SEX ORGAN CHANGES AND BREEDING PERFORMANCE OF MALE RATS EXPOSED TO ALTITUDE: EFFECT OF EXERCISE AND PHYSICAL TRAINING

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Summary. Exposure of male rats at rest to 21,500 ft simulated altitude 4 hr daily for 4 weeks induced testicular changes such as small to large vacuolated areas, pale fibrillar acellular areas and prematurely sloughed germinal cells in the seminiferous tubules. These changes, noted first following three exposures, increased in incidence for the next 25 days. Within 1 week, degenerate germinal cells were scattered among normal spermatozoa in the caput and caudal portions of the epididymis and after 4 weeks as many as one-fourth of the cells were abnormal. Similar changes were observed in the rats exercised at altitude. The weights of the seminal vesicles of rats at rest were not significantly reduced by altitude exposure, but exercise at altitude produced a 50% reduction within 1 week. There were no significant weight or cellular changes in the testes or seminal vesicles in rats exercised at ground level. Exercise training for 3 weeks at ground level before exercise at altitude did not ameliorate the testicular changes, but significantly reduced the loss in weight of the seminal vesicles. Successful mating ensued in 2 to 4 weeks in 90% of the resting rats exposed to altitude. The untrained rats exercised at altitude failed to mate, but 3 weeks of ground level physical training before exercise at altitude enabled 38% of the rats to mate successfully.

INTRODUCTION

Exposure of unacclimatized man to altitudes above 13,000 ft and rats to altitudes of 25,000 ft is known to produce severe structural and functional changes in the reproductive system (Van Liere & Stickney, 1963). Early attempts to establish the mechanism responsible for such effects have been unsuccessful. For example, administration of gonadotrophic hormones, vitamin E, short and long term altitude acclimatization have been ineffective

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in ameliorating the testicular changes induced in rats exposed repeatedly to altitudes of 25,000 to 30,000 ft (Gordon, Tornetta, D'Angelo & Charipper, 1943; Saha, 1954; Dalton, Jones, Peters & Mitchell, 1945; Altland, 1949a). The severity of the testicular changes produced by such marked hypoxia may have overwhelmed the effects of the agents under study. For this reason we have studied the effects of 4-hr daily exposures to less severe hypoxia (21,500 ft barometer pressure 328 mm Hg, pO_2 67.3 mm Hg, O_2 equivalent 8.9%) on the male reproductive system. At this altitude it is possible to produce mild testicular changes and better to evaluate factors which influence these changes. We have determined the effects of exercise on the incidence and severity of the reproductive changes, including breeding performance, and we have determined the effects of ground level physical training on these parameters. An understanding of basic mechanisms involved in the effect of physical training on physiological processes at altitude may be timely in view of the impending Olympics at Mexico City in 1968 (Carlile & Carlile, 1966).

METHODS

Three-month-old male Sprague-Dawley rats were exposed 4 hr daily to 21,500 ft simulated altitude in a well-ventilated cylindrical decompression chamber 9×20 ft maintained at 23 to 25° C. Exercise was provided by a rotating drum with thirty compartments, each holding one rat, as previously described (Altland & Highman, 1961). The drum was rotated 4.5 times/min forcing the rats to cover 6.9 m/min. During exercise training at ground level the rats were exercised for 6 hr with a 5-min rest period at 30-min intervals. At altitude the rats were exercised for 4 hr. Groups of rats were studied after 1, 3, 7, 14 and 28 days.

Breeding experiments were conducted by placing fertile females with experimental males during the intervals between exercise and altitude treatment. Each rat was housed, commencing on Day 3 of the experiment, with two fertile females which were replaced on Day 17 by two other fertile females. The exposures were terminated after 4 weeks. Four groups of rats were used: group I was untrained and not exercised during altitude exposure; group II was untrained and exercised at altitude; group III was trained for 3 weeks before exercising at altitude; and group IV was untrained and maintained at ground level.

The rats were killed immediately after the experimental treatment by ether anaesthesia and they were bled out by cutting the aorta. The testes, epididymides, seminal vesicles (coagulating gland removed) and adrenal glands were dissected free of fat and weighed on a Roller-Smith torsion balance. The testes and epididymides were fixed in Bouin's fluid. Routine paraffin sections were stained with haematoxylin and eosin or the PAS method (Lillie, 1965).

Student's 't'-test was used for statistical analyses.

RESULTS

The mean body weight of unexercised rats exposed 4 hr daily to 21,500 ft for 4 weeks was not significantly reduced (Table 1). The mean body weights of

untrained rats exercised at altitude from 1 to 4 weeks were significantly reduced ($P < 0.01$) 15 to 20% below pre-exposure values, whereas the mean body weights of pre-trained rats exercised at altitude were not significantly reduced. During 3 weeks of exercise training at ground level the rats lost an average of 5% of their body weight.

TABLE 1

EFFECTS OF TRAINING ON MEAN BODY, ORGAN WEIGHTS AND HAEMATOCRIT OF RATS EXERCISED 4 HR/DAY AT 21,500 FT SIMULATED ALTITUDE

Condition	No. rats	Body weight		Organ weight			
		Before (g)	After (g)	Testis ^a wt (mg)	Seminal vesicle (mg)	Adrenal (mg)	Haematocrit (%)
Controls	10	363 ^b ± 10	366 ± 13	1741 ± 24	1184 ± 93	23.5 ± 0.5	46.2 ± 0.3
Exercise at ground level ^c 3 weeks	7	349 ± 12	330 ± 8	1761 ± 41	1079 ± 114	30.9* ± 2.2	49 ± 1
Exposure to 21,500 ft 4 weeks	13	367 ± 11	352 ± 9	1570* ± 36	920 ± 60	29.5* ± 0.7	71.5* ± 0.8
Exercise at 21,500 ft 1 week							
Untrained	7	375 ± 12	315 ± 10	1517* ± 39	560* ± 68	30.6* ± 2	37* ± 2
Trained ^c	9	334 ± 5	324 ± 10	1594** ± 50	954 ± 59	32.3* ± 1.7	51* ± 1
2 weeks							
Untrained	12	345 ± 10	276 ± 11	1418* ± 30	565* ± 46	33.0* ± 0.7	47.4 ± 1.9
Trained	11	307 ± 5	315 ± 5	1455* ± 51	979 ± 65	35.4* ± 3	52.7* ± 1.7
4 weeks							
Untrained	6	383 ± 13	327 ± 9	1292** ± 83	578* ± 103	42* ± 2.9	57.5* ± 3
Trained	5	340 ± 21	360 ± 20	1551** ± 54	888** ± 54	33.1* ± 1.1	64* ± 0.8

^a Each testis value represents average of left and right testis weight.

^b Values represent Mean ± S.E.

^c Training involves exercise 6 hr/day for 3 weeks at ground level.

* Significantly different from controls $P < 0.01$ (t test).

** Significantly different from controls $P < 0.05$ (t test).

Exposure to 21,500 ft 4 hr daily produced no significant testicular weight changes during the first 2 weeks. After 4 weeks, however, the mean testicular weight was reduced (Table 1), and the calculated mean testis : body weight ratio was also reduced. The weights of the testis of rats exercised at ground level were unaltered. The mean testes weights of both trained and untrained rats exercised at altitude were significantly reduced ($P < 0.01$), but due to a

loss of weight after exercise the testis : body weight ratios were unchanged, except for a reduction at 4 weeks.

No significant changes were found in seminal vesicle weights in four groups of rats (5 to 13/group) after 3, 7, 14 and 28 days' exposure to 21,500 ft. Likewise, there were no changes in seminal vesicle weights in rats exercised for 21 days at ground level. The mean weights of the seminal vesicles and the mean seminal vesicle : body weight ratios of all untrained rats exercised at altitude, however, were significantly reduced (Table 1). Exercise training, before daily exercise at altitude, prevented this loss in the weight of the seminal vesicles during at least the first 14 days. After 28 days there was a slight reduction, but it was less than in untrained rats ($P < 0.05$).

TABLE 2
INCIDENCE OF TESTIS PATHOLOGY IN RATS EXPOSED TO 21,500 FT OR EXERCISED 4 HR DAILY AT 21,500 FT OR AT GROUND LEVEL

No. of days exposed	No. of rats	Seminiferous tubules (cross-section)				Epididymis	
		Vacuolation		Severe cell loss		No. of rats with many immature sex cells	
		No. of rats	Average No. tubules	No. of rats	Average No. tubules	Caput	Cauda
<i>Exercise at ground level*</i>							
14	5	1	$1 \pm 0.8 \ddagger$	1	1 ± 0.8	0	0
28	4	3	1 ± 0.5	1	1 ± 0.2	0	0
<i>Exposure to 21,500 ft*</i>							
3	5	3	1 ± 0.8	0	0	3	0
7	6	5	2 ± 0.5	1	1 ± 0.2	4	1
14	13	13	8 ± 2.4	1	1 ± 0.1	10	6
28	13	13	16 ± 2.8	10	6 ± 2.5	10	8
<i>Exercise at 21,500 ft</i>							
3 Untr.*	9	5	2 ± 0.3	0	0	5	1
3 Tr.†	9	5	2 ± 0.3	1	1 ± 0.1	5	1
7 Untr.	3	3	8 ± 3.7	1	1 ± 0.3	3	1
7 Tr.	3	3	4 ± 1.0	1	1 ± 0.3	3	1
14 Untr.	8	8	17 ± 3.7	5	1 ± 0.2	4	4
14 Tr.	5	5	15 ± 5.6	2	1 ± 1.0	5	5
28 Untr.	6	6	13 ± 2.0	5	5 ± 3.0	5	5
28 Tr.	5	5	23 ± 7.0	4	4 ± 3.2	3	4

* Untr. = Untrained rats.

† Tr. = Rats trained 6 hr daily in exerciser for 21 days.

‡ Mean $\pm$ S.E.

Two early changes in the testes due to altitude hypoxia were identified. First, small to large acellular spaces (vacuoles) were found in various portions of the seminiferous tubules (Pl. 1, Figs. 1 and 2), chiefly along the basement membrane, often displacing germinal cells toward the lumen. Occasionally, however, the vacuoles were centrally located and caused no apparent displacement of cells (Pl. 1, Fig. 2). The vacuoles were found as early as 3 days in some rats and with continued exposure their incidence increased until all rats showed them by 14 or more days (Table 2). Usually only one vacuole was found in each tubule cross-section. Degenerate spermatogonia and spermatocytes

PLATE 1

Changes in the rat testis and epididymis produced by 4-hr daily exposures to 21,500 ft.

FIG. 1. Portion of seminiferous tubule of rat exposed 3 days. Small vacuolated area present at basement membrane. Spermatids and spermatocytes displaced. $\times 290$.

FIG. 2. Portion of seminiferous tubule of rat exposed 7 days. Large vacuolated area not adjacent to basement membrane. Displacement of germinal cells not evident. $\times 290$.

FIG. 3. Portion of seminiferous tubule of rat exposed 28 days. Large pale fibrillar acellular area. Sloughing of germinal cells into lumen. $\times 290$.

FIG. 4. Abnormal germinal cells and apparently normal spermatozoa in the caput epididymidis of a rat exposed 28 days. Note the condensed, hyperchromatic, spherical masses in the spermatids. A few binucleate cells are shown. $\times 390$.

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cytes usually found after testicular artery occlusion (Oettlé & Harrison, 1952) were not found in the seminal vesicles in these rats. Secondly, large pale fibrillar acellular areas tapering towards the lumen were found in the seminiferous tubules. This lesion is attributed to sloughing of spermatocytes and spermatids into the lumen (Pl. 1, Fig. 3) and was found occasionally during the first 2 weeks of altitude exposure, but much more frequently after 4 weeks (Table 2). The testicular changes found after 4 hr daily exercise for 14 to 28 days at ground level were minor (Table 2).

A small number of spermatids with nuclei condensed into hyperchromatic, spherical masses was scattered among the spermatozoa in the caput epididymidis of all rats after as few as three exposures to 21,500 ft. The abnormal cells increased in number after 1 week and occupied as much as one-fourth of the lumen in some portions of the caput epididymidis within 4 weeks (Pl. 1, Fig. 4). In the caudal epididymis abnormal germinal cells first appeared at 1 week and subsequently increased in number in some rats (Table 2).

TABLE 3
FERTILITY OF MALE RATS EXPOSED 4 HR DAILY FOR 4 WEEKS TO
21,500 FT SIMULATED ALTITUDE

	<i>No. rats</i>	<i>Day of mating*</i>
Control	16	1, 2, 2, 3, 3, 4, 5, 6, 6, 6, 7, 9, 10, 14
Altitude exposure		
Untrained, no exercise	10	15, 15, 16, 17, 26, 27, 28, 28, 28
Untrained, exercise	13	None mated
Trained, exercise	13	13, 14, 14, 16, 18

* Mating day was determined by allowing 21 days for gestation.

When untrained rats were exercised at altitude, no testicular changes were found after 1 day and only minor changes after 3 days (Table 2). The incidence and extent of altitude-induced lesions in the testes were not significantly enhanced by exercise or influenced by exercise training before the exercise altitude exposures (Table 2).

No differences in the number and histological appearance of interstitial cells were found between rats exposed to altitude only and trained or untrained rats exercised at altitude.

The results of the fertility studies are shown in Table 3. All but two unexposed control male rats mated successfully (determined by timing litters) during the first 2 weeks with fertile females. No untrained, unexercised rats mated successfully during the first 2 weeks of exposure, but 90% were fertile during the last 2 weeks. Exercise combined with altitude exposure prevented successful mating of untrained rats during the entire 4 weeks of exposure. Ground level exercise training of rats before exercise at altitude enabled 38% of the rats to mate during the 4 weeks of exposure.

The changes in mean haematocrit values are shown in Table 1. The untrained rats exercised at altitude were slightly anaemic within 1 week, but recovered by 2 weeks. The haematocrit values of exercised rats exposed to altitude never reached the peak level found in unexercised rats exposed to

altitude. They were significantly higher in trained than in untrained rats at 1 and 2 weeks ($P < 0.05$), but at 4 weeks there was no significant difference.

There was a significant increase in mean adrenal weights in all groups of rats exercised and exposed to altitude (Table 1). The elevations in adrenal weights of untrained and trained rats exercised at altitude were similar, except for a greater increase in untrained rats at 4 weeks.

DISCUSSION

Pathological changes were produced in the testes of untrained, unexercised rats by daily 4-hr exposures to 21,500 ft for 4 weeks. In addition, abnormal germinal cells appeared in the lumen of the epididymis. These changes were not severe enough to affect reproduction adversely since an entire spermatogenic cycle in the rat may require as long as 48 days (Roosen-Runge, 1962), and most of the spermatozoa involved in mating were under the influence of hypoxia for only a relatively short time during development. Also, from studies on preservation of spermatozoa of domestic animals, the viability of spermatozoa under hypoxic conditions is known to be high. It is apparent that when abnormal germ cells occupied as much as one-fourth of the caput epididymidis conception was not impaired. It is not known how many abnormal cells were released during an ejaculation at 4 weeks, but their presence in the caudal epididymis indicated that some could have been released earlier.

The failure of any of the untrained rats to mate successfully during the periods between exercise at altitude may be due in some degree to their low seminal vesicle weights, since subnormal secondary sexual organs are generally regarded as detrimental. Recently, however, it has been shown that there was no significant change in the total capacity for sexual activity in rats after the removal of the seminal vesicles (Beach & Wilson, 1963).

Exercise reduces the altitude tolerance of male rats by about 4000 ft (Altland & Highman, 1962), and rats are infertile when they are exposed 4 hr daily to 25,000 ft (Altland, 1949a). This suggests that exercise at 21,500 ft may have caused infertility by augmenting the effect of hypoxia similar to that found at 25,000 ft. Additional factors may be involved, however, since the severity of the testicular changes in exercised rats at 21,500 ft is less than in resting rats at 25,000 ft.

Periods of exercise at ground level 6 hr daily for 21 days produced no significant changes in the testes or seminal vesicles. Härkönen, Kontinen, Kormano & Niemi (1963) also found the testes extremely resistant to severe exercise. They reported that Leydig cells changed little, except after prolonged exhaustive exercise. In their study the weights of the seminal vesicles were generally not significantly altered in unexhausted rats.

Exercise training for 21 days at ground level did not prevent the pathological changes in the testes of rats exercised at altitude, but it did prevent or significantly retard the weight loss of seminal vesicles. This suggests that exercise training may aid in the maintenance of male hormone activity necessary for reproduction during periods of heavy work at high altitudes. The mechanism of action of hypoxia on the endocrine system is not well understood. It has

previously been shown that gonadotrophic hormone treatment (extracts of pregnant urine and pregnant mare serum gonadotrophin) restored normal seminal vesicle weights in rats exposed periodically to low atmospheric pressures, but damaged spermatogenic tissue was not repaired (Gordon *et al.*, 1943). Gordon *et al.* (1943) postulated that in prolonged periods of exposure to hypoxia the effects were exerted directly on endocrine organs such as the thyroid and the testis. However, the possibility of a direct effect upon the trophic mechanism of the pituitary could not be excluded.

The absence of any testicular changes in rats exposed 4 hr daily to 18,000 ft for as long as a year reported previously (Altland, 1949b) and the presence of minor changes found in this study at 21,500 ft, suggest that 21,500 ft is near the critical level for development of pathology. The O₂ concentration in the testes has not been determined at this altitude, but the arterial O₂ saturation of rats exposed to an O₂-N₂ mixture equivalent to this altitude is approximately 63% (Altland, Brubach, Parker & Highman, 1967). It should be noted that there is a species difference in the capacity of the testes to withstand altitude hypoxia. Testes of mice were normal after 1 year of 4-hr daily exposure to 25,000 ft simulated altitude (Altland & Highman, 1951). Baird & Cook (1962) also found that male mice were highly resistant to altitude hypoxia. They reported that, when male mice were allowed to adapt to the hypoxic environment for 1 to 3 weeks before breeding, then, even under extremely severe conditions of hypoxia, functional impairment of male fertility was not seen. No data are available on the tissue or arterial O₂ tensions of mice exposed to altitude.

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