Recovery of reproductive functions in male rats after intermittent exposure to hypoxia

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The recovery of reproductive functions and fertility during recovery at sea level after exposure to a simulated altitude of 25,000 ft for five days (6 h/day) was studied in male rats. The weight of the reproductive organs, which was reduced immediately after exposure, returned to control levels in 7–10 weeks time except for testis and epididymis. The histological changes and the sperm parameters also recovered completely within 7 weeks of rest at sea level. Fertility showed a biphasic response. After an initial reduction in fertility, 100% fertility was observed at 7 weeks of recovery; then there was a drop in fertility which again at 10 weeks showed complete recovery after 12 weeks of rest. The testosterone level showed a significant decrease immediately after exposure and recovered within 7 weeks of rest.

Keywords: Hypoxia; Reproductive functions

Exposure to high altitudes is known to cause adverse effects on the male reproductive system in animals and human beings [1, 2]. Impaired testicular function has been observed in experimental animals exposed to intermittent hypoxia [3–6]. There is a marked decrease in sperm counts, an increase in abnormal forms of sperms and a decrease in sperm motility in men on exposure to high altitudes [7]. In rabbits exposed to 4000 m, Monge [1] reported that spermatogenesis ceased and spermatogonia were not normal, as seen by the occurrence of some multinucleate cells [2].

Acute hypoxic exposure has been found to reduce the synthesis of testicular hormone in mice [8] and rats, Fahim *et al.* [9].

Though the sperm parameters [7] and hormone levels [10] have been reported to return to normal levels on descent to sea level, detailed information regarding the recovery of fertility and reproductive organs could not be found. Hence, the present study was undertaken to evaluate the recovery of fertility and the reproductive organs after intermittent exposure of rats to an altitude of 7620 m for 6 h daily for 5 days.

Material and methods

Adult Sprague–Dawley male rats having body weights of 200–300 g were used. They were divided into six groups having six animals each. One group (group A) served as control and the remaining 5 groups (groups B, C, D, E and F) were exposed intermittently to a simulated altitude of 7620 m for a duration of five days in a small animal decompression chamber for 6 h/day. The rate of ascent was maintained at 3000 ft/min and the air inflow was 4 l/min. The temperature was maintained at 32 ± 1°C.

Animals of group B were sacrificed immediately after exposure and the remaining groups were sacrificed after rest at sea level for 1 week (group C), 4 weeks (group D), 7 weeks (group E) and 10 weeks (group F) after exposure. The body weights of these animals were recorded before exposure, at the end of exposure, at weekly intervals during recovery and also at the time of sacrifice.

At the time of sacrifice blood from all the animals was collected from the heart under ether anaesthesia. The plasma was separated and kept at −20°C for estimation of circulating levels of testosterone and cortisol. Then all the organs were dissected out and the adhering tissues were removed. The weights of all the re-

productive organs (including the accessory glands), lungs, liver, heart, kidney and adrenals were recorded. Small pieces of reproductive tissues were preserved in Bouin's fluid for histological studies and the remaining portions were used for biochemical studies.

Sperm analysis. At the time of sacrifice the fluid containing spermatozoa was collected from vas deferens and examined for motility and survival, as reported in a previous study [4]. The sperm concentration in cauda epididymis was estimated by the method of Pakrashi *et al.*

Fertility studies. Rats belonging to group F exposed to 7620 m for 5 days (6 h/day) were kept in individual cages and tested for fertility every week. An estrous female was introduced into each cage in the evening and checked for the presence of sperms by vaginal smearing. If mating had not taken place, another estrous female was introduced in the evening and this was repeated on all days of the week. The females that mated during each week were marked and kept in separate cages. These animals were laparotomized on day 10 of pregnancy and the number of implants were counted and recorded. The delivery was observed and the number of young ones delivered was noted.

Histology. Sections of testis, cauda epididymis, vas deferens, seminal vesicle, ventral prostate and adrenals were studied by light microscopy.

Biochemical studies. The levels of sialic acid, GPC, protein, alkaline and acid phosphatases in various reproductive organs were studied in all the groups using the methods of Aminoff [11], Lowry *et al.* [12] and Hawk *et al.* [13], respectively. The cholesterol content of testis and adrenal was estimated by the method of Sackett [14] in all the groups.

Hormone analysis. Circulatory levels of cortisol [15] and testosterone [16] were analysed by radioimmunoassay. 1,2,6,7-^{3}H-cortisol and 1,2,6,7-^{3}H-testosterone with a specific activity of 80–105 Ci/mmole were purchased from the Radiochemical Centre, Amersham. The cortisol serum was obtained by immunizing rabbits

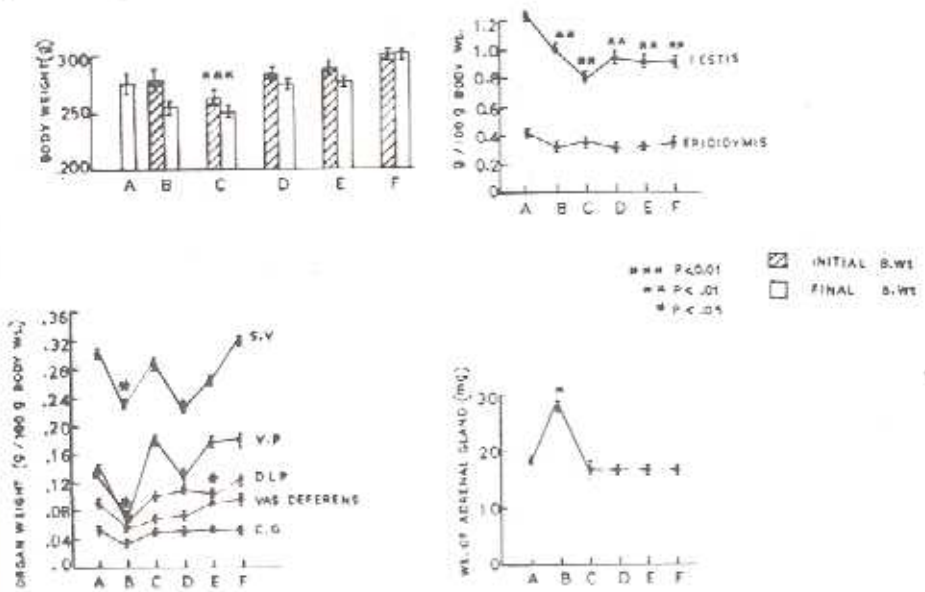


Figure 1. Body weight and organ weight during recovery at sea level after intermittent exposure to hypoxia

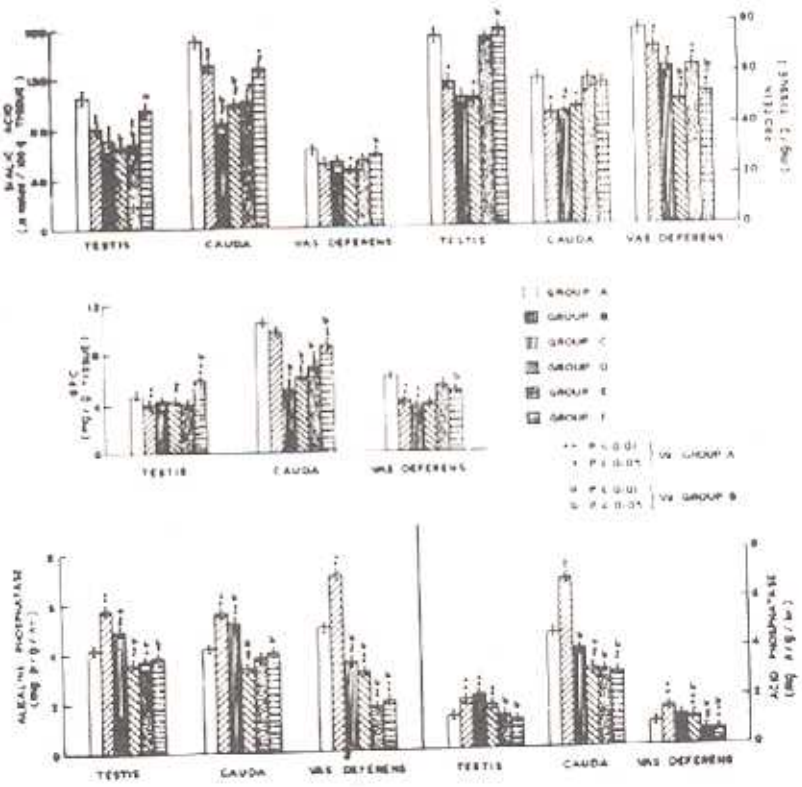


Figure 2. Sialic acid, glyceryl phosphoryl choline, protein, acid and alkaline phosphatase level in rat tissue during recovery at sea level after intermittent exposure to hypoxia

against cortisol–3-carboxymethyl oxime bovine serum albumin conjugate and standardized. Plasma testosterone was estimated in 0.25 µl of plasma using testosterone–3-carboxymethyl oxime bovine serum albumin monoclonal antibody obtained from the World Health Organization and standardized. Statistical analyses were done using Student's 't' test.

Results

Body weight. The body weight of rats showed a significant decrease immediately after exposure, but was found to return to control value after 10 weeks of recovery at sea level (Figure 1).

Organ weights. The weight of testis decreased significantly in group G (immediately after exposure). A further decrease was observed in group C (1 week after recovery). Thereafter, there was a tendency towards recovery (group D and group E), but values did not reach control levels even after 10 weeks of recovery. The weights of epididymis, vas deferens and accessory reproductive organs, after showing a significant decrease in group B (immediately after exposure), gradually increased during recovery, reaching control values at 10 weeks of recovery (Figure 2).

Sperm parameters. The spermatozoal motility was found to be drastically decreased from 55.00 ± 1.2% in control group to 5.00 ± 0.5% in group B. In group C the motility was 7.00 ± 0.5% and then there was an increase in the percentage of motility to almost control val-

Table 1. Spermatozoal characteristics during recovery at sea level after exposure to simulated altitude of 25,000 ft for 6 h/day for 5 days.

Parameters studied	Group A (control)		Group B (immediately after exposure)		Group C (1 week recovery)		Group D (4 weeks recovery)		Group E (7 weeks recovery)		Group F (10 weeks recovery)	
Sperm conc cauda ($\times 10^6$)	20.4	2.0	5.08	0.57***	8.10	0.61**	7.82	0.52**	9.2	0.63**	13.8	0.84
Motility	55.00	1.2	8.00	0.5	7.00	0.5***	25.00	2.0	25.00	4.0	50.00	1.4
Survival	55.00	1.2	3.50	0.4***	5.00	1.0*	15.00	0.8*	25.00	1.2	35.00	1.3

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Table 2. Fertility in rats during recovery at sea level after exposure to simulated altitude of 25,000 ft for 6 h/day for 5 days.

Group	No. of animals	No. of animals mated	No. of animals with implants	Fertility
1 week	6	6	2	2/6 – 33.3%
4 weeks	6	5	1	1/6 – 16.6%
7 weeks	5	5	5	5/5 – 100%
10 weeks	5	5	3	3/5 – 60%
12 weeks	5	5	5	5/5 – 100%

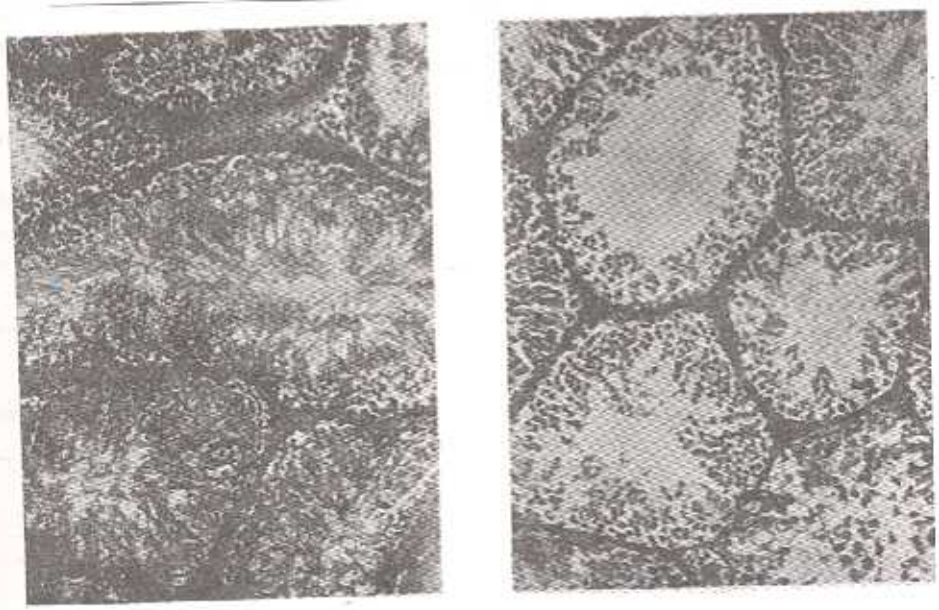


Figure 3. Transverse section of testis of control rats (a) and rats exposed to hypoxia (b)

ues after 10 weeks of recovery following exposure. The survival time also showed a decrease from 55 ± 1.2 min in control group to 3.5 min in group B. There was a gradual increase in the survival time in groups D and E. Group F had a sperm survival time of 35 min (Table 1).

The sperm concentration per cauda in control group (group A) was 20.4×10^6 and in the

group sacrificed immediately after exposure (group B) it decreased to 5.08×10^6. Groups C, D and E (1, 4 and 7 weeks after exposure) had values of 8.10, 7.82 and 9.2×10^6, whereas group F (10 weeks) showed recovery (13.8×10^6), but it was still below the control values.

Fertility rate. During the first week of recovery all the animals mated but only 33.3% animals were fertile. At 4 weeks of recovery only 16.6% of the animals were fertile. There was complete recovery of fertility at 7 weeks after exposure as all the animals were fertile, having normal size implants. Again after 10 weeks of recovery only 60% of the animals were fertile and the number of implants per animal was also reduced (Table 2).

Biochemical variables. The levels of sialic acid in testis, cauda epididymis and vas deferens showed a significant decrease immediately after exposure (group B). During the 1st and 4th weeks of recovery (groups C and D) there was a further fall which returned to normal levels after 10 weeks of recovery (group F).

The GPC levels in testis, cauda epididymis and vas deferens also showed a fall immediately after exposure (group B). In testis there was a gradual increase during recovery (groups C and D) and reached control values after 7 weeks of recovery (group E). In cauda epididymis there was a further fall in the GPC level during the 1st, 4th and 7th weeks of recovery (groups C, D and E) but at 10 weeks of recovery there was an increase but not to the control level. In vas deferens there was a further decrease during the first week of recovery but at four weeks of recovery the value reached control level.

Testis, cauda epididymis and vas deferens showed a significant decrease in protein level immediately after exposure (group B). In testis and cauda epididymis there was a gradual increase which reached control level after 10 weeks of recovery, whereas in vas deferens there was a gradual decline up to 4 weeks of recovery, after which there was an increase without reaching control value.

Alkaline phosphatase level in testis, cauda epididymis and vas deferens showed a significant increase immediately after exposure (group B). At 1 and 4 weeks of recovery the level of alkaline phosphatase in testis and vas deferens fell below control values, which remained so even at 10 weeks of recovery. In cauda epididymis also the alkaline phosphatase level was low but it was not statistically significant compared to control value.

Testis, cauda epididymis and vas deferens showed an increase in acid phosphatase level immediately after exposure (group B). In testis the acid phosphatase level remained significantly high at 1 week of recovery (group C).

Table 3. Testosterone and cortisol in plasma and cholesterol in testis and adrenal during recovery at sea level after exposure to simulated altitude of 25,000 ft for 6 h/day for 5 days.

Control		Immediately after exposure	1 week	4 weeks	7 weeks	10 weeks
Plasma testosterone	2.15	0.494***	0.56***	1.11*	1.46	3.75*
(ng/ml)	0.32	0.067	0.04	0.243	0.36	0.56
Plasma cortisol	3.5	5.74***	7.83***	5.03*	0.42	3.6
(pg/ml)	0.24	0.55	0.96	0.67	0.38	0.43
Testis cholesterol	2.93	4.02**	3.98**	3.88**	3.56	3.35
(mg/gm)	0.20	0.108	0.08	0.16	0.21	0.23
Adrenal cholesterol	37.17	23.29**	24.81***	28.19**	35.93	39.35
(mg/gm tissue weight)	1.72	1.33	0.29	1.07	0.82	1.15

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

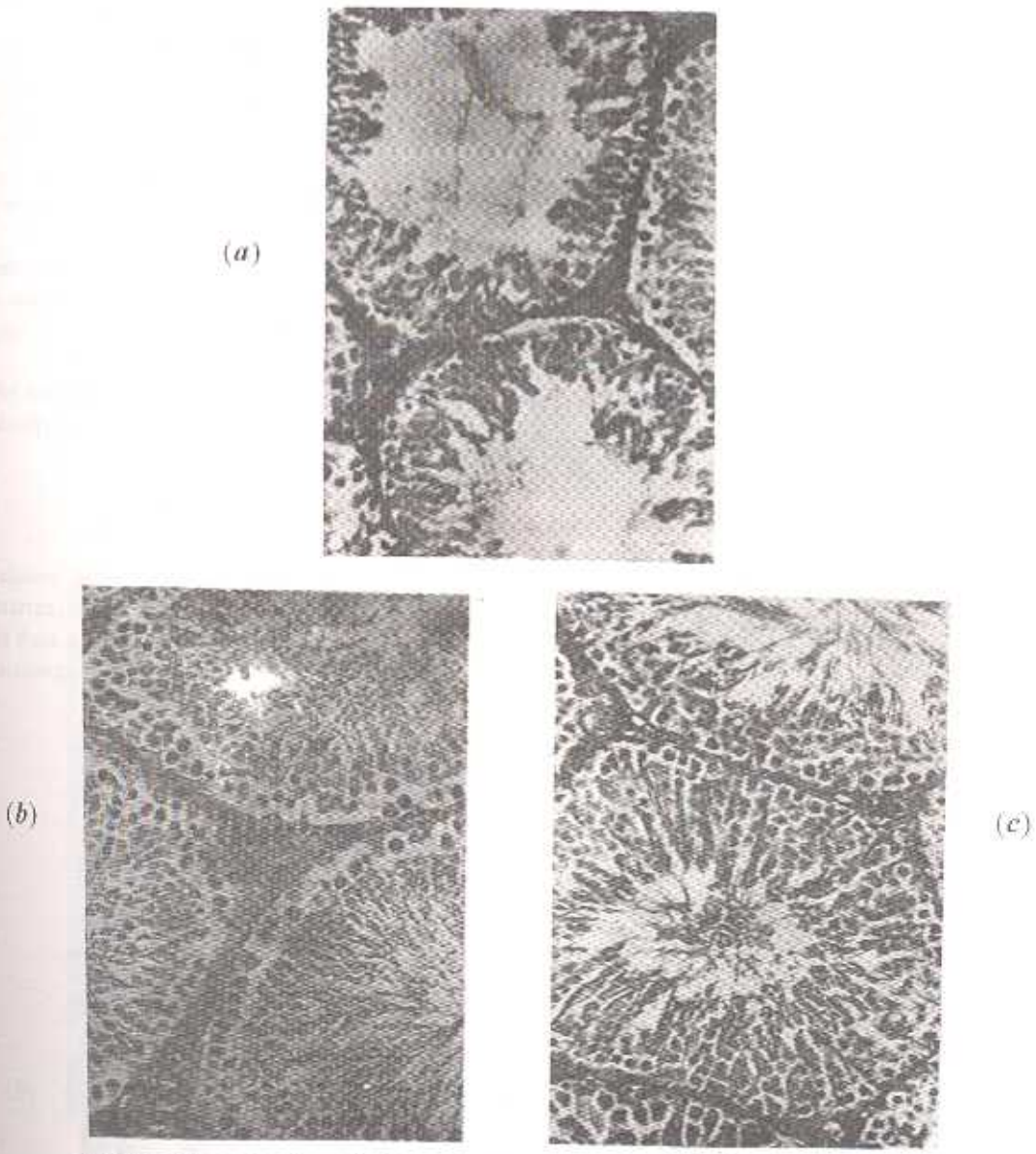


Figure 4. Transverse section of testis of rats, recovery after 1 week (a), 7 weeks (b) and 10 weeks (c).

Then there was a decrease and the level came back to control value in groups E and F (7 and 10 weeks of recovery). In cauda and vas deferens the acid phosphatase level showed a decrease and the values were below the control values at 4, 7 and 10 weeks of recovery (Figure 2).

The cholesterol level in testis was significantly increased immediately after exposure

(group B), but showed a gradual decline during recovery, reaching control values after 7 weeks of recovery. In the case of adrenal, the cholesterol level decreased immediately after exposure and during recovery there was an increase. At 7 weeks of recovery the cholesterol level in adrenal was comparable with control values. The plasma testosterone in control animals ranged between 1.19 and 3.09 ng/ml with a mean of 2.15 ± 0.32 ng/ml. The testosterone values in animals exposed to hypoxia (group B) were found to be significantly lower ($p < 0.001$) compared to the control values. The plasma testosterone values remained significantly lower in group C (1 week) and group D (4 weeks). After 7 weeks of recovery (group E) the testosterone level reached the control level. The plasma cortisol level showed a significant increase after exposure to hypoxia ($p < 0.001$) compared to control values. The cortisol level increased further in group C (1 week recovery) and then there was a decrease in group D (4 weeks of recovery). After 7 weeks of recovery (group E) the cortisol level reached the control level (Table 3).

Histological changes The histological changes observed in the reproductive organs after exposure to hypoxia were similar to those observed earlier [4].

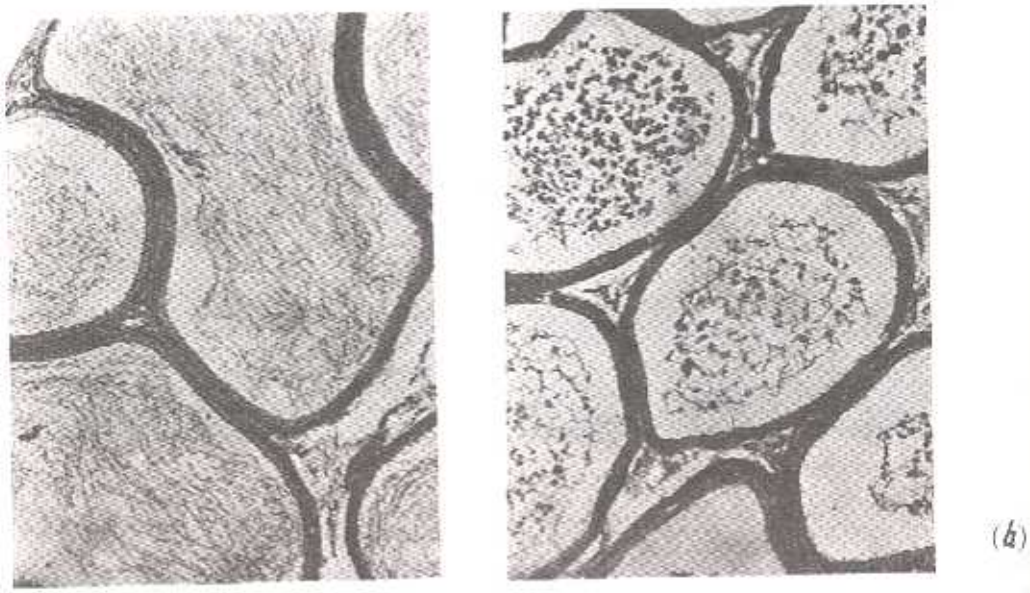
After 1 week of rest spermatogenesis was normal. There was fine vacuolation in testis. The tubules were full of spermatozoa. When the resting period was extended to 4 weeks, spermatogenesis appeared to be affected again, but after 7 weeks of rest there was a complete recovery in the structure of testis (Figures 3 and 4).

The cauda epididymis and the vas deferens recovered partially from the effects of hypoxia by 1 week of rest and the recovery was complete after 7 weeks of rest (Figures 5–8).

The structure of the seminal vesicle and adrenal showed complete recovery from hypoxic effects by about 4 weeks of rest.

Discussion

Intermittent exposure of rats to a simulated altitude of 7620 m resulted in certain changes in the male reproductive organs such as decrease in weight, arrest of spermatogenesis,



(*a*) (*b*)

Figure 5. Transverse section of cauda of control rats (a) and rats exposed to hypoxia (b)

(*a*)

(*b*)

(*c*)

Figure 6. Transverse section of cauda of rats: recovery after 1 week (a), 7 weeks (b) and 10 weeks (c).

appearance of multinucleated cells in testis and atrophic changes in epididymis, vas deferens and accessory reproductive organs. The changes observed in the present study confirm the earlier observations [4, 5]. The changes in the histology of the male reproductive organs and in sperm physiology may be due to the direct effect of hypoxia on these tissues, leading to reduced metabolism with resultant atrophic changes, or due to reduced androgen status.

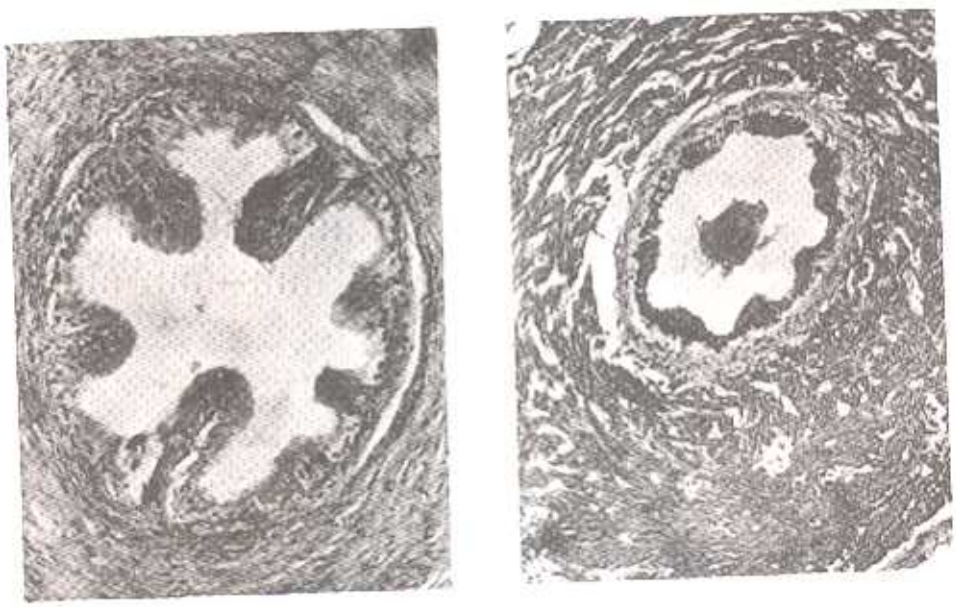


Figure 7. Transverse section of vas deferens of control rats (a) and rats exposed to hypoxia (b)

All the histological and biochemical changes observed after exposure to hypoxia were found to recover completely by 7–10 weeks of recovery at sea level. But the weight of testis and epididymis showed further fall after one week of recovery and afterwards there was an increase but the control level was not reached even after 10 weeks of rest in the case of testis, suggesting that the damage to the spermatogonia is long-lasting and takes more time to recover.

The decrease in testosterone level after hypoxia confirms the earlier reports [8, 9, 17]. The simultaneous increase in testicular cholesterol level, the changes in the structure of reproductive organs and also in androgen-dependent biochemical parameters corroborate the decrease in testosterone level.

The fall in testosterone level may be due to the direct effect of hypoxia on testis and/or due to the effect on pituitary–gonadal axis. It has been reported that hypoxia decreases the LH level [10, 18], which in turn may be the cause for reduction in testosterone level. During the 1st and 4th weeks of recovery the testosterone levels showed an increase and reached control values after 7 weeks of recovery. The testicular cholesterol level, which shows an increase immediately after exposure and a decline during recovery, also reaches control values after 7 weeks of recovery. Testicular cholesterol and plasma testosterone values show a negative correlation ($r = 0.382$). The increase in testicular cholesterol values after exposure might be due to the nonutilization of cholesterol for the synthesis of testosterone, which is decreased after exposure to hypoxia. This is in agreement with the observation of Kar and Roy [19] and Choudhury and Mukherjee [20], who reported increase in cholesterol content of testis with inhibition of testosterone synthesis. Marshall and Coombs [21] also associated the reduction in testicular cholesterol content of several species of birds during the breeding season with enhanced rates of androgen production.

The results of fertility studies showed that after an initial reduction in fertility during the first 6 weeks of recovery, the fertility returned back to normal after 7 weeks of recovery at sea level. But during the 8th, 9th and 10th weeks of recovery there was a slight fall in the fertility

(*a*)

(*b*)

(*c*)

Figure 8. Transverse section of vas deferens of rat recovery after 1 week (a), 7 weeks (b) and 10 weeks (c)

rate. According to Zenick *et al.* [22], when the treatment corresponds to one spermatogenic cycle (5 days), studies at 1, 4, 7 and 10 weeks after treatment will be indicative of the damage to the spermatozoa, spermatid, spermatocyte or spermatogonia, respectively. In this study the exposure to hypoxia is for 5 days, corresponding to one spermatogenic cycle, and the results

suggest that exposure to hypoxia causes damage to spermatozoa, spermatid and spermatogonia but not to spermatocytes. These results of fertility studies also prove our observation of sperm parameters and the histological evidence that the spermatogonia are affected as a result of exposure to hypoxia.

The increase in cortisol level immediately after exposure observed in this study confirms the earlier observations [23]. During recovery at sea level the cortisol level shows an increase after 1 week of recovery, followed by a decrease after 4 weeks of recovery before it comes back to the control level after 7 weeks. The increase in cortisol level after exposure to hypoxia is reflected by the decrease in cholesterol level in adrenals and hypertrophy of adrenal cortex.

In conclusion, it may be stated that the present study shows that the stress of hypoxia causes a decrease in the serum testosterone level, adverse changes in the reproductive organs and a reduction in fertility. When the animals are allowed to recover at sea level, all these changes come back to normal at 7 to 10 weeks after exposure. But the fertility rate after attaining 100% at 7 weeks of recovery shows slight fall at 10 weeks suggesting that the damage has been done to the spermatogonia and that more than 10 weeks are needed for complete recovery.

References

1. Monge C. Chronic mountain sickness. *Physiol Rev* 1943;**32**:166.
2. Abelson AE. Altitude and Fertility. *Human Biol* 1976;**48**:83.
3. Gordon AS, Tornetta FJ, D'Angelo SA, Charipper HA. Effects of low atmospheric pressures on the activity of the thyroid, reproductive system and anterior lobe of the pituitary of the rat. *Endocrinology* 1943;**33**:366.
4. Riar SS, Malhotra MS. Changes in sex organs of male rats on exposure to hypoxia (7576 m). *Ind J Exp Biol* 1977;**15**:1052.
5. Riar SS, Malhotra MS, Bhatt S. Effect of hypoxia (6060 m) and cold (−5°C) on sexual organs of male rabbits. *Int J Biometeorol* 1979;**24**:31.
6. Havazhagan G, Riar SS, Kain AK, Jaya Bardhan, Pauline Thomas. Effects of ascorbic acid supplementation on male reproductive system during exposure to hypoxia. *Ind J Biometeorol* 1989;**33**:165.
7. Donayre J, Guerra-Garcia R, Monocloa F, Sobrevilla L. Endocrine studies at high altitude. iv. Changes in the semen of man. *J Reprod Fertil* 1968;**16**:55.
8. Clegg EJ. Androgen production in male mice at simulated high altitude. *J Physiol* 1966;**182**:43.
9. Fahim MS, Messiha FS, Girgis SM. Effect of acute and chronic simulated high altitude on male reproduction and testosterone level. *Arch Androl* 1980;**4**:217.
10. Humpler F, Skrabal F, Bartsch G. Influence of exposure to moderate altitude on the plasma concentrations of cortisol, aldosterone, renin, testosterone and gonadotropins. *Eur J Appl Physiol* 1980;**45**:167.
11. Aminoff D. Method for quantitative estimation of N-acetyl neuraminic acid and their application to hydrolysate of sialomucoids. *Biochem J* 1961;**81**:384.
12. Lowry OH, Rosebrough NJ, Farr, AL., Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951;**193**:265.
13. Hawk PB, Oser BL, Summerson WH. Practical Physiological Chemistry. New York McGraw Hill, 1957.
14. Sackett GE. Modification of Bloor's method for the determination of cholesterol in whole blood or blood serum. *J Biol Chem* 1925;**64**:203.
15. Klemn W, Gupta DA. A routine method for the radioimmunoassay of plasma cortisol without chromatography. In Gupta D, ed. *Radioimmunoassay of steroid hormones*. Weinheim, Verlag Chemie, 1975, 143.
16. Ismail A, Niswender GD, Midgley AR. Radioimmunoassay of testosterone without chromatography. *J Clin Endocrinol* 1972;**34**:177.
17. Sawhney RC, Chhabra, PC, Malhotra, AS, Singh T, Riar, SS, Rai RM. Hormone profiles at high altitude in man. *Andrologia* 1985;**17**:178.
18. Bungham CRM, Hackett PH. Effect of high altitude on endocrine functions in the Sherpas of Nepal. *J Endocrinol* 1978;**79**:147.
19. Kar AB, Roy SN. The effect of testosterone propionate on total cholesterol content of the testis in young rats. *Acta Endocrinologia* 1955;**18**:267.
20. Chowdhury AR, Mukherjee AK. Effect of exogenous testosterone propionate and progesterone on testicular ascorbic acid and cholesterol in relation to spermatogenesis. *Indian J Expl Biol* 1976;**14**:701.
21. Marshall SJ, Coombs C. Lipoid changes in the gonads of wild birds. Their possible bearing on hormone production, sexual display and the breeding season. *Nature* 1952;**169**:261.
22. Zenick H, Blackburn, K, Hope E, Oudiz, D, Goeden H. Evaluating male reproductive toxicity in rodents – A new animal model. In Legator SM, Rosenberg MJ, Zenick H, eds. *Environmental Influences on Fertility, Pregnancy and Development. Strategies for Measurement and Evaluation*. New York:Alan R. Liss., 1984.
23. Ruttner BA, Macmillan BT, Michael SD, Altland PD. Plasma gonadotropins, prolactin and corticosterone concentrations in male mice exposed to high altitude. *J Reprod Fertil* 1980;**60**:431.