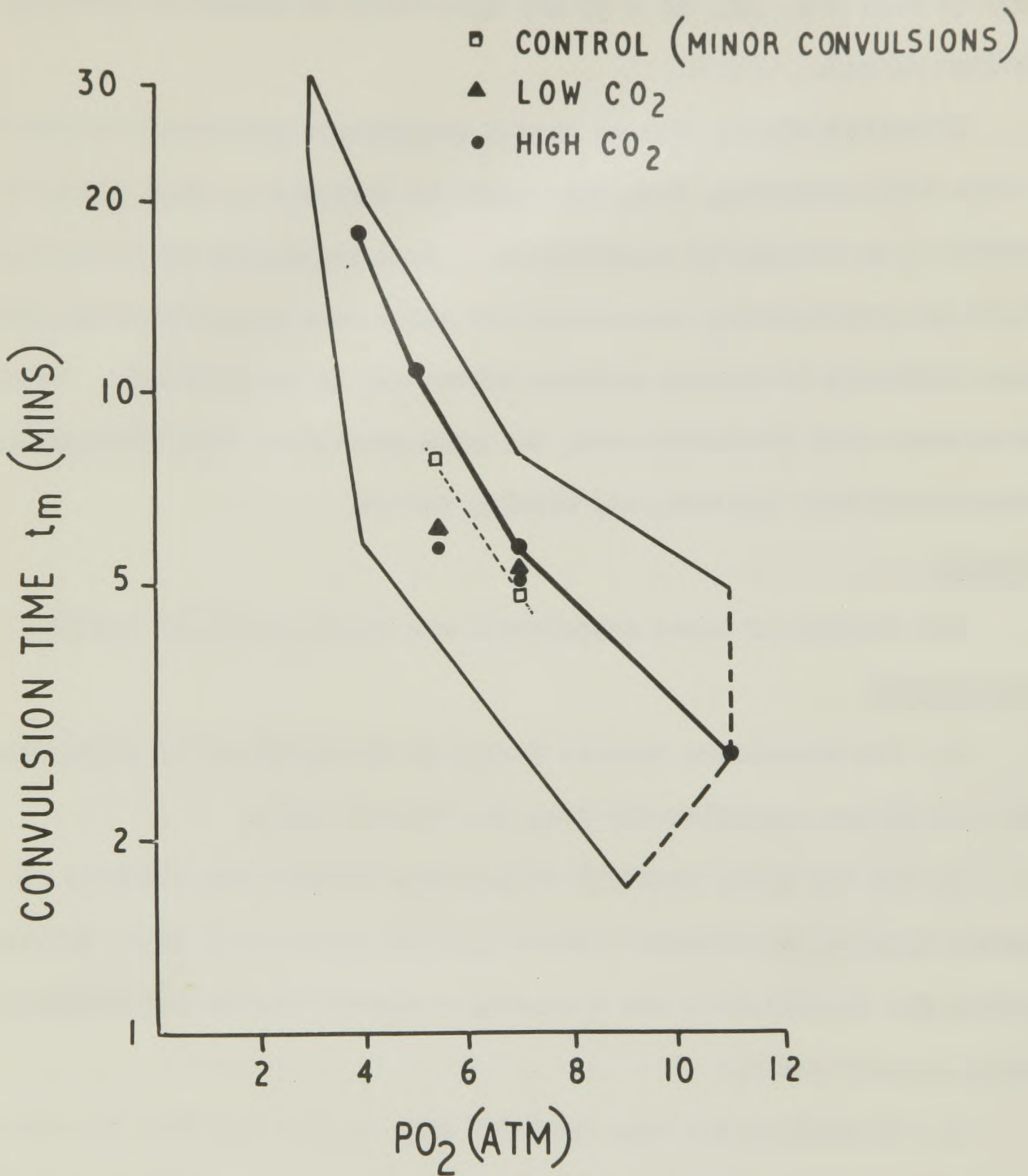
RESULTS

The results of these experiments are shown in Tables 8 and 9.

CONCLUSIONS

1. The convulsion time at 7 atm. in the presence of carbon dioxide did not differ significantly from its control value.
2. At the lower pressure of 5.4 atm. however the addition of carbon dioxide had a marked effect upon the convulsion time, the mean values for treatments E and F showing a highly significant difference from their control value.
3. At neither 5.4 atm. nor at 7 atm. was it possible to distinguish in these experiments between the effects of carbon dioxide pressures which differed by a factor of two, since there was no significant difference between treatments A and B, or E and F.
4. A significant effect of 0.03 atm. carbon dioxide was detected at a total pressure of 5.4 atm. but a similar PCO_2 appeared to be without effect at a total pressure of 7 atm.

FIGURE 9



Minor Convulsions in the presence of Carbon Dioxide:
 The data obtained at 5.4 and 7.0 atm. is shown superimposed upon a fan which illustrates the dose-response curve for mice and the range of results obtained with various levels of Carbon Dioxide by Marshall and Lambertsen (1961).

5. Neither rows nor columns contributed a significant proportion of the total variation.

MINOR CONVULSIONS

The results so far presented have been for major convulsions only, however, minor convulsions were also noted in this series. The data for minor convulsions are presented graphically in Figure 9 superimposed upon results obtained by Marshall and Lambertsen (1961).

It is clear that there is excellent agreement between the two sets of data and that the present work confirms the potentiating effect of carbon dioxide so far as the effect seen at 5.4 atm. is concerned; but shows an absence of effect, rather than a protective effect at 7 atm.

POTENTIATION OF CONVULSIONS

In an attempt to elucidate the nature of the change in convulsion time under the influence of carbon dioxide, a series of divided exposures was carried out similar to those previously described.

Method (divided exposures)

Exposures were made in the medium pressure chamber, using groups of three mice, with a constant flow of gas passing through. The pressures were chosen so that the first exposure was at half the pressure of the second exposure, thus two gas mixtures could be used, 1% carbon dioxide in oxygen at the lower pressure and 0.5% carbon dioxide in oxygen at the higher pressure. The effect of this was to keep a constant PCO_2 while doubling the total pressure. Two series of exposures were used:

(a) Initial exposures of 5, 10 or 20 minutes at 3 atm., followed by exposure to 6 atm. were taken in a random order until fifteen exposures had been made for each of the three different exposures.

Table 8
The effects of carbon dioxide upon oxygen convulsions

Source of Variation	Sum of Squares (S.O.S.)	Degrees of Freedom (D.F.)	Mean Square (M.S.)	Variance Ratio (F)
Between columns	0.0353	5	0.0071	1.1
" rows	0.0419	5	0.0084	1.3
" treatments	1.2638	5	0.2528	39.5 ***
Residual	0.1274	20	0.0064	
Total	1.4684	35		
Detailed comparisons				
Control 7 atm. v A	0.0131	1	0.0131	2.1
" " v B	0.0242	1	0.0242	3.8
" 5.4 atm. v E	0.3832	1	0.3832	60.3 ***
" " v F	0.2398	1	0.2398	37.7 ***

*** Significant at the 0.1% level

Table 9
Convulsion times with CO₂/O₂ mixtures

Treatment	Total pressure (atm.)	P.CO ₂ (atm.)	Mean log t _c	t _c (mins.)	
A	7 atm.	0.07	0.85	7.0	High CO ₂
B	7 atm.	0.04	0.82	6.6	Low CO ₂
C	7 atm.	--	0.91	8.1	7 atm. control
D	5.4 atm.	--	1.37	23.7	5.4 atm. control
E	5.4 atm.	0.05	1.02	10.4	High CO ₂
F	5.4 atm.	0.03	1.09	12.3	Low CO ₂

(b) Initial exposures of 10, 20 and 40 minutes at 2.7 atm. followed by exposure to 5.4 atm. were made in a similar manner.

Results

The results of these divided exposures in the presence of carbon dioxide are presented in Table 10 and analysis of these results by the method given for partial exposures in Chapter 4, is presented in Table 11.

Conclusions

1. The most obvious conclusion which can be drawn from these results is that there is no significant difference between any of the values of $\log t_2$, either with respect to the length of time t_1 , spent at the pressure P_1 , or to the pressure P_2 at which these results were obtained.

2. Nevertheless, $\log t_2$ is significantly less than the convulsion time obtained in the absence of carbon dioxide (Tables 4 and 5).

3. The provoked threshold for convulsions in the presence of ca. 0.03 atm. carbon dioxide appears therefore to be above a total pressure of 3 atm.

4. The slope of the dose-response curve for oxygen convulsions is altered in the presence of carbon dioxide as evidenced by the value of K in Table 11. However, since the first exposure t_1 had no significant effect, it is perhaps more logical to consider only the second exposure as contributing to convulsions. These give a mean value for $K = 4.7$.

The approximate empirical equation for exposures in the presence of 0.03 atm. of carbon dioxide is thus $P \cdot \log t = 4.7$.

5. The crossover point at 7 atm. implies that the intercept is also altered by carbon dioxide.

Table 10				
Divided exposures with carbon dioxide				
$PCO_2 = 0.03 \text{ atm. } P_1 = 3.0 \text{ atm. } P_2 = 6.0 \text{ atm.}$				
t_1 (mins.)	t_2 (mins.)	$\log t_2$	σ	n
5	6.3	0.80	0.08	14
10	5.4	0.73	0.12	13
20	5.8	0.76	0.09	15
$PCO_2 = 0.027 \text{ atm. } P_1 = 2.7 \text{ atm. } P_2 = 5.4 \text{ atm.}$				
t_1	t_2	$\log t_2$	σ	n
10	8.5	0.93	0.12	14
20	7.5	0.88	0.13	15
40	6.5	0.81	0.18	15

Table 11				
Summation of partial exposures with carbon dioxide				
$P_1 \log (t_1 + t_2) + (P_2 - P_1) \log t_2 = K$				
$y_1 + y_2 = K$				
$PCO_2 = 0.03 \text{ atm. } P_1 = 3.0 \text{ atm. } P_2 = 6.0 \text{ atm.}$				
t_1	t_2	y_1	y_2	K
5	6.3	3.15	2.40	5.6
10	5.4	3.57	2.19	5.8
20	5.8	4.23	2.28	6.5
$PCO_2 = 0.027 \text{ atm. } P_1 = 2.7 \text{ atm. } P_2 = 5.4 \text{ atm.}$				
t_1	t_2	y_1	y_2	K
10	8.5	3.42	2.52	5.9
20	7.5	3.88	2.36	6.2
40	6.5	4.50	2.20	6.7

CHAPTER 6. NITROUS OXIDE

The earliest observations of the physiological interaction between raised pressures of oxygen and gaseous anaesthetics were made by Bert (1878) who showed that convulsions in animals might be abolished by aether or chloroform anaesthesia. The experiments which follow were made in order to see whether sub-anaesthetic doses of nitrous oxide had any detectable effect upon oxygen convulsions and if so, to attempt to quantify this effect.

METHOD

Groups of three mice were exposed in the medium pressure chamber in the presence of soda-lime, to a mixture of N_2O and oxygen. The five different treatments detailed below were combined in a 5 x 5 Latin square and the results subjected to an analysis of variance.

Treatment	N_2O	PO_2	Total Pressure
A	0.07	7.0	7.07
B	0.14	7.0	7.14
C	0.27	7.0	7.27
D	0.54	7.0	7.54
E	--	7.0	7.0

Table 12
The effect of N₂O upon oxygen convulsions

Source of Variation	Sum of squares (S.O.S.)	Degrees of freedom (D.F.)	Mean square (M.S.)	Variance ratio (F)
Rows	0.0491	4	0.0123	1.8
Columns	0.0254	4	0.0063	-
Treatments	0.0729	4	0.0182	3.3 *
Residual	0.0867	12	0.0072	
Total 0.2341		24		
Detailed Comparison				
Control E. v. D.	0.0881	1	0.0881	12.2 **
* Significant at 5% level ** Significant at 1% level				

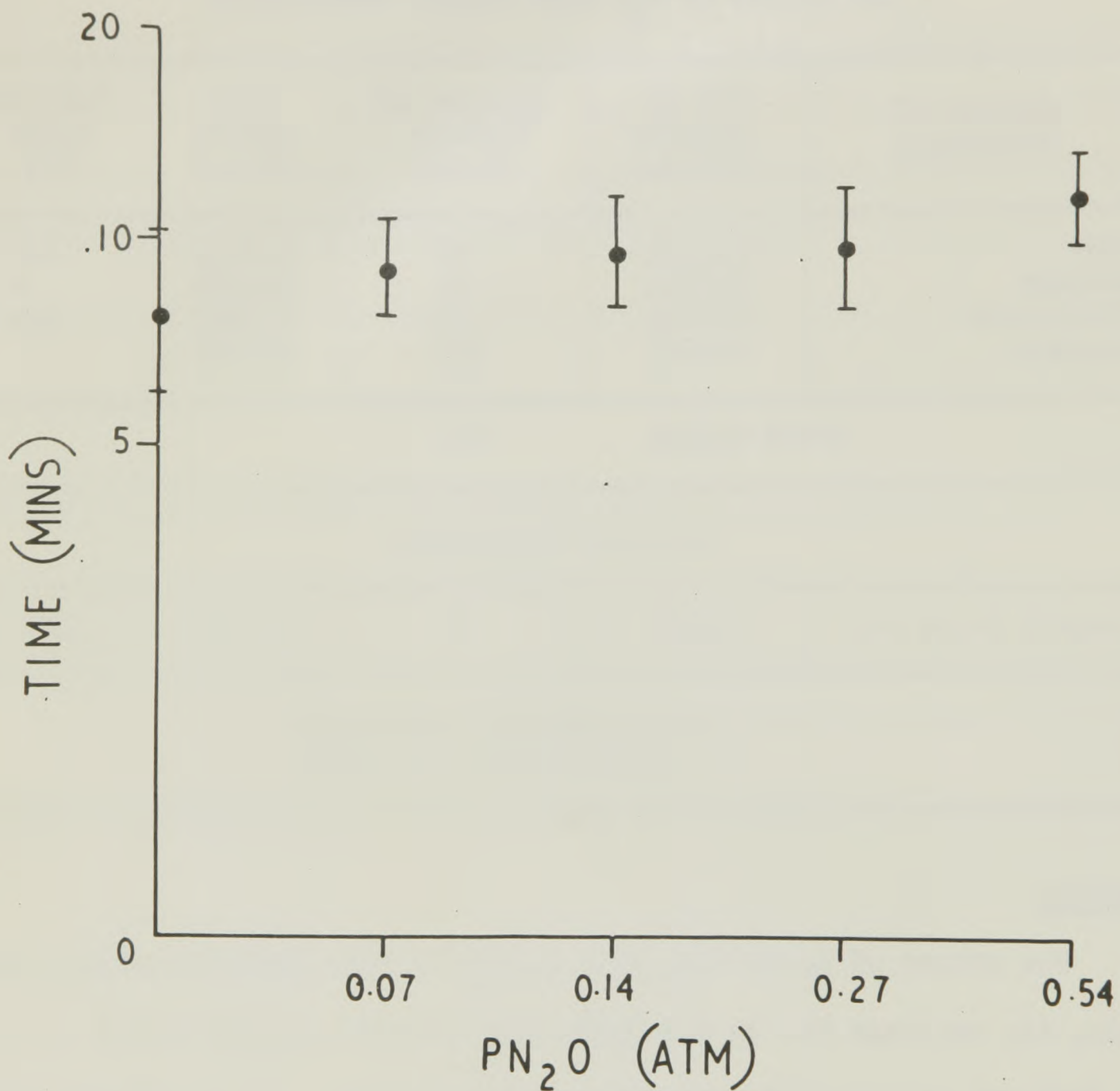
RESULTS

The effect of up to 0.54 atm. of N₂O is thus just detectable, however, the variance due to the difference between rows is almost as large as that due to the difference between treatments. It is thought that this was due to having used animals from three different batches.

Upon detailed comparison, the only N₂O level which showed a significant difference from the control level was the highest, i.e. 0.54 atm., although as can be seen in Figure 10 the trend is for all the convulsion times to be longer in the presence of Nitrous oxide.

The level of N₂O which gives significant protection against

FIGURE 10



Convulsion times with Nitrous Oxide: The mean convulsion times obtained with 7.0 atm. of Oxygen and four different levels of Nitrous Oxide are shown together with their standard deviations σ .

convulsions is one at which hyperactivity was shown by nearly all the animals. It is a level which represents about one-third of the anaesthetic pressure as judged by the loss of the righting reflex. Miller, Paton and Smith (1967).

PROTECTIVE ACTION

A number of exposures was made to investigate the form which this protective action of Nitrous oxide might take.

Method

Exposures were planned in a similar manner to those previously described using four different oxygen pressures spanning the range from 6.4 to 10 atm. Animals were exposed in pairs to the following treatments.

Treatment	N_2O	PO_2	Total Pressure
A	0.54	8.5	9.04
B	--	7.2	7.2
C	0.54	7.2	7.74
D	--	10.0	10.0
E	0.54	6.4	6.94
F	--	6.4	6.4
G	--	8.5	8.5
H	0.54	10.0	10.54

Results

Due to the large number of animals which showed collapse, chronic convulsions or simply absence of major convulsions it was not possible to analyse this data as fully as had been hoped. However, each type of treatment has been characterised by its mean and standard deviation as shown in Table 13.

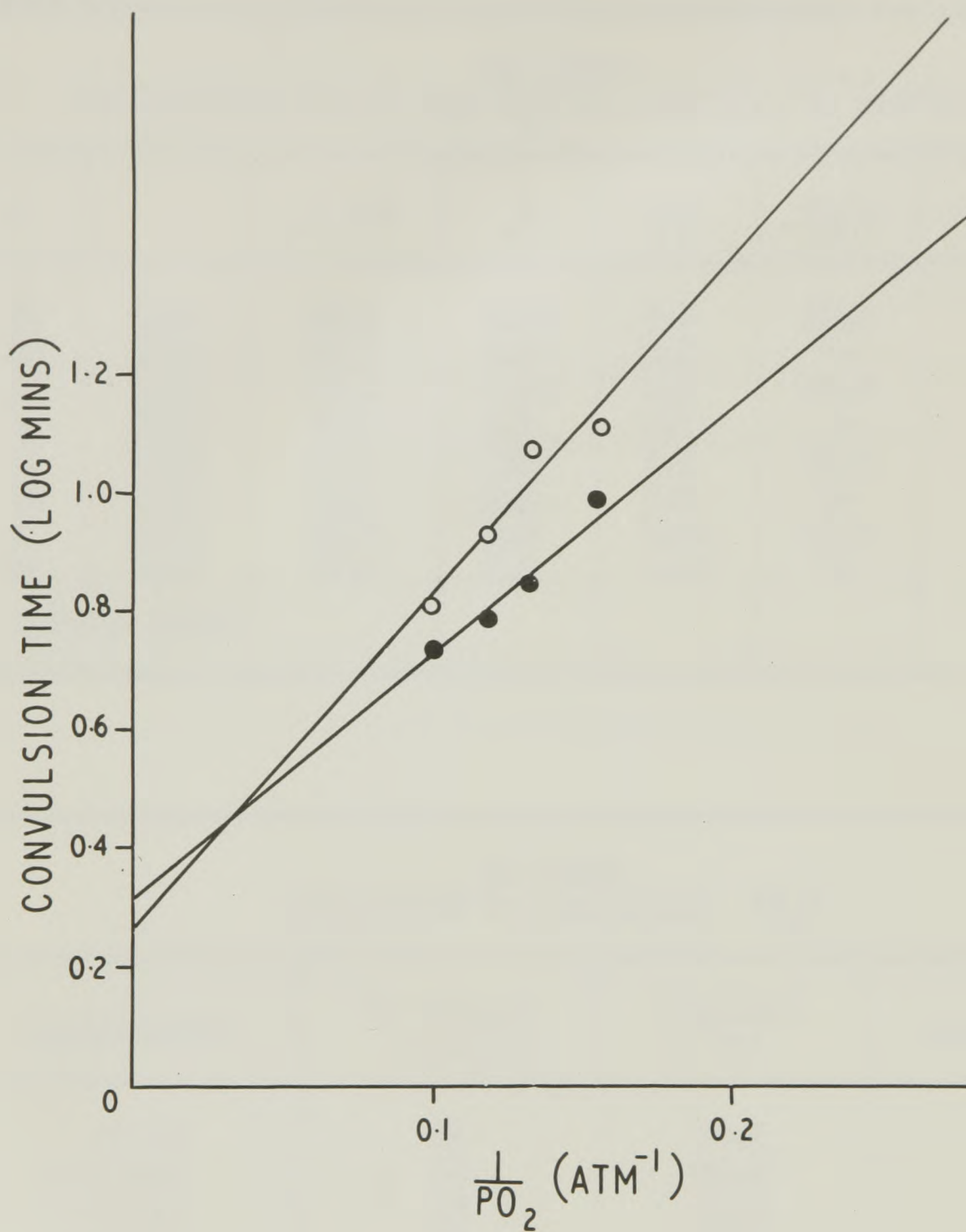
Table 13
The effect of 0.54 atm. of H₂O upon oxygen convulsions

Treatment	PH ₂ O	PO ₂	t _o	log t _o	σ	n
E	0.54	6.4	13.1	1.12	0.07	11
F	—	6.4	9.6	0.98	0.14	16
C	0.54	7.2	12.0	1.08	0.11	11
B	—	7.2	7.1	0.85	0.11	16
A	0.54	8.5	8.5	0.93	0.08	11
G	—	8.5	6.2	0.79	0.09	16
H	0.54	10.0	6.5	0.81	0.03	5
D	—	10.0	5.5	0.74	0.10	14
Total						100/128

Table 14
H₂O: Comparison of Treatments

Comparison	Students 't'	Degrees of freedom	Probability P
E v F	3.00	25	1% **
C v B	5.18	25	0.1% ***
A v G	4.11	25	0.1% ***
H v D	1.58	17	20% Not Significant

FIGURE II



Dose-response curve in the presence of 0.54 atm. of Nitrous Oxide: The control figures are shown as solid circles, while the open circles represent the effect of adding 0.54 atm. of N₂O.

It can be seen from Table 13 that about one-third of the animals failed to reach a clear end-point at the lower pressures, when Nitrous oxide was present. At 10 atm. of oxygen however two-thirds of the animals failed to reach an end-point.

CONCLUSIONS

1. The presence of 0.54 atm. of Nitrous oxide in the inspired oxygen delays the onset of convulsions.
2. The way in which the dose-response curve for oxygen is modified is by an increase in the slope (vid. Figure 11). The ratio of the slopes of the two regression lines illustrated is $\frac{5.66}{4.14} = 1.37$.

CHAPTER 7. NITROGEN

In the course of studying dives using a mixture of oxygen and nitrogen Lanphier (1955) noticed an apparent sensitisation to oxygen convulsions.

Since the reason for this sensitisation is not known the following experiment was designed to test whether it was possible to detect both a shortening of the convulsion time, such as is produced by carbon dioxide; and since Nitrogen at pressure is narcotic, a protective effect similar to that shown by Nitrous oxide.

METHOD

Pairs of mice were exposed in the high-pressure chamber to 7 atm. of oxygen both in the presence and the absence of Nitrogen. The Nitrogen pressures chosen were 2 atm., comparable with that used by Lanphier, and 15 atm., that is about half the anaesthetic pressure for mice. Miller Paton and Smith (1967). This study was combined with an investigation into the effect of the rate of compression upon convulsion time, compression either being in one minute (fast) or in five minutes (slow). The four treatments plus a control were arranged in a 5 x 5 Latin square.

RESULTS

As can be seen from Table 15 few animals gave a clear end-point at 22 atm., the five animals out of twenty which convulsed did so in a shorter time than the control animals, however, the remainder presumably failed to convulse due to the presence of the Nitrogen, thus giving strong qualitative evidence for the protective effect of 15 atm. of Nitrogen.

No significant effect attributable to the rate of compression could be demonstrated, the results for treatments A and B were therefore pooled.

There was a significant difference between Treatments (A + B) and the control value C the value given by Students 't' test being 2.554, which just exceeds a probability of 0.02 for 18 degrees of freedom.

Table 15
The Effects of Nitrogen upon oxygen convulsions

Treatment	N ₂ (atm.)	PO ₂ (atm.)	Convulsion time (t _c)	log t _c	σ	n	Rate of compression
A	2	7	12.1	1.08	0.1	6	Fast
B	2	7	13.6	1.13	0.1	6	Slow
C	-	7	16.6	1.22	0.1	8	Fast
D	15	7	11.1	1.05	0.1	3	Fast
E	15	7	11.2	1.05	-	2	Slow
						Total	25/50

CONCLUSIONS

1. There was a significant shortening of the convulsion time in the presence of 2 atm_s. of Nitrogen.
2. There was a marked fall in the number of animals which convulsed in the presence of 15 atm_s. of Nitrogen.
3. No differences could be detected which could be ascribed to the rate of compression.

CHAPTER 8. HELIUM

Mixtures of oxygen and helium are of importance in Deep Diving, since apart from hydrogen, which has been little used, helium is the only practicable diluent for the essential oxygen. During the course of some simulated dives made in 1964 which formed part of the Deep Diving programme of the Royal Navy, the author observed some unusual signs in several subjects who were exposed to helium pressures in excess of 18 atm.

HELIUM TREMOR

These signs took the form of intense tremors of all voluntary muscles; they were associated with inco-ordination of gait and other movements. Compression to depth was made at a rate of some 3-4 atm./minute and the tremor was fully developed by about one minute after arrival at depth. The subsequent course was surprising in that a gradual improvement occurred during exposure at depth, the diver appearing normal after thirty minutes to one hour. The oxygen pressure throughout such an exposure was not greater than 1 atm.

Although the observations did not correspond with the signs of oxygen toxicity, they were thought to show that there might be some interaction between helium and oxygen.

HELIUM POTENTIATION

Initially, pairs of animals were exposed in the high pressure chamber to a mixture of 7 atm. of oxygen with 34 atm. of Helium, or to a denser mixture of 7 atm. of oxygen with 6.8 atm. of Nitrogen. The result of this was quite clear, the eighteen animals exposed to the Helium-oxygen mixture convulsed in 4.6 minutes while the eight animals exposed to the

oxy-Nitrogen mixture convulsed in 11.2 minutes. The former convulsion time was significantly shorter than the time for 7 atm. of oxygen given by the dose-response curve of 9.2 minutes; while the convulsion time with the mixture of oxygen and nitrogen showed no significant difference. It had been thought possible that at 41 atm. total pressure the density of the breathing medium might be sufficient to cause a significant alteration in the alveolar PCO_2 and that such an alteration could have the effect of shortening the convulsion time. For this reason the control exposures were made with nitrogen at a level which does not cause narcosis but which would give a mixture of greater density than the mixture of oxygen and Helium.

Since no shortening of convulsion time was found with the oxy-nitrogen mixture, while such a shortening was found with the oxy-helium mixture it was concluded that the major part of the effect observed was due either to the presence of Helium or to pressure per se.

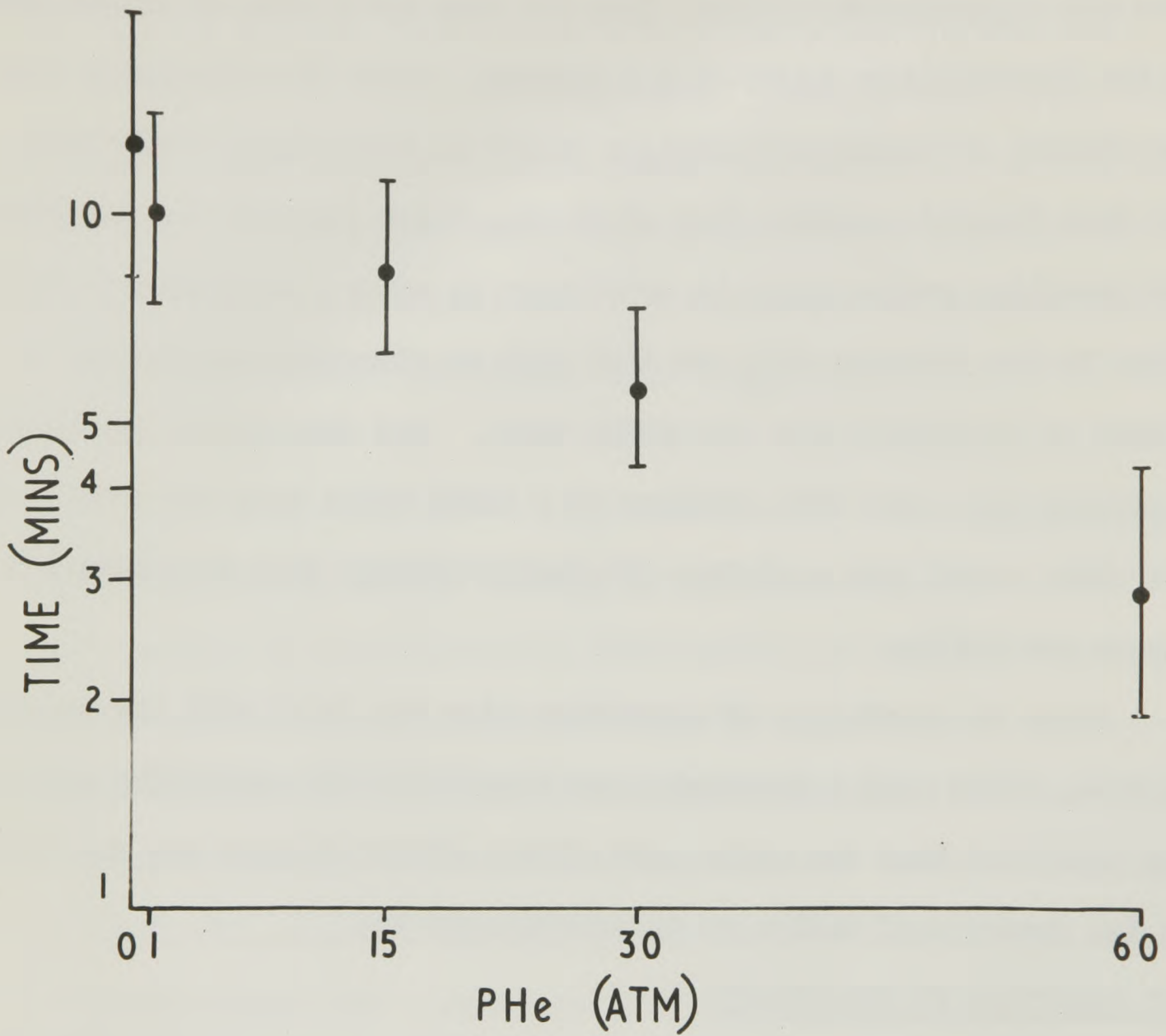
THE MAGNITUDE OF THE EFFECT

A further investigation of the effects of Helium was made using a 5 x 5 Latin square design, in which pairs of mice were exposed, in the high-pressure chamber, either to 7 atm. of oxygen (control), or to 7 atm. of oxygen with either 1, 15, 30 or 60 atm. of Helium.

The results are shown in Table 16.

Of the treatments given, only that with one atmosphere of Helium failed to show a significant difference from the control; the remaining three were all different from each other and from the control value at the 0.1% level of significance.

FIGURE 12



The effect of Helium upon the convulsion-time:
The mean convulsion times t_c and their standard deviations σ are shown for exposures to mixtures of 7.0 atm. of Oxygen and four different levels of Helium.

Treatment		Convulsion Time		σ	n
PO ₂ (atm.)	P He (atm.)	t _c (mins.)	log t _c		
7	1	10.1	1.00	0.13	19
7	15	8.3	0.92	0.13	17
7	30	5.6	0.75	0.11	18
7	60	2.8	0.44	0.18	16
7	-	12.7	1.11	0.19	18

It was also found that the convulsion time in the presence of 60 atm. of Helium, viz. 2.8 minutes, was shorter than was ever found using high pressures of oxygen alone.

The decrease in convulsion time was found to be approximately linear with pressure as shown in Figure 12.

HELIUM AT HIGH PRESSURES

A number of attempts was made to see whether Helium could be used to provoke convulsions at oxygen pressures below the threshold of about 3 atm. Two main complicating factors prevented a clear answer being obtained at high pressures. The first of these was the very rapid temperature rise during compression, due to adiabatic heating. The simplest way to have controlled this might have been to have slowed the rate of compression. However, this would have made the assessment of the severity of the exposure and the comparison of different exposures more difficult. The second complication was the occurrence of hyperactivity and convulsions or tremor, similar to that seen in man. These

signs seemed to be absent below about 45 atm. while above this pressure they became more common and more intense.

A few clear results were, however, obtained:

1. Two animals exposed to 1 atm. of O_2 and 68 atm. of Helium did not convulse during a 30 minute exposure.

2. Two animals exposed to 60 atm. of Helium with 2 atm. of oxygen did not convulse during 40 minutes.

3. Two animals exposed to 60 atm. of Helium with 3 atm. of oxygen did not convulse during 40 minutes, and finally

4. Two animals exposed to 60 atm. of Helium and 4 atm. of oxygen developed minor and chronic but not major convulsions.

The tentative conclusion reached was that Helium potentiated the action of oxygen in producing convulsions, but did not itself produce convulsions when the level of oxygen was below the threshold.

The nature of the disturbances produced by compression is not clear but rapid temperature changes may be a factor in their production.

THE OXY-HELIUM DOSE-RESPONSE CURVE

The next series of experiments were designed to show the way in which the convulsion time depended upon both the PO_2 and the P_{He} to see in effect whether the dose-response curve for oxy-helium was different from the dose-response curve for oxygen with respect to slope or to intercept. Animals were, therefore, exposed in pairs to two levels of oxygen and two of Helium as indicated in the table of results, Table 17.

(a) 2.5 atm. of O_2 + 25 atm. of Helium followed by the addition of 2 atm. of oxygen, and similarly

Table 17
Oxy-Helium dose-response curve

Treatment		t_c (mins.)	$\log t_c$	σ	n
P_{O_2} (atm.)	P He (atm.)				
5.4	15	21.4	1.33	0.18	8
5.4	30	9.0	0.95	0.14	8
7.2	15	9.2	0.96	0.13	8
7.2	30	6.6	0.82	0.09	8

Since virtually the same convulsion time was produced either by 5.4 atm. O_2 + 30 atm. of Helium, or by 7.2 atm. O_2 + 15 atm. of Helium; it is clear that Helium alters the slope of the oxygen dose-response curve, having a greater effect at the lower oxygen pressure than at the higher oxygen pressure.

DIVIDED EXPOSURES

A final series of exposures was made, using divided exposures in which pairs of animals were exposed to a mixture of oxygen and Helium at a Pressure P_1 for a time t_1 ; more oxygen was then introduced until the Pressure P_2 was reached and the animals then observed until major convulsions occurred.

The three types of exposure were as follows:

(a) 2 atm. of O_2 + 15 atm. Helium for either 7.5, 15 or 30 minutes, followed by the addition of 5 atm. of O_2 to a total pressure of 7 atm. oxygen + 15 atm. of Helium.

(b) 2.5 atm. of O_2 + 15 atm. of Helium; followed by the addition of 5 atm. of oxygen, and similarly

(c) 3.0 atm. O_2 + 15 atm. Helium, followed by the addition of 5 atm. of oxygen.

The results of these experiments are presented in Table 18.

CONCLUSIONS

The conclusions to be drawn from these exposures are quite straightforward since there is no significant difference between any of the values of t_2 . This means that over the range of pressure studied the addition of 15 atm. of Helium did not markedly alter the provoked threshold which in previous experiments was found to be in the region of 3 atm. of oxygen.

To summarise the conclusions from all the experiments upon the interactions of oxygen and Helium:

1. Helium has a marked potentiating effect upon oxygen convulsions.
2. This effect has been shown at total pressures of 20.4 atm. and greater.
3. The presence of Helium alters the slope of the dose-response curve for oxygen convulsions.
4. The change in the logarithm of the convulsion time is approximately in inverse proportion to the pressure of Helium added.
5. Helium does not seem to alter the threshold to oxygen convulsions.
6. No specific physiological effects which could be attributed to the action of Helium were seen during these experiments; the foregoing conclusions are, therefore, equally valid for the effects of pressure per se.

Table 18 Divided exposures with oxy-helium mixtures 2 atm. to 7 atm. O ₂ plus 15 atm. He				
t ₁ (mins.)	t ₂ (mins.)	log t ₂	σ	n
7.5	7.3	0.86	0.35	13
15	8.8	0.94	0.13	13
30	8.7	0.94	0.08	12
2.5 atm. to 7 atm. O ₂ + 15 atm. He				
t ₁ (mins.)	t ₂ (mins.)	log t ₂	σ	n
7.5	7.5	0.88	0.12	15
15	8.6	0.94	0.10	13
30	9.7	0.99	0.16	14
3.0 atm. to 7 atm. O ₂ + 15 atm. He				
t ₁ (mins.)	t ₂ (mins.)	log t ₂	σ	n
7.5	7.2	0.86	0.13	12
15	6.0	0.78	0.17	15
30	6.2	0.79	0.22	16

CHAPTER 9. THE NATURE OF THE CONVULSIVE PROCESS

EVIDENCE FROM THE DOSE-RESPONSE CURVE

Empirical equations have been published by a number of authors which describe the dose-response relationship between the ambient oxygen pressure and the end-point being investigated. These are summarised in Table 19.

It is clear that most of these equations may be written in the form:

$$P^n t = \text{constant.}$$

This is the general equation for a rectangular hyperbola which describes an inverse relationship between the ambient PO_2 and the time taken for signs of toxicity to appear at this pressure. Such a relationship gives a straight line when plotted on log./log. co-ordinates and the present data for major convulsions in mice have been analysed in this way to give the empirical equation:

$$\log (t_c - 3.5) = 4.3 - 4.0 \log P \dots\dots\dots (i)$$

where t_c = major convulsion time in minutes

P = ambient PO_2 in atm.

A similar equation describes the incidence of minor convulsions:

$$\log (t_m - 3.5) = 3.2 - 3.3 \log P \dots\dots\dots (ii)$$

where t_m = minor convulsion time in minutes

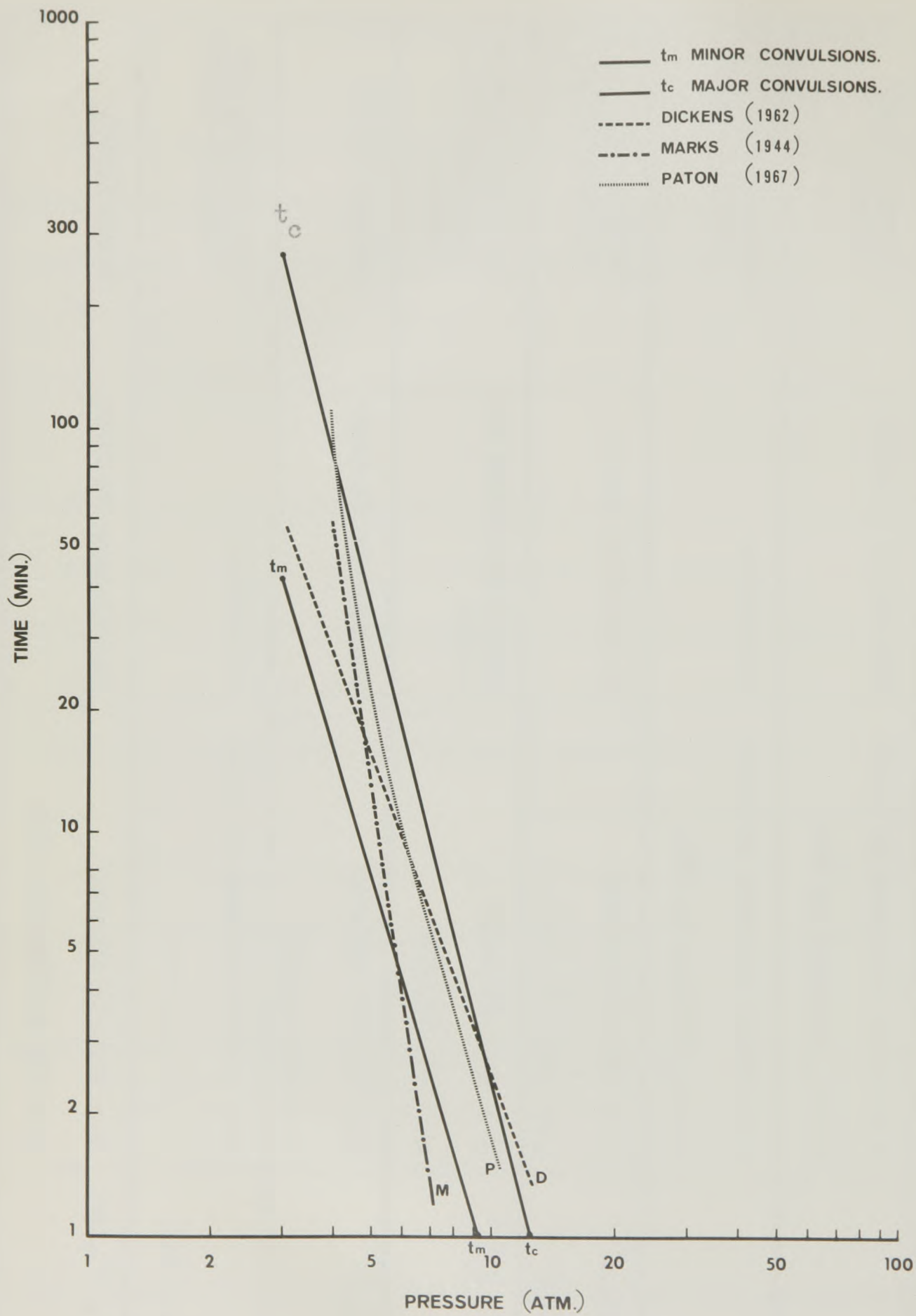
P = ambient PO_2 in atm.

The data summarised by equation (i) above are in close agreement with that presented by Paton (1967), while as has been previously mentioned, there is close agreement between the data for minor convulsions and that presented by Marshall and Lambertsen (1961). The equation

Table 19
Empirical dose-response equations

Empirical Equation	Units	Species	Author
$y = 1.73 x^{-3.8}$ (p 3.8 t = 54)	x = lethal exposure in hours y = O ₂ pressure in atm.	Intestinal protozoa	Cleveland L.R. (1925)
$y = 2.08 x^{-1.81}$ (p 1.8 t = 120)	x = lethal exposure in hours y = O ₂ tension in atm.	Drosophila	Williams and Becher (1944)
$\log t = 2.368 - 0.0152^d$ ((a - 39.7) t = 1086)	t = time in minutes d = depth in feet	Convulsions in man	Haldane and Spurway (1944)
$\log t = 3.78 - 2.73 \log P$ (p 2.73 t = 102)	t = time in hours p = PO ₂ in atm.	Lethal exposures in mice	Gerschman Gilbert and Caccamise (1958)
$\log t = 3 - 2.6 \log P$ (p 2.6 t = 1000)	t = time in minutes p = atm. oxygen	Convulsions in mice	Dickens (1955)
$p^2 t = 126$ or $(PO_2 - 0.21)^{2.2} t = 102$	t = lethal exposure in hours p = PO ₂ in atm.	Drosophila	Fenn Hemming and Philpott (1967)
$(p - 30)^2 (t - 3) = 20,000$	t = time in minutes p = PO ₂ in lbs./sq. ins.	Convulsions in mice	Paton (1967)

FIG. 13



Dose-response relationships for mice: Empirical relationships derived by other authors are compared with the equations derived for Major convulsions t_c and Minor convulsions t_m .

given by Dickens (1962) and the data of Marks (1944) are intermediate in slope and position between equations (1) and (11) above (Figure 13).

Such a description of the data however ignores two of the major factors observed, the apparent threshold to convulsions and the minimum response time, for unless suitable asymptotes are chosen extrapolation in one direction would predict convulsions at atmospheric pressure while in the other would predict instant convulsions at high oxygen pressures.

Pressure/rate relationship

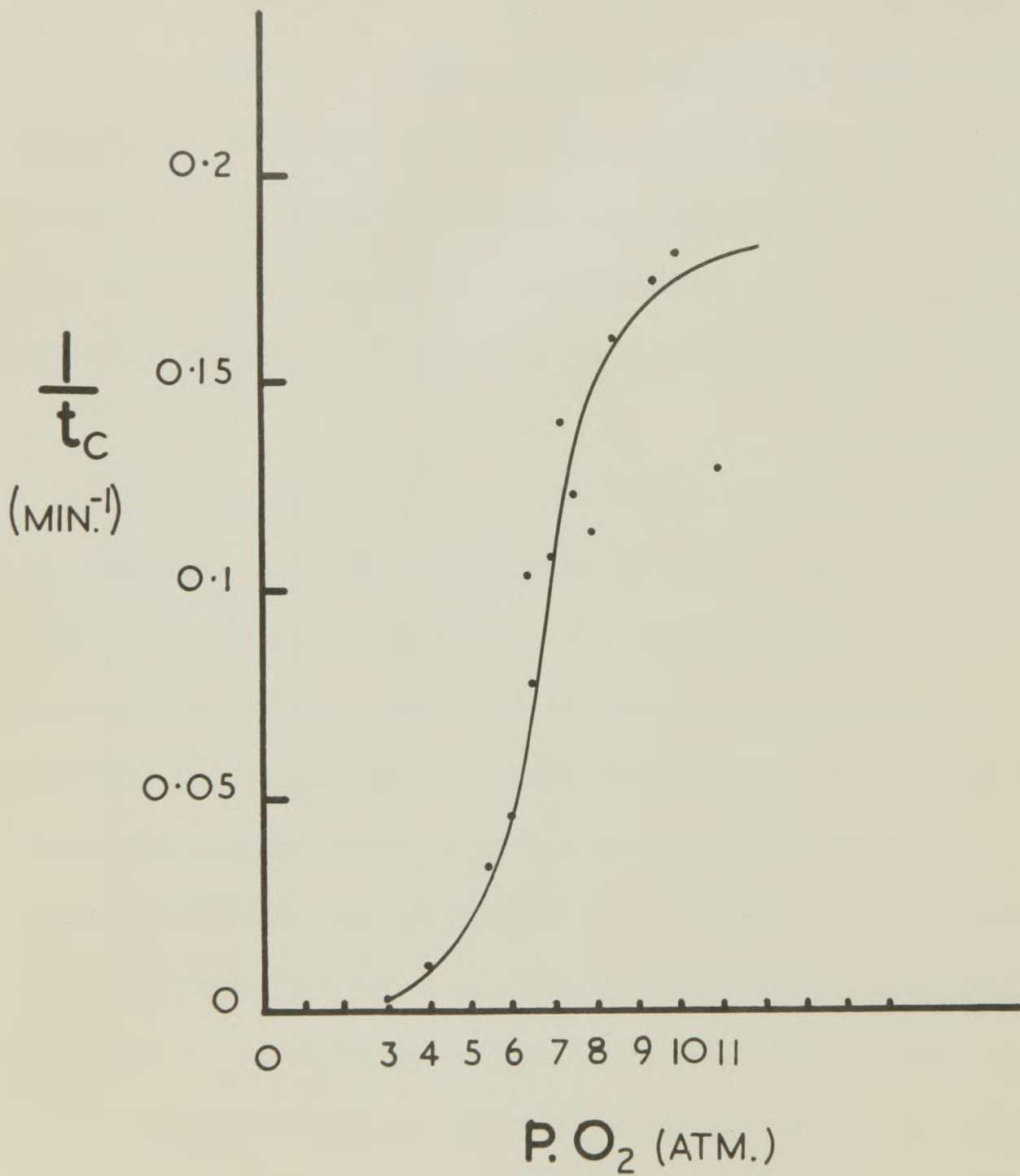
In an attempt to overcome these objections the following interpretation was considered. If it is assumed that the dose-response curve results from a process which leads to convulsions and further that the shape of the curve represents the way in which the rate of this process is altered by changes in ambient PO_2 ; then one may regard the convulsion time seen as a measure of this rate and interpret the minimum response time as resulting from an increase in the rate to some limiting value.

It is convenient to plot the rate of such a process as a fraction of the limiting rate against the concentration of substrate. In the present case, assuming that the convulsion time t_c is inversely related to the rate of the process, then the fractional rate may be represented by the ratio $\frac{t_{\text{minimum}}}{t_c}$.

Since no accurate estimate is available for the minimum response time the general form of the relationship may be seen if $\frac{1}{t_c}$ is plotted against the PO_2 (Figure 14).

The sigmoid curve given by this method of plotting the data shows an approximately linear increase in $\frac{1}{t_c}$ for an increase in PO_2 from 4 atm.

FIG. 14



Pressure-Rate Relationship: The Oxygen Dose-Response curve (Figure 6) is shown re-plotted as a Pressure-rate relationship, as is described in the text (Chapter 9).

to 10 atm. Each end shows a pronounced curvature which by extrapolation might be expected to be asymptotic to $\frac{1}{t_c} = 0$ at low oxygen pressures and to $\frac{1}{t_c} = \text{maximum}$ at high oxygen pressures. The experiments which have been described show quite clearly that such extrapolation is unreliable since no effects could be detected below the threshold pressure of 3 atm., while at high pressures there was an obliteration of the end-point, the animals collapsing without convulsing.

Pressure/log. time relationship

Oxygen uptake

The empirical equations used to describe the present data are of the general form $P \log t = K$ (vid. Figure 7), the precise equations used being:

- (i) $\log t_c = -0.1 + 8.1 \left(\frac{1}{P}\right)$ for major convulsions, and
- (ii) $\log t_m = +0.1 + 5.1 \left(\frac{1}{P}\right)$ for minor convulsions.

In each case the first term on the right-hand side of the equation represents a time, less than one minute, which has been assumed to include a lag due to the finite time of compression; and also the time necessary for the tissue oxygen levels to reach a new equilibrium following a step-change in ambient oxygen levels. Since compression itself usually occupied some 50-60 seconds, most of the lag time may be accounted for as being due to the impossibility of a truly instantaneous compression, however, if any of the lag is to be accounted for as being due to oxygen-uptake time then this time is less than one minute.

Extrapolation

Extrapolation, in either direction, of the relationship

$\log t_0 = -0.1 + 8.1 \left(\frac{1}{P}\right)$ beyond the values used in deriving it leads to the same dilemma as with other attempts to describe the dose-response data. Where the line cuts the ordinate at -0.1 for major convulsions, the minimum response time must be assumed to be zero, if as has just been discussed this constant is taken to represent the compression plus uptake time. On the other hand, extrapolation towards lower pressures gives no indication of a threshold pressure which would be represented by a maximum convulsion time. It is, therefore, clear that all the descriptions of the dose-response curve so far used are inadequate, since they do not satisfactorily account for two of the three aspects of the relationship, viz. the threshold and the minimum response time, while all may be used, with different degrees of confidence, to describe the curvature of the relationship.

It is suggested that the most probable explanation for this inadequacy is that other separate factors, not so far taken into account, are responsible for the appearance of a threshold and for the minimum response time.

The Threshold

Rather unexpectedly perhaps the present studies have shown that exposure of mice to pressures below the 'threshold pressure' had no detectable effect upon subsequent exposures at a higher pressure.

A summary of these results is given in Table 20 overleaf.

These efforts to define a provoked threshold for mice give a value extremely close to an ambient pressure of 3 atmospheres of oxygen. It can be seen that convulsions were never provoked at initial pressures of less than 3 atm., regardless of what other gas was present, and that

Table 20
The Provoked Threshold

Gas mixture breathed	Pressure of first exposure (atm.)	Effect upon Convulsions
O ₂	1.0	-
O ₂	2.0	-
O ₂	3.0	+
O ₂	4.0	+
O ₂ + CO ₂	2.7	-
O ₂ + CO ₂	3.0	-
O ₂ + He	2.0	-
O ₂ + He	2.5	-
O ₂ + He	3.0	-

provocation was absent at 3 atm. in the presence of either Carbon Dioxide or Helium. This does not disagree with the findings of Matteo and Nahas (1965) that mice which were first exposed to 3 atm. or 4 atm. of oxygen, for times shorter than those leading to convulsions, subsequently convulsed in significant numbers during a second exposure. It is also consistent with the observation of Marshall and Lambertsen (1961) that animals did not convulse at a PO₂ of 3 atm. of oxygen alone, but did convulse at that pressure in the presence of Carbon Dioxide; if one takes into account the difference in exposure times. In the restricted time of 90 minutes Marshall and Lambertsen found the threshold for mice to be 4 atm. while the present work has found a threshold of 3 atmospheres after an exposure of some 5 to 6 hours. It should be noted that there is no evidence that either Carbon Dioxide or Helium lower the provoked threshold.

The Minimum Effective Pressure

In spite of the failure to show sub-liminal effects it is quite likely that some occur, since other signs of oxygen toxicity undoubtedly occur at lower pressures, for example, pulmonary damage. The term threshold is thus not particularly apt and it is suggested that it might be preferable to talk of a minimum effective pressure, or E.P.50 defined as that ambient oxygen pressure at which one half of the population will convulse following an indefinitely long exposure.

Tissue Oxygen Levels

No justification has so far been given for equating ambient oxygen pressure with the convulsant dose; for while this is both usual and convenient it implies a relationship between ambient oxygen pressure and oxygen tension at the critical site which is in fact not known. The assumption used in the present work has been that this relationship is linear, since simple physico-chemical considerations would lead one to expect a linear increase in the concentration of oxygen in the tissues with increasing pressure.

Measurements of various oxygen levels available in the literature are summarised in Table 21, from which it can be seen that these figures are inadequate to test the nature of any relationship. The initial difficulty is the identification of the critical site at which changes in oxygen tension will give rise to signs of toxicity. Since there is considerable evidence, Chance et al (1965) Haugaard (1968) that the development of convulsions is associated with derangement of tissue glycolysis or energy transfer, it is perhaps not too fanciful to place this critical site within the cell at the surface of the mitochondria, Lehninger (1964) Lemberg (1969).

Table 21
Measurements of oxygen tension

PO_2 Ambient (atm.)	PO_2 Arterial mm. Hg.	PO_2 Venous mm. Hg.	PO_2 Other	Species	Source
1	500	65	-	Man	McDowall (1965)
1	-	72	-	Man	McDowall (1965)
1	520	61	-	Dog	Harper (1965)
1	-	54	-	Man	Wollman (1964)
1	515	-	175 mm. Hg. (C.S.F.)	Dog	Schoemaker (1965)
2	1106	125	-	Man	McDowall
2	1165	240	-	Man (post endarterectomy)	McDowall
2	1100	72	-	Dog	Harper
2	-	150	-	Man	McDowall
3	1355	-	631 mm. Hg. (C.S.F.)	Dog (37°C)	Schoemaker
3	1550	-	722 mm. Hg. (C.S.F.)	Dog (hypothermia) (23°C)	Schoemaker
3.5	ca. 2000	100	-	Man	Lambertsen (1953)

The usual assumption that the tissues are in equilibrium with the venous blood is, in such a situation, misleading, since the mitochondria may be regarded as "oxygen sinks". There is thus an oxygen gradient from the cell-membrane, which is assumed to be in equilibrium with the venous blood, to the mitochondria; which will be at some lower oxygen tension. In a discussion of various experimental estimates of the PO_2 gradient within tissues Forster (1965) quotes values for the oxygen gradient of some 15-30 mm. Hg. and calculates that the data of Chance Cohen Jobsis and Schoener (1962) give a value for living rat cerebral cortex of 15 mm. Hg. If these results may properly be applied to the oxygen levels responsible for convulsions, the most satisfactory guide to tissue oxygen levels will be the venous PO_2 which will be greater than the oxygen tension at the critical site by some 15 to 30 mm. Hg.

An estimate of the level at which toxicity occurs may be made from the following observations:

(a) Venous PO_2 levels at 1 atm. of oxygen range from about 50 to 70 mm. Hg. vid. Table 21.

(b) Lambertsen et al (1953) found that convulsions might occur at 3.5 atm. of oxygen when the jugular mixed venous blood showed an oxygen tension of less than 100 mm. Hg.

It may therefore be inferred that the oxygen tension which will produce convulsions in man is represented by a venous PO_2 of between 70 and 100 mm. Hg. This is probably equivalent to a tension at the critical site some 15 to 30 mm. Hg. less, that is of the order of 40 to 85 mm. Hg.

The Threshold and O_2 Consumption

In exposures to raised oxygen pressures, in spite of the fact that the

arterial PO_2 may be many times the normal level, the quantity of oxygen per unit volume of blood is only increased by the gas which dissolves in the plasma since the Haemoglobin is fully saturated Bert (1878) Gesell (1923). Boerema Meijne Brummelkamp Bouma Mensch Kamerlings Stern Hanf and Van Aalderen (1960) have shown that at about 3 atm of oxygen the quantity dissolved becomes sufficient to meet the metabolic requirements of the tissues; while at lower pressures the venous PO_2 is little different from normal (Lambertsen et al (1953)), above 3 atm. the amount of dissolved oxygen is greater than the amount needed by the tissues and the venous PO_2 is raised. It is therefore suggested that the apparent threshold to convulsions depends to a great extent upon the fact that the tissue oxygen tension can only rise when the quantity of oxygen available exceeds the quantity being consumed. According to this view the rate of oxygen consumption will largely determine the threshold to toxicity, inherent differences in oxygen consumption between individuals or species may thus partly explain the different degrees of tolerance to oxygen which are observed.

While such a view may have the merit of simplicity it should be noted that such figures as are available show raised venous oxygen tensions even at 2 atm. ambient pressure.

This situation might perhaps be explained by alterations in perfusion of the tissue, for instance by the opening of shunt pathways so that oxygenated blood by-passes the tissues without losing all of its oxygen. However, it is clear from the polarographic studies of Bean (1961), using dogs and albino rats, that while he found considerable local variations

in the oxygen level in different parts of the brain, all were above normal levels at an ambient oxygen pressure of 5 atm. Indeed, Bean concluded from these findings that the level of oxygen in the jugular venous blood was not a reliable index of the variations in oxygen level observed in the brain.

The Process to Convulsions

It was concluded in Chapter 4, as a result of the summation of partial exposures that the process to convulsions might be considered to be approximately exponential. The reasons for this view are presented below:

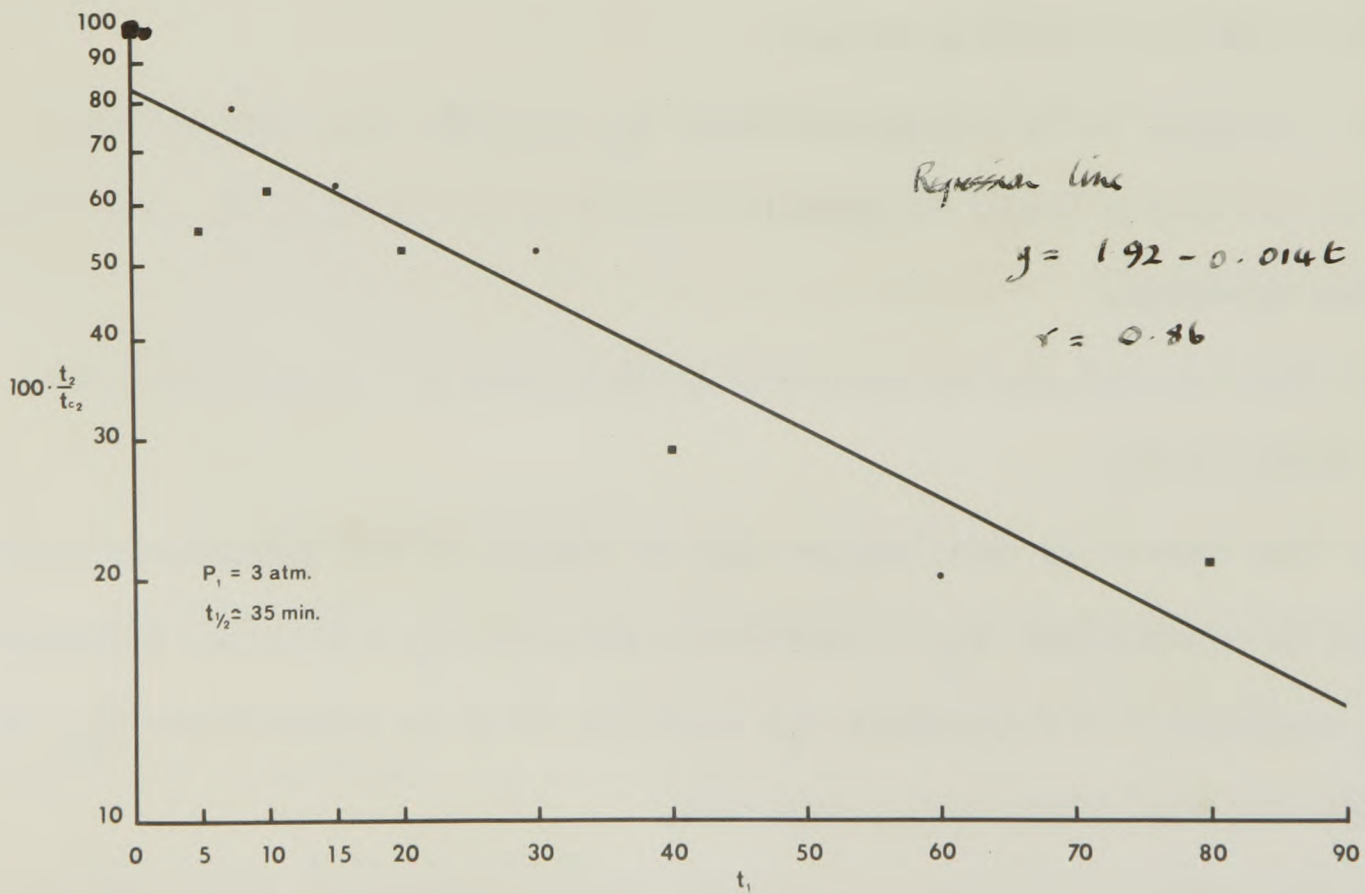
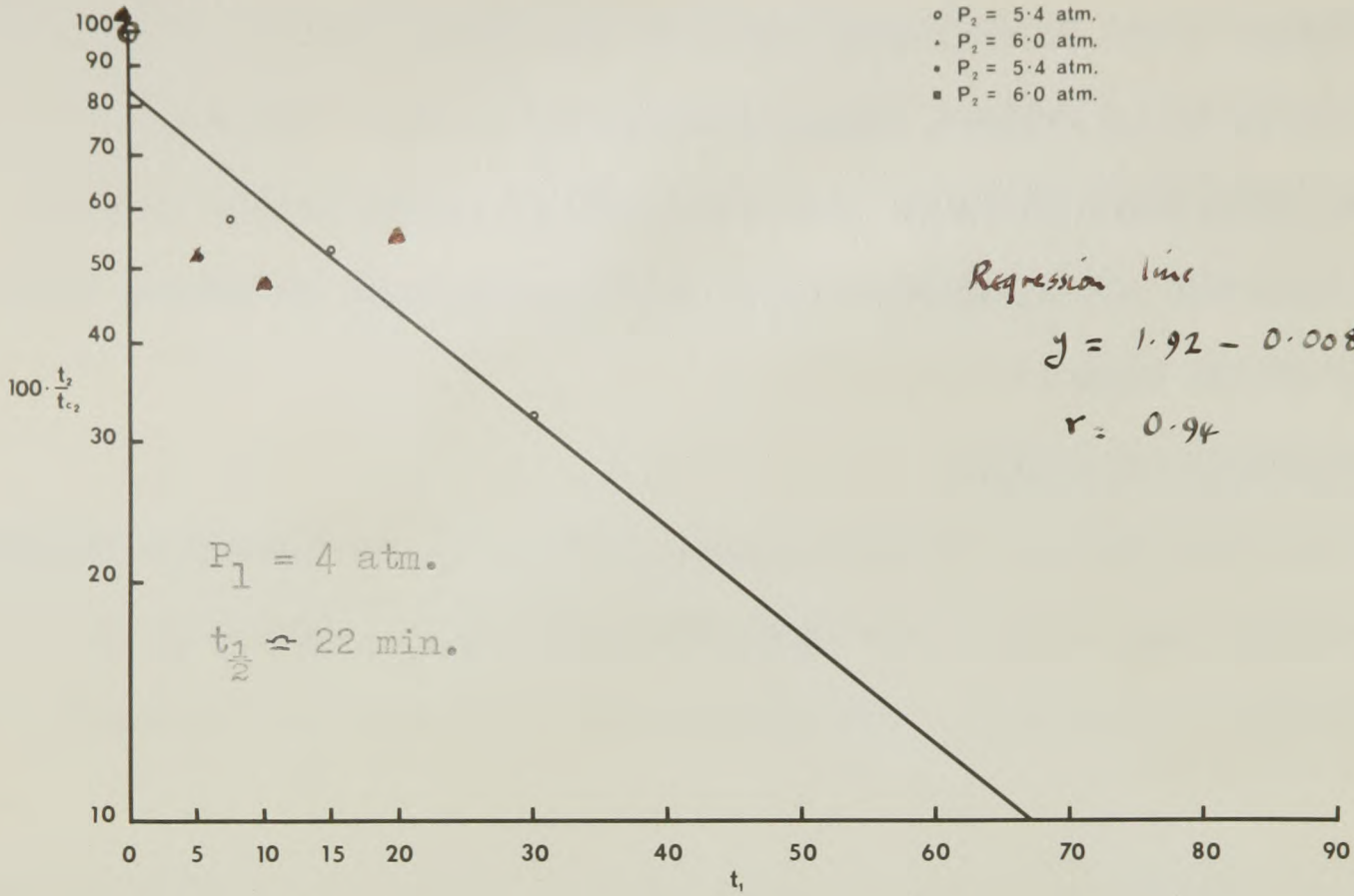
It is assumed that there is some process leading to convulsions which is defined in the following manner

1. Exposure to an oxygen pressure P_1 , greater than the minimum effective pressure, leads to changes which can be detected at some other effective pressure.
2. This change may be measured by titration to convulsions at a second pressure P_2 .
3. The amount of this change may be judged by the difference between the time to convulsions t_2 , measured at pressure P_2 , following exposure for t_1 minutes at the pressure P_1 ; and the time to convulsions t_{c2} at P_2 in the absence of any prior exposure.
4. An increase in the time of the first exposure t_1 will lead to a decrease in the observed time t_2 , since partial exposures have been shown to be additive (Chapter 4).
5. The manner in which t_2 will decline towards zero from an initial value of t_{c2} (for the pressure P_2) may therefore be taken as a reflection

FIG. 15

KEY

- $P_2 = 5.4$ atm.
- $P_2 = 6.0$ atm.
- ◐ $P_2 = 5.4$ atm.
- ◑ $P_2 = 6.0$ atm.



The Process to Convulsions: The Partial exposures contained in Table 5 have been analysed semi-logarithmically to give estimates of the rate of the convulsive process at 3.0 and 4.0 atm.

of the way in which the effect of the exposure t_1 at pressure P_1 increases with a lengthening of the time t_1 (this effect will increase from zero towards a maximum represented by the time t_{o1} at which convulsions would occur at the initial pressure P_1).

Inspection of the values of t_2 in Table 5 shows a decrease with increasing values of t_1 , the change being most marked initially. In order to compare values from different exposures these were converted to a standard form by expressing each time t_2 as a percentage of the convulsion time t_{o2} for the particular pressure at which t_2 was being measured a semi-logarithmic plot of $100 \left(\frac{t_2}{t_{o2}} \right)$ against t_1 is shown in Figure 15 from which it may be seen that the half-time for the process at 3 atm. is about 35 minutes, while at 4 atm. it is about 22 minutes.

Since a linear relationship exists between the logarithm of $(t_1 + t_2)$ and the logarithm of t_2 , it is clear that the graph of t_1 against the logarithm of t_2 can only be approximately linear, but it may give a satisfactory estimate of the initial rate of the process.

Recovery

As was shown by Donald (1947) in man and by a number of authors for animals Bean (1945), recovery from oxygen convulsions occurs in spite of the fact that in isolated enzyme systems the reactions may be found to be reversible, irreversible or partially reversible Haugaard (1968).

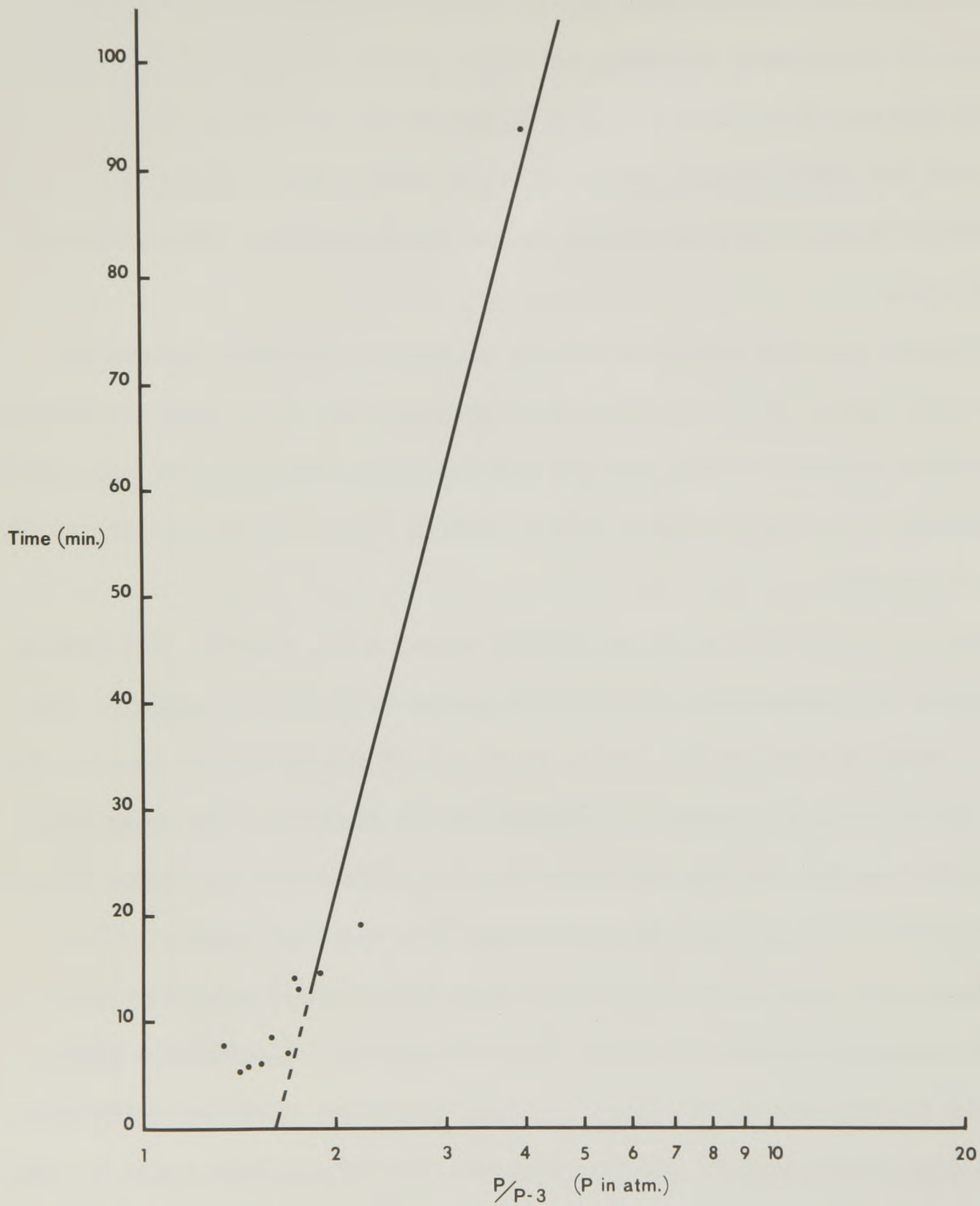
Permanent injury may however result from exposure to oxygen as was shown by Bean and Siegfried (1945) who produced neurological damage in rats by repeated exposures. These findings have been confirmed by Van Den Brenk and Jamieson (1964) and characteristic lesions in animals showing oxygen induced paralyses were described by Balentine and Gutsche (1966).

Two types of lesions were found: Type A consisting of the focal necrosis of individual neurones, and Type B where there was complete or partial necrosis of nuclear groups with damage to the myelin sheaths, the axons and the glial cells. Both types of lesion were usually symmetrical and bilateral and were most common in the basal ganglia, brain stem and spinal cord.

Delayed recovery from the effects of oxygen has been reported by Matteo and Nahas (1965) who found in exposures with mice, that the effect of a single exposure, which was not sufficient to cause convulsions, could be detected as a sensitisation to any further exposure for as long as six days afterwards.

Kaufman Owen and Lambertsen (1956) compared the effects of exposing two groups of guinea-pigs, the control group, to 3 atm. of oxygen; the second group to alternating oxygen pressures of 3 atm. for 30 minutes followed by 0.2 atm. of oxygen in Nitrogen for 10 minutes. The mean time to convulsions for the control group was 5.2 hours while for those animals with intermittent exposure the convulsion time was 17.0 hours. This experiment was interpreted as showing that "the rate of recovery from oxygen poisoning exceeds the rate of development". (Lambertsen 1955). Implicit in this statement however is the assumption that the rates concerned are linear, and if this is true the rate of recovery would be some 1.8 times the rate of development. Since evidence has previously been presented to show that the process to convulsions is non-linear, the rate of recovery cannot exceed the rate of development. Although it is not known whether the recovery process is or is not linear; if non-linear and exponential, a rate of recovery some two-thirds of the rate of development of convulsions could account for the convulsion time observed.

FIG. 16



Simultaneous Recovery: The Convulsion Time is shown plotted against $P/P-3$ where P is the ambient oxygen pressure in atmospheres.

Data obtained from *Drosophila* were analysed by Fenn Philpott Meehan and Henning (1967) with the intention of testing whether or not simultaneous recovery might be taking place during exposure. The method used was a modification of that suggested by Blair (1932) for the degree of excitability of tissue under the influence of an applied voltage, taking into account a simultaneous recovery process. In the case suggested by Blair the equation simplified to:

$$\log \frac{V}{V-R} = kt$$

where R = rheobase
V = applied voltage
t = time

In the form used by Fenn et al the rheobase was equated with the 'threshold' for *Drosophila* which was taken as 0.21 atm. and the equation became:

$$\log \left(\frac{PO_2}{(PO_2 - 0.21)} \right) = kt$$

A plot of $\log \left(\frac{PO_2}{(PO_2 - 0.21)} \right)$ against the time to the end-point was used to test the fit of the data to this equation. The results were thought to be inconsistent with the view that simultaneous recovery occurred in *Drosophila*.

A similar plot of $\log \frac{PO_2}{(PO_2 - 3)}$ against convulsion time for mice Figure 16 shows a curvature above about 7 atm. oxygen pressure. However, the original derivation of the equation by Blair assumed that the effect 'p' was directly proportional to the applied voltage 'V'. The rate of recovery was taken as being proportional to the magnitude of the effect 'p'. In the present case, the effect at higher pressures was not directly proportional to the ambient pressure but seemed to approach a

limiting value as is suggested by Figure 14. One would therefore expect a plot of the logarithm of $\frac{P_{O_2}}{(P_{O_2} - 3)}$ against time to be linear at low pressure, but to deviate progressively from linearity at higher pressures. It can be seen from Figure 16 that the findings are consistent with this view.

The next important step in the investigation is to determine the effect of the initial concentration of the gas on the rate of reaction. This is done by plotting the logarithm of the concentration of the gas against the inverse of the initial concentration.

This has been represented diagrammatically in Figure 17.

In general, it may be seen that an interaction with another gas would modify the characteristics of the reaction. This is particularly true in the case of the reaction between hydrogen and oxygen. The rate of reaction is affected by the presence of a third gas, and this is particularly true in the case of the reaction between hydrogen and oxygen. The rate of reaction is affected by the presence of a third gas, and this is particularly true in the case of the reaction between hydrogen and oxygen.

The slope of the line being constant is of course the constant in the equation $\log \frac{P}{P_0} = kt$. The slope of the line being constant is of course the constant in the equation $\log \frac{P}{P_0} = kt$. The slope of the line being constant is of course the constant in the equation $\log \frac{P}{P_0} = kt$.

EVIDENCE FROM INTERACTIONS

General

It has been concluded from the experiments described in earlier chapters that the convulsion time following exposure to oxygen at raised pressures may be modified by the presence of other gases. Prior exposure to oxygen and exposure to oxygen in the presence of added carbon dioxide or of Helium all accelerate the onset of convulsions, while Nitrous oxide delays convulsions and Nitrogen appears slightly to accelerate the onset at low pressures and to delay it at higher pressures. The most convenient summary of these actions is obtained by plotting the logarithm of the convulsion time for the various gases to be considered against the inverse of the ambient oxygen pressure.

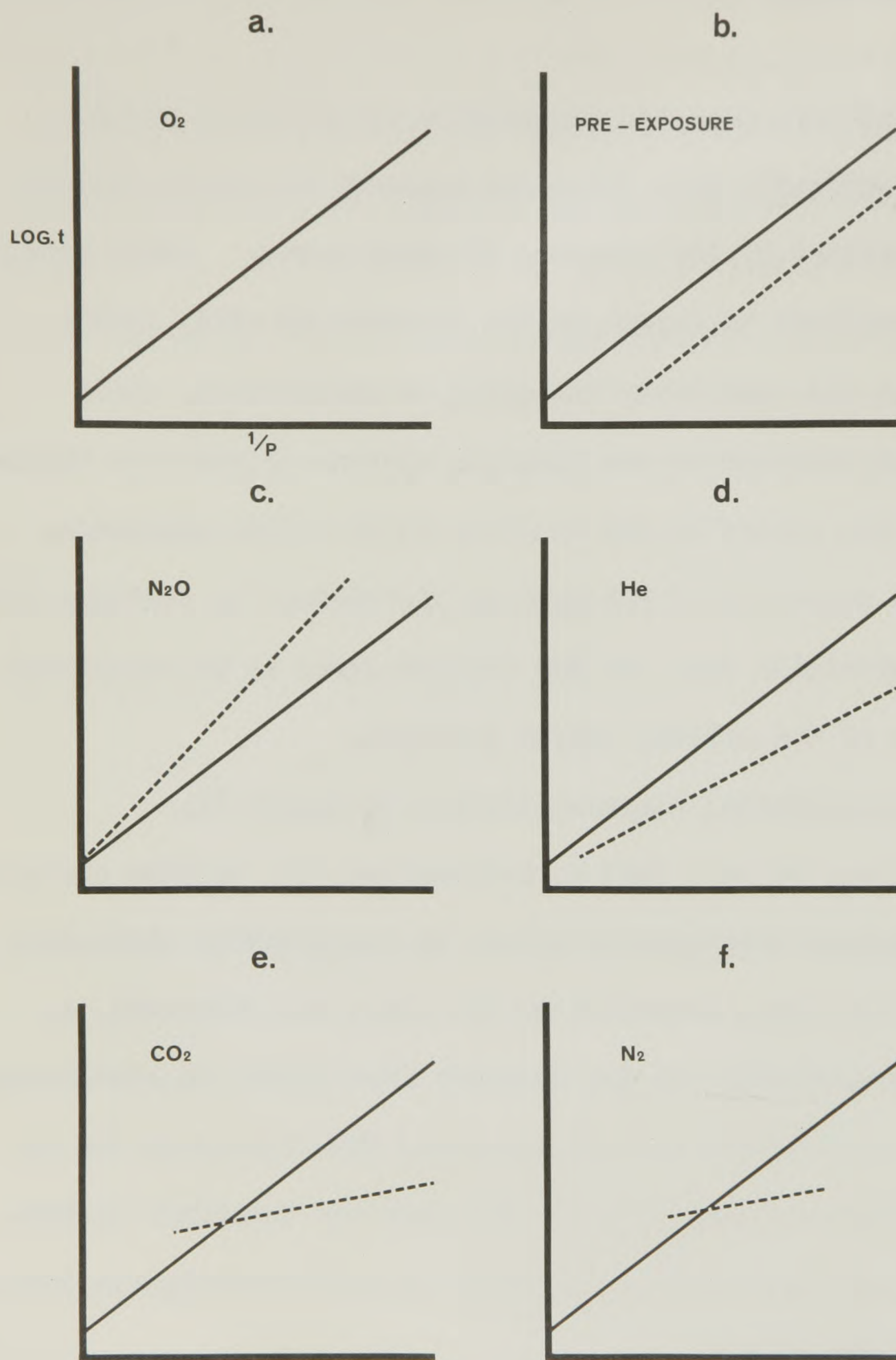
This has been represented diagrammatically in Figure 17.

In general, it may be seen that an interaction with another gas could modify the dose-response relationship either by quantitative alteration in the position of the line, described by its slope and intercept, or qualitatively by an alteration in the response from linear to non-linear. There is, however, no evidence for any departure from linearity due to the interactions being discussed and all the data may therefore be described in terms of the alteration in position of the dose-response line.

The Significance of the Slope

The slope of the line being considered is of course the constant in the equation $P \log t = K$. Any alteration in this slope therefore describes a change in the relationship between the convulsion time and the ambient oxygen pressure. A proportional change in the convulsion time as a result of an interaction would lead to a parallel displacement

FIG. 17



Diagrammatic Summary of the interactions between oxygen and other gases. In each case the solid line represents the oxygen dose response curve, the interrupted line the dose-response in the presence of the interacting gas.

of the dose-response line such as is seen in Figure 17 (b); however, a proportional change in an exponential rate, such as has been suggested for the process to convulsions, would lead to an alteration in the slope of the dose-response line. Thus it is suggested that the most likely explanation for such a change in slope is that it results from the influence of the interacting gas upon the rate of the process to convulsions. In the case of Helium, Figure 17 (d), the reduction in slope seems to be approximately proportional to the pressure of Helium present. The evidence for Nitrous oxide, Figure 17 (c) is less conclusive, but points to a similar relationship. In the cases of carbon dioxide and Nitrogen, not only is there an alteration in the slope but also in the intercept, that is the minimum response time is considerably delayed. This is to be expected if the effect of anaesthetic or narcotic gases is to slow the rate of the convulsive process, since even the fastest rate will of course be retarded and the minimum response time prolonged. Whilst such an effect may also result from exposure to Nitrous oxide, it was not detected with the pressures used.

Sites of Action

Surgical attempts to define a site from which convulsions may be said to originate have been made by Bean and Rottschaefer (1938) Gersh and Pfeiffer (1944) and Mantegazzini (1954). The work of the earlier authors supported the view that convulsions were non-specific in origin while that of Mantegazzini suggested that oxygen convulsions might originate sub-cortically.

Not only is there therefore some doubt as to the anatomical site of action of oxygen in producing convulsions, but more specifically doubt as

to what cells or parts of cells are susceptible.

It has been assumed that the site of action of oxygen is intracellular, possibly at the mitochondrial surface. Such a view depends upon the assumption that the toxic effects of oxygen result from an interference with normal tissue oxidations. As Haugaard (1968) remarks however, 'The molecular mechanisms involved in the oxidation of labile constituents by oxygen have yet to be understood'.

Although it is perhaps unsatisfactory to implicate interference with some imperfectly understood mechanism, it is suggested that the hypothetical process to convulsions represents the rate-limiting step of a cyclic or chain reaction involved in tissue oxidations and that the kinetics of this process may assist in identifying the critical site or sites. Interactions with this process may represent interference at the same or adjacent sites or, in the case of anaesthetics, possibly at the cell membrane affecting oxygen diffusion into the cell. The evidence presented seems to show that Nitrous oxide and Helium, which alter the slope, behave differently from Carbon dioxide and Nitrogen, which alter both the slope and the intercept.

Modes of Action

Carbon Dioxide

As has already been mentioned, there is evidence that carbon dioxide may enhance the toxic effects of oxygen in *Drosophila*, Williams and Beecher (1944). The main effect in mammals, however, seems to be a result of vasodilatation which leads to higher tissue oxygen levels, Lambertsen et al (1953) (1955). The finding that Carbon dioxide levels which produced significant effects at 5.4 atm. of oxygen had no detectable effect

at 7.0 atm. of oxygen implies that such a view is an over-simplification. If it is thought that convulsions occur earlier due to vasodilatation, some point will be reached at which the vascular effects are maximal; the result at 7 atm. therefore suggests that vasodilatation is maximal at this pressure in the absence of Carbon dioxide and hence that vasodilatation occurs as a result of increasing oxygen pressure. It may be that the increased density of the respired gas leads to a rise in endogenous Carbon dioxide levels due to increased respiratory work and poor mixing of gas in the lungs.

Nitrous Oxide

Having concluded from the experimental findings that Nitrous oxide slows the rate of the process to convulsions, it is a matter for speculation as to how and where this interaction could take place, since the site of action of anaesthetics is also uncertain. Paton and Speden (1965).

Nitrogen

The effect of Nitrogen is so similar to that of Carbon dioxide that it is tempting to blame gas density, leading to Carbon dioxide retention, for the acceleration of convulsions seen with 2 atm. of Nitrogen. There is, however, little evidence for this, indeed Rashbass (1955) showed that normal alveolar Carbon dioxide levels could be maintained in men breathing air at 10 atm., although whether this would be true for mice at 9 atm. is not known.

Helium

The possibility that the addition of Helium acts only by increasing the pressure must be considered. An acceleration of convulsions by Helium was observed by Bennett (1967) in rats; a finding that he

attributed to carbon dioxide retention. However, Miller et al (1967) showed effects in Triturus at equivalent pressures of Helium, Neon or hydrostatic pressure which suggested that convulsions seen at similar high pressures of Helium might be a function of pressure rather than of the gas breathed. Fenn (1967) has reported some experiments in which 41 atm. hydrostatic pressure inhibited the growth of *Streptococcus faecalis* to the same degree as 41 atm. of Helium. There is thus evidence to support the view that the effect observed with Helium is due to pressure per se, which as discussed by Fenn may perhaps act by preventing the volume increase resulting from glycolysis. The effect upon oxygen convulsions is detectable at a total pressure of 20.4 atm., which suggests that the effects of pressure upon other physiological processes might be detected at similar pressures. The equivalent depth in the sea is about 200-250 metres, approximately the depth at which the author has observed tremor in men and about half the pressure at which tremor was seen in mice.

Conclusions

The toxic effects of oxygen at raised pressures which result in convulsions in mice may be described in the following way:

1. When exposed to an ambient oxygen pressure above some minimum effective pressure (threshold) there is an increasing tendency for animals to convulse which may be described by a hypothetical process towards convulsions.
2. This process follows an approximately exponential course with time; the rate of the process being proportional to the ambient oxygen pressure.

3. The apparent threshold may be due in part to the limiting effect of oxygen consumption upon the tissue PO_2 but a process of recovery may also be involved. The rate of the recovery process may be thought of as being independent of pressure but proportional to the concentration of some hypothetical product of the convulsive process.

4. The relationship between the convulsive and the recovery processes is such that at pressures below the minimum effective pressure the critical concentration of product necessary to initiate convulsions is never reached. At the minimum effective pressure the rate of production is equal to the rate of removal of product, while above this pressure the rate of production exceeds the rate of removal.

5. The rate of the convulsive process increases towards some limiting value with increasing oxygen pressures and it may be speculated that the minimum response time may therefore be due to progressive inactivation of one or more enzymes by oxygen at pressure.

6. The interactions studied lead to the view that the convulsive process may be accelerated or retarded by other gases and that there are two principal modes of interaction, namely:

(a) Interference with cellular functions by the anaesthetic gases,

(b) The effect of pressure upon the physical state or reactivity of enzymes or reactants, leading to disruption of normal processes: and possibly a third consequent upon changes in intracellular pH.

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