Observations on Rats Exposed to a Space Cabin Atmosphere for Two Weeks

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The effects of breathing 98 per cent oxygen at 258 mm, Hg were studied in male albino rats maintained for two weeks in a closed system environmental chamber. Three separate experiments were conducted, in each of which temperature, humidity and CO_2 concentration were carefully regulated. Control animals were maintained in identical cages in room air. All but one of the 140 rats exposed to oxygen survived for a mortality rate of less than one per cent and a total exposure time of 1,960 rat-days. No significant differences as compared to controls were noted in growth rates or in pulmonary, hepatic, renal and thyroid function. A very modest reduction in hematocrit observed in each experiment may be attributable to a mild suppression of erythropoiesis.

THE ADVENT OF MANNED SPACE exploration has resulted in consideration of a host of biomedical problems of interest to both the environmental physiologist and the engineer. One of the areas receiving increasing concern involves the choice of a gaseous environment for prolonged space flights. Use of our ambient, sea level oxygen-nitrogen atmosphere or one closely simulating it, would obviously be physiologically sound. However, the structural and weight penalties, as well as the complexities of monitoring and control imposed by a multigas system at sea level pressure, have necessitated the search for a compromise solution. As a result pure oxygen at reduced pressure (5 PSI) has been utilized in all manned space flights in Project Mercury and is programmed for use in Project Gemini.

Because engineering commitments involved in spacecraft design require early decision making considerable gaps still exist in our knowledge concerning the physiologic properties of this unnatural gaseous milieu. Recent experiments on human volunteers designed to clarify man's response to the hyperoxic, low pressure environment of the space capsule have resulted in conflicting data concerning possible hematologic[12,28] renal and visual abnormalities.[13,17] While man is the optimal test subject for resolving these differences animal experimentation permits use of greater numbers of subjects as well as microscopic examination of tissues to determine structural derangement.

The present investigation was consequently designed to study the physiologic and histologic response of albino rats to a fourteen-day exposure to essentially pure oxygen at 258 mm. Hg. As a companion project ultrastructural effects in liver and kidney tissue were studied with the electron microscope and are reported separately.[23]

MATERIALS AND METHODS

Three experiments were performed in each of which the subjects were male Sprague-Dawley rats weighing 140-180 grams (obtained from the Holtzman Co., Madison, Wisconsin, for use in Experiment I and from Laboratory Supply Co., Indianapolis, Indiana, for use in Experiments II and III). The animals were exposed to the conditions shown in Table I in a closed system environmental chamber modified for altitude studies following its previous use in ground level experiments on oxygen toxicity.[9] The chamber is a cylindrical aluminum structure, 1.78 m. in diameter, consisting of a main animal cell 2.06 m. long with a total volume of 5.12 $m.^3$ and an entry lock 1.37 m. in length. Within the chamber the animals were housed in individual wire mesh cages, with food and water available *ad libitum.*

The experimental environment was established by flushing the chamber at ground level with oxygen to a concentration of 80 per cent, after which the capsule was evacuated slowly to a total pressure of 258 mm.

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TABLE I. SUMMARY OF EXPERIMENTAL ENVIRONMENTAL CONDITIONS

PARAMETER	MEAN VALUES ± S. E.		
	Experiment I	Experiment II	Experiment III
Total pressure (mm. Hg)	258	258	258
Oxygen concentration (%)	97.6 ± 0.1	97.6 ± 0.3	98.3 ± 0.1
CO_2 partial pressure (mm. Hg)	0.61 ± .02	0.49 ± .03	0.56 ± .03
Temperature (°C)	23.9 ± 0.1	24.7 ± 0.3	23.6 ± 0.4
Relative humidity (%)	45.0 ± 0.3	44.8 ± 0.1	47.4 ± 0.5
Vapor pressure (mm. Hg)	10.0	10.5	10.5
Duration (days)	14	14	14

Hg and again flushed with oxygen until a concentration of 99 per cent was achieved. Purging was re-instituted during each experiment whenever oxygen concentration fell below 97 per cent. Cylinders of aviators' breathing oxygen served as the source.

The chamber atmosphere was recirculated at a flow rate of 250-280 liters/min through lithium hydroxide for carbon dioxide removal (Figure 1). Sampling for gas analyses was continuous and achieved by means of a compressor-aspirator pump (Dia pump, Air Shields, Inc.) which withdrew and compressed the sampled gas to sea level pressure prior to its passage through the various gas analyzers.

Oxygen concentration was determined by a Beckman F3 paramagnetic oxygen analyzer, carbon dioxide with a Beckman LB-1 medical gas analyzer and nitrogen with a Model 300 AR Nitralyzer. Total pressure was sensed with a direct readout Wallace and Tiernan pressure gauge. Relative humidity was determined by a Durotherm-Hygrometer placed in the main re-circulating flow line. Temperature was sensed with copper-constantan thermocouples placed within the chamber, with direct readout of their output on a Honeywell-Brown "Electronik" recorder. The instruments were continuously monitored by chamber technicians and their values recorded every two hours. The gas analyzers were recalibrated every eight hours. Samples were also obtained intermittently for analysis on a Bendix Time-of-Flight Mass Spectrometer and contained no detectable contaminants. Flame photometry analyses for lithium indicated less than one microgram per cubic meter of chamber atmosphere.

Entries into the chamber (via the lock) were made every one-to-two days to replenish water and food and to observe the animals. Growth rates were determined by weighing the same randomly selected group of rats at the outset and conclusion of each experiment and every two-to-three days during the course of Experiments II and III.

Blood for chemical and hematologic analyses was withdrawn at the conclusion of each experiment by aortic puncture following induction of light ether anesthesia. For arterial oxygen determinations (obtained in Exp. III only) pentobarbital (10 mg/100 gm body weight) was administered intraperitoneally to insure a more uniform level of anesthesia. One group of rats was anesthetized in this manner within the chamber at the conclusion of day 14, while another group was returned to room air for six to eight hours prior to anesthesia and blood sampling for gas analysis.

Hematocrits, hemoglobin, red and white blood cell counts and reticulocytes were determined by standard methods. Arterial oxygen was measured with an Instrumentation Laboratories direct readout meter. Protein-bound iodine (PBI) was determined by the method of Moran,[18] blood urea nitrogen (BUN) by the auto-analyzer method,[25] serum phosphorus by the method of Fiske and Subbarow[10] and total serum bilirubin by the method of Evelyn and Malloy.[16] Serum glutamic-oxaloaectic transaminase (SGOT), was measured by the method of Reitman and Frankel[21] and alkaline phosphatase was determined by the Bodansky method.[1]

Necropsies were performed in all animals with gross examination of organs, followed by formalin fixation and microscopic examination of sections stained with hematoxylin and eosin.

During the course of each experiment a group of control rats similar in weight and age to those in the chamber was simultaneously maintained in a room air environment in which temperature was kept at 23-25° C and relative humidity at 40-60 per cent. These rats were housed in cages identical with those in use in the

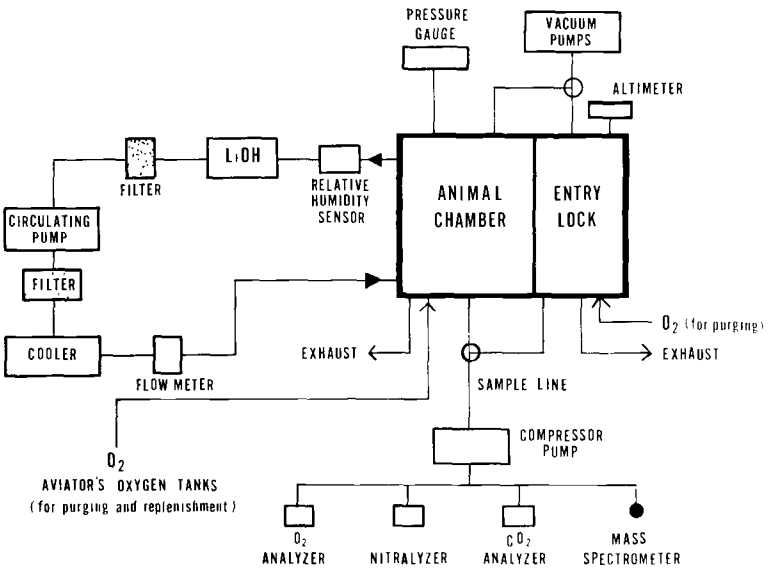

Fig. 1. Schematic diagram of closed environmental system. Sampling through the various gas analyzers was continuous. Samples were obtained intermittently for analysis on the mass spectrometer.

TABLE II. SURVIVAL OF RATS BREATHING 98% OXYGEN AT 258 mm. Hg FOR TWO WEEKS

	Experiment I		Experiment II		Experiment III		Total	
	C	O_2	C	O_2	C	O_2	C	O_2
Number of Rats	48	48	46	51	40	41	134	140
Number Surviving	48	48	46	50	40	41	134	139
Mortality (%)	0	0	0	2.0	0	0	0	0.7

C = control group
O_2 = Oxygen group

chamber and were treated exactly as was the experimental group in terms of food and water changes, weighing, anesthesia, blood withdrawal and analysis and pathologic examination.

In Experiment I propyl thiouracil was added at a level of 0.1 per cent to the diet of 12 control and 12 chamber rats to determine whether oxygen at reduced pressure interfered with the normal pituitary-thyroid response mechanism.

Statistical analyses were performed by the method of student's "t" distribution in Tables III, IV and VI and by the method of least squares in Figures 2 and 3.

RESULTS

As indicated in Table II, all the animals but one survived the two-week exposure to oxygen for an overall mortality rate of less than one per cent and a total exposure time of 1,960 rat-days. No deaths occurred in either Experiment I or Experiment III. The sole fatality occurred on day 14 of Experiment II and while, as indicated below, pulmonary lesions were readily identifiable the hyperoxic environment may not necessarily be implicated as the responsible causal factor.

During the periods of animal handling and observation within the chamber no abnormalities of behavior or appearance were noted. Specifically there was no roughening of body hair, lethargy, motor paralysis or gross change in respiratory rate. Occasional animals

TABLE III. TWO WEEK BODY WEIGHT GAIN IN EXPERIMENTAL AND CONTROL RATS

Mean weights (gm) ± S. E.

	Number Rats	Initial Weight	Final Weight	Weight gain	p (weight gain)
Experiment I					
Control	10	152.3 ± 1.3	196.6 ± 3.3	44.3 ± 2.7	P > 0.4
Oxygen	12	153.9 ± 1.8	197.1 ± 3.4	43.2 ± 3.2	
Experiment II					
Control	15	149.5 ± 0.8	222.2 ± 2.3	72.7 ± 2.2	P > 0.5
Oxygen	23	149.7 ± 0.5	220.3 ± 2.8	70.7 ± 2.0	
Experiment III					
Control	25	172.2 ± 1.2	195.7 ± 1.7	23.5 ± 1.5	P > 0.2
Oxygen	25	171.0 ± 1.4	197.5 ± 3.2	26.6 ± 3.2	

TABLE IV. HEMATOLOGIC RESPONSE MEAN VALUES ± S. E.

	Hematocrit Volume %			Hemoglobin* gm/100 ml	Red Cell Count* $X10^6$	Reticulocytes* %	White Cell Count* $X10^3$
	Experiment I	Experiment II	Experiment III				
Control	42.3 ± .8	43.7 ± .6	43.8 ± .3	14.5 ± .1	7.2 ± .1	2.8 ± .4	5.2 ± .2
Number Rats ( )	(11)	(12)	(26)	(6)	(6)	(6)	(6)
Oxygen	41.3 ± .5	42.4 ± .5	42.5 ± .5	13.9 ± .2	6.9 ± .1	2.7 ± .4	5.4 ± .3
Number Rats ( )	(12)	(21)	(18)	(8)	(8)	(6)	(8)
P	N.S.	N.S.	P < 0.05	P < 0.025	N.S.	N.S.	N.S.

*Determined in Experiment III only
N.S. = Not significant, P > 0.05

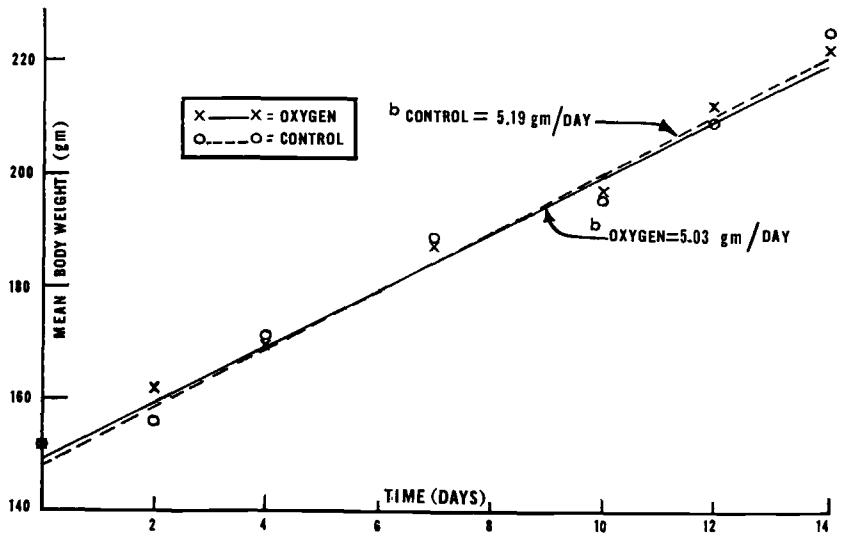

Fig. 2. Rate of growth as indicated by weight gain in Experiment II. The number of animals in each group is shown in Table III. Straight lines were fitted by the least squares method.

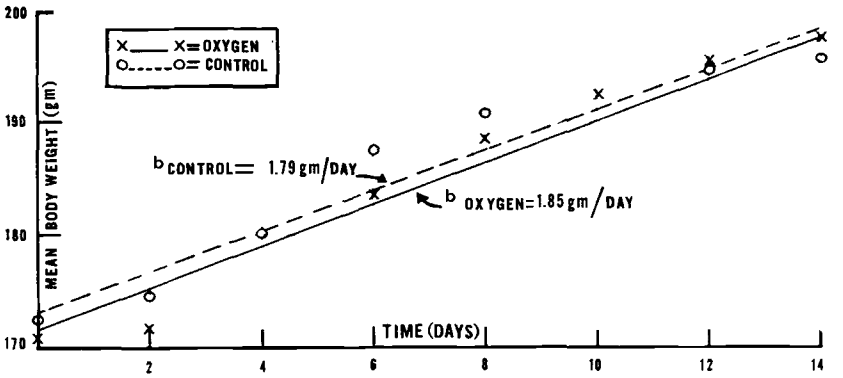

Fig. 3. Rate of growth in 25 control and 25 oxygen-exposed rats studied in Experiment III.

manifested audible wheezing; however, this was present with equal frequency in controls.

The growth rates as indicated by overall body weight gain (Table III), were essentially the same for exposed and control rats in each experiment, although the collective groups did vary from one experiment to the next. In Experiment II (Figure 2), in which weights were determined every 2-3 days, the regression coefficient for weight gain, b = 5.19 gm/day in controls and 5.03 gm/day in the exposed group. Comparison of these slopes by analysis of covariance[6] revealed no significant statistical difference ($P > 0.05$). Similar analysis of the growth data in Experiment III (Figure 3) also failed to reveal a statistically significant variation from controls ($P > 0.8$).

The hematologic studies are summarized in Table IV. In each experiment a slight fall in hematocrit was noted. However, this decline reached a level of probable statistical significance in the final experiment only, during which hemoglobin was also decreased. Nevertheless in no instance were these reductions of the magnitude generally considered necessary to produce detectable physiologic embarrassment.

The mean value for arterial oxygen tension (Pa_{O_2} Table V) in the chamber animals on day 14 of their exposure was approximately 147 mm. Hg. Although alveolar oxygen tension was not measured directly, this value can be calculated in subjects breathing oxygen in accordance with the following equation:[20]

$$P_{A_{O_2}} = P_B - P_{A_{H_2O}} - P_{A_{CO_2}} - P_{A_{N_2}}$$

in which P_B = total barometric pressure, and $P_{A_{O_2}}$, $P_{A_{H_2O}}$, $P_{A_{CO_2}}$ and $P_{A_{N_2}}$ represent respectively the alveolar tension of oxygen, water vapor, carbon dioxide and nitrogen. Assuming that $P_{A_{H_2O}}$ and $P_{A_{CO_2}}$ remain normal at 47 and 40 mm. Hg respectively, then

$$P_{A_{O_2}} = 258 - 47 - 40 - 5 = 166 \text{ mm. Hg}$$

The alveolar-arterial oxygen gradient derived by this method is 19 mm. Hg. While this value is greater than that reported as normal in room air (5-10 mm. Hg), it does not reflect increased venous to arterial shunting; the shape of the oxygen dissociation curve is such that at oxygen tensions of 160-170 mm. Hg a normal degree of venous admixture has a greater dilutional effect.[2]

TABLE V. ARTERIAL BLOOD OXYGEN TENSION (Pa_{O_2}) IN RATS ON DAY 14 OF EXPOSURE TO 98% OXYGEN AT 258 mm Hg, AND 6 - 8 HOURS AFTER RETURN TO ROOM AIR

GROUP	Number of Rats	Pa_{O_2} (mm Hg) Mean ± S.E.
Oxygen (day 14)	8	146.9 ± 5.2
Post-oxygen (6 - 8 hrs.)	8	95.8 ± 2.5
Control	10	95.3 ± 2.6

TABLE VI. BLOOD CHEMISTRIES AND ENZYMES. MEAN VALUES ± S. E.
(Number of rats studied in parentheses)

TEST	CONTROL	OXYGEN	P
BUN Mg/100ml*	17.2 ± .7 (9)	18.8 ± 1.0 (9)	$p > 0.2$
Phosphorus Mg/100ml*	5.1 ± .3 (12)	5.4 ± .3 (10)	$p > 0.4$
Total Bilirubin Mg/100ml*	0.08 ± .02 (12)	0.11 ± .02 (10)	$p > 0.4$
P.B.I. - Exp I μg/100ml	3.2 ± .3 (11)	3.0 ± .2 (12)	$p > 0.2$
P.B.I. - Exp II μg/100ml	3.0 ± .2 (12)	2.8 ± .1 (10)	$p > 0.2$
Alkaline Phosphatase units/100ml**	35.3 ± 2.0 (15)	35.9 ± 3.3 (9)	$p > 0.5$
SGOT units*	78.0 ± 5.2 (10)	85.6 ± 1.5 (10)	$p > 0.2$

* obtained in Exp II only
** obtained in Exp III only

In animals returned to room air following their two-week sojourn in oxygen, arterial oxygen tension was equivalent to that seen in controls (Table V). Dependence on the hyperoxic environment for maintenance of adequate oxygenation thus did not develop.

Blood chemistries and serum enzyme values are shown in Table VI. The various parameters of liver and kidney function investigated did not vary significantly from control levels. Of interest is the fact that although oxygen exposure at one atmosphere pressure results in a prompt fall in serum PBI,[8] this response does not occur at a pressure of 258 mm. Hg.

At necropsy the lungs in the oxygen exposed rats were pink, normal in consistency and floated in formalin. On histologic examination the alveolar spaces were free of edema or cellular exudate (Figure 4). However, in both controls and experimental animals focal perivascular and peribronchial accumulations of lymphocytes, as well as patchy areas of interstitial pneumonitis (Figure 5), were readily identifiable. These lesions, characteristic of chronic murine pneumonia,[14] were present with essentially the same fre-

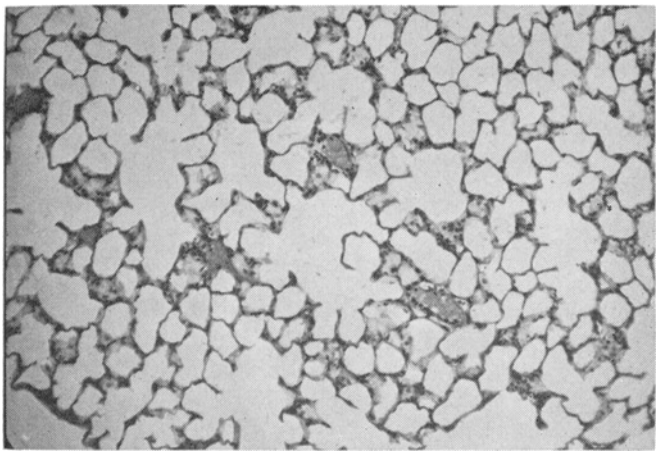

Fig. 4. Lung from oxygen exposed rat demonstrating normal degree of aeration as well as absence of intra-alveolar fluid or cellular exudate. X 103

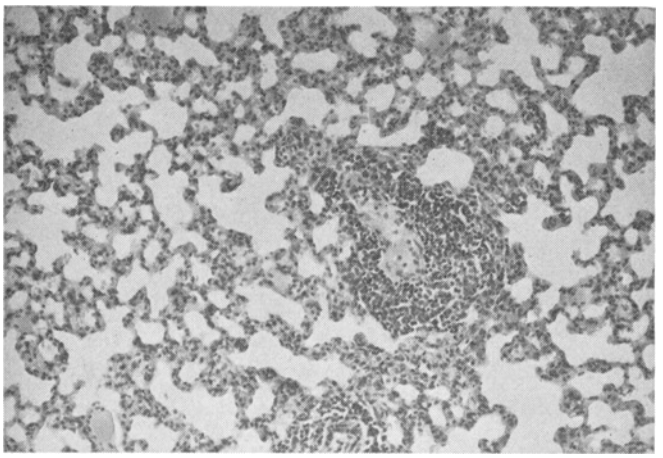

Fig. 5. Lung from control rat demonstrating perivascular accumulations of lymphocytes and inflammatory thickening of alveolar septa. Similar patchy changes were also observed in occasional oxygen-exposed rats. X 103

quency in control and experimental animals.

Light microscopic examination of the heart, liver, kidneys and thyroid was unremarkable. In the oxygen exposed animals given propyl thiouracil the typical goitrogenic response was noted. Grossly, the thyroids were increased about three times in size. On histologic evaluation they manifested absence of colloid with tall columnar epithelium, papillary infolding and increased vascularity characteristic of thyrotropin stimulation.

The pulmonary findings in the sole animal not surviving oxygen exposure were those of a lobar pneumonia with the alveoli in all lobes laden with polymorphonuclear leukocytes, lymphocytes and macrophages. These changes suggest a severe infectious process probably unrelated to the gaseous environment.

DISCUSSION

The experiments presented above represent an attempt to delineate the effects of continuous exposure to oxygen at a pressure of 258 mm. Hg. Although recent evaluations of this environment in human volunteers are in agreement as to the lack of demonstrable pulmonary damage,[5,19,22] unanimity of opinion does not exist with regard to effects on other organ systems. For example, Helvey, et al.[12] reported hemolytic anemia and abnormalities on urinalysis (proteinuria and cylindruria); yet, in another study, Mammen, et al.[17] noted disturbances in dark adaptation but could not substantiate the presence of hemolysis or renal dysfunction. On the other hand, Herlocher, et al.[13] reported a very mild reduction in hematocrit but normal dark adaptation curves and negative findings on urinalysis.

The results of the present investigation indicate that rats can survive and grow in an atmosphere of essentially pure oxygen at reduced pressure without adverse effect. In addition to confirming previous studies demonstrating survival of rodents in an environment almost devoid of inert gas,[4,15] the evidence presented supports the conclusion that an increase in the tension of inspired oxygen to a level of 5 PSI is well tolerated. These findings are, however, at variance with those of Dickerson,[3] who reported a mortality rate of 25 per cent in twenty rats exposed to oxygen at 258 mm. Hg for up to 30 days. However, since "their deaths could not be attributed to oxygen toxicity on the basis of lung findings," the possibility that some factor other than oxygen was the primary etiologic agent cannot be excluded.

Of particular interest in the present study, in view of the predominance of lung damage when oxygen is inhaled at 760 mm. Hg,[27] is the absence of detectable alterations in pulmonary structure on histologic examination, as well as the lack of evidence of increased veno-arterial shunting to suggest atelectasis. The focal inflammatory changes demonstrated in both control and experimental lungs reaffirm the need for detailed histologic evaluation when studying rats, as well as the requirement for adequate numbers of controls. Only by careful examination of both groups, as previously emphasized by Innes, et al.,[14] can naturally occurring chronic inflammatory processes be properly differentiated from experimentally acquired pulmonary lesions.

The mild fall in hematocrit reported is in accord with that noted by Zalusky, et al.[28] in humans. While hemolysis cannot be ruled out the absence of reticulocytosis or hyperbilirubinemia does not support this possibility. On the other hand a very mild suppression of erythropoeisis may be present, although the pronounced effects seen with higher tensions of oxygen[26] are obviously not operative.

With regard to the liver and kidney the results indicate no significant alteration in structure or function within the limits of the observations undertaken. These data would appear in conflict with previous studies from this laboratory indicating that exposure to oxygen at reduced pressure for one week can induce ultrastructural changes in the form of mitochondrial alterations in both liver and kidney.[7,24] More recent studies have shown, however, that at two weeks (the point at which liver and kidney function were determined) there is evidence of some restitution of normal morphology.[23] Furthermore, the functional significance of these mitochondrial changes has not as yet been ade-

quately clarified. More sensitive indices than levels of serum enzymes or blood metabolites may ultimately be required to fully evaluate the functional integrity of hepatic and renal cells.

The response of the thyroid gland to oxygen is of interest in view of the clear-cut differentiation in effects at 760 mm. Hg as compared to 258 mm. Hg. At the former pressure serum protein-bound iodine (PBI) declines within the first 24 hours of exposure and progressively decreases thereafter.[8] However, at the reduced pressure of the current study PBI remains unaltered when determined at the conclusion of a two-week exposure. In addition the typical goitrogenic response to propyl thouracil indicates that pituitary release of thyrotropin proceeds normally and that the thyroid retains its usual degree of responsiveness to this trophic agent.[11]

In summary, rats can tolerate a two-week exposure to oxygen at 5 PSI without development of pulmonary, hepatic, renal or thyroid abnormalities. A slight fall in hematocrit without evidence of hemolysis was the sole abnormality defined. The relative safety of this environment for exposures of greater than two weeks' duration will require further study.

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