Effect of Oxygen Tension on Drug Levels and Pharmacological Action in the Intact Animal[1] (34143)

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It is now well established that molecular oxygen is required for oxidative drug metabolism occurring in the microsomes. This data has been accumulated from *in vitro* studies, but little, if anything, is known of the requirements for oxygen in microsomal drug metabolism in intact animals. However, there are observations which indicate that the pharmacological effects of drugs change in animals made hypoxic or exposed to hyperbaric oxygen. It was noted by Bean (1) in 1931 that high pressure oxygen lightened anesthesia, and Jamieson (2) demonstrated that the pentobarbital (35 mg/kg) sleep times of mice were shortened if treated mice were exposed to oxygen at 4, 5, or 6 ATA, but not at 3 ATA. The toxicity of digitalis (3) and carbon tetrachloride (4) are also reduced by hyperbaric oxygen. On the contrary, the toxicity of digitalis (5), strychnine (6), and reserpine (7) increase at elevated altitudes. The data accumulated from previous studies would suggest that the effects and toxicity of drugs decrease in hyperbaric oxygen and increase in altitude-induced hypoxia. However, exceptions appear to occur since the hexobarbital sleep time was reduced in mice pretreated in an altitude chamber (8, 9).

Although many of these investigators suggest that changes in the rate of drug metabolism may be responsible for these results, only very few measurements of tissue levels of drugs and rates of metabolism have been reported. This study was undertaken to determine the effect of oxygen and pressure on tissue concentrations of drugs and in this manner, indirectly, possibly gain information concerning their rates of biotransformation.

Material and Methods. Male Charles-River rats, weighing 140–200 g were used throughout the study. Animals were pretreated for 45 min with varying concentrations and pressures of oxygen at a gas flow of 2 liters/min, removed from the chamber, injected with the drug to be studied, and replaced in the chamber of the oxygen concentration and pressure being studied. The temperature of the chamber was maintained at 25 ± 1°. For the studies of altitude-induced hypoxia, the animals were pretreated at reduced pressure (0.44 ATA) for 90 min and were returned to the low pressure chamber following injection of the drugs studied. The animals exposed to 5% oxygen and 95% nitrogen at 1 ATA were pretreated only 45 min. The drugs studied were administered intraperitoneally at the doses recorded in Tables I and II. The barbiturate sleep times and zoxazolamine paralysis times were determined as the period from the loss to the return of the righting reflex. The plasma and brain concentrations of hexobarbital (10) and zoxazolamine (11) were measured at the time the righting reflex returned as well as after a fixed period of time after injection of the drug. The plasma levels of warfarin (12), sulfadiazine (13), phenylbutazone (14), isoniazid (15), and *p*-aminopropiophenone (13) were only determined after a fixed period of time. The control animals were handled in a similar manner, except they were kept outside of the hyperbaric or altitude chamber.

Results. The effect of increased oxygen

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[1] Supported in part by Grant GM 15956 from the U. S. Public Health Service.

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TABLE I. Effect of Oxygen and Pressure on Sleep and Paralysis Times after Administration of Barbiturates and Zoxazolamine.

Drug	Dose (mg/kg)	Atmosphere	Sleep or paralysis time (min ± SE; no. of animals)	
			Control	Experimental
Hexobarbital	150	100% O_2–4 ATA	60 ± 4 (9)	42 ± 5 (11)[a]
Barbital	200	100% O_2–4 ATA	238 ± 16 (6)	168 ± 10 (4)[a]
Zoxazolamine	80	100% O_2–4 ATA	127 ± 8 (9)	124 ± 10 (8)
	60	100% O_2–4 ATA	62 ± 4 (6)	57 ± 6 (6)
Hexobarbital	150	100% O_2–1 ATA	63 ± 10 (5)	60 ± 6 (5)
	200	100% O_2–1 ATA	88 ± 7 (6)	89 ± 4 (6)
	200	100% O_2–3 ATA	97 ± 3 (6)	100 ± 5 (6)
	175	Air–4 ATA	82 ± 19 (6)	83 ± 10 (6)
Hexobarbital	175	Air–0.44 ATA	54 ± 7 (6)	67 ± 7 (6)[a]
Barbital	200	Air–0.44 ATA	217 ± 35 (4)	422 ± 38 (5)[a]
Zoxazolamine	80	Air–0.44 ATA	149 ± 6 (6)	246 ± 10 (5)[a]
Hexobarbital	150	5% O_2–95% N_2–1 ATA	52 ± 8 (6)	67 ± 3 (6)[a]

[a] $p = < 0.05$.

tension on hexobarbital sleep times are shown in Fig. 1. As shown, 100% oxygen at 4 ATA decreases the hexobarbital sleep time at low doses and that the percentage reduction decreases as the dose of hypnotic is increased until at 250 mg/kg the hyperbaric oxygen effect is no longer evident.

Table I shows the effect of oxygen and pressure on sleep and paralysis times after administration of barbiturates and zoxazolamine. The decreased hypnotic effect of hexobarbital requires 100% oxygen at 4 ATA since no shortening of the sleep time was obtained with 100% oxygen at 1 ATA or 3 ATA. Increased pressure alone is also not the mechanism, since air at 4 ATA has no effect. A shortened sleep time in animals exposed to hyperbaric oxygen is also seen when barbital is the hypnotic agent.

The relationship of the pharmacological effect of hexobarbital and zoxazolamine to tissue levels of the drugs is shown in Table II. At the time of awakening, the brain levels of hexobarbital are significantly higher in

TABLE II. The Effect of Hyperbaric Oxygen on Brain and Blood Concentrations of Drugs.[a]

Drug	Dose (mg/kg)	Elapsed time after administration (min)	Plasma (μg/ml ± SE)		Brain (μg/g ± SE)	
			Control	Hyperbaric	Control	Hyperbaric
Hexobarbital	175	At awakening	65.3 ± 7.0	76.1 ± 9.1	53.6 ± 7.2	67.1 ± 6.6[b]
	175	40	78.9 ± 1.8	77.5 ± 3.3	87.3 ± 7.6	85.3 ± 7.1
Zoxazolamine	80	At awakening	24.4 ± 1.1	25.8 ± 3.1	72.2 ± 3.1	79.5 ± 3.7
Warfarin	2	60	15.4 ± 1.2	19.0 ± 2.6		
Phenylbutazone	110	60	255 ± 7	259 ± 5		
Sulfadiazine	150	60	396 ±23[c]	330 ±13[b]		
Isoniazid	110	40	63 ± 4	52 ± 2[b]		
p-Aminopropiophenone	20	60	109 ±19	57 ± 7[b]		

[a] Animals were pretreated for 45 min with 100% oxygen at 4 ATA, injected with the drug to be studied, and returned to the chamber at the same conditions.

[b] $p = < 0.05$.

[c] Whole blood.

TABLE III. Effect of Altitude-Induced Hypoxia on Brain and Plasma Drug Levels.[a]

Drug	Dose (mg/kg)	Elapsed time after administration (min)	Plasma (μg/ml ± SE) Control	Plasma (μg/ml ± SE) Hypoxic	Brain (μg/g ± SE) Control	Brain (μg/g ± SE) Hypoxic
Hexobarbital	150	30	63.8 ± 4.0	68.8 ± 3.6	123.0 ± 11.4	100.3 ± 10.7
Zoxazolamine	80	At awakening	30.8 ± 2.2	23.5 ± 1.3[b]	74.3 ± 5.2	52.2 ± 6.5[b]
Warfarin	2	90	18.6 ± 1.9	17.3 ± 1.2		
Phenylbutazone	110	90	242 ±16[c]	268 ±10		
Sulfadiazine	150	90	394 ±22	429 ±33		
Isoniazid	100	90	50.2 ± 3.6	42.5 ± 1.1		

[a] Animals were pretreated for 90 min in chamber with air at 0.44 ATA, injected with the drug to be studied, and returned to the chamber at the same conditions.

[b] $p = < 0.05$.

[c] Whole blood.

the animals exposed to hyperbaric oxygen, but the levels are similar to the controls if the animals are studied after a fixed period of time has elapsed. Hyperbaric oxygen has no effect on the paralysis time induced by zoxazolamine and again no difference is seen in brain levels at a fixed period of time. These results suggest that hyperbaric oxygen has no effect on hexobarbital or zoxazolamine metabolism. Similarly, after a fixed period of time has elapsed, there are no differences in the plasma levels of warfarin and phenylbutazone, but the levels of sulfadiazine, isoniazid, and *p*-aminopropiophenone are significantly lower in animals exposed to hyperbaric oxygen (Table II).

In acute altitude-induced hypoxia the sleep times of hexobarbital and barbital as well as zoxazolamine paralysis are markedly prolonged (Table I). In the hypoxic animals the prolongation of the sleep time increases proportionately to the increase of the dose of hexobarbital (Fig. 2). The decrease in oxygen tension produced by exposure to altitude does not influence the rate of metabolism of any studied drugs (Table III).

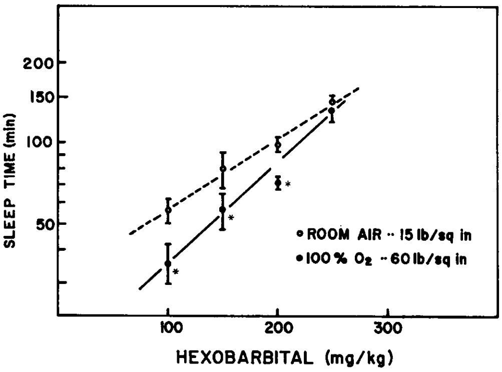

FIG. 1. Effect of 100% oxygen at 4 ATA on dose–response to hexobarbital-induced sleep times.

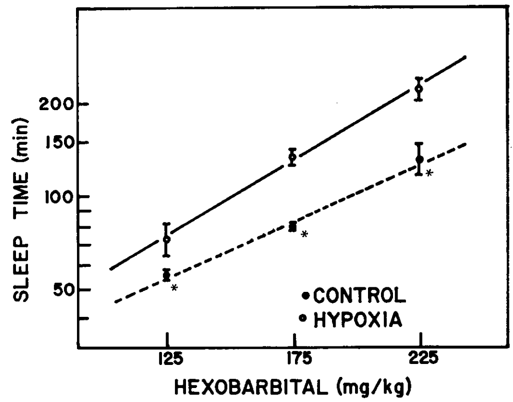

FIG. 2. Effect of hypoxia (air, 0.44 ATA) on dose–response to hexobarbital-induced sleep times.

Discussion. The levels of hexobarbital in brain (Table II) demonstrate that the shortened sleep time of the barbiturate-treated animal exposed to hyperbaric oxygen is not due to changes in the rate of biotransformation of this hypnotic agent. A similar decrease in sleep time with barbital, which is excreted primarily unchanged, supports this contention. A markedly increased pO_2 is required since the decreased sleep time is not produced at less than 4 ATA and is also not seen with air at 4 ATA so must be due to an elevated pO_2 rather than pressure.

Barbiturates are weak acids and thought to be effective only in the undissociated form.

Therefore, we would expect its effect to increase rather than decrease when the extracellular pH is lowered as occurs in animals treated with hyperbaric oxygen (16). Hyperbaric oxygen decreases cerebral blood flow (17) so that an increased rate of removal from the brain circulation also cannot explain our results.

Any explanation must take into account the finding that the animals exposed to hyperbaric oxygen awaken at a higher brain level of hexobarbital than nonexposed animals. It was demonstrated over 30 years ago (18) and subsequently confirmed by several investigators (19, 20) that barbiturates and other anesthetic agents suppress brain respiration *in vitro*. Barbiturates suppress the rate of oxidation of $NADPH_2$ by the cytochrome system (21) and inhibit oxidative phosphorylation. The suppression of $NADPH_2$ oxidation by barbiturates has the double effect of suppressing the citric acid cycle and the formation of ATP. Hyperbaric oxygen, although raising the brain pO_2 (22), does not increase metabolism by the normal intact brain or isolated tissues. If, however, an increased pO_2 could increase oxygen uptake and utilization by barbiturate-depressed cells, such a mechanism could explain our results. A similar mechanism would also explain the potentiation of barbiturate hypnosis caused by hypoxia. However, no data are currently available concerning this possibility.

A question also arises whether the changes produced by exposure to altitude are due to decreased pO_2 or pressure. It has been shown that morphine is less toxic to rats kept in an altitude chamber at reduced pressure but with pO_2 equivalent to ground level (23). Similarly, hypoxia rather than reduced pressure is responsible for potentiation of barbiturate hypnosis (Table I).

Hyperbaric oxygen does not change the rate of metabolism of hexobarbital nor phenylbutazone nor warfarin, drugs also metabolized by hepatic microsomes (Table II). However, the rate of metabolism of sulfadiazine, isoniazid, and *p*-aminopropiophenone are significantly accelerated. Sulfadiazine and isoniazid are converted by supernatant primarily to acetylated metabolites (24). The *p*-aminopropriophenone metabolism is poorly understood, but probably oxidation of the NH_2 group and glucuronide formation is the major pathway (25). Acetylation seems to be only a minor route. An increase in the rate of excretion is also a possibility and currently under investigation. Hypoxia had no effect on the plasma level of any of the drugs studied.

Summary. A markedly increased pO_2 shortens hexobarbital sleep times in the intact rat while decreased pO_2 lengthens it. These changes are not related to the rate of hexobarbital biotransformation. A decreased pO_2 has no effect on the rates of metabolism of the drugs studied, but an increased pO_2 increases the rate of disappearance of INH and sulfadiazine from the plasma.

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Received May 5, 1969. P.S.E.B.M., 1969, Vol. 132.