THE EFFECTS OF DISCONTINUOUS EXPOSURE TO 18,000 FEET SIMULATED ALTITUDE ON THE ALBINO RAT

By

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INTRODUCTION

Studies of the effects of discontinuous exposure of animals and humans to anoxia produced by decrease of atmospheric pressure have been made in increasing numbers in recent years. The stimulus which has brought about research in this particular field is the desire for information applicable to the condition of anoxia encountered in human aviation. The most satisfactory method for investigation of the effects of these conditions is exposure of experimental animals or human subjects to simulated altitude (i.e., lowered barometric pressure) in the decompression chamber. In this apparatus, it is possible to observe the effects of decreased pressure and low temperature, either together, so as to simulate actual altitude conditions, or separately, so as to facilitate consideration of the effects of one of these factors alone.

In the present study, altitude alone was considered, the temperature being held fairly constant by passing a stream of water over the exterior of the chamber during exposures.[1]

The altitude considered in the present work is 18,000 feet, or the equivalent of a barometric pressure of 379 mm Hg. The effects on rats of repeated exposures to this altitude are of interest, since this altitude represents a barometric

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[1]The mean temperature of the chamber during exposure periods was 22°C, and the extremes were 14°C and 26°C. This variation was considerably less than the variation of the ambient temperature of the animal room, in which all the animals were kept between exposures.

pressure high enough to permit of easy acclimatization for rats, and yet low enough to produce a considerable degree of anoxia (pO_2 c. 80 mm Hg).

The previous work in altitude physiology has been concerned with two types of exposure, namely, "continuous" and "discontinuous" exposure. In the continuous exposure, experimental animals are sustained at the test altitude for the duration of the test, possibly with "descents" to "ground level" for short periods for the purpose of feeding and cleaning of cages. In discontinuous exposure, the animals are taken to "altitude" for a specified time interval each day throughout the test period. The former (continuous exposure) has been of interest because of its simulation of living conditions at high altitude in the mountains, while the latter (discontinuous exposure) is more nearly like the conditions encountered in aviation. In the present work, animals were exposed to the test altitude for 1 hour per day. This, then, represents a study of the effects of discontinuous anoxia. It has been reported by Van Liere and Stickney (1942) and other workers, that discontinuous exposure to anoxia produces acclimatization in much the same manner as continuous exposure, the degree of acclimatization attained being proportional to the degree of anoxia produced and the length of the exposure period. If this is the case, it is possible to use the general findings of continuous exposure experiments in the consideration of the effects of discontinuous exposure to lowered barometric pressure, as long as the difference in method of exposure is borne in mind, and no attempt is made to apply the findings of the former type of experiment too strictly to consideration of the latter.

As there are available two excellent reviews of the history of experimentation in altitude physiology, no attempt will be made here to treat this subject exhaustively. The two sources recommended for such review are Barometric Pressure, by Paul Bert, and the review of Langley (1943). References will be made throughout the text of this paper to previous work as it is related to the subject under discussion.

METHODS

One hundred and two Sprague-Dawley albino rats were divided into 4 groups and paired according to body weight before the start of the experiment. Thus, group 1 contained 26 marked animals slated for exposure to low pressure, and group 3 contained 26 control animals, each one having the same body weight as an animal with the same ear marking in group 1. Groups 2 and 4 were paired in the same manner, but each of these groups contained only 25 animals. Each group was then placed in a separate large wire cage and fed water and Purina Dog Chow ad libidum.

The first exposure of the test animals in the decompression chamber was made on the day following the pairing and was repeated 7 days a week for 9 weeks. The exposure of group 2 started 1 week prior to that of group 1 and the former group was sacrificed 1 week earlier than the latter. Two advantages are gained from this "staggering": (1) The time permitted for the dissection of such a large number of animals is extended. (2) Since the average weights of the two experimental groups (1 and 2) at any time represent the weights after the same length of exposure, but are a week apart chronologically, the effects of minor environmental changes on the average weights of the animals are partially eliminated.

Exposure of the experimental animals to simulated altitude was carried out in a large steel decompression chamber.[2] At approximately 5:00 P. M. every day, the entire rack of cages was taken to the chamber, the test animals put inside and the controls left outside the chamber. Ascent to 18,000 feet (379 mm Hg) was made at a rate of 3,000 feet per minute, and the animals were kept at this altitude for 1 hour with a ventilation rate of 30 cubic feet of air per minute. After 1 hour's exposure, the animals were brought back to ground level at a rate of 3,000 feet per minute and returned to the animal room. They were then given food and water in sufficient quantity to assure them an excess until the following exposure period.

All of the animals were weighed once a week on the afternoon of the same day of the week (Tuesday) as the original pairing.

At the end of 9 weeks' exposure, the animals were sacrificed. Thirty-five of the experimental animals taken at random and the corresponding controls were anaesthetized with nembutal and dissected. The dissection procedure was as follows: The abdomen was opened and a 10 ml hypodermic syringe containing a drop or two of heparin solution was introduced into the left ventricle of the heart by means of a #20 hypodermic needle. Blood was drawn from the beating heart slowly

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[2]The decompression chamber used in this study is one on the grounds of the National Institute of Health, Bethesda, Maryland, and is the property of the U. S. Public Health Service (Industrial Hygiene Research Laboratory).

until no more could be obtained. The volume of blood in the syringe was recorded. This was taken as a rough index of the blood volume of the animal (Selye, 1937). The blood in the syringe was then used for erythrocyte count, using the Neubauer counting chamber, and for hemoglobin, using the Heilige-Wintrobe hemoglobinometer. Organs were then dissected and placed in 0.7% salt solution as follows: left adrenal, spleen, right adrenal, thymus, thyroid, left testis, right testis, seminal vesicle,[3] and pituitary.

Before fixation, the organs of 28 of the animals were weighed on the analytic balance. This was accomplished by the use of a glass weighing vial filled with mineral oil as a tare. The organ to be weighed was cleaned of liquid adhering to the surface by rolling on filter paper and was then dropped into the weighed bath of mineral oil, into which it sank, preventing evaporation of water from the surface during weighing. Weight of the organ was then computed by difference.

These organs were cleaned of connective tissue and placed in Zenker's formol fixative, with the exception of the right adrenal and right testis, which were fixed in the osmicating fluid of Flexner and Grollman (1939).

After fixation and dehydration, all of the organs taken were again weighed by the same method, and those for which fresh weights were available were used for estimation of a conversion factor by means of which fresh weights could be

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[3] Before removal of the seminal vesicle, the neck of the organ was ligated with cotton thread in order to retain its fluid content in toto.

calculated for those organs which had not been weighed in a fresh condition.[4]

Table I

Organ	Fresh Wt. Av.	Fixed Wt. Av.	Factor[1]	No.
Thyroid	0.0241	0.0190	1.2684	28
Left Adrenal	0.0170	0.0140	1.2143[2]	28
Right Adrenal	0.0190	0.0237	0.8017[2]	24
Testes	1.6353	1.3993	1.1687	28
Seminal Vesicle	1.3194	1.0609	1.2437	26
Thymus	0.3505	0.2217	1.5810	20
Spleen	0.5659	0.4040	1.4007	25
Pituitary	0.0079	0.0053	1.4906	13

[1]Factor = Fresh Wt./Fixed Wt. or Fresh Wt. = Fixed Wt. x Factor.

[2]Difference between factors for left and right adrenals is due to difference in fixing technique.

After dissection of the above organs, a small amount of air was injected into the left ventricle and the heart was compressed between the fingers. This forced blood and bubbles of air into the aorta. Upon ceasing the compression, in the normal heart the air bubbles remain in the aorta in approximately the same position. However, in animals with insufficiency of the aortic valves, regurgitation occurs and the

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[4]This method was suggested, in a personal communication, by Dr. A.J. Dalton, of the National Cancer Institute, Bethesda, Maryland.

bubbles are seen to flow backward toward the ventricle. This was taken as an indication of valvular insufficiency.

The stomach was then removed, opened, washed free of contents, and examined for blood and for gastric ulcers. These are plainly visible, when present, as hemorrhagic spots, or as small lesions surrounded by an elevated area. The carcass of the animal was then examined for anatomical abnormalities, and records kept of those found.

After the dissection described above of 70 animals, 32 rats remained alive. These animals were used for tests to determine the value of the acclimatization attained in increasing resistance to acute anoxia. Twelve of these animals (6 experimental, 6 control) were subjected to acute anoxia by decompression to an equivalent of 50,000 feet (87 mm Hg) on the day following their last acclimatizing exposure; while 20 (10 experimental, 10 control) were allowed to rest at ground level in the animal room for 1 week after the last exposure to 18,000 feet and then exposed to acute anoxia at 40,000 feet (141 mm Hg).[5] Records of respiratory rate of each animal, survival time and cause of death were made during these acute exposures.

Statistical analysis of organ changes, and of other numerical measurements, was performed utilizing the Fisher t-test (Fisher, 1938) to show reliability of differences between test and control group averages.

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[5]During the latter exposure, the author was decompressed with the animals, wearing demand oxygen equipment and staying in such a position that the oxygen expelled from the mask was carried out the evacuation line and did not increase the oxygen tension of the chamber. This was done with the purpose of better observing the animals (respiratory rate and cause of death) during exposure.

The testes, thyroid, and adrenal glands of ten of the experimental animals dissected under nembutal, selected at random, and of the corresponding control animals, were used for histological examination. Paraffin sections were made at ten micra. Tissues fixed in Zenker's fluid were stained with Mallory's triple stain and osmicated tissues were mounted in Canada balsam without counter-staining. Sections of corresponding organs of the paired control and experimental animals were mounted on opposite ends of the same microscope slide for facility of comparison.

OBSERVATIONS

1. <u>General Condition</u>. Little difference was noted between the general physical condition of the animals exposed to 18,000 feet simulated altitude and that of the animals maintained at sea level during the course of the

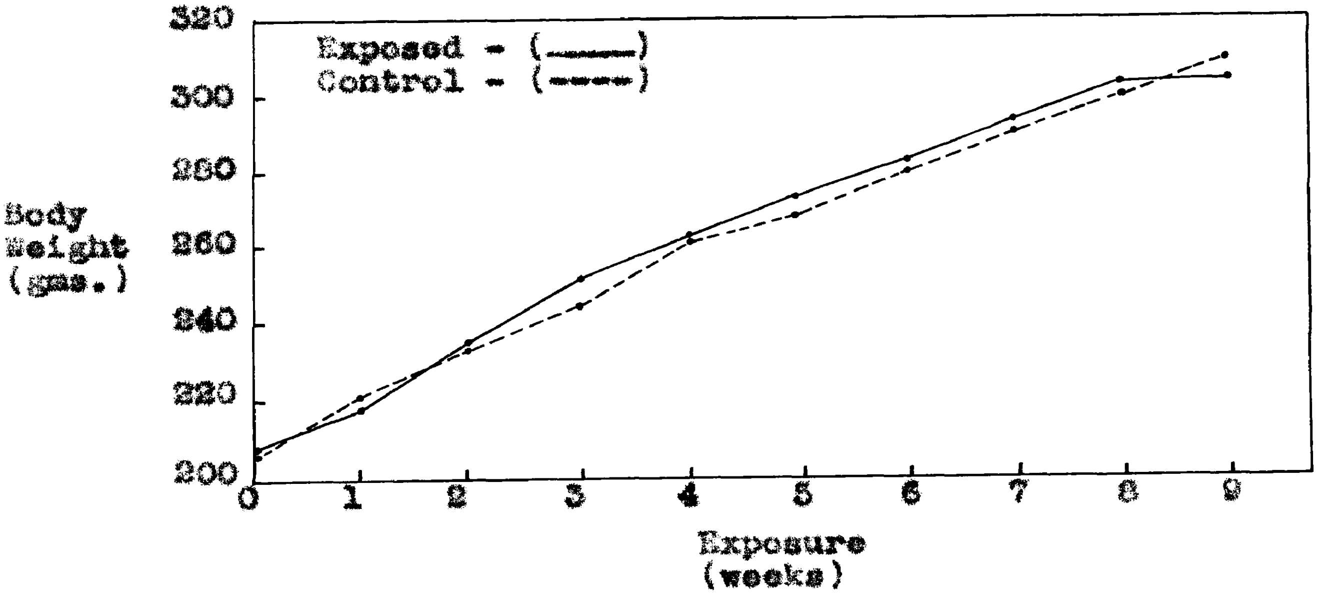

Figure 1. -- Growth of exposed and control animals.

experiment. The average body weight of the exposed animals closely paralleled that of the control animals throughout the 9 weeks' test period. A graphic representation of the average body weights of the two groups is presented in Figure 1, and the body weights of all animals may be found in Table III (Appendix).

After two weeks of exposure, the ears and the plantar surfaces of the feet of the test animals were, practically universally, found to be slightly darker in color than those of the controls.

The general behavior of the two groups was much the same, except that the exposed animals became more excited than did the controls when the rack of cages was rolled to the altitude chamber. During the "ascent" and "descent," the test animals were rather active, running about their cages, washing and scratching their bodies frequently; but during the hour while they were at altitude, both the animals inside and outside the chamber were quiescent, most of them sleeping. One difference was observed in their behavior during this period. The control animals tended, as rats do habitually, to sleep in closely crowded bunches, piled one on top of the other. Usually they were so concentrated at each end of their cages. This was not the practice of the exposed animals while at altitude, as they spread themselves out rather uniformly over the floor of the cage. When the test rats slept in the animal room, however, they tended to "pile up" just as did the controls. It is likely that this behavior in rarified atmosphere is an attempt to allow themselves as much opportunity for ventilation as possible.

2. Effects on Body Organs. The accumulated data on the organs of each of the animals studied is presented in Table III (Appendix). A summary of the effects of exposure is given in Table II (p. 12).

A. The Blood and Circulatory System.

Blood volume was found to be appreciably increased in the animals exposed to 18,000 feet altitude. As is shown in the accompanying table, an increase of approximately 21% occurred. Statistical analysis of this change showed a probability of only 1 in 1,000 that this distribution would occur by chance.

The erythrocyte count of the exposed animals was not significantly increased above that of the controls. The average counts of both groups, 8,020,000 and 8,040,000, respectively, are within the normal range for albino rats of the age group represented (approximately 20 weeks).

The hemoglobin content of the blood, however, was significantly increased (14.5%). Statistical analysis shows this increase might be expected to occur by chance in only 2 in 100 cases.

Insufficiency of the aortic semilunar valves was found in 34.3% of the exposed animals examined, while only one of the corresponding 35 control animals (2.9%) showed this deficiency. It is possible that this one represents experimental error in that rupture of these valves might possibly be effected during the dissection technique.

The mean weight of the spleen was not changed significantly by the exposure to altitude.

B. Endocrine Organs. (Weight)

(1) Thyroid. The mean weight of the thyroid gland was found to be 9.5% greater in the animals exposed to low pressure than that of the control animals. This distribution would be expected to occur by chance in 5% of cases.

(2) Adrenal Glands. The mean weight of the adrenal glands (sum of right and left adrenals) was not appreciably different in the two groups.

(3) Testes. The mean weight of the testes (sum of left and right testes) of the exposed animals was found to be slightly (3.8%) decreased in the experimental

Table II

SUMMARY OF EFFECT OF DISCONTINUOUS EXPOSURE TO

18,000 FT. 1 HOUR/DAY FOR 9 WEEKS ON THE ALBINO RAT

	CONTROL ANIMALS			EXPOSED ANIMALS				
	Datum[1]	No.	Body Wt.	Datum[1]	No.	Body Wt.	Change[2]	P[3]
Blood Volume	6.86	37	308.6	8.28	34	303.9	20.7% Increase	.001
Red Cell Count (millions/mm^3)	8.02	17	319.7	8.04	15	316.7	None	>.50
Hemoglobin(g%)	15.2	15	290.6	17.4	15	289.1	14.5% Increase	.02
Spleen Wt. (g)	0.5900	27	310.1	0.5947	27	308.0	None	>.50
Thyroid Wt. (g)	0.0231	29	309.0	0.0253	28	305.9	9.5% Increase	.05
Adrenal Wt.(g)	0.0350	35	308.0	0.0343	35	303.4	None	>.50
Testis Wt. (g)	3.4408	32	310.8	3.3101	35	303.4	3.8% Decrease	.15
Seminal Vesicle Wt. (g)	1.3782	26	307.8	1.3720	27	306.6	None	>.50
Thymus Wt. (g)	0.3349	25	313.6	0.3305	24	308.3	None	>.50
Pituitary Wt. (g)	0.0091	11	317.8	0.0076	11	311.3	16.5% Decrease	.03
Survival Acute Anoxia[4]	2/6	6	317.5	2/6	6	300.8	None	>.50
Survival Acute Anoxia[5]	8/10	10	317.3	3/10	10	312.7	62.5% Decrease	.03
Aortic Valve Insufficiency	1 (2.9%)	35	308.0	12 (34.3%)	35	303.4	91.7% Increase	.0001
Gastric Ulcers	7 (20%)	35	308.0	15 (42.9%)	35	303.4	53.3% Increase	.03
Lesions of Lungs	3 (8.6%)	35	308.0	16 (45.7%)	35	303.4	81.3% Increase	.0001

[1]Average figures for organ weights, blood volume, and hemoglobin, or occurrence (ratio or per cent) of stated condition.

[2]Effect of exposure on experimental animals.

[3]Probability of occurrence of difference (2) by chance. Determined by Fisher t.

[4]Exposure to acute anoxia the day after discontinuance of 18,000 ft. exposures.

[5]Exposure to acute anoxia one week after discontinuance of 18,000 ft. exposures.

animals, but this decrease is not statistically significant.

(4) Seminal Vesicle.[6] The seminal vesicle weight, including fluid content, was unchanged by the experimental procedure.

(5) Thymus. The mean weight of the thymus was found to be insignificantly different in the two groups.

(6) Pituitary. The mean weight of the pituitary gland was significantly decreased in the exposed animals (16.5%) below that of the controls. The probability of the occurrence of the difference by chance is 3%.

C. Thyroid Gland, Adrenal Gland, and Testis (Histology)

(1) Thyroid Gland. Eight of the ten thyroid glands of experimental animals examined differed from the glands of the controls by the presence of a large number of follicles engorged with "colloid." The average follicle size was also greater in the glands of experimental animals than in those of the controls.

(2) Adrenal Gland. The differences observed between the adrenal glands of exposed and control animals were slight.

a. In each case, however, of glands examined, the cross-sectional area of cortex comprised a greater portion of the entire adrenal section in the gland of exposed animals than in the corresponding controls. This observation might be subject to doubt in that the relative amount of cortex and medulla represented in an adrenal section is dependent

[6] Although it is not an endocrine organ, the seminal vesicle will be discussed with the testes throughout this paper because of its relationship to testicular function.

upon the distance of the section from the center of the gland. As serial sections were not made, an attempt was made to use those sections nearest the central portion of the gland. Thus the fact that in all cases the relationship between exposed and control sections was the same makes it seem likely that the observation is a true one, indicating a slight degree of adrenal cortical hypertrophy, or possibly a decrease in medullary tissue. This latter hypothesis is strengthened by the observation that the cells of the adrenal medulla of exposed animals appeared to be very compact as compared with these cells of the control animals.

b. In osmicated sections, the darkened area of intense osmic acid staining comprised a greater portion of the adrenal cortex of exposed animals than of the controls. In all cases (control and exposed glands) the most deeply stained area was near the capsul. This would indicate the presence of a greater proportion of lipoid elements in the adrenal cortex of the exposed animals, presumably indicating an increase in endocrine activity of the gland.

(3) Testis. The following differences were observed between the testes of exposed and control animals.

a. In all cases but one, the cross-sectional areas of the seminiferous tubules of the testes of exposed animals were smaller than those of the controls.

b. In 50% of cases, the interstitial cells of the testis of exposed animals were much larger than those of the controls. In no case were they smaller.

c. The sections of exposed animals were invariably more deeply stained with osmic acid than those of the controls. This staining was not limited to the interstitial cells but appeared to be in large irregularly shaped patches around the periphery of the seminiferous tubules.

D. Other Factors.

(1) Gastric Ulceration. The incidence of gastric ulcers was appreciably higher in the animals exposed to altitude (43%) than in the control animals (20%). All cases of actively hemorrhagic gastric ulcers encountered were in the experimental group.

(2) Pulmonary Lesions. The occurrence of lesions of the lungs was much more common in the exposed animals (16 cases in 36) than in the control animals (3 cases in 35).

(3) Miscellaneous Pathology. The occurrence of pathology not considered to be related to exposure to altitude is given in footnote of Table III (Appendix).

(4) Resistance to Acute Anoxia.

a. Of "acclimatized" and control animals subjected to acute anoxia the day after the last regular exposure, the resistance to high altitude (50,000 feet) was identical in each group (exposed simultaneously). Four out of 6 animals died with convulsions. However, it was found that the principle causes of death differed in the two groups. The principle causes of death in the previously exposed group appeared to be hemorrhage of the lungs and the cerebral arteries,[7] while every case of death in the control animals

[7] One case of cardiorrhexis was encountered.

showed ventricular spasm and auricular engorgement of the heart, as well as lung and cranial hemorrhage.

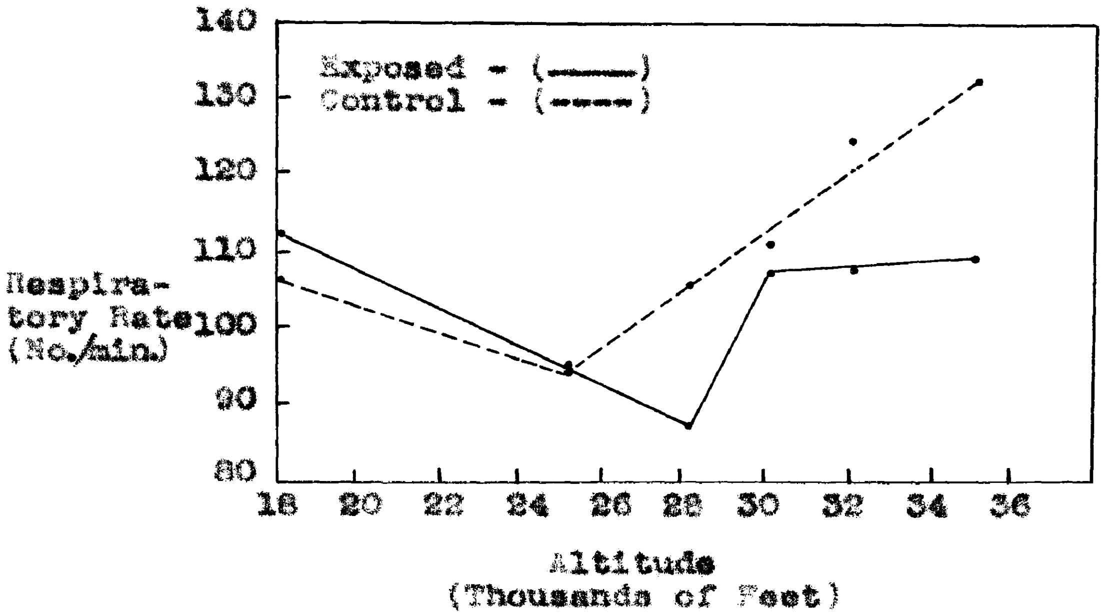

Figure 2. -- Respiratory rates of exposed and control animals, exposed to acute anoxia.

b. When kept at sea level pressures for 1 week after the last regular exposure to 18,000 feet, it was found that mortality in the previously treated animals was greatly increased upon exposure to acute anoxia. Thus, of 10 test animals subjected to 40,000 feet altitude, 7 died in convulsions, the principle pathology observed being cerebral hemorrhage. However, of 10 control animals exposed at the same time, only two did not survive, both of these animals showing ventricular spasm on autopsy. In this group also a pronounced difference was observed between test and control animals with respect to the change in respiratory rate in response to anoxia. This difference is shown in the accompanying figure (Figure 2).

DISCUSSION

Anoxia is one of the many stimuli which produce in the organism a syndrome characterized as the "alarm reaction." This syndrome has been described by Selye (1937) in great detail. In general, it may be described here as a non-specific response of the organism to damage in any of a number of forms. It is typified by increase in activity and thickening of the adrenal cortex, decrease in size of the thymus, and changes in the blood, as well as a number of other changes. If the stimulus is repeated, adaptation is brought about. The adaptation occurring from a particular stimulus, however, is specific in its protection against the particular damaging agent used as a stimulus and to none other. After continuation of the stimulus for an indefinite period, however, adaptation is lost and the animal is no longer capable of combating the damaging agent. Following this regression in adaptation, the organism dies of exhaustion with symptoms similar to those of acute alarm.

In general, the above syndrome is exemplified by animals exposed to anoxia. However, there are certain anatomical changes which occur specifically with anoxia produced by low barometric pressure. These will be discussed with reference to the present study.

1. <u>The Adrenal Cortex</u>. It has been shown that the adrenal cortex undergoes hypertrophy when the organism is subjected to a sufficient degree of stress of any sort. Ingle (1938)

has shown that continued muscular work produces hypertrophy of the adrenal cortex of the rat. Low temperature, intoxication, and many other unrelated stimuli also produce this symptom. The effect of anoxia on the adrenal cortex has been reported by many workers. Thorn et al (1942) reported an increase of approximately 17% by weight in the adrenals of rats exposed to 18,000 feet for 4 hours a day, 5 days a week, over a period of 3-4 weeks, and Dohan (1942), Evans (1936), Langley et al (1942), Hailman (1944), Dernehl et al (1943), Sundstroem et al (1942), and Dalton (1944), have all reported hypertrophy of the adrenal cortex as a consistent symptom of "acclimatization" to altitude. However, increase in activity of the adrenal cortex is necessary only during the period of adaptation. As soon as adaptation is attained, the organism is no longer in need of excess cortical secretion. This is shown by the work of Langley and Clarke (1942) who found that adrenalectomized rats exposed to 20,000 feet altitude require an increased amount of adrenal cortical extract during the period of acclimatization, but after this has occurred, the dose required for survival is the same as for adrenalectomized rats at sea level. Also Dalton (1944) has shown that the adrenals of rats exposed to 25,000 feet (282 mm Hg) decrease in weight after the initial hypertrophy.

This, perhaps, is an explanation of the findings reported in the present study. Since exposure was continued for 9 weeks before the adrenals were examined, any adrenal hypertrophy occurring in the early stages (first 3-5 weeks) during the adaptation phase, may have been lost after adaptation was attained. This is substantiated by the work of Nelson and

Burrill (1944) who found no hypertrophy of the adrenals of rats exposed to 18,000 feet, 1 hour per day, for approximately 7 weeks. The histological appearance of the glands indicates, however, that some increase in activity is present in the adrenal cortex of the exposed animals after 9 weeks exposure, associated with slight hypertrophy. This hypertrophy, however, appears to be at the expense of the medulla and does not result in an increase in the overall size and weight of the gland.

2. Testis and Seminal Vesicle. Decrease in the weight of the testis in continuous exposure has been reported by Sundstroem et al (1942) and Dohan (1942). Decrease in weight of both seminal vesicle and testis brought about by discontinuous exposure to 25,000 feet is reported by Gordon et al (1943). The foregoing reports have been based, however, on a more acute extent of anoxia than was employed in the present study. The effects as reported in the present study, (i.e., no statistically significant weight change in either testis or seminal vesicle) substantiate the work of Nelson and Burrill (1944) based on 19 exposed animals. However, the histological picture presented (p. 15) by the testes of exposed animals observed in the present study, is one of low activity. Probably, in view of the enlarged interstitial cells present in many cases, this low activity is due to retention of testicular hormone by these cells. Thus it seems probable that the decrease in average testis weight of 3.8% (see Table II) is a real decrease in spite of the low statistical significance. ($p=0.15$)

3. Thyroid Gland. Little reference is to be found in the literature concerning the effects of altitude on the weight of the thyroid gland. Streuli (1918) and Sarach et al (1941) have reported thyroidectomized animals to have greater tolerance for anoxia than normal animals. Van Liere and Stickney (1943) have reported enlargement of the thyroids of dogs kept at sea level for 3 months following 3 months' exposure to 18,000 feet for 8 hours a day. Hughes (1944) has shown that both thyroidectomized and functionally athyroid (by treatment with thiouracil) animals are more resistant to anoxia than normal animals.

The increase in weight reported for the thyroid in the present study is subject to two interpretations. Thyroid enlargement may be due to (1) increased activity or (2) decreased activity. If increased activity is the cause of the increase in weight, the value to the organism is difficult to explain, except insofar as the resultant increase in metabolic rate might increase hemopoesis, heart rate, respiratory rate and other vital functions. However, much more likely is the explanation that the increase in weight is due to decreased release of thyroxin, with resultant accumulation of "colloid," as was suggested by Gordon et al (1943). This would produce a lowered oxygen consumption of real value to an animal subjected to anoxia. The histological examination of the organs (p. 14) indicates the latter to be the nature of the change in thyroid activity.

4. Pituitary Gland. The regulating effect of the pituitary gland on the other endocrine organs is well known. No statistically significant changes in the weight of this organ

have been previously reported, to the author's knowledge, in studies of anoxia. However, it is evident that a stimulus involving the endocrine system generally in such a pronounced manner as does anoxia, should have some reflection in changes of the hypophysis. For example, decrease in activity of the testis and thyroid should be related to a similar decrease in the cells of the pituitary which produce the thyrotropic and gonadotropic hormones, namely, the acidophils and basophils of the anterior pituitary. If this decrease is sufficient to bring about a corresponding decrease in the weight of the organ, the results would be the same as those found in this study (i.e., decrease in pituitary weight). Thus, it seems probable that the decrease in weight reported here is due to the above loss in cell numbers.

5. The Blood and Circulatory System. An increase in blood volume of animals exposed to altitude has not, to the author's knowledge, been reported previously.[8] The value of this increase to the organism under conditions of low oxygen pressure is obvious. It is possible by this means to supply more blood, and, therefore, more oxygen to all the tissues of the body than is possible with a normal blood volume.

Also, the increase in hemoglobin permits of a greater oxygen carrying capacity per unit volume of blood. This increase has also been reported by Van Liere and Stickney (1942) for dogs, and by Campbell (1927)(1928). However, for human subjects living at high altitude (c. 15,000 feet) Hur-

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[8] Dalton, A. J., in a personal communication, has mentioned increase in blood volume in rats exposed to lowered barometric pressure.

tado (1932) reports no elevation of average hemoglobin content of the blood.

It may seem inconsistent that the erythrocyte count is not correspondingly increased. In fact, Van Liere and Stickney (1942) report an increase of 84% in erythrocyte count of dogs exposed to 18,000 feet for 17 weeks and Thorn et al (1942) report a similar increase in both hemoglobin per cent and red cell count for dogs exposed in the altitude chamber. However, the benefit to the organism of increase in hemoglobin content of the blood without recourse to increase in the number of erythrocytes is obvious, since an increase in the number of red cells is accompanied by a corresponding increase in the viscosity of the blood (Hurtado, 1932). This is decidedly a detrimental factor because of the increased work demanded of the circulatory system. Campbell (1927) makes the statement that increase of hemoglobin per cent of the blood is not an essential result of acclimatization and should be regarded only as a possible consequence. However, he points out that this change is of value to the heart when it does occur.

A number of workers have studied the effect on the heart of low barometric pressure. Van Liere (1941)(1943) was unable to produce cardiac hypertrophy (measured by change in weight) in guinea pigs at 20,000 feet altitude either when exercised or at rest, nor in dogs at 18,000. This, however, does not show that dilation or valvular rupture do not occur.

The fact that the heart is placed under a considerable strain under the conditions of the present experiment is borne out by the fact that damage of the aortic valve was found to have occurred in approximately 1/3 of the experimental animals. In this group the average hemoglobin percentage (20.6) is well above the average for the entire experimental group (17.4), but the average red cell count (8,300,000) was very near the average of the experimental group (8,040,000). It is possible that the elevated hemoglobin found in the animals with valvular insufficiency represents an attempt on the part of the organism to nullify the cardiac defect.

As the lethal effects of acute anoxia seem to be due to failure of the circulatory system at one point or another, it will be discussed here.

The observation that the major injuries evident in the "acclimatized" animals after exposure to acute anoxia were hemorrhage in the lungs and brain, leads to the following alternative hypotheses.

A. Previous exposure to mild anoxia weekens the arterial vessels to such an extent that the increase in blood pressure (resulting from an attempt on the part of the organism to meet the demands of acute anoxia) imposes on these vessels a strain which they are unable to bear.

B. The "acclimatized" animals respond to acute anoxia with a higher arterial pressure than do the control animals.

The high incidence of pulmonary lesions in discontinuously exposed animals not exposed to acute anoxia (discussed below, (p. 26) indicates that the former hypothesis is the more likely

No explanation is offered of the ventricular spasm encountered so universally in the case of the control animals when unable to survive acute anoxia, and never encountered in the presumably acclimatized or "deacclimatized" animals. Campbell (1927) states that in all animals affected by low O_2 tensions, heart failure is the most constant phenomenon.

It is evident that adaptation to mild anoxia enables the organism to resist exposure to acute anoxia at least as well as can the normal animal, but when any advantages this adaptation may have imparted have "passed off," (Campbell, 1927), during a stay at low altitude, the organism is less well fitted to resist extremely high altitude than is the normal animal. This phenomenon may conceivably be due to deleterious changes in the circulatory system incurred during the original adjustment to low pressure.

The lack of increase in spleen weight in the acclimatized animals is probably correlated with the lack of increase of circulating erythrocytes, as reported above. Studies made at 18,000 feet, 8 hours per day for 3 months by Van Liere and Stickney (1943) have shown an increase in spleen weight of dogs along with an increase of red cell count.

6. Gastric Ulcers. The high incidence of stomach ulcers in the exposed group is presumably due to anoxia of the stomach mucosa, making it less resistant to the action of the digestive enzymes, coincident with increased stimulation of secretion by the vagus nerve. If this is the case, the HCl content of the stomach should increase during anoxia. Van Liere and Vaughan (1941) have shown with Pavlov pouch dogs

that in these animals no change in the basal secretion of the stomach is produced by exposure to 28,000 feet altitude.

7. Pulmonary Lesions. No reference to disturbances of the respiratory tract as a result of chronic exposure to anoxia has been found in the literature. The results found in the present study indicate that some phase of the effects of exposure to low barometric pressure--possibly hyperventilation, pulmonary occlusion, increased arterial pressure, or a combination of these factors and others--have a deleterious effect on the lung tissues. The two types of lesions found were purulent cysts and small hemorrhagic spots.

To interpret the findings discussed above with reference to the phases of the "alarm reaction," it is necessary to reconsider them together.

The phase of acute alarm is engendered in the organism exposed to low barometric pressure, probably during the first few exposures. The beginnings of the adaptative response to anoxia (i.e., adrenal hypertrophy, hemoconcentration, etc.) can be demonstrated after a single exposure (Sundstroem, 1942) (Thorn, 1942).

The adaptation phase is commonly referred to as "acclimatization" when it results from exposure to low pressure. However, if acclimatization is taken to mean that its result is an organism more fit to survive under anoxic conditions than is the normal animal, it is seriously doubted by the author that this occurs to any practical degree in discontinuous exposure to anoxia. Certainly it does not occur in the present study as is evidenced by the exposures to acute anoxia cited above. Any beneficial adaptative response which had occurred in the exposed rats was only sufficient to counteract the deleterious changes in the circulatory system. In fact, if the animals were allowed to lose the beneficial adaptative changes by a week's stay at ground level, the deleterious effects remaining were sufficient to cause an appreciable decrease in the altitude "ceiling" of the test animals as compared with the normal controls. Thus, it seems probable that no practical benefit to the organism can be expected from acclimatization by discontinuous exposure to even a mild degree of anoxia.

The appearance of regression of adaptation to discontinuous anoxia had not been reported in the literature to the author's knowledge, even in prolonged experiments (12 weeks) using a test altitude of 25,000 feet (Dalton, 1944). However, such effects have been described in studies of continuous exposure to anoxia (Sundstroem, 1942), so it is possible that still longer investigation of repeated exposures might produce these results. If the last phase above were shown to occur in discontinuous exposure, the comparison of anoxia with the other stimuli known to elicit the alarm reaction would be even more complete.

SUMMARY

1. Exposure of 51 albino rats to discontinuous anoxia by decreased barometric pressure equivalent to 18,000 feet for 1 hour per day for 9 weeks produced no obvious changes in appearance, weight, or behaviour as compared with 51 carefully paired control animals.

2. Statistical analysis of changes in body organs, supplemented with histological observations, of exposed animals compared with controls revealed the following.

A. A significant increase of 20.7% in mean blood volume.

B. No increase in mean erythrocyte count.

C. A significant increase of 14.5% in mean hemoglobin content (g%) of the blood.

D. No change in mean weight or histological appearance of the spleen.

E. A significant increase of 9.5% in mean thyroid weight, associated with increased "colloid" content of the follicles.

F. Increased activity, by histological evidence, of the adrenal cortex, not associated with increase in mean weight of the total adrenal gland.

G. Decreased activity, by histological evidence, of the testes, associated with a probably significant decrease in mean testis weight of 3.8%.

H. No change in mean weight or appearance of the seminal vesicle.

I. No change in mean weight or appearance of the thymus.

J. A significant decrease of 16.5% in the mean weight of the pituitary gland.

K. A significant increase of 91.7% in the incidence of aortic valve insufficiency.

L. A significant increase of 53.3% in the incidence of gastric ulcer.

M. A significant increase of 81.3% in the incidence of lesions (of various types) of the lungs.

3. The physiological significance of these changes is discussed.

4. The exposed animals were found to have the same resistance to acute anoxia (50,000 feet equiv.) as the controls on the day following the last regular exposure. However, after resting at ground level for 1 week, the previously exposed animals showed a 62.5% decrease in survival when exposed to acute anoxia (40,000 feet equiv.) as compared with the controls.

5. Deleterious changes in the circulatory system, such as weakening of the heart valves and arterial walls caused by discontinuous exposure to anoxia is suggested as an explanation of this decreased resistance to acute anoxia of the exposed animals after loss of "acclimatization."

LITERATURE CITED*

Barach, A. L., M. Eckman, and N. Molumut. Modification of resistance to anoxia, with special reference to high altitude flying. Am. J. Med. Sci., 1941, 202:336-341.

Bert, P. La pression barometreque; recherches de physiologie experimentale. Paris, G. Masson, 1878.

Campbell, J. A. Further observations on oxygen acclimatisation. J. Physiol., 1927, 63:325-342.

Campbell, J. A. Tissue oxygen tension and haemoglobin. J. Physiol., 1928, 65:255-272.

Dalton, A. J., E. R. Mitchell, B. F. Jones, and V. B. Peters. Changes in the adrenal glands of rats following exposure to lowered oxygen tension. J. Nat'l Cancer Inst., 1944, 4:527-536.

Dernehl, C. V., J. K. Cline, H. G. Swann, and W. D. Collings. The effect of excess panththothenic acid, para-amino benzoic acid and inositol on adaptation of rats to lowered atmospheric pressures. Texas Reports on Biol. and Med., 1943, 1:143-152.

Dohan, F. C. Effect of low atmospheric pressure on the adrenals, thymus, and testes of rats. Proc. Soc. Exp. Biol. and Med., 1942, 49:404-408.

Evans, G. The adrenal cortex and endogenous carbohydrate formation. Amer. J. Physiol., 1936, 114:297-308.

Fisher, R. A. Statistical Methods for Research Workers. Edinburgh and London, Oliver and Boyd, 1938.

Flexner, L. B. and A. Grollman. The reduction of osmic acid as an indication of adrenal cortical activity in the rat. Anat. Rec., 1939, 75:207-216.

Gordon, A. S., F. J. Tornetta, and H. A. Charipper. Effect of low atmospheric pressure on the reproductive system of the male rat. Proc. Soc. Exp. Biol., 1943, 53:6-7.

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*For a complete coverage of the literature of aviation medicine through 1942, the reader is referred to A Bibliography of Aviation Medicine, by E. C. Hoff and J. F. Fulton. Springfield and Baltimore, Charles C. Thomas, 1942.

Gordon, A. S., F. J. Tornetta, S. A. D'Angelo, and H. A. Charipper. Effects of low atmospheric pressures on the activity of the thyroid, reproductive system, and anterior lobe of the pituitary in the rat. Endocrinology, 1943, 33:6, 366-383.

Hailman, H. F. The effect of vitamins of the B complex on the resistance of the organism to anoxia. Amer. J. Physiol., 1944, 141:176-185.

Hughes, A. M. Resistance to the anoxia of high altitude afforded by thiouracil. Fed. Proc. Amer. Soc. Exp. Biol., 1944, 3:219.

Hughes, A. M. and E. B. Astwood. Thyroid function and resistance to anoxia in the rat. J. Av. Med., 1944, 15:152-157.

Hurtado, Alberto. Studies at high altitude - blood observations on the Indian natives of the Peruvian Andes. Amer. J. Physiol., 1932, 100:487-505.

Ingle, D. J. The time for the occurrence of cortico-adrenal hypertrophy in rats during continuous work. Amer. J. Physiol., 1938, 124:627-630.

Langley, L. L. and R. W. Clarke. The reaction of the adrenal cortex to low atmospheric pressure. Yale J. Biol. Med., 1942, 14:529-546.

Langley, L. L. An historical introduction to the physiology of anoxia. Bull. Hist. Med., 1943, 14:321-340.

Nelson, Dorothy, and M. W. Burrill. Repeated exposure to simulated high altitude: weights of adrenals, testes and seminal vesicles in the white rat. Fed. Proc. Amer. Soc. Exp. Biol., 1944, 3:34.

Selye, H. Studies on adaptation. Endocrinology, 1937, 21:169-188.

Stickney, J. C. and E. J. Van Liere. Erythrocyte and hemoglobin values in acclimatization produced by discontinuous anoxia. J. Aviat. Med., 1942, 13:170-176.

Streuli, H. Beitrage zur Physiologie der Drusen. Biochem. Z., 1918, 87:359-417.

Sundstroem, E. S. and G. Michaels. The adrenal cortex in adaptation to altitude, climate and cancer. Berkeley and Los Angeles, University of California Press, 1942.

Thorn, G. W., B. F. Jones, R. A. Lewis, E. R. Mitchell, and G. F. Koepf. The role of the adrenal cortex in anoxia; the effect of repeated daily exposure to reduced oxygen pressure. Amer. J. Physiol., 1942, 137:606-619.

Van Liere, E. J. Effect of anoxic anoxia on the heart. J. Aviat. Med., 1941,12:131-135.

Van Liere, E. J. and P. E. Vaughan. Basal secretion of Pavlov pouch dogs as influenced by oxygen want. Amer. J. Digest Dis., 1941, 8:155-156.

Van Liere, E. J. and J. C. Stickney, Acclimatisation during discontinuous exposure to anoxia. Fed. Proc. Amer. Soc. Exp. Biol., 1942, 1:87.

Van Liere, E. J. and J. C. Stickney. The organ weight-body weight ratio in dogs following exposures to discontinuous anoxia. J. Aviat. Med., 1943, 14:194-199.

APPENDIX

	Thyroid Weight		Adrenal Weight		Testis Weight		Seminal Vesicle Weight	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
1	0.0188	0.0280	0.0367	0.0351	4.0594	3.5497	1.5654	1.5595
2	0.0189	0.0209	0.0303	0.0363	3.0249	3.1673	1.3101	1.2165
3	0.0261	0.0231	0.0463	0.0328	3.3565	2.5050	1.3479	1.1639
4	0.0268	0.0242	0.0278	0.0240	3.2466	2.9568	1.1733	1.1021
5	0.0237	0.0282	0.0389	0.0295	3.3722	3.1670	1.3167	1.5353
6	0.0223	------	0.0306	0.0321	3.6923	3.7085	1.5243	1.7828
7	0.0192	0.0302	0.0346	0.0287	3.1919	3.5456	1.2823	1.4801
8	0.0241	0.0213	0.0365	0.0378	3.6392	3.6120	1.4505	1.1356
9	0.0260	0.0264	0.0336	0.0336	3.6801	3.2765	0.9198	1.0700
10	0.0234	0.0237	0.0319	0.0319	3.3868	3.3489	1.2066	------
11	0.0208	0.0259	0.0364	0.0330	3.1103	3.5777	1.6345	1.5169
12	0.0254	0.0215	0.0417	0.0335	3.5874	2.9414	1.5351	1.4367
13	0.0338	0.0286	0.0395	0.0427	3.6152	3.4045	1.4622	1.2038
14	0.0343	0.0293	0.0425	0.0362	3.8083	3.7503	1.5959	1.2627
15	0.0215	0.0302	0.0316	0.0610	3.4663	3.1502	1.4411	1.4844
16	0.0233	0.0255	0.0339	0.0396	3.4688	2.8718	1.7008	1.7047
17	0.0161	0.0255	0.0387	0.0407	3.5162	4.2230	------	1.2240
18	0.0301	0.0232	0.0427	0.0398	3.8677	3.5830	1.5689	1.6646
19	0.0258	0.0298	0.0366	0.0350	3.3804	3.5634	------	1.6963
20	0.0233	0.0250	0.0374	0.0464	3.6746	3.7760	1.7220	1.6413
21	0.0191	0.0234	0.0532	0.0246	3.0779	3.8767	1.2674	1.1643
22	0.0198	0.0210	0.0381	0.0314	2.9413	2.9627	1.0167	1.1345
23	0.0240	0.0219	0.0298	0.0323	3.0246	2.7816	1.5134	1.2432
24	0.0257	0.0211	0.0281	0.0203	3.1403	3.0768	1.1920	1.2045
25	0.0194	0.0231	0.0358	0.0271	3.2846	2.7201	------	1.5785
26	0.0214	0.0214	0.0286	0.0296	3.3373	2.7748	0.9926	------
27	0.0186	0.0288	0.0254	0.0274	3.2346	3.2045	1.3948	1.3092
28	0.0214	0.0285	0.0340	0.0347	------	3.1889	1.4754	1.3519
29	0.0161	0.0280	0.0194	0.0329	------	3.2903	1.2222	1.1774
30	------	------	0.0320	0.0436	3.3826	3.6364	------	------
31	------	------	0.0330	0.0328	3.3840	3.0160	------	------
32	------	------	0.0310	0.0349	------	4.1138	------	------
33	------	------	0.0315	0.0375	3.7040	3.4282	------	------
34	------	------	0.0344	0.0284	3.3920	2.6966	------	------
35	------	------	0.0418	0.0346	4.0461	3.4058	------	------

*Red cell count expressed in million cells per cubic millimeter.

Miscellaneous

Controls

12 Renal calculus in urinary bladder.
17 Infected toe.
21 Dilated aorta.
24 Cyst in liver.

Autopsy Data

Thymus Weight		Spleen Weight		Pituitary Weight		Blood Volume		Red Cell Count* / Hemoglobin g%/?	
Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp	Cont.	Exp.
0.3881	0.2979	0.6314	0.5457	------	------	5.0	7.5	6.64	6.53
0.2286	0.3442	0.4905	0.5371	0.0101	0.0092	6.5	9.0	7.25	7.96
0.3968	0.2620	0.5303	0.5044	------	------	7.0	8.0	7.76	----
0.2740	0.3865	0.5133	0.4990	0.0069	0.0072	6.0	8.5	8.37	7.79
0.2473	0.2395	0.6013	0.6113	------	------	8.0	10.0	----	----
0.3292	0.3545	------	0.5875	------	------	7.5	10.0	7.10	8.30
0.2877	0.2266	0.6167	0.5241	------	------	7.0	7.0	8.31	8.15
0.3813	0.2063	0.6672	0.7892	------	------	10.0	10.0	8.24	5.56
0.2873	0.2522	0.5229	0.6030	------	------	6.3	----	7.73	----
0.2845	0.3601	0.6302	0.6313	0.0098	0.0065	8.5	9.5	7.74	8.55
0.3328	0.3130	0.5593	0.6257	0.0106	0.0105	8.3	8.5	7.25	8.13
0.3480	0.2319	0.7665	0.5725	0.0098	0.0081	7.5	9.0	8.61	9.90
0.2931	0.5161	0.6616	0.6026	0.0097	0.0071	10.0	7.0	8.75	7.88
0.3217	0.3747	0.6861	0.6653	------	------	8.3	10.0	8.41	9.01
0.2775	0.3650	0.6209	0.7414	0.0077	0.0086	8.0	8.5	7.72	7.52
0.2695	0.2849	0.6249	0.6577	0.0085	0.0083	8.0	9.5	8.28	8.67
0.3804	------	0.6080	0.9105	------	------	8.5	8.0	----	----
0.3706	0.4112	0.7695	0.6778	------	------	9.5	10.5	----	----
0.3976	0.4307	0.6178	0.6174	0.0092	0.0094	9.5	10.0	8.30	7.99
0.3162	0.3396	0.5629	0.6821	0.0099	------	6.5	11.5	9.94	8.61
0.3217	0.3394	0.4774	0.5118	------	------	5.5	6.5	8.2	10.0
0.5002	0.3498	0.6592	0.5210	------	------	5.0	6.5	9.2	11.4
0.3084	0.3637	0.6290	0.4311	------	------	5.3	7.8	7.6	7.8
0.2876	0.3868	0.5433	0.5578	------	------	4.0	6.0	8.2	6.2
0.5444	0.2958	0.4559	0.4325	------	------	5.5	6.3	8.2	6.4
------	------	------	------	0.0079	0.0052	6.0	5.0	7.6	11.4
------	------	0.4874	------	------	0.0039	7.3	7.3	9.0	11.9
------	------	0.6139	0.4743	------	------	7.8	7.8	8.6	6.0
------	------	0.3821	0.5529	------	------	7.0	8.5	6.2	7.2
------	------	------	------	------	------	5.5	8.8	9.0	9.3
------	------	------	------	------	------	5.5	8.0	7.5	9.8
------	------	------	------	------	------	5.5	8.0	7.9	8.3
------	------	------	------	------	------	4.5	9.5	6.5	8.6
------	------	------	------	------	------	8.0	----	5.1	6.0
------	------	------	------	------	------	4.5	----	4.5	8.5

Pathology

Experimentals

9 Strangulated right spermatic cord. Fatty heart.
20 Tumor of left iliac lymph node.
27 Liver cyst.
33 Congested kidneys and edema of lumbar region.

Table IV

	Before Exp		1st Week		2nd Week		3rd Week		4th Week	
	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp
1	184	180	188	204	205	216	217	241	225	252
2	194	196	216	208	231	225	242	245	255	253
3	183	189	199	199	213	212	225	231	230	237
4	196	199	206	209	218	219	221	235	228	241
5	198	194	208	210	218	224	230	238	243	245
6	185	191	208	205	221	221	231	238	246	240
7	194	197	211	214	227	235	237	249	250	259
8	197	206	206	215	228	237	240	252	253	259
9	208	218	220	221	240	222	244	255	256	262
10	190	198	208	206	223	233	231	239	244	247
11	203	208	226	222	241	235	254	247	259	254
12	217	210	233	219	248	238	258	251	272	262
13	218	206	229	215	250	228	257	235	272	244
14	206	207	222	218	257	241	246	254	253	266
15	216	212	237	224	257	240	259	260	274	273
16	216	216	230	226	254	242	274	259	288	264
17	217	218	232	236	250	250	257	262	267	267
18	223	222	237	232	254	243	257	262	274	267
19	237	225	252	237	278	247	286	255	296	264
20	221	222	242	237	255	254	257	275	271	284
21	225	225	239	241	258	258	265	267	274	275
22	216	232	250	247	242	256	249	273	259	285
23	231	235	254	241	268	254	285	268	294	270
24	241	234	259	244	279	260	289	275	306	269
25	234	242	252	255	263	272	278	293	288	294
26	244	252	263	262	276	280	282	298	293	299
27	177	177	192	184	202	190	215	221	232	236
28	191	191	206	199	215	218	233	234	254	252
29	182	182	198	187	209	219	233	236	241	256
30	180	180	198	198	213	216	220	230	242	242
31	181	181	193	189	205	214	226	234	239	251
32	185	185	195	194	206	220	211	238	237	257
33	185	185	202	199	217	220	236	232	266	249
34	189	189	203	208	213	222	224	242	243	261
35	191	191	205	202	214	226	218	244	238	259
36	196	196	207	210	215	226	230	246	252	256
37	200	200	215	213	233	231	253	250	274	266
38	203	203	210	215	219	229	232	247	249	256
39	206	206	220	215	232	237	243	250	268	267
40	193	191	208	201	221	215	225	235	245	254
41	203	203	211	212	218	230	237	245	255	261
42	206	206	217	213	225	243	235	258	257	267
43	211	211	224	226	242	243	250	270	260	284
44	219	219	228	222	240	238	247	265	286	267
45	220	220	241	232	254	252	271	270	296	284
46	213	212	225	224	242	240	255	261	268	273
47	217	217	227	226	242	249	256	266	274	275
48	221	221	228	229	241	245	248	259	269	267
49	218	218	222	225	230	245	252	264	266	276
50	227	227	245	232	254	253	277	272	299	285
51	237	236	248	242	256	265	273	284	289	298
AV	207	208	221	218	235	236	246	253	262	265

Body Weights

5th Week		6th Week		7th Week		8th Week		9th Week	
Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp
256	257	257	257	248	279	254	280	265	287
273	267	270	268	285	293	291	293	308	300
238	249	243	244	246	258	249	262	270	265
242	253	245	265	258	265	255	286	276	300
254	256	254	256	276	270	275	272	298	280
255	256	260	263	275	286	277	285	289	294
262	268	261	282	275	299	277	304	295	309
268	270	269	284	277	300	282	304	310	310
270	274	275	289	285	299	290	300	309	305
260	257	261	266	274	283	272	284	292	294
277	262	283	272	287	292	305	293	326	300
290	262	291	272	306	292	309	293	332	300
279	253	288	269	302	286	306	286	329	285
263	276	260	276	282	299	305	310	300	318
294	284	293	294	305	315	313	330	331	324
308	273	312	279	322	301	332	301	360	305
286	287	289	298	298	324	277	322	320	338
286	281	292	286	305	304	309	304	330	314
309	279	315	285	326	311	331	307	349	317
285	295	292	303	299	322	304	328	325	355
284	284	289	288	302	307	301	305	326	317
280	291	286	294	293	316	299	324	325	331
309	284	310	289	328	314	336	319	354	331
327	285	331	293	339	313	343	318	365	328
301	306	302	318	319	339	320	342	350	345
306	315	308	324	322	350	318	355	340	364
240	249	255	255	264	280	282	269	278	286
257	259	273	273	275	273	289	292	278	298
249	269	270	287	270	283	283	303	289	297
246	258	269	253	270	271	269	284	285	276
242	264	267	272	267	280	280	296	281	290
232	268	254	277	260	287	280	304	279	272
263	258	269	266	293	274	312	295	306	285
247	271	266	277	270	276	265	294	279	291
239	270	248	276	256	262	270	297	267	292
253	264	272	276	285	271	305	292	298	285
276	276	304	285	311	288	330	306	329	297
252	266	274	277	274	284	294	299	280	296
270	275	286	291	294	286	314	300	315	295
248	263	262	278	277	277	301	292	287	288
256	272	265	279	286	284	305	296	302	289
256	276	279	285	286	288	310	303	304	298
269	301	292	306	294	314	316	337	309	326
271	277	263	283	287	282	298	297	293	288
294	295	317	308	327	307	325	313	345	312
273	286	293	296	297	296	312	315	306	311
283	286	294	293	307	293	330	314	325	316
277	277	294	285	296	263	313	297	312	294
263	296	284	299	288	309	307	322	305	321
296	296	323	312	330	310	330	313	349	330
292	309	309	317	312	313	330	334	327	332
270	275	282	283	290	294	300	303	310	305