A STUDY OF THE EFFECTS OF THE SPACECRAFT ENVIRONMENT ON DRUG ACTION

final report to

NASA MANNED SPACECRAFT CENTER
HOUSTON, TEXAS

ON WORK PERFORMED
JULY 1, 1969 — OCTOBER 31, 1970

CONTRACT NO NAS 9-9606

ARTHUR D. LITTLE, INC.
CAMBRIDGE, MASSACHUSETTS

OCTOBER 31, 1970

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Arthur D. Little, Inc.
Cambridge, Massachusetts 02140

October 31, 1970

FOREWORD

This program for a study of the effects of the spacecraft environment on drug action was carried out during the period July, 1969 to November, 1970, and was the subject of our unsolicited proposal of January 31, 1969.

Dr. Elliot Harris, DB5, of the NASA Manned Spacecraft Center, acted as technical officer for this agency. Drs. F. J. Bullock, D. W. Yesair, D. Shooter, and J. Howes, Mrs. L. Remington, R. J. Bruni, K. Siemen, S. Locke, and Mrs. M. Callahan comprised the Arthur D. Little, Inc. project team. Professor Louis S. Harris, University of North Carolina Medical School, served as a consultant to Arthur D. Little, Inc. on certain aspects of the work.

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SUMMARY

A study of possible effects of 5 psia oxygen on oxidative drug metabolism has been carried out in the rat. Several drugs in the Apollo medical kit were studied. Exposures to 5 psia oxygen of approximately four, eight, twelve, and twenty-eight days were carried out. This environment had no effect on liver size, amount of liver microsomal protein, or cytochrome P-450 and cytochrome b_5 levels in the rat liver.

In vitro metabolic rates for oxidation of the following materials were studied using rat liver microsomes from rats exposed to 5 psia oxygen: meperidine (Demerol®), propoxyphen (Darvon®), cyclizine (Marezine®), hexobarbital, and aniline. Use of the first three substrates provides a measure of microsomal N-demethylase activity, hexobarbital provides a measure of barbiturate oxidase activity, and aniline provides a measure of activity of an aryl hydroxylase. From Lineweaver-Burk plots of the rate data, the kinetic parameters K_m, and V_{max} were obtained for most of these substrates. No differences in oxidative metabolic rates were found between oxygen-exposed groups and control groups of rats.

Some pharmacological studies were carried out using rats exposed to 5 psia oxygen. An exposure of twenty-eight days did not effect secobarbital-induced sleeping times or meperidine (Demerol®) analgesia in the rat. An eight-day exposure produced no effect in propoxyphen (Darvon®) analgesia in the rat. Some study of amphetamine was done, but results are inconclusive. Pharmacological studies were carried out one hour after animals were removed from the altitude chamber.

Potentiation of hexobarbital and secobarbital activity

by diphenoxylate (Lomotil®) was demonstrated in the rat. *In vitro* barbiturate metabolism by liver microsomes was unchanged in diphenoxylate (Lomotil®)-treated rats. The potentiation does not appear to involve inhibition of barbiturate metabolism by diphenoxylate (Lomotil®). Diphenoxylate (Lomotil®) does not potentiate chloral hydrate-induced sleeping times in the rat. Exposure of animals to 5 psia oxygen for six days does not result in further potentiation of secobarbital activity by diphenoxylate (Lomotil®).

A cylindrical Plexiglas® environmental chamber adequate for controlling and monitoring the necessary oxygen-rich atmosphere was built. The gas recirculation system included provision for removal of carbon dioxide. A relatively inexpensive commercial gas chromatograph was modified to provide for automatic sampling of chamber entrance and exit gas at thirty-minute intervals. Unmodified space suit sleeves and gloves were interfaced with the chamber and shown to provide a practical means of carrying out certain pharmacological studies at pressures down to 450 mm. It was not possible to carry out such studies in a routine way at 260 mm using the unmodified space suit sleeves and gloves. Similarly, studies of possible interaction of pain-induced stress and oxygen stress could not be carried out in this chamber.

It appears that a 5 psia 100 percent oxygen environment is not sufficiently stressful to alter action of the particular drugs studied in the rat or to alter their biotransformation rates.

I. INTRODUCTION

A summary of medical experience in Apollo 7 through 11 spaceflights has recently appeared.[1] The medical kits carried on various manned space flights have included the following systemically acting agents: Demerol, Marezine, Dexedrine, Darvon Compound, Actifed, Lomotil, Achromycin, Ampicillin, Seconal, Benedryl, Tylenol, Scopolamine-Dexedrine, and Aspirin. Drugs used by astronauts during various Apollo missions through Apollo 11 include Marezine, Dexedrine, Darvon Compound, Actifed, Lomotil, Aspirin, Seconal, Tylenol, and Scopolamine-Dexedrine. The general approach to the use of drugs by astronauts during space flights has been cautious but in-flight medical problems requiring their use have occurred.

There has been little systematic study of possible effects of the spacecraft environment of drug action. The cabin atmosphere in the United States' spacecraft of the Apollo series is presently a mixture of 64 percent oxygen and 36 percent nitrogen at launch. The crew is denitrogenated for three hours prior to launch and following launch the nitrogen is gradually purged from the cabin atmosphere. Oxygen analysis during Apollo 7 showed a gradual enrichment from pO_2 about 170 mm[a] to pO_2 about 255 mm during ten days. pO_2 reached about 230 mm during the first three days. Total cabin pressure is 5.0 psia. Accordingly, the cabin atmosphere during the major part of the flights consists of 90-96 percent oxygen. Possible effects of this high oxygen atmosphere on drug action were the concern of this project.

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a Values refer to oxygen partial pressures in mm Hg.

It was our view in initiating work on the question of effects of the spacecraft environment on drug action that the [illegible] drug metabolism needed consideration. Many of the drugs contained in Apollo medical kits are known to be metabolized by cellular processes largely in the microsomal fraction of the liver. Rat liver mitochondria have been reported to become enlarged and contain increased numbers of cristae in animals exposed to 5 psia oxygen for prolonged periods.[2] There are species differences in this response, but similar ultrastructural abnormalities in hepatocellular organelles are seen in dogs and monkeys.[3] No evidence has been produced to exclude ultrastructural changes in the microsomal fraction of liver and the highly lipidic nature of microsomes might make them a reasonable site for appearance of effects produced by hyperbaric oxygen. Effects of hyperbaric oxygen on lipid metabolism are well documented.[4]

The intimate involvement of cytochrome P-450 in oxidative drug metabolism is known.[5] Since changes in levels of cytochromes P-450 and b_5 may frequently be correlated with changes in rates of drug metabolism, determinations of these cytochromes have been included in the overall profiling of the effects of 5 psia oxygen. Assessment of effects of the environment on drug metabolism was based largely on studies of *in vitro* metabolism by microsomal preparations, but some studies of drug action *in vivo* were carried out with animals exposed to 5 psia oxygen. The particular drugs studied were selected from the Apollo 7 medical kit. The results of these various studies and the details involved in their execution are described in the following sections.

III. STUDY OF THE EFFECT OF 5 psia OXYGEN ON DRUG METABOLISM *IN VITRO*.

A. Organization of Results

The presentation of results of our *in vitro* metabolism experiments will be organized as follows. The individual exposed groups are organized according to length of exposure period in Table 1. Included are the weight ranges of each group, number of animals in each group, liver weights, microsomal protein yield, and cytochromes P-450 and b_5 levels. Data for each control group are given for comparison.

Supplementary comments and data pertinent to each drug study are included in the section devoted to that drug. A single summarizing discussion is given at the end of the section.

B. Presentation of Metabolism Rates

Throughout this report, rates will be expressed in terms of millimicromoles of substrate metabolized (or millimicromoles of metabolite appearing)/mg microsomal protein/minute. These units are commonly used by other workers.[6] There proved to be no differences in liver weights between control and oxygen-exposed groups that could not be attributed to small differences in the sizes of the animals. The yield of microsomal protein obtained from each group of livers was similar (see Table 1). Accordingly, the rate data need not be converted to, for example, units of rate/animal/time for comparison of groups. Most experiments have been done on at least two different groups of animals for each time period and all metabolic results are included in the various tables to follow, even if the rate data did not subsequently give "good" Lineweaver-Burk fits.

TABLE 1

DATA CHARACTERIZING LIVER PREPARATIONS FOR INDIVIDUAL GROUPS EXPOSED TO 5 psia OXYGEN

	Wt. range (g)	Liver weight (g)	Ratio*	No.[a] Animals	Microsomal yield[b]	Cytochrome[c] P-450	Cytochrome b_5[c]	Assayed for
				84-Hour Exposures				
Group 1	150 ± 16.0	8.2 ± 0.55	.053	5	25.5 ± 1.2	0.224 ± .021	0.242 ± .010	Meperidine N-
Group 1(C)[d]	150 ± 16.0	9.8 ± 0.81	.063	4	25.1 ± 0.5	0.218 ± .050	0.240 ± .020	demethylase
Group 2	171.5 ± 8.2	8.4 ± 0.66	.049	6	22.7 ± 2.6	0.207 ± .032	0.224 ± .022	Meperidine N-
Group 2(C)	147.8 ± 36.7	6.8 ± 2.4	.046	6	22.7 ± 3.2	0.205 ± .038	0.243 ± .026	demethylase
Group 3	210.5 ± 18.2	21.0 ± 1.5	.100	3(2)	28.5 ± 0.5	0.165 ± .02	0.221 ± .009	Aniline hydroxy-
Group 3(C)	190.8 ± 6.5	18.9 ± 1.0	.099	3(2)	29.06± 0.68	0.175 ± .038	0.210 ± .009	lase
Group 4	219 ± 10.6	10.0 ± 0.78	.046	3(2)	26.4 ± 2.09	0.159 ± .012	0.219 ± .004	Aniline hydroxy-
Group 4(C)	213 ± 12.9	10.2 ± 1.06	.048	3(2)	22.6 ± 2.35	0.177 ± .012	0.270 ± .017	lase
Group 5	140 ± 15.0	8.3 ± 0.41	.057	6	20.8 ± 0.68	0.133 ± .030	0.153 ± .010	Propoxyphen N-
Group 5(C)	140 ± 15.0	6.2 ± 0.49	.043	5	21.2 ± 0.38	0.148 ± .010	0.160 ± .020	demethylase
Group 6	221 ± 19.8	10.7 ± 1.1	.049	2(3)	27.2 ± 1.8	0.189 ± .038	0.265 ± .016	Barbiturate
Group 6(C)	203 ± 37.7	9.9 ± 1.0	.049	2(3)	26.8 ± 1.9	0.181 ± .017	0.215 ± .021	oxidase
Group 7	206.5 ± 10.1	10.1 ± 0.5	.049	2(3)	26.0 ± 2.4	0.256 ± .04	0.263 ± .014	Meperidine, cyclizine
Group 7(C)	204.3 ± 10.9	10.1 ± 0.7	.050	2(3)	20.9 ± 5.3	0.257 ± .04	0.288 ± .019	N-demethylase

TABLE 1 - Continued

	Wt. range (g)	Liver weight (g)	Ratio*	No.[a] Animals	Microsomal yield[b]	Cytochrome P-450[c]	Cytochrome b_5[c]	Assayed for
188 Hour Exposures								
Group 8	165 ± 21.5	8.0 ± 1.5	.049	3(2)	27.0 ± 1.9	0.164 ± .030	0.263 ± .046	Meperidine, cyclizine
Group 8(C)	148 ± 31.1	7.4 ± 1.5	.050	3(2)	23.1 ± 2.0	0.174 ± .030	0.277 ± .030	N-demethylase
Group 9	302 ± 20.8	13.6 ± 1.0	.045	3(2)	24.8 ± 1.8	0.152 ± .010	0.257 ± .010	Meperidine, Propoxy-
Group 9(C)	275 ± 12.2	12.7 ± 1.4	.046	3(2)	22.3 ± 2.3	0.169 ± .050	0.255 ± .040	phen N-demethylase
Group 10	296.0 ± 12.8	13.7 ± 1.7	.046	3(2)	37.9 ±11.4	0.143 ± .030	0.207 ± .040	Meperidine, cyclizine
Group 10(C)	278.0 ± 18.5	12.2 ± 1.5	.044	3(2)	21.2 ± 0.5	0.176 ± .040	0.211 ± .030	N-demethylase
Group 11	202.3 ± 11.9	9.7 ± .1	.048	3(2)	26.5 ± 2.2	0.138 ± .056	0.197 ± .010	Aniline hydroxy-
Group 11(C)	188.6 ± 6.7	9.14± 1.2	.048	3(2)	26.6 ± 2.7	0.161 ± .015	0.205 ± .034	lase
284 Hour Exposures								
Group 12	202.7 ± 22.7	9.02± 0.9	.045	3(2)	25.1 ± 3.4	0.178 ± .070	0.219 ± .017	Aniline hydroxy-
Group 12(C)	190.8 ± 20.0	7.02± 1.2	.037	3(2)	30.6 ± 1.5	0.169 ± .090	0.224 ± .014	lase
Group 13	258.7 ± 19.7	11.8 ± 1.3	.046	3(2)	31.3 ± 5.8	0.151 ± .010	0.207 ± .020	Cyclizine, propoxy-
Group 13(C)	259.7 ± 12.9	13.02± 1.2	.050	3(2)	38.6 ± 3.42	0.160 ± .016	0.215 ± .020	phen N-demethylase
308 Hour Exposures								
Group 14	172.0 ± 8.7	8.1 ± .7	.047	3(2)	30.4 ± 1.6	0.173 ± .017	0.214 ± .001	Meperidine, cyclizine
Group 14(C)	230.0 ± 25.4	12.2 ± 0.6	.053	3(2)	27.9 ± 3.2	0.152 ± .008	0.215 ± .005	propoxyphen N-demethylase
Group 15	190.7 ± 19.8	10.0 ± 2.1	.053	4	26.05± 1.57	0.163 ± .013	0.216 ± .023	Barbiturate oxidase
Group 15(C)	196.8 ± 8.2	9.9 ± 1.2	.050	4	28.97± 0.92	0.151 ± .028	0.226 ± .024	

TABLE 1 - Continued

	Wt. range (g)	Liver weight (g)	Ratio*	No.[a] Animals	Microsomal yield[b]	Cytochrome P-450[c]	Cytochrome b_5[c]	Assayed for
				660 Hour Exposures				
Group 16	190 ± 18.5	6.5 ± .6	.034	6	23.5 ± 1.01	0.150 ± .020	0.320 ± .020	Aniline hydroxy-
Group 16(C)	245 ± 16.4	10.5 ± 1.3	.043	6	24.5 ± 4.10	0.180 ± .020	0.270 ± .070	lase
Group 17	241.7 ± 20.4	10.0 ± 1.0	.042	6	26.8 ± 3.1	0.190 ± .00	0.240 ± .010	Propoxyphen,
Group 17(C)	244.3 ± 15.8	12.7 ± 1.3	.053	6	27.4 ± 3.8	0.150 ± .010	0.270 ± .050	Meperidine N-demethyl-
								ase
				960 Hour Exposures				
Group 18	190.8 ± 19.7	10.1 ± 0.9	.053	6	24.5 ± 2.8	0.210 ± .02	0.290 ± .04	Barbiturate
Group 18(C)	196.8 ± 8.2	11.3 ± 1.3	.057	6	24.2 ± 2.3	0.199 ± .01	0.270 ± .03	oxidase

* Ratio of mean liver weight/mean body weight.

a Designations such as 3(2) signify three groups with two animals per group.

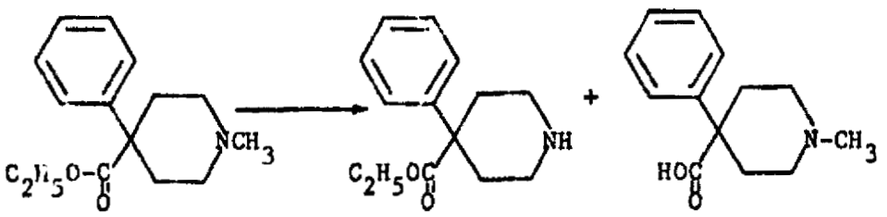
b Units are milligrams/g of liver

c Units are optical densities - see page 61 for experimental details

d The bracketed (C) indicates the control group

C. Meperidine (Demerol) N-Demethylase

The two major metabolic pathways for meperidine (1) are N-demethylation and ester hydrolysis as indicated below.



1

Microsomal N-demethylase activity was studied by following the rate of appearance of formaldehyde. The rate of formaldehyde production was found to be linear with time out to about 10 minutes (Figure 1). Experiments carried out with microsomes from individual rats showed individual variability in our preparations to be within reasonable limits (±10%). Accordingly, in later phases of the work we felt justified in pooling microsomal preparations. This gave us the opportunity to study several drugs with a single group of oxygen-exposed animals.

Seven minutes was found to be an appropriate time at which to aliquot for determination of metabolism rates. The rate data obtained in this way with meperidine using rats exposed to 5 psia oxygen for varying periods up to 28 days are given in Table 2. Rates obtained at several substrate concentrations were used to construct Lineweaver-Burk plots. Some plots are given in Figure 2.

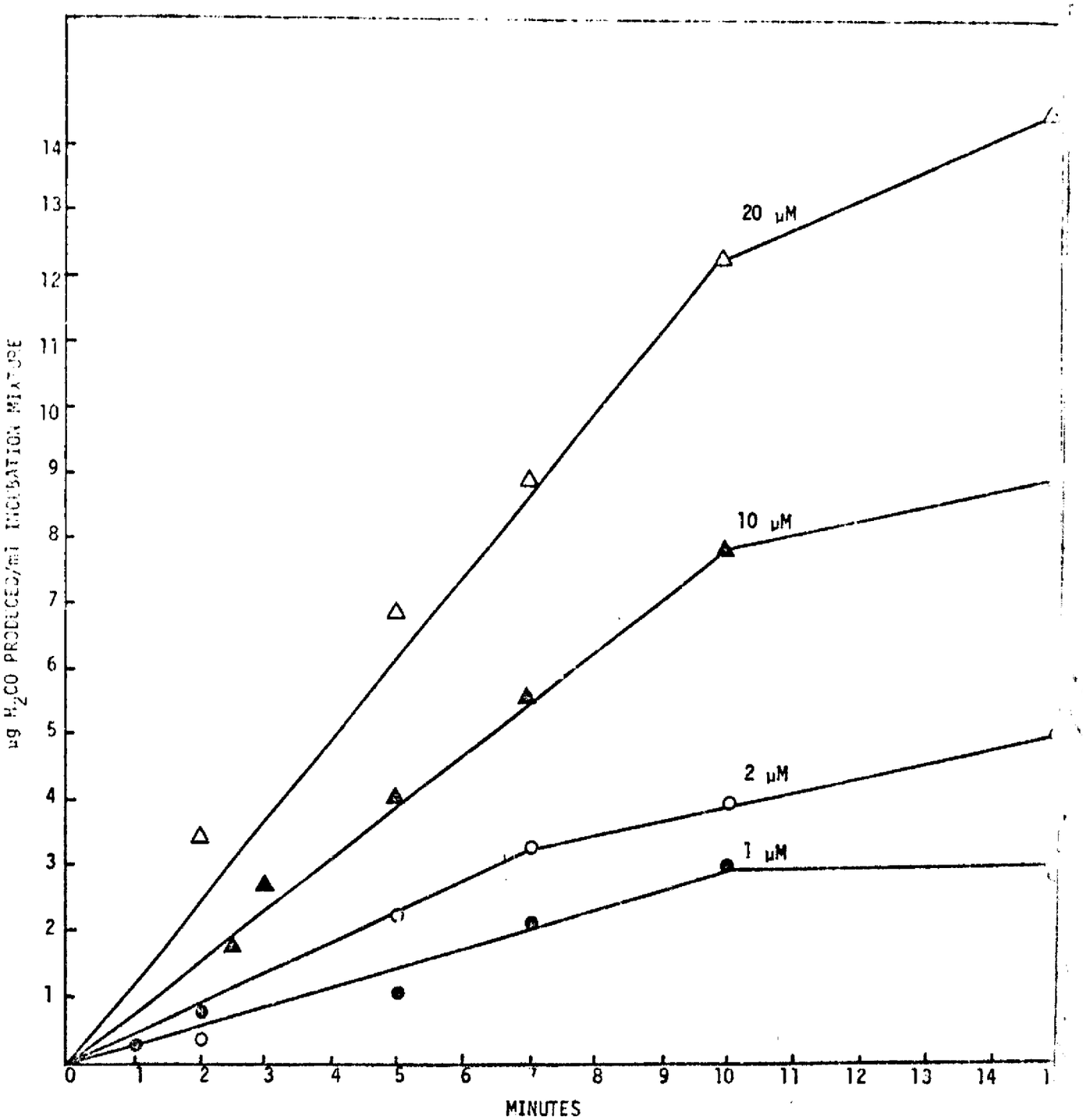


FIGURE 1: Rate of Production of Formaldehyde from Meperidine at Several Substrate Concentrations.

TABLE 2

METABOLIC RATES OF MEPERIDINE *IN VITRO* USING LIVER MICROSOMES FROM RATS EXPOSED TO 5 psia OXYGEN AND TO NORMAL ATMOSPHERIC CONDITIONS

Substrate[a] Level (μM)	(mμM H_2CO/mg protein/min)	(mμM H_2CO/mg protein/min)
	88 Hour Exposures	
	Control Group 1	Oxygen-exposed Group 1
1	1.29 ± 0.77	1.05 ± 0.44
2	1.10 ± 0.78	1.25 ± 0.53
10	2.01 ± 0.70	2.48 ± 0.67
20	1.47 ± 0.53	2.43 ± 0.65
	Control Group 2	Oxygen-exposed Group 2
1	1.57 ± 0.23	0.96 ± 0.26
2	1.77 ± 0.43	1.50 ± 0.41
10	1.62 ± 0.37	1.35 ± 0.27
20	2.08 ± 0.53	1.49 ± 0.30
	188 Hour Exposures	
	Control Group 8	Oxygen-exposed Group 8
1	1.40 ± 0.40	2.40 ± 1.53
2	1.73 ± 0.65	2.67 ± 1.10
10	2.40 ± 0.80	2.86 ± 0.46
20	2.60 ± 0.35	2.38 ± 0.42
	Control Group 9	Oxygen-exposed Group 9
1	1.34 ± 0.63	1.39 ± 0.54
2	1.91 ± 1.13	1.77 ± 0.44
10	2.50 ± 1.04	2.12 ± 0.65
20	2.82 ± 1.18	2.45 ± 0.83
	Control Group 10	Oxygen-exposed Group 10
1	2.42 ± 0.59	1.82 ± 0.13
2	2.42 ± 0.22	1.97 ± 0.10
10	3.15 ± 0.13	2.70 ± 0.33
20	3.07 ± 0.22	2.70 ± 0.25

TABLE 7 (Continued)

308 Hour Exposures

	Control Group 14	Oxygen-exposed Group 14
1	1.49 ± 0.314	1.63 ± 0.33
2	1.83 ± 0.40	1.94 ± 0.54
10	--	2.39 ± 0.39
20	2.39 ± 0.63	2.81 ± 0.94

660 Hour Exposures

	Control Group 17	Oxygen-exposed Group 17
1	0.6 ± 0.1	0.6 ± 0.3
2	0.8 ± 0.2	0.8 ± 0.5
10	1.3 ± 0.3	1.0 ± 0.6
20	1.6 ± 0.3	1.3 ± 0.4

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a Substrate concentrations are per 6 ml of incubation mixture, see Experimental, page 59 for further details.

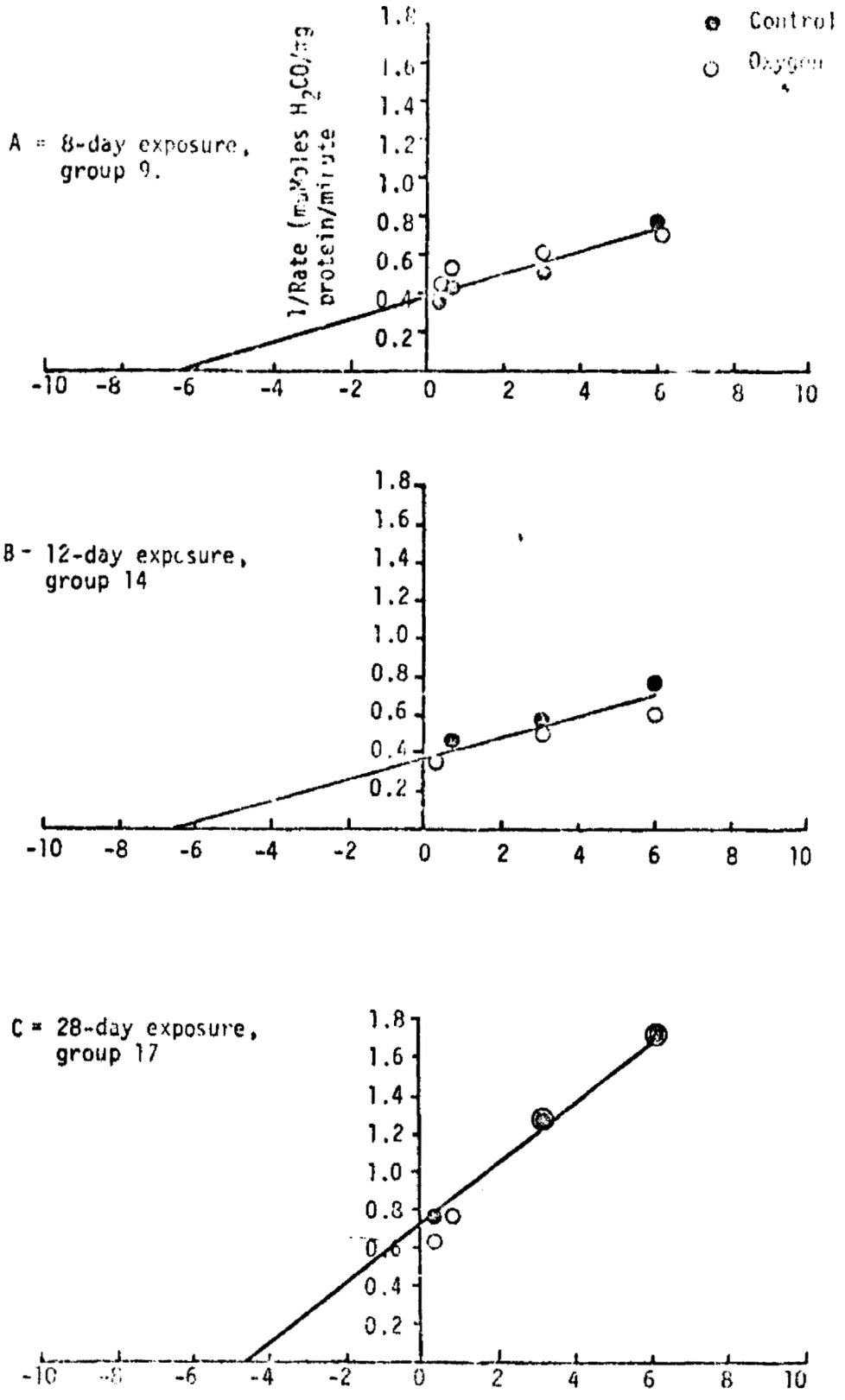
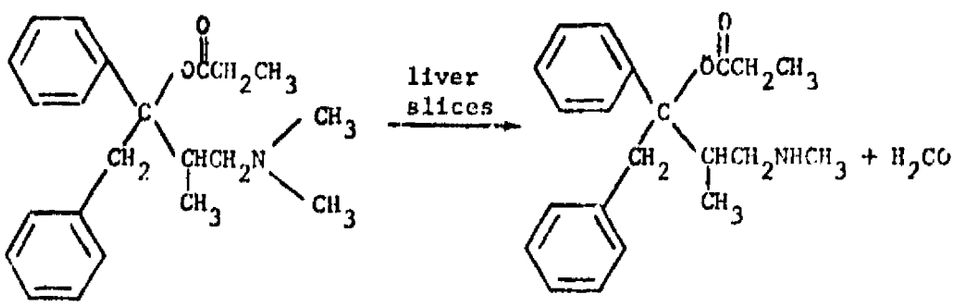


FIGURE 2: Lineweaver-Burk Plots for Meperidine Metabolism In Vitro.

D. Propoxyphen (Darvon) N-Demethylase

A previous study[7] demonstrated that incubation of propoxyphen with rat liver slices accomplished N-dealkylation. This reaction occurs with liver microsomes and was used to study the effects of 5 psia oxygen on propoxyphen metabolism *in vitro*.



A preliminary study established that the rate of production of formaldehyde by N-demethylation was linear out to seven minutes and that the rate was substrate-dependent (Figure 3). At 20 μmoles substrate/ml incubation mixture, there was clearly inhibition by substrate. Rates of *in vitro* metabolism in various groups of rats exposed to 5 psia oxygen are given in Table 3. Some Lineweaver-Burk plots of the rate data are shown in Figure 4. Because inhibition was observed at high substrate levels, the data for 20 μmoles of substrate are not plotted.

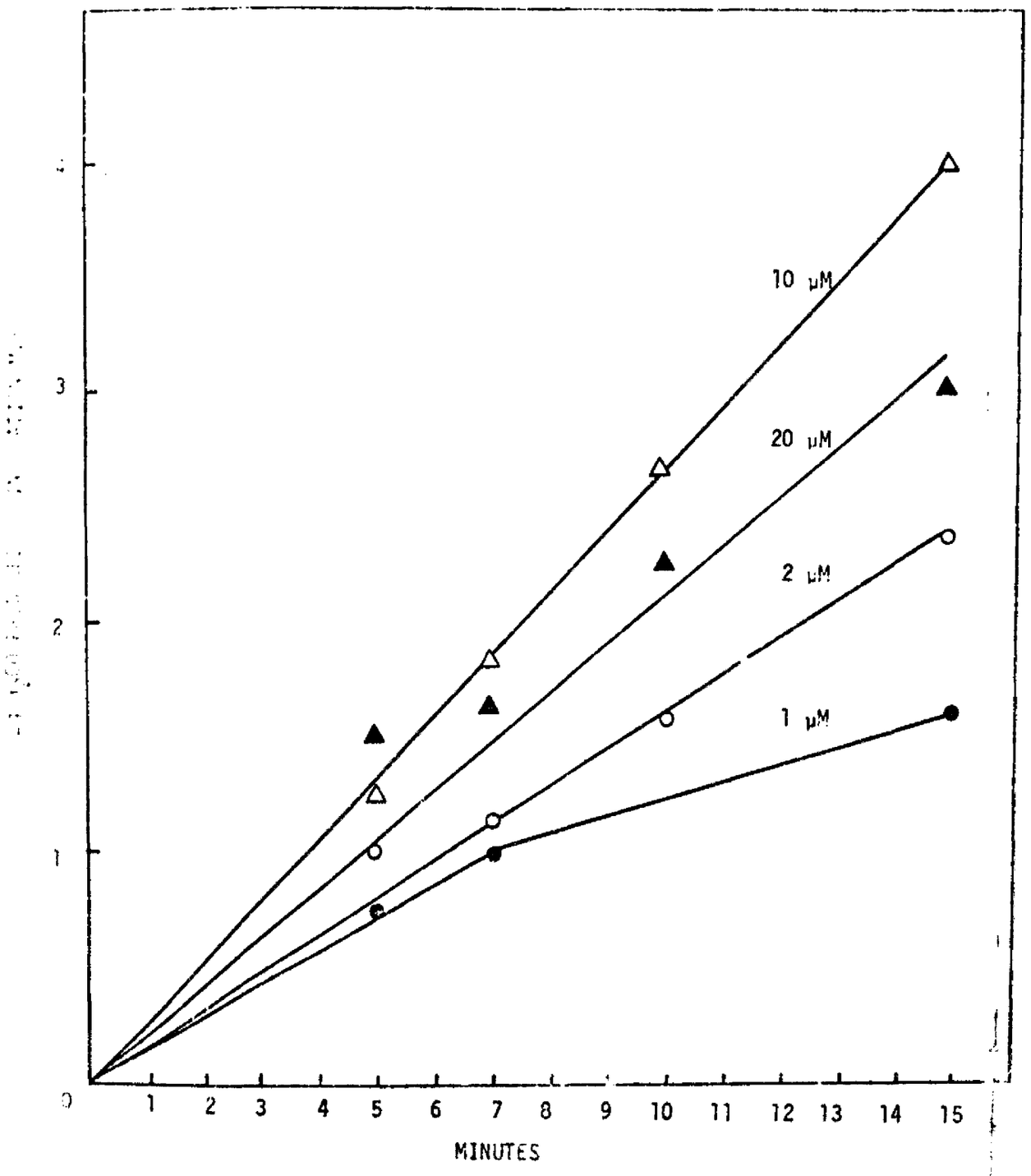


FIGURE 3: Rate of Production of Formaldehyde from Propoxyphen at Several Substrate Concentrations.

TABLE 3

METABOLIC RATE OF PROPOXYPHEN *IN VITRO* BY LIVER MICROSOMES FROM RATS EXPOSED TO 5 psia OXYGEN AND TO NORMAL ATMOSPHERIC CONDITIONS.

Substrate[a] Level (μM)	(mμM H_2CO/mg protein/min	(mμM H_2CO/mg protein/min
	88 Hour Exposures	
	Control Group 5	Oxygen-exposed Group 5
1	0.55 ± 0.34	0.59 ± 0.40
2	1.20 ± 0.75	1.10 ± 0.42
10	1.53 ± 0.65	1.81 ± 0.66
20	1.36 ± 0.74	1.37 ± 0.73
	188 Hour Exposures	
	Control Group 9	Oxygen-exposed Group 9
1	1.70 ± 0.57	1.40 ± 0.39
2	2.06 ± 0.40	1.69 ± 0.50
10	2.36 ± 0.90	1.73 ± 0.41
20	1.82 ± 0.69	1.34 ± 0.32
	284 Hour Exposures	
	Control Group 13	Oxygen-exposed Group 13
1	1.24 ± 0.22	1.42 ± 0.53
2	1.75 ± 0.13	2.11 ± 0.32
10	2.02 ± 0.24	1.82 ± 0.22
20	1.52 ± 0.06	1.32 ± 0.47
	308 Hour Exposures	
	Control Group 14	Oxygen-exposed Group 14
1	1.60 ± 0.37	1.53 ± 0.25
2	2.31 ± 0.13	1.79 ± 0.24
10	2.48 ± 0.70	2.59 ± 0.78
20	2.54 ± 0.38	2.02 ± 0.24

TABLE 3 - Continued

660 Hour Exposures

	Control Group 17	Oxygen-exposed Group 17
1	0.6 ± 0.2	0.6 ± 0.3
2	0.8 ± 0.3	0.8 ± 0.4
10	1.1 ± 0.4	1.3 ± 0.4
20	0.8 ± 0.4	0.9 ± 0.1

a Substrate concentrations are per ml of incubation mixture, see Experimental, page 59, for details.

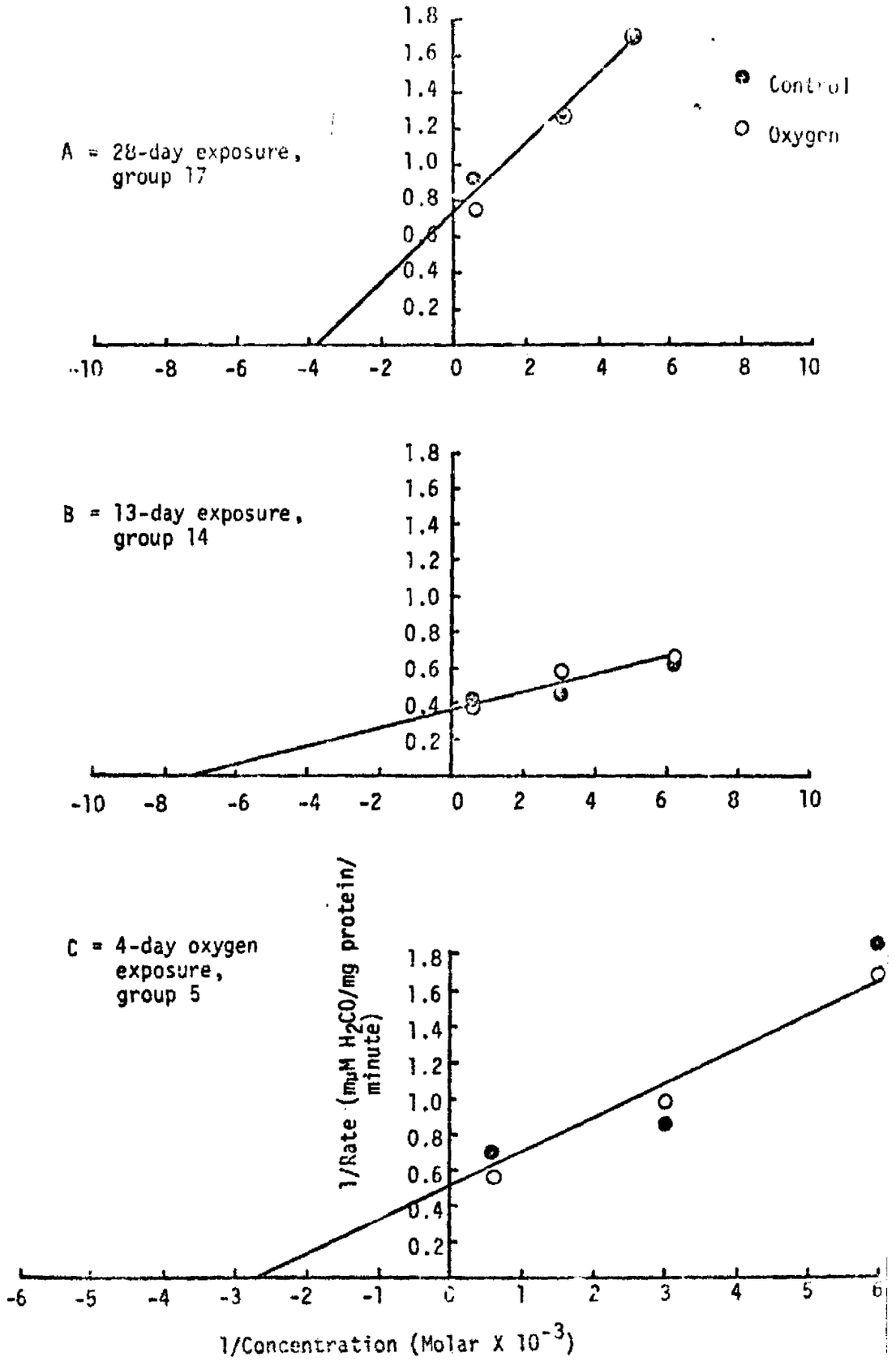
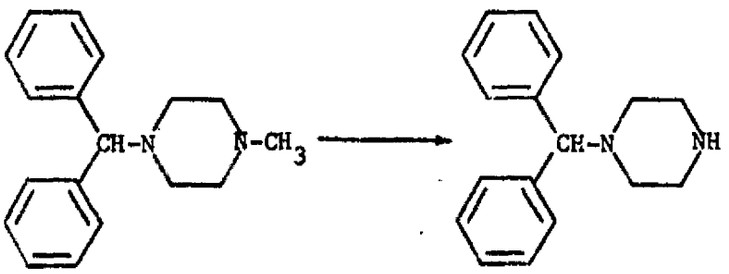


FIGURE 4: Lineweaver-Burk plots for Propoxyphen Metabolism In Vitro.

E. Cyclizine (Marezine) N-Demethylase

Cyclizine has been demonstrated to be metabolized by N-demethylation in the rat and dog.[8] In our work with this drug using rat liver microsomes we found that the rate of production of formaldehyde did not show a substrate dependence for initial rates. The rate of production of formaldehyde at several substrate concentrations is shown as Figure 5 for two age ranges of rats. It was not possible to make a Lineweaver-Burk plot of the data. Comparisons of groups were made by a point by point comparison of rates at several substrate concentrations. The rates in Table 4 were determined by sampling each incubation mixture at seven minutes as has been described for other drugs.



F. Barbiturate Oxidase

Secobarbital is the barbiturate being carried and used on manned spaceflights. This barbiturate was found not to be metabolized *in vitro* by rat liver microsomes at a rate sufficient to permit reliable kinetic studies. To permit a study of the effect of 5 psia oxygen on hepatic barbiturate oxidase to be carried out we used hexobarbital as substrate. Hexobarbital was known to be rapidly metabolized by rat liver microsomes *in vitro*.[9]

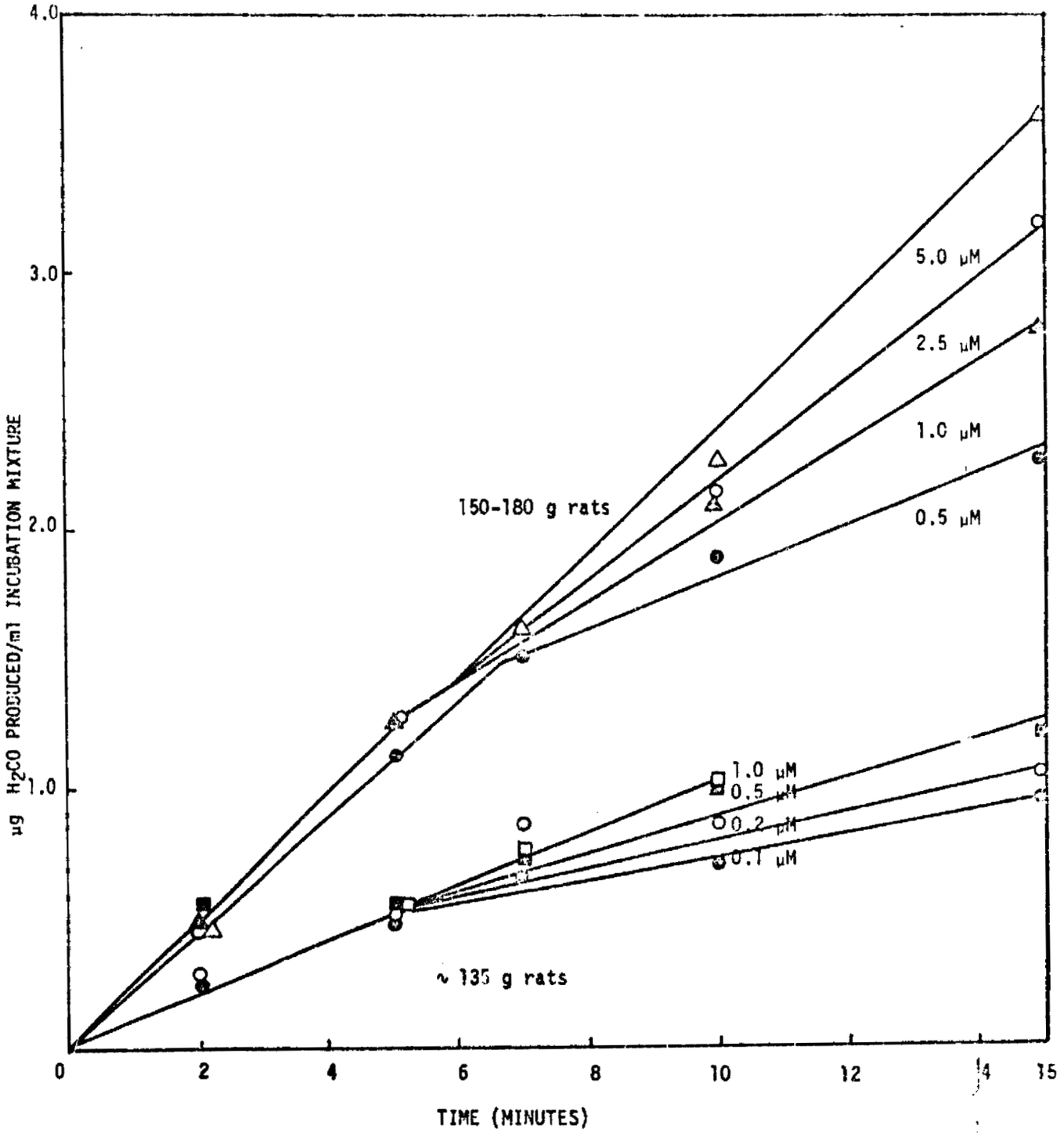


FIGURE 5: Rate of Production of Formaldehyde from Cyclizine at Several Substrate Concentrations.

TABLE 4

METABOLIC RATES OF CYCLIZINE *IN VITRO* USING LIVER MICROSOMES FROM RATS EXPOSED TO 5 [illegible] OXYGEN AND TO NORMAL ATMOSPHERIC CONDITIONS

Substrate[a] Level (μM)	(mμM H_2CO/mg protein/min)	(mμM H_2CO/mg protein/min)
	88 Hour Exposures	
	Control Group 7	Oxygen-exposed Group 7
0.1	0.434 ± 0.16	0.566 ± 0.06
0.2	0.769 ± 0.17	0.707 ± 0.05
0.5	1.05 ± 0.36	1.05 ± 0.15
1.0	1.88 ± 0.92	1.79 ± 0.66
	184 Hour Exposures	
	Control Group 8	Oxygen-exposed Group 8
0.1	1.20 ± 0.35	1.80 ± 0.92
0.2	0.80 ± 0.28	0.07 ± 0.11
0.5	1.06 ± 0.58	0.87 ± 0.12
1.0	1.60 ± 0.57	1.00 ± 0.20
	188 Hour Exposures	
	Control Group 10	Oxygen-exposed Group 10
0.1	1.90 ± 0.12	0.51 ± 0.13
0.2	1.98 ± 0.01	0.66 ± 0.01
0.5	1.91 ± 0.68	0.76 ± 0.50
1.0	1.04 ± 0.43	1.25 ± 0.45
	284 Hour Exposures	
	Control Group 13	Oxygen-exposed Group 13
0.1	0.88 ± 0.25	0.73 ± 0.11
0.2	1.16 ± 0.49	9.91 ± 0.28
0.5	1.49 ± 0.48	1.46 ± 0.74
1.0	1.23 ± 0.06	1.15 ± 0.17

TABLE 4 - Continued

308 Hour Exposures

	Control Group 14	Oxygen-exposed Group 14
0.1	0.54 ± 0.07	0.50 ± 0.04
0.2	0.67 ± 0.24	0.85 ± 0.17
0.5	1.33 ± 0.18	1.34 ± 0.45
1.0	2.09 ± 0.82	2.08 ± 0.24

a Substrate concentrations are per 6 ml of incubation mixture, see Experimental, page 59, for details.

The rate of hexobarbital metabolism was linear with time out to ten minutes and showed good substrate dependence. The metabolic rates for a number of oxygen-exposed groups are given in Table 5. To obtain these rates each incubation mixture was sampled at seven minutes. Some of the rate data are plotted in Lineweaver-Burk form in Figure 6.

It should be noted that the final group studied was exposed to 5 psia oxygen for 40 days rather than for the usual 28 days studied with other drugs.

G. Aniline Hydroxylase

A study of the effect of 5 psia oxygen on aniline hydroxylase was introduced into this program of work when we were unable to demonstrate *in vitro* metabolism of amphetamine. The details of our efforts with amphetamine are described in the Experimental section, page 58.

Aniline hydroxylase activity was assayed according to the method of Kato and Gillette.[10] Incubation mixtures were sampled at seven minutes during which time the rate of production of p-aminophenol with time was linear (Figure 7).

Rates of production of p-aminophenol observed with groups of rats exposed to 5 psia oxygen for periods up to 28 days are given in Table 6. Lineweaver-Burk plots of the rate data are given in Figure 8.

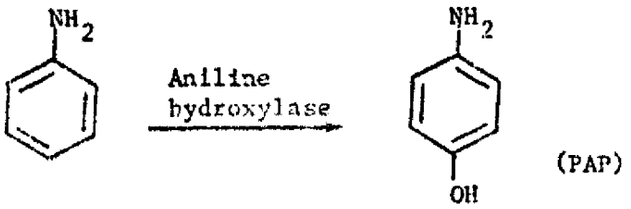


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TABLE 5

METABOLIC RATES OF HEXOBARBITAL *IN VITRO* USING LIVER MICROSOMES FROM RATS EXPOSED TO 5 psia OXYGEN AND TO NORMAL ATMOSPHERIC CONDITIONS

Substrate[a] Level (ml)	mμM Hexobarb. metabolized/ mg protein/min	mμM Hexobarb. metabolized/ mg protein/min
	88 Hour Exposures	
	Control Group 6	Oxygen Exposed Group 6
0.2	0.58 ± 0.17	0.71 ± 0.06
0.3	0.86 ± 0.03	1.03 ± 0.05
0.5	1.32 ± 0.19	1.37 ± 0.21
1.0	1.78 ± 0.36	1.67 ± 0.04
	308 Hour Exposures	
	Control Group 15	Oxygen Exposed Group 15
0.2	0.46 ± 0.03	0.36 ± 0.16
0.3	0.54 ± 0.09	0.71 ± 0.13
0.5	0.98 ± 0.39	0.92 ± 0.43
1.0	1.44 ± 0.23	1.46 ± 0.61
	960 Hour Exposures	
	Control Group 18	Oxygen Exposed Group 18
0.2	0.67 ± 0.03	0.69 ± 0.12
0.3	1.19 ± 0.13	1.10 ± 0.16
0.5	1.70 ± 0.10	1.80 ± 0.20
1.0	2.10 ± 0.09	2.20 ± 0.22

[a]Substrate concentrations are per ml of incubation mixture, see Experimental, page 57 for details.

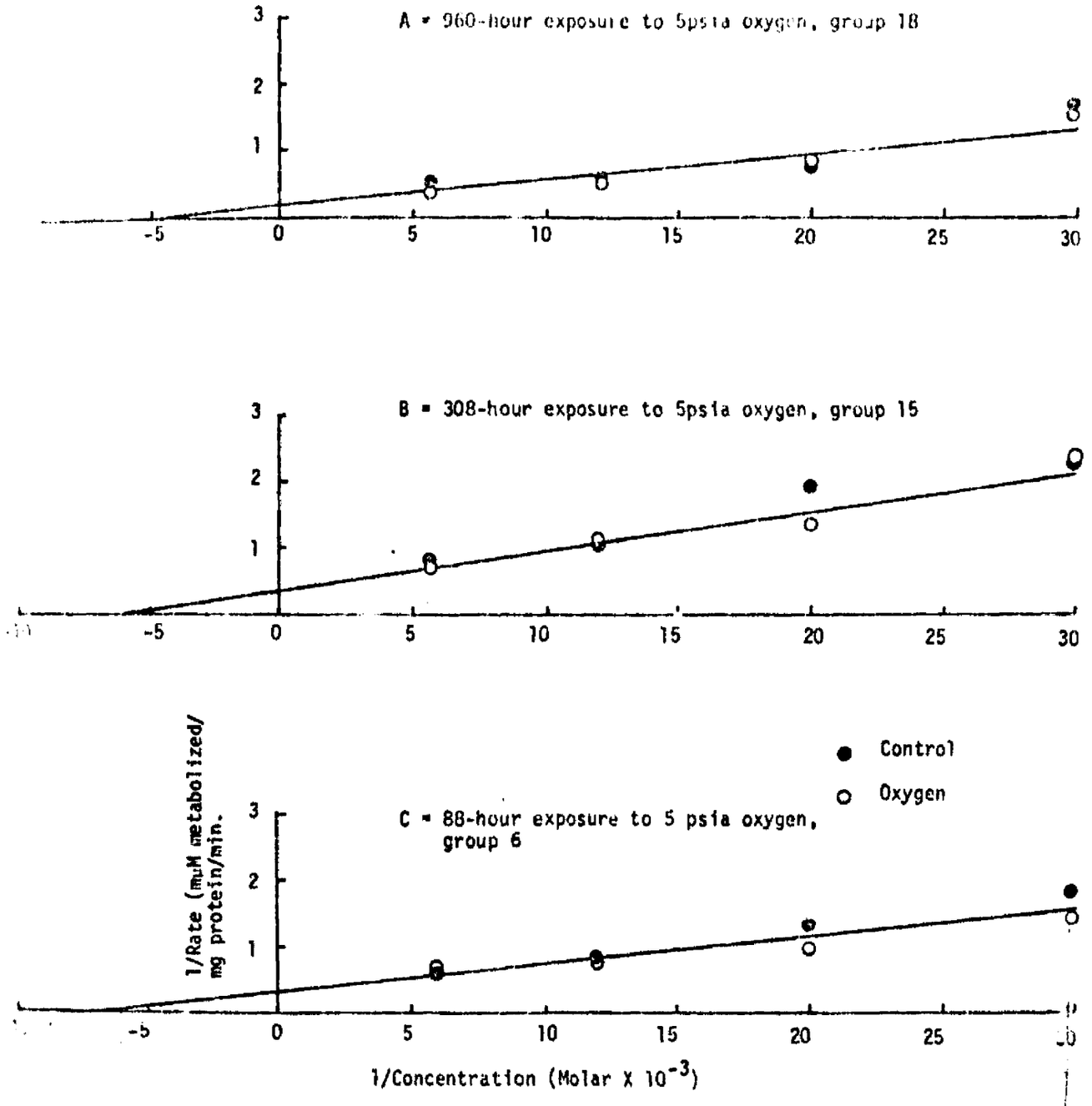


FIGURE 6: Lineweaver-Burk plots for Hexobarbital Metabolism In Vitro.

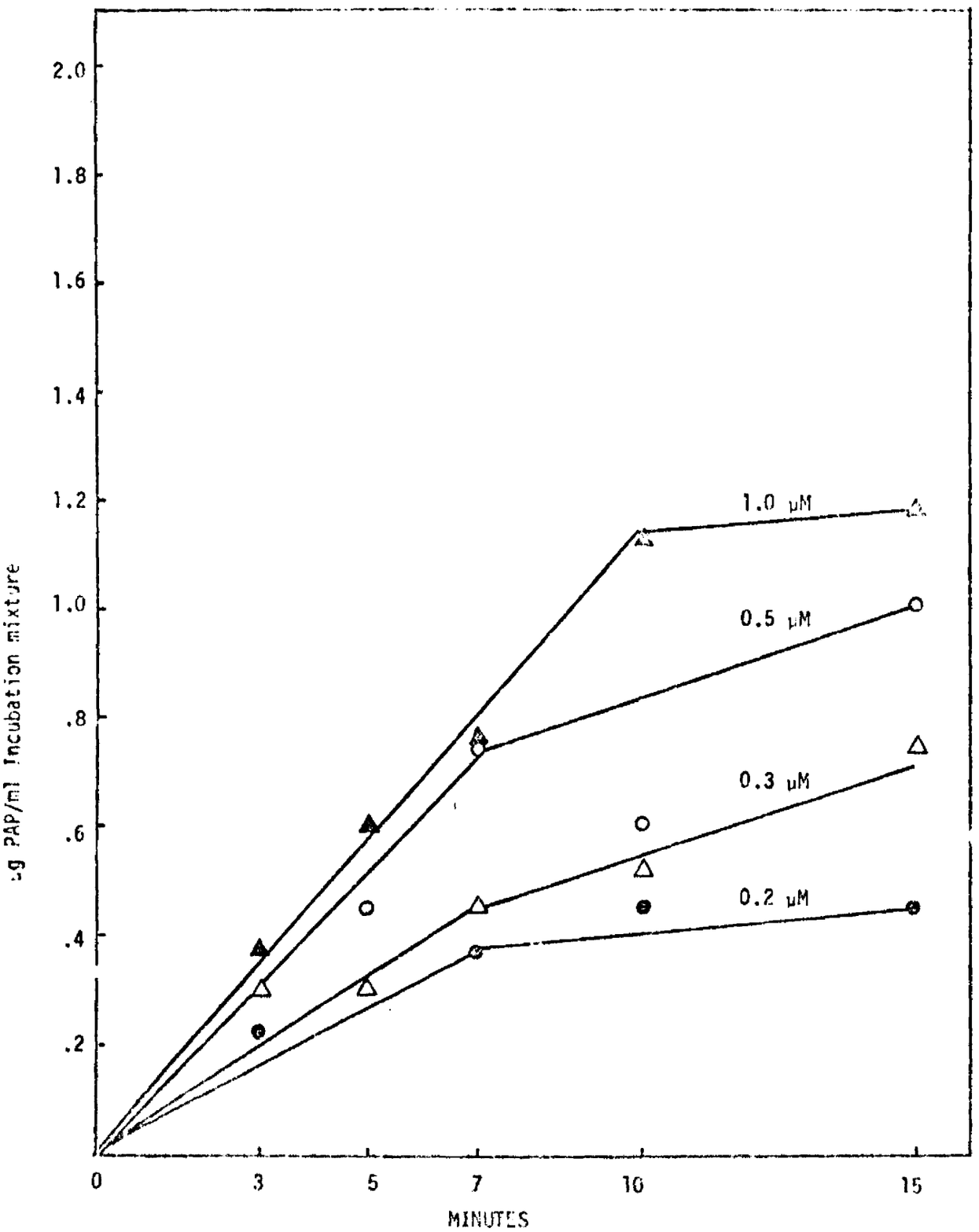
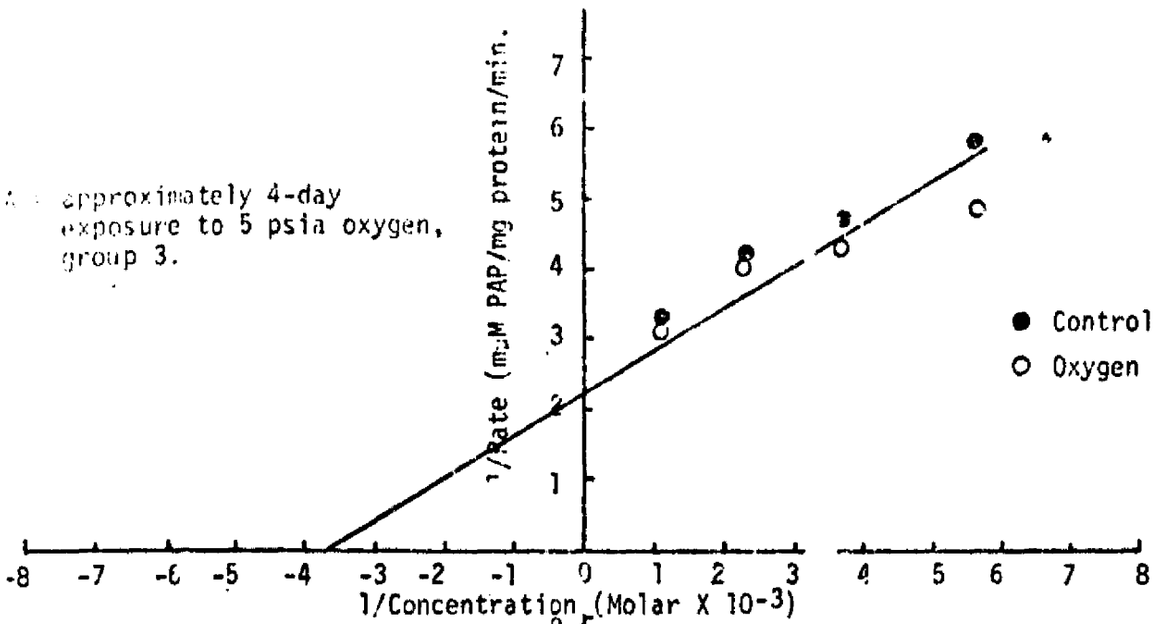
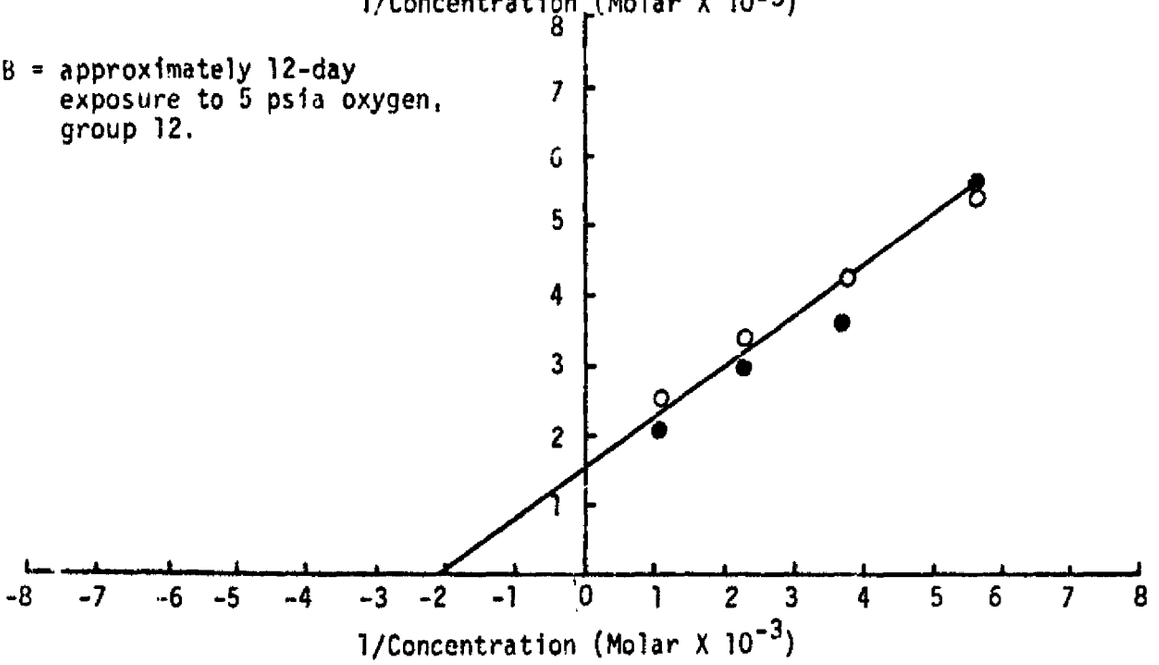


FIGURE 7: Rate of p-Aminophenol Production from Aniline at Several Substrate Concentrations

A = approximately 4-day exposure to 5 psia oxygen, group 3.



B = approximately 12-day exposure to 5 psia oxygen, group 12.



C = approximately 28-day exposure to 5 psia oxygen, group 16.

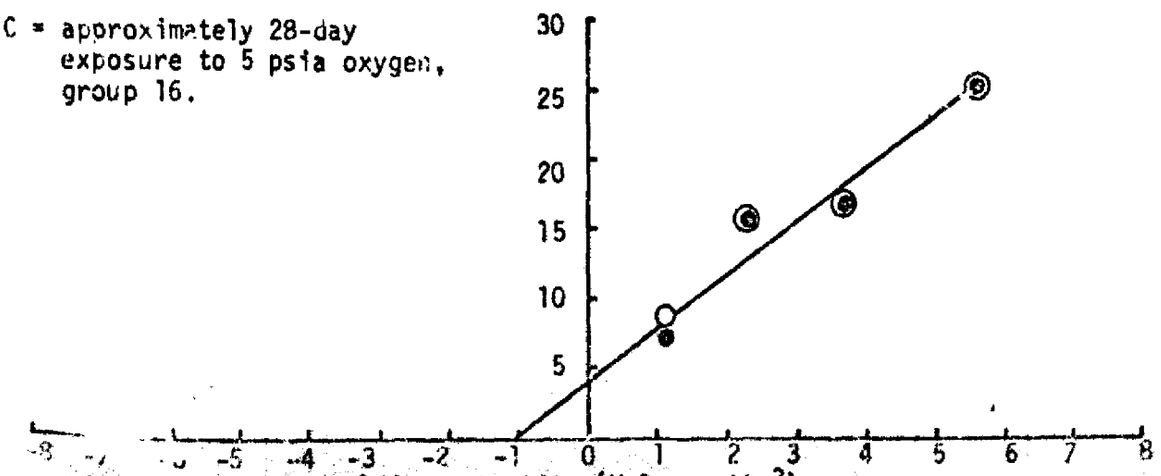


FIGURE 8: Lineweaver-Burk plots of *In Vitro* Metabolism Rates of Aniline.

TABLE 6

METABOLIC RATES OF [illegible]OXYLATION *IN VITRO* USING LIVER MICROSOMES FROM RATS EXPOSE[illegible]GEN AND TO NORMAL ATMOSPHERIC CONDITIONS

Substrate[a] Level (μM)	μMoles metabolized/mg/min	MμMoles metabolized/mg/min
	88 Hour Exposures	
	Control Group 3	Oxygen-Exposed Group 3
1.0	0.171 ± 0.026	0.207 ± 0.008
1.6	0.213 ± 0.035	0.236 ± 0.020
2.6	0.242 ± 0.027	0.248 ± 0.018
5.3	0.306 ± 0.206	0.321 ± 0.0[illegible]
	Control Group 4	Oxygen-Exposed Group 4
2.0	0.226 ± 0.061	0.196 ± 0.045
3.0	0.256 ± 0.034	0.226 ± 0.045
5.0	0.216 ± 0.017	0.197 ± 0.001
10.0	0.236 ± 0.042	0.206 ± 0.029
	188 Hour Exposures	
	Control Group 11	Oxygen-Exposed Group 11
1.07	0.143 ± 0.031	0.115 ± 0.013
1.6	0.165 ± 0.020	0.127 ± 0.033
2.6	0.227 ± 0.065	0.239 ± 0.024
5.3	0.301 ± 0.047	0.248 ± 0.017
	284 Hour Exposures	
	Control Group 12	Oxygen-Exposed Group 12
1.07	0.178 ± 0.065	0.186 ± 0.042
1.62	0.277 ± 0.087	0.232 ± 0.001
2.62	0.327 ± 0.091	0.288 ± 0.026
5.3	0.488 ± 0.034	0.389 ± 0.061

TABLE 6 - Continued

660 Hour Exposures

	Control Group 16	Oxygen-exposed Group 16
1.07	0.043 ± .022	0.048 ± .026
1.6	0.065 ± .019	0.063 ± .027
2.6	0.079 ± .012	0.075 ± .015
5.3	0.135 ± .011	0.123 ± .017

a Substrate concentrations are per ml of incubation mixture, see Experimental, page 58, for details.

H. Summary and Discussion of *In Vitro* Metabolism Studies

1. Observations of gross effects on liver. During the course of this work, there were no systematic differences in growth rates of oxygen-exposed and non-exposed groups of rats. This is in agreement with the results of a previous study[11] where, by use of individual capsules, rats were kept continuously exposed to 5 psia oxygen for periods of several months. Using the ratio of liver weight/body weight as a means of comparison, no systematic differences in liver size between groups appeared with animals exposed to 5 psia oxygen for up to 308 hours. In twelve animals exposed to 5 psia oxygen for 660 hours, livers appeared slightly smaller, but in six animals exposed for 960 hours, there were essentially no differences between groups. There were no systematic differences in the amounts of microsomal protein obtained from liver after any length of oxygen exposure studied. Cytochrome P-450 and b_5 levels were not significantly different in oxygen-exposed control groups of rats. Previous studies have shown that microsomal protein yields and levels of cytochromes P-450 and b_5 in rats exposed to enzyme inducers are significantly increased over those of control rats.[12]

In summary, we encountered no evidence that exposures to 5 psia oxygen resulted in gross changes in the livers of rats.

2. *In vitro* metabolism rates. *In vitro* metabolism studies were carried out with meperidine, propoxyphen, cyclizine, aniline, and hexobarbital using microsomal preparations from rats exposed to 5 psia oxygen for approximately 4, 9, 12, and 28 days.

N-Demethylase activity *in vitro* with three different substrates, meperidine, propoxylase, and cyclizine, and at least

four different substrate levels was unchanged after exposure of rats to 5 psia oxygen for periods of up to 660 hours. Similarly, hexobarbital oxidase and aniline hydroxylase activity was unchanged by exposures to 5 psia oxygen. Kinetic parameters derived from the various Lineweaver-Burk plots are given in Table 7.

Examination of the rate data in the previous sections reveals that there is considerable day to day variation in the experiments. These variations are also evident on comparison of the various Lineweaver-Burk plots obtained for the same drug. As has recently been pointed out by Fouts in a detailed experimental paper,[13] these batch variations of absolute specific activity are real. Day to day variations can depend on the time of sacrifice, age of the animals, and method of homogenizing the liver tissue. The rate of shaking during the incubation was also shown to have an effect on absolute rates.[13] It is clear that because of these day to day and experimental variations in absolute specific activities, conclusions regarding effects of particular treatments must be drawn from comparisons of treated and control groups in experiments carried out on the same day.

Comparisons of standard errors in our *in vitro* experiments with those of other laboratories was possible only for hexobarbital oxidase and aniline hydroxylase activities. The rates for hexobarbital metabolism in Table 5 are on the average ±16 percent. Those for aniline hydroxylase of Table 6 are ±17 percent on the average. This includes data from every experiment carried out during the course of the work. Fouts[13] gives the value ±10 percent in his studies with hexobarbital oxidase and aniline hydroxylase.

We conclude that within the limits of detectability imposed by the experiment, exposure to 5 psia oxygen for up to 28 days has no effect on metabolism rates of the drugs studied

here in the rat. The error limits of these experiments is small enough to detect effects likely to be of potential clinical significance. We feel, based on our results, that if exposure to the spacecraft environment changes drug action, it will be by a mechanism not involving a change in metabolic rates. It appears that while stress may have an effect on metabolic rates of drugs, prolonged exposure to oxygen at 260 mm is not sufficiently stressful to produce an effect.

TABLE 7

KINETIC PARAMETERS FOR *IN VITRO* METABOLISM OF MEPERIDINE, PROPOXYPHEN, ANILINE, AND HEXOBARBITAL BY RAT LIVER MICROSOMES

	Km	Vmax	
Meperidine	0.15 - 0.22 mM	1.39 - 2.78	nanomoles/mg/min
Propoxyphen	0.14 - 0.30 mM	1.40 - 2.78	" "
Hexobarbital	0.15 - 0.20 mM	2.85 - 5.00	" "
Aniline	0.27 - 0.92 mM	2.50 - 6.65	" "

IV. PHARMACOLOGICAL STUDIES

Studies of pharmacological activity of drugs on animals exposed to 5 psia oxygen while the animals were at altitude proved to be an extremely difficult task with our chamber facility. A detailed description of the results of our efforts is to be found on pages 63-64. Suffice it to say here that immobilization of the arm assemblies and gloves when they were used at 260 mm chamber pressure was severe and it was not possible to carry out useful work in a routine way. In view of this, the following procedures were adopted for the pharmacological work.

Exposures of animals were carried out as previously described for the *in vitro* metabolism studies - that is, the chamber was opened every third or fourth day for about one hour for cleaning and servicing of cages. Pharmacological studies were carried out one hour after animals were removed from the chamber. This holding period was used in an attempt to insure that no effects attributable to excitement of the animals during repressurization would be introduced. This compromise has some undesirable features, but it seemed certain that effects not reversible within one hour would be detected.

The results of these studies are detailed in the following sections. As previously, results with each drug or drug combination are given separately with the final section containing a single summarizing discussion.

A. Studies of Potentiation of Barbiturate Activity by Diphenoxylate (Lomotil).

This combination of drugs has been used by United States astronauts on prolonged space flights. The manufacturer's

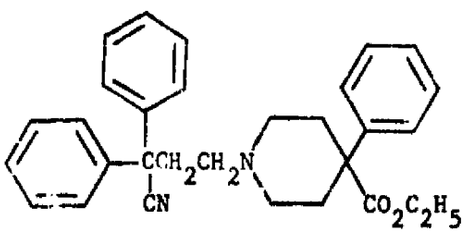
literature on diphenoxylate noted that this drug should be used with caution, if at all, in individuals simultaneously receiving addicting drugs or barbiturates because of potentiating effects.[14] No data on interactions between barbiturates and diphenoxylate appeared in the open literature. The manufacturer informed us that no pharmacological work on this combination had been done. Although no adverse drug reactions have been reported with this combination,[15] it was of interest in the context of our program of research to determine if possible potentiating effects were exaggerated by prolonged exposure to 5 psia oxygen.

We initiated our study using rats dosed in air. Preliminary dose-ranging with secobarbital, using male Sprague-Dawley rats, established that a dose of 20 mg/kg (i.p.) and 30 mg/kg (p.o.) produced a convenient length sleeping time for control animals. With hexobarbital, a convenient length sleeping time was produced with 80 mg/kg (i.p.). Sleeping times were recorded as the interval between loss and return of the righting reflex. The criteria for the loss and return of the reflex were the inability and ability, respectively, of the animal to right himself within 30 seconds when placed on his back.[16]

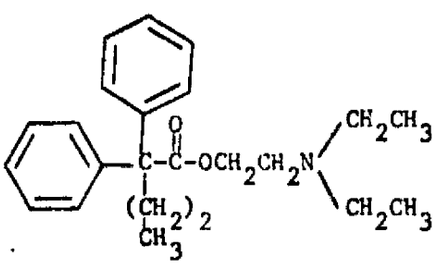
The selection of the initial dose of diphenoxylate was based on the results of Janssen *et al.*[17] The influence of an oral dose of diphenoxylate (R 1132) on fecal excretion by Wistar rats was studied by Janssen by quantitation of the number of fecal pellets passed over a period of 24 hours. At 10 mg/kg significant effects were produced which persisted for at least 48 hours after a single oral dose. At 1 mg/kg a smaller, but measurable, effect was seen on the first day following dosage, but none on the second day. Diphenoxylate was devoid of significant analgesic activity in mice and rats following subcutaneous doses of up to 80 mg/kg of body weight.

To avoid the tedious and time-consuming task of dose ranging with diphenoxylate, we elected to use the initial oral dose of 10 mg/kg found effective in rats by Janssen. Barbiturate was given both orally and intraperitoneally. Potentiation was clearly observable with both secobarbital and hexobarbital and the sleeping times observed in a number of experiments are given in Table 8.

The structural similarity of diphenoxylate (1) to SKF-525A (2), a known potent inhibitor of hepatic drug metabolism, prompted the speculation that the potentiation of barbiturate action involved an inhibition of drug metabolism. Studies of barbiturate levels in rat plasma and *in vitro* studies with microsomal enzymes were carried out to explore this possibility.



1



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The study of the effect of diphenoxylate on plasma barbiturate levels of the rat was carried out as follows: rats were dosed intraperitoneally with secobarbital and sacrificed in groups of two at each of the twelve time periods - 1, 2, 5, 10, 20, 30, 40, 60, 90, 120, 180, and 240 minutes - after dosing. The next day, a second group of twenty-four rats dosed seventeen hours previously with diphenoxylate (10 mg/kg, p.o.) was carried through the same experiment. The citrated plasmas from each animal were analyzed separately for barbiturate. Recoveries of known amounts of

TABLE 8

EFFECT OF DIPHENOXYLATE ON BARBITURATE-INDUCED SLEEPING TIMES IN RATS DOSED IN AIR

Group	No. Animals	Route & Dose Diphenoxylate	Barbiturate	Dose and Route of Administration	Time between Diphenoxylate & Barbiturate Dosage	Control Sleep Times (Min)	Diphenoxylate-dosed Sleep Times (Min)
1[a]	10	10 mg/kg p.o. in physiological saline, 2% Tween	Secobarbital	30 mg/kg p.o.	16 hr	65 ± 4.0	107 ± 12.0
2[b]	10	"	"	20 mg/kg p.o.	"	41 ± 3.0	188 ± 36.0
3[c]	10	10 mg/kg p.o. in 1% CMS	"	35 mg/kg p.o.	"	41 ± 4.5	90 ± 4.0
4[c]	10	"	"	"	"	50 ± 7.0	75 ± 8.0
5[d]	19	10 mg/kg p.o. in 1% CMC	Hexobarbital	80 mg/kg i.p.	18 hr	43 ± 10.4	66 ± 14.9
6[e]	6	"	"	"	15 min	47	87
7[e]	6	"	"	"	6 hr	43	77
8	5	"	"	"	20 hr	-	64 ± 26.0

a) 100-112 g rats; b) 100-160 g rats, groups 1 and 2 fasted 8 hours but given water *ad lib.* before dosing; c) groups 3 and 4 were not fasted and given access to food and water throughout the experiment; d) 100-125 g rats; e) 100-120 g rats.

barbiturate from the plasma of rats dosed with diphenoxylate indicated that neither this drug nor any of its unknown metabolites interfered with the barbiturate determination of Brodie *et al.*[18] The experiment was done twice and the plasma levels of barbiturate found for each group at the various time periods studied is given in Table 9. The general trend of a slower decline of barbiturate concentrations in plasma of the diphenoxylate-treated rats seems real but the standard error of some of the points is high. This seems likely due to considerable individual variability among rats. Analysis of the urines collected from each group of two animals up until the termination of the experiment (4 hours after dosing) showed no impressive difference in the amount of barbiturate eliminated - about 1 percent of the total dose was excreted unchanged in urine in the control group during this period as compared with about 1.4 percent of the dose in the diphenoxylate-treated group. From these data, the increased sleeping time of diphenoxylate-treated rats seems related to a slower removal of drug from plasma.

Attempts to demonstrate inhibition of metabolism *in vitro* were carried out with rat liver microsomal preparations. We used hexobarbital rather than secobarbital in the study because use of this drug gave much better rates of metabolism, as we discussed on page 19. The schedule of dosing and sacrifice of the animals was done such that it closely resembled the protocol for studies *in vivo*. Groups of rats were dosed with diphenoxylate (10 mg/kg, p.o., in 1 percent carboxymethylcullulose) and fasted for 16 hours before being sacrificed. The control group was dosed with 1 percent carboxymethylcellulose and similarly fasted before sacrifice. A homogenate was prepared with livers from 6 to 8 animals in each group and microsomes prepared in the usual way. Hexobarbital at levels of 1.0 μmole, 0.5 μmole, 0.3 μmole, and 0.2 μmole/6 ml of incubation mixture

TABLE 9

SECOBARBITAL BLOOD LEVELS IN CONTROL AND DIPHENOXYLATE-DOSED RATS*

Time after dosage (Mins)	Number of Animals		Secobarbital in plasma (µg/ml ± S. E.)		
	Control	Diphenoxylate-dosed	Control	Diphenoxylate-dosed	Change in the Mean (%)
1	4	2	10.6 ± 9.1	11.0 ± 5.2	
2	4	2	12.4 ± 2.5	16.0 ± 1.2	
5	4	2	24.5 ± 5.0	12.8 ± 0.6	
10	4	2	22.2 ± 1.0	20.8 ± 3.7	
20	4	2	17.2 ± 0.2	14.1 ± 5.0	
30	4	4	13.8 ± 1.2	19.1 ± 1.1	+ 38
40	4	4	17.3 ± 4.2	22.8 ± 9.4	+ 32
60	4	4	19.1 ± 6.8	17.7 ± 4.8	- 7.4
90	4	4	11.6 ± 3.4	14.5 ± 7.1	+ 25
120	4	4	8.1 ± 3.4	11.5 ± 5.2	+ 42
180	4	4	7.2 ± 3.3	14.6 ± 9.8	+ 100
240	4	2	5.5 ± 2.1	9.0 ± 5.9	+ 63

*Rats were male, Sprague-Dawley, mean weight 125 g. All animals were dosed with 20 mg/kg, i.p. with secobarbital after 17 hours fasting but with water given *ad lib*. The diphenoxylate-treated groups were given drug (10 mg/kg p.o.) in 1% carboxymethylcellulose, then fasted 17 hours before being dosed with secobarbital, i.p. Rats were anesthetized with ether, then blood collected by puncture of the vena cava and citrated. The sleeping time (mean ± S. D.) for the control group was 36 ± 6.3 minutes and for the diphenoxylate-treated group, 60 ± 9.9 minutes.

was run, with each microsomal incubate being sampled at 3, 7, 10 and 15 minutes for each drug level. The total microsomal protein was 20 mg/6 ml in each incubation mixture. Metabolic rates were determined at each substrate level from that portion of the rate data which was linear (about 7 minutes). The rates for each group are shown in Table 10. The rates of hexobarbital metabolism in control and diphenoxylate-dosed rats were not distinguishable at 10 mg/kg of diphenoxylate. A Lineweaver-Burk plot of the mean rates of Table 9 is given as Figure 9. The kinetic constants derived from this plot, Km = 0.151 mM, V_{max} = 2.5 nanomoles/mg protein/minute, are in acceptable agreement with those tabulated on page 32.

In summary, we have been unable to associate the potentiation of barbiturate sleeping times by diphenoxylate in the rat with the inhibition of barbiturate metabolism.

TABLE 10

IN VITRO RATES OF HEXOBARBITAL METABOLISM IN CONTROL AND DIPHENOXYLATE-TREATED RATS

	Substrate Level[a]			
Rats	0.2 µmoles	0.3 µmoles	0.5 µmoles	1.0 µmoles
Control	0.45 ± 0.16	0.38	0.92 ± 0.53	1.25 ± 0.23
Diphenoxylate-treated group	0.60 ± .05	0.64	0.89 ± 0.53	1.28 ± 0.17

a Substrate concentrations are µmoles of drug/6 ml incubate. Rates are given as mµmoles hexobarbital metabolized/mg and protein/min ±S.D. Rats were dosed 10 mg/kg (p.o.) with diphenoxylate as described in the text. Each microsomal preparation was prepared by pooling liver homogenates from 6-8 rats. Male Sprague-Dawley rats weighed 140-150 g.

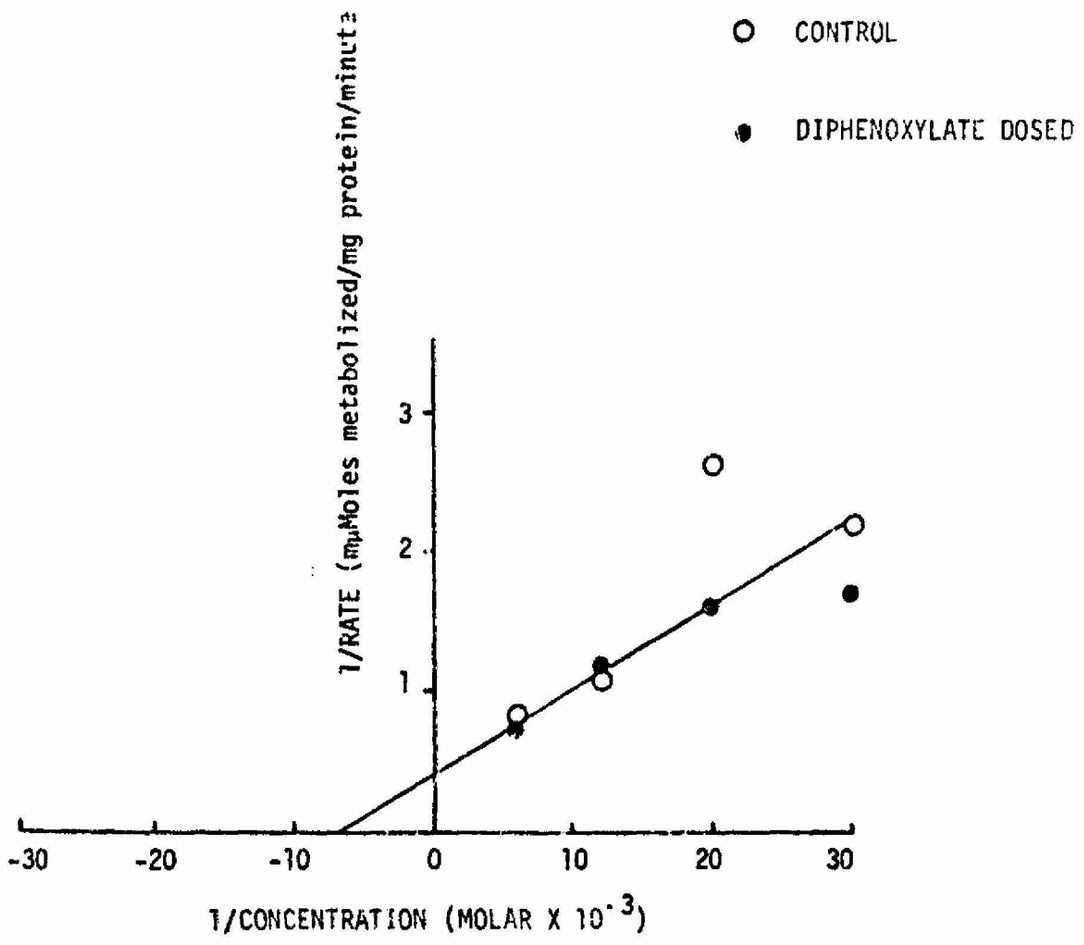

FIGURE 9: Lineweaver-Burk plots for Hexobarbital Metabolism *In Vitro* in Control and Diphenoxylate-Dosed Rats.

1. Study of effect of exposure to 5 psia oxygen on potentiation of barbiturate activity by diphenoxylate. For this study a group of five rats was exposed to 5 psia oxygen as described previously. On the third day, the chamber was opened for a short period for servicing. On the evening of the fifth day, the chamber was again opened, the rats were given diphenoxylate (10 mg/kg, p.o.), then the chamber again brought to altitude. Non-oxygen exposed controls were dosed with diphenoxylate at the same time. On the sixth day (20 hours after diphenoxylate dosing) the rats were removed from the chamber and after one hour dosed (80 mg/kg, p.o.) with hexobarbital. Barbiturate-induced sleeping times observed in the oxygen-exposed group and the control groups are summarized in Table 11. There was no difference between oxygen-exposed and control groups.

TABLE 11

POTENTIATION OF HEXOBARBITAL-INDUCED SLEEPING TIMES BY DIPHENOXYLATE IN AIR AND AFTER 6 DAYS EXPOSURE TO 5 psia OXYGEN.

Control+	Oxygen-exposed+
109	37
43	39
58	61
64	86
48	90
Mean = 64.4 ± 26.3	Mean = 62.2 ± 25.1

+ Sleeping times are in minutes. Rats were male, Sprague-Dawley. Both groups were given diphenoxylate, 10 mg/kg, p.o., 20 hours prior to hexobarbital treatment (80 mg/kg, p.o.).

B. Study of Possible Potentiation of Chloral Hydrate-Induced Sleeping Times by Diphenoxylate in the Rat.

During the course of our work, it came to our attention that NASA was contemplating use of chloral hydrate rather than hexobarbital on future space flights. Possible interaction between this drug and diphenoxylate was excluded by the following brief study. A group of six rats was dosed with diphenoxylate (10 mg/kg, p.o.) as had been done in the barbiturate study. Chloral hydrate (200 mg/kg, i.p.) was given 20 hours after dosing with diphenoxylate and sleeping time quantitated from times for loss and gain of the righting reflex. The sleeping times for the two groups are given as Table 12. The groups were indistinguishable.

TABLE 12

CHLORAL HYDRATE-INDUCED SLEEPING TIMES IN CONTROL AND DIPHENOXYLATE-DOSED RATS.

Control+	After diphenoxylate+ (10 mg/kg, p.o.)
29	40
32	27
23	29
19	23
38	31
25	22
Mean = 27 ± 6.8	Mean = 28.7 ± 6.3

+ Sleeping times are in minutes. Rats were male, Sprague-Dawley, dosed 200 mg/kg, i.p., with chloral hydrate.

C. Effect of 5 psia Oxygen on Hexobarbital-Induced Sleeping Times in the Rat.

For this study, rats were exposed to 5 psia oxygen in the usual way, then removed from the chamber for study. Hexobarbital sleeping times[16] were studied after rats had been exposed to 5 psia oxygen for 140 hours (approximately 6 days) and for 700 hours (approximately 30 days). The dose of hexobarbital was 80 mg/kg, i.p., given 1 hour after animals were removed from the chamber. The sleeping times observed for each group and the control groups are summarized in Table 13.

There was no significant difference between control and oxygen-exposed groups.

TABLE 13

HEXOBARBITAL-INDUCED SLEEPING TIMES IN CONTROL RATS AND RATS EXPOSED TO 5 psia OXYGEN FOR 140 AND 700 HOURS.

140-Hour Exposure		700-Hour Exposure	
Control+	Oxygen-exposed+	Control+	Oxygen-exposed
38.5	61.5	22.0	22.0
29.0	23.0	22.0	25.0
29.5	41.5	34.5	26.5
76.5	27.0	35.0	36.0
23.5	64.0	36.0	46.5
29.0	21.5	43.0	56.5
26.5	39.5		
44.5	32.0		
Mean =	Mean =	Mean =	Mean =
37.1 ± 6.2	39.9 ± 4.5	32.1 ± 3.4	35.3 ± 5.3

+ Sleeping times are in minutes. Rats were male, Sprague-Dawley, dosed 80 mg/kg, i.p., with hexobarbital.

D. Effect of 5 psia Oxygen on Propoxyphen (Darvon) Analgesia in the Rat.

In view of the fact that it was not possible for us to study the analgesics without removing the animals from the chamber, we used the Eddy hot plate technique[18] for these studies. The hot plate was maintained at 58°. Analgesic activity was measured over a period of two hours using a twenty-second cut-off time (defined as 100 percent response). The groups were compared in terms of "percent analgesia" observed over the total two-hour test period. Percent analgesia is determined from the area under a curve found by plotting the response times found at various time periods in the way illustrated in Figure 10.

The mean response times found with propoxyphen in rats exposed to 5 psia oxygen for 188 hours are given in Table 14. There were six animals per group and propoxyphen was given 50 mg/kg, i.p., one hour after animals were removed from the chamber.

The intensity and time course of propoxyphen activity in the control and oxygen-exposed groups were very similar.

E. Effect of Exposure to 5 psia on Meperidine-Induced Analgesia in the Rat.

The study of the effect of exposure to 5 psia oxygen on meperidine-induced analgesia was carried out with groups of rats exposed for 88 hours and 700 hours. Exposures were carried out as previously described. Analgesia was studied using the Eddy hot plate technique with animals which had been removed from the chamber. Analgesic activity was measured over a two-hour period using, as above, a twenty-second cut-off time. Meperidine was given 25-30 mg/kg, i.p. Cut-off times observed at various

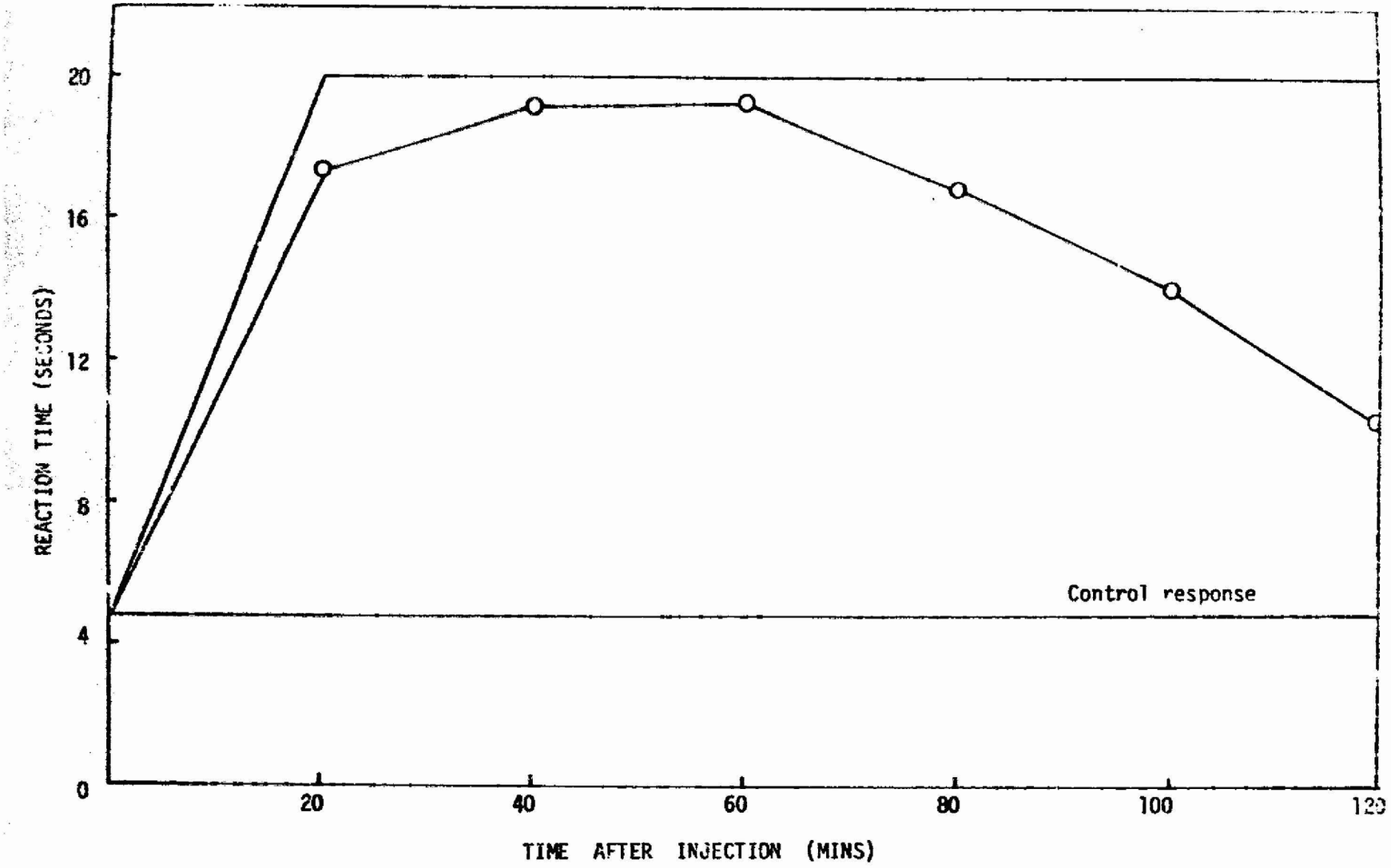

FIGURE 10: Illustration of procedure for determining percent analgesia. Data is from Table 13, oxygen-exposed group.

TABLE 14

PROPOXYPHEN-INDUCED ANALGESIA IN CONTROL RATS AND RATS EXPOSED TO 5 psia OXYGEN FOR 188 HOURS.

	Mean Reaction Time++	
Time after Injection+	Control*	Oxygen-exposed*
0	4.5	4.6
20	17.4	15.6
40	19.1	18.7
60	19.3	18.8
80	16.8	17.4
100	14.0	14.3
120	10.2	9.7
Percent analgesia	79.1	72.8

\+ Time in minutes

++ Time in seconds

* There were six animals per group. Rats were male, Sprague-Dawley.

time periods after dosage are given for control and oxygen-exposed groups in Table 15. Values for percent analgesia were calculated as described for propoxyphen on page 45.

Again, the intensity and time course of meperidine-induced analgesia in control and oxygen-exposed groups does not appear different.

F. Effect of Exposure to 5 psia Oxygen on Amphetamine Activity in the Rat.

For this study, increase in spontaneous activity was used as a measure of amphetamine activity. Spontaneous activity was measured in a circular activity cage with six beams of light going to six photocells spaced evenly around the cage. Each time the rat broke one of the beams, a single count was recorded. One cage contained two control animals which were treated with vehicle, while the other two cages held animals given 5 mg/kg, i.p., of d-amphetamine sulfate. For the oxygen-exposed group, oxygen-exposed animals untreated with amphetamine, were used as controls. This was done in an attempt to minimize artifacts which might result from the repressurization.

Only a single dose of amphetamine has been studied to date. Readings were made at 10, 25, 40, 55, 70, and 90 minutes. The mean number or counts observed per time period are given for exposed and non-exposed groups in Table 16. The means of activity of the amphetamine-treated animals are from two groups of two animals. Because of the small number of animals studied, no standard errors have been calculated.

Data from such studies as this are usually examined by

TABLE 15

MEPERIDINE-INDUCED ANALGESIA IN CONTROL RATS AND RATS EXPOSED TO 5 psia OXYGEN FOR 188 HOURS AND 700 HOURS

188 Hour Exposures

Time after Injection (min)	Mean Reaction Time*	
	Controls	Oxygen-Exposed
0	4.6	4.6
20	8.4	11.7
40	11.8	9.2
60	7.4	9.6
90	6.0	6.1
120	5.3	5.6
Percent Analgesia	18.3%	21.6%

700 Hour Exposures

Time after Injection (min)	Mean Reaction Time**	
	Controls	Oxygen-Exposed
0	5.9	6.1
20	20.0	17.5
40	19.0	15.2
60	12.2	13.7
80	9.9	11.1
100	8.6	9.5
120	6.8	8.7
Percent Analgesia	52.7%	48.3%

* Rats dosed 25 mg/kg i.p.

** Rats dosed 30 mg/kg i.p.

TABLE 16

AMPHETAMINE-INDUCED INCREASE IN SPONTANEOUS ACTIVITY IN CONTROL RATS AND RATS EXPOSED TO 5 psia OXYGEN FOR 188 HOURS.

	Non-Exposed		Oxygen-Exposed	
Treatment -	Control	5 mg/kg Amphetamine	Control	5 mg/kg Amphetamine
No. of animals -	2	4	2	4
Time period (min)	No. of Counts			
0-10	1054	706	330	635
10-25	521	1133	302	1220
25 40	476	1173	48	1060
40-55	221	888	24	1161
55-70	67	1272	27	1090
70-90	7	866	7	1779

considering the percentage increase in counts of treated animals relative to the controls. In these terms, substantial differences were observed between control and oxygen-exposed groups. The differences seem almost wholly attributable to the low spontaneous activity of the oxygen-exposed control group, however. In terms of counts per time period of the amphetamine-treated groups, there seems to be little difference between control and oxygen-exposed groups.

G. Summary and Discussion of Pharmacological Studies

Studies of the effects of 5 psia oxygen on barbiturate-induced sleeping times have been carried out and the results given in a previous section. We were unable to demonstrate a significant difference between rats exposed to 5 psia oxygen for up to twenty-eight days and control rats, one hour after the test animals were removed from the chamber.

There has evidently been only one previous study of the effect of hyperbaric oxygen on barbiturate sleeping times in rats.[20] This was a study of acute exposure for 90 minutes to oxygen at pressures up to four atmospheres. No effect was found at an oxygen pressure of one atmosphere or three atmospheres. A decrease in sleeping times was seen after a similar exposure to four atmospheres of oxygen. Supplementary studies of barbiturate brain levels were carried out and they were found to be the same in exposed and non-exposed groups. It was concluded that a change in the rate of biotransformation of barbiturate was not involved in producing the effect. In our study, neither an *in vivo* nor an *in vitro* effect of oxygen at 260 mm on the action of barbiturate could be demonstrated.

We were similarly unable to demonstrate that exposure to 5 psia oxygen changed the activity of meperidine in the rat using the hot plate technique one hour after test animals were removed from the chamber. An exposure period of eight days did not change the activity of meperidine or propoxyphen in the rat in a similar study. Of possible interest with regard to this result is the very recent report of Greenbaum *et al.*[21] These investigators showed that exposure to an atmosphere of helium and oxygen at 266 psig was without effect on morphine analgesia in the rat. Oxygen partial pressures did not exceed that of the atmosphere. Their very elegant experimental arrangement did not require the exposure to be interrupted for a study of the drug, and the hot plate technique was also used. In another paper,[22] it was also shown that the antiinflammatory activity of corticosterone was unaltered by the same helium-oxygen atmosphere. Of interest here, the stress associated with pressurization did not influence the activity of the drugs studied.

The single case where we may have found a difference between control and oxygen-exposed groups is in the study of amphetamine, but the case is not yet convincing. It is quite possible that the effect of repressurization artificially resulted in decreased spontaneous activity of the control groups. As we did not wish to pursue a study of the effects of repressurization on spontaneous activity in the rat, this uncertainty in interpretation of the result remains.

It is our feeling that our finding of no difference between exposed and non-exposed rats in the studies carried out is a stronger result than could be claimed if differences were found. This conclusion is reached if the uncertainty of the effect of repressurization is considered. Differences could always have been attributed to the need for this operation.

As it turns out, an exact cancellation of oxygen-induced effec by effects from repressurization would be extraordinary.

A final possibility is that all oxygen-induced effects are reversed within the first one or two hours after the animals are removed from the chamber. This possibility remains, but in any event, effects readily reversible within this time may not be profound to begin with.

An important and interesting question, not adequately addressed in the program, is whether stress induced by pain will interact with the stress imposed by the oxygen environment in altering drug effects. The considerable difficulty experienced in handling animals at altitudes are described on page 63. These problems prevented our being able to stress the animals in the chamber without substantial modification of its design. The approach of removing animals from the chamber for studies of cumulative effects of other stresses seemed undesirable. Physiological and pharmacological effects of stress are usually studied after animals have been subjected to a chronic stress of up to several hours. If this were done outside the chamber, the effects of the oxygen environment could disappear during this period. Consequently, such a study was not attempted.

The major conclusion from our program of work would seem to be that an oxygen environment at 260 mm is insufficiently stressful by itself to result in altered pharmacological action. It may yet prove that stress in an oxygen environment will have greater pharmacological effect that the same stress in an ordinary environment but there is no information on this point available at present.

V. EXPERIMENTAL DETAILS

A. Exposure of Animals to 5 psia Oxygen

The details of construction and performance of our altitude chamber are described in Section VI. Here we have summarized the procedures used in exposing the animals and the ambient operating conditions of the chamber.

Total atmospheric pressure maintained in the chamber was 260 mm. Carbon dioxide concentration was generally below 1 mm, but occasionally went as high as 2.5 mm when the lithium hydroxide absorbent required replacement. Nitrogen, doubtless introduced into the chamber by leaks, varied from 2 mm to 10 mm. Water vapor concentration was usually in the range of 7-14 mm (relative humidity about 37-74 percent). The humidity tended to drift toward the upper level between changes of the lithium hydroxide absorbent. Oxygen concentration varied between 91 and 96 percent during the exposure period.

To initiate exposure, groups of animals were placed in the chamber and it was brought to 260 mm during about 20 minutes. During this period, a high rate of oxygen introduction was used to keep the partial pressure of oxygen in the range 200-230 mm. The gas flow rate was about 900 liters/hour during this period and oxygen concentration of the exit gas from the chamber was monitored continuously. When the chamber reached 260 mm, pumping was continued and the oxygen flow rate reduced to about 500 liters/hour. This permitted nitrogen to be purged quite rapidly and pO_2 could be brought to 240 mm in an additional 30 minutes. At this point, the system was switched to the Thomas pump used in the gas recirculating system and the gas flow rate reduced to about 200 liters/hour.

It proved to be impractical to change water bottles and remove animals from the chamber using the space suit sle. and gloves with the air lock while the chamber was at 260 mm. The chamber was therefore brought to ground level for these operations. The rate of introduction of air was such that the chamber pressure was returned to one atmosphere within about 5 minutes. Animals were removed, water bottles replaced, trays used to collect excreta changed, and the chamber again brought to altitude as described above. This cycle could be completed in about one hour and total exposure times recorded in previous sections of this report are corrected for the time during which exposure was interrupted. Uninterrupted exposures of up to 4 days could be carried out. As work progressed, it was necessary to have two or more groups of animals on different exposure schedules in the chamber simultaneously. This resulted in the chamber being opened at three-day intervals on occasion.

Operation of the recirculating pump was somewhat noisy and for this reason all control animals were kept in the same roo. as the altitude chamber. It was hoped that this would minimize any possible effects due to noise being introduced into our experiments.

The room was air conditioned and when room temperature was controlled, no increase in temperature within the altitude chamber occurred because of heat produced by the animals.

Male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories. Rats were isolated in suspended cages for at least five days prior to use. This was done since it has been shown[23] that certain types of wood shavings commonly used in animals' bedding will induce production of microsomal enzymes resulting in high rates of drug metabolism *in vitro* and *in vivo*.

A support, constructed from galvanized iron, was used to suspend cages in the environmental chamber. Animal cages were constructed from stainless steel and had a wire mesh floor (Figure 11). This permitted excreta to drop from the cage into alumirum trays suspended about two inches below the floor of the animal cage. The aluminum tray was filled with animal bedding. This isolated the animal from the bedding and permitted the bedding to be changed by changing aluminum trays.

Control rats were kept in similar suspended cages. Control and oxygen-exposed rats were always from the same shipment from the breeder. This was done in an effort to keep uncontrolled variables from being introduced into the experiment.

Rat weights ranged from 150-300 g, although in each individual experiment animal weights from control and oxygen-exposed groups were approximately the same. For the 28-day exposure period, exposures were started with 90 g rats. At the end of the exposure period these rats were about 250 g. Rats for other exposure periods were usually about 125-150 g at the beginning of exposure.

B. Preparation of Microsomes

Rats were weighed then sacrificed by cervical dislocation or decapitation and exsanguinated. Livers were excised from control and oxygen-exposed rats immediately placed in cold 1.15% KCl[24] and kept on ice. Each liver was weighed and homogenized in 25 ml cold 1.15% KCl and the homogenate spun at 9000 x g for 30 minutes using a Lourdes centrifuge which was kept in a cold room. The supernatant was poured through a double layer of gauze to remove floating lipids. It was then centrifuged in a refrigerated Beckman Model L ultracentrifuge (head 30) for 1-1/4 hours at a 100,000 x g maximum field. The supernatant was de-

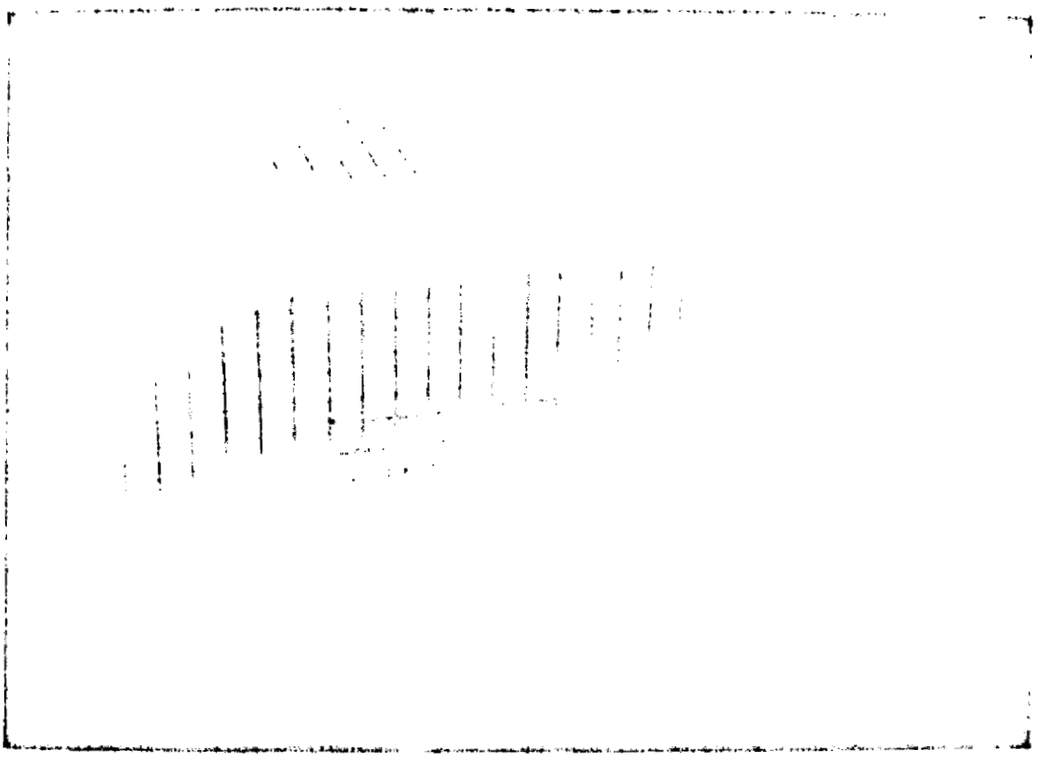

FIGURE 11: Cage Used for Rats in Altitude Chamber

fuged and the pellets representing the microsomal fraction were pooled into six groups representing two livers each and resuspended in cold 0.05 M phosphate buffer, pH 7.4.

A protein determination was carried out in duplicate on each pooled liver preparation using the Folin Phenol procedure.[25] Each microsomal preparation was then diluted with buffer to 20 mg protein/ml. Incubation mixtures to be used for *in vitro* metabolism studies were prepared immediately. Each mixture contained 20 mg microsomal protein, 20 μmoles nicotinamide (Mann Research Labs), 20 μmoles glucose-6-phosphate (Sigma Chemical Co.), 25 μmoles of magnesium chloride, 2.25 μmoles of NADP (Sigma), 2 units of glucose-6-phosphate dehydrogenase (Sigma) and drug (0.1-20 μmoles per 6 ml). All incubations were carried out in air using a shaking rate of 105-110 oscillations per minute.

Solutions of cofactors in buffer were prepared fresh for each experiment. The NADPH generating system (glucose-6-phosphate, NADP and glucose-6-phosphate dehydrogenase) was assayed whenever a new bottle of any of these reagents was used. The method was that of Kornberg and Horecker.[26] This precaution saved effort in tracing causes of the few inactive microsome preparations encountered during this work.

C. Barbiturate Oxidase

The kinetics of metabolism of hexobarbital was followed by observing the rate of disappearance of substrate using the analytical method of Brodie *et al.*[18]

Incubation mixtures containing 0.2, 0.3, 0.5 and 1.0 micromoles of hexobarbital per 6 ml of the microsomal mixture described above were prepared. Each mixture (12 ml) was kept at zero degrees on ice at all times prior to incubation. At zero time a 5 ml aliquot of the mixture was taken and placed in 30 ml

of heptane containing 1.5% of isoamyl alcohol with 5 ml 0.05 molar phosphate buffer pH 5.3 and saturated with NaCl. After removal of the zero time sample, the incubation mixture was placed in a 37° shaking water bath (New Brunswick Scientific Co., Model G-77) and incubated for seven minutes. At this point another 5 ml aliquot of the incubation mixture was taken and placed in the heptane/buffer mixture to stop the reaction. All samples were extracted by shaking in a reciprocal shaking apparatus for 30 minutes. After extraction, each tube was centrifuged at 700 x g for seven minutes in an International Model PP-2 centrifuge.

For determination of barbiturate a 7 ml aliquot of the upper organic phase was added to 3 ml of 0.8 M phosphate buffer, pH 11 and shaken for 10 minutes. Samples were centrifuged, the organic phase aspirated off and the optical density of the aqueous phase read at 240 nm and 280 nm. From the optical density difference at 240 nm and 280 nm concentration of barbiturate was determined. Hexobarbital that was metabolized was calculated from the difference between this value and that found for the zero time sample.

D. Aniline Hydroxylase

The following is based on the procedure of Kato and Gillette[10]. Incubation mixtures (6 ml) were prepared as previously described and contained 1.1, 1.6, 2.6 and 5.3 micromoles of aniline per 6 ml of mixture. The aniline had been recently distilled. Each sample was incubated at 37° in the shaking water bath and a 5 ml aliquot taken at 7 minutes. This aliquot was added to 15 ml of peroxide free ether,[27] sodium chloride was added to saturate the aqueous phase, and the mixture shaken for 10 minutes. It was then centrifuged for 7 minutes at 15,000 rpm. To a 10 ml aliquot of the ether layer was added 1 ml of a 1% phenol solution and 1 ml 0.5 M Na_3PO_4 and the mixture shaken for

3 minutes. The ether layer was aspirated and the color allowed to develop for 20 minutes. One ml of water was added to the colored solution and the optical density read at 660 nm for determination of p-hydroxyaniline.

The color generated by the metabolite, p-hydroxyaniline, with phenol in basic solution is due to the formation of indophenol.

E. N-Demethylase Activity

The rate of formaldehyde production from the N-demethylation of propoxyphen, meperidine and cyclizine was determined using the Nash procedure.[28] Incubation mixtures were prepared as previously described, but with the addition of 15 micromoles of semicarbazide hydrochloride/ml of incubation mixture. This traps the formaldehyde as its semicarbazone, preventing its further oxidation to carbon dioxide.

Drug levels were as follows: propoxyphen and meperidine at 1, 2, 10 and 20 micromoles per 6 ml of incubation mixture; cyclizine at 0.1, 0.2, 0.5 and 1 micromoles per 6 ml of incubation mixture.

The mixture was incubated at 37° in the shaking water bath and at seven minutes, a 5 ml aliquot taken. This was immediately added to 2 ml of 10% $ZnSO_4$ (w/v using anhydrous $ZnSO_4$) to stop the reaction. Each sample was agitated with a vortex mixer and after 5 minutes again agitated. Saturated barium hydroxide solution (4 ml) was added to each sample, the tube contents agitated and centrifuged at 15-17,000 rpm for 10 minutes. If the supernatant was cloudy, known amounts of 10% $ZnSO_4$ solution or saturated barium hydroxide solution were added until it became clear.

To a 5 ml aliquot of the clear supernatant, 2 ml of freshly prepared double strength Nash reagent[28] was added and the mixture incubated for 30 minutes at 60°. The yellow color which developed was quantitated by reading optical density at 412 nm. The yellow color is due to the formation of 3,5-diacetyl pyridine from 2,3-butanedione and formaldehyde in the presence of ammonium acetate in the Nash reagent.

F. Amphetamine Hydroxylase

Attempts were made to follow metabolism of amphetamine by rat liver and guinea pig liver microsomes by measuring disappearance of substrate.[29] The assay procedure was that of Sulser *et al.*[30] in which H^3-*d*-amphetamine is determined by scintillation counting. We were unable to demonstrate metabolism *in vitro* with the rat or guinea pig. After this work had begun, an independent report appeared which claimed that the rat hydroxylated amphetamine *in vivo*, but also that rat liver microsomes *in vitro* did not metabolize this drug Rat liver slices were also inactive. It seemed possible that inactivity was due to the fact that amphetamine existed predominantly as its salt at pH 7.4. The salt might not enter microsomal lipid. Since studies with other substrates[31] had shown that increasing the pH of the incubation mixture to 8.2 permitted metabolism to be observed *in vitro* with organic bases not metabolized at pH 7.4 by microsomes, we altered the pH of the incubation mixture. With rat liver microsomes at pH 7.8 or 8.2 no metabolism of amphetamine occurred.

We confirmed the report[32] that *in vitro* metabolism of amphetamine is facile with rabbit liver microsomes, but the rabbit was too big for convenient study in our chamber. We also found rapid metabolism of *d*-amphetamine with liver microsomes prepared from the Dutch rabbit. Substrate levels were 0.05, 0.1,

0.2 and 0.5 micromoles of drug per 7 ml of incubate. There was a good substrate dependence in the rates observed. The Dutch rabbit is much smaller than the albino rabbit and could have been used in our chamber.

It was ultimately decided that doing all our work with one species was preferable and that aniline would be used to study the aromatic hydroxylase system of rat liver.

C. Determination of Cytochromes b_5 and P-450

Cytochrome b_5 was determined by the method of Stittmatter and Velick.[33] Cytochrome P-450 was determined as described by Omura and Sato.[34] An aliquot of the microsomal protein suspension (~20 mg/ml) was diluted with 0.05 molar pH 14 phosphate buffer to a concentration of 2 mg/ml. Three ml of this dilution was aliquoted into two matched cuvettes. To the sample cell was added a few milligrams of sodium hydrosulfite and, after mixing the contents, the difference spectrum was recorded (Figure 12). The sample cuvette was then placed in the reference beam and the reference cuvette treated as follows: a few milligrams of sodium hydrosulfite was added and a gentle stream of carbon monoxide bubbled through for 20 seconds. This cuvette was then placed in the sample beams and the difference spectrum recorded (Figure 12).

Cytochrome b_5 levels are expressed as the optical density difference between 427 nm and 407 nm/10 mg microsomal protein/3 ml. Cytochrome P-450 levels are expressed as optical density differences between 450 nm and 490 nm/10 mg microsomal protein/3 ml in this standard procedure. This is adequate for purposes of this study since we are interested in comparing groups rather than in absolute amounts of cytochrome.

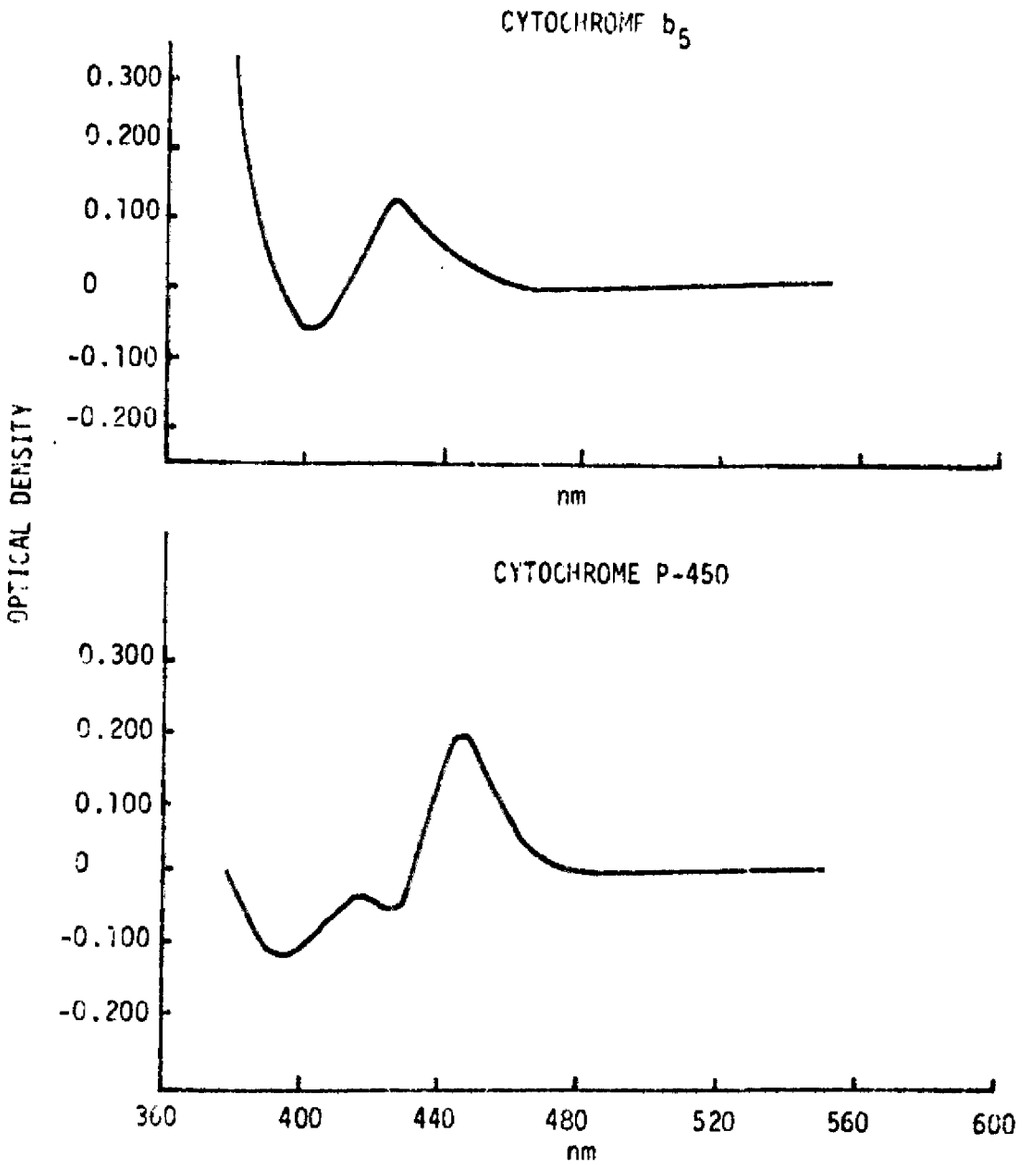

FIGURE 12: Difference Spectra of Cytochromes

H. Attempts to Handle Animals at Altitude

The purpose of this section is to record in detail our experience in attempting to work with available space suit sleeves and gloves at 5 psia chamber pressure. The rigidity of the sleeves and the unavailability of rotatable arm bearings made lateral and vertical arm movements at altitude almost impossible. Movements involving bending of the elbow joint were difficult, but possible. Rotation of the wrist joint was done with little effort and finger dexterity with the Gemini gloves was acceptable. Prolonged use of the Gemini gloves at 5 psia resulted in rupture of the glove bladder. Apollo gloves cocked to one side at 5 psia and it was not possible to use them at this pressure.

Opening and closing the air lock door was difficult at 5 psia using the gloves and sleeves. With use of a ratchet wrench and a vacuum manipulator (Model HE-103-3 from Vacuum Atmospheres Corporation) the air lock door could be opened and shut with ease. Also with the use of the manipulator, animal cages could be moved in and out of the chamber.

To utilize the space in the chamber, cages were arranged in two tiers of four cages along one side of the chamber. With either the sleeves or vacuum manipulators moving cages to and from the second tier was not practical. Water bottles could be changed and food added in cages directly in front of port holes, but with our arrangements there was insufficient mobility for handling all eight cages.

It proved that all necessary operations - moving cages, changing water bottles, transferring animals within the chamber and injection of drugs into animals with a hypodermic syringe - could be carried out in a practical way at 450 mm chamber pressure, corresponding to about 12,000 feet altitude.

Giving animals intraperitoneal injections at altitude was done without the need for actual handling of the animals. This was done by placing the animals individually in cages (Figure 13) which permitted the animal to be immobilized by lowering an adjustable cage top (Figure 13). The animal was injected intraperitoneally through the cage, the cage top was raised and the animal observed for drug effects. Gain and loss of the righting reflex after administration of barbiturate could be determined by watching the animal's response to tilting of the cage.

At lower pressures some operations were still possible, but in our judgment not practical without further modification of sleeves. In the course of our work, we did the following brief study to gain experinece in injecting animals at altitude in the chamber. Five animals in individual cages (Figure 13) were taken to 450 mm (~12,000 feet) for 18 hours. During this period, a flow of air through the chamber at 900 liters per hour was maintained. Monitoring of the exit gas by GLC showed no accumulation of CO_2. Without interrupting exposure, the animals were given hexobarbital (80 mg/kg, i.p.) and sleeping time determined from loss and gain of the righting reflex. Five control animals in similar cages were given drug and studied by the same procedure. Sleeping times for both groups are given in Table 17. This experiment was carried out in about 3 hours, demonstrating the practicality of such an experimental approach for certain low pressure studies.

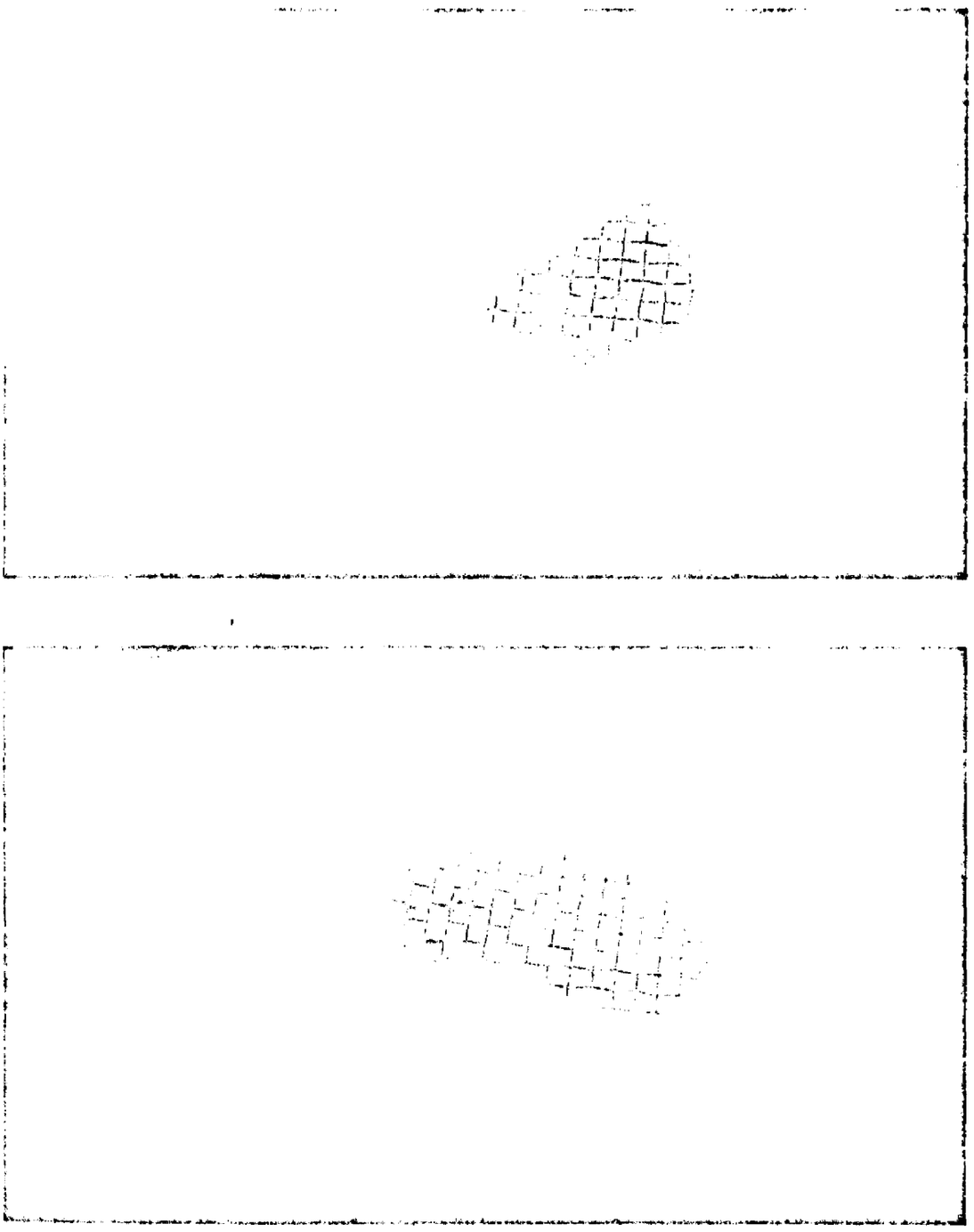

FIGURE 13: Cage Used for Intraperitoneal Injection of Rats at Altitude

TABLE 17

HEXOBARBITAL-INDUCED SLEEPING TIME IN CONTROL RATS AND IN RATS AT 450 mm FOR 18 HOURS

Altitude Exposed	Controls
Sleeping Time (mins)	Sleeping Time (mins)
42	23
25	26
25	47
42	29
45	33
Mean = 35.8±9.9	Mean = 31.6±9.4

VI. A SYSTEM FOR SIMULATING THE SPACECRAFT ATMOSPHERE

A. Introduction

It was recognized from the outset of this program that construction of a chamber adequate for the proposed work had to be carried out at minimum cost. This consideration ruled out use of a conventional altitude chamber and necessitated experimentation with a novel approach. The approach was to construct a glove box which would permit exposure and manipulation of a large number of rodents during the course of the program. The only previous experience with low pressure glove boxes of which we were aware was in the Lunar Receiving Laboratory at the NASA Manned Spacecraft Center, Houston. Specially designed space suit sleeves and gloves were used in the facility. Cost of these sleeves and gloves was high and for our purposes conventional space suit equipment, borrowed from NASA, was interfaced with our chamber.

Also for reasons of cost, the chamber was constructed fro. Plexiglass®. This construction material proved to be completely satisfactory and its transparency offered a considerable advantage. Plexiglass® ports were arranged along one side of the chamber permitting animal cages to be stacked along its other side without putting them out of reach.

The chamber was equipped with an air lock and an environmental control and life support system. This system was designed to maintain a group of animals for at least 30 days. The following sections describe the various components of the system. Our experience in carrying out various tasks at altitude using the space suit sleeves and gloves is detailed on Page 64.

FIGURE 14: Front View of Plexiglass® Chamber Showing Four Ports, Two Equipped with Vacuum Manipulators

achieve a satisfactory vacuum tight seal with the O-ring already installed in the Plexiglass® flange of the main chamber body. Both end plates were suitably drilled to mate with the Plexiglass® flanges of the chamber body and a large hole was machined centrally in one end plate to accommodate the air lock. Additional holes were also provided for the gas feed-throughs and to accommodate chamber safety devices.

The Plexiglass® air lock on the commercial chamber was not suitable for the larger ADL chamber which would be operated at a pressure below ambient. Therefore, a circular aluminum air lock (18" long x 15" diameter) was purchased from Vacuum Atmospheres Corporation, North Hollywood, California (Figure 15). This air lock did not require modification except for the removal of a sliding shelf to permit the entry of the large animal cages. It was equipped with air inlet and evacuation valves and a full diameter opening door at either end.

To prevent slight distortion of the end plates when the chamber was operated at 5 psia, both end plates were reinforced. The reinforcement took the form of two lengths of channel beam (type 6061T5 alloy, tensile strength of 45,000 psi) bolted to each end plate which allowed each section of beam to carry a uniform load of 1.3 tons (see Figure 15). Each beam was bolted to the plate with 6 high tensile steel bolts, uniformly spaced and able to carry a load of 500 pounds. All the bolt holes were reinforced with steel helicoils into which the bolts were screwed. The channel beams on the end supporting the air lock could not be placed at the optimum separation because of interference from the air lock flange. This channel beam reinforcement was, nevertheless, sufficient to prevent distortion of the end plate.

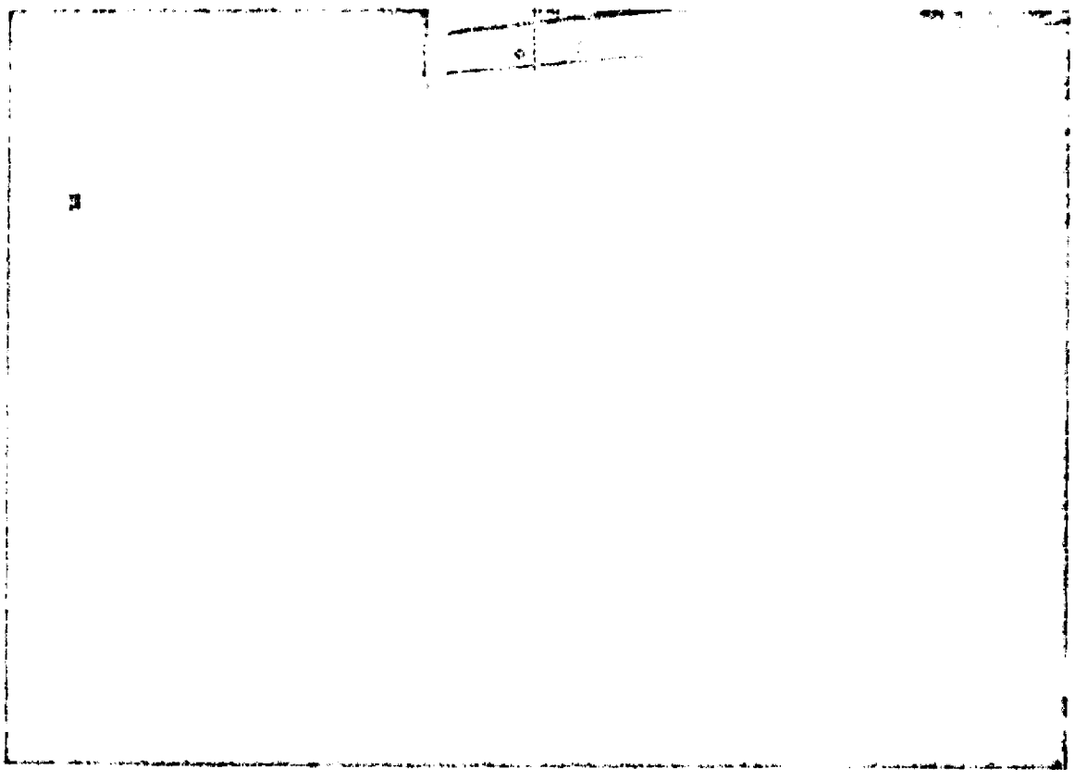

FIGURE 15: View of Channel Beam Reinforcement of Aluminum End-Plates and Air Lock

Three pairs of space suit gloves and sleeves were obtained on loan from NASA and were interfaced with the chamber. It did not appear feasible to cut the sleeves and it was decided to attach the sleeves at their full length, although this length was not optimum, in the manner described below.

A picture of the complete sleeve and glove attached to the glove port flange is shown in Figure 16. The assembly consisted of a flange with notched shoulder which fitted inside the Plexiglass® glove port with just sufficient clearance to attach the shoulder end of the sleeve. The convoluted shoulder was a tight fit over the aluminum support and was held in place by a band which forced the material into the notch and held it firmly. A gas tight seal was obtained by the use of RTV silicone sealant (General Electric, Waterford, New York) between the rubber sleeve and the aluminum. The sleeve assembly is subjected to an estimated 300-pound pull when the chamber is under vacuum. As a further precaution, nylon lacing was used to connect the nylon webbing on the sleeve pins, set at intervals around the circumference of the flange shoulder (Figure 17). This gave a strong gas tight assembly which gave no trouble during extended operation.

C. Gas Supply and Circulation System

A line diagram of the system is shown in Figure 18. This system was designed to supply oxygen at controlled pressure and flow rate to the space simulation chamber, to circulate gas through the absorber system for removal of carbon dioxide and water, and finally to purge gas from the system. The gas purge prevented build-up of nitrogen from leaks and of toxic products produced by the animals which were not removed in the absorber units.

FIGURE 16: Complete Sleeve and Glove Attached to the Glove Port Flange

FIGURE 17: Nylon Lacing Used to Reinforce Sleeve Assembly

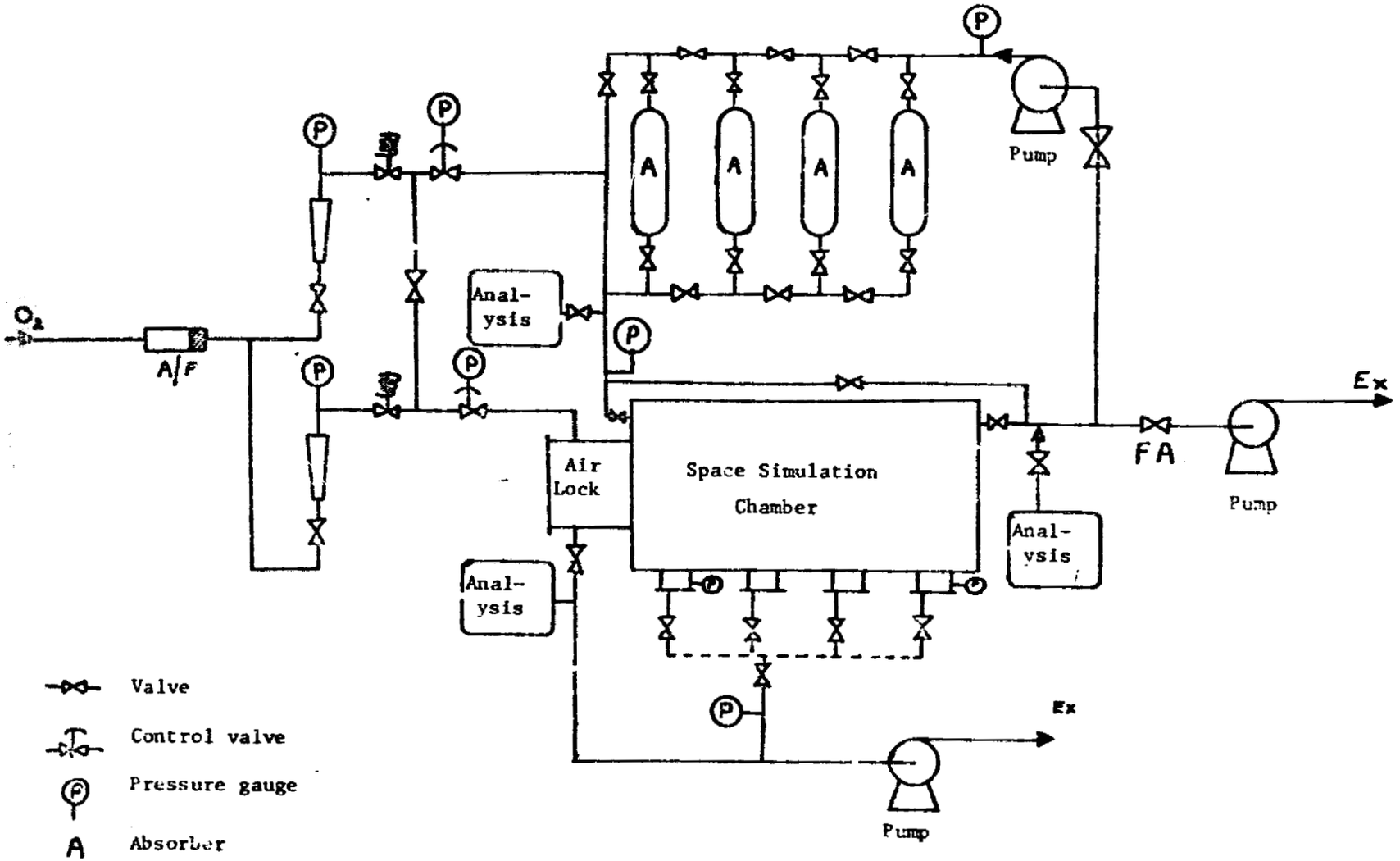

FIGURE 18: Line Diagram of Life Support System

The system was constructed using brass valves and other fittings connected by copper tubing. A 3/8" bore was used for the main lines to the chamber and 1/4" bore for all other lines. A double ended diaphragm pump with a single motor (Thomas model 4907CA22, Thomas Industries, Sheboygan, Wisconsin) was used for the combined circulation and purge pump. All parts of the pump except the pumping chamber itself are isolated from the gas system by a rubber diaphragm, thus preventing the access of oil and other contaminants into the system. Rapid initial pump down of the system was achieved with a wide bore connection on the air lock using the air lock pump which was a rotary vacuum pump (Kinney model KC-5, Kinney Vacuum, Boston, Massachusetts). The pump fluid was a phosphate based material (Kinlube 220, Kinney Vacuum, Boston, Massachusetts) recommended for pumping oxygen. System pressure was maintained by the gas pressure regulators (Matheson model 49, Matheson Gas Products, East Rutherford, New Jersey) which could be set at any value from 760-0 Torr. Maximum gas flow rate through the system was determined by the pump capacity on the exit (purge) line and could be throttled back by means of a fine adjustment valve in advance of the pump. The circulation system could be similarly throttled if required. Valves were placed at each end of the chamber to isolate it from the gas handling system and a bypass was available to operate the system gas supply separately from the chamber itself. The circulation loop contained four absorbers which could be operated in series or parallel and isolated from the system to permit changing absorbent without interrupting chamber operation. Closing the chamber isolation valves also permitted an experiment to proceed in the chamber for a limited period of time in the event of a malfunction in the gas supply system which required it to be shut down.

The air lock had its own pressure regulator, gas supply system and purge pump so that it could be operated independently with the inner air lock door closed. This allowed the animals to be transferred to and from the chamber without interruption of operation. The air lock pump also had a separate line which provided a means of pumping the glove port covers. With the glove port covers in place the gloves could be collapsed by producing a vacuum on the outside thus relieving the stress on the gloves when they were not in actual use. Oxygen was provided from a supply of medical grade liquid oxygen (99.5% from Linde Carbonic, Division of General Dynamics, purchased in LC3 cylinders), regulated to 10 psig, and was passed through a molecular sieve filter to remove any oil, particulates, or other impurities before entering the system. Three sampling lines to the analytical system were provided to monitor gas inlet to the chamber, exit from the chamber, and from the air lock.

Commercially available absorbers were used (Matheson model 449, Matheson Gas Products, East Rutherford, New Jersey), although these were considered somewhat smaller than optimum. Each absorber contained a removable cartridge which held about 400 grams of anhydrous lithium hydroxide. An 0.5 micron filter (Gelman type E, Gelman Instrument Co., Ann Arbor, Michigan) was placed at the exit side of each absorber to prevent lithium hydroxide dust from being carried into the chamber.

The gas supply system worked very well, the only problem being some corrosion of the copper lines after 6-8 months of operation. This corrosion was more pronounced in sections exposed to high humidity and was thought to be due to formation of a copper-ammonia complex. Operation of the circulation pump and other components was still reliable at the end of the program.

D. Design and Operation of Life Support System

The system design was based on an internal chamber volume of 1200 liters (42.4 cubic feet). The chamber could be pumped down to 5 psia in about 25 minutes, using a large capacity pump attached at the air lock. It could be repressurized to 1 atm. in 5-10 minutes. Separate pumpdown of the air lock alone took only a few minutes. With the chamber isolated from the gas handling system and pumps, no pressure loss was noticeable over a period of six hours. This corresponds to a leak rate of less than 3 liters/hour.

The specific considerations given to chamber design and a review of actual performance achieved are outlined in the following sections.

1. Mass Balance

The system was intended to provide life support for 40 rats for periods up to 30 days in an atmosphere of pure oxygen at 5 psia. The rates of carbon dioxide and water production assumed, the estimated leak rate of nitrogen through the gloves and the assumed rate of contaminant generation are given in Appendix 1. From these data, ammonia appeared to be the most serious contaminant. Consequently, design considerations were aimed at satisfactory removal of ammonia.

Estimated daily gas consumption and production rates are given in Table 18, together with the minimum gas flow rates required to remove trace contaminants from the system and prevent them exceeding their maximum allowable concentration (MAC). The flow rate calculated to remove total water was based on the assumption that all water produced by the rats (liquid and vapor)

must be removed as water vapor in the gas stream. The calculation for partial water vapor removal assumes that only the water vapor produced from perspiration and respiration must be removed in the gas stream. The MAC for water of 10 mm is not a true MAC, but based on the desirable relative humidity level of about 50%.

TABLE 18

ESTIMATED HOURLY GAS PRODUCTION AND CONSUMPTION RATES IN THE CHAMBER

		1/hr	Minimum Required Flow Rate 1/hr (STP)	MAC
Addition				
Oxygen		14	14	258 mm (5 psi)
Removal				
Carbon Dioxide		14	1400	2.6 mm
Water	Total	88	2270	10.0 mm
	Partial	30	775	10.0 mm
Nitrogen		6	300	5.2 mm
Trace Contaminants		0.034	340	10.0 ppm

A combined circulation and purge system with carbon dioxide absorbers in the loop was selected as most suitable for our needs. The alternative, a straight through system, was discarded in view of the very high oxygen requirements (at least 1400 liters/hour) necessary to keep the carbon dioxide at an acceptable level.

For a combined circulation and purge system, the minimum purge flow rate (300 liters/hour) was fixed by the rate of nitrogen leaking into the system because the nitrogen could not be removed in any other way. The figures in Table 18 showed that a slightly higher than minimum circulation rate (340 liters/hour) would also take care of the trace contaminants expected

to be produced in the system. Estimates of water vapor concentration were felt to be least certain. It was decided to deal with this problem by direct experimentation after construction of the system.

Consideration was also given to the question of gas velocities. At a combined flow rate of 1400 liters/hour (4200 liters/hour at 5 psi), the linear gas velocity through the chamber would be about 0.0056 feet/second, which was insufficient to disturb the animals. The linear velocity in the 3/8" diameter circulation would be about 10 feet/second (depending on absorber configuration). These gas velocities would not result in a noticeable pressure drop through the system.

A design purge rate of 400 liters/hour and a recirculation rate of 1000 liters/hour seemed adequate for meeting anticipated contaminant problems. Initial maximum flow rates achieved in the chamber as it was constructed proved to be 850 liters/hour in the circulation loop and 300 liters/hour in the oxygen supply. Increased flow rates for the circulation loop could have been achieved by the use of larger bore components. That for the oxygen supply could have been increased by use of a conventional vacuum pump with increased pumping speed in addition to the Thomas pump used in the recirculation system. As the chamber was operated, the number of animals actually exposed in the chamber never exceeded 24 and no difficulty was encountered due to inadequate gas flow rates. Therefore, these possible changes were not made.

2. Carbon Dioxide Absorption

It was decided that sufficient absorption capacity to remove 10 liters/hour of carbon dioxide should be placed in the circulation loop. Anhydrous lithium hydroxide was chosen as the carbon dioxide absorbent because of its high capacity and its ability to absorb water simultaneously. Values obtained from the literature for the absorption of carbon dioxide by one pound of lithium hydroxide varied from 131-390 liters. This figure is obviously dependent on bed design and in many cases insufficient details of the design were given. One example[35] gives a fairly conservative figure of 200 liters of carbon dioxide/pound of lithium hydroxide (440 liters carbon dioxide/kg) and provides the information shown in Table 19 below.

TABLE 19

DESIGN PARAMETERS FOR CARBON DIOXIDE ABSORBERS

		Literature Reference 35	Available Absorbers
Carbon dioxide partial pressure	mm	51	2.6
Gas flow rate	l/hr	10.5	250
Bed volume	mls	10	850
Linear gas velocity	ft/min	2.9	4.2
Bed aspect ratio	(bed length/ bed diameter)	3.1	3.8

Available absorber units gave the design parameters also shown in Table 19. These values appeared close to the literature values provided allowance was made during construction for the use of absorbers in series or in parallel. This was done. The

theoretical absorption capacity of the lithium hydroxide in the absorber was calculated to be 150 liters of carbon dioxide. According to the literature, 280 liters of water could be expected to be removed simultaneously. It was anticipated that the lithium hydroxide would become saturated with water before becoming saturated with carbon dioxide.

The absorber system was designed to be operated with two units in series, or up to four units in parallel without appreciable pressure drop. Operation showed that the life of the absorber system was not limited by CO_2 absorption, but by water absorption in the lithium hydroxide as anticipated. Leakage from the water bottles contributed to the water removal problem and was a significant source of water vapor. In a run with 18 rats in the chamber, four absorber canisters (in parallel) became saturated with water after 48 hours use. The lithium hydroxide was caked inside the absorbers and contained about 55-60 wt% at saturation, which is quite close to the theoretical value (75 wt%). At 50 wt% absorption of water the lithium hydroxide appeared quite dry. During the 48 hours operation the water vapor partial pressure rose from about 7 mm to 14 mm in this run. Water vapor content in the inlet oxygen was estimated to be around 200 ppm, therefore making a negligible contribution to the total water content. Calculations showed that the absorbers were removing an average of 15 liters/hour of water vapor making the total water vapor removed from the chamber by absorption and purging about 30 liters/hour.

Carbon dioxide removal by the absorbers was equivalent to 2.8 liters/hour when 23 rats were in the chamber. It was estimated that a total of 4 liters/hour was produced in the chamber. This is equivalent to 4 liters/day/rat (1.4 mls/gm rat/day) which is about 70% of the figure for CO_2 production obtained from the literature.

The ratio of water vapor to carbon dioxide absorbed (4.0 v/v) is within limits calculable from published data (2.1-6.5, v/v).

E. Analytical Measurements

Three sampling lines monitoring gas composition were controlled by electrically operated solenoid valves actuated automatically or manually. Details of this arrangement are shown in Figure 19. Each sample line was operated with a 3-way solenoid so that gas passed directly to the pump or, on actuation of the solenoid, passed through the analytical measuring system and then to the pump. The automatic timing circuit (Figure 20) allowed inlet gas or exit gas to be sampled during each consecutive half hour. Midway through this half-hour period, the solenoid valve controlling the sample valve on the gas chromatograph was actuated to inject a sample into the gas chromatograph. The air lock was not sampled automatically but air lock, inlet, or exit gas could be manually sampled and analyzed at any time by the gas chromatograph. The gas being sampled passed through a small chamber containing the humidity and oxygen sensors then through the gas chromatograph sample valve before going to the pump. The pressure in the sampling system was the same as that in the chamber, 260 mm.

The main analysis was by gas chromatography (Fisher-Hamilton, Model 29, Fisher Scientific Co., Pittsburgh, Pa.) This instrument is a two-column unit with the columns thermostated near room temperature and possesses a thermal conductivity detector. A flow diagram of the gas chromatograph is shown in Figure 21.

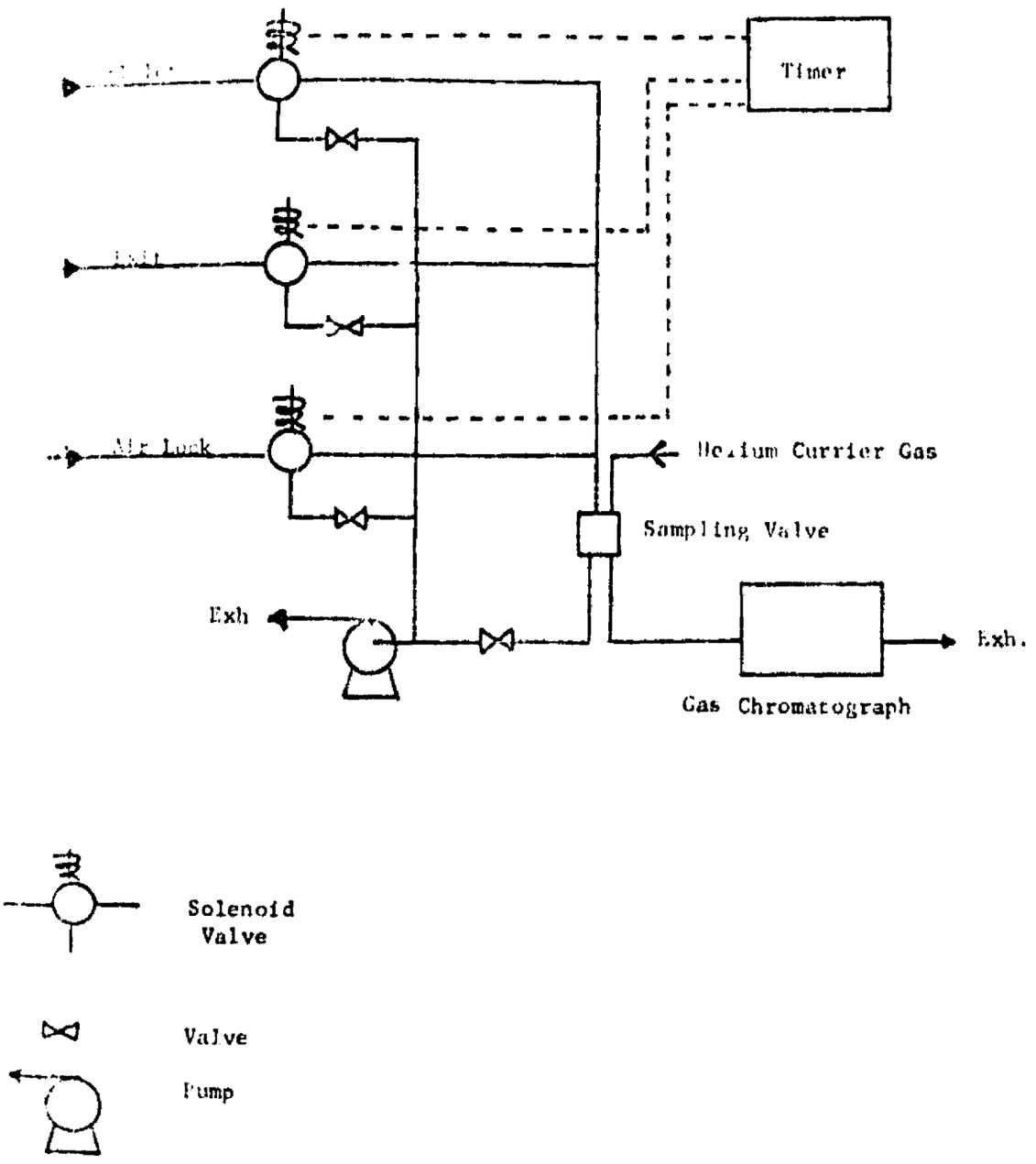

FIGURE 19: Line Diagram of Chamber Gas Sampling System

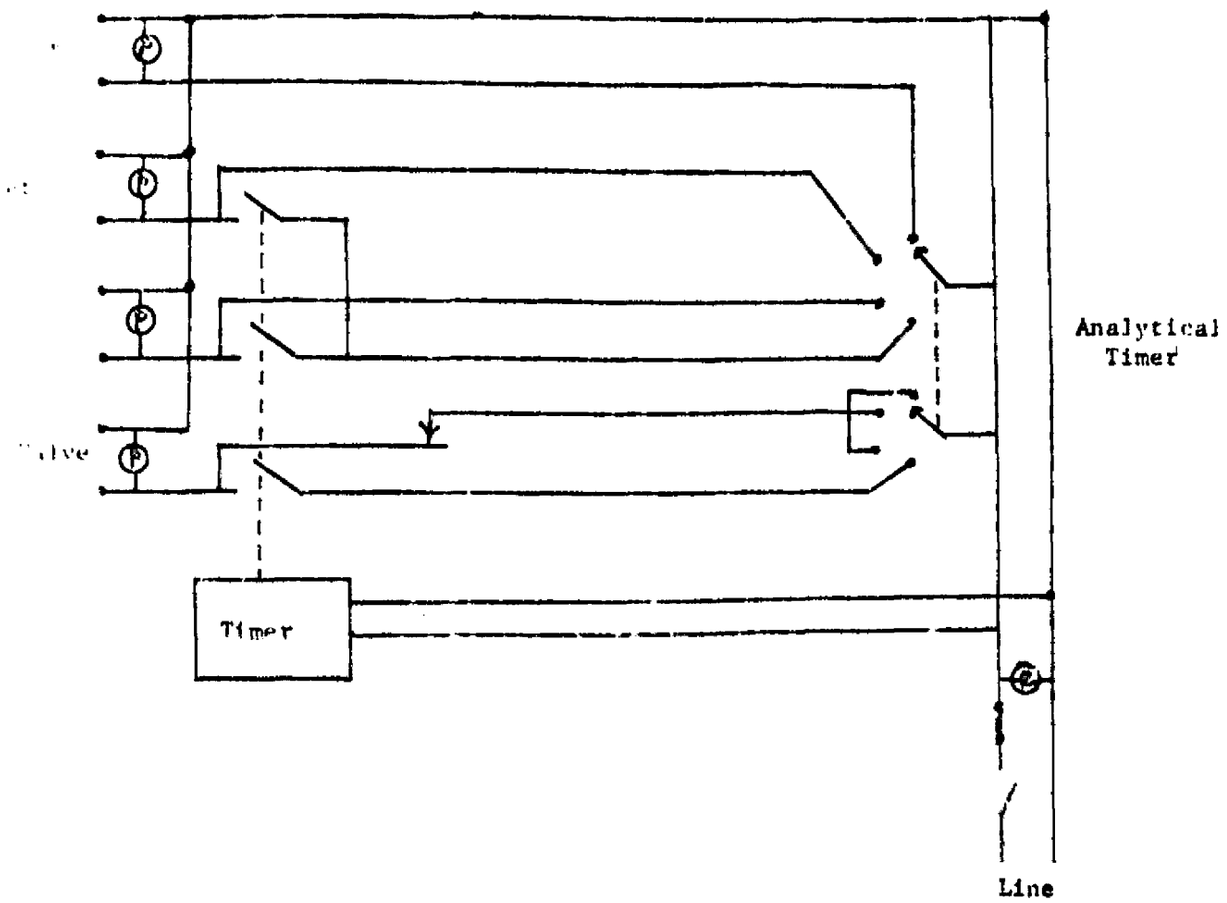

FIGURE 20: Circuit for Timing Solenoid Valve Operation

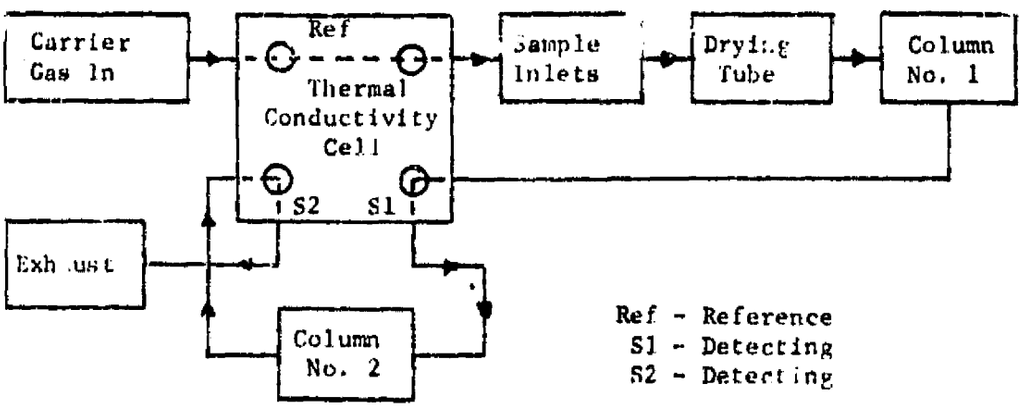

FIGURE 21: Flow Diagram of Gas Chromatography

Helium carrier gas flows through the reference side of a double thermal conductivity cell and then through the sample inlet and drying tube 1 (molecular sieve). This column holds back carbon dioxide while the rest of the composite sample is recorded as it passes detector S_1. While the sample is passing through column 2 (30 percent DEHS* on Chromosorb P), the column 2 peak appears and is detected at S_2. This relatively simple system was able to separate carbon dioxide, oxygen and nitrogen, which were the main components of interest. A reference trace (from the manufacturer's catalog) and a typical chromatogram are shown in Figure 22. The system would also have detected the presence of methane or carbon monoxide. Neither of these components was detected. As received, the gas chromatograph had only a manual sampling valve. This was automated by attaching the valve stem to the core of a heavy duty solenoid, and actuating it by the timing circuit previously described (see Figure 20).

To supplement the gas chromatographic analysis, separate analyzers were used to give a continuous record of oxygen and water vapor concentration. The Model 3600 oxygen analyzer was a polarographic type (IMI, Becton Dickinson, Newport Beach, Calif.) with 0-100 percent oxygen full scale. The water vapor analyzer was a Panametrics Model 1000 (Panametrics, Waltham, Mass.), able to measure water vapor concentration from a few ppm to 20 mm. Output from these two units was recorded on a dual pen Varian recorder (Model 5A, Varian Associates, Palo Alto, Calif.).

After minor adjustments to the solenoid which actuated the sampling valve, the gas chromatograph operated reliably during the program. Towards the end of the program, some deterioration of the molecular sieve column was observed (decrease in peak

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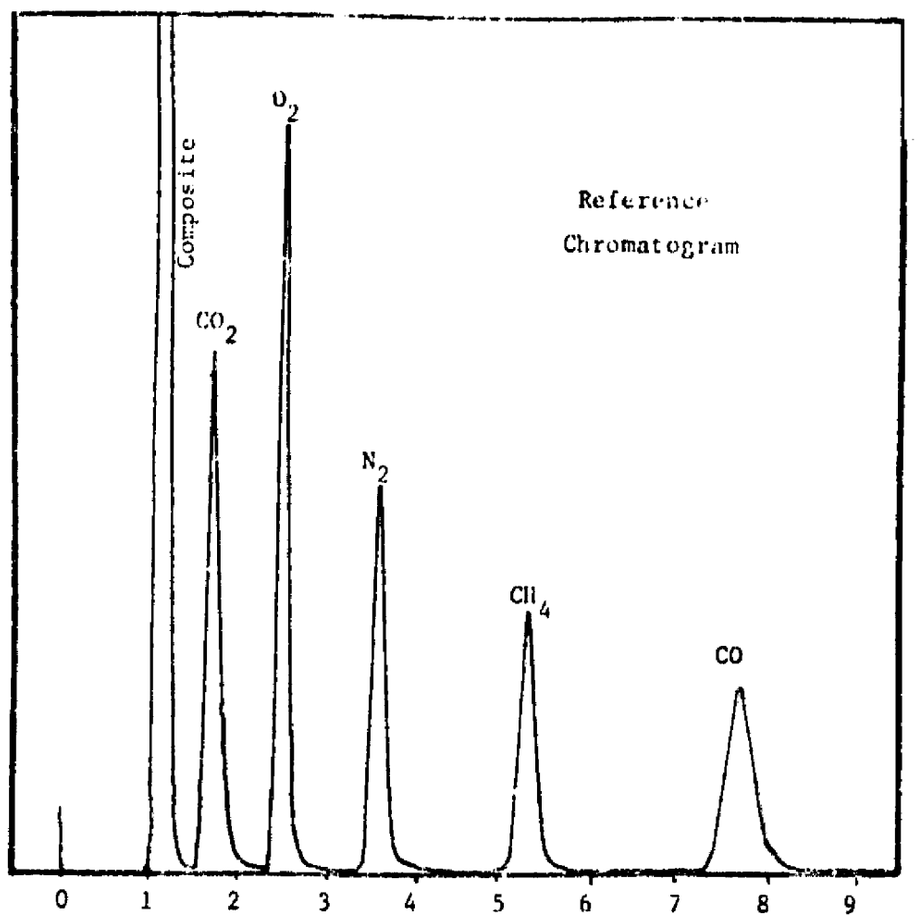
* DEHS = Di-2-ethylhexylsebacate



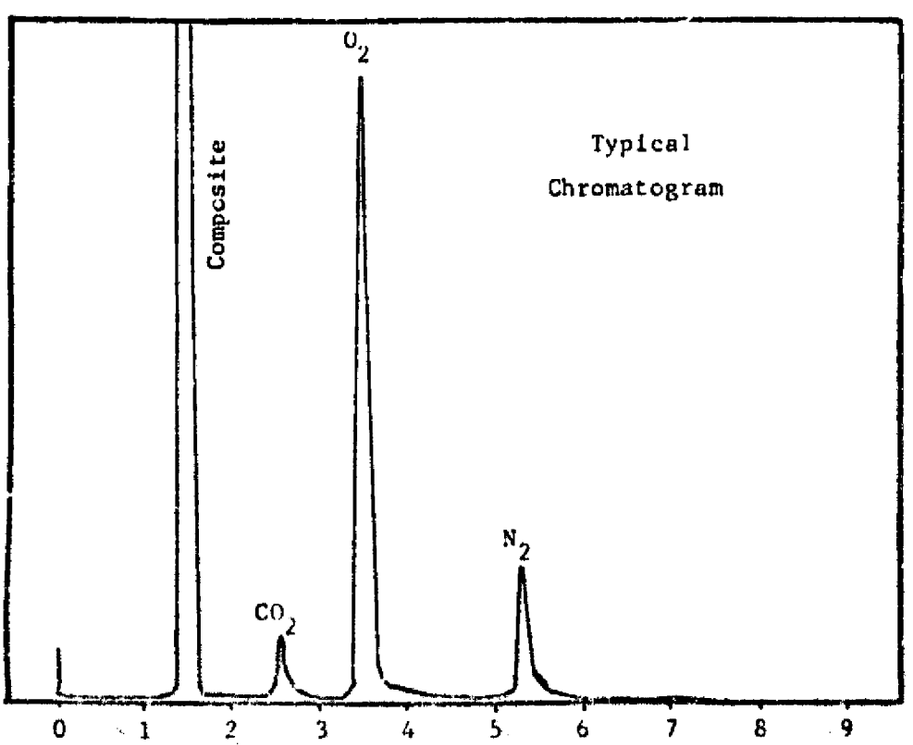


FIGURE 22: Reference and Typical Chromatograms

retention times) probably due to some contaminant from the chamber. No problems were encountered with the Panametrics water vapor instrument. The oxygen analyzer maintained its calibration for about four days. Recharge of the polarographic sensor was required every 2-3 weeks which was somewhat inconvenient. No problems were encountered with the mechanical operation of the analytical section except for one solenoid valve which became noisy near the end of the program after more than six months' operation. This was replaced at a convenient point although it was still operating reliably.

F. Safety Features

The main hazards associated with the use of a system such as this are (i) implosion of the chamber due to a fault or stress tracking in the Plexiglas®, and (ii) ignition in the presence of higher than atmospheric concentrations of oxygen. The Arthur D. Little, Inc. chamber was evacuated to 2.5 psia without incident and it was assumed that the chamber could be operated satisfactorily at 5 psia. To minimize ignition hazards, all potential sources of ignition were excluded from the chamber. Provision was made for rapidly filling the chamber with nitrogen in the event of ignition. The danger from static electricity build-up was considered to be small because of the relatively high humidity in the chamber. Additional safety features were incorporated to compensate for any malfunction which might develop in the life support system. Alarms were incorporated to bring the operators' attention to the malfunction. These features consisted of the following:

(1) High pressure alarm set to operate at about 8 psia, and actuate a solenoid valve to shut off the oxygen supply along with an audio

and visual alarm. This alarm had to be reset manually when the pressure was decreased below 8 psia again. (A circuit diagram is shown in Figure 23).

(2) High temperature alarm set to operate at about 100°F and connected to the oxygen supply solenoid and audio visual alarm in the same manner as the high pressure switch.

(3) Low pressure switch set to operate at about 3 psia and actuate a solenoid valve in the line to the vacuum pump, cutting off the pump. An increase in chamber pressure above 3 psia automatically restored the pumping again.

(4) Positive pressure blowout plug, 1.5 inch diameter, placed over a hole in the chamber end-plate. This plug was held in place by the negative pressure normally present in the system with an O-ring forming a vacuum tight seal. Even slight positive pressure inside the chamber caused the plug to fall out and release this pressure. The plug was attached to the chamber by a chain so that it could not become a projectile in the event of a rapid pressure rise.

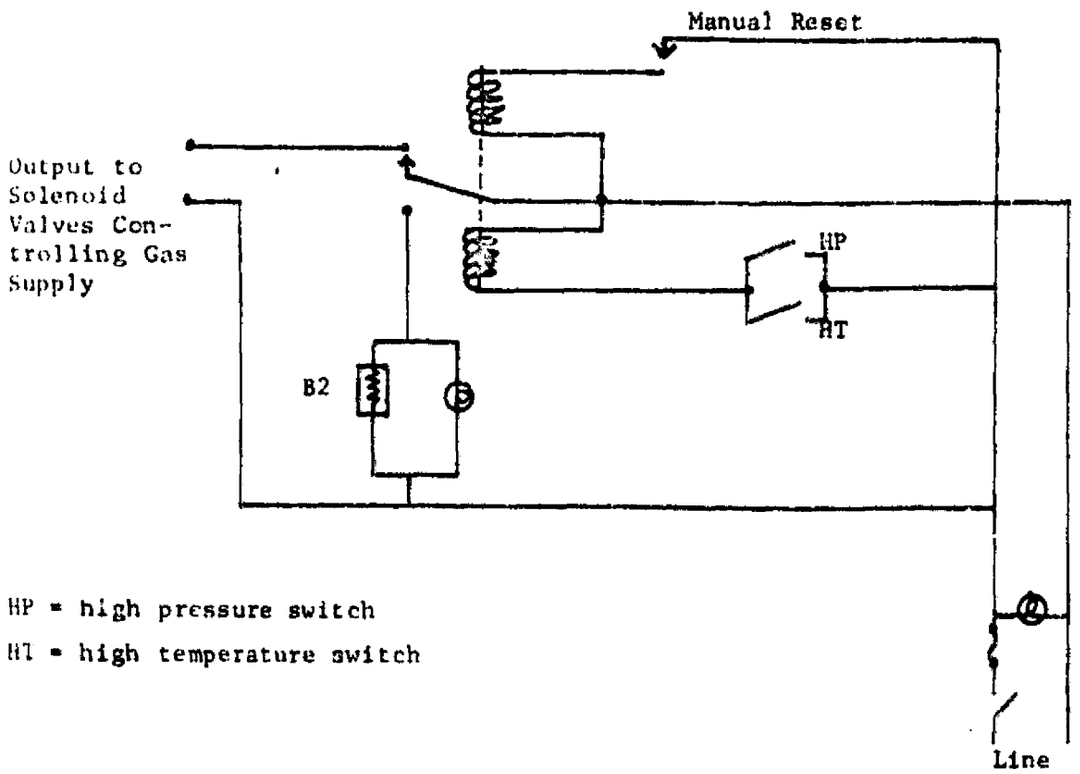


FIGURE 23: Circuit for High Pressure and High Temperature

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APPENDIX

Assumptions Made in Calculation of Gas Production and Consumption Rates.

1. Oxygen

The rate of oxygen consumption was assumed equal to the rate of CO_2 production. In fact, it should be slightly lower.

2. Carbon dioxide

CO_2 production in rat is 2.0 ml/g/hr, or 8.2 liters/day for a 200-gram rat. Forty rats were expected to produce 328 liters of CO_2/day. To maintain the carbon dioxide concentration at 1 percent (2.6 mm) by purging alone would require a removal rate of 1400 liters/hr.[1]

3. Water vapor

Figures for the rate of water production by rats were not found in the literature. It was assumed that the ratio of water to carbon dioxide for rats is the same as that for man, although this assumption was recognized to be tenuous. Konecci[2] gave a figure of 2.62 for the weight ratio of water to carbon dioxide (equivalent to a 6.5 volume ratio). Calculation gave the water production rate of 88 liters/hour. At a relative humidity of 50 percent (10 mm water vapor pressure), the required purge rate was estimated at about 2270 liters/hour. However, only about one-third of the total water production is water vapor produced by respiration and perspiration. The remaining two-thirds

is eliminated as liquid water which must evaporate before it can contribute to the relative humidity. Although a relative humidity of 50 percent was not felt to be critical, it was a desirable figure at which to aim.

4. Nitrogen

Nitrogen would only appear through leaks in the system. It appeared that the major leak would be through the gloves and leaks from other parts of the chamber were ignored. The glove manufacturer (David Clark Company, Worcester, Mass.) suggested that the leak rate of a glove would be in the region of 50 mg/min, equivalent to 6 liters/hour for a pair of gloves. The suggested maximum allowable concentration of nitrogen is 2 percent.[3] Assuming the leakage through the gloves to be 100 percent nitrogen, it was calculated that a minimum removal rate of 300 liters/hour would be required.

5. Trace Contaminants

Conkle[4] gives figures for an altitude chamber occupied by sub-human primates, fowls, and rodents. The other two references give figures for man.[5,6] Conkle did not give figures for rate of generation of contaminants. Therefore, it was more convenient to use the data given by Auerbach and Russell which gave estimated generation rates and maximum allowable concentrations for a number of contaminants. These data are reproduced in Table A-1. A column has also been added to this table for the ratio of the estimated generation rate (EGR) to the maximum allowable concentration (MAC). The largest figure in this column represented the contaminant which would require the highest purge rate to keep it within acceptable limits. All other

contaminants can then be expected to remain below the maximum allowable concentration.

TABLE A-1

TRACE CONTAMINANT GENERATION RATES AND MAXIMUM ALLOWABLE CONCENTRATIONS FOR A SPECIFIC TWO-MAN MISSION.

	Estimated Generation Rate (EGR) (lb/hr x 10^8)	Space Maximum Allowable Conc. (MAC) (PPM)	EGR / MAC
Ammonia	8,300	10	830
Benzene	830	5	166
Carbon monoxide	230	20	11.5
Cyclohexane	170	80	2.1
Dioxane	83	20	4.15
Ethanol	830	200	4.15
Formaldehyde	83	1	83
Hydrogen	1,640	41,000	0.04
Hydrogen fluoride	250	1	250
Hydrogen sulfide	3.58	2	1.8
Methane	144,000	200	720
Methanol	830	40	27.5
Methylene chloride	83	100	0.83
Ozone	8.3	0.02	415
Sulfur dioxide	83	1	83
Toluene	830	40	21

From the above table, ammonia appeared to be the most serious contaminant. The value in the above table was translated into terms of liters per unit of body weight to obtain an equivalent figure for rats. Assuming the two men weighed 140 kg, this calculation resulted in a removal rate of 340 liters/hour necessary to maintain 10 ppm ammonia. It was recognized that the figure could be higher for rats, but this estimate was the best that could be made with available data.

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