Br. J. Pharmac. Chemother. (1967), **29**, 350–366.

EXPERIMENTS ON THE CONVULSANT AND ANAESTHETIC EFFECTS OF OXYGEN

BY

W. D. M. PATON

From the Department of Pharmacology, University of Oxford

(*Received December* 5, 1966)

Poisonous effects of oxygen at high pressures are of two types: an action on the central nervous system, causing convulsions and other signs, and an action on the pulmonary alveoli, causing irritative changes and effusion (Lorrain Smith, 1899). Neither of these actions is properly understood. The experiments described below were primarily designed to study the convulsant action of oxygen by analysing the effect on it of various drugs known to have central effects, so as to define to some extent what type of convulsant activity oxygen exerts.

Marks (1944) has already initiated work of this type. He began by showing that in a group of animals the logarithm of the convulsion time was normally distributed in all the species he tested; it was thus possible to express his results in terms of the 50% convulsion time, obtained by interpolation of cumulative response curves plotted on probit-log paper, and of its standard error. Using this index of sensitivity he found considerable species variation, in the order of descending sensitivity: kittens, mice, adult cats, rabbits, rats and guinea-pigs; man was on the average slightly more sensitive than the mouse but also showed considerable individual variation. Of drugs active on the central nervous system which Marks tested (chloralose, urethane, barbitone, phenobarbitone, dilantin, bromide, atropine, metrazol, picrotoxin, strychnine, benzedrine, methedrine, caffeine and hyoscine) only the barbiturates and bromide had appreciable anti-convulsant effect, whereas dilantin was inactive and picrotoxin and hyoscine had some accelerant action. Marks also observed that certain inorganic ions (nickel, cobalt and zinc) had some anti-convulsant effect.

METHODS

For most of the experiments mice were used, not of homogeneous origin, weighing 20–30 g. They were compressed in groups of six or eight in a small compression chamber provided with Perspex windows so that each animal could be seen at all times. Each animal was distinctively marked so that individual convulsion times could be determined. They were compressed to pressures between 30 and 300 lb/sq in, taking about 1¼ min to reach the final pressure and being exposed to this for periods up to 40 min. Most of the experiments were done at 65 or 75 lb. In every experiment, except where specially noted, only half the animals were injected; the others served as controls, so that individual differences in technique of compression and between batches of mice were compensated. Control animals were not injected; the fact that several of the drugs injected had no effect on convulsion times indicates that the injection procedure was without effect also. Some carbon dioxide accumulation occurred under these conditions, at a rate of about 0.7% of an atmosphere in 10 min. This was disregarded, however, since both test and control animals were equally exposed to it. Before compression, after the animals had been placed in the chamber,

it was flushed through with oxygen for a minute so that there was less than 2% nitrogen in the atmosphere in which the animals were exposed. All pressures quoted are gauge pressures, in excess of atmospheric pressure.

In some experiments rats were used and in this case one or two rats at a time were compressed in a similar way.

Injections were made either subcutaneously into the skin of the back or intraperitoneally, normally in volumes of 0.1 or 0.2 ml. saline.

Statistical analysis

The nature of the experimental results—that is, cumulative curves of convulsion times in groups of mice—calls for probit analysis. Full statistical analysis of this kind seemed inappropriate, however, since the experiments were exploratory and many of the cumulative curves were incomplete. When it was found, in agreement with Marks (1944), that the logarithm of the convulsion time is normally distributed, all the results were plotted on logarithmic probability paper and the 50% convulsion time (CT_{50}) estimated by eye. Figure 1 shows the results of such a plot for two sets of mice and one set of rats. The reciprocal slope of the line (λ) for 74 mice of batches 1 and 2, expressed as $\log_{10}$ convulsion time/probit, is 0.378 (equivalent to a ratio of convulsion times of 2.39 between 31% and 69% response). This is very close to Marks's figure of 0.375. If it is assumed that this slope is constant, and an average probit weight of 0.5 is assigned to each observation, then the standard error of the estimate of the logarithm of the CT_{50} is 0.152. This implies that, provided slope does not change, a prolongation (or shortening) of CT_{50} by a factor of two or more for a group of 12 animals would be significant. This has been adopted as a working criterion. Since with some mice—for example, batch 3—the slope was steeper, this

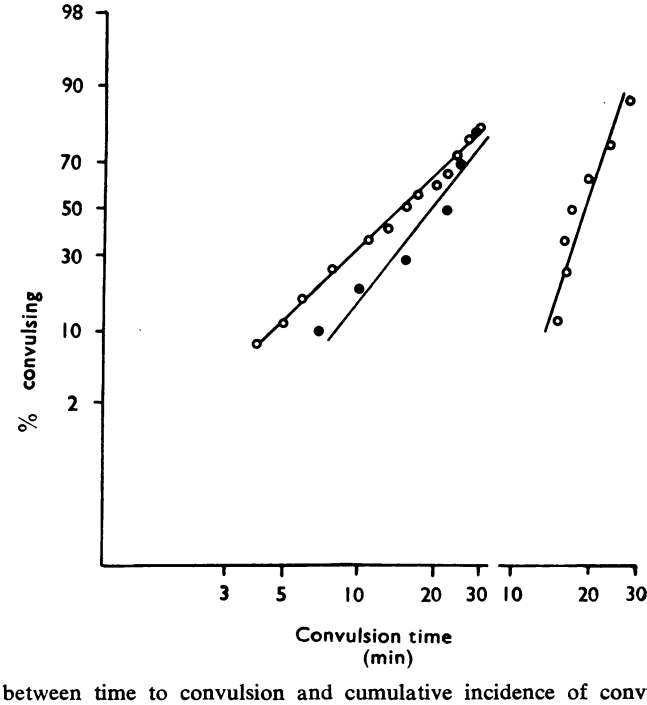

Fig. 1. Relation between time to convulsion and cumulative incidence of convulsions in two sets of mice and in rats exposed to 65 p.s.i.g. oxygen. Ordinate: % convulsions, scaled by probits. Abscissa: convulsion time (min), logarithmic scale. Curves left to right: ○——○ = mice (batch 1-2 (74)); ●——● = mice (batch 3 (54)); ○——○ = rats (16).

criterion is correspondingly conservative. Paramethadione, trimethadione and mephenesin steepened the slope of the probit/log-time relationship (Fig. 3), and further experiments with these drugs may call for special analysis. Figure 1 also shows results for rats, the reciprocal slope being 0.117, implying, with the same provisos, that a prolongation of CT_{50} by 25% or more would be significant.

Where the probit plot indicated a significant drug effect, this was checked by a test for the significance of the difference between the mean log convulsion times; for this purpose, entries of >30 or >40 in Table 5 were taken as $=30$ or $=40$. Where a difference emerged between control and treated groups, for $P<0.05$, the 50% convulsion times are shown in bold type in Table 5. A similar procedure was used in the variance analysis of Table 6. For the variance analyses of experiments using rats (Tables 1 and 7), convulsion times rather than their logarithms were used, since the distributions did not differ significantly from normality.

Drugs

The drugs used were sodium bromide, diethazine hydrochloride (Diparcol, May & Baker), mephenesin (Myanesin, B.D.H.), trimethadione (Abbott), paramethadione (Abbott), chlorpromazine hydrochloride (May & Baker), cobalt acetate, morphine sulphate, procaine amide, hexamethonium bromide, dimercaprol, and methyl fluoroacetate.

RESULTS

The characteristics of oxygen poisoning in mice

Signs of oxygen poisoning

A wide variety of signs appear when a mouse is exposed to high pressures of oxygen, in addition to obvious convulsions. They include attempting to climb up the sides of the chamber, apparent vertigo with attempts to twist round continuously in one direction or another, vigorous head tremor, apparent retching attempts, backing away, tail erection, and sitting up as though begging like a dog. None of these were regarded as end-points and only two types of end-point were used—that is, either an obvious convulsion or a remarkable kind of violent over-activity in which the mouse moves violently all over the cage, completely out of control and scattering its companions. Sometimes minor incidents of these two types took place followed by recovery, but the end point was not taken unless the convulsions or over-activity persisted.

One gained the impression, particularly from the very striking head tremor which sometimes occurred, that oxygen is capable of activating centres producing semi-automatic but co-ordinated movements. The same suggestion has been made as a result of the study of "lips" as it occurs in humans. This would imply that the effect of oxygen may be somewhere in the basal ganglia or mid-brain rather than the cortex. The tail erection was not uniform but was nevertheless common and slightly reminiscent of morphine poisoning.

Variability of convulsion time

Marks's result, that the logarithm of the convulsion time is distributed normally, has been amply confirmed both in mice and in rats by the simple test of the linearity of a plot of % incidence of convulsions against logarithm of convulsion time on probability paper (Fig. 1). It was also confirmed that rats were in general more resistant than mice. Further, there seemed to be a significant difference in the variability of the two species. Whereas mouse convulsion times can range at 65 lb/sq. in. between 5 and more than 40 min, rat convulsion times are considerably more regular. Significant difference was also found sometimes between batches of mice, even though they did not appear to differ

appreciably in physical characteristics or behaviour. In all experiments with drugs in mice, a control group of untreated animals from the same batch was used.

It is not possible to use individual mice repeatedly, since the death rate after exposure to high oxygen tensions is too high. With the rat, however, if the exposure is terminated soon after the end-point, repeated exposures are possible. Table 1 gives the convulsion

TABLE 1

Convulsion times of rats at 65 p.s.i.g. (min)	
Test 1	Test 2
15	16
16	23
14	12
16	19
24	15
29	18
16	15
15	15
20	17
17	15
17	18
21	19
17	27
28	24
35	31
29	32
Mean 20·6	19·8

	Sum of squares	d.f.	Variance
Between tests	5	1	5
Between animals	954	15	63·6
Residual	207	15	13·8
	1,166	31	

$$\text{Variance ratio } \frac{\text{between animals}}{\text{residual}} = 4{\cdot}61 : P < 0{\cdot}01$$

times for 16 animals, each given two exposures. The accompanying analysis shows that most of the variation in the results is attributable to the individual animal. The results imply that some advantage could be gained, in testing drugs against oxygen poisoning, by using rats with a cross-over procedure; although fewer animals can be used at a time than if mice are employed, the standard deviation of an individual convulsion time can be reduced to around 3–4 min, against 10–15 min for mice.

Relation of convulsion time to oxygen pressure

An attempt was made to establish a dose-response curve for oxygen. Groups of four mice were compressed at pressures between 30 and 200 lb; the 50% convulsion times are shown in Table 2a and Fig. 2. The convulsion time shortens quite rapidly at first, and then abruptly at about 100 lb/sq. in. it ceases to decrease any further and remains at an approximately constant value of 4–5 min. There is a difficulty when assessing convulsion time at the highest pressures, which will be discussed in more detail later; for it was observed that with pressures of 100 lb or more the convulsions became somewhat attenuated and at 200 lb the animals passed from a state of normality through a very brief convulsive episode into prostration. Nevertheless the time at which such convulsive phenomena occurred was virtually the same at all pressures between 100 and 200 lb/sq in. The dose-response relationship can be approximately matched by a hyperbolic equation:

$$(\text{pressure} - 30)^2 \times (\text{time} - 3) = 20{,}000$$

where pressure is in lb/sq. in. gauge and time in min. In rats a similar curve was obtained of almost identical shape but slightly shifted to the right, giving a minimum convulsion time of about 6 min (Table 2b and Fig. 2).

The significance of these dose-response curves will be considered in the discussion.

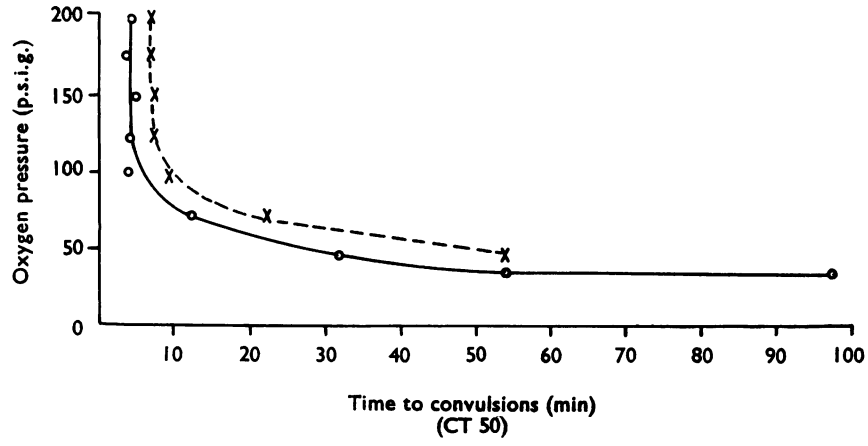

Fig. 2. Relation between oxygen pressure (p.s.i.g.) and 50% convulsion time for mice (○——○) and rats (×——×).

TABLE 2A

MICE

Pressure (p.s.i.g.)	Convulsion times (min)	50% convulsion time (min)
200	4, 4, 4; one mouse prostrated, no convulsion signs	4
175	3, 3½, 3½, 4	3·5
150	4½, 4½, 5½, 6	4·5
125	3½, 3½, 5½, 6	3·5
100	4, 4, 5½, 9	4
75	5, 5, 12, 12, 13, 15, 18, 23	12
50	25, 32, 32, 52	32
40	73, 98, >120, >120	98
30	>120, >120, >120, >120	>120

TABLE 2B

RATS

Pressure (p.s.i.g.)	Convulsion times (min)	Mean convulsion time (min)
200	6, 6½	6¼
175	6, 7½	6¾
150	7, 7½	7¼
125	6½, 8	7¼
100	8, 9½	8¾
75	22, 22	22
50	45, 65	55
40	>120	>120

Lethality of oxygen at high pressure

It is doubtful if the convulsions produced by oxygen are themselves the cause of death in mice and rats exposed to high oxygen tensions. First, death quite frequently occurs in animals which have not convulsed. Secondly, it is known from experience with electrical convulsions that convulsion is not normally fatal unless so severe and continuous as to suspend respiration or to break the vertebral column. Neither of these conditions applies to the animals tested, although the mortality was over 50%. The cause of death was not studied in detail but in a few animals examined, post mortem, there was obvious severe pulmonary haemorrhage and lesions corresponding to the Lorrain Smith (1899) effect obtained at sub-convulsive tensions. These pulmonary lesions may well have been the determining factor.

The mortality clearly depended on the duration of exposure to oxygen although for most experiments only two exposure times were used, 30 and 40 min (Table 3).

TABLE 3
RELATIONSHIP OF DEATH RATE TO EXPOSURE TIME (MICE)

Pressure, 65 p.s.i.g.	30 min	40 min
Survived	32	6
Died	48	32
Death rate	60%	84%

It has occasionally been suggested that oxygen convulsions may represent a protective mechanism against the toxicity of oxygen. If this implies that for equal exposure times, animals which convulse early will have a lower death rate than those who do not, it is certainly not true. On the contrary, the earlier the convulsion time the higher the mortality (Table 4). Thus, even if they are not primarily lethal, oxygen convulsions must be regarded as at least a sign of oxygen toxicity and possibly as actually contributing to the lethal effect. An appearance of a protective action might be produced if, as soon as the convulsion took place, the exposure to oxygen was terminated; for it has already been shown that the mortality is less if the total time in oxygen is diminished.

TABLE 4
RELATIONSHIP OF DEATH RATE TO TIME OF ONSET OF CONVULSIONS (MICE)

Pressure, 65 p.s.i.g. Exposure time, 30 min

	Time to first convulsion (min)			
	0–10	10–20	20–30	>30
Survived	7	4	12	13
Died	16	20	9	4
Death rate	70%	83%	43%	23%

The drugs which were found to delay convulsion did not appear to depress mortality rate any more than could be attributed to the delay in convulsion time. This implies that they did not interfere with the lethal action of oxygen in any other way, although the point was not tested. There was a suggestion that morphine, although it had no effect on convulsions, reduced the mortality rate.

Anaesthetic action of oxygen at high pressures

Oxygen does not always act as a convulsant. If given alone convulsions are, at all pressures save the highest, the dominant effect. But at the higher pressures it is notable that the convulsion is greatly reduced in vigour, so that at 175 or 200 lb/sq. in. it is only represented by some convulsive movements of the limbs accompanying a general prostration. This might, of course, be regarded as a general "depressant" action of oxygen; but two further points are of interest. First, if the animals previously received a dose of bromide (800–1,000 mg/kg), which itself produced no loss of liveliness in the animals, the convulsive phase at high pressures of oxygen disappeared altogether and the animals passed instead, after a few minutes, into peaceful anaesthesia. They then remained so with unimpaired respiratory movements until decompression. This "depressant" action therefore closely resembles that of a volatile anaesthetic. Secondly, oxygen is known to be approximately twice as soluble in fat as nitrogen (Lawrence, Loomis, Tobias & Turpin, 1946), so that its anaesthetic activity should be exerted at a pressure about half that for nitrogen (Miller, Paton & Smith, 1965). Presumably the pretreatment with bromide potentiates an anaesthetic effect, so that the anaesthetic pressure for oxygen by itself must be above 150 lb/sq. in. (11 atmospheres absolute); that for nitrogen is of the order of 29 atm for mice. Oxygen can thus be regarded as falling into place with the other anaesthetics as having an anaesthetic tendency in direct relation to its lipoid solubility. It seems probable, therefore, that the additional depressant action of oxygen on the central nervous system is not to be regarded as a peculiar toxic action of the gas, but as a typical anaesthetic effect, such as scores of other gases are known to exert. This action does not of course manifest itself normally, because the tensions are too low and are in any case further reduced by the oxidative processes of the body.

Effect of drugs in delaying oxygen convulsions (Table 5)

Sodium bromide

Marks's finding that sodium bromide could delay oxygen poisoning has been confirmed. A just detectable effect could be obtained with 200 mg/kg given subcutaneously 1 hr before exposure (Table 6); a substantial effect was obtained with 800 mg/kg intraperitoneally 30 min before exposure (Fig. 3). Although this dose had a considerable effect on the oxygen poisoning, before exposure the mice receiving bromide were indistinguishable from the controls, and were not obviously sedated. When convulsions were observed in treated animals, they were attentuated in comparison with the normal response. It must be remarked, however, that the six-fold increase in convulsion time described by Marks with 5 mg doses could not be repeated. It is possible that there were particular circumstances in Marks's experiment which might lead to considerably greater estimates of protection than in the experiments reported here.

The anticonvulsive effect of bromide was demonstrable at all pressures investigated, up to 150 p.s.i. At the highest pressures, and with high doses of bromide, the convulsions were extinguished, and the animals passed directly into a state resembling anaesthesia. The dose-response relation for oxygen convulsions thus assumes an interesting form in the presence of bromide; as the tension rises, convulsion time becomes shorter, until the depressant action of oxygen itself annuls the convulsions. Convulsant phenomena often appeared during decompression, possibly simply because of the removal of the depressant effect of oxygen.

TABLE 5

EFFECT OF DRUGS ON CONVULSION TIMES OF MICE IN OXYGEN AT RAISED PRESSURES

Drug	Dose (mg/kg)	Route	Time (min)		Pressure (p.s.i.g.)	Convulsion time (min)	Estimated CT_{50}	24-hr death rate
Sodium bromide	200	S.C.	60	Control	65	4, 5, 9, 10½, 11, 13, 14, 24, 25, 26, 28, 33	13	12/12
				Test	65	3, 6, 9, 14, 17, 17, 17, 18, 26, 30, >40, >40	17	10/12
	400	S.C.	90	Control	65	6, 7, 8, 9, 10, 11, 14, 22, 23, 26, >30, >30	**12**	4/12
				Test	65	7, 15, 17, 28, >30, >30, >30, >30, >30	**32**	3/9
	800	I.P.	30	Control	65	7, 9, 19, 21, 21, 22, 23, 23, 25, 25, 25, >30	20	5/12
				Test	65	18, 25, >30×10	c. 40	5/12
				Control	75	16, 22, 25, 26	21	
				Test	75	34, 40, >40, >40	40	
				Control	100	5, 5, 5, 7	**5**	
				Test	100	10, 10½, 13, 14	**11½**	
				Control	150	5, 5, 5, 6½	5	
				Test	150	>15×4	>15	
	1,000	I.P.	30	Test	100	>20×2, anaesthesia	—	
				Test	150	>10×2, anaesthesia	—	
Diethazine	40	S.C.	30	Control	65	3, 4, 4½, 5, 8, 9, 12, 16, 29½, >30, >30, >30	**10**	9/12
				Test	65	5½, 16, 16½, 21, 29, 29½, 30, >30×5	**30**	5/12
Chlorpromazine	40	I.P.	15	Control	65	3, 4, 9½, 10, 14, 14, 14, 16, 16, 16, 26, >30	**12**	10/12
				Test	65	14, 14, 16, 19, 25, 30, >30×6	**29**	11/12
Paramethadione	100	I.P.	15	Control	65	7, 10, 13, 22, 25, 25, 30, >30	**18**	4/8
				Test	65	25, 25, 29, 29, >30×4	**29**	2/8
Trimethadione	20	I.P.	15	Control	65	5, 12, 15, 18	12	
				Test	65	13, 15, 16, 23	15	

TABLE 5—*continued*

Trimethadione	50	I.P.	30	Control	65	15, 20, 22, 24, 25, 29, >40, >40	24	
				Test	65	22, 24, 27, 34, 34, >40, >40, >40	31	
	100	I.P.	30	Control	75	5, 5, 10, 12, 12, 13, 15, 18, 20, 20, 23, 29	14	
				Test	75	12, 12, 14½, 15, 15, 17, 17½, 18, 20, 22, 23, 28	17	
(Tests with trimethadione not individually significant; but, if the three runs are pooled, treated are significantly different from controls)								
Mephenesin	40	S.C.	30	Control	65	5, 6, 6, 30	6	0/4
				Test	65	4½, 5, 13, 30	6	2/4
	80	S.C.	30	Control	65	4, 8½, 12, 13, 14, 22, 33, >40	**13**	4/8
				Test	65	20, 24, 26, 29, 33, 39½, >40, >40	**30**	5/8
	200	I.P.	20	Test	65	>30×4	>30	0/4
Morphine	0·5	S.C.	30	Control	65	5½, 19, >30, >30		3/4
				Test	65	9, 11, >30, >30		0/4
	2	S.C.	30	Control	65	10, 24, 28, >30		1/4
				Test	65	6, >30, >30, >30		1/4
	10	S.C.	30	Control	75	8, 9, 10, 12, 15, 16, 22, 24	12	6/8
				Test	75	8, 9, 10, 12, 16, 25, 26, 30	12	4/8
	20	S.C.	30	Control	65	18, 18, 20, 26		3/4
				Test	65	9, 24, 24, 26		2/4
Procaine amide	500	I.P.	10	Control	65	4, 17, 25, >30		
				Test	65	13, 20 (18, 18, animals prostrated)		
Hexamethonium	40	S.C.	30	Control	65	7, 7, 8, 8, 11, 11, 15, 21, 27, >30, >30, >30	13	7/12
				Test	65	6, 7, 7, 9, 13, 13, 16, 18, 20, 27, 28, >30	13	8/12
Cobalt acetate	5	S.C.	30	Control	65	5½, 16, 17, 18, 18, 23, 24, 27, 30, 35, 39, >40	22	7/12
				Test	65	10, 16, 18, 19, 22, 24, 32, 34, >40×4	26	6/12
Dimercaprol	80	S.C. in arachis oil	30–50	Control	65	3, 4, 14, 15, 16, 18	12	6/6
				Test	65	4½, 11, 13, 21, 24, 39	14	6/6

TABLE 6

EFFECT OF DRUG COMBINATIONS ON CONVULSION TIMES OF MICE IN OXYGEN AT 65 p.s.i.g.

	Mean log CT (n=8)	Geometric mean convulsion time (min)	Mean log CT (n=16)	Geometric mean convulsion time (min)
Saline	1·2846	19·3	1·3133	20·6
Diethazine, 20 mg/kg	1·2670	18·5	1·3137	20·6
Mephenesin, 60 mg/kg	1·2959	19·8	—	
Bromide, 200 mg/kg	1·3655	23·2	1·4127	25·9
Diethazine+mephenesin	1·3198	20·9	—	
Diethazine+bromide	1·4490	28·1	1·4111	25·8
Mephenesin+bromide	1·3444	22·1	—	
Diethazine+mephenesin+bromide	1·3955	24·9	—	

Mean log CT for all without diethazine	(48) 1·34870±0·0265 (S.E.)	Difference 0·0286	=6·8% prolongation
all with diethazine	(48) 1·37727±0·0226 (S.E.)		Not significant
Mean log CT for all without mephenesin	(32) 1·34153±0·0347 (S.E.)	Difference 0·0223	=5·3% prolongation
all with mephenesin	(32) 1·36388±0·0316 (S.E.)		Not significant
Mean log CT for all without bromide	(48) 1·31160±0·0213 (S.E.)	Difference 0·1030	=26·8% prolongation
all with bromide	(48) 1·41458±0·0211 (S.E.)		$P<0·05$
Mean log CT for diethazine+mephenesin+bromide	(8) 1·3955±0·0404 (S.E.)	Difference 0·1009	=26·2% prolongation
saline	(8) 1·2846±0·0942 (S.E.)		$P>0·05$
		Variance ratio	=5·4, $P<0·05$

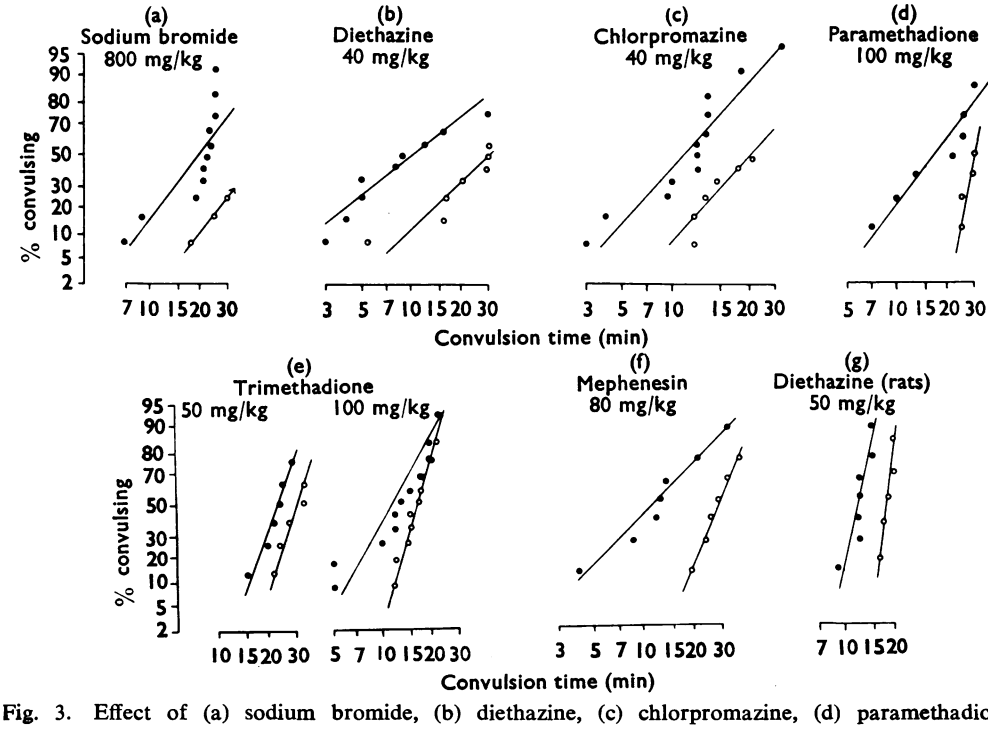

Fig. 3. Effect of (a) sodium bromide, (b) diethazine, (c) chlorpromazine, (d) paramethadione, (e) trimethadione and (f) mephenesin on incidence of convulsions in mice exposed to oxygen at 65 p.s.i.g., and (g) of diethazine on rats exposed to oxygen at 75 p.s.i.g.

Diethazine

This substance, used clinically in the treatment of Parkinson's disease, proved to have a significant effect in doses of 40 mg/kg given subcutaneously 30 min before exposure. A smaller dose, 20 mg/kg, also prolonged convulsion time, but the effect was only significant at the $P=0.2$ level. The dose of diethazine used had no obvious effect of itself on the mice.

Diethazine was also shown to be effective as an anticonvulsant in the rat, at pressures ranging from 80 to 200 p.s.i. (Table 8 and Fig. 3). Table 7 gives the results of a test using six rats in a cross-over test; the segregation of the variation between the different animals thus made possible considerably increased the precision of the test.

TABLE 7

TEST OF DIETHAZINE AGAINST OXYGEN CONVULSIONS (80 p.s.i.g.) IN RATS BY CROSS-OVER METHOD

Rat No.	Convulsion times (min) Control	Convulsion times (min) Diethazine		Sum of squares	d.f.	V	F	P
1	13*	17	Between treatments	397	1	397	8·86	<0·05
2	14	21*	Between days	184	1	184	4·11	>0·05
3	14*	38	Between animals	371	5	74·2	1·66	>0·05
4	15	26*	Residual	179	4	44·8		
5	17*	47	Total	1,231	11			
6	25	18*						

* Denotes test on 2nd day.

Mean ratio of convulsion times diethazine/control = 1·79.

TABLE 8

EFFECT OF DRUGS ON CONVULSION TIMES OF RATS IN OXYGEN AT RAISED PRESSURES

Drug	Dose	Route	Time	Pressure (p.s.i.g.)	Convulsion time (min) Control	Convulsion time (min) Test	Death rate Control	Death rate Test
Diethazine	50	I.P.	15	80	9, 12, 12, 12, 12, 14, 14, 15	16, 17, 18, 19½, 19½, >20	7/8	0/6
				100	11½, 21	21, 21		
				150	6	10½		
				200	8½	12		
Methyl-fluoroacetate	0·8	I.P.	30	100	25, 26	14, 15		
				150	11	6½		

Mephenesin

This compound, known to depress internuncial transmission in the brain stem and spinal cord, was able to delay oxygen poisoning in a dose of 80 mg/kg or more; this dose reduced, but did not abolish, the spontaneous activity of the animals. With 200 mg/kg, the animals appeared as though anaesthetized and exposure to oxygen had no action on them at all.

Trimethadione and paramethadione

These two drugs developed for the treatment of petit mal epilepsy were both able to delay oxygen convulsions in doses of 50–100 mg/kg. The animals receiving these doses were not in any way depressed or detectably different from controls. When convulsions occurred, they appeared normal in character.

Chlorpromazine

In a dose of 40 mg/kg, chlorpromazine had a considerable effect in delaying convulsions. The dose was sufficient to render the animals lethargic.

Combination of Drugs

Table 6 gives the results and part of the analysis of an experiment in which eight different procedures involving diethazine, mephenesin and bromide in various combinations were used, a series of runs being made in which each of eight animals was allotted randomly to each procedure and then exposed to oxygen at 65 p.s.i. Eight runs were made in full, making a total of 64 animals tested. Four of the procedures were repeated further in the same way using eight animals for each procedure. Low doses of diethazine, mephenesin and bromide were chosen, so that when combined, their depressant effect should not be too great. This had the result that the differences between pairs of groups were not significant. But since a balanced design had been used, results could be pooled. This showed that the effect of bromide was significant, with an increase of convulsion time of 27%. The increase produced by diethazine, 7%, and that by mephenesin, 5%, were not significant; prolongations of the order of 10–15% would have been detectable. The delay in convulsion time produced by the administration of all three drugs was not significant, although the change in variance produced by this treatment was significant. The magnitude, 26%, of the prolongation was so close to that attributable to the bromide alone that it seems improbable that the other drugs were contributing at all, or that any striking true potentiation of antagonism exists between them.

Cobalt

Marks reported that various ions protected against oxygen, including cobalt acetate. This could not be confirmed; the prolongation observed was a very slight one and not significant.

Dimercaprol

One of the possibilities for the action of oxygen is that it poisons sulphydryl enzymes in the central nervous system. It is possible, therefore, that some protective effect could be obtained by treating the animals with substances provided by sulphydryl groups. Marks in fact tested this approach by administering glutathione. In the present experiments dimercaprol was given in a dose of 80 mg/kg. This was probably too large a dose and caused partial prostration of part of the animals; but it failed to yield any good evidence of protection.

Morphine

Morphine has a mixed action on the central nervous system, displaying both stimulant and depressant activities which vary with different species, and it seemed possible that it might in fact sensitize to oxygen poisoning, particularly when the tail erection occasionally produced by oxygen was noted. No significant effect, however, could be obtained with doses between 0.5 and 20 mg/kg.

Procaine amide

Diethazine and chlorpromazine have some local anaesthetic activity and it was possible that their effects were attributable to this. But a large dose of procaine amide, the stable analogue to procaine, which would almost certainly, in the dose given, have a comparable local anaesthetic effect, proved to offer no protection to the animals although a dose large enough to produce ataxia and other systemic effects was given.

Hexamethonium

It has been suggested that some of the features of oxygen poisoning, such as the rise in blood pressure and pallor of the skin, are mediated by a peripheral vasoconstriction, and that this is, to some extent, a protective reaction against the raised oxygen tension. Such a reaction might well be mediated through the autonomic ganglia and if so should be abolished by a sufficient dose of the ganglionic blocking agent, hexamethonium. In fact, however, a large dose, 35 mg/kg subcutaneously, has no significant effect.

Potentiation of oxygen poisoning by fluoroacetate

Some of the results obtained, which are discussed later, suggest that oxygen might be acting on an enzyme in a somewhat similar way to the action of fluoroacetate. A preliminary experiment was made to test whether fluoroacetate sensitized to oxygen or not. Three rats were injected with 100 μg methyl fluoroacetate intraperitoneally in 0.1 ml. alcohol and compressed to 100 or 150 lb. In each case convulsions appeared earlier in the fluoroacetate-treated rats than in controls.

DISCUSSION

The assessment of oxygen poisoning and drugs influencing it

The variability of the response to oxygen poisoning introduces difficulty in the distinguishing of the action of drugs on it. Rats are, on the whole, more regular in their response than mice but it is harder to do large numbers of them. Although one can use the same rat repeatedly, with judicious spacing of compressions, it is not legitimate (without further test) to compare such a repeatedly poisoned animal with animals receiving their first exposure. Yet it is presumably the latter which are of most interest as corresponding to humans meeting high oxygen tensions for the first time in some emergency. Mice can be tested conveniently in fairly large numbers and are also among the animals nearest in sensitivity to man. But they are very variable in response, the end point is sometimes imprecise, and a very large proportion of them fail to survive the

compression so that as a rule the mice can only be used once. Clearly the two species each offer advantages for different types of experiment.

The dose-response curve pattern is probably of considerable importance for the conduct of the test of a drug. If there is a definite threshold pressure before poisoning ensues and if the pressure of the exposure is fairly close to this, then a moderate degree of protection may bring the animal into the completely immune zone, conferring an apparent infinite degree of protection. At higher pressures, however, the same degree of protection might be obtained (in terms of the extra pressure of oxygen tolerated before convulsing in a given time) although the convulsion time at a given pressure might be hardly altered. It is suspected that this may account for the difference between Marks's results and those reported here, since he worked at lower pressures and with what were in the main longer convulsion times. A second factor which might also contribute is that as exposure is prolonged the condition of the animals deteriorates (no doubt due to the advancing pulmonary lesions) and it is probable that if convulsions have not taken place within 60 or 90 min that they will rarely do so. Thus a misleadingly long convulsion time might be obtained where the progress of oxygen toxicity in the nervous system has become overlaid by the pulmonary damage.

The mechanism of oxygen poisoning

If the curve of convulsion time against oxygen pressure is studied the most striking point is the hyperbolic shape and the fact that there is a minimum convulsion time. Such a minimum time in cats has been reported by Pratt (personal communication quoted by Taylor, 1949) for pressures taken up to 150 lb. This minimum convulsion time has certain consequences. The first is that it makes it unlikely that oxygen itself is the stimulus to convulsions; for in that case doubling the tension of oxygen (from 100 to 200 lb) must surely produce a much quicker convulsion. We may contrast the behaviour to oxygen with that of, say, metrazol, where a dose given intraperitoneally will produce a convulsion within 45 sec. Although oxygen must be regarded as being "injected" into the pulmonary circulation and would therefore be expected to act even more quickly than metrazol, in fact it seems always to act at least six times more slowly. Equally it seems unlikely that the effect of oxygen is due to some product, such as an abnormal product of oxidation which is formed in direct proportion with the oxygen tension. It also becomes unlikely that the delay to oxygen poisoning represents to more than a small extent delay in access of oxygen to some site. So long as the minimum latency is considerably greater than the circulation time (as it is) it is inevitable that the higher the ambient oxygen pressure the sooner a given region in the central nervous system will reach a given oxygen tension. The definite irreducible latency can only imply that some process takes place which requires a minimum finite time to produce convulsions.

From these considerations the suggestion immediately arises that oxygen acts not directly but by poisoning some enzyme, and that this leads either to the accumulation of a convulsant substrate of the enzyme or to the depletion of some anticonvulsant product of the enzyme's activiy. Such a hypothesis requires a minimum latency of action and an oxygen tension (that at which the enzyme is completely inhibited) above which no further intensification of convulsing action can occur. It also of course, like other

theories, implies that there would be a threshold oxygen tension for convulsion—that is, that at which the enzyme has come to be so far inhibited that it just cannot maintain its normal metabolic traffic. Thirdly, the hyperbolic nature of the dose-response curve is compatible with an enzyme inhibition of the type postulated.

Two more direct tests were done, one with the particular possibility of the poisoning of a sulphydryl enzyme particularly in mind. First was the experiment with dimercaprol, which might buffer the effect of oxygen; this failed to show any significant protection. But there is no certainty that the drug will have reached the point where its action would be required, so that not much significance can be attached to this negative result. The second experiment was a demonstration, in a preliminary way, that fluoroacetate sensitized the animal to oxygen. This is not a proof that the same enzyme systems are inactivated by the two procedures, since there are well-known examples of agents summating while acting at different points. Nevertheless this result suggests a hypothesis for the effects of oxygen poisoning—that it inhibits an enzyme concerned with the citric acid cycle of the brain.

The second main problem in the mechanism of oxygen poisoning is the site of initiation of the convulsions. Three points can be brought into consideration here. First, the stimulant action tends to arouse not only clonic convulsions and intense motor activity but also fairly well co-ordinated tremors, athetosis and varied spastic states. They suggest that it might indeed be some structure below the cortex which is the primary site of attack. Secondly, Marks showed that the hydantoins do not antagonize oxygen poisoning, and Taylor (unpublished) has shown the same for primidone. Both these drugs are known to be effective against grand mal epilepsy and against electrically excited cortical convulsions, and the hydantoins are almost specific in this respect. This action suggests that oxygen may have an extra-cortical site of action. Thirdly mephenesin and diethazine have some antagonistic effect. Neither of these is normally regarded as anticonvulsant but they probably function at internuncial sites or in basal ganglia. The same may be true as regards paramethadione and trimethadione. Although this evidence is far from decisive, it all points to the conclusion that oxygen acts at the basal ganglia rather than at the cortical level.

It has been argued above that the existence of a minimum latency for oxygen convulsions suggests that it acts, not directly, but by poisoning some enzymic system as a result of which either a convulsant precursor accumulates or a depressant product is depleted, or both of these. A wide range of enzymes are known to be susceptible to oxygen (Bean, 1945; Dickens, 1962). One particular possibility would be the system involving glutamic acid and γ-aminobutyric acid, now known to be (respectively) excitant and depressant to a considerable range of central neurones. The pattern of neural effects produced by oxygen, and the nature of the drugs effective against them, point to a subcortical site of action; the γ-aminobutyric acid content is said to be higher in subcortical regions than elsewhere (Roberts, 1962). Treatment with fluoroacetate, known to inhibit the tricarboxylic acid cycle, predisposes to oxygen convulsions; the metabolism of γ-aminobutyric acid is closely bound in with the tricarboxylic acid cycle (Elliott, 1965). The action of oxygen could well be, therefore, to disturb the normal balance in the subcortex between glutamic acid and γ-aminobutyric acid. Wood & Watson (1963) have indeed found a fall in γ-aminobutyric acid in the brain in animals convulsed with

oxygen. The greatest problem, however, is that γ-aminobutyric acid (and no doubt other amino acids) exist in the brain in five states (free intracellularly and extracellularly, loosely bound, firmly bound and covalently bound) (Elliott, 1965); to develop this approach, it will be necessary to identify which states of the amino acids determine their physiological effect, and to develop methods for measuring changes therein.

The anaesthetic effect of oxygen is probably chiefly of theoretical importance, by extending to a gas of physiological importance the generalization connecting lipoid solubility and anaesthesia. It may also be significant for distinguishing different facets of oxygen action. It is known, for instance, that high oxygen tension can depress nerve conduction (Dickens, 1962); the analysis of oxygen's actions will be simplified if such an effect can be associated with an anaesthetic action rather than straining to correlate it with the mechanism of convulsant action.

SUMMARY

1. The sensitivity of mice and rats to oxygen has been measured by determining the time to produce convulsions at various oxygen tensions. The observation by Marks that the logarithm of the convulsion time is distributed normally has been confirmed. Rats are generally less sensitive than mice, and much less variable.

2. As the oxygen tension is raised, the convulsion time shortens, until a minimum convulsion time of 3–4 min for mice and about 6 min for rats is reached at about 100 p.s.i.g. Above this tension, the convulsion time is not reduced further. There is also a minimum tension below which convulsions cannot be produced, so that the pressure-convulsion time relation takes a hyperbolic form.

3. The earlier a convulsion occurs, the higher the lethal effect of oxygen.

4. At very high tensions (150 p.s.i.g.) the convulsion produced by oxygen is very brief and is followed by a state resembling anaesthesia. If the animal is pretreated with bromide in a dose insufficient to produce behavioural changes, and then exposed to high oxygen tensions, the convulsion is abolished and the animal passes directly and rapidly into this anaesthetized state. It is suggested that this represents a true anaesthetic action of oxygen, comparable with that of the inert gases; the fat solubility of oxygen, approximately twice that of nitrogen, is compatible with this suggestion.

5. Significant prolongations of convulsion time of mice in oxygen at 65 p.s.i.g. were obtained with sodium bromide, diethazine, chlorpromazine, paramethadione and trimethadione, and mephenesin. Cobalt acetate, hexamethonium, dimercaprol, procaine amide and morphine were ineffective. Diethazine was also shown to be effective in rats. Preliminary evidence was obtained that methyl fluoroacetate potentiated oxygen convulsions in rats.

6. It is suggested, in the light of these results, that oxygen, in producing convulsions, acts not directly but by poisoning some enzyme system, so leading to accumulation of a convulsant substrate or depletion of a depressant product; and that the site of initiation of convulsions is subcortical.

This work was done during a visit to the Royal Naval Physiological Laboratory, Alverstoke, in July 1953, at the invitation of the Superintendent, Dr. H. J. Taylor, to whom it is a pleasure to express my thanks for his hospitality. The skilled assistance of Mr. A. W. Dossett is also gratefully acknowledged.

REFERENCES

BEAN, J. W. (1945). Effects of oxygen at increased pressure. *Physiol. Rev.*, **25**, 1–147.

DICKENS, F. (1962). The toxic effect of oxygen on nervous tissue. Chapter 35. In *Neurochemistry*, pp. 851–869, ed. ELLIOTT, K. A. C., PAGE, I. H. & QUASTEL, J. H. 2nd edition. C. C. Thomas, Springfield.

ELLIOTT, K. A. C. (1965). γ-Aminobutyric acid and other inhibitory substances. *Br. med. Bull.*, **21**, 70–75.

LAWRENCE, J. H., LOOMIS, W. F., TOBIAS, C. A. & TURPIN, F. H. (1946). Preliminary observations on the narcotic effect of xenon with a review of values for solubilities of gases in water and oils. *J. Physiol.*, **105**, 197–204.

LORRAIN SMITH, J. (1899). The pathological effects due to increase of oxygen tension in the air breathed. *J. Physiol. Lond.*, **24**, 19–35.

MARKS, H. P. (1944). Interim report on oxygen intoxication in animals, and the effect of drugs on sensitivity to oxygen intoxication. Medical Research Council. Royal Naval Personnel Research Committee Report 44/101.

MILLER, K. W., PATON, W. D. M. & SMITH, E. B. (1965). Site of action of general anaesthetics. *Nature, Lond.*, **206**, 574–577.

ROBERTS, E. (1962). γ-Aminobutyric acid. Chapter 26. In *Neurochemistry*, ed. ELLIOTT, K. A. C., PAGE, I. H. & QUASTEL, J. H. 2nd edition. C. C. Thomas, Springfield.

TAYLOR, H. J. (1949). The role of carbon dioxide in oxygen poisoning. *J. Physiol. Lond.*, **109**, 272–280.

WOOD, J. D. & WATSON, W. J. (1963). Gamma-aminobutyric acid levels in the brain of rats exposed to oxygen at high pressures. *Can. J. Biochem. Physiol.*, **41**, 1907–1913.