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EFFECT OF REDUCED BAROMETRIC PRESSURE ON DRUG ACTION AND METABOLISM IN MICE*

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Abstract—Mice kept at a simulated altitude of 18,000 ft for 5 days had a shorter duration of loss of righting reflex with hexobarbital, zoxazolamine and mephenesin, but not with pentobarbital. Exposure to reduced pressure did not alter brain receptor sensitivity to these drugs. Decreased hexobarbital sleeping time was produced 3 days after initial simulated altitude exposure and was reversed within 3 days after return to normobaric conditions. The hepatic microsomal metabolism of aniline, hexobarbital, nitroanisole and dichlorophenolindophenol was increased in mice kept in a hypobaric chamber while that of methylaniline was not altered. Mice exposed to reduced pressure had a loss of total body weight, liver weight, and liver water content. Hepatic RNA-P and DNA content was increased, but there was no change in the hepatic microsomal protein or cytochrome P-450 content. A decreased hexobarbital sleeping time in different mice strains was observed at a simulated altitude of 18,000 ft but not at 8000 ft. The results suggest that the alterations in drug action observed may be due primarily to increased drug metabolism produced by hypobaric hypoxia.

ALTERATIONS in the action and metabolism of drugs may be produced by the administration of a variety of drugs or chemicals.[1] Similar effects are seen when animals are subjected to different types of "nonspecific" stresses such as dehydration, temperature changes, hind-limb ligation, starvation, hypoxia and altitude.[2–6]

Although there have been many investigations on the effects of altitude exposure on the pharmacologic activity or toxicity of drugs, few reports have appeared on the effect of altitude on drug metabolism. We have previously described some alterations in drug metabolism which resulted from a 5-day exposure to a simulated altitude of 18,000 ft.[7] However, there is no information concerning changes in other parameters usually associated with drug metabolism such as the concentration of hepatic cytochrome P-450, DNA, RNA, and microsomal protein. In this present study we have undertaken an examination of these parameters and other factors in order to obtain

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information regarding the possible mechanism(s) involved in alteration of drug metabolism by a hypobaric environment.

MATERIALS AND METHODS

Male C-57 or Swiss–Webster mice weighing between 20 and 30 g were used in the experiments. The mice were exposed to reduced pressure in a walk-in chamber divided into two compartments; one compartment of the chamber was evacuated to a pressure equivalent to either 18,000 ft (0·5 atmosphere) or 8000 ft (0·74 atmosphere) while the other was maintained at ambient pressure (approximately 1·0 atmosphere) and used to house the control group. The temperature in the chamber was maintained at 25° and the humidity at 50 per cent. All animals were kept in a wire cage in groups of 12–25 and allowed food and water *ad lib.* for the duration of the experiment. The chamber was repressurized at the completion of the experiment, the mice were removed and either sacrificed or injected with the drug under investigation. Control mice were acutely depressurized to either 0·5 atmosphere or 0·74 atmosphere and then immediately repressurized in order to expose them to the same stress of repressurization as the experimental group. In later experiments it was found that omission of this procedure did not produce results which differed from those in which the control group was subjected to this stress. However, in order to maintain continuity of results, all of the controls reported in this paper were subjected to this stress procedure.

Enzyme assay. Mice were sacrificed by a blow on the head and the livers immediately removed. All steps in the preparation of the homogenates were carried out at 0–4°. The liver was homogenized with a glass homogenizer in 4 vol. of cold 0·1 M tris–HCl buffer, pH 7·5. The homogenate was then centrifuged at 9000 *g* for 15 min and the resultant supernatant centrifuged at 100,000 *g* for 60 min. The microsomal pellet was resuspended in the tris–HCl buffer so that each ml contained 5·0 mg of microsomal protein. Oxidative enzyme activity was determined in a medium containing 0·33 mM $NADP^+$, 5 mM $MgCl_2$, 8 mM glucose 6-phosphate, 1 unit of glucose 6-phosphate dehydrogenase, 1 mg microsomal protein per ml and one of the following substrates: 1 mM aniline or methylaniline; 4 mM hexobarbital; 3 mM nitroanisole. The total volume of the mixture was brought to 5 ml with tris–HCl buffer and incubated at 37° for 15 min. The hydroxylation of aniline was determined by measuring the formation of *p*-aminophenol.[8] Hexobarbital metabolism was assayed by the method of Cooper and Brodie.[9] The formation of *p*-nitrophenol was used to follow the demethylation of *p*-nitroanisole[10] and the degree of *N*-demethylation of methylaniline was estimated by measuring the formation of formaldehyde as described by Cochin and Axelrod.[11] The activity of NADPH-dichlorophenolindophenol reductase was determined by the method of Williams and Kamin.[12]

Loss of righting reflex. Mice were injected intraperitoneally with the following drugs in order to determine the effect of simulated altitude on the duration of loss of righting reflex: sodium hexobarbital (125 mg/kg), sodium pentobarbital (50 mg/kg), zoxazolamine (100 mg/kg), and mephenesin (200 mg/kg). Sodium hexobarbital and sodium pentobarbital were dissolved in saline; zoxazolamine was dissolved in a stoichiometric quantity of HCl, and mephenesin was dissolved in an aqueous 10% propylene glycol solution. In some experiments when the animals had regained their righting reflex they were sacrificed by a blow to the head and the brain was rapidly removed for drug assay.

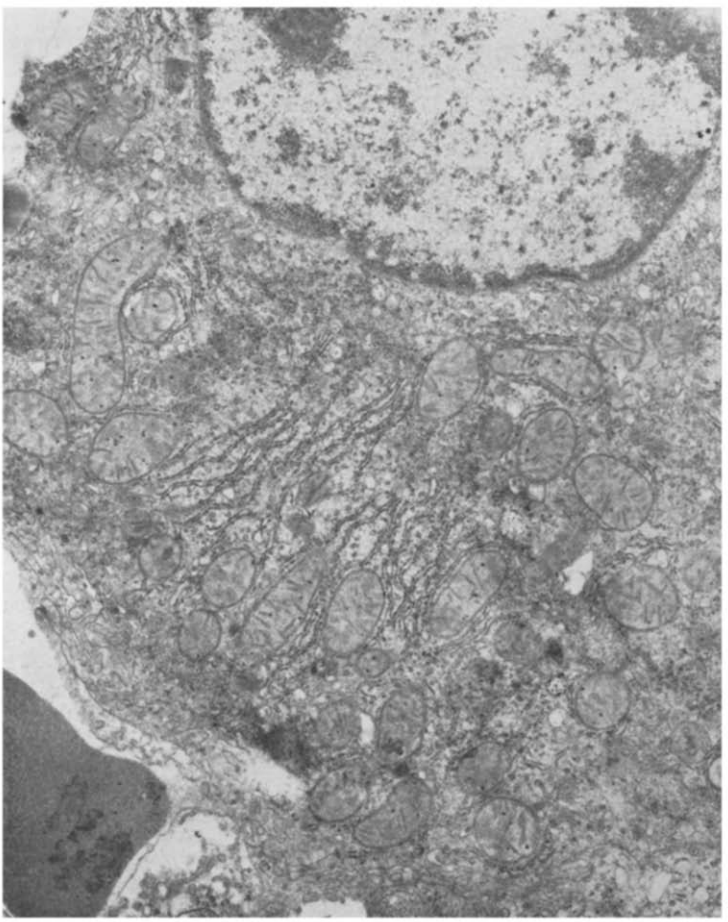

FIG. 2. Liver cell from control group (× 20,500).

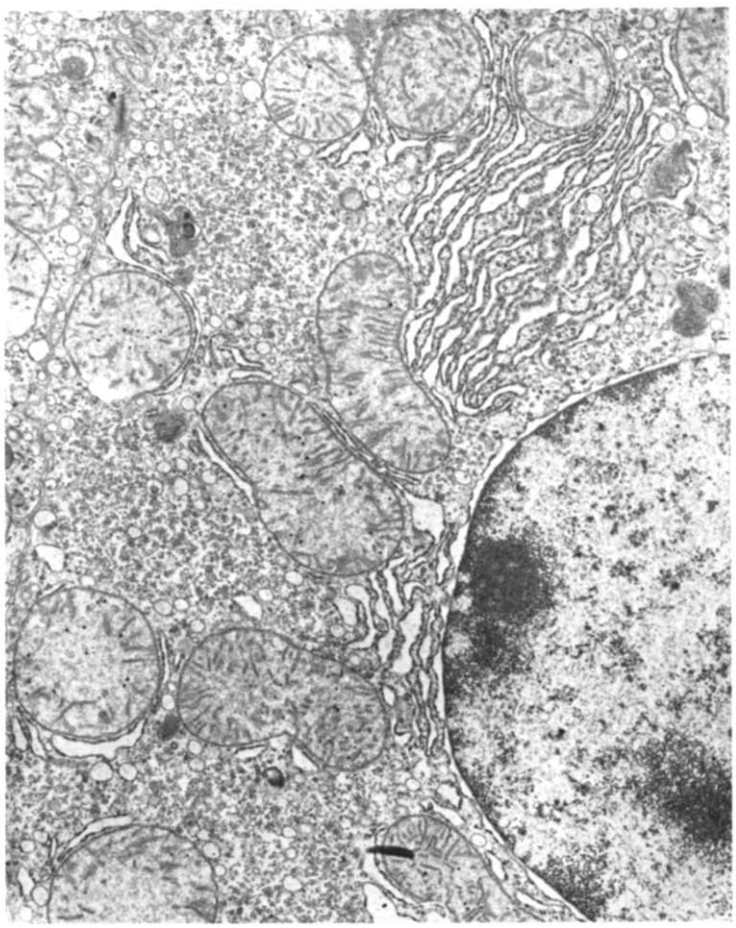

FIG. 3. Liver cell from altitude-exposed group showing swollen mitochondria and general swelling of endoplasmic reticulum ($\times$ 20,500).

Chemical analysis. Microsomal protein was determined by the method of Lowry *et al.*[13] using bovine serum albumin as the standard. The concentration of cytochrome P-450 was assayed in the microsomal pellet.[14] Brain levels of hexobarbital and pentobarbital were measured using the method of Cooper and Brodie;[9] zoxazolamine was determined as described by Juchau *et al.*;[15] and mephenesin was assayed by the procedure of Maass *et al.*[16] DNA and RNA-P content were estimated in liver homogenates using calf thymus DNA as the standard.[17]

Electron microscopy. Representative samples from mouse liver were fixed in Karnovsky's solution and post-fixed in 2% OsO_4 in phosphate buffer, pH 7·3. The tissue was then embedded in Epon 812 (Shell Epikote 812) and thin sections were cut on a Porter Blum ultratome. The sections were stained with uranyl acetate–lead citrate and viewed in an RCA EMU-3G electron microscope.

Statistics. All results are expressed as the mean ± standard error (S.E.) unless otherwise indicated. A student's *t*-test was used to evaluate the difference between groups and a value of $P < 0{\cdot}05$ was taken as being statistically significant.

RESULTS

Induction of alteration of hexobarbital sleeping time by reduced pressure. It has been previously shown that a 5-day exposure to a simulated altitude of 18,000 ft will result in a decreased hexobarbital sleeping time in mice; however, the time required for the production of this effect was not investigated.[7] In order to determine the time for onset of this effect, mice were kept at a simulated altitude of 18,000 ft, groups of 12 were removed at different time intervals, injected with 125 mg/kg of sodium hexobarbital, and their sleeping time measured. There was no significant decrease in sleeping time between simulated altitude-exposed and control mice until the third day of exposure, when it was approximately 80 per cent of the control value (Fig. 1). In one experiment mice were removed from the altitude chamber after 5 days' exposure

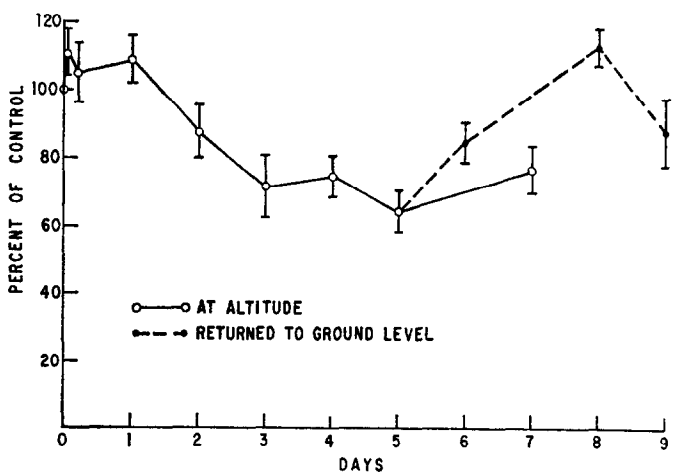

FIG. 1. Loss of righting reflex in mice at different periods after exposure to an altitude of 18,000 ft. Each point is the mean and S.E. of 12 observations. Per cent of control = [sleep time of control mice (min)/sleep time of altitude-exposed mice (min)] × 100. Value for 100 per cent control was 82·6 ± 4·2 min.

and the sleeping time of groups of the mice was determined on successive days thereafter. Sleeping time had returned to control values within 3 days after the animals had been removed from the hypobaric environment. These results are similar to those reported by Robinson *et al.*[18] for mice exposed to a simulated altitude of 19,000 ft.

Differences in sleeping time in mice at a simulated altitude of 8000 *ft or between different strains at a simulated altitude of* 18,000 *ft.* All of our previous work has been performed at a simulated altitude of 18,000 ft. Under these conditions the mice would be subject to hypoxia, since the oxygen tension at this altitude is approximately one-half that at sea level. We, therefore, decided to see if relatively nonhypoxic hypobaric conditions would also result in an alteration of hexobarbital sleeping time. Our results sh•w that a 5-day exposure of C-57 mice to a simulated altitude of 8000 ft does not produce any change in the duration of sleeping time between the exposed and

TABLE 1. HEXOBARBITAL SLEEPING TIME IN MICE EXPOSED TO 8000 ft AND DIFFERENT STRAINS EXPOSED TO 18,000 ft*

Strain	Altitude	Sleeping time (min) Ground	Altitude	N	P
C-57	8,000	68·2 ± 4·6	58·9 ± 4·3	12	N.S.
C-57	18,000	85·1 ± 5·8	55·1 ± 3·8	20	< 0·001
Swiss–Webster	18,000	64·8 ± 5·3	38·7 ± 3·4	10	< 0·001

* Mice exposed to altitude for 5 days, then injected with hexobarbital (125 mg/kg) i.p. N = the number of animals per group; N.S. = not significant.

the control group (Table 1). We also explored the possibility that the alteration of hexobarbital sleeping time by reduced pressure might not be produced in a different strain of mice, since it has been demonstrated that the duration of drug response will vary among different mice strains.[19] For these experiments we used Swiss–Webster mice exposed to a simulated altitude of 18,000 ft for 5 days. Table 1 shows that there was a significant difference in the sleeping time between the treated and the control mice when either C-57 or Swiss–Webster mice were used.

Effect of reduced pressure on total body weight, liver weight, liver water content, microsomal protein, cytochrome P-450, DNA and RNA liver content in mice. The purpose of these experiments was to determine if hypobaric exposure would affect various factors which are involved, directly or indirectly, with drug metabolism in the liver. At the end of the 5-day exposure the body weight, liver weight, and liver water content of the exposed mice were significantly smaller than those of the control mice (Table 2). Body weight was 27 g for the controls as compared to 23 g for the exposed group, a difference of approximately 15 per cent. Similarly, the liver weight and the liver water content of the group exposed to reduced pressure were 15 and 5·6 per cent lower, respectively, than those found for the controls. Although there were significant differences found in the above parameters, there was no significant difference in the liver microsomal protein or cytochrome P-450 content between the two groups. However, the liver DNA and RNA-P content was higher in the group exposed to reduced pressure (Table 2).

TABLE 2. EFFECT OF ALTITUDE (18,000 ft) ON TOTAL BODY WEIGHT, LIVER WEIGHT, LIVER WATER CONTENT, MICROSOMAL PROTEIN, CYTOCHROME P-450, DNA AND RNA LIVER CONTENT IN MICE*

	Ground	Altitude	N	P
Body weight (g)	27 ± 0·03	23 ± 0·5	10	< 0·001
Liver (g wet wt.)	1·23 ± 0·04	1·04 ± 0·03	24	< 0·001
Liver water content (%)	69·6 ± 0·67	64·0 ± 0·88	12	< 0·001
Liver microsomal protein (mg protein/g wet wt.)	12·9 ± 0·3	14·0 ± 0·7	8	N.S.
Cytochrome P-450 (nmoles/mg protein)	0·770 ± 0·046	0·801 ± 0·046	11	N.S.
DNA (μg/100 g protein)	180 ± 6	210 ± 14	12	< 0·025
RNA-P (μg/100 g protein)	858 ± 19	921 ± 21	12	< 0·025

* Mice maintained at altitude for 5 days. N = the number of observations in each group; N.S. = not significant.

Effect of reduced pressure on duration of loss of righting reflex induced by different drugs. A 5-day exposure to a simulated altitude of 18,000 ft resulted in an alteration in the duration of loss of righting reflexes produced by three of the four drugs tested (Table 3). There was a significant decrease observed with sodium hexobarbital (125 mg/kg), zoxazolamine (100 mg/kg), and mephenesin (200 mg/kg) between exposed and control groups. However, no significant difference was seen between the two groups when sodium pentobarbital (50 mg/kg) was injected.

Brain levels of drugs in simulated altitude-exposed mice upon awakening. In order to determine if exposure to reduced pressure resulted in a change in the sensitivity of the brain receptors, the levels of hexobarbital, pentobarbital, zoxazolamine, and mephenesin were assayed in brains of mice upon awakening. The results show that no significant differences were found in the brain levels of any of the four drugs between the simulated altitude-exposed and the control mice upon awakening (Table 4).

Alteration of drug-metabolizing activity in liver microsomal preparations by reduced pressure. In a previous communication we reported that a 5-day exposure to a simulated altitude of 18,000 ft will result in an alteration of drug-metabolizing activity *in vitro*.[7] However, our present findings show that a change in liver weight and liver

TABLE 3. EFFECT OF ALTITUDE (18,000 ft) ON THE LOSS OF RIGHTING REFLEX IN MICE PRODUCED BY HEXOBARBITAL, PENTOBARBITAL, MEPHENESIN AND ZOXAZOLAMINE*

Drug	Dose (mg/kg)	Loss of righting reflex (min)		N	P
		Ground	Altitude		
Hexobarbital	125	82·6 ± 4·2	60·4 ± 5·2	12	< 0·01
Pentobarbital	50	84·0 ± 9·6	88·5 ± 6·7	11	N.S.
Mephenesin	200	50·3 ± 3·2	35·0 ± 4·2	10	< 0·01
Zoxazolamine	100	38·4 ± 2·0	31·5 ± 2·5	20	< 0·025

* Mice exposed to 18,000 ft for 5 days, then injected with indicated drugs. N = the number of animals per group; N.S. = not significant.

TABLE 4. EFFECT OF ALTITUDE (18,000 ft) ON BRAIN LEVELS OF HEXOBARBITAL, PENTOBARBITAL, MEPHENESIN AND ZOXAZOLAMINE OF MICE UPON AWAKENING*

Drug	Dose (mg/kg)	Brain levels (μg/g) Ground	Altitude	N
Hexobarbital	125	15·3 ± 1·4	13·4 ± 1·1	6
Pentobarbital	50	13·6 ± 1·3	14·4 ± 1·3	6
Mephenesin	200	72·6 ± 7·1	71·8 ± 10·0	6
Zoxazolamine	100	67·5 ± 2·9	65·1 ± 5·4	6

* None of the values was significantly different between groups. N = the number of observations in each group.

water content is produced under these conditions. The possibility arose that our original results might be in error, since they were reported on a wet weight basis. We re-examined some of the drugs previously tested using the 100,000 *g* microsomal pellet and calculated our results on the basis of milligrams of microsomal protein. Using this preparation, the oxidative metabolism of aniline, hexobarbital, and nitroanisole, as well as the reduction of dichlorophenolindophenol, was increased in the liver microsomal preparations from the simulated altitude-exposed mice, while the oxidation of methylaniline was not altered (Table 5). These results agree with those previously reported.

TABLE 5. EFFECT OF ALTITUDE (18,000 ft) ON DRUG METABOLISM IN MOUSE LIVER MICROSOMES*

Drug	Ground (nmoles/mg protein/30 min)	Altitude	N	P
Aniline	51·6 ± 2·1	73·0 ± 0·2	6	< 0·001
Hexobarbital	95·0 ± 11·0	131·0 ± 8·0	12	< 0·01
Nitroanisole	65·1 ± 1·8	77·9 ± 1·3	12	< 0·001
Methylaniline	57·6 ± 1·3	53·2 ± 1·7	18	N.S.
Dichlorophenolindophenol	91·0 ± 2·6†	151·2 ± 4·8†	12	< 0·001

* Drug metabolism from individual liver microsomal preparations was performed in triplicate. N = the number of animals per group; N.S. = not significant.
† $\times 10^{-3}$.

Electron microscopy. Comparison of electron photomicrographs prepared from livers of controls and simulated altitude-exposed mice revealed some striking differences (Figs. 2 and 3). There was a marked swelling of the mitochondria in the group maintained under hypobaric conditions, but no indication of any alteration of the cristae. A considerable dilation of the endoplasmic reticulum was also produced, resulting in the appearance of large spaces in the hepatic cell.

DISCUSSION

Chronic exposure to a simulated altitude of 18,000 ft results in changes in the rate of drug metabolism *in vitro*. Of the five drugs tested in these experiments, four were metabolized at a faster rate by hepatic microsomes from mice exposed to reduced pressure while one of the drugs was not affected. These results are similar to those previously reported using a 9000 *g* supernatant fraction.[7] Exposure to altitude or hypoxia does not always result in a uniform change in drug metabolism. Mustala and Azarnoff[20] could not find any differences in plasma or brain levels of hexobarbital or zoxazolamine in rats exposed to altitude. On the other hand, it has been reported that a 3-hr exposure to hypoxic conditions will produce a decrease in the metabolism of hexobarbital and aminopyrine in male rats but not in females.[5]

The body weight, liver weight, and liver water content were smaller in the simulated altitude-exposed mice than in the control group. The difference in body and liver weight is probably due to anorexia during the initial period of exposure.[21] A decreased food intake in male rats and mice results in an increased hexobarbital sleeping time and a decreased metabolism of this drug *in vitro*.[4,5] Starvation of male rats with or without subsequent refeeding produces alterations in either the K_m or V_{max}, or both, for different substrates oxidized by the hepatic drug-metabolizing system.[22] In the only experiment in which changes in K_m, V_{max} and pharmacologic activity of the same drug were tested, starvation of male rats produced a decrease in the K_m and the V_{max} for hexobarbital as well as an increased hexobarbital sleeping time. Others have shown that food deprivation in male mice increases hexobarbital sleeping time and decreases oxidative metabolism of drugs such as hexobarbital, chlorpromazine, and acetanilide, and that these changes are promptly restored to control rates after a 24-hr refeeding.[4] Thus initial hypoxic anorexia alone or in combination with a later restoration of normal feeding would not appear to account for the decreased drug action and increased drug metabolism observed in our experiments. Similarly, the dehydration of the mice as reflected by liver water loss could not explain our results, since water deprivation results in a reduction rather than an increase in hexobarbital metabolism.[23]

In our experiments a decreased hexobarbital sleeping time in simulated altitude-exposed mice did not appear until the third day of exposure (Fig. 1). There were also no significant differences in sleeping time in mice exposed to this environment for 1 or 8 hr. This is in contrast to the results of others who have reported a prolongation of hexobarbital sleeping time in rats acutely exposed to altitude or hypoxia.[20] This difference may be due to the species used, since rats given phenobarbital (50 mg/kg) during chronic simulated altitude exposure in our laboratory have been observed to be more sedated than drug-treated controls.*

The length of time required for the appearance of a decreased hexobarbital sleeping time induced by reduced pressure is similar to that observed when the same effect is produced by administration of phenobarbital.[24] It has been demonstrated that this action of phenobarbital is produced by a stimulation of hepatic protein and cytochrome P-450 synthesis.[1] Acute or chronic hypobaric exposure will result in an increase in the activity of tryptophan oxygenase and tyrosine-α-ketoglutaric acid transaminase.[25,26] It is, therefore, conceivable that hypobaric exposure could also result in an increase in

* J. H. Landez, personal communication.

the synthesis of hepatic drug-metabolizing enzymes. Although the increases in DNA and RNA-P found in our experiments would tend to support this hypothesis, the absence of any changes in microsomal protein or cytochrome P-450 does not. It should be pointed out that induction of increased hepatic drug metabolism may or may not be paralleled by an increased cytochrome P-450 content. A correlation is observed between induction with phenobarbital and increased hepatic cytochrome P-450 content.[27] However, 3-methylcholanthrene produces no such correlation and also appears to induce the synthesis of a hemoprotein with spectral characteristics different from cytochrome P-450.[28] Stimulation of drug metabolism could also be effected by changes in other parameters of the enzyme system. For example, phenobarbital,[29] 3-methylcholanthrene,[30] and starvation[22] alter the K_m and V_{max} for different substrates of hepatic drug-metabolizing enzymes. The possibility also exists that increased synthesis of other components of the enzyme system such as NADPH-cytochrome P-450 reductase could act to enhance drug metabolism.

Exposure to a simulated altitude of 18,000 ft decreased the sleeping time induced by hexobarbital, mephenesin, and zoxazolamine, but not that with pentobarbital. The fact that brain levels of these drugs were the same for both exposed and control mice at the time of awakening would indicate that the differences in sleeping time observed were not due to alterations in the sensitivity of brain receptors.

The electron photomicrographs indicate that some structural changes in the liver were produced by the hypobaric environment. These changes are rather general in nature and similar to those reported to occur in liver and other tissues subjected to hypoxic conditions.[31] Under our environmental conditions the mice were exposed to hypoxia and reduced pressure. There are few experiments on the effect of decreased oxygen on drug metabolism in the intact animal. Cumming and Mannering[32] reported impairment of hexobarbital metabolism when arterial blood P_{O_2} fell below 45 mm Hg in rats. The fact that there was no alteration in sleeping time found in mice exposed to a simulated altitude of 8000 ft, where hypoxia would be minimal, suggests that the differences we found in drug action at a simulated altitude of 18,000 ft may be due primarily to hypoxia. Our experiments do not permit us to differentiate between effects which may have been due to hypoxia and those from reduced pressure. That reduced pressure may also exert some effect is evidence by several reports showing that drug action is altered under a hypobaric environment containing ground-equivalent oxygen.[33–35]

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