Survey of A Steroidal Precursor Isolated from the Fat of UROMASTIX HARDWICKLL and its Effect on the Testicular Function in Rats

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ABSTRACT

The effect of steroidal-precursor, obtained from the unsaponifiable matter of the fat of *Uromastix hardwickii* has been studied in the testis of rats specially on the activity of the Leydig cells, the epithelium of the seminiferous tubules, spermatogenesis and degenerative changes in the germinal epithelium, if any. The appearance of the nuclei, the distribution of chromatin matter, nuclear activity, cytoplasmic activity and cell mitosis and meiosis have been critically observed. The various effects produced by the steroidal-precursor have been compared with those of the control rats and testosterone, testosterone propionate and methyl testosterone treated ones over a period ranging from 10 days to 40 days.

Androgens are derived from acetate-cholesterol chain (Ungar and Dorfman, (1953)[1]. The adipose tissues (fat bodies) of *Uromastix hardwickii* are found to be quite rich in cholesterol and its esters (Kar and Chauhan, 1977)[2]. The seasonal changes in testicular structure and function and the effects of gonadotropins in the fresh water turtle has been studied recently (Callard *et al,* 1976)[3], while Kholkute (1977)[4] studied the effect of *Hibiscus rosa* on spermatogenesis and accessory reproductive organs in rats. Hence, it was thought worthwhile to screen the effect of the steroidal precursor obtained from the unsaponifiable matter of the fat of *U. hardwickii* over the activity of the Leydig cells and on the activity of the epithelium of the seminiferous tubules. The vertebrate restis converts various non-steroidal and steroidal precursors into biologically active androgens (Presslock, 1977)[5]. A comparison of the isolated fraction with that of other androgens like testosterone, testosterone propionate and methyl testosterone on the testicular tissue has been observed. Specially the effect of the extract and other hormones was tested in male albino rats (Haffkine strain) so as to reveal their activity over the cellular function of the interstitial cells, spermatogenesis, degenerative changes in the germinal epithelium and development of the Leydig cells. The appearance of the nuclei, the distribution of chromatin matter, nuclear activity, cytoplasmic activity and mitosis have also been observed.

Androgen were reported to be absorbed through the intact skin and to exert a strong action when applied locally to the chick's comb, [6] or to the penis of the castrate rat.[7] Very likely the effect depends on the rate of absorption of the androgens, - testosterone being most rapid, testosterone propionate is least, whereas methyl testosterone is intermediate. Absorption may be immensely hindered by the mixing of cholesterol with the active steroid perhaps due to the mechanism of substratecompetition.[8]

EXPERIMENTAL

Male albino rats (Haffkine strain) of 50 days age and weighing 55 to 75g were employed in the present study.

Isolation of Steroidal Precursor

The unsaponifiable matter was separated by the standard method.[9] 4.05g of it was dissolved in the minimum quantity of petroleum ether (60-80°C) and poured on the top of a column (size 70 × 2.5cm) packed with 80gm of activated silica gel. The various eluants used were Petroleum Ether (60-80°C), Petroleum ether - Ethyl Acetate (97:3); Petroleum ether - Ethyl acetate (94:6); Petroleum ether - Ethyl acetate (90:10) and finally chloroform. In al 45 fractions each of 50ml. were collected in numbered and tared tubes. Thin-layer chromatography of all the fractions with appropriated solvent systems and spraying with sulphuric acid and water (1:1) revealed the presence of four fractional out of which the first fraction named as 'A' has been employed in the present study. The steroidal precursor (Fraction 'A') is white amorphous powder having a melting point 161°C, (acetate derivative, m.pt. 182–183.5°C). It gave a strong positive test for sterol [10, 11] which was further ascertained by infra-red spectroscopy.

Dosage

The suspension of the steroidal precursor and the androgens were made in propylene glycol in the following concentrations:-

Steroidal precursor	—	10mg/ml
Testosterone and Methyl Testosterone	–	1mg/ml
Testosterone Propionate	—	50mg/ml

The animals were divided into five groups each consisting of 25 rats, The first group received the steroidal precursor at a level of 25 mg/kg weekly, the second group, Testosterone at 1.5mg/kg daily, the third group testosterone propionate at 100mg/kg weekly, the fourth group Methyl testosterone at 1.5mg/kg daily, whereas the fifth control group were injected with an appropriate dose of the solvent only.

All the animals were injected with the said dosage of drugs up to a period of forty (40) days, because during this period at least one complete development of the sperms from the spermatogonium would take place.

Fixation of the Testicular Tissue

The animals were anaesthetised with ether. The testicular tissue was dissected, washed in Ringer's solution and was fixed in Carnoy's fluid (6:3:1) (Davenport, 1960). [12] Tissues were dehydrated and embeded in paraffin wax (60–62°C). Sections of 6u thickness were cut and

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stained by different staining techniques. Iron-haematoxylin stain [6] was, however, preferred to see the nuclear chromatin. The sections were also stained by haematoxylin - eosin[6] method. The cytoplasmic details were studied by osmium/ethyl gallate method (Wigglesworth, 1959).[13]

The detailed histological and cytological studies of the intertitial cells have been made. Further, the effect of the steroidal precursor upon the formation of spermatogonia, spermatocytes, spermatids and sperm were studied. These observed effects were compared to those other testicular stimulating hormones viz; testosterone, methyl testosterone and testosterone propionate respectively. Lastly, a comparison of the total effect on the seminiferous epithelium and the intertitial cells have been observed. All the experiments were performed in triplicate.

RESULTS

In control rats spermatogenesis has been observed to commence in 20 days and usually the interstitial cells have a small nuclei, the nucleolus is situated in the centre and the chromatin is found in the aggregate state. The various stages of spermatogenesis, primary and secondary spermatocytes, and spermatids have been noticed.

In steroidal-precursor-treated rats, (25mg/kg weekly) at 20 days the interstitial cells increased in size than the control ones. The nuclei were sharp and sperm bundles and spermatogenesis could be seen (Fig. 1). However, after a duration of 30 days the interstitial cells were found

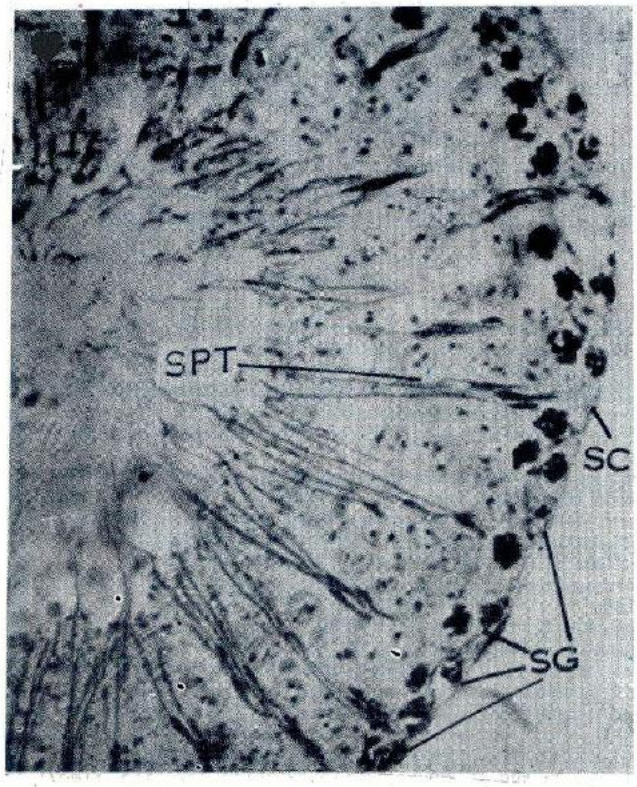

Fig. 1

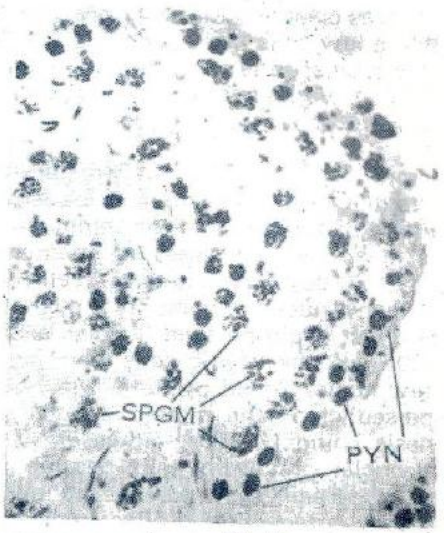

Fig. 2

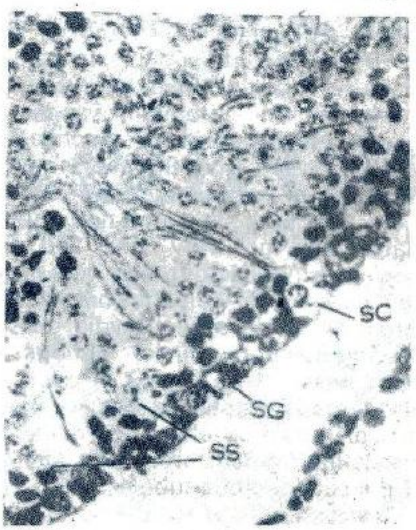

Fig. 3

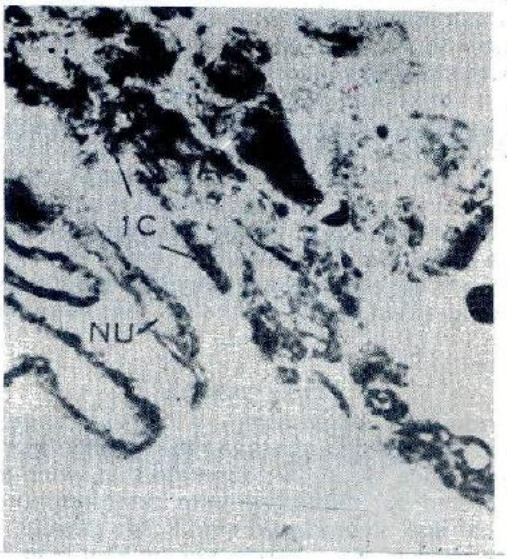

Fig.4

to have large nuclei with distributed chromatin matter. The cytoplasm was clear and the secretory cells could be observed. Only a few cells had pycnotic nuclei (Fig. 2). At 40 days, the tubular zone showed primary and secondary spermatocytes. Various stages of mitotic and meiotic cell divisions were observed (Fig. 3).

In testosterone (1.5mg/kg per day) treated rats at 10 days the interstitial cells were found to be large having sharp nuclei with distributed nuclear chromatin. The cytoplasm was clear and all stages of spermatogenesis observed. But at 20 days- the interstitial cells decreased in size with many nuclei turning into pycnotic ones. Degenerative effect could be seen in some seminiferous tubules. At 30 days, the Leydig cells were found to be affected. A few nuclei turned pycnotic while others still actively dispersed chromatin matter. Various stages of spermatogenesis could be seen. However, at 40 days most of the nuclei of Leydig cells became pycnotic. Many of the cytoplasm seen around the nuclei were pycnotic in nature. A degenerative effect in the epithelium of the seminiferous tubules could be noticed. (Fig. 4).

In testosterone-propionate - (100mg/kg per week) treated rats at 10 days the interstitial cells were of normal size. The nuclei were not very sharp, but however sharper than the control ones. The chromatin was distributed in nuclei. The interstitial cells were not so active as those of steroidal-precursor-treated and the testosterone-treated ones. Various stages of spermatogenesis could be seen. At 20 days the Leydig cells were not enlarged. The chromatin matter was not very clear and distributed. spermatogenesis was noticed but there was some cellular effect observed in the nuclei were the chromatin could not be differentiated. At 30 days, the Leydig cells were not very sharp having pycnotic nuclei. Various stages of spermatogenesis were observed, but in many cases the nuclei were also turned pycnotic. At 40 days, the interstitial cells displayed a distinct drug-effect with markedly pycnotic nuclei. The cellular details were also affected in the epithelium of the seminiferous tubules.

At 10 days, in rats treated with methyl testosterone (1.5mg/kg per day the interstitial cells showed sharp nuclei which were marked with distinct chromatin matter. Cytoplasm could be seen with secreted activities. At 20 days, the Leydig cells were of moderate size. In many cases the chromatin was distributed. However, neither the cell size nor the nuclear size were as big as that of the steroidal-precursor treated ones. At 30 days, the Leydig cells were found to be quite big with large cytoplasmic area. Nuclei were sharp but chromatin was not distributed. Various mitotic divisions could be observed. At 40 days, the interstitial cells became small. Nuclei were found to be pycnotic. Spermatogenesis could be seen. A distinct drug-effect on the epithelial cells were noticed.

DISCUSSION

The androgens when given in higher dosage for a prolonged period can damage the testicular tissue.[8] Moore and Price [14 15, 16] observed that when the rats were selected for treatment with testosterone before 40 days of age, it produced significant damage to the testicular tissue in rats when they were treated at 70 days or later i.e. a period when a rat attains the sexual maturity. However, a relatively higher dose of methyl testosterone (10mg/kg per day) was most active with respect to Leydig cell damage.[15] Very likely the rate of absorption of free testosterone is most rapid and testosterone propionate is least, whereas methyl testosterone is intermediate.[15]

In the present investigation it has been found that the steroidal fraction isolated from the fat of **U. hardwickii** activates the interstitial cells and has no adverse effect over the testicular tissue and spermatogenesis as that of mesterolone.[16] The steroidal fraction very likely serves as a precursor and it perhaps forms the tropic material which stimulates the interstitial cells. Possibly, it enriches the acetate-cholesterol chain and androgen formation in the body of the animals. It has also been observed that a prolonged administration of the androgens produced damage to the testicular tissue which may have been caused by the inhibition of gonadotropin secretion from the pituitary. However, it has been observed critically that the steroidal - precursor did not cause any such damage to the testicular tissue at any stage ranging from 10 to 40 days.

From the results of the effect of androgens on the testicular tissue of rats at various stages it has been observed that testosterone damaged it considerably as reported earlier by Moore and Price.[14] According to Hertz [17] a very high dose of methyl testosterone is required to cause damage to the testicular function, but in the present study a significant drug-effect could not be observed even up to a period of 40 days.

As the results of the steroidal-precursor were very encouraging on the testicular function in rats, it may be worthwhile to carry out further studies on its chemical characterization and identification together with more elaborate histologicel studies.

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