SAM-TR-67-86

PHYSIOLOGIC AND BIOCHEMICAL OBSERVATIONS ON THE RHESUS MACACA MULATTA EXPOSED TO OXYGEN-RICH ATMOSPHERES

FRODE ULVEDAL, Ph.D.
H. E. BROWN, Ph.D.

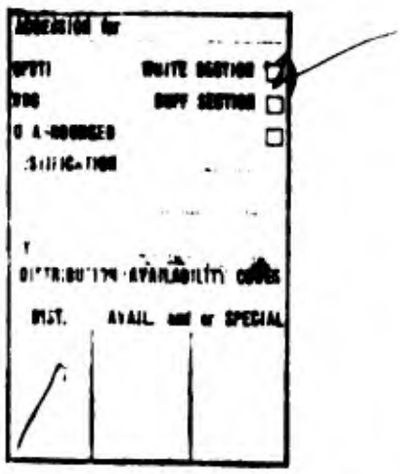
September 1967

USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

Qualified requesters may obtain copies of this report from DDC. Orders will be expedited if placed through the librarian or other person designated to request documents from DDC.

When U. S. Government drawings, specifications, or other data are used for any purpose other than a definitely related government procurement operation, the government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

This document has been approved for public release and sale; its distribution is unlimited.



PHYSIOLOGIC AND BIOCHEMICAL OBSERVATIONS ON THE RHESUS MACACA MULATTA EXPOSED TO OXYGEN-RICH ATMOSPHERES

FRODE ULVEDAL, Ph.D.

H. E. BROWN, Ph.D.

FOREWORD

The experiments described in this paper were conducted in the Environmental Systems Branch under task No. 793002. The work was accomplished between 17 May 1964 and 24 March 1965. The report was submitted for publication on 28 June 1967.

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences-National Research Council.

We are also indebted to the many chamber technicians, laboratory technicians, veterinary officers, and other personnel who participated in our research and without whose continuous help the task could not have been accomplished.

This report has been reviewed and is approved.

GEORGE E. SCHAFER
Colonel, USAF, MC
Commander

ABSTRACT

Adolescent male rhesus monkeys *(Macaca mulatta)*, weighing 2.9 to 3.4 kg, were exposed to 100% oxygen atmospheres at 258 and 380 mm. Hg pressures for 30 and 22 days, respectively, and the results obtained were compared to the observations made in a ground-level control study using the same chamber system. Physiologic, hematologic, enzymatic, biochemical, and anatomic measurements were obtained throughout the experiments. Several changes and trends were common to all three experiments; however, some were observed only for the oxygen-exposed animals and were dependent on the concentration of oxygen in the atmosphere.

PHYSIOLOGIC AND BIOCHEMICAL OBSERVATIONS ON THE RHESUS MACACA MULATTA EXPOSED TO OXYGEN-RICH ATMOSPHERES

I. INTRODUCTION

Subhuman primates have been used in medical and biologic experiments studying the tolerance to various space environmental conditions in advance of human experiments. The use of these animals has certain advantages in experiments of this kind in that anatomic, biochemical, and physiologic measurements can be considerably more detailed than with human volunteers. Several long-duration experiments were, therefore, performed using the rhesus *Macaca mulatta* in an attempt to evaluate in detail the possible effects of two proposed spacecraft environments consisting of oxygen as the single gas atmosphere. Because several scientific disciplines were covered in this study, the results from liver enzyme evaluations, histologic findings, and the assays for lung surfactant will be reported separately. This paper will report on some of the physiologic and biochemical observations which were made during these experiments.

II. SUMMARY

The results reported in this paper show that the rhesus *Macaca mulatta* can tolerate a 100% oxygen atmosphere at a total pressure of 258 mm. Hg with only minor changes to the various physiologic and anatomic variables In a 100% oxygen atmosphere at 380 mm. Hg total pressure, the changes in the various physiologic and anatomic variables become somewhat more pronounced; in certain cases, these changes are of statistical significance. Most of the hematologic changes observed in these animals were believed to be adaptive changes due to an enriched oxygen environment rather than symptoms of oxygen toxicity, though this possibility cannot be ruled out.

III. METHODS

Adolescent male rhesus monkeys *(Macaca mulatta)* were used for this study. They ranged in weight from 2.9 to 3.4 kg. at the onset of each experiment. The animals, all in good health, were accustomed to daily handling and other treatment procedures which would be used during the experiment while still in the holding colony. The animals were fed once a day with standard primate chow (Ralston Purina) along with supplemental vitamins. Furthermore, the animals received fresh fruit or vegetables each day on a rotating schedule; one day they were given half an apple, the next day one carrot, and on the third day a third of one potato. Fresh water was available all the time through spigots. At the time the animals were removed from the holding colony, they were placed in individual cages in a rectangular altitude chamber. The chamber measured approximately 25.6 $m.^3$ in volume and had a pass lock about one-third the size of the main chamber. The individual cages measured 40.6 cm. in length, 50.8 cm. in height, and 30.5 cm. in width. The animals were kept in the chamber for 14 days at ground-level conditions. The purpose of this procedure was to adapt the animals to the individual, more restrictive cages, as well as to the 10-hour light period maintained in the chamber, the noise from the various chamber blowers and pumps, and the general traffic in and out of the chamber. The chamber and drop trays were cleaned

every day before feeding time. Weights (to the nearest 25 gm.) and rectal temperatures (to the nearest 0.1° C.) were measured and recorded twice weekly (Mondays and Thursdays) and were used as a general index of well-being of the animals throughout the experiments. Furthermore, veterinary officers inspected the animals twice weekly during the entire period they were in the chamber. After the initial adaptation period, the experimental phase of the profile was begun.

Three experiments were performed in this study with each experiment divided into three periods: a pre-experimental period, an experimental period, and a postexperimental period. The first experiment was conducted entirely at ground-level atmospheric conditions and consisted of a 14-day pre-experimental period, a 30-day experimental period, and a 14-day postexperimental period. This was done to ascertain if normal chamber environment would have any effect on the variables to be measured and to establish baselines for the altitude experiments.

During the second experiment the animals were taken to 258 mm. Hg (27,000 ft.) pressure with a 100% oxygen atmosphere and kept in that environment continuously for 30 days, after a 14-day pre-experimental control period. The initial change from normal atmosphere to 100% oxygen atmosphere was accomplished in 2 hours. After the 30-day exposure, there was a 14-day postexperimental recovery period in the chamber. The third experiment of this series lasted for 22 days and was conducted in a 100% oxygen atmosphere at 380 mm. Hg (18,000 ft.) pressure, after a 14-day pre-experimental period, with a 30-day recovery period in the chamber at ground-level conditions after the exposure period. This experiment had been programmed with a 30-day exposure period followed by a 14-day recovery period, similar to the other two experiments. On the basis of the results obtained during this experiment, however, it was decided to shorten the exposure period and thereby lengthen the recovery period to ascertain the time required for the observed changes to return to normal.

These three experiments are summarized in table I, which indicates the dates of the various experiments and the atmospheric conditions which were maintained during the experiments.

The instruments used to measure the environmental atmospheres were: Beckman models F-3 and E-2 for oxygen, Beckman 15A (infrared) for carbon dioxide, Cambridge dew point hygrometer for relative humidity, Nitralyzer 305 AR for nitrogen, YSI model 46 Tele-thermometer for rectal and atmospheric temperatures, and a Wallace and Tiernan absolute pressure gage.

Every seventh day, 5 ml. of blood were drawn from either of the saphenous veins and immediately divided into vials containing either heparin (Sigma) or oxalate, or vials without an anticlotting agent for serum collection; all of these vials were kept ice-cold until the various assays were performed. The blood sampling schedule was arranged so that half the animals were bled on Mondays and the other half bled on Thursdays, thus providing two assay points per week.

Animals were sacrificed, two at a time, at regular intervals during the experimental periods corresponding to one-third, two-thirds, and completion of exposure, and similarly during the postexperimental periods. In addition to the blood studies performed on the necropsied animals, organs were weighed and samples taken for histologic studies, liver enzyme studies, and lung surfactant studies. Bacteriologic studies were also done throughout this series of experiments for enteric pathogenic microorganisms. Seven primates were similarly examined while they were in the veterinary holding colony.

The assays performed on whole blood were routine hematology (RBC, WBC, hematocrit, hemoglobin, differential counts) (31), sulfhemoglobin, methemoglobin (7), glutathione, and glutathione stability (2). The assays performed on washed red blood cells were glucose-6-phosphate dehydrogenase (32) and hexokinase (15). The hexokinase assay was performed only on red blood cells from animals

TABLE I

Atmospheric conditions during the three experiments

Experiment	Duration	Barometric pressure (mm. Hg)	Temperature (°C.)	Relative humidity	Oxygen	Carbon dioxide	Nitrogen
I					(percent)		
Pre-exp.	17 May–31 May 1964	750.0 ± 2.9*	25.1 ± 1.8	—	20.95	0.03	78.08
Exp.	31 May–30 June 1964	749.1 ± 1.6	25.7 ± 0.1	39.4 ± 2.5	20.95	0.03	78.08
Postexp.	30 June–15 July 1964	748.8 ± 1.3	25.6 ± 0.5	39.5 ± 1.3	20.95	0.03	78.08
II							
Pre-exp.	22 Aug.–5 Sept. 1964	752.4 ± 2.4	25.8 ± 0.8	—	20.95	0.03	78.08
Exp.	5 Sept.–7 Oct. 1964	258.0 ± 0.2	26.0 ± 0.5	21.4 ± 0.7	99.50 ± 0.24	0.13 ± 0.05	0.23 ± 0.07
Postexp.	7 Oct.–21 Oct. 1964	754.2 ± 4.1	25.0 ± 0.8	49.0 ± 0.9	20.95	0.03	78.08
III							
Pre-exp.	17 Jan.–31 Jan. 1965	752.9 ± 4.9	26.0 ± 1.7	62.5 ± 5.5	20.95	0.03	78.08
Exp.	31 Jan.–22 Feb. 1965	380.0 ± 0.0	26.4 ± 0.3	59.3 ± 2.4	99.21 ± 0.25	0.25 ± 0.09	0.38 ± 0.10
Postexp.	22 Feb.–24 Mar. 1965	754.0 ± 1.4	26.0 ± 1.4	63.4 ± 5.0	20.95	0.03	78.08

*Standard deviation.

exposed to 100% O_2 at 380 mm. Hg. The following assays were performed on serum or plasma: glutamic oxalacetic transaminase (GOT) (24), glutamic pyruvic transaminase (GPT) (24), alkaline phosphatase (14, 20), creatinine (10), total protein (16), albumin, and albumin to globulin (A/G) ratio, as well as electrophoresis patterns for the individual plasma globulins (25).

IV. RESULTS

Visual observations and physical examinations of the animals gave the impression that they remained healthy, vigorous, and active throughout the experiments. Their appetites were excellent and at no time did they become docile. These observations are supported by the weight and rectal temperature measurements which were obtained twice weekly throughout the experiments and as shown in table II. The ground-level control animals gained on the average 2.6% and 6.4% for the experimental period and postexperimental period, respectively, while the animals exposed to 100% oxygen at 258 mm. Hg pressure gained 4.0% and 9.3% during the same periods. The animals exposed to 100% oxygen at 380 mm. Hg pressure gained an average of 4.1% during the 22 days in the experimental atmosphere, but the remaining animals lost weight postexperimentally so that the net gain compared to the pre-experiment mean was only 1.4%. The return to normal atmosphere might, therefore, have affected these animals. As is also indicated in table II, the postexperimental weight gains for the ground-level control animals and the animals exposed to 100% oxygen at 258 mm. Hg pressure were statistically higher when compared to the pre-experimental weights, while the weight gains for the animals exposed to 100% oxygen at 380 mm. Hg pressure were not significantly changed for either period. It should be mentioned that there was statistically no difference, initially, between the animals sacrificed during the postexperimental periods and the pre-experimental periods. Furthermore, the animals were randomly selected from the colony and randomly placed in their individual cages in the chamber. The order in which the animals were sacrificed was, therefore, also on a random basis. The rectal temperatures for these animals were within normal range, indicating again that the animals were in good health.

Table III gives the average changes for the hematologic variables as observed during these

TABLE II

Body weights and rectal temperatures

Condition	Pre-experimental		Experimental			Postexperimental		
	Number of animals	Mean	Number of animals	Mean	P*	Number of animals	Mean	P*
	Body weights (kg.)							
Ground-level	10	3.14 ± 0.21†	10	3.22 ± 0.34	NS	4	3.34 ± 0.12	.05
258 mm. Hg	10	3.22 ± 0.26	10	3.35 ± 0.21	NS	4	3.52 ± 0.21	.05
380 mm. Hg	22	2.94 ± 0.07	20	3.06 ± 0.26	NS	6	2.98 ± 0.31	NS
	Rectal temperatures (°C.)							
Ground-level	10	39.1 ± 0.2	10	39.0 ± 0.3	NS	4	39.1 ± 0.2	NS
258 mm. Hg	10	39.5 ± 0.3	10	39.3 ± 0.3	NS	4	39.3 ± 0.1	.05
380 mm. Hg	22	39.3 ± 0.3	20	39.1 ± 0.4	NS	6	39.2 ± 0.2	NS

*P = Level of significance when compared with pre-experimental mean.

†Standard deviation.

TABLE III

Hematologic variables

Condition	Pre-experimental		Experimental			Postexperimental		
	Number of animals	Mean	Number of animals	Mean	P*	Number of animals	Mean	P*
Hemoglobin (gm./100 ml.)								
Ground-level	10	12.9 ± 0.8†	10	12.1 ± 0.7	.05	4	12.8 ± 0.6	NS
258 mm. Hg	10	12.2 ± 0.5	10	11.4 ± 0.6	.01	4	11.2 ± 0.7	.02
380 mm. Hg	22	12.2 ± 0.8	20	11.3 ± 0.7	.01	6	11.3 ± 0.7	.01
Hematocrit (%)								
Ground-level	10	41.9 ± 1.4	10	40.6 ± 2.1	NS	4	41.9 ± 1.4	NS
258 mm. Hg	10	42.2 ± 1.4	10	38.6 ± 2.0	.01	4	38.0 ± 1.5	.01
380 mm. Hg	22	41.8 ± 1.8	20	38.6 ± 2.0	.01	6	37.9 ± 1.5	.01
RBC (× 10^6/mm.^{3})								
Ground-level	10	5.03 ± 0.44	10	4.60 ± 0.30	NS	4	5.00 ± 0.45	NS
258 mm. Hg	10	5.04 ± 0.24	10	4.85 ± 0.32	.01	4	4.89 ± 0.09	NS
380 mm. Hg	22	5.12 ± 0.36	20	4.80 ± 0.47	.02	6	4.17 ± 0.22	.01
WBC (× 10^3/mm.^{3})								
Ground-level	10	16.80 ± 4.45	10	12.15 ± 2.82	.05	4	13.46 ± 1.53	.10
258 mm. Hg	10	15.32 ± 2.93	10	12.75 ± 3.82	.01	4	10.05 ± 2.25	.01
380 mm. Hg	22	12.98 ± 3.08	20	11.09 ± 3.78	.10	6	8.20 ± 0.49	.01
Reticulocytes (%)								
Ground-level	10	1.13 ± 0.39	10	1.18 ± 0.12	NS	4	1.42 ± 0.34	NS
258 mm. Hg	10	1.22 ± 0.12	10	1.34 ± 0.30	NS	4	1.26 ± 0.10	NS
380 mm. Hg	22	1.27 ± 0.44	20	1.35 ± 0.20	NS	6	1.24 ± 0.62	NS
Neutrophils (%)								
Ground-level	10	40.1 ± 12.7	10	30.3 ± 9.4	.10	4	30.0 ± 9.0	NS
258 mm. Hg	10	23.3 ± 5.6	10	23.5 ± 10.6	NS	4	12.6 ± 1.5	.01
380 mm. Hg	22	30.4 ± 12.5	20	29.1 ± 12.6	NS	6	11.5 ± 2.8	.01
Lymphocytes (%)								
Ground-level	10	57.5 ± 12.7	10	67.8 ± 9.3	.05	4	68.7 ± 8.6	.10
258 mm. Hg	10	72.5 ± 6.1	10	70.6 ± 12.3	NS	4	81.3 ± 2.7	.01
380 mm. Hg	22	63.5 ± 12.7	20	64.1 ± 14.0	NS	6	79.7 ± 4.2	01

TABLE III (contd.)

Condition	Pre-experimental		Experimental			Postexperimental		
	Number of animals	Mean	Number of animals	Mean	P*	Number of animals	Mean	P*
Monocytes (%)								
Ground-level	10	1.3 ± 1.0	10	2.0 ± 0.6	.05	4	1.0 ± 0.8	NS
258 mm. Hg	10	1.6 ± 0.3	10	3.0 ± 1.0	.01	4	2.3 ± 0.8	.10
380 mm. Hg	22	2.4 ± 1.0	20	2.1 ± 1.1	NS	6	2.2 ± 0.4	NS
Eosinophils (%)								
Ground-level	10	1.2 ± 1.6	10	2.3 ± 2.8	.10	4	1.5 ± 1.7	NS
258 mm. Hg	10	3.4 ± 1.2	10	3.7 ± 2.0	NS	4	4.7 ± 2.9	NS
380 mm. Hg	22	4.4 ± 3.5	20	5.6 ± 3.8	NS	6	6.8 ± 3.2	NS
Bands (%)								
Ground-level	10	0.3 ± 0.0	10	0.2 ± 0.0	NS	4	0.0 ± 0.0	NS
258 mm. Hg	10	0.0 ± 0.0	10	1.5 ± 0.7	NS	4	0.0 ± 0.0	NS
380 mm. Hg	22	0.0 ± 0.0	20	0.0 ± 0.0	NS	6	0.0 ± 0.0	NS
Basophils (%)								
Ground-level	10	0.0 ± 0.0	10	0.4 ± 0.7	NS	4	0.0 ± 0.0	NS
258 mm. Hg	10	1.0 ± 0.0	10	0.2 ± 0.0	NS	4	0.0 ± 0.0	NS
380 mm. Hg	22	1.5 ± 0.7	20	1.0 ± 0.0	NS	6	0.0 ± 0.0	NS
Methemoglobin (gm./100 ml.)								
Ground-level	10	0.16 ± 0.03	10	0.16 ± 0.02	NS	4	0.13 ± 0.02	.05
258 mm. Hg	10	0.16 ± 0.04	10	0.17 ± 0.03	NS	4	0.21 ± 0.06	NS
380 mm. Hg	12	0.41 ± 0.08	10	0.49 ± 0.24	NS	6	— —	—
Sulfhemoglobin (gm./100 ml.)								
Ground-level	10	0.55 ± 0.03	10	0.54 ± 0.03	NS	1	0.56 ± 0.01	.02
258 mm. Hg	10	0.66 ± 0.03	10	0.62 ± 0.02	NS	4	0.75 ± 0.01	.01
380 mm. Hg	12	0.97 ± 0.04	10	0.91 ± 0.14	NS	6	— —	—

*P Level of significance when compared with pre-experimental mean.
†Standard deviation.

experiments. Several of the changes were highly significant even though all the values are within the accepted normal range (16, 18, 26). From the average values, it can be observed that the hemoglobin values decreased during the experimental periods by 5.6%, 6.9%, and 7.8% for the ground-level experiment, 258 mm. Hg experiment, and 380 mm. Hg experiment, respectively. The hemoglobin values for the control animals returned to normal during the postexperimental period, whereas the values for the oxygen-exposed animals did not. Figure 1 shows the average values in each of the three experiments for the two animals which were followed throughout and necropsied on the last day of their respective postexperimental periods. These representative animals show a similar trend for hemoglobin concentration as well as the different responses to increased concentrations of oxygen. While the initial decreases may have been due to blood loss, the stabilization for the ground-level animals as compared to the animals exposed to 100% oxygen at 258 and 380 mm. Hg pressures separates this effect from the continued effect as caused by the atmosphere. Undoubtedly, the oxygen concentration at 380 mm. Hg is detrimental since the trend in hemoglobin concentration is downward throughout, while the trend in those animals maintained at 258 mm. Hg levels off and then begins to return to normal while the animals were still exposed to the elevated oxygen atmosphere.

The hematocrit values for these animals, which are also indicated in figure 1, parallel the hemoglobin trends. Even though there are decreases in these two variables during the equivalent ground-level experimental period, they are either statistically insignificant or only of borderline significance, while the equivalent values for the oxygen-exposed animals are highly significant, showing that the atmosphere affected these variables. Furthermore, they were also significant during the postexperimental periods. Percentagewise, the hematocrit of the animals exposed to 100% oxygen at 258 mm. Hg pressure dropped an average of 8.5%, with a 7.7% drop for the animals exposed to 100% oxygen at 380 mm.

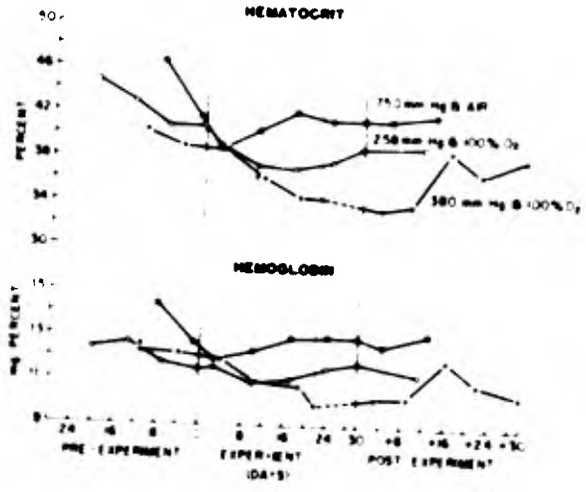


FIGURE 1

Hematocrit and hemoglobin values obtained during the three experiments as followed for the two primates sacrificed on the last day of their respective postexperimental periods.

Hg pressure compared to 3.2% for the ground-level animals during the experimental periods. Postexperimentally, there was no decrease in hematocrit values for the ground-level animals, while for the oxygen-exposed animals at 258 and 380 mm. Hg pressures, the decreases were 10.0% and 9.4%, respectively. It should be remembered that at 380 mm. Hg pressure, the animals were exposed to the oxygen atmosphere for 22 days, 8 days less than the exposure time for the animals at 258 mm. Hg pressure.

The red cell counts were more variable, and it is, therefore, difficult to ascertain a trend (fig. 2). The curves plotted from the average red blood cell counts from the ground-level animals and the animals exposed to the oxygen atmosphere at 258 mm. Hg pressure parallel each other and show initial declines, but these values returned toward normal even during the respective experimental phases. The red cell counts for the oxygen-exposed animals at 380 mm. Hg pressure also show this initial decline, but instead of returning to normal, there is a further decline which continues into the postexperimental recovery period. This decrease

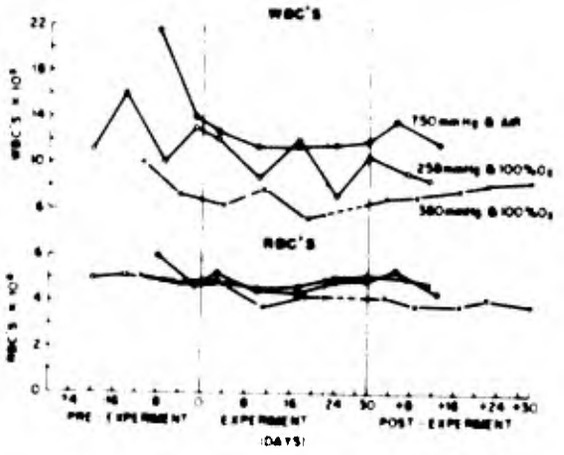


FIGURE 2

White blood cell and red blood cell counts obtained during the three experiments as followed for the two primates sacrificed on the last day of their respective postexperimental periods.

postexperimentally was 18.6% and is significant ($P < .01$) when compared to the preexperimental value (table III).

The white blood cell counts decreased significantly for all three groups during the experimental periods, with the ground-level animals showing a return toward the original level postexperimentally. Postexperimentally, there were further and significant decreases for the two groups of oxygen-exposed animals as indicated in table III and figure 2.

None of the average values for the reticulocyte counts were significantly different; however, it should be noted that while the counts for the ground-level animals showed slight but progressive increases for the three periods, the postexperimental averages for the oxygen-exposed animals were lower than during the experimental period (table III). The results of the differential counts are shown in table III and indicate that with few exceptions, there were no major changes due to the experimental parameters in the environments. Likewise, the assays for methemoglobin and sulfhemoglobin did not show any significant changes due to the treatment, be it either habitation in the chamber or the atmosphere at decreased pressures. Admittedly, there are differences in the baseline values for the three groups of animals, and postexperimental values are missing for the 380 mm. Hg group, which makes any comparison difficult.

In table IV are the results from the assays of glucose-6-phosphate dehydrogenase (G-6-PD), glutathione (GSH), and glutathione stability, as well as for hexokinase. The preexperimental, experimental, and postexperimental average values for the ground-level group show progressive increases in G-6-PD activity, as does the trend for the two primates indicated in figure 3. The oxygen-exposed animals showed the opposite trend, and G-6-PD activity decreased during the experimental periods with return to normal activity postexperimentally. These changes, however, are not significant or are only of borderline significance, but they do indicate a different trend for the oxygen-exposed animals. In the graphs for the animals at 258 and 380 mm. Hg pressures, it is difficult to observe that the two curves go in the same direction, but they do, as verified mathematically.

The hexokinase activity in the red blood cells was determined only during the 380 mm. Hg experiment and shows a significant decrease during the exposure to the oxygen atmosphere with a significantly elevated average level of activity postexperimentally.

The results for GSH and GSH stability (table IV) did not indicate that any major changes took place with these SH-donors during these experiments. Figure 3, which presents data from the same two animals throughout the experiment, likewise shows no change.

The values obtained for plasma protein, albumin, and the globulin fractions are shown in table V and indicate normality for all groups during the various experimental phases. There are a few changes; for example, the albumin levels in the plasma increased percentagewise for the oxygen-exposed animals during the postexperimental recovery periods. Likewise,

TABLE IV

Hematologic variables in the red blood cell

Condition	Pre-experimental		Experimental			Postexperimental		
	Number of animals	Mean	Number of animals	Mean	P*	Number of animals	Mean	P*
Glucose-6-phosphate dehydrogenase (enzyme units/100% RBC)								
Ground-level	10	164.6 ± 17.5†	10	169.5 ± 12.4	NS	4	186.3 ± 10.6	.02
258 mm. Hg	10	188.7 ± 15.8	10	172.9 ± 14.4	.05	4	184.0 ± 8.5	NS
380 mm. Hg	22	158.9 ± 15.4	20	151.6 ± 13.1	NS	6	154.2 ± 10.0	NS
Hexokinase (Δ OD/min./100% RBC)								
Ground-level	No data							
258 mm. Hg	No data							
380 mm. Hg	22	9.49 ± 3.08	20	7.24 ± 2.69	.02	6	12.27 ± 1.14	.01
Glutathione (mg./100% RBC)								
Ground-level	10	121.2 ± 14.1	10	122.5 ± 11.1	NS	4	114.7 ± 11.7	NS
258 mm. Hg	10	113.6 ± 8.8	10	121.9 ± 9.0	.10	4	121.7 ± 11.2	NS
380 mm. Hg	22	117.9 ± 12.7	10	119.5 ± 15.1	NS	6	108.4 ± 16.2	NS
Glutathione stability (mg./100% RBC)								
Ground-level	10	105.0 ± 17.4	10	106.1 ± 15.5	NS	4	106.6 ± 11.4	NS
258 mm. Hg	10	102.5 ± 6.4	10	114.4 ± 8.0	.01	4	111.4 ± 6.4	.05
380 mm. Hg	22	116.9 ± 12.4	20	120.8 ± 11.8	NS	6	107.8 ± 15.0	NS

*P = Level of significance when compared with pre-experimental mean.
†Standard deviation.

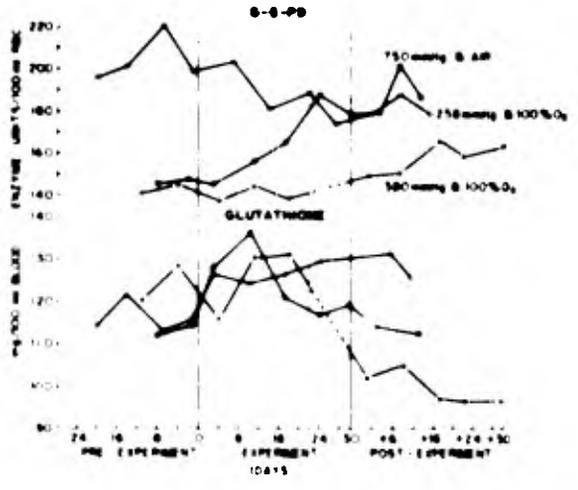


FIGURE 3

Glucose-6-phosphate dehydrogenase activity and glutathione values in red blood cells as followed for the two primates sacrificed on the last day of their respective postexperimental periods.

the A/G ratios increased postexperimentally for the animals at 258 and 380 mm. Hg pressures.

Table VI shows the data for alkaline phosphatase, GOT, GPT, and creatinine in the plasma of the primates used in these experiments. The alkaline phosphatase activity decreased somewhat during the experimental periods for the three groups of animals, but only to a borderline significant degree (P = .05) for the animals at 380 mm. Hg pressure. The same can be said for the GOT and GPT except that the changes were not significant; however, the changes for these three variables revert toward pre-experimental level after the exposure periods. Plasma creatinine values were significantly elevated for the 380 mm. Hg group during the exposure to the oxygen atmosphere and remained high during the recovery period in normal atmosphere. Similar but insignificant changes were evident for the 258 mm. Hg group, while the plasma creatinine level for the ground-level control animals remained stable throughout.

TABLE V

Plasma proteins and electrophoresis patterns

Condition	Pre-experimental		Experimental			Postexperimental		
	Number of animals	Mean	Number of animals	Mean	P*	Number of animals	Mean	P*
Total protein (gm./100 ml.)								
Ground-level	10	7.42 ± 0.23†	10	7.41 ± 0.34	NS	4	7.25 ± 0.15	NS
258 mm. Hg	10	7.26 ± 0.30	10	7.45 ± 0.46	NS	4	7.36 ± 0.25	NS
380 mm. Hg	22	7.33 ± 0.40	20	7.35 ± 0.40	NS	6	7.11 ± 0.24	NS
Albumin (%)								
Ground-level	10	61.1 ± 1.9	10	57.4 ± 4.2	.10	4	60.4 ± 4.0	NS
258 mm. Hg	10	57.5 ± 3.2	10	60.5 ± 6.2	NS	4	61.8 ± 3.8	.05
380 mm. Hg	22	60.4 ± 3.8	20	60.1 ± 4.1	NS	6	62.8 ± 1.8	.01
A/G ratio								
Ground-level	10	1.6 ± 0.1	10	1.4 ± 0.2	.05	4	1.6 ± 0.2	NS
258 mm. Hg	10	1.4 ± 0.2	10	1.6 ± 0.4	.10	4	1.6 ± 0.3	.10
380 mm. Hg	22	1.6 ± 0.2	20	1.5 ± 0.2	NS	6	1.7 ± 0.1	.05
Alpha 1 (%)								
Ground-level	10	2.7 ± 0.3	10	3.2 ± 0.6	.05	4	2.8 ± 0.4	NS
258 mm. Hg	10	3.7 ± 0.4	10	3.3 ± 0.4	.05	4	3.2 ± 0.3	.05
380 mm. Hg	22	3.7 ± 0.8	20	3.5 ± 0.7	NS	6	3.4 ± 0.4	NS
Alpha 2 (%)								
Ground-level	10	3.9 ± 1.3	10	4.5 ± 1.2	NS	4	4.2 ± 1.5	NS
258 mm. Hg	10	4.5 ± 0.5	10	4.2 ± 0.6	NS	4	4.5 ± 0.6	NS
380 mm. Hg	22	5.0 ± 1.2	20	4.5 ± 1.0	NS	6	5.3 ± 0.4	NS
Alpha 3 (%)								
Ground-level	10	4.0 ± 1.8	10	4.2 ± 1.5	NS	4	3.7 ± 0.5	NS
258 mm. Hg	10	4.2 ± 1.9	10	4.1 ± 1.6	NS	4	3.8 ± 1.3	NS
380 mm. Hg	22	4.2 ± 2.5	20	4.2 ± 2.4	NS	6	2.5 ± 2.2	NS
Beta (%)								
Ground-level	10	10.2 ± 2.4	10	10.1 ± 1.9	NS	4	8.6 ± 2.1	NS
258 mm. Hg	10	8.7 ± 2.3	10	8.3 ± 1.5	NS	4	8.3 ± 2.5	NS
380 mm. Hg	22	9.9 ± 2.8	20	10.3 ± 0.1	NS	6	10.9 ± 2.5	NS
Fibrinogen (%)								
Ground-level	10	3.3 ± 0.7	10	4.2 ± 1.2	.10	4	3.3 ± 0.9	NS
258 mm. Hg	10	2.5 ± 0.3	10	2.2 ± 0.9	NS	4	2.4 ± 0.5	NS
380 mm. Hg	22	3.0 ± 0.6	20	3.1 ± 0.9	NS	6	2.4 ± 0.5	.02
Gamma (%)								
Ground-level	10	15.2 ± 1.4	10	17.1 ± 1.8	.02	4	16.3 ± 1.3	NS
258 mm. Hg	10	19.3 ± 3.0	10	17.4 ± 3.3	NS	4	16.9 ± 1.5	.10
380 mm. Hg	22	13.3 ± 3.5	20	14.2 ± 3.1	NS	6	12.7 ± 0.8	NS

*P = Level of significance when compared with pre-experimental mean.

†Standard deviation.

TABLE VI

Plasma biochemical variables

Condition	Pre-experimental		Experimental			Postexperimental		
	Number of animals	Mean	Number of animals	Mean	P*	Number of animals	Mean	P*
Alkaline phosphatase (mg. % phenol)								
Ground-level	10	44.6 ± 7.1†	10	41.6 ± 7.4	NS	4	48.7 ± 4.6	NS
258 mm. Hg	10	39.0 ± 8.1	10	37.1 ± 6.6	NS	4	37.7 ± 9.4	NS
380 mm. Hg	22	58.0 ± 15.7	20	48.2 ± 11.3	.05	6	50.1 ± 16.7	NS
GOT (S-F units/ml. serum)								
Ground-level	10	30.9 ± 12.3	10	28.2 ± 5.8	NS	4	28.3 ± 4.1	NS
258 mm. Hg	10	35.9 ± 5.2	10	35.1 ± 7.7	NS	4	45.2 ± 10.6	.10
380 mm. Hg	22	39.2 ± 12.1	20	37.8 ± 8.6	NS	6	41.1 ± 13.7	NS
GPT (S-F units/ml. serum)								
Ground level	10	18.6 ± 6.0	10	18.8 ± 3.7	NS	4	18.0 ± 2.2	NS
258 mm. Hg	10	27.1 ± 4.7	10	27.1 ± 5.9	NS	4	32.5 ± 2.6	.02
380 mm. Hg	22	28.8 ± 9.0	20	25.2 ± 7.4	.10	6	25.5 ± 6.5	NS
Creatinine (mg. %)								
Ground-level	10	0.79 ± 0.09	10	0.85 ± 0.08	NS	4	0.84 ± 0.08	NS
258 mm. Hg	10	0.97 ± 0.08	10	0.95 ± 0.13	NS	4	1.04 ± 0.13	NS
380 mm. Hg	22	0.84 ± 0.22	20	0.94 ± 0.10	.05	6	1.00 ± 0.17	.10

*P = Level of significance when compared with pre-experimental mean

†Standard deviation.

Figures 4 through 9 show graphically the organ weights expressed per kilogram body weight for each pair of primates as they were necropsied on corresponding days during the three experiments, and table VII summarizes the organ weights for all the animals as they were sacrificed by experimental periods. The results in table VII are analyzed in two ways. First, the data for the various experimental periods are compared to the data obtained from seven colony control primates of corresponding sex, age, and weight; and secondly, the data for the oxygen-exposed animals are compared within group to the ground-level control animals for the same periods. The ground-level control animals are different from the colony control animals.

The liver values when reported as grams per kilogram body weight show progressive decreases as the oxygen concentration increases (fig. 4). For the animals exposed to 380 mm. Hg pressure, this decrease is quite rapid, reaching a low point about the eighth exposure day.

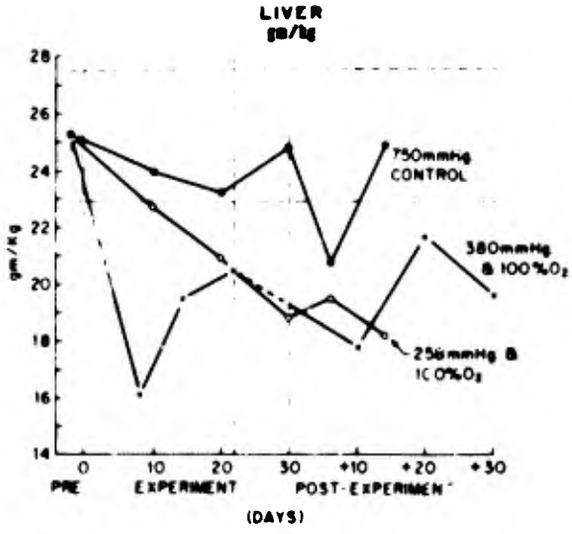


FIGURE 4

Average liver weights reported in grams per kilogram body weight for each pair of primates as they were necropsied throughout the three experiments.

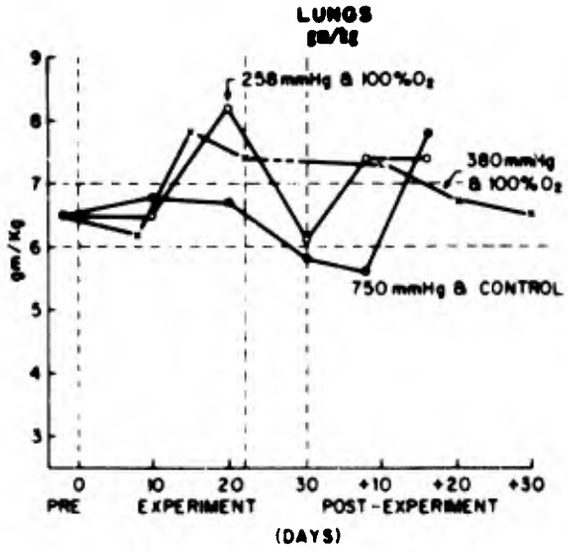


FIGURE 5

Average lung weights reported in grams per kilogram body weight for each pair of primates as they were necropsied throughout the three experiments.

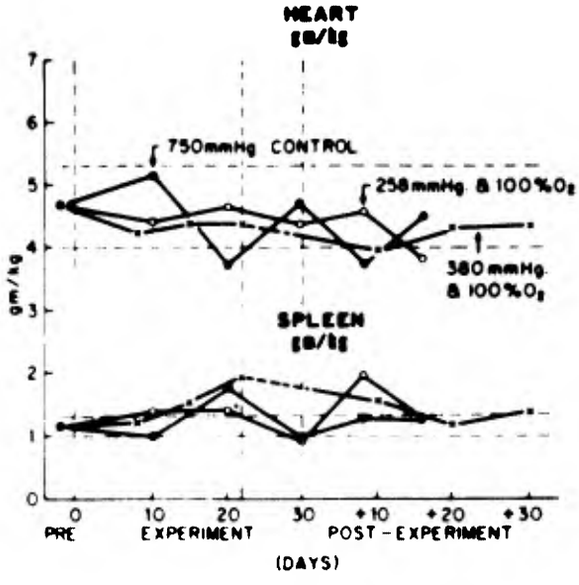


FIGURE 6

Average heart and spleen weights reported in grams per kilogram body weight for each pair of primates as they were necropsied throughout the three experiments.

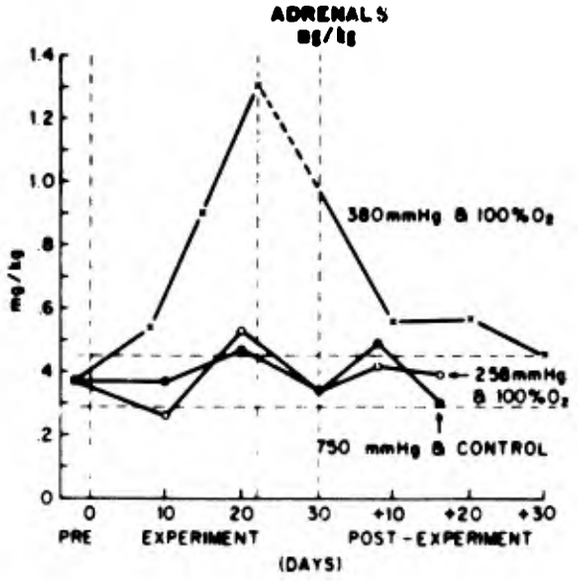


FIGURE 7

Average adrenal weights reported in milligrams per kilogram body weight for each pair of primates as they were necropsied throughout the three experiments.

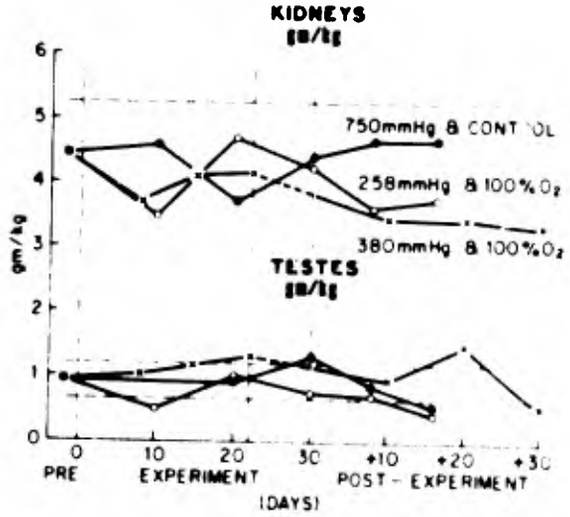


FIGURE 8

Average kidney and testes weights reported in grams per kilogram body weight for each pair of primates as they were necropsied throughout the three experiments.

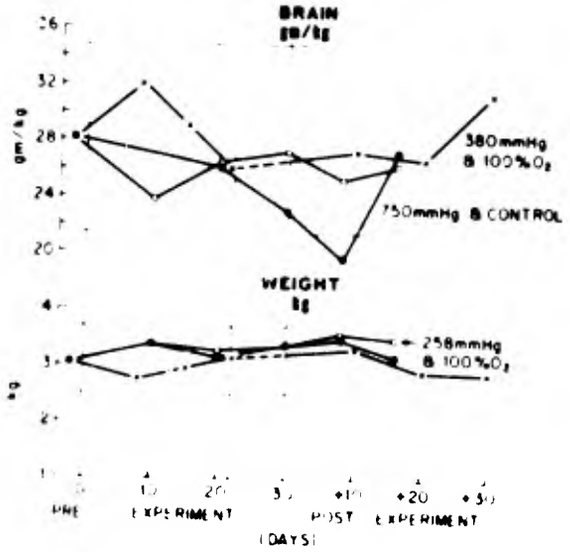


FIGURE 9

Average brain weights reported in grams per kilogram body weight and the average weight (in kilograms) for the two primates as recorded on their respective necropsy days

TABLE VII

Organ weights

Condition	Colony controls		Experimental				Postexperimental			
	Number of animals	Mean	Number of animals	Mean	P*	P†	Number of animals	Mean	P*	P†
				Liver (gm./kg.)						
Ground-level	7	25.20 ± 2.29‡	6	24.09 ± 3.75	NS	—	4	22.77 ± 2.62	NS	—
258 mm. Hg		(26.88)§	6	20.88 ± 2.22	.02	.10	4	18.86 ± 1.93	.01	.10
380 mm. Hg			14	20.42 ± 2.70	.01	.05	6	19.81 ± 3.35	.01	.20
				Lung (gm./kg.)						
Ground-level	7	6.49 ± 0.52	5	6.68 ± 0.61	NS	—	4	6.68 ± 1.63	NS	—
258 mm. Hg		(7.67)	6	6.90 ± 1.15	NS	NS	4	7.39 ± 0.27	.01	NS
380 mm. Hg			14	6.77 ± 0.79	NS	NS	6	6.87 ± 0.81	NS	NS
				Heart (gm./kg.)						
Ground-level	7	4.62 ± 0.65	6	4.64 ± 1.17	NS	—	4	4.39 ± 0.61	NS	—
258 mm. Hg		(4.57)	6	4.49 ± 0.34	NS	NS	4	4.19 ± 0.47	NS	NS
380 mm. Hg			14	4.01 ± 0.42	.05	NS	6	4.22 ± 0.77	NS	NS

TABLE VII (contd.)

Condition	Colony controls		Experimental				Postexperimental			
	Number of animals	Mean	Number of animals	Mean	P*	P†	Number of animals	Mean	P*	P†
Spleen (gm./kg.)										
Ground-level	7	1.14 ± 0.14	6	1.28 ± 0.54	NS	—	4	1.31 ± 0.11	.10	—
258 mm. Hg		(1.27)	6	1.21 ± 0.26	NS	NS	4	1.62 ± 0.43	.10	NS
380 mm. Hg			14	1.30 ± 0.38	.20	NS	6	1.37 ± 0.27	.10	NS
Adrenals (mg./kg.)										
Ground-level	5	0.37 ± 0.08	5	0.42 ± 0.10	NS	—	3	0.42 ± 0.23	NS	—
258 mm. Hg		(0.30)	3	0.41 ± 0.13	NS	NS	3	0.43 ± 0.06	NS	NS
380 mm. Hg			12	0.65 ± 0.32	.05	.05	6	0.54 ± 0.13	.05	NS
Kidneys (gm./kg.)										
Ground-level	7	4.45 ± 0.80	5	4.11 ± 0.80	NS	—	4	4.65 ± 0.31	NS	—
258 mm. Hg		(4.86)	6	4.13 ± 0.58	NS	NS	4	3.69 ± 0.32	.10	.01
380 mm. Hg			14	4.05 ± 0.53	NS	NS	6	3.45 ± 0.31	.02	.01
Testes (gm./kg.)										
Ground-level	6	0.93 ± 0.29	4	1.12 ± 0.57	NS	—	4	0.64 ± 0.20	.10	—
258 mm. Hg		(0.61)	5	0.70 ± 0.26	.20	NS	4	0.58 ± 0.18	.05	NS
380 mm. Hg			14	1.06 ± 0.24	NS	NS	6	0.98 ± 0.14	NS	.20
Brain (gm./kg.)										
Ground-level	7	28.28 ± 6.65	4	24.69 ± 4.14	NS	—	4	24.97 ± 3.23	NS	—
258 mm. Hg		(28.20)	5	25.58 ± 2.26	NS	NS	3	25.43 ± 0.54	NS	NS
380 mm. Hg			14	27.19 ± 3.43	NS	NS	6	28.86 ± 4.76	NS	.20
Weight (kg.)										
Ground-level	7	3.08 ± 0.59	6	3.35 ± 0.41	NS	—	4	3.35 ± 0.27	NS	—
258 mm. Hg		(3.04)	6	3.37 ± 0.21	NS	NS	4	3.54 ± 0.21	.10	NS
380 mm. Hg			14	3.07 ± 0.28	NS	.20	6	2.99 ± 0.36	NS	.20

*P: Level of significance compared to the colony control animals

†P: Level of significance when the animals exposed to 258 and 380 mm. Hg pressures are compared to their respective ground-level control animals

‡Standard deviation

§Values calculated from Kennard and Willner (15)

This decline is followed by a rebound toward normal values while the animals are in the oxygen atmosphere, at least for this group. Animals sacrificed prior to the eighth day followed this pattern and the points fit the curve. For the oxygen-exposed animals at 258 mm. Hg pressure, the liver weights follow closely a declining straight line, which continues to decline postexperimentally. None of the liver weights for the oxygen-exposed animals returned to within normal range postexperimentally. These observations are verified statistically as reported in table VII where it can be noted that the decreases for the oxygen-exposed animals are highly significant when compared to the colony control animals. These decreases are also over and beyond the confinement to the chamber because the decreases are also significant or borderline significant when compared to the ground-level control animals. The fact that these graphs depict only two animals per point causes greater fluctuations than desired, but even so, it should be noted that pre-experimental liver weights were not even reached 30 days postexposure for the animals exposed to 380 mm. Hg pressure.

Likewise, the graphs depicting the lung weights (fig. 5) show that the oxygen-exposed animals had heavier lungs per unit body weight than did their ground-level controls. The average lung (table VII) is heavier for the oxygen-exposed animals as expected, but the difference is not statistically significant. While the ground-level control animals maintained fairly constant lung weights throughout, the animals exposed to 258 mm. Hg pressure had significantly heavier lungs postexperimentally, while the postexperimental increase in lung weight was insignificant for the animals exposed to 380 mm. Hg pressure.

The hearts of oxygen-exposed animals (table VII and fig. 6) were found to be lighter in weight by period than those of both the colony control animals and the ground-level control animals, and the weights seem to become lighter with increasing oxygen levels so that, at 380 mm. Hg pressure with 100% oxygen, the difference when compared to the colony control animals is borderline significant (P .05). Compared to the colony control animals, the spleens are increased in weight for all three experimental groups, and the weights postexperimentally are still heavier. These postexperimental values are of borderline significance when compared to the colony control animals but are not significant when compared to the ground-level control animals in the respective period.

The graphs depicting the adrenal weights (fig. 7) show interesting patterns. The primates exposed to 100% oxygen at 258 mm. Hg pressure apparently did not experience more stress than the ground-level control animals, while the primates exposed to 100% oxygen at 380 mm. Hg pressure had increasing adrenal weights for as long as the oxygen exposure lasted. Furthermore, the return to normal range was slow and was reached only with the last two animals which were necropsied on the 30th postexperimental day. In table VII where the adrenal weights are summarized, it can again be seen that it is not more stressful for the animals to be exposed to 258 mm. Hg pressure with a 100% oxygen atmosphere than to be kept in the same cage system at ground-level conditions. The adrenal weights for the animals at 380 mm. Hg pressure increased an average of 75.7% during the oxygen exposure, however, and this is statistically significant when compared to the ground-level control animals during this period. Postexperimentally, the adrenal weights are still above either control group but apparently returning toward normal.

The kidneys, like the hearts, decreased in weight for all three experiments while the animals were in the chamber as compared to the colony control animals, with the animals at 380 mm. Hg pressure having the greatest decrease (table VII). Postexperimentally, however, the ground-level control animals regained their kidney weight, while the oxygen-exposed animals showed further and significant decreases. This occurred regardless of whether the results are compared to the colony control animals or the ground-level control animals during the postexperimental period. This is

also observed in figure 8 where it is evident that the ground-level control animals have an upward trend, while the oxygen-exposed animals have downward trends. The testicular weights are also reported in table VII and depicted in figure 8. Results are variable and inconclusive as would be expected in adolescent primates. No significant trends or major changes were observed in this study for the brain weights (table VII, fig. 9).

The body weights for the paired primates as they were necropsied are shown in figure 9 and indicate an upward trend for both the ground-level control animals and the animals exposed to 100% oxygen at 258 mm. Hg pressure. As a matter of fact, these curves are almost identical. The animals exposed to 100% oxygen at 380 mm. Hg pressure, on the other hand, appear to lose weight during the first week of exposure, but then gain weight without catching up with the weights for the other two groups of animals. The body weights obtained on necropsy days are summarized in table VII, in which it is evident that all the animals belong to the same weight group since none of the values are significantly different. The oxygen-exposed animals at 380 mm. Hg pressure, on the average, did not gain weight during the exposure period and actually lost weight post-experimentally. This is also the case when compared to the total average weights as shown in table II.

V. DISCUSSION

In this series of experiments the opportunity existed for studying healthy young male primates while they were maintained in environments of increased oxygen concentrations for a long time and also for providing additional baseline or normal biologic values to the literature on these research animals. This study was designed, in part, to establish whether hyperoxia of these magnitudes would have deleterious effects on the more common clinical and biochemical variables. During the ground-level control study, baseline values, therefore, were collected for these adolescent male primates housed in individual cages. The nutritional requirements for the animals were met, as verified by the continuous weight gain seen throughout this experiment which was similar to the weight gain seen for the colony control animals. Hurst (12) reported a weight gain average of 0.13 kg. per month for the male rhesus *Macaca mulatta*, starting at 2.38 through 4.31 kg. This gain is essentially what is reported here, except for the lack of increased weight postexperimentally for the animals exposed to 380 mm. Hg pressure; the remaining animals in this group actually lost an average of 80 gm.

The 5 ml. of blood removed from each animal weekly might have been too much for a primate of this size, even though Krise (17) indicated that this volume of blood could be removed without effect. As seen in figure 1 and table III, there were considerable decreases in hematocrit, hemoglobin, and red cell counts, as well as changes in other variables concerned in hematopoiesis (1). These changes started to take place during the pre-experimental phases and for the ground-level animals either returned to normal or plateaued. This accumulating loss of blood had to reach a certain level before erythropoietic activity was stimulated as seen by the increasing activity of glucose-6-phosphate dehydrogenase, which indicates under these conditions an increasingly younger red cell population. Admittedly, it could also mean that the older cells were destroyed at a faster rate, but no evidence was found for this theory. On the contrary, the increases in G-6-PD activity are perceptible at the time when hematocrit and hemoglobin values return toward normal and also by the slight but continuous upward trend of the reticulocytes. Since the animals in all three experiments were of the same size, weight, and sex, and were bled the same amount, the changes seen in the hematologic variables for the oxygen-exposed animals were over and beyond those due to the blood loss and, therefore, were caused by the oxygen atmospheres. Decreased pressure (21) with hypoxia normally stimulates the erythropoietic activity in mammalian organisms, while in this study we see an inhibition of erythropoiesis with increased oxygen environments. The changes seen may be considered to be only

adaptive trends to the new hyperoxic environment, as suggested by Campbell in 1928 (5). Even though some of them are statistically different from the values obtained in the colony control animals and the ground-level control animals, it is agreed that these changes in the red blood cells ought not to be considered signs of oxygen toxicity at the present time. All the values are still within published normal range for this species (17, 19, 26, 27). This may be due to the fact that the duration of exposure in these oxygen atmospheres was not long enough to drive the hematologic variables to greater changes. On the basis of the study reported here, there is some evidence that alveolar oxygen partial pressures of about 171 and 293 mm. Hg exert effects on erythropoiesis during 30- and 22-day exposure periods. The mild decreases in hematocrit associated with the low reticulocyte count and reduction of red blood cells at the termination of exposure might be interpreted as evidence of suppression. Cooperberg and Singer (6) reported similar findings in guinea pigs exposed to 70% oxygen for periods of up to 36 days. In these animals, there was evidence of increasing activity in the marrow after the second week of exposure, suggesting a compensatory mechanism. The major component in the hematocrit reduction in our animals had occurred over the first 2 weeks of residence in the oxygen atmosphere; thereafter, there was an indication of stimulation.

There is no evidence in these data indicating increased hemolysis after exposure to hyperoxia in our animals; even the fibrinogen values were normal. Except for a few reports (5, 10, 16), this has been the experience of several other investigators (4, 5, 8, 22, 28). Recent studies by Mengel et al. (18) have shown that the exposure to 100% oxygen at 2 to 3 atmospheres' pressure resulted in severe hemolysis in both mice and men. Abnormal stromal lipid peroxidation, along with aberrations in the glycolytic pathway of the red cell, have been implicated as the mechanisms leading to hemolysis. The extreme differences in the partial pressure of oxygen under these conditions as compared with ours probably account for the absence of similar abnormalities in the present study.

Although higher concentrations of oxygen have unequivocally shown pronounced effects on erythropoiesis, the results presented here indicate that arterial oxygen partial pressures of 170 to 300 mm. Hg in the rhesus *Macaca mulatta* have only a minor influence over these periods of exposure. There are, however, some data in this laboratory which indicate that reticulocytosis is decreased in rabbits after acute hemorrhage in an oxygen atmosphere at 380 mm. Hg total pressure (30). For space missions not exceeding the duration of this study, one would expect some alteration hematopoietically. Missions of longer duration would require further laboratory study since the possibility of compensatory reactivation of the bone marrow may occur, thus ameliorating the early suppressive phase. Our studies further indicate that the virtual absence of nitrogen exerts little influence on erythropoiesis.

The loss in liver weights, as depicted in figure 4, for the oxygen-exposed animals is not readily explainable, especially since the ground-level control animals did not show the same trend. As indicated in table VII, the average values for the oxygen-exposed animals are significantly lower than those for the colony control animals and the ground-level control animals throughout the experiment. One explanation which might account for a portion of this decrease is that glycogen storage in the livers was depleted and not replaced owing to an oxygen effect on vital enzyme reactions; however, glycogen was not measured and there is no proof that this was the case. Another possible explanation for this finding is that the livers lost their lipid content, also owing to altered enzyme activity. These explanations are feasible on the basis of histologic and electron microscopic slides (9, 23).

The lungs of the oxygen-exposed animals are heavier than those of their ground-level controls. The postexperimental mean values for the animals exposed to 258 mm. Hg pressure are significantly heavier. The only reasonable explanation for the apparent insignificant increase for the animals exposed to 380 mm. Hg pressure may be attributable to the

relatively small number of animals and the individual tolerance to the oxygen atmospheres. For the animals at 380 mm. Hg pressure, about 20 days of normal atmospheric condition were necessary before the lung weights had returned to normal range.

Two possibilities exist to explain the decreasing heart sizes. Less activity in the single, more restrictive cages in the chamber may be one factor causing a cardiovascular deconditioning and, therefore, atrophy of the heart muscle. The other possibility may be increased oxygenation of the blood, requiring less blood to be pumped by the heart. The first factor may be correct for the ground-level control animals; however, since the changes were greater in the oxygen-exposed animals, the difference must have been caused by the oxygen, since hypoxia or decreased pressure normally will cause the heart muscle to become heavier owing to additional work.

Postexperimentally, the spleens for the three experimental groups increased ($P < .10$); so the weight gains cannot be attributable to either the oxygen atmosphere or the decreased pressure. Again, the question of inactivity must be answered whereby less circulating blood is required. This blood could be stored in the spleens, and importance is added to this theory since the spleens increased in weight during the experimental periods also.

The only surprising thing about the adrenal weights is that the oxygen-exposed animals at 258 mm. Hg pressure did not vary from the ground-level control animals, indicating that somewhere between 258 and 380 mm. Hg oxygen pressures lies the critical level for oxygen stress for these animals under these exposure conditions. The animals exposed to 380 mm. Hg had adrenal weights which kept increasing throughout the entire exposure period and did not reach normal levels before the 30th day postexperimentally. If any effect can be seen at the 258 mm. Hg exposure, it must be a slight and transitory inhibiting effect during the initial period of exposure, masking any possible stress situation during that time.

In 1941 Kennard and Willner (13) reported the organ weights from male rhesus monkeys (*Macaca mulatta*) with the same average body weight as the colony control animals in this experiment and found that increasing body weight also increased absolute organ weights. Also, the loss in body weight, as seen for the animals in the 380 mm. Hg experiment, was accompanied by a loss in the weight of spleens, pancreata, and testes; the weights of the remaining organs were little affected. Loss in body weight, therefore, does not explain the decreases observed in the liver, heart, and kidney weights. Otherwise, the values for the organ weights for male rhesus monkeys were essentially the same as reported for our colony control animals. The decreases observed in kidney weights of the oxygen-exposed animals must remain unexplained until further studies can be performed in this area. In 1959 Hale et al. (9) reported on organ weights in male rats after 24 weeks of exposure to ambient air at 380 mm. Hg pressure and found that liver and kidney weights were pressure dependent, each varying directly with pressure. They also stated that organ weights may vary because of differences in tissue structure, extracellular or intracellular water or fat, or differences in blood content. Since the higher blood content of the organs of the altitude animals would be a factor which would increase their weight, provided no other changes occurred, the findings that organ weights in these animals were either similiar to or lighter than those in the controls indicate that tissue constituents or water content must have been reduced.

REFERENCES

1. Berlin, N. I., T. A. Waldmann, and S. M. Weissman. Life span of the red blood cell. Physiol. Rev. 39:577 (1959).

2. Beutler, E., R. J. Dern, C. L. Flanagan, and A. S. Alving. The hemolytic effect of primaquine. VII. Biochemical studies of drug sensitive erythrocytes. J. Lab. Clin. Med. 45:286 (1955).

3. Beutler, E., O. Duron, and B. M. Kelly. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61:882 (1963).

4. Boycott, A. E., and C. L. Oakley. The regulation of marrow activity. Experiments on blood transfusion and on the influence of atmospheres rich in oxygen. J. Path. Bact. 36:205 (1933).

5. Campbell, J. A. Further observations on oxygen acclimatization. J. Physiol. (London) 63:325 (1928).

6. Cooperberg, A., and K. Singer. The reaction of the bone marrow to high oxygen tension in normal and anemic guinea pigs. J. Lab. Clin. Med. 37:946 (1951).

7. Evelyn, K. A., and H. T. Malloy. Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. J. Biol. Chem. 126:655 (1938).

8. Gyllensten, L., and G. Swanbeck. Influence of concentrated oxygen on the circulating erythrocytes in growing mice. Acta Path. Microbiol. Scand. 45:229 (1959).

9. Hale, H. B., R. B. Mefferd, Jr., G. Vawter, G. E. Foerster, and D. Criscuolo. Influence of long-term exposure to adverse environment on organ weights and histology. Amer. J. Physiol. 196:529 (1959).

10. Hawke, P. B., B. L. Oser, and W. H. Summerson. Practical physiological chemistry. 12th ed. Philadelphia: Blakiston, 1947.

11. Helvey, W. M., G. A. Albright, F. B. Benjamin, L. S. Gail, J. M. Peters, and H. Rind. Effects of prolonged exposure to pure oxygen on human performance. Republic Aviation Corporation RAC 393-1 (ARD-807-701), Nov. 1962.

12. Hurst, C. M. Monthly weight increases in growing rhesus monkeys. ARL TR 6: 24, Holloman AFB, N. Mex., Dec. 1961.

13. Kennard, M. A., and M. D. Willner. Findings in 216 routine autopsies of *Macaca mulatta*. Endocrinology 28:955 (1941).

14. Kind, P. R. N., and E. J. King. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Path. 7:322 (1954).

15. Kornberg, A. Reversible enzymatic synthesis of diphosphopyridine nucleotide and inorganic pyrophosphate. J. Biol. Chem. 182:779 (1950).

16. Krause, R. E., and J. W. Haynes. Practical methods in clinical chemistry. USAF School of Aviation Medicine, Gunter Branch, Gunter AFB, Ala., May 1958.

17. Krise, G. M. Hematology of the normal monkey. Ann. N. Y. Acad. Sci. 85:803 (1960).

18. Mergel, C. E., L. G. Zipkle, B. W. O'Malley, and B. D. Horton. Further studies of the mechanism of in vivo RBC damage by oxygen. Aerospace Med. 36:154 (1965).

19. Migeon, C. J., A. B. French, L. T. Samuels, and J. Z. Bowers. Plasma 17-hydroxycorticosteroid levels and leucocyte values in rhesus monkey including normal variation and effect of ACTH. Amer. J. Physiol. 182:462 (1955).

20. Powell, M. E. A., and M. J. H. Smith. The determination of serum acid and alkaline phosphatase activity with 4 aminoantipyrine (A.A.P.). J. Clin. Path. 7:245 (1954).

21. Pugh, L. G. C. E. Blood volume and hemoglobin concentration at altitudes above 18,000 feet (5500 meters). J. Physiol. (London) 170:344 (1964).

22. Reinhard, E. H., C. V. Moore, R. Dubach, and L. J. Wade. Depressant effects of high concentrations of inspired oxygen on erythropoiesis. J. Clin. Invest. 23:683 (1944).

23. Schaffner, F., D. V. Roberts, F. L. Ginn, and F. Ulvedal. Electron microscopy of monkey liver after exposure of animals to pure oxygen atmosphere. Proc. Soc. Exp. Biol. Med. 121:1206 (1966).

24. Sigma Chemical Company, St. Louis, Mo. A simplified method for the colorimetric determination of glutamic-oxalacetic and glutamic pyruvic transaminases at approximately 505 mμ. Sigma Technical Bulletin, 15 Jan 1961.

25. Spinco Paper Electrophoresis System, Model R. Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif. Serum protein analysis, procedure B. Technical Bulletin, 1962.

26. Spector, W. S. Handbook of biological data. WADC-TR-56-273, Wright-Patterson AFB, Ohio, Oct. 1956.

27. Stackhouse, S. P. Intraspecies biologic and behavioral variability. SAM-TR-66-58, June 1966.

28. Tinsley, J. C., C. V. Moore, R. Dubach, V. Minnich, and J. Grinstein. The role of oxygen in regulation erythropoiesis. Depression of the rate of delivery of new red cells to the blood by high concentrations of inspired oxygen. J. Clin. Invest. 28:1544 (1949).

29. Ulvedal, F. Preliminary observations on testicular function in roosters and mice exposed to increased partial pressure of oxygen. SAM-TR-66-40, Apr. 1966.

30. Ulvedal, F. Unpublished data.

31. USAF Medical Service Manual, AFM 160-51. Laboratory procedures in clinical hematology, May 1962.

32. Zinkham, W. H., R. E. Lenhard, and B. Childs. A deficiency of glucose-6-phosphate dehydrogenase activity in erythrocytes from patients with favism. Bull. Hopkins Hosp. 102:169 (1958).

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)
USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

2a REPORT SECURITY CLASSIFICATION
Unclassified

2b GROUP

3 REPORT TITLE
PHYSIOLOGIC AND BIOCHEMICAL OBSERVATIONS ON THE RHESUS MACACA MULATTA EXPOSED TO OXYGEN-RICH ATMOSPHERES

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)
17 May 1964 - 24 Mar. 1965

5 AUTHOR(S) (Last name, first name, initial)
Ulvedal, Frode
Brown, H. E.

6 REPORT DATE	7a TOTAL NO OF PAGES	7b NO OF REFS
September 1967	20	32

8a CONTRACT OR GRANT NO	9a ORIGINATOR'S REPORT NUMBER(S)
b PROJECT NO 7930	SAM-TR-67-86
c Task No. 793002	9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d	

10 AVAILABILITY LIMITATION NOTICES
This document has been approved for public release and sale; its distribution is unlimited.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas

13 ABSTRACT

Adolescent male rhesus monkeys (Macaca mulatta), weighing 2.9 to 3.4 kg., were exposed to 100% oxygen atmospheres at 258 and 380 mm. Hg pressures for 30 and 22 days, respectively, and the results obtained were compared to the observations made in a ground-level control study using the same chamber system. Physiologic, hematologic, enzymatic, biochemical, and anatomic measurements were obtained throughout the experiments. Several changes and trends were common to all three experiments; however, some were observed only for the oxygen-exposed animals and were dependent on the concentration of oxygen in the atmosphere.

DD FORM 1 JAN 64 1473

Unclassified
Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Physiology Spacecraft atmospheres Oxygen toxicity Primates in research						

INSTRUCTIONS

1. **ORIGINATING ACTIVITY**: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2a **REPORT SECURITY CLASSIFICATION**: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2b **GROUP**: Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. **REPORT TITLE**: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. **DESCRIPTIVE NOTES**: If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. **AUTHOR(S)**: Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. **REPORT DATE**: Enter the date of the report as day, month, year, or month, year. If more than one date appears on the report, use date of publication.

7a **TOTAL NUMBER OF PAGES**: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b **NUMBER OF REFERENCES**: Enter the total number of references cited in the report.

8a **CONTRACT OR GRANT NUMBER**: If appropriate, enter the applicable number of the contract or grant under which the report was written.

8b, 8c, & 8d **PROJECT NUMBER**: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9a **ORIGINATOR'S REPORT NUMBER(S)**: Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9b **OTHER REPORT NUMBER(S)**: If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. **AVAILABILITY LIMITATION NOTICES**: Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. **SUPPLEMENTARY NOTES**: Use for additional explanatory notes.

12. **SPONSORING MILITARY ACTIVITY**: Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13 **ABSTRACT**: Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS)*, *(S)*, *(C)*, or *(U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14 **KEY WORDS**: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.