AMRL-TR-67-78

RESPONSES OF ANIMALS TO OXYGEN AT REDUCED PRESSSURE

J. D. MacEWEN, Ph. D.
R. P. GECKLER, Ph. D.
Aerojet-General Corporation

K. C. BACK, Ph. D.
Aerospace Medical Research Laboratories

FEBRUARY 1968

AEROSPACE MEDICAL RESEARCH LABORATORIES
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

NOTICES

When US 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.

Federal Government agencies and their contractors registered with Defense Documentation Center (DDC) should direct requests for copies of this report to:

DDC
Cameron Station
Alexandria, Virginia 22314

Non-DDC users may purchase copies of this report from:

Chief, Storage and Dissemination Section
Clearinghouse for Federal Scientific & Technical Information (CFSTI)
Sills Building
5285 Port Royal Road
Springfield, Virginia 22151

Organizations and individuals receiving reports via the Aerospace Medical Research Laboratories' automatic mailing lists should submit the addressograph plate stamp on the report envelope or refer to the code number when corresponding about change of address or cancellation.

Do not return this copy. Retain or destroy.

The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 89–544, "Laboratory Animal Welfare Act," August 24, 1967.

700 - April 1968 - CO455 - 33-701

AMRL-TR-67-78

RESPONSES OF ANIMALS TO OXYGEN AT REDUCED PRESSSURE

J. D. MacEWEN, Ph. D.

R. P. GECKLER, Ph. D.

K. C. BACK, Ph. D.

FOREWORD

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit. This report is concerned with the chronic response of animals exposed to 100% oxygen at 5 psia. The experimental program has been accomplished by the Aerojet-General Corporation under Contract AF 33(657)-11305 for the Toxicology Branch, Toxic Hazards Division, Biomedical Laboratory, Aerospace Medical Research Laboratories. The contract was initiated in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," and Task No. 630201, "Toxicology." K. C. Back, Ph. D., was the technical contract monitor for the Aerospace Medical Research Laboratories.

J. D. MacEwen, Ph. D., was the Principal Investigator for the contractor in the conduct of the research program. Acknowledgement is made to N. M. Breene, E. H. Vernot and R. Farquhar for assistance in the preparation of this report.

This report is identified as Aerojet-General Corporation Report No. 3266.

This technical report has been reviewed and is approved.

WAYNE H. McCANDLESS
Technical Director
Biomedical Laboratory
Aerospace Medical Research Laboratories

ABSTRACT

As extensions of previous short-term experiments on the toxicity of oxygen at reduced pressure in animals, long-term continuous exposures of beagle dogs, rhesus monkeys and albino rats and mice were undertaken. The exposures were for 230 days to 100% oxygen at 5 psia.

No mortality occurred in the exposed dogs or monkeys. More rats and mice died in the control groups than in the experimental groups, indicating no effects on mortality due to the experimental conditions. The growth rates of control and experimental rats were almost identical, further indicating lack of deleterious effects.

Histopathologic examination of tissues was conducted on all four animal species. No differences were observed between exposed animals and their controls in monkeys, rats or mice. Minimal differences consisting of mild bronchitis and mild congestion were seen in the dogs exposed to 100% oxygen at reduced pressure. One exposed dog exhibited severe pulmonary changes associated with acute interstitial bronchial pneumonia. Periodic measurements of blood constituents showed no significant differences attributable to prolonged oxygen exposure at reduced pressure.

TABLE OF CONTENTS

LIST OF TABLES

SECTION I

INTRODUCTION

Although single-gas (100% oxygen at reduced pressure) systems were selected for the Mercury, Gemini and Apollo space programs, there have been expressions of doubt that such a system would be adequate for space programs in which longer manned missions will be undertaken. Also, as advances are made in developing regenerative life support systems and materials are recycled, there arises a need for assurance that materials used in the construction of space cabins are nontoxic and that any volatile substances given off by these materials do not cause toxic effects. Contaminants must also be removed before they accumulate to potentially hazardous concentrations. Biological products arising from human metabolism or from other biological components of the total system (e.g., bioregenerative subsystems such as photosynthetic gas exchangers waste treatment subsystems) must not be allowed to accumulate for the same reasons.

The requirements for (1) providing an initial safe respiratory gas mixture at an appropriate pressure and (2) maintaining the purity of the gas have led to the initiation of a significant experimental effort throughout the nation. In the past, considerable information has been obtained concerning the toxicity of oxygen at atmospheric (References 1 through 5) and hyperbaric (References 6, 7) pressures, but little is known concerning the toxic response of man or other species to oxygen under hypobaric conditions.

Herlocher et al (Reference 8) measured the physiological response of men exposed to essentially pure oxygen at 258 mm Hg pressure (5 psia) for a 30-day period. Their clinical observations indicated that no significant changes had occurred which were not associated with the prolonged confinement of these men. Robertson et al (Reference 13) studied the hematopoietic condition of the subjects included in the study reported by Herlocher and found that no significant changes occurred during the 30-day exposure period, except a slight change in hematocrit values. The hematocrit values for the experimental subjects decreased approximately 9.1%.

Since human exposures to pure oxygen environments at reduced pressure will become longer as space flight technology improves, it is important to determine both the effects of selected gas mixtures on organisms and whether these mixtures affect the toxicity of trace contaminants.

One of the primary objectives of the research program of the Air Force being conducted in the Toxic Hazards Research Unit (THRU) facilities at Wright-Patterson Air Force Base is the evaluation of the inhalation toxicity of potential space cabin atmosphere contaminants under conditions, insofar as feasible, resembling those found in space cabins. The experimental animal exposure chambers used can maintain respiratory gases, either pure oxygen or mixtures, ranging from 5 psia to ambient pressure (approximately 14.7 psia). The inhalation toxicity of contaminants in almost any gas mixture which may be suggested for space cabin use can, therefore, be determined. This report describes two of a series of experiments performed to obtain baseline data on the long-term effects of 100% oxygen at 5 psia, so that the true effects of contaminating materials may be derived in other studies, i.e., effects of oxygen toxicity per se will not be mistaken for effects of atmospheric contaminants. In the experiments to be described, experimental animals were exposed to oxygen at reduced pressure for 230 days. Other experiments in the series have been concerned with the 14- and 90-day exposures (References 14 through 18).

SECTION II

MATERIALS AND METHODS

ANIMAL EXPOSURE FACILITIES

One of the altitude chambers in the Aerospace Medical Research Laboratories THRU facilities was used for the animal exposures. The altitude facilities are described in detail in Reference 19. In brief, these specially designed chambers can operate at pressures between 5 psia (258 mm Hg) and 14.7 psia (760 mm Hg) and are equipped to operate with either 100% oxygen, ambient air, or mixtures of these gases over a range of 20% to 100% oxygen in the atmosphere of the chamber. Temperatures and relative humidity are automatically controlled to 72 + 5F and 50 + 10%, respectively. Gas flows through the chamber may be varied from 0 to 125 cfm, although usual operation is at 20 cfm. The flow rate of 20 cfm can maintain carbon dioxide concentrations below 0.5% (with a usual operating range of 0.1 to 0.2%) when the chamber has a full complement of animals. Each chamber is equipped with an air lock to effect entry into the chamber without disruption of experimental conditions. Liquid oxygen, used as a source of the atmosphere, is converted to gaseous form, and its temperature and relative humidity is adjusted before it is passed into the chamber. No recycling or purification of the atmosphere within the chamber is attempted. The gas is passed from the chamber into the outside atmosphere through exhaust ducts.

EXPERIMENTAL CONDITIONS

The operating conditions of the chamber used for the experiment are listed in Table I.

Table I
Exposure Chamber Operating Conditions

Atmosphere Composition	100% oxygen
CO_2	0.1 (0.07-0.72)%
Total Pressure	260 mm Hg
O_2 Partial Pressure w/Leak Rate	258 mm Hg
N_2	0.5-1.4%
Flow Rate of O_2	25 (18-25) cfm
Temperature	72 (71-76) F
Humidity	50 (44-74) *% RH

*R.H. Levels above 60% occurred only for short periods during daily cleaning operations.

The atmospheric composition was, for all practical purposes, 100% oxygen. The leak rate, humidity, and CO_2 contributed approximately 2 mm Hg pressure. The CO_2 was kept below 0.5% and usually was approximately 0.1%. The concentration occasionally exceeded 0.5% when more than one individual entered the chamber to collect biological data. Carbon dioxide was continuously monitored, using a nondispersive infrared analyzer, and oxygen was monitored by a polarographic sensor checked and calibrated with a gas chromatograph.

EXPERIMENTAL ANIMALS

Experimental animals used in these experiments were beagle dogs, rats, mice and rhesus monkeys (Macaca mulatta). When entered into the experiment, dogs weighed 6 to 7 kilograms. Monkeys weighed 3 to 4 kilograms. Rats (Sprague-Dawley Strain) weighed 125 to 150 grams. Mice which were used in the experiment weighed 20 to 25 grams and were of the Harlan ICR Strain. All animals received routine inspection and quarantine when received from commercial sources. The appropriate clinical baseline data were determined four times before beginning the animal exposures.

During the course of an experiment, all animals were observed routinely at 30-minute intervals. When deaths occurred, animals were removed from the exposure chamber immediately for post mortem examination and tissue sampling. Clinical laboratory determinations on blood from the beagle dogs and rhesus monkeys were done on a monthly basis. These determinations were made on rats and compared with control values only at the time of necropsy. Only gross observations such as mortality, gross pathology and histopathology were made on mice. Clinical chemistry determinations included the following: hematocrit, hemoglobin, red blood cell count, white blood cell count, sodium (Reference 19), potassium (Reference 20), calcium (Reference 20), total protein (Reference 21), albumin (Reference 21), SGOT* (Reference 22), SGPT** (Reference 22), alkaline phosphatase (Reference 23), total phosphorus (Reference 24) and LDH*** (Reference 25). At necropsy, tissue samples for histopathological examination were taken from all species studied.

---

* Serum glutamic oxaloacetic transaminase
** Serum glutamic pyruvic transaminase
*** Lactic dehydrogenase

Rat body weights were determined before the start of each experiment, biweekly during the experiment, and at necropsy. Beagle dog and monkey body weights were determined before the start and at the end of the experiment. Accurate weighing of larger animals within the altitude exposure chambers was not feasible.

At necropsy, weights were taken of heart, lung, liver, spleen and kidney. These weights were then used in calculating organ/body weight ratios.

DATA ANALYSIS

Means and standard deviations for each biological determination were calculated for each group of animals whenever measurements were made. The overall means and standard deviations were also computed for each determination for the preexposure or baseline period and the exposure period. The raw data, means and standard deviations were evaluated, and particular data were selected for detailed statistical analysis as appeared appropriate. No effort was made to analyze data exhaustively when a series of measurements made on animals exposed to 100% oxygen at reduced pressure was obviously uniform and essentially the same as that of the unexposed control animals.

SECTION III

EXPERIMENTAL RESULTS

GENERAL

The experiments reported were designated numbers 128 for animals introduced into the altitude dome and 129 for animals held at ambient pressures; i.e., the controls. Forty male mice, 65 male rats, 8 beagle dogs, and 4 monkeys were placed in one of the altitude domes for exposure to the test environment. Dogs and monkeys were equally divided with respect to sex. Forty mice, 46 rats, 4 dogs, and 2 monkeys were held under ambient air conditions. Clinical chemistry and hematology baseline values were determined for the monkeys and dogs at least 4 times during the month before exposure. Rat baseline weights were recorded 14 and 7 days before the experiments were begun.

During the first 3 months of the experiment, determinations similar to those mentioned above were done on a biweekly basis, after which the determinations were made monthly. To make a uniform presentation of the data, the first, third and fifth biweekly determinations have been deleted, and the tabular data in this report represent data obtained at monthly intervals. Statistical evaluation of the data, however, included all samples analyzed. Except for overall summaries and data of particular significance, the data obtained are located in the appendix. A preliminary report of the data was made at the 2nd Annual Conference on Atmospheric Contamination in Confined Spaces (Reference 26).

MORTALITY AND GROWTH DATA

Cumulative mortality data after 230 days of exposure are shown in Table II. Note that control rats and mice fared somewhat worse than the exposed animals. The unexpected increased mortality in control rats was due to a mild outbreak of murine pneumonia during the last month of the experimental period. This was confirmed by both observed weight loss and histopathologic examination.

After the 230th day of the experiment, both exposed and control animals were sacrificed and necropsied on different schedules as needed for electron microscopic studies of lungs, kidneys and livers. Other special studies were performed to obtain cellular enzyme data. The results of the special studies were presented at the 2nd Annual Conference

TABLE II

Mortality Experience of Experimental Animals

Exposed to Reduced Pressure-100% O_2 Conditions and Their Controls

(No. Deaths/No. Tested)

	Exposed	Control
Mice	8/40	11/40
Rats	3/65	12/46
Dogs	0/8	0/4
Monkeys	0/4	0/2

on Atmospheric Contamination in Confined Spaces (References 27 through 30). Half of the exposed animals were held for an additional 40 days in the vivarium for histopathologic comparison with those animals necropsied immediately after exposure termination.

The mean body weights of rats (see Table III) do not show any significant differences in growth rates between the animals exposed to 100% oxygen at reduced pressure and their controls, except for the final weights. As mentioned above, this difference was due to the occurrence of murine pneumonia in the control rats.

HEMATOLOGY AND CLINICAL CHEMISTRY

The hematology and clinical chemistry data obtained from both control and exposed dogs are summarized in Table IV. Similarly, data from monkeys are presented in Table V. The data are presented as mean values with their accompanying standard deviations. Statistical evaluation of these combined data gave no evidence that continuous exposure of these animals for 230 days to 100% oxygen at 5 psia had detectable effects. Also, differential blood counts, performed periodically on dogs and monkeys, failed to show any effects due to the reduced pressure 100% oxygen experimental conditions.

When the data from the individual monthly sampling periods (Tables X through XIII, Appendix) were evaluated by analysis of variance, a number of statistical differences appeared between exposed and control animals, even though the individual means were within normal values. The differences in hematocrit, red blood cell count and serum electrolytes became evident only when the two groups were compared sample period by sample period. These differences cannot be ascribed to the 100% oxygen-reduced pressure exposure, but rather to changes in the control animals' environment which could not be as well maintained as the exposure chambers. Also, in both the control and exposed dogs, alkaline phosphatase showed a decrease with time compared to preexposure values. Such a decrease has been observed repeatedly in other experiments and is a phenomenon of aging in the beagle. A similar response could not be demonstrated statistically in the monkey.

PATHOLOGY

Pathology data from the animals used in the experiment were of two types; one, the standard histopathology data obtained by light microscopy; the other, special studies involving electron microscopy. The results of these studies are presented in References 27, 28, 30, and 31.

TABLE III

Mean Body Weights of Male Rats Exposed to O_2

At Reduced Pressure and Their Controls

(Weight in Grams)

Sample Period	Exposed Rats N = 65 Mean	Std. Dev.	Control Rats N = 46 Mean	Std. Dev.
1	116	9	112	7
2	133	11	-	-
3	170	13	207	16
4	207	19	207	16
5	253	20	234	28
6	329	30	303	26
7	348	30	323	27
8	367	28	355	22
9	418	44	388	19
10	431	31	424	28
11	449	38	444	29
12	444	36	446	36
13	481	38	452	38

TABLE IV

Hematology and Blood Chemistry Values - Dogs

(Mean Value ± Standard Deviation)

Parameter	Control Group			Exposed Group		
	Period			Period		
	Baseline	Exptl.	Post Exp.	Baseline	Exptl.	Post Exp.
HCT, Vol. %	43 (3)	47 (3)	49 (3)	43 (3)	44 (3)	48 (1)
HGB, gm. %	13.9 (1.0)	16.2 (1.5)	16.9 (0.2)	14.1 (0.9)	14.6 (2.6)	16.1 (0.5)
RBC, x 10^6	4.5 (0.5)	6.1 (0.5)	6.5 (0.1)	4.3 (0.4)	5.7 (0.4)	6.1 (-)
WBC, x 10^3	12.4 (1.6)	13.4 (2.7)	11.2 (1.6)	11.5 (2.3)	12.3 (2.3)	9.8 (0.8)
Na, mEq/l	146 (3)	147 (3)	142 (1)	145 (2)	145 (3)	146 (3)
K, mEq/l	4.9 (0.3)	5.2 (0.3)	4.4 (0.2)	4.9 (0.3)	4.9 (0.4)	4.4 (0.2)
Ca, mEq/l	5.6 (0.3)	5.6 (0.3)	5.2 (0.2)	5.6 (0.1)	5.5 (0.3)	5.3 (0.2)
T. Prot., gm.%	5.4 (0.4)	5.8 (0.7)	5.7 (0.3)	5.5 (0.3)	5.8 (0.6)	5.4 (0.3)
Alb., gm. %	3.4 (0.2)	4.0 (0.4)	3.7 (0.1)	3.4 (0.1)	3.6 (0.3)	3.5 (-)
SGPT, units/ml	22 (8)	30 (6)	25 (13)	23 (9)	32 (6)	23 (3)
SGOT, units/ml	31 (7)	33 (8)	29 (10)	32 (7)	34 (9)	47 (26)
Alk. Phos., units/ml	2.7 (1.1)	1.2 (0.9)	0.8 (0.4)	2.3 (0.8)	1.0 (0.7)	2.2 (01.4)
T. Phos., mg.%	6.4 (0.6)	6.3 (1.3)	4.1 (0.5)	6.5 (0.5)	5.5 (1.2)	3.3 (0.5)
LDH, units/ml	125 (54)	213 (107)	190 (53)	153 (84)	228 (104)	407 (304)

TABLE V

Hematology and Blood Chemistry Values - Monkeys

(Mean Value ± Standard Deviation)

Parameter	Control Group			Exposed Group		
	Period			Period		
	Baseline	Exptl.	Post Exp.	Baseline	Exptl.	Post Exp.
HCT, vol. %	38 (2)	41 (5)	--	38 (3)	38 (2)	42 (1)
HGB, gm. %	11.0 (0.5)	12.4 (1.6)	--	11.3 (0.7)	11.8 (0.7)	13.6 (0.8)
RBC, x 10^6	3.99 (0.90)	4.80 (0.49)	--	3.95 (0.83)	4.61 (0.44)	5.4 (0.10)
WBC, x 10^3	12.7 (1.7)	15.5 (2.9)	--	13.9 (2.6)	13.8 (2.3)	11.3 (12.1)
Na, mEq/l	150 (4)	147 (3)	--	148 (4)	147 (4)	144 (-)
K, mEq/l	4.9 (0.5)	4.8 (0.6)	--	5.0 (0.6)	4.8 (0.6)	4.3 (0.1)
Ca, mEq/l	5.8 (0.4)	5.2 (2.4)	--	5.7 (0.4)	5.5 (0.3)	5.3 (0.1)
T. Prot., gm. %	7.1 (0.5)	7.4 (0.5)	--	7.3 (0.5)	7.6 (0.6)	7.4 (0.2)
Alb., gm. %	4.2 (0.4)	4.7 (0.3)	--	4.4 (0.3)	4.8 (0.3)	4.4 (0.2)
SGPT, units/ml	28 (28)	33 (7)	--	31 (14)	31 (10)	25 (3)
SGOT, units/ml	39 (11)	51 (5)	--	50 (16)	46 (13)	40 (-)
Alk. Phos., units/ml	28.2 (5.7)	19.9 (7.5)	--	31.3 (4.3)	18.2 (5.5)	9.7 (2.8)
T. Phos., mg.%	5.6 (1.4)	7.2 (1.3)	--	5.1 (1.3)	5.8 (1.4)	4.5 (0.4)
LDH, units/ml	253 (74)	351 (155)	--	366 (122)	421 (166)	360 (20)

Hagebusch (Reference 31), studying tissues by light microscopy, noted that only in dogs could differences be observed between the control group and the groups exposed to oxygen at reduced pressure. No differences ascribable to the exposure could be shown in rats, mice or monkeys, although he indicated that future work might reveal a response in the rat. In the beagle, minimal differences consisting of mild bronchitis and mild congestion were seen in exposed animals only. One dog exposed to 100% oxygen at reduced pressure exhibited severe pulmonary changes associated with acute interstitial and bronchial pneumonia. The minimal pulmonary changes seen in the other exposed dogs were probably due to the beginning spread of infection from the sick animal. In the animals returned to ambient air after 230 days and held for an additional 40 days, the pathologic changes observed were less than in those examined at 230 days, indicating healing.

Electron microscopy studies (References 27, 28, 30) gave evidence of an adaptive process during the exposure period. The livers of rats, dogs and monkeys showed signs of mitochondrial alteration due to exposure to 100% oxygen, not only at 5 psia but at higher pressures as well. Mitochondria in the tubular cells of the kidney also appeared affected. Similar effects were found in lung tissue. Hepatic changes appeared to be non-specific and were not sufficient to interfere with the health of the animals.

CELLULAR BIOCHEMISTRY

Riesen (Reference 29) conducted a special study covering certain aspects of the cellular biochemistry of some animals after various periods of exposure. The study was aimed primarily at mechanisms of tissue respiration, particularly oxidative phosphorylation.

In rats exposed to 5 psia-100% oxygen, an upward temporary trend occurred in the overall exposure index after 3 to 7 days when compared to control animals under ambient conditions. According to Riesen, this signifies a "beneficial" effect. Soon after the first week, this trend disappeared and the index was within the normal range. No other differences were noted in determinations of P/O* or NAD/NADH** ratios for rats, monkeys or dogs.

---

* Number of atoms of inorganic phosphorus incorporated into organic phosphates per atom of oxygen consumed.

** Nicotinamide adenine dinucleotide, Oxidized/Reduced

SECTION IV

DISCUSSION AND CONCLUSIONS

The blood changes described in the preceding section appear to be of little consequence to the general health of the experimental animals. Individual determinations on control and exposed animals were indistinguishable from one another and fell within normal limits. The trend of increasing SGPT values in dogs exposed to 100% oxygen at reduced pressure previously reported (Reference 16) for 90-day continuous exposures was not repeated in this experiment.

Hematocrit, RBC, and serum electrolyte values were found to be slightly higher in control dogs and monkeys when compared with exposed animals. Detailed examination of data revealed that the exposed animals were relatively stable, with expected normal fluctuation. The slightly higher values obtained in the control dogs and monkeys, although still within normal limits for these determinations, are believed to be due to problems of temperature regulation in the animal room. The exposed dogs and monkeys exhibited lower SGOT and SGPT than their controls throughout the experimental period. Again, this finding is believed to result from the more stable regulated environment of the exposure chambers.

The stability of hematologic parameters in the exposed animals is a significant finding. As previously noted, one of the important findings in short-term human exposures to 100% oxygen reduced pressure conditions has been the reduction of hematocrit and red blood cell levels. A transitory effect of this type may have occurred in the exposed animals in the experiment described herein, but no measurable differences were found when the animals were first tested after 2 weeks of exposure.

The 4 animal species continuously exposed to 100% oxygen at 5 psia pressure for 230 days showed no biologically significant clinical or pathologic changes attributable to their environment.

APPENDIX

ANIMAL EXPOSURE DATA

TABLE VI

List of Units for Hematology and Clinical Chemistry Determinations

Determination	Units
Hematocrit (HCT)	Vol. %
Hemoglobin (HGB)	gm. %
Red Blood Cell Count (RBC)	x 10^6
White Blood Cell Count (WBC)	x 10^3
Sodium (Na)	mEq/l
Potassium (K)	mEq/l
Calcium (Ca)	mEq/l
Total Protein (T. Prot.)	gm. %
Albumin (ALB.)	gm. %
Serum Glutamic Pyruvic Transaminase (SGPT)	Units/ml
Serum Glutamic Oxaloacetic Transaminase (SGOT)	Units/ml
Alkaline Phosphatase (Alk. Phos.)	Units/ml
Total Phorphorus (T. Phos.)	mg. %
Lactic Dehydrogenase (LDH)	Units/ml

TABLE VII

Differentiation of Leucocytes - Dogs

(Mean* Values in Percentage of Total Leucocytes Counted)

Cell Class	Preexposure Period		Exposure Period	
	Control	Exptl.	Control	Exptl.
Neutrophiles	53 (6)	53 (9)	52 (9)	50 (12)
Lymphocytes	39 (6)	37 (9)	42 (11)	43 (12)
Monocytes	4 (2)	4 (3)	3 (2)	3 (3)
Basophiles	< 1	< 1	< 1	< 1
Eosinophiles	3 (2)	3 (2)	2 (2)	3 (3)
Combined Immature	2 (2)	2 (2)	1 (1)	1 (1)

*Mean (± SD)

TABLE VIII

Differentiation of Leucocytes - Monkeys

(Mean* Values in Percentage of Total Leucocytes Counted)

Cell Class	Preexposure Period		Exposure Period	
	Control	Exptl.	Control	Exptl.
Neutrophiles	23 (9)	18 (8)	21 (8)	19 (9)
Lymphocytes	67 (9)	70 (9)	71 (10)	74 (10)
Monocytes	4 (2)	3 (3)	3 (2)	2 (2)
Basophiles	< 1	< 1	<1	<1
Eosinophiles	6 (3)	9 (7)	4 (3)	5 (5)
Combined Immature	< 1	<1	< 1	<1

*Mean (± SD)

TABLE IX

Periodic Mean Blood Values of Dogs Exposed to Reduced Pressure-100% O_2 Conditions

Parameter	Sampling Period - BASELINE*				Sampling Period - EXPERIMENTAL*								
	1	2	3	4	1	2	3	4	5	6	7	8	9
HCT	42	43	43	44	41	43	44	46	41	44	45	45	46
HGB	13.5	13.9	14.1	14.7	13.4	14.1	14.3	14.6	14.2	15.2	15.3	16.4	14.9
RBC	4.21	4.75	4.46	4.55	5.26	5.27	5.65	5.69	5.69	5.99	6.01	6.15	6.19
WBC	10.8	13.2	11.3	10.7	11.7	10.0	12.5	12.6	13.7	11.5	12.3	13.2	12.8
Na	144	145	144	147	146	144	145	145	144	145	146	141	143
K	4.9	4.9	4.9	4.9	5.4	5.0	5.0	4.7	4.7	4.9	5.1	4.5	4.7
Ca	5.5	5.8	5.6	5.5	6.0	5.4	5.4	5.5	4.9	5.4	5.5	5.3	5.6
T. Prot.	5.5	5.6	5.5	5.3	5.4	5.6	5.4	5.8	6.4	6.4	6.2	6.1	6.0
Alb.	3.4	3.3	3.6	3.3	3.5	3.7	3.9	3.7	3.6	3.6	4.0	3.6	3.5
SGPT	16	19	25	29	32	31	32	30	34	35	27	38	35
SGOT	35	29	29	35	33	33	34	40	39	37	22	44	32
Alk. Phos.	2.8	2.2	2.0	2.1	1.7	1.4	0.7	1.1	0.5	0.5	0.4	0.3	0.3
T. Phos.	6.4	6.3	6.4	6.7	6.4	5.9	6.3	4.5	5.0	5.0	5.6	4.1	3.7
LDH	210	126	120	155	170	255	169	298	283	214	269	390	175

*N = 8

TABLE X

Periodic Mean Blood Values of Experimental Control Dogs

Parameter	Sampling Period - BASELINE*				Sampling Period - EXPERIMENTAL*								
	1	2	3	4	1	2	3	4	5	6	7	8	9
HCT	43	43	44	42	44	42	48	45	47	49	46	47	48
HGB	13.5	13.9	14.1	14.3	14.6	13.9	16.3	14.8	16.7	16.7	16.1	16.9	16.8
RBC	4.26	4.71	4.53	4.33	5.80	5.32	6.02	5.51	6.35	6.44	5.80	6.53	6.41
WBC	12.1	14.0	11.1	12.3	14.1	11.2	14.6	11.4	16.4	13.7	11.9	15.7	13.4
Na	144	145	144	150	146	148	146	146	148	151	142	144	146
K	4.8	4.9	4.9	4.9	5.5	5.4	5.6	5.0	5.2	5.2	5.6	5.0	5.1
Ca	5.4	5.7	5.6	5.9	5.4	6.0	5.7	5.5	5.5	5.6	5.4	5.0	5.7
T. Prot.	5.5	5.5	5.4	5.0	5.0	5.5	5.7	5.6	6.3	4.9	5.7	6.3	6.3
Alb.	3.5	3.3	3.6	3.3	3.5	3.7	3.7	4.0	3.7	4.4	3.8	3.7	3.9
SGPT	18	26	22	32	25	30	32	32	33	32	31	32	30
SGOT	36	29	26	34	34	34	34	38	34	30	28	38	30
Alk. Phos.	3.8	2.5	2.2	2.2	2.4	2.4	1.9	2.0	1.7	0.7	1.1	0.3	0.6
T. Phos.	6.2	6.4	6.5	6.6	6.8	5.9	8.0	6.1	7.2	7.7	6.3	6.7	5.3
LDH	168	152	100	80	290	205	190	190	80	140	200	140	170

* N = 4

TABLE XI

Periodic Mean Blood Values of Monkeys Exposed to Reduced Pressure-100% O_2 Conditions

Parameter	Sampling Period - BASELINE*				Sampling Period - EXPERIMENTAL*								
	1	2	3	4	1	2	3	4	5	6	7	8	9
HCT	41	37	36	39	38	39	40	39	38	36	36	39	38
HGB	11.5	11.1	11.2	11.4	11.3	12.0	11.8	11.5	12.3	11.5	11.4	13.2	11.5
RBC	4.11	4.00	3.72	3.99	4.35	4.52	4.64	4.65	4.72	4.57	4.63	5.01	4.70
WBC	13.6	13.9	15.5	12.4	16.2	12.0	11.9	13.3	17.6	12.6	16.1	12.6	13.5
Na	145	148	147	154	148	147	145	146	147	147	152	142	143
K	5.0	5.2	4.7	5.3	4.9	4.3	4.2	4.3	4.5	4.1	4.9	4.0	4.6
Ca	5.4	5.9	5.6	6.0	6.1	5.5	5.2	5.4	5.2	5.4	5.8	5.2	5.4
T. Prot.	7.1	6.8	7.5	7.6	7.3	8.0	7.5	7.3	8.1	7.8	7.7	7.7	7.5
Alb.	4.4	4.3	4.1	4.7	5.0	4.6	5.0	4.9	4.6	4.6	5.3	4.8	4.5
SGPT	28	24	32	40	40	32	32	33	34	26	19	31	26
SGOT	58	46	45	51	45	63	50	50	48	40	27	55	38
Alk. Phos.	33	26	32	34	20	22	19	22	13	16	14	17	15
T. Phos.	5.7	5.8	4.4	4.6	6.0	5.7	6.0	6.7	5.5	4.9	5.4	4.8	5.7
LDH	442	322	378	340	250	550	550	475	310	360	348	573	528

*N = 4

TABLE XII

Periodic Mean Blood Values of Experimental Control Monkeys

Parameter	Sampling Period - BASELINE*				Sampling Period - EXPERIMENTAL*								
	1	2	3	4	1	2	3	4	5	6	7	8	9
HCT	40	39	36	38	39	39	41	42	42	43	42	46	--
HGB	11.2	11.2	10.7	11.1	11.1	11.8	11.4	12.7	12.3	13.6	13.2	15.2	--
RBC	4.18	4.09	3.74	3.86	4.38	4.68	5.26	4.60	4.84	5.05	4.93	5.54	--
WBC	10.8	13.9	12.7	13.6	17.4	16.0	16.3	12.4	17.1	15.4	13.1	19.0	--
Na	149	150	149	155	148	149	146	149	147	144	152	144	144
K	4.7	4.9	4.6	5.4	5.4	4.1	4.6	4.5	5.1	4.9	5.1	4.3	5.3
Ca	5.6	6.1	5.7	6.0	6.0	5.5	5.7	5.8	5.2	5.8	5.8	5.8	5.8
T. Prot.	7.0	6.7	7.4	7.2	7.5	7.2	6.9	7.4	7.9	7.6	7.8	7.4	7.9
Alb.	4.3	4.2	4.1	4.2	4.8	4.8	4.3	4.8	4.5	4.7	5.3	4.8	5.0
SGPT	23	33	28	83	27	35	35	32	32	31	25	31	30
SGOT	48	48	55	69	48	60	45	41	67	50	31	50	62
Alk. Phos.	24	33	29	27	20	19	20	23	16	24	20	17	19
T. Phos.	5.9	5.9	5.0	5.5	6.8	7.0	7.5	6.9	8.1	6.7	7.4	7.4	4.7
LDH	230	255	390	330	190	520	250	190	400	340	450	495	610

*N = 2

TABLE XIII

Periodic Differentiation of Leucocytes - Dogs

(Mean Values in Percentage of Total Leucocytes Counted)

	Preexposure Period				Exposure Period								
EXPERIMENTAL	1	2	3	4	1	2	3	4	5	6	7	8	9
Neutrophiles	57	53	49	54	44	49	54	56	46	54	40	56	53
Lymphocytes	35	37	37	41	45	41	45	37	50	39	53	40	39
Monocytes	4	4	7	3	7	5	<1	3	1	4	2	3	2
Basophiles	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Eosinophiles	3	3	3	2	4	4	1	2	3	2	5	1	3
Comb. Immat.	2	4	4	<1	<1	<1	<1	1	<1	<1	1	<1	2
CONTROLS													
Neutrophiles	55	53	48	56	44	54	60	51	59	57	46	50	47
Lymphocytes	36	40	42	36	48	37	39	44	37	33	50	48	47
Monocytes	4	3	5	3	6	6	1	2	1	4	2	2	2
Basophiles	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Eosinophiles	3	3	2	4	3	4	<1	<1	3	4	3	<1	2
Comb. Immat.	2	1	4	1	<1	<1	1	2	1	2	<1	1	2

TABLE XIV

Periodic Differentiation of Leucocytes - Monkeys

(Mean Values in Percentage of Total Leucocytes Counted)

	Preexposure Period				Exposure Period								
EXPERIMENTAL	1	2	3	4	1	2	3	4	5	6	7	8	9
Neutrophiles	19	13	18	24	16	17	22	10	20	21	16	30	16
Lymphocytes	69	75	66	68	78	76	75	84	73	69	74	64	78
Monocytes	2	4	8	1	3	2	1	3	2	4	1	3	1
Basophiles	<1	1	1	<1	<1	<1	<1	<1	1	1	<1	<1	<1
Eosinophiles	10	9	8	7	3	6	2	3	5	6	9	4	6
Comb. Immat.	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
CONTROLS													
Neutrophiles	25	24	26	20	30	14	17	28	23	27	14	21	
Lymphocytes	68	66	63	70	61	78	79	62	73	68	74	74	
Monocytes	3	4	7	1	6	2	2	4	1	1	4	3	
Basophiles	<1	<1	1	1	1	1	1	1	<1	1	1	<1	
Eosinophiles	5	7	4	9	3	6	2	4	5	4	8	3	
Comb. Immat.	<1	<1	<1	<1	<1	<1	<1	2	<1	<1	<1	<1	

Sample Clotted

REFERENCES

1. Bean, J. W.: "Hypophyseal and Adrenocortical Factors in Pulmonary Damage Induced by Oxygen at Atmospheric Pressure," Am. J. Physiol., 172:169-174, 1953.

2. Bert, P.: La Pression Barometrique: Recherches de Physiologie Experimental, G. Masson, Paris, 1878.

3. Comroe, J. H., and Drips, R. D.: "Oxygen Toxicity," J. A. M. A., 128:710-717, 1945.

4. Ohlsson, W. T. L.: "A Study on Oxygen Toxicity at Atmospheric Pressure," Acta Medica Scandinavica, 128: Supplement 190, 1947.

5. Weir, F. W., Bath, D. W., Yevich, P., and Oberst, F. W.: "Study of Effects of Continuous Inhalation of High Concentrations of Oxygen at Ambient Pressure and Temperatures," Aerospace Med., 36:117-120, 1965.

6. Bean, J. W.: "Effects of Oxygen at Increased Pressure," Physiol. Rev., 25:1-47, 1945.

7. Smith, F. J. C., Heim, J. W., Thompson, R. M., and Drinker, C. K.: "Bodily Changes and Development of Pulmonary Resistances in Rats Living Under Compressed Air Conditions," J. Exper. Med., 56:63-78, 1932.

8. Herlocher, J. E., Quigley, D. G., Behar, V. S., Shaw, E. G., and Welch, B. E.: "Physiologic Response to Increased Oxygen Partial Pressure I. Clinical Observations," Aerospace Med., 35:613-618, 1964.

9. Robertson, W. G., Hargraves, J. J., Herlocher, J. E., and Welch, B. E.: "Physiologic Response to Increased Oxygen Partial Pressure II. Respiratory Studies," Aerospace Med., 35:618-622, 1964.

10. Helvey, W. M., Albright, G. A., Benjamin, F. B., Gaul, L. S., Peters, J. J., and Rind, H.: Effects of Prolonged Exposure to Pure Oxygen on Human Performance, Republic Aviation Corporation RAC 393-1 (ARD-807-701), November, 1962.

11. Mammen, R. E., Critz, G. T., Dery, D. W., Highly, F. M., and Hendler, E.: "Effects of Sequential Exposure to Acceleration and Space Capsule Atmospheric Conditions," Aerospace Med., 34: Abstr. 260, 1963.

12. Morgan, T. E., Jr., Cutler, R. G., Shaw, E. G., Ulvedal, F., Hargraves, J. J., Moyer, J. E., McKenzie, R. E., and Welch, B. E.: "Physiologic Effects of Exposure to Increased Oxygen Tension at 5 psia." Aerospace Med., 34:720-726, 1963.

13. Zalusky, R., Ulvedal, F., Herlocher, J. E., and Welch, B.E.: "Physiologic Response to Increased Oxygen Partial Pressure III. Hematopoiesis," Aerospace Med., 35:622-626, 1964.

14. Harper, D. T.: "Pathology of Animals Exposed to a Pure Oxygen Atmosphere at Reduced Pressure for Prolonged Periods," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1966.

15. MacEwen, J. D.: "Oxygen Toxicity at Near-Ambient Pressures," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1965.

16. McNerney, J. M.: "Preliminary Results of Toxicity Studies in 5 psia 100% Oxygen Environment," Proceedings of the Conference on Atmospheric Contamination in Confined Spaces, 30 March - 1 April 1965, AMRL-TR-65-230, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1965.

17. Patrick, R. L.: "Pathologic Effects of Exposure to Pulmonary Irritants at Ambient Air vs. 5 psia 100% Oxygen Atmosphere for Periods up to 90 Days," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1966.

18. Robinson, F. R.: "Pathological Evaluation of Oxygen Toxicity at Near-Ambient Pressures," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1966.

19. MacEwen, J. D.: Toxic Hazards Research Unit; Design and Construction Phase, AMRL-TR-65-125, (AD624 473), Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, September, 1965.

20. Kingsley, G. R., and Schaffert, R. R.: "Direct Micro-determination of Sodium, Potassium and Calcium," Anal. Chem., 25:1738, 1953.

21. Kingsley, G. R.: "The Determination of Serum Total Protein, Albumin, and Globulin by the Biuret Reaction," J. Biol. Chem., 131:197, 1939.

22. Reitman, S., and Frankel, S.: "A Colorimetric Method for the Determination of Serum Glutamic Oxaloacetic Transaminase and Glutamic Pyruvic Transaminase," Am. J. Clin. Path., 28:56, 1957.

23. Klein, B., Read, P. A., and Babson, A. L.: "Rapid Method for the Quantitative Determination of Serum Alkaline Phosphatase," Clin. Chem., 6:169, 1960.

24. Dryer, D. L., Tammes, A. R., and Routh, J. I.: "The Determination of Phosphorus and Phosphatase with N-Phenyl-p-Phenylenediamine," J. Biol. Chem., 225:177, 1957.

25. Cabaud, P. G., and Wroblewski, F.: "Colorimetric Determination of Lactic Dehydrogenase Activity of Body Fluids," Am. J. Clin. Path., 10:235, 1933.

26. Back, K. C.: "Toxicity Studies on Animals Exposed Continuously for Periods up to 235 Days to a 5 psia 100% Oxygen Atmosphere," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1966.

27. Kistler, G.: "Electron Microscopic Investigations of Oxygen Effects on Lung Tissue," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1966.

28. Mautner, W.: "Electron Microscopic Investigations of Oxygen Effects on Kidney Tissue," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1966.

29. Riesen, W. H.: "Cellular Biochemistry of Oxygen Toxicity," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1966.

30. Schaffner, F.: "Electron Microscopic Investigations of Oxygen Effects on Liver Tissue," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1966.

31. Hagebusch, O. E.: "Pathology of Animals Exposed for 235 Days to a 5 psia 100% Oxygen Atmosphere," 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, 4 and 5 May 1966, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, December, 1966.

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)	2a. REPORT SECURITY CLASSIFICATION
*Aerojet-General Corp. Dayton, Ohio **Aerospace Med. Res. Labs. & AMD, AFSC Wright-Patterson AFB, OH	UNCLASSIFIED
	2b. GROUP N/A

3. REPORT TITLE

RESPONSES OF ANIMALS TO OXYGEN AT REDUCED PRESSURE

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report, May 1965 - May 1966

5. AUTHOR(S) (First name, middle initial, last name)

*J. D. MacEwen, PhD
*R. P. Geckler, PhD
**K. C. Back, PhD

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
February 1968	28	31

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
AF 33(657)-11305	Aerojet-General Corp. No. 3266
b. PROJECT NO. 6302	
c. Task No. 630201	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	AMRL-TR-67-78

10. DISTRIBUTION STATEMENT

Distribution of this document is unlimited. It may be released to the Clearinghouse, Department of Commerce, for sale to the general public.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Aerospace Medical Research Laboratories Aerospace Medical Division, Air Force Systems Command, WPAFB, Ohio 45433

13. ABSTRACT

As extensions of previous short-term experiments on the toxicity of oxygen at reduced pressure in animals, long-term continuous exposures of beagle dogs, rhesus monkeys and albino rats and mice were undertaken. The exposures were for 230 days to 100% oxygen at 5 psia. No mortality occurred in the exposed dogs or monkeys. More rats and mice died in the control groups than in the experimental groups, indicating no effects on mortality due to the experimental conditions. The growth rates of control and experimental rats were almost identical, further indicating lack of deleterious effects. Histopathologic examination of tissues was conducted on all four animal species. No differences were observed between exposed animals and their controls in monkeys, rats or mice. Minimal differences consisting of mild bronchitis and mild congestion were seen in the dogs exposed to 100% oxygen at reduced pressure. One exposed dog exhibited severe pulmonary changes associated with acute interstitial bronchial pneumonia. Periodic measurements of blood constituents showed no significant differences attributable to prolonged oxygen exposure at reduced pressure.

DD FORM 1 NOV 65 1473

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Space Cabin Toxicology						
Oxygen						
Clinical Pathology						
Rodents						
Dogs						
Monkeys						