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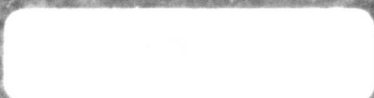
ADRENOCORTICAL STEROIDS, BODY ORGAN WEIGHTS,
AND HEMATOLOGY OF RATS EXPOSED TO A PURE
OXYGEN ENVIRONMENT AT 210 MM. HG. ABSOLUTE



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



**ADRENOCORTICAL STEROIDS, BODY ORGAN WEIGHTS, AND HEMATOLOGY OF RATS
EXPOSED TO A PURE OXYGEN ENVIRONMENT AT 210 MM. HG, ABSOLUTE**


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FOREWORD

The research reported in this paper was accomplished in the Physiology Branch under task No. 775801 between September 1965 and January 1967. The paper was submitted for publication on 15 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.

This report has been reviewed and is approved.


JAMES B. NUTTALL
Colonel, USAF, MC
Commander

ABSTRACT

Continuing interest in the possible use of low pressure, normoxic environments in spacecraft has led to investigation of the effect of such environment upon hematologic parameters, organ weights, and plasma corticosteroids.

Experiments were conducted with rats exposed up to 11 months in an environment containing 98% oxygen at 210 mm. Hg, absolute. Blood levels of corticosterone and body and organ weights of experimental animals were measured after specified periods of exposure. Hematologic parameters (hematocrit, hemoglobin, red cell, white cell, eosinophil, and reticulocyte counts) were also determined.

Except for the first few days of exposure to altitude, the results of plasma corticosterone determinations gave no evidence of prolonged stress to the animals. In general, changes in body organ weights and hematologic parameters were minor and did not appear to affect the animals adversely even after 11 months of continuous exposure.

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I. INTRODUCTION

The present study is a continuation of work previously reported (1) and is a result of continuing interest in the possible use of low pressure, normoxic environments for spacecraft.

There is considerable disagreement concerning the effects of such environments upon hematologic parameters. Rats exposed to 190 to 200 mm. Hg, absolute, with near 100% oxygen were found to have hemoglobin levels 120% of normal, red cell counts up to 134% of normal, and reticulocytes up to 160% of normal during the first 41 days of exposure—with a gradual return to normal by 58 days (2). In other studies, however, rats exposed 4 to 7 days in a similar environment showed no changes in hematocrit, hemoglobin, or red cell counts (3), nor was there a change in hemoglobin after 24 days' exposure (4). With slightly hyperoxic conditions, 258 mm. Hg, absolute, and 100% oxygen, humans showed anisocytosis, microcytosis, a slight decrease in hemoglobin concentration, and an increase in osmotic fragility after 2 weeks of exposure (5). Hematopoiesis was not changed in 4 men exposed for 30 days (6).

Urinary 17-hydroxycorticosteroids (17-OHCS) were normal in humans exposed 2 weeks to 100% oxygen at 258 mm. and 196 mm. Hg (5). Rats exposed to 100% oxygen at 226 mm. and 187 mm. Hg responded to cortisone injections with an increase in urinary nitrogen comparable to levels of controls. Baseline nitrogen excretion, however, was 50% greater than in controls, suggesting possible increased glucocorticoid secretion (7).

The present study was conducted to determine the effect of a low pressure normoxic environment upon hematologic parameters, organ weights, and plasma corticosteroids.

II. METHODS

Housing and care

Charles River CD* strain rats were used as experimental subjects. Experimental and control animals were kept in the same room under similar conditions of temperature, light, and humidity. The rats were housed in clear plastic cages, from 2 to 5 to a cage depending upon age and size of the animals. Rats with litters were placed in separate cages. Pine or cedar shavings were used as bedding. Feeding and transfer of rats to clean cages were carried out twice weekly. Experimental animals were cared for similarly to the controls in every respect except that the altitude animals received water in open, galvanized iron cups as opposed to water bottles for controls. Purina laboratory chow was fed to nonpregnant animals, and Purina mouse breeder ration was fed to pregnant and nursing females. Liquid vitamins were placed in the drinking water.

Chamber and environmental conditions

Experimental animals were maintained in an altitude chamber having an interior volume of about 300 cubic feet. The chamber was equipped with a man lock and a small instrument lock. Total pressure was automatically controlled at 210 mm. Hg. Humidity ranged from 40% to 50% with $\text{CO}_2 < 2$ mm. Hg. Chamber and room temperature was maintained at $76 \pm 1^\circ \text{F}$. For the long-term run,

chamber oxygen levels were $\geq 98\%$ of the total dry gas the first 7 months, and $> 90\%$ the last 4 months owing to the necessity of transferring to a less airtight chamber. The transfer was made under the same low pressure conditions as prevailed in the chamber. In all short-term experiments, oxygen levels were $> 98\%$.

Experimental design

A group of 13 young adult females was placed in the altitude chamber and maintained there 11 months during which time the animals were bred and produced a total of 23 litters. The F_1 generation used in the present experiment remained in the chamber until sacrificed at 60 days of age. A control colony was maintained at the same time as the experimentals with the females being bred and producing F_1 generation controls.

For shorter-term experiments, 60 young adult females were placed in the chamber and sacrificed in groups of 10 after 1, 2, 4, 8, 16, or 32 days in the experimental environment.

Measurements

Body organ weights (heart, liver, kidney, spleen, and lungs) were recorded for animals exposed 8, 16, and 32 days, for those exposed 11 months, and for F_1 generation rats born and raised in the chamber. In addition, adrenals were weighed in rats exposed 8, 16, and 32 days, and testes were weighed in F_1 generation males.

Hematologic measurements (hematocrit, hemoglobin, red and white cell counts, and eosinophil and reticulocyte counts) were determined for animals exposed for periods of 1, 2, 4, 8, 16, and 32 days, and for those exposed 11 months to the experimental environment.

Blood levels of corticosterone were measured in rats exposed 1, 2, 4, 16, and 32 days. The rats were decapitated with the aid of a small guillotine, the blood being drained into a beaker. Corticosterone was determined in the plasma using the sulfuric acid-induced fluorescence technic of Sweat (8).

III. RESULTS

Results for body organ weights are listed in tables I and II. Body weights of rats exposed up to 32 days showed significant increases over those of controls by day 8 ($P < .05$). Since they were young adults, this increase was most likely an increase in body fat. It seems probable that decreases in heart, liver, and kidney weights per 100 gm. of body weight on day 32 of exposure merely reflected this increase in body weight. Spleen weights of experimental rats after 8 days, and lung weights after 16 days, and after 11 months of exposure, were greater than those of controls ($P < .05$). Liver weights were less than those of controls and testes weights per 100 gm. of body weight were greater ($P < .05$) for 60-day-old males born and raised under the experimental conditions than were those of controls. Chamber-born females did not differ from controls.

Hematologic parameters are listed in table III. Reticulocytes showed significant ($P < .05$) increases in rats exposed 1, 16, and 32 days, and nonsignificant increases on days 2, 4, and 8. Females exposed 11 months, however, had a lower reticulocyte percentage than did controls. Red cell counts were below control levels ($P < .05$) in rats exposed 16 and 32 days. The opposite results appeared in the rats exposed 11 months. Hematocrit and hemoglobin increased after 2 days and decreased after 16 days' exposure ($P < .05$).

Corticoids

Plasma corticosterone levels are shown in figure 1 for rats exposed up to 32 days. Analysis of variance showed no significant time or treatment effects. A high level of interaction ($P < .01$), however, indicated a treatment effect that varied with time. As a result, individual points were compared using the t-test. Corticosterone levels of altitude-exposed rats were higher than controls from day 2 through day 4 ($P < .01$). By day 16 corticosterone levels of the altitude rats were below those of the controls ($P < .05$) and by day 32 differences had disappeared.

TABLE I

Body and organ weights (gm.) of rats exposed to a pure oxygen environment at 210 mm. Hg., absolute

	Controls (10)*	At 8 days (10)	At 16 days (10)	At 32 days (10)	Controls (9)
Body	301.6 ± 4.6	319.1 ± 5.3†	321.7 ± 3.9†	329.9 ± 5.5†	439.3 ± 20.8
Heart	1.006 ± 0.046	1.006 ± 0.057	0.945 ± 0.019	0.937 ± 0.019	1.167 ± 0.051
Liver	10.966 ± 0.320	11.597 ± 0.275	11.186 ± 0.209	10.644 ± 0.243	13.508 ± 0.705
Kidney	2.172 ± 0.082	2.185 ± 0.049	2.155 ± 0.050	2.104 ± 0.042	2.613 ± 0.133
Spleen	0.670 ± 0.023	0.865 ± 0.878†	0.655 ± 0.040	0.632 ± 0.034	0.761 ± 0.032
Lungs	1.700 ± 0.082	1.978 ± 0.055§	2.106 ± 0.103†	1.833 ± 0.063	1.712 ± 0.218§
Adrenal	0.0683 ± 0.0051	0.0649 ± 0.0017	0.0611 ± 0.0020	0.0701 ± 0.0030	
	After 11 months (10)	Control males (7)	Chamber-born males (8)	Control females (6)	Chamber-born females (10)
Body	438.5 ± 16.1	313.6 ± 15.0	268.8 ± 12.2‡	216.3 ± 12.6	204.1 ± 6.3
Heart	1.166 ± 0.051§	1.056 ± 0.065	0.960 ± 0.038	0.782 ± 0.059	0.726 ± 0.030
Liver	13.496 ± 0.471	14.101 ± 0.612	11.123 ± 0.958†	9.330 ± 0.630	8.626 ± 0.366
Kidney	2.661 ± 0.098	2.531 ± 0.202	2.350 ± 0.122	2.017 ± 0.306	1.720 ± 0.079§
Spleen	0.687 ± 0.055	0.734 ± 0.036	0.645 ± 0.083	0.557 ± 0.035	0.558 ± 0.031§
Lungs	2.623 ± 0.327§	1.426 ± 0.063	1.364 ± 0.086§	1.137 ± 0.036	1.105 ± 0.054§
Adrenal		2.977 ± 0.154 (testes)	2.894 ± 0.061 (testes)		

*Number in parentheses is number of rats in standard sample.

†Greater than control $P < .05$.

‡Less than control $P < .05$.

§Fever than standard sample.

IV. DISCUSSION

Except for the first few days of exposure to altitude, the results of plasma corticosterone determinations gave no evidence of prolonged stress to the animals. Below-normal corticosterone levels by day 16 indicated not only that above-normal stimulation of the adrenal gland had ceased but that the gland had become temporarily less sensitive as a result of earlier stimulation.

It would be difficult to define precisely the cause of stress during the first few days of exposure. It could have been a result of either decreased barometric pressure or a lack of inert gas—two factors which cannot be varied

independently in a normoxic environment. On the other hand, it is possible that the rats were merely apprehensive as a result of changes in sound conduction (9), or from the slight breathlessness and decreased respiratory effort brought about by decreased total pressure (10).

Body organ weights showed no discernible pattern of change with exposure to the experimental environment. The most noteworthy change was the enlargement of the lungs of females exposed 11 months. These lungs were outwardly similar to controls, except for size. Histologic studies (11) indicated some arteriolar hypertrophy but no definite signs of damage to the lung structure.

TABLE II

Organ weights/100 gm. body weight of rats exposed to a pure oxygen environment at 210 mm. Hg, absolute

Organ	Controls (10)*	At 8 days (10)	At 16 days (10)	At 32 days (10)	Controls (9)
Heart	0.334 ± 0.016	0.336 ± 0.026	0.296 ± 0.006	0.285 ± 0.006†	0.266 ± 0.004
Liver	3.630 ± 0.067	3.634 ± 0.056	3.479 ± 0.054	3.226 ± 0.065†	3.073 ± 0.073
Kidney	0.720 ± 0.024	0.685 ± 0.015	0.670 ± 0.011	0.639 ± 0.014‡	0.608 ± 0.023
Spleen	0.222 ± 0.006	0.272 ± 0.021†	0.205 ± 0.011	0.191 ± 0.009	0.175 ± 0.008
Lungs	0.564 ± 0.028	0.615 ± 0.019§	0.650 ± 0.026†	0.556 ± 0.020	0.399 ± 0.051§
Adrenal	0.0225 ± 0.0017	0.0203 ± 0.0005	0.0191 ± 0.0007	0.0214 ± 0.0010	
	After 11 months (10)	Control males (7)	Chamber males (8)	Control females (6)	Chamber females (10)
Heart	0.270 ± 0.012§	0.335 ± 0.010	0.359 ± 0.015	0.358 ± 0.013	0.357 ± 0.011
Liver	3.090 ± 0.057	4.510 ± 0.100	4.109 ± 0.247	4.305 ± 0.069	4.223 ± 0.095
Kidney	0.599 ± 0.017	0.800 ± 0.028	0.873 ± 0.026	0.921 ± 0.114	0.832 ± 0.028§
Spleen	0.156 ± 0.012	0.230 ± 0.010	0.239 ± 0.027	0.258 ± 0.008	0.257 ± 0.017§
Lungs	0.615 ± 0.090† (F < .10)§	0.456 ± 0.014	0.477 ± 0.018§	0.551 ± 0.021	0.545 ± 0.027§
Adrenal		0.952 ± 0.015 (testes)	1.084 ± 0.030† (testes)		

*Number in parentheses is number of rats in standard sample.

†Greater than control P < .05.

‡Less than control P < .05.

§Fewer than standard sample.

Hematocrit and hemoglobin increase after 2 days' exposure indicated a transient hemoconcentration, possibly caused by dehydration resulting from an increased evaporation rate. Red blood cells appear to have a higher rate of destruction after 16 days' exposure, while after the 11-month exposure the reverse was found to be true.

In general, although some physiologic changes were detected, the rats adapted quite well to the experimental environment, and suffered no measurable ill effects even after 11 months of continuous exposure. Eight months' exposure of rats, dogs, and monkeys to pure oxygen at 258 mm. Hg, absolute, resulted in essentially similar findings (12).

TABLE III

Hematologic parameters of rats exposed to a pure oxygen environment at 210 mm. Hg, absolute

Determination	Controls (10)*	At 1 day (10)	At 2 days (10)	At 4 days (10)	At 8 days (9)
Hematocrit	40.50 ± 0.56	41.00 ± 0.48	43.15 ± 0.80†	40.60 ± 0.55	41.61 ± 0.55
Hemoglobin	13.58 ± 0.13	13.66 ± 0.16	14.39 ± 0.28†	13.63 ± 0.18	13.91 ± 0.16
Red cell count (mm. ³ × 10 ⁶)	6,196.0 ± 48.1	6,180.0 ± 84.2	6,670.0 ± 177.5	6,148.0 ± 126.0	6,408.3 ± 211.2
White cell count (mm. ³)	5,930.0 ± 263.5	5,825.0 ± 385.0	—	6,075.0 ± 387.2	6,100.0 ± 300.0
Eosinophils (mm. ³)	143.0 ± 11.1	96.4 ± 8.8	97.2 ± 10.6	129.5 ± 14.5	124.4 ± 13.2
Reticulocytes (% total red cells)	1.58 ± 0.13	2.14 ± 0.10†	1.70 ± 0.18	1.85 ± 0.09	2.02 ± 0.13
Determination	At 16 days (9)	At 32 days (8)	Controls (6)	After 11 months (6)	
Hematocrit	37.50 ± 0.69‡	40.81 ± 0.28	40.75 ± 0.78	42.00 ± 1.13	
Hemoglobin	12.77 ± 0.24‡	13.44 ± 0.13	13.93 ± 0.20	14.05 ± 0.28	
Red cell count (mm. ³ × 10 ⁶)	4,685.6 ± 153.7‡	4,747.5 ± 60.2‡	6,013.3 ± 49.9	6,591.7 ± 212.2†	
White cell count (mm. ³)	6,322.2 ± 737.3	5,737.5 ± 43.0	6,091.7 ± 394.0	5,408.3 ± 365.0	
Eosinophils (mm. ³)	170.5 ± 25.6	107.5 ± 8.0	216.7 ± 53.2	139.3 ± 19.1	
Reticulocytes (% total red cells)	2.27 ± 0.11†	2.27 ± 0.14†	1.97 ± 0.10	1.43 ± 0.17‡	

*Number in parentheses is number of rats in sample.

†Greater than controls $P < .05$.

‡Less than controls $P < .05$.

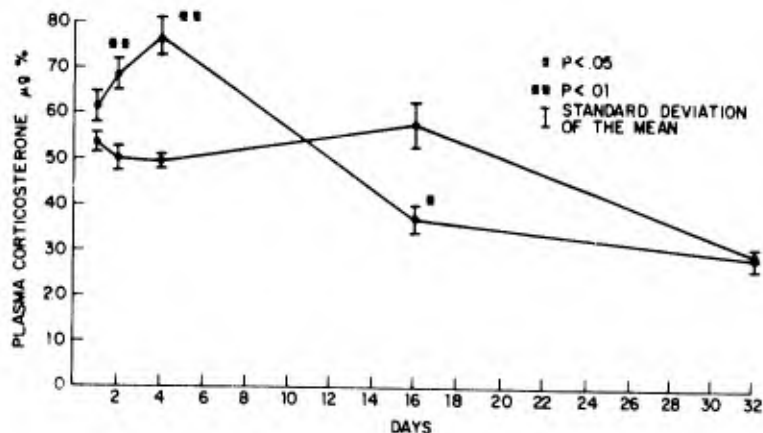


FIGURE 1

Plasma corticosterone levels of rats exposed to a pure oxygen environment at 210 mm. Hg, absolute.

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13. ABSTRACT

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Experiments were conducted with rats exposed up to 11 months in an environment containing 98% oxygen at 210 mm. Hg, absolute. Blood levels of corticosterone and body and organ weights of experimental animals were measured after specified periods of exposure. Hematologic parameters (hematocrit, hemoglobin, red cell, white cell, eosinophil, and reticulocyte counts) were also determined.

Except for the first few days of exposure to altitude, the results of plasma corticosterone determinations gave no evidence of prolonged stress to the animals. In general, changes in body organ weights and hematologic parameters were minor and did not appear to affect the animals adversely even after 11 months of continuous exposure.

14 KEY WORDS Physiology Stress physiology Hematology Oxygen environment Normoxic environment Adrenocortical steroids Body organ weights Pressure effects Corticosterone	LINK A		LINK B		LINK C	
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