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Effect of castration and androgen treatment on the androgen dependent parameters in the accessory glands of the slender loris, *loris Tardigradus lydekkerianus* (Cabra).

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Abstract. The accessory glands of reproduction in slender loris exhibit reduction in secretory activity following castration for 15 days and 30 days. Administration of androgens (testosterone—propionate and 5α dihydrotestosterone) to castrated animals had a differential effect in maintaining the biochemical parameters like citric acid and fructose.

Keywords. Loris Tardigradus lydekkerianus; slender loris; castration; androgen treatment.

1. Introduction

Of the several secretions of the male sex accessory glands of mammals, fructose and citric acid are important and are found in large quantities in the semen. Their synthesis and secretion are entirely regulated by the androgens secreted by the testis (Price and William Ashman 1961; Prasad *et al* 1973a,b). Amongst the non-human primates, besides the monkey (Dinakar *et al* 1974a,b) not much information about the prosimian primates is available. The present investigation deals with the secretions and their androgen control in the accessory sex glands of the male slender loris, *Loris tardigradus lydekkerianus* (cabra).

2. Materials and Methods

Lorises, also known by the native names as ceylon sloths, sherminds, unhappen luma and thevangu, occur in some of the forested areas of Southern India and Ceylon from sea level to an elevation of about 1800 inches. Lorises are found mostly in casurina, tamarind and pongamia trees, quite close to rural habitation. They were collected from the forests around Bangalore, brought to the laboratory, maintained in cages and provided with food and water.

Adult male lorises used for the experimental groups were divided into nine groups of 3/5 animals in each (table 1). They were castrated by opening the inguinal passage under asceptic conditions using sodium pentobarbitone (Nembutol, Abbot laboratories) as anesthetic. Through a small incision in the inguinal passage, the testis were exposed, carefully freed from the epididymis after ligating the efferent ductules and testicular blood vessels without damaging the vascular supply to the epididymis and ductus deferens. The different doses of testosterone propionate (TP) used were 125 μg, 250 μg

Table 1. Changes in the weights (mg) of the accessory glands in the castrated, testosterone propionate (TP), and 5α-dihydrotestosterone (5α-DHT) treated lorises (Mean ±S.E.)

Treatment	Body weight (g)	Seminal vesicles	Prostate gland	Cowper's glands
Intact control	279(5)	293·4 ± 6·3	75·4 ± 14·9	104·0 ± 3·2
♂ for 15 days	252(3)	195·4 ± 9·0^c	55·2 ± 7·0^c	85·4 ± 12·4^c
♂ for 30 days	260(3)	192·1 ± 4·8^b	53·3 ± 3·6^b	83·9 ± 11·1^b
♂ + 500 μg of TP/day	241(5)	315·0 ± 38·4^b	137·0 ± 9·0^b	122·7 ± 9·2^b
♂ + 250 μg of TP/day	283(5)	393·0 ± 48·0^c	71·7 ± 3·5^a	174·1 ± 3·1^b
♂ + 125 μg of TP/day	259(5)	292·2 ± 89·7^a	70·7 ± 19·3^a	114·5 ± 13·7^a
♂ + 250 μg of DHT/day	285(5)	299·1 ± 25·6^a	71·3 ± 10·5^a	117·0 ± 18·0^a
♂ + 50 μg of DHT/day	284(5)	286·0 ± 20·4^a	56·5 ± 6·4	116·0 ± 21·5^a
♂ + 5 μg of DHT/day	272(5)	216·6 ± 10·4	58·9 ± 3·8	74·3 ± 7·9

Figures in parentheses represent the number of animals used in each group. Levels of significance compared with intact control animals. $^aP < 0·05$; $^bP < 0·01$; $^cP < 0·05$. ♂ = Castrated.

and 500 μg/day and of 5α- dihydrotestosterone (5α-DHT) were 5 μg, 50 μg and 250 μg/day. The androgens were administered subcutaneously in 0·2 ml of olive oil daily for 15 days from the next day of castration. Intact and castrated lorises of comparable body weights received the vehicle only and they served as controls.

The lorises were autopsied 24 hr after the last injection; one castrated group without androgen treatment was autopsied 30 days after castration. The accessory glands were removed, cleaned of fat and connective tissue and weighed to the nearest 0·1 mg in a torsion balance; fructose was estimated in the accessory glands by the method of Roe (1934) as modified by Linder and Mann (1960) and citric acid by the method of Ettinger *et al* (1952). The results were analysed statistically using student's *t* test.

3. Results

Response of the accessory glands of reproduction in the lorises to the administration of different dosages of TP/5α-DHT in terms of change in weight is shown in table 2. Changes in the content and concentration of fructose in the accessory glands resulting from castration and androgen replacement are shown in table 3. In loris, fructose is secreted by the prostate gland in maximum quantities. The seminal vesicles and Cowper's glands also secrete some quantities of the fructose. Castration for 15 days resulted in significant decrease of fructose concentration in the accessory glands and a further decrease was observed in 30-day castrated animals. More than 500 μg of TP or 5 μg of 5α-DHT/day maintained the fructose concentration in the seminal vesicles but there was a three-fold increase in the fructose concentration in the animals treated with 50 μg of 5α-DHT. Cowper's gland required 250 μg of TP or more than 50 μg of 5α-DHT for the maintenance of normal fructose concentration and in the prostate gland, it required 250 μg of TP/5α-DHT/day.

Changes in the levels of citric acid in the accessory glands of loris resulting from castration and androgen replacement is shown in table 3. Citric acid is mainly secreted

Table 2. Effect of administration of TP and DHT on the content and concentration of fructose in the accessory glands of the castrated slender loris (Mean ± S. E)

	Seminal vesicles		Prostate glands		Cowper's glands	
	A	B	A	B	A	B
Intact control	199·0 ± 2·8	69·4 ± 15·6	267·9 ± 99·2	140·3 ± 51·9	132·5 ± 11·7	79·2 ± 50·6
♂ for 15 days	149·6 ± 20·3[d]	42·5 ± 22·7[d]	137·7 ± 1·3[d]	84·0 ± 15·3[d]	94·5 ± 6·6[d]	54·8 ± 6·0[d]
♂ for 30 days	34·1 ± 4·4[d]	16·3 ± 12·3[d]	62·2 ± 9·4[d]	18·1 ± 8·0[d]	50·6 ± 19·5[d]	30·8 ± 15·0[d]
♂ + 500 μg of TP/day	300·0 ± 21·4[b]	131·8 ± 28·8[a]	302·9 ± 151·9[a]	126·2 ± 33·2	267·6 ± 121·3[a]	66·2 ± 12·7[c]
♂ + 250 μg of TP/day	241·2 ± 137·1[d]	64·1 ± 14·0[a]	218·2 ± 94·8	87·5 ± 24·0	157·4 ± 51·6[a]	63·0 ± 15·9
♂ + 125 μg of TP/day	214·8 ± 74·3	52·9 ± 5·7	97·1 ± 43·7	85·3 ± 29·9	108·2 ± 6·6	52·0 ± 7·9
♂ + 250 μg of DHT/day	267·2 ± 50·2[b]	179·1 ± 95·0[a]	690·4 ± 318·2[a]	347·9 ± 88·0[a]	282·0 ± 37·2[b]	288·9 ± 68·0[a]
♂ + 50 μg of DHT/day	213·0 ± 82·4[a]	162·2 ± 59·3[a]	164·5 ± 45·7	68·5 ± 2·6	144·3 ± 25·7[a]	69·9 ± 24·8[c]
♂ + 5 μg of DHT/day	203·0 ± 16·6[a]	55·7 ± 29·3	131·3 ± 47·4	54·8 ± 12·9	51·6 ± 19.5	23·8 ± 5·4

Levels of significance compared with intact control animals. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P \leqslant 0.05$, [d]$P < 0.05$ A = μg of Fructose/organ, B = μg of Fructose/100 mg of tissue.

Table 3. Effect of administration of TP and DHT on the content and concentration of citric acid in the accessory glands of the slender loris (mean ± S. E)

	Seminal vesicles		Prostate glands		Cowper's glands	
	A	B	A	B	A	B
Intact control	501·3 ± 282·3	430·1 ± 42·0	478·5 ± 136·6	375·8 ± 44·4	373·6 ± 223·1	195·0 ± 32·8
♂ for 15 days	174·6 ± 115·9[d]	129·5 ± 88·0[d]	166·1 ± 20·7[d]	134·9 ± 46·8[d]	133·7 ± 22·7[d]	65·0 ± 0·0[d]
♂ for 30 days	Absent	Absent	Absent	Absent	Absent	Absent
♂ + 500 μg of TP/day	481·5 ± 39·4[c]	481·9 ± 39·4[a]	131·3 ± 21·9	157·8 ± 0·0	302·0 ± 58·6	135·8 ± 2·1
♂ + 250 μg of TP/day	231·1 ± 105·1	129·8 ± 0·0	73·4 ± 25·0	126·2 ± 10·4	58·6 ± 12·1	126·3 ± 0·0
♂ + 125 μg of TP/day	110·9 ± 51·4	118·0 ± 7·0	46·3 ± 12·5	92·9 ± 16·0	42·5 ± 20·2	15·1 ± 2·0
♂ + 250 μg of DHT/day	799·6 ± 217·2[b]	143·8 ± 38·0	378·1 ± 26·6	353·6 ± 23·0	225·9 ± 24·9	310·4 ± 87·6[a]
♂ + 50 μg of DHT/day	437·9 ± 264·0	134·6 ± 94·9	167·9 ± 1·9	191·5 ± 9·0	101·3 ± 32·1	232·4 ± 58·8[a]
♂ + 5 μg of DHT/day	305·8 ± 55·3	71·6 ± 43·1	166·7 ± 1·5	107·8 ± 32·8	41·8 ± 10·4	78·6 ± 3·8

Levels of significance compared with intact controls [a] $P < 0.05$, [b] $P < 0.01$, [c] $P \leqslant 0.05$, [d] $P < 0.05$ A = μg of citric acid/organ. B = μg of citric acid/100 mg of tissue.

by the seminal vesicles in maximum quantities. Like fructose, citric acid in the accessory glands also depends on the androgens. Prostate and Cowper's glands also contribute citric acid to the seminal plasma in the slender loris. In the 15-day castrated animals the concentration of citric acid had decreased significantly and it was completely absent from the glands of 30-day castrated animals. Requirement of androgens for the maintenance of citric acid concentration by the different glands varied. 500 μg of TP or 250 μg of 5α-DHT maintained the citric acid concentration in the seminal vesicles while more than 500 μg of TP or more than 250 μg of 5α-DHT was required for the maintenance of the citric acid concentration in the prostate and Cowper's glands.

4. Discussion

A decrease in the weights and secretory activity of the male reproductive system of slender loris has been observed following castration (Manjula and Kadam 1980) for 15 days. These tissues showed a further significant decrease in weight after 30 days of castration. A similar decrease has been observed in laboratory rodents (Price and William Ashman 1961; Mann 1964; Gupta *et al* 1974), hamster (Ortiz 1953; Karkun *et al* 1974), and rhesus monkey (Dinakar *et al* 1974a,b).

Fructose has been found in the semen of man, monkey, ram, guinea pig, rat and other mammals (Mann 1964). Androgen is necessary for the production of fructose (Mann 1964; Gassner and Hopwood 1952) in the male reproductive system. Fructose is secreted mainly by the prostate gland in the slender loris, but the seminal vesicles and Cowper's gland also contribute. In the castrated guinea pig and rats (Harold and Clara 1955a,b) a lower dosage of testosterone (0·5μg) was sufficient to restore the fructose content. In the accessory glands of mouse, fructose level had decreased significantly following castration for 3 days (Mawhinney *et al* 1970). In the castrated loris, a higher dose of androgens, 500 μg of TP and 250 μg of 5α-DHT, was required by the prostate and Cowper's glands to restore the secretory activity in 15-day castrated animals. The seminal vesicles required 250 μg of TP and a lower dose of 5α-DHT (5 μg). Thus 5α-DHT appears to be more potent than TP in restoring and maintaining the secretory activity of the accessory glands. Similarly in the rhesus monkey, the seminal vesicles required four implants of 5α-DHT and eight implants of testosterone (Dinakar *et al* 1974a,b). The content of fructose in dorsolateral prostate was maintained at control levels with 500 μg of TP or 250 μg of 5α-DHT in rats (Gupta *et al* 1974). In loris, as in monkey and rat, 5α-DHT is more potent, whereas in hamster (Karkun *et al* 1974) testosterone appears to be more potent. This shows that testosterone and 5α-DHT were not equipotent in stimulating the production of fructose in the slender loris.

Citric acid is also an androgen-dependent parameter in the accessory glands. Most of the higher mammals (Barron and Huggins 1946a,b; Humphrey and Mann 1948, 1949; Schersten 1929, 1936), have a high concentration of citric acid in semen. A direct relationship exists between the plasma testosterone and citric acid in seminal plasma in man and it is a good index of androgen secreted by the gonads (Dondero *et al* 1972).

Decrease in the levels of citric acid in the seminal vesicles of rabbit following castration has been observed (Humphrey and Mann 1948) and it reappears following administration of testosterone. In the accessory glands of loris, castration for 15 days resulted in a significant decrease in the levels of citric acid and it was completely absent

in the seminal vesicles of 30-day castrated animals, prostate and Cowper's glands also showed significant reduction in the citric acid content. In the castrated loris, seminal vesicles required a low dosage of 5α-DHT (50 μg) and a high dosage of TP (500 μg) for the maintenance of citric acid content. The prostate and Cowper's glands required more than 500 μg of TP or 250 μg of 5α-DHT, of both the androgens, 5α-DHT is more potent in low doses than TP in maintaining citric acid content in the seminal vesicles thus indicating greater sensitiveness to low doses of 5α-DHT, than to testosterone itself.

Androstendione and 5β-androsten 3-01-17-one have been found in the seminal vesicles after administration of testosterone (Harding and Samules 1962; Kinson 1962; Evalkar *et al* 1964) and 5α-androstane 17-01-3-one is found bound to the macromolecular fraction of the homogenate of the seminal vesicles in the ventral prostate of the rat (Unjem and Tveter 1969). In the slender loris the results indicate that 5α-DHT is more potent than TP and hence it is possible that similar DHT binding sites to macromolecules are present in the accessory glands of loris also.

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