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EFFECTS OF MICROQUANTITIES OF TESTOSTERONE ON THE EPIDIDYMIS AND ACCESSORY GLANDS OF THE CASTRATED RHESUS MONKEY, *MACACA MULATTA*

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SUMMARY

Release of testosterone from silastic implants over a period of 90 days resulted in variable stimulation of the epididymis and accessory glands of reproduction in the castrated rhesus monkey. While the weights of the seminal vesicles, prostate gland and bulbo-urethral glands were maintained at the same level as the intact control animals by four or eight implants of testosterone, those of the epididymis and ductus deferens were not affected by either dose of testosterone.

Fructose in the seminal vesicles was stimulated significantly above intact control levels by eight implants of testosterone.

There was no regional variation in the levels of sialic acid in the caput, corpus and cauda epididymides, but the concentrations of phospholipid and total lipid were significantly higher in the caput epididymidis.

Our observations suggest there may be differential threshold requirements of androgens for the maintenance of the epididymis and accessory glands in the male rhesus monkey.

INTRODUCTION

Spermatozoa undergo physiological and morphological changes leading to their functional maturation during their transit through the epididymis (Orgebin-Crist, 1969; Hamilton, 1972). The epididymis is, therefore, an ideal extragonadal site for control of fertility in the male by selective alteration of its function. The factors that regulate the function of the epididymis have recently been elucidated in a few laboratory rodent species (Rajalakshmi & Prasad, 1968, 1969, 1971; Orgebin-Crist, 1969; Prasad, Rajalakshmi & Reddy, 1972; Hamilton, 1972; Prasad, Rajalakshmi, Gupta & Karkun, 1973), but have not been studied in detail in any primate, apart from the effects of foetal castration on the differentiation of the epididymal epithelium, and of vasectomy on the ultrastructure of the epididymis in adult rhesus monkeys (Alexander, 1972*a*, *b*).

The present studies constitute one of a series of investigations on the mechanisms regulating the functional integrity of the epididymis of the rhesus monkey, *Macaca mulatta*.

MATERIALS AND METHODS

Three subadult, male, rhesus monkeys (*Macaca mulatta*), weighing between 6 and 9 kg, were used for each experimental group. The animals were housed singly and were fed twice daily a standard monkey pellet produced by Hindustan Lever, India, supplemented by fresh fruit. They had unrestricted access to water. No special lighting régime other than natural light was provided.

Monkeys were castrated by the scrotal route, under aseptic conditions, using i.v. sodium pentobarbitone (Nembutal, Abbott Laboratories) (25 mg/kg body wt) as anaesthetic. Immediately after castration, four or eight silastic capsules containing testosterone (Sigma Chemical Co.) were inserted subcutaneously, using a modified trochar (gauge 10), in the subscapular and lumbar regions in groups of four capsules each.

The silastic capsules were prepared as follows: silicone rubber polymer (Medical Grade Silastic; Dow Corning, lot no. HH 1701, outer diameter 0·125 in, inner diameter 0·062 in) in 2·5-cm lengths, was sterilized and filled with approximately 15 mg testosterone; the ends were sealed with Silastic Medical Adhesive Silicone Type A, and the capsules were weighed and numbered. The position of the capsules was noted at the time of insertion and at autopsy. There was very little migration of the capsules during the experimental period. At autopsy, 90 days after castration, the capsules were removed, cleared of adhering tissue and dried to constant weight. From the difference in the weight of the capsules at the beginning and at the end of the experiment, the amount of hormone released per day was computed arithmetically. The control animals, intact and castrated, received sham-implants.

At autopsy, the epididymis, ductus deferens, seminal vesicles, prostate and bulbo-urethral glands were removed, cleared of fat and connective tissue in ice-cold 0·9 % saline solution and weighed to the nearest 0·2 mg on a Roller-Smith torsion balance. Sialic acid in the epididymis, ductus deferens and bulbo-urethral glands was estimated by the method of Warren (1959). Tissues were homogenized in 0·28 M-H_2SO_4 and hydrolysed at 80 °C for 1 h. Sialic acid was estimated from a known volume of the supernatant and the optical density read on a Beckman DB recording spectrophotometer. Since the epididymis, ductus deferens and bulbo-urethral glands of the rhesus monkey contain deoxyribose, the amount of sialic acid present was calculated using Warren's formula No. 2, applying a correction factor for 2-deoxyribose. Content of sialic acid is expressed as μmol sialic acid present in both sides of the organ concerned; concentration is expressed as μmol sialic acid/100 g tissue. Lipids from the epididymis were extracted (Folch, Lees & Sloane-Stanley, 1957) and total phosphorus was estimated by the modified method of Marinetti (1962). Total lipid was determined gravimetrically. Different phospholipid components were separated by thin-layer chromatography using the solvent system, chloroform:methanol:7·34 M-ammonia (230:90:15, by vol.) (Abramson & Blecher, 1964). Fructose from the seminal vesicles was estimated by the method of Roe (1934) as modified by Lindner & Mann (1960). The data were analysed statistically using Student's *t*-test.

RESULTS

The silastic capsules recovered at autopsy were encased in connective tissue sheaths. The average release rate of testosterone/capsule/day was 40–48 μg, the cumulative release rate being about 185 μg/day in the case of monkeys with four implants and about 325 μg/day in the case of those with eight implants.

Changes in the weight of the epididymis, ductus deferens and accessory glands after the different treatments are shown in Table 1. Ninety days after castration, the seminal vesicles showed a reduction in weight of 72 % compared with the intact controls, followed by the bulbo-urethral glands (68 %), the ductus deferens (58 %), the epididymis and the prostate gland (56 %).

Exposure to the lower dose of testosterone resulted in a marked increase in the weight of the bulbo-urethral glands, representing an increase of 83 % over the levels of the castrated controls; increases in the prostate gland (60 %), seminal vesicles (54 %) and ductus deferens (27 %) were also found. The weights of the bulbo-urethral glands and the prostate gland were maintained at the levels of the intact controls by four capsules of testosterone. In monkeys with eight implants, weights of the seminal vesicles, prostate and bulbo-urethral glands were maintained at the levels of the intact controls. The weights of the epididymis and the ductus deferens, on the other hand, did not increase above the levels of the castrated control animals after exposure to either level of testosterone.

Changes in the content and concentration of sialic acid in the different regions of the epididymis, the ductus deferens and the bulbo-urethral glands are shown in Table 2. In the intact controls, there was no significant difference in either the content or concentration of sialic acid in the different regions of the epididymis, but both were markedly less in the ductus deferens. The content of sialic acid in the different regions of the epididymis decreased after castration while the concentration was not changed. Castration resulted in a decrease in both the content and concentration of sialic acid in the ductus deferens. The content of sialic acid in the caput epididymidis and ductus deferens was partially maintained in monkeys with four implants and reached intact control levels with eight implants. The content of sialic acid in the corpus epididymidis was maintained at intact control levels in monkeys with four implants, while that of the cauda epididymidis was maintained at less than half that of the intact control levels with four or eight implants of testosterone.

The concentration of sialic acid in the caput and corpus epididymides was increased significantly above the intact control levels ($P < 0{\cdot}05$) in monkeys carrying eight implants, while in the ductus deferens it was maintained at intact control levels with four or eight capsules. There was no change in the concentration of sialic acid in the cauda epididymidis with four or eight capsules.

The content of sialic acid in the bulbo-urethral glands decreased significantly after castration ($P < 0{\cdot}05$) but was maintained at intact control levels with four or eight implants. The concentration of sialic acid in the bulbo-urethral glands did not change after castration or exposure to testosterone.

Changes in phospholipid and total lipid in the epididymis are shown in Tables 3 and 4. In the intact control monkeys the concentration of phospholipid in the caput epididymidis was higher ($P < 0{\cdot}05$) than in the cauda or corpus epididymides.

Table 1. *Maintenance of the epididymis and accessory glands of castrated, rhesus monkeys by testosterone implants*† (*means* ± S.E.M.)

Treatment group	Caput epididymides (mg)	Corpus epididymides (mg)	Cauda epididymides (mg)	Ductus deferens (mg)	Seminal vesicles (g)	Prostate gland (mg)	Bulbo-urethral glands (mg)
Castrated control	664·0 ± 182·8***	892·0 ± 248·2**	753·7 ± 154·1***	676·3 ± 183·9**	3·9 ± 1·6**	1829·7 ± 791·8*	124·0 ± 16·1*
Castrated + 4 implants	773·7 ± 250·6**	936·7 ± 117·5**	652·7 ± 132·1***	857·3 ± 153·5*	6·0 ± 1·2*	2920·3 ± 336·6	226·7 ± 60·6
Castrated + 8 implants	596·0 ± 89·2***	802·0 ± 69·9***	640·7 ± 41·8***	860·7 ± 37·2**	12·7 ± 1·7	3212·3 ± 332·5	265·3 ± 51·8
Intact control	1454·0 ± 172·7	2197·7 ± 441·0	1622·7 ± 240·6	1615·7 ± 346·7	13·9 ± 4·3	4166·0 ± 1030·7	392·7 ± 159·0

† Four or eight silastic capsules were implanted subcutaneously immediately after castration. Release rate of testosterone: 185 μg/day from four capsules and 325 μg/day from eight capsules.

Each group comprised three monkeys. Levels of significance compared with intact control animals: * $P > 0.1$; ** $P > 0.05$; *** $P < 0.05$.

Table 2. *Changes in content and concentration of sialic acid in the epididymis, ductus deferens and bulbo-urethral glands of castrated rhesus monkeys treated with testosterone implants* (*means* ± S.E.M.)

	Caput epididymidis		Corpus epididymidis		Cauda epididymidis		Ductus deferens		Bulbo-urethral glands	
Treatment group	Content	Concn.	Content	Concn.	Content	Concn.	Content	Concn.	Content	Concn.
Castrated control	0·76 ± 0·14**	122·60 ± 21·61	0·54 ± 0·18	81·53 ± 17·72	0·68 ± 0·17*	108·84 ± 3·67	0·28 ± 0·02***	26·60 ± 1·20	0·24 ± 0·06***	189·94 ± 26·13
Castrated + 4 implants	0·90 ± 0·20**	130·61 ± 22·65	1·55 ± 0·89	157·71 ± 84·71	0·67 ± 0·19*	99·65 ± 8·80	0·42 ± 0·08	50·74 ± 11·84	0·52 ± 0·16	223·91 ± 8·95
Castrated + 8 implants	1·32 ± 0·10	233·14 ± 43·45***	1·74 ± 0·13	223·83 ± 32·94***	0·77 ± 0·16*	117·55 ± 16·32	0·53 ± 0·03	61·29 ± 2·90	0·71 ± 0·13	281·43 ± 66·18
Intact control	1·39 ± 0·22	98·13 ± 19·27	2·04 ± 0·61	88·63 ± 10·88	1·84 ± 0·54	115·67 ± 17·26	0·69 ± 0·09	47·10 ± 18·11	0·75 ± 0·11	229·37 ± 53·76

Content expressed as μmol/organ; concentration expressed as μmol/100 g tissue.

Levels of significance compared with intact control animals: * $P > 0.1$; ** $P > 0.05$; *** $P < 0.05$.

Table 3. *Changes in content and concentration of phospholipid in the epididymis of castrated rhesus monkeys treated with testosterone implants* (*means* ± S.E.M.)

	Caput epididymidis		Corpus epididymidis		Cauda epididymidis	
Treatment group	Content	Concn.	Content	Concn.	Content	Concn.
Castrated control (3)	3·56 ± 0·95****	5·54 ± 1·31***	4·88 ± 1·91**	5·18 ± 0·60*	4·66 ± 1·20***	6·02 ± 0·72***
Castrated + 4 implants (3)	5·15 ± 1·97****	6·33 ± 0·80***	7·27 ± 1·56**	7·53 ± 0·97	5·98 ± 2·08**	8·79 ± 1·89
Castrated + 8 implants (3)	5·30 ± 1·37****	8·68 ± 1·32*	7·78 ± 0·40**	9·96 ± 1·26	3·73 ± 1·05***	6·06 ± 1·92
Intact control (5)	21·28 ± 0·88	13·00 ± 1·32	23·12 ± 4·71	8·55 ± 1·10	18·41 ± 2·66	9·54 ± 0·62

Content is expressed as mg/organ and concentration as mg/g tissue. Levels of significance compared with intact control animals: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$. Numbers in parentheses are the number of monkeys/group.

Table 4. *Changes in content and concentration of total lipid in the epididymis of castrated rhesus monkeys treated with testosterone implants* (*means* ± S.E.M.)

	Caput epididymidis		Corpus epididymidis		Cauda epididymidis	
Treatment group	Content	Concn.	Content	Concn.	Content	Concn.
Castrated control	44·89 ± 14·32*	65·74 ± 3·99	36·29 ± 8·53	45·17 ± 13·65	20·51 ± 4·71*	27·75 ± 1·90
Castrated + 4 implants	47·92 ± 7·47	87·50 ± 42·33	44·44 ± 4·72	50·64 ± 12·87	15·41 ± 2·73**	24·04 ± 1·74
Castrated + 8 implants	24·94 ± 4·25*	42·26 ± 6·50**	29·96 ± 1·06	34·96 ± 2·82	31·82 ± 18·11	52·68 ± 31·01
Intact control	126·97 ± 44·89	81·93 ± 14·15	60·47 ± 33·86	25·04 ± 6·64	45·74 ± 11·21	28·33 ± 5·16

Content expressed as mg/organ and concentration as mg/g tissue. Levels of significance compared with intact control animals: * $P > 0.1$; ** $P > 0.05$.

Table 5. *Changes in the pattern of individual phospholipids in the epididymis of castrated rhesus monkeys treated with testosterone implants*

Treatment group	... Castrated control			Castrated + 4 implants			Castrated + 8 implants			Intact control		
Fractions	a	b	c	a	b	c	a	b	c	a	b	c
PI + PS	0·32	5·49	2·61	12·22	5·78	7·88	3·84	8·57	5·30	6·00	6·77	12·08
LPC + LPE	1·41	11·21	6·13	3·12	2·70	5·28	3·12	1·75	4·82	1·84	2·65	2·32
SPH	16·38	21·16	13·85	15·67	20·25	15·44	13·03	10·96	13·52	15·88	13·48	10·70
PC	49·32	37·13	49·35	33·10	45·86	44·21	46·59	50·68	45·77	44·04	46·39	51·35
PE	30·48	22·44	24·76	30·85	24·79	21·74	26·92	28·10	25·88	27·29	26·35	22·82
PGP	0·80	3·36	3·30	3·17	0·00	3·96	3·33	1·63	2·34	3·06	3·15	2·47
PA	0·28	1·58	0·00	1·87	1·79	1·71	3·19	0·64	2·39	1·89	1·21	2·13

† a = Caput epididymidis; b = corpus epididymidis, c = cauda epididymidis. Abbreviations: PI + PS, phosphatidyl inositol + phosphatidyl serine; LPC + LPE, lysophosphatidyl choline + lysophosphatidyl ethanolamine; SPH, sphingomyelin; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PGP, polyglycerol phosphatide; PA, phosphatidic acid.

Values are expressed as percentage of the total phospholipid.

However, the differences in the content of phospholipid in the three regions were not statistically significant. Castration resulted in a marked, significant decrease in the content and concentration of phospholipid in all regions of the epididymis. The concentration of phospholipid in the cauda and corpus epididymides was maintained at intact control levels with four or eight implants. However, the concentration of phospholipid in the caput epididymidis and the content of phospholipid in all three regions was not maintained by either dose of testosterone.

The content of total lipid decreased markedly in the caput and cauda epididymides after castration and did not show any increase in monkeys with either four or eight implants. The content and concentration of total lipid in the corpus epididymidis and its concentration in the caput and cauda epididymides showed no change after castration or treatment with testosterone. The concentration of total lipid in the intact control animals was significantly higher in the caput ($P < 0{\cdot}05$) than in the corpus or cauda epididymides.

Table 6. *Changes in content and concentration of fructose in the seminal vesicles of castrated rhesus monkeys treated with testosterone implants* (*means* ± S.E.M.)

	Fructose	
Treatment group	Content (mg/organ)	Concentration (μg/100 mg tissue)
Castrated control	0·24 ± 0·11***	6·17 ± 0·90**
Castrated + 4 implants	1·50 ± 0·68NS	22·19 ± 8·45NS
Castrated + 8 implants	25·33 ± 8·00*	189·01 ± 46·90***
Intact control	2·09 ± 0·04	15·43 ± 2·04

Levels of significance compared with intact control animals: * $P < 0{\cdot}05$; ** $P < 0{\cdot}02$; *** $P < 0{\cdot}001$. NS, not significant.

Variations in the individual phospholipids are given in Table 5. In the intact monkey, high levels of phosphatidyl choline and phosphatidyl ethanolamine were present, followed by sphingomyelin, phosphatidyl inositol + phosphatidyl serine, lysophosphatidyl choline + lysophosphatidyl ethanolamine, polyglycerol phosphatide and phosphatidic acid. Neither castration nor administration of testosterone resulted in any changes in the ratios of the individual phospholipid fractions.

The content and concentration of fructose in the seminal vesicles are shown in Table 6. The content of fructose decreased markedly ($P < 0{\cdot}001$) after castration while the concentration was reduced to about 50% that of the intact control animals ($P < 0{\cdot}02$). The content and concentration of fructose were maintained at intact control levels in monkeys with four implants and were nearly 12 times those of the intact controls in animals with eight implants.

DISCUSSION

Our results show that castration results in a pronounced regression of the weight and secretory activity of the epididymis and accessory glands of the monkey, similar to the condition seen in laboratory rodents (Price & Williams-Ashman, 1961;

Mann, 1964). However, the rate of regression is slow and resembles the condition in the hamster (T. Karkun, M. Rajalakshmi & M. R. N. Prasad, personal communication).

Release of testosterone from silastic capsules causes a variable stimulation of the epididymis and accessory glands. The cumulative release rates are approximations which do not indicate the daily variations in the release of the hormone from the different capsules. A factor which may affect the rate of release of steroids may be the encapsulation of the silastic implants in connective tissue sheaths in monkeys, a condition not observed in the rat or hamster in our laboratory. Continuous exposure of an animal to hormones released from indwelling silastic capsules elicits a better response from the target organs than injection of hormones, the circulating levels of which are subject to fluctuations depending upon the metabolic clearance of the compound from the system. The levels of androgen released into the circulation from silastic capsules in the present study should be compared with the normal levels of testosterone in the systemic plasma (230–1200 ng/100 ml plasma) of adult male rhesus monkeys (Resko & Phoenix, 1972; Bennett, Dufau, Catt & Tullner, 1973). Resko (1967) could not detect any measurable amounts of testosterone by gas–liquid chromatography after castration of adult rhesus monkeys. Using radioimmunoassay, 186–613 pg testosterone/ml plasma were measured in monkeys 55 weeks after castration (Resko & Phoenix, 1972).

Our results indicate that the levels of androgen released from silastic capsules were high enough to stimulate the accessory glands. The weights of the bulbo-urethral glands and prostate gland were maintained at intact control levels with four capsules while that of the seminal vesicles was maintained with eight capsules. However, the weight of the different regions of the epididymis was not maintained even at 50 % of the control levels with either four or eight implants of testosterone.

The secretory activity of the seminal vesicles, on the other hand, was maintained at intact control levels with four implants while there was hyperstimulation to twelve times that of the intact control level with eight implants.

The epididymis of the monkey contains large amounts of sialic acid. Bose & Kar (1968) reported no change in the concentration of sialic acid in the epididymis 30 days after castration and obtained no increase after administration of 2 mg testosterone propionate/day for 21 days. Our studies also showed that the concentration of sialic acid in the different regions of the epididymis did not decrease even 90 days after castration. However, the content of sialic acid was significantly reduced in all the regions of the epididymis and in the ductus deferens. The content of sialic acid was maintained at intact control levels in the corpus epididymidis with four capsules and in the caput epididymidis and ductus deferens with eight capsules, whereas the content of sialic acid in the cauda epididymidis was maintained at less than half that of the intact control levels with four or eight capsules. These results indicate a differential stimulatory effect of testosterone released from silastic capsules on the accessory glands on the one hand, and the epididymis and ductus deferens on the other. While the weights of the prostate, seminal vesicles and bulbo-urethral glands were optimally increased with four capsules, that of the epididymis was not affected by testosterone. Similarly, the secretory activity of the epididymis showed a differential response in the maintenance of the secretory function of the caput

epididymidis, the corpus epididymidis and the ductus deferens while that of the cauda epididymidis was only marginally stimulated.

These results are in agreement with the observations of Prasad *et al.* (1973) who reported that the epididymis and the accessory glands of the hamster and the rat exhibit a differential response to exogenously administered testosterone in maintenance of weight and secretory activity. They concluded that the epididymis has a higher threshold requirement of androgen, particularly the cauda epididymidis, in comparison with the other regions of the epididymis, the ductus deferens and the accessory glands.

Differential thresholds of androgens seem to exist for maintenance of different cell organelles in the epididymis of rat; stereocilia require much lower levels of androgen to maintain their structural integrity than is required to maintain the secretory activity of the epithelium (Rajalakshmi, Singh & Prasad, 1971).

Our results show that the concentrations of phospholipid and total lipid were greater in the caput epididymidis than in the corpus or cauda epididymides of the intact monkey. The decrease of phospholipid from the caput towards the cauda epididymidis may be due to utilization of phospholipids by the spermatozoa of the monkey as a source of energy similar to that found in the bull and ram (Lardy & Phillips, 1941; Hartree & Mann, 1961; Scott, Voglmayr & Setchell, 1967). High levels of phospholipid in the caput epididymidis indicate that this region is the site of maximal synthesis of glycerylphosphoryl choline. Castration resulted in a marked reduction in the content and concentration of phospholipid and content of total lipid in all regions of the epididymis. While the content of phospholipid in the caput and corpus epididymides was maintained at less than one-third the intact control levels with four or eight implants, the concentration of phospholipid in the corpus and cauda epididymides was maintained at control levels with four implants. These results are similar to the observations of Turner & Johnson (1971) and Prasad *et al.* (1972) who reported a decrease in phospholipid in the rat epididymis after ligation of the efferent duct.

The failure of testosterone to maintain the content of phospholipid and total lipid in the epididymis may be due to the decreased weights of the different regions of the epididymis and absence of spermatozoa in animals with four or eight implants. In the absence of spermatozoa in the epididymis, microdoses of androgen maintained the concentration of phospholipid at the same level as in the intact controls only in the corpus and cauda epididymides. The difference in the response of the various regions of the epididymis may mean that the synthetic and secretory activities of the caput epididymidis are affected to a greater degree by the presence of spermatozoa and testicular fluid than by the presence of androgens. The corpus and cauda epididymides on the other hand appear to be more dependent on the presence of androgen for the maintenance of their functional integrity than on the presence of spermatozoa. These results are similar to the observations of Gustafsson (1966) and Prasad *et al.* (1973).

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