EFFECT OF HYPOBARIC HYPOXIA ON SPERMATOGENESIS, LEYDIG CELLS AND Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN TOAD

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Summary : The effect of different grades of hypobaric hypoxia for 48 hours was studied on spermatogenesis, Leydig cells and Δ^5-3 β –hydroxysteroid dehydrogenase activity in toad (*Bufo melanostictus*). Maximum inhibition of testicular activity was noted in 7,315 m exposed animals. The impairment of testicular function at high altitude is possibly due to inhibition of gonadotrophin secretion.

Key words : hypoxic stress spermatogenesis Leydig cell Δ^5-3β-hydroxysteroid dehydrogenase enzymes

INTRODUCTION

The deleterious effect of hypoxic exposure on reproduction in man has been well documented (Monge, 1942 ; 1948). Several investigators have observed testicular degeneration in rat (1,6,10) following exposure to lowered pressures. Extensive works on mammals has shown that high altitude or reduced pressure causes gonadal inhibition but little attention has been directed towards the effect of high altitude exposure on lower vertebrates. No work seems to have been done so far to measure the activity of Δ^5-3 β-hydroxysteriod dehydrogenase in the testis of both lower and higher vertebrates after exposure to high altitude. The present work has been undertaken to study the effect of acute exposure to different grades of hypobaric hypoxia on spermatogenesis, histology of Leydig cells and Δ^5-3 β-hydroxysteroid dehydrogenase (Δ^5-3 β-HSD) activity in toad.

MATERIAL AND METHODS

Adult male toads (*Bufo melanostictus*) weighing about 50-60 *g* were used in this experiment during the month of September. The toads were divided into 4 groups. The first 3 groups were exposed to 3,658; 5,487 and 7,315 meters altitude respectively. The Po_2

level was measured 101, 79 and 61.6 mm Hg respectively in the decompression chamber of these three altitude groups of animals. The 4th group was used as control. The experimental animals were exposed for 48 hr in a decompression chamber, set up in the laboratory according to the method of Purushottam and Ghosh (17). The different grades of altitude were maintained by adjusting the air pressure inside the chamber. Temperature inside the chamber was maintained at 32°±1°C. Experimental animals were sacrificed immediately after the exposure period along with the controls. Testes were dissected out and their weights were recorded. One testis of each animal was used for histochemical localization of Δ^5-3 β-HSD and the other was fixed in Bouin's fluid for histological examination. Paraffin sections (5μ) were stained with haematoxylin and eosin. Quantitative studies of spermatogenesis were performed according to Biswas *et al.* (3) dividing the process into the following five stages :

Stage 0	:	Primary spermatogonia in resting phase.
Stage I	:	Small cell nest of secondary spermatogonia consisting of not more than ten cells.
Stage II	:	Large cell nest of secondary spermatogonia consisting of more than ten cells.
Stage III	:	Primary spermatocytes.
Stage IV	:	Secondary spermatocytes.

The number of cell nest/tubule in all the stages were counted from 30 seminiferous tubules of each testis. The mean value of the different stages in seminiferous tubules of each testis was taken as an index of the spermatogenic activity. Nuclear area of 50 round Leydig cell nuclear area from each testis were drawn on mm2 graph paper with the help of camera lucida and were measured according to the method of Deb *et al.* (8) For histochemical localization of the enzyme Δ^5-3β-HSD, fresh frozen sections (20 μ) were cut on a cryostat and mounted on coverslips. Δ^5-3 β-HSD in the testicular sections were determined by incubation for 60 minutes at 37°C in a substrate medium (dehydroepiandrosterone) described by Deane *et al.* (7). Parallel sections were incubated in a substrate-free medium for the same time served as controls. Blood eosinophil count was also included in the present study and was done by usual Leishman's staining method.

RESULTS

The results are presented in Table I. There was a significant change in the testicular weight of the animals exposed to 7,315 meters in comparison to control. The quantitative study of spermatogenesis revealed that the primary spermatogonia (Stage 0) was increased in 5,487 and 7,315 meters exposed animals, whereas it was decreased in 3,658 meters group. The cell nest representing secondary spermatogonia (Stages I and II) were decreased in number in the testis of toads exposed to different altitudes. The cell nests

TABLE I : Changes of testicular weight and Leydig cell nuclear area in the animals after exposure to 3,658 : 5,487 and 7,315 meters high.

	Control (n=10)	*3,658 m (n=10)*	*5,487 m (n=11)*	*7,315 m (n=11)*
Body weight *gms* (Absolute)	57.9±2.0	54.3±2.1	53.3±2.1	52.7±1.2
Testicular weight *mg* (Absolute)	143.7±5.1	128.6±9.8	132.0±7.9	113.0±5.4
Testicular wt. *mg*/100 *g* body wt.	250.6±10.3	240.2±19.8	247.3±10.8	216.1±12.2*
Leydig cell nuclear area in sq. mm	15.5±0.9	14.1±0.3	16.2±0.4	12.9±0.5**

Each value represent±mean standard error.

n = number of animal in each group.

Leydig cell nuclear area = Camera lucida X800.

* Compare with control group (Student's t-test), $P<0.05$;

** $P<0.02$.

TABLE II : Changes of spermatogenesis in the animals after exposure to 3,658; 5,487 and 7,315 meters high.

Stage of spermatogenesis (Nest/Tubule)	*Control (n=8)*	*3,658 m (n=8)*	*5,487 m (n=8)*	*7,315 m (n=8)*
0	1.2±0.15	0.72±0.1 0.1**	1.0±0.27	1.58±0.08*
I	1.9±0.21	1.37±0.13*	1.24±0.2**	1.35±0.15*
II	0.78±0.07	0.64±0.09	0.61±0.1	0.52±0.098*
III	1.3±0.13	1.76±0.18	1.94±0 24*	1.93±0.26*
IV	0.45±0.04	0.35±0.07	0.31±0.14*	0.31±0.04*

Each value represents mean ±standard error.

n = number of animal in each group.

* Compare with control group (Student's t-test), $P<0.05$

** $P<0.02$

consisting of secondary spermatocytes (Stage IV) were found to decrease significantly in 5,487 and 7,315 meters exposed animals as compared to controls. On the other hand, primary spermatocytes (Stage III) were increased in all three altitude groups. The Leydig cell nuclear area was decreased significantly in 7,315 meters exposed group only and the testicular Δ^5-3 -HSD activity was also decreased in this group of animals (Figs. 1 and 2) in comparison to the control group. In addition to this, the percentage of circulating eosin phil was also decreased significantly in 7,315 meters exposed animals (2.5±0.4 in 12 animals) in comparison to controls (4.7±0.7 in 9 animals)

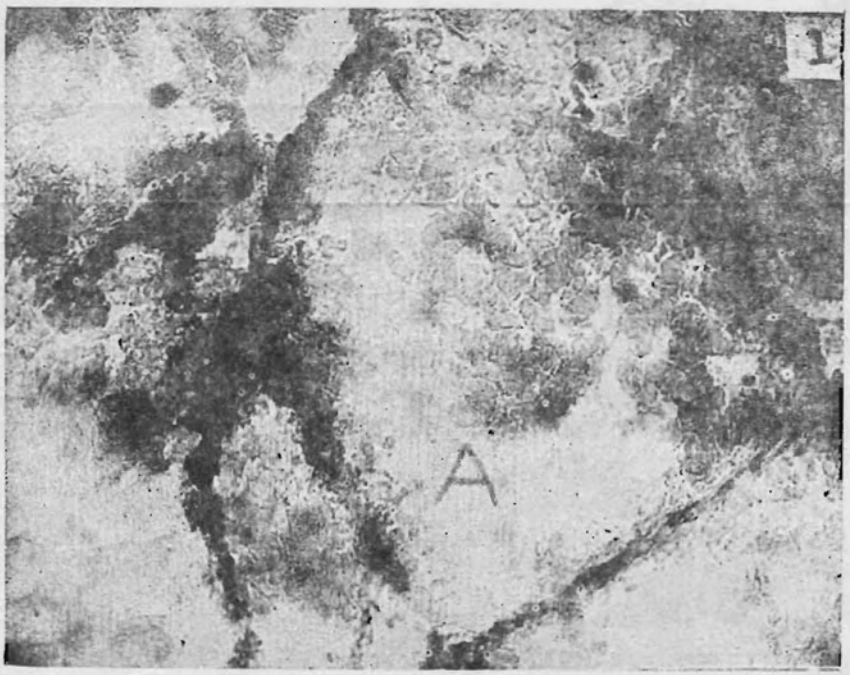

Fig. 1 : Δ^5–3β–hydroxysteroid dehydrogenase in the testis of normal toad. Deeply stained areas are Leydig cells. 'A' marked areas are seminiferous tubules. X 250.

Fig. 2 : A decreased Δ^5–3β–hydroxysteroid dehydrogenase in the testis of 7,315 m exposed toad. X 250.

DISCUSSION

The present experiment shows that hypobaric hypoxic exposure lowered spermatogenic activity as well as Leydig cell function (nuclear area and Δ^5-3β-HSD enzyme) in toad. Significant decrease of testicular weight in 7,315 meters exposed animals is thought to be due to degenerative changes in the testis. Similar observation in hypoxic rats has also been reported by several investigators (1,10,19).

Maximum inhibition of spermatogenic activity was noted in the toads exposed at 7,315 meters. The significant decrease of both secondary spermatogonia (Statge I) and secondary spermatocytes (Stage IV) developed in the toads as a result of exposure to both 5,487 and 7,315 meters was thought to be due to inhibition of transition from their primary stages, although the distribution of the number of cell nest/tubule in these groups of animals seems to be similar. A conclusive explanation cannot be drawn at the moment from this significant decrease of primary spermatogonia (Stage 0) in 3,658 meters exposed animals, although it is not reflected on the testicular weight. It can be suggested that this level of hypoxia may have reduced the formation of the primary spermatogonia (Stage 0). The testicular inhibition in toad is in close agreement with he findings of (6,10,18) in their studies with hypoxic rats. Riar *et al.* (19) have shown that hypoxic exposure causes deterioration in sperm quality in rat. It is well known that the seminiferous tubules are highly sensitive to oxygen lack, both in man and animals (9,11). The changes described in the testis of toad after acute exposure to hypoxia may be due to damage of the seminiferous epithelium.

Leydig cell nuclear area is reported to have relation with the steroidogenic activity of the testis (3,4). Δ^5-3β-HSD, a key enzyme of steroidogenesis (21), is known to have a direct relation with the steroidogenic activity of the Leydig cells (4,20). Inhibition of Δ^5-3β-HSD activity and decrease in Leydig cell nuclear area after exposure to 7,315 meters indicates suppression of testicular steroid hormone synthesis in toads. Riar *et al.* (18) have shown that simulated high altitude exposure reduces the Leydig cell activity in rat. Acute hypoxic exposure also reduces the synthesis of testicular hormone in mice (5).

In this study an attempt was made to show the status of adrenal gland at simulated high altitude and it's relation with the mechanism of the inhibition of testicular activity. Acute altitude stress has been found to stimulate the release of excess adrenal steroid hormones (13) and this may result from increased ACTH secretion. Since the adrenal gland in toad is fused with the kidney, it is difficult to evaluate the status of adrenal gland directly. Blood eosinophil count, an indirect method for studying the adrenocortical activity (24), was decreased in the toads exposed to 7,315 meters (2) and the result is consistent with the findings in man (22). It has also been suggested that ACTH has a reciprocal relation with gonadotropic hormone secretion (16). A fall in plasma leutenizing hormone

was found by Sobrevilla and Ress Midgley (23) to occur in men after exposure to hypoxic environment. On the other hand Guerra-Carcia (12) has reported reduced urinary excretion of testosterone in man after acute exposure to the hypoxia of high altitude and it seems likely that the fall in excretion of testosterone is due to a diminished plasma level of lutenizing hormone)

The present investigation, therefore, suggests that the depression of testicular functions in toads after hypobaric hypoxic exposure was due to inhibitory response of pituitary gonadotropins.

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