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Effect of Cyproterone Acetate on Structure and Function of Rhesus Monkey Reproductive Organs

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ABSTRACT A low dose of Cyproterone acetate (CPA; 1 mg/kg body weight/day for 70 days) was administered to adult male rhesus monkeys to assess its effects on testicular and epididymal structure and function in a nonhuman primate species. CPA caused extensive degenerative changes in morphology of seminiferous, efferent duct, and epididymal epithelia, including decrease in diameter of seminiferous and epididymal tubules and their lumen, height of epididymal epithelium, and an increase in intertubular connective tissue. The protein profile of spermatozoa showed alterations during their epididymal transit in control and CPA-treated monkeys. In CPA-treated animals, 19 polypeptides were acquired and nine were eliminated during epididymal transit in contrast to acquisition of 12 and loss of 14 polypeptides in control animals. Treatment with CPA also resulted in the appearance of 14 new polypeptides in epididymal cytosol and luminal fluid, probably of lysosomal origin. The protein pattern of caput and cauda epididymal tubule cytosol, maintained in organ culture and exposed to 100 μM CPA for 3 days, showed absence of eight polypeptides.

These results indicate that even at the low dose used in this study, CPA has caused spermatogenic arrest, degenerative changes in the epididymal structure, and alterations in epididymal and sperm protein profile. Suppression of serum testosterone levels indicates the need for androgen supplementation if CPA is to be used for male contraception.

Immature testicular spermatozoa of mammals undergo changes in morphology, surface properties, and biochemical composition during epididymal transit, resulting in acquisition of progressive motility and ability to recognize zona and undergo fertilization reactions (Robaire and Hermo, 1988). These changes are related to the epididymal microenvironment, which is formed by the absorptive and secretory functions of the epididymal epithelium. Epididymal proteins have been implicated in the process of sperm maturation in rodents, farm animals, and men (Eddy et al., 1985). In contrast, only one report, using denaturing conditions of electrophoresis, records the changes in protein composition of spermatozoa during epididymal maturation or the role of epididymal proteins in this process in nonhuman primates (Young et al., 1985). Limited information is reported (Haider et al., 1983; Arslan et al., 1986) on the gross changes in protein composition of tissue cytosol of different segments of monkey epididymis using nondenaturing conditions of electrophoresis.

In the evaluation of new fertility regulating agents for the male, preclinical assessment of the drug of choice needs to be carried out in a nonhuman primate model before the drug can be cleared for human use since extrapolation of data obtained in rodents is of limited relevance for clinical trials. The rhesus monkey was used in the present study since baseline data was available on the hormone profile and semen parameters in this species. The close similarity in the pattern of metabolic capabilities of human and rhesus monkey ejaculated spermatozoa (Rajalakshmi et al., 1983) and the use of this species in the pharmacokinetic and pharmacodynamic evaluation of newly developed long acting androgen (Rajalakshmi and Ramakrishnan, 1989) also favoured the use of rhesus monkey as the animal model for the present study. But our knowledge of the structure and the functions of the epididymis of rhesus monkey is limited (Arslan et al., 1976; Prakash et al., 1979; Ramos and Dym, 1979) compared to similar information available for rodents even though the epididymis is considered as an ideal extragonadal site for targeting male fertility regulating agents.

Cyproterone acetate is a potent antiandrogen and a number of studies in rodents indicate that it exerts adverse effects on different aspects of male reproductive functions (Prasad and Rajalakshmi, 1977; Ratnasooriya, 1982). Since CPA was in use in the treatment of male hypersexuality (Davies, 1974), acne (Cunliffe et al., 1969), and hirsuitism (Hammerstein and Cupceancu, 1969) and had evoked no adverse side effects even at high doses, limited clinical trials were conducted using low doses of CPA as a male contraceptive. But the results of these trials did not support the data obtained in animals; Koch et al. (1976) and Roy et al.

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(1976) reported inhibition of motility of ejaculated spermatozoa and reduction in glycerylphosphoryl choline in seminal plasma and the appearance of immature and morphologically abnormal spermatozoa in increasing numbers in the ejaculate. These results could be due to the effects of CPA either on inhibition of spermatogenesis, or epididymal sperm maturation or a combined effect on both testicular and epididymal structure and function.

One of the drawbacks in using CPA for male contraception in humans had been the decrease in circulating testosterone levels even though Phase I clinical trials did not report adverse effects on libido and sexual function (Koch et al., 1976; Roy et al., 1976). However, any contraceptive formulation that decreases serum testosterone levels would be unacceptable for family planning programs, particularly in developing countries, due to its implications on nitrogen balance in subjects of marginal nutritional status, as is prevalent in these countries. The development of a new and long-acting androgen ester of testosterone (Rajalakshmi and Ramakrishnan, 1989) with highly promising pharmacokinetic profile has renewed interest in the use of CPA for male contraception in combination with this androgen ester when it is cleared for clinical use. Before such trials can be undertaken in human volunteers, it was essential to evaluate the effects of a low dose of CPA on spermatogenesis, morphology of epididymal epithelium, changes in polypeptide profile of spermatozoa during epididymal maturation and the role of epididymal proteins in this process. These in vivo studies were complemented with in vitro experiments in which the protein profile of cytosol of epididymal tubules in culture was analyzed and the data compared with the changes caused by exposure of explants to CPA.

MATERIALS AND METHODS

Animals

Fourteen adult male rhesus monkeys (*Macaca mulatta*), weighing 8–10 kg, were procured from jungles ~250 km east of Delhi and quarantined in the Primate Research Facility of AIIMS for 3 months. The animals were subjected to tuberculin test once in 15 days and dewormed. Hematological analyses were done once a month. The animals were allowed to acclimatize in the Experimental Animal Facility for 15 days. Blood samples were collected at 10 A.M. and 10 P.M. on day 16 and the presence of nocturnal rise in serum testosterone, indicative of sexually active animals, was confirmed before the start of experimentation. The animals were electroejaculated (Mastroianni and Manson, 1963) and spermiogram studied (Belsey et al., 1980) to confirm their sexual maturity.

Protocol of Experiment

Eight animals were used for in vivo and six for in vitro studies.

In vivo studies

Two groups of four animals each were treated during November-January as follows: Group 1: untreated monkeys injected intragluteally 0.2 ml of benzyl benzoate: olive oil (1:50) for 70 days, and Group 2: CPA (1 mg/kg body weight/day) injected intragluteally in 0.2 ml of benzyl benzoate:olive oil for 70 days.

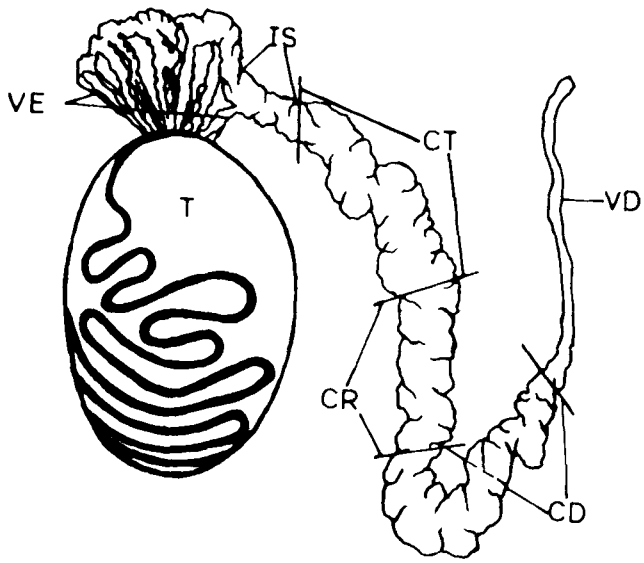


Fig. 1. Schematic diagram of the division of monkey epididymis into initial segment, caput, corpus and cauda epididymides. T, testis; VE, Efferent duct; IS, initial segment; CT, caput epididymidis; CR, Corpus epididymidis; CD, cauda epididymidis; VD, vas deferens.

Blood was collected at 10 A.M. on day 71, and the animals were castrated (testes and epididymides removed) under ketamine (Themis Chemicals Ltd., India; 10 mg/kg body weight) anaesthesia.

Histology of the epididymis

The epididymis was divided into initial segment (1 cm), caput (2.2 cm), corpus (2.5 cm), and cauda (3.5 cm) epididymides (Fig. 1). Pieces of epididymal segments, efferent duct, and testis were fixed in Karnovsky's fixative, processed for histology, and embedded in araldite. Sections 1.5–2.0 μm in thickness were stained with toluidine blue. Tubular and lumen diameter, height of epithelium, and width of intertubular connective tissue were measured (100 measurements/tissue) for each parameter. Statistical analyses were done by Student's t-test.

Protein analysis

The contralateral epididymis was divided into different segments and connective tissue layers removed mechanically under stereomicroscope. The tubules were cut into pieces in 0.05 M Tris hydrochloric acid (Tris-HCl) buffer (pH 7.2) containing 1 mM ethylene diamine tetraacetate (EDTA) and gently stirred to release spermatozoa. The tubule pieces were removed and the fluid centrifuged at 2,000 rpm for 20 minutes. The supernatant containing luminal fluid was stored at −20°C as aliquot. The sperm pellet was washed with buffer until the supernatant was clear, resuspended in buffer, and known concentration of sperm were aliquoted and kept at −20°C. The purity of sperm suspension and luminal fluid was confirmed under microscope. The tubule pieces were repeatedly washed in buffer until free of spermatozoa and homogenized using a Polytron homogeniser. The homogenate was centrifuged at 105,000 × g for 90 minutes and the cytosol stored as aliquot at −20°C.

Proteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE; Laemmli, 1970) with necessary modifications. Equal volume of cold 2% trichloroacetic acid was added to the aliquot and incubated for 1 hour at 4°C followed by sequential washing twice with double distilled water and once with ethanol. The precipitate was dissolved in 0.625 M Tris-HCl buffer (pH 7.2) containing 1 mM EDTA, 2% SDS, 5% B-mercapto-ethanol and 40% sucrose, heat denatured at 90°C for 10 minutes in a water bath followed by loading of 165 μg protein on 10% acrylamide gels. Electrophoresis was done using 0.025 M Tris-glycine buffer (pH 8.3) as the electrode buffer. The gels were stained with Coomassie Brilliant Blue R 250 and destained with 10% acetic acid at room temperature. Serum and molecular weight markers were processed simultaneously.

In vitro studies: Organ culture of epididymal tubules

Caput and cauda epididymidal tubules were cultured (Kaur et al., 1991) for 4 days. The explants, exposed to 100 μM of CPA for 72 hours in culture, were processed for protein analysis.

Protein analysis of cultured epididymidal tubules

Control and explants exposed to CPA were homogenized, centrifuged at 105,000 × g for 90 minutes, and the cytosol obtained was aliquoted and stored at −20°C. Proteins were separated by SDS-PAGE. Serum, culture medium, and molecular weight markers were processed simultaneously.

RESULTS

The rhesus monkey testis consists of seminiferous tubules with characteristic distribution of germ cells and Sertoli cells. The interstitial tissue contains Leydig cells, blood vessels, nerve fibers, and lymphatic tissue (Fig. 2a).

The efferent duct epithelium consists of ciliated and nonciliated cells with occasional basal cells (Fig. 3a). The nonciliated cells are short and columnar with basal nuclei. The microvilli at the luminal surface are arranged to resemble a brush border. The goblet-shape ciliated cells have apical nuclei with distinct nucleoli; cilia are prominent at the apical end.

The epididymal epithelium consists predominantly of principal cells along with few basal and apical cells whose distribution varies in different regions. The principal cells are tall and columnar. The nuclei with prominent nucleoli are seen in the basal third of the cells in the initial segment and caput epididymidis (Figs. 4a, 5a) and more toward the middle portion of principal cells in corpus and cauda epididymides (Figs. 6a, 7a). Nuclear infoldings that begin to appear in caput epididymidis become prominent in corpus and cauda epididymides. Supranuclear cytoplasm has abundant empty spaces in the initial segment and caput epididymidis, but these decrease in corpus and cauda epididymides. The apical region in the initial segment and caput epididymidis has dense granules and pinocytotic vesicles. In the initial segment, the stereocilia are very long, joined together at the apical end, and extend into the lumen, almost obliterating it. The height of stereocilia gradually decreases from caput epididymidis onward and resembles a brush border in the cauda epididymidis. An increase in the number of spermatozoa in the lumen is seen from initial segment to cauda epididymidis. Cauda epididymidal epithelium is characterized by the presence of intraepithelial crypts, formed by the folding and fusion of its epithelium. The basal cells increase in number from initial segment to cauda epididymidis. Apical cells are few in all regions.

Measurements of different morphometric parameters of epididymal regions indicated the occurrence of a gradual decrease in height of epithelium, increase in diameter of tubules and their lumen, and width of intertubular connective tissue from initial segment to cauda epididymidis (Table 1).

Effect of CPA on the Histology

Spermatogenesis is adversely affected by CPA (Fig. 2b). All stages of spermatogenesis except spermatogonia and Sertoli cells are exfoliated into the lumen of the seminiferous tubules. The cytoplasm of Sertoli cells contains metachromatically stained droplets that may represent lipid material. Seminiferous tubular diameter has decreased significantly ($p < 0.001$). The basal lamina appears wavy.

CPA treatment resulted in degenerative changes in the efferent ducts (Fig. 3b). These include extensive vacuolation and desquamation of cells, nuclear pycnosis, and loss of cellular demarcation.

CPA caused loss of epithelial integrity in the majority of initial segment tubules. Only pale euchromatic nuclei with nucleoli are seen distributed without any orderly arrangement (Fig. 4b). In the few tubules where epithelium is still present, the vacuolated cytoplasm contains degenerating material. The lumen contains desquamated epithelium.

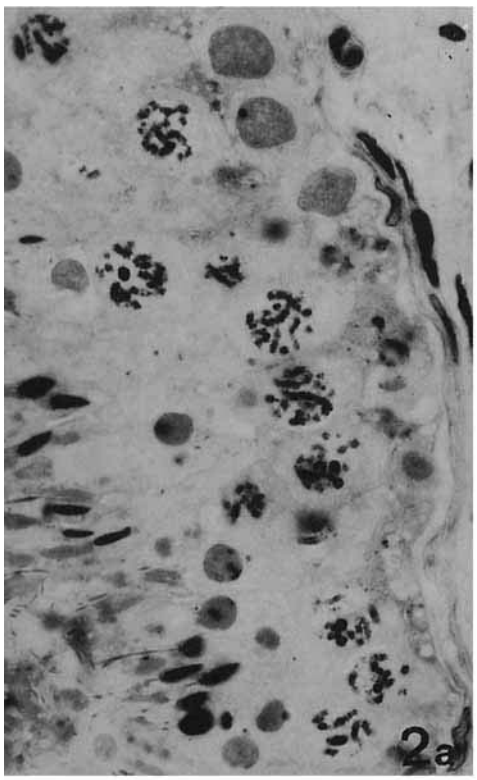
In caput and corpus epididymides, the changes seen are: (1) a decrease in height of epithelium, (2) degeneration of epithelium with loss in luminal margin in majority of tubules, (3) extensive vacuolation of infra- and supranuclear cytoplasm, and (4) pycnosis of majority of principal cell and basal cell nuclei (Figs. 5b, 6b). The nuclear infoldings seen in normal animals are almost completely absent in treated animals.

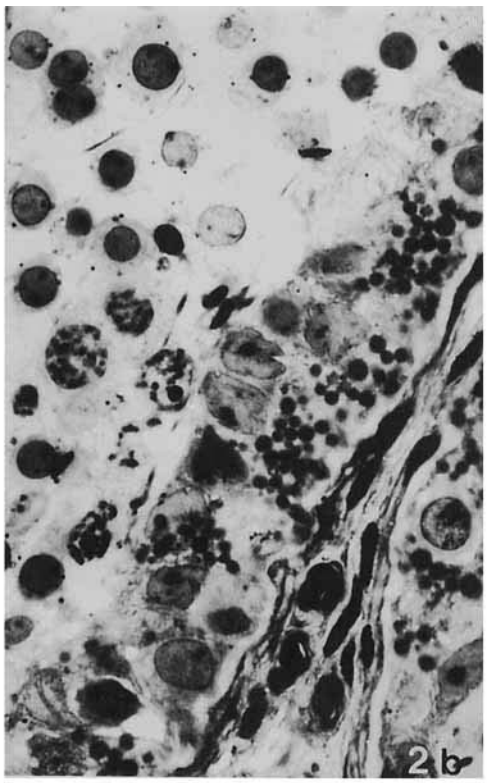
The effects of CPA in the cauda epididymidis are: (1) degeneration of principal cell cytoplasm including vacuolation and accumulation of darkly staining degenerative material (Fig. 7b), (2) decrease in nuclear infoldings, and (3) occasional pycnosis of principal and basal cell nuclei.

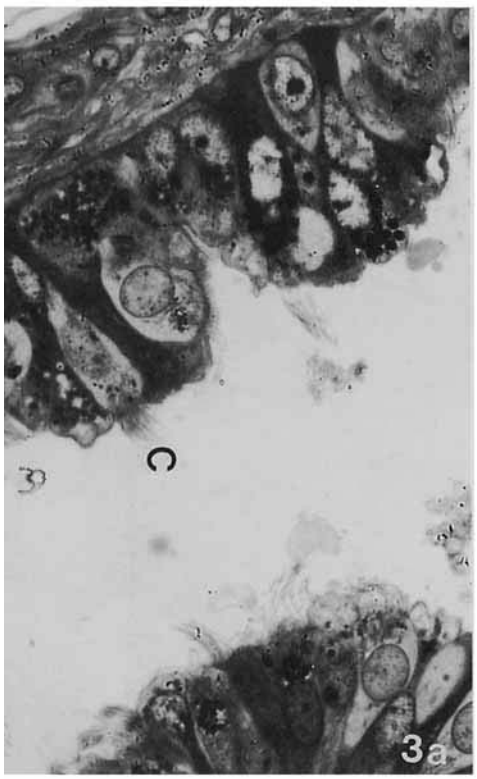
The diameter of the tubule and the lumen and height of epithelium of all regions decreased in treated animals (Table 1). The lumen in caput, corpus, and cauda epididymides contain desquamated cells and spermatozoa. The intertubular connective tissue of all regions of epididymis of treated animals increased significantly ($p < 0.01$; Table 1) and showed increased affinity for toluidine blue. This effect was less in cauda epididymidis.

Effect of CPA on Polypeptide Profile

The polypeptide pattern of the spermatozoa during epididymal transit in CPA treated monkeys (Fig. 8) was analyzed in relation to that of luminal fluid and cytosol in treated and control animals (Table 2). Only those polypeptides that were absent in serum were con-







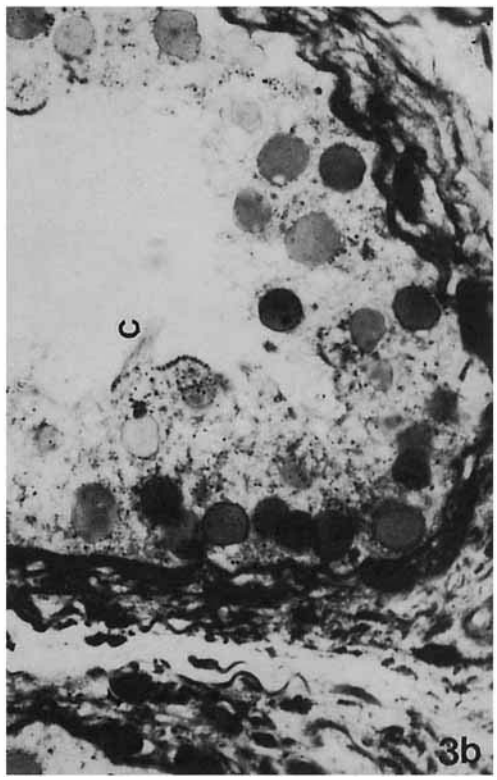
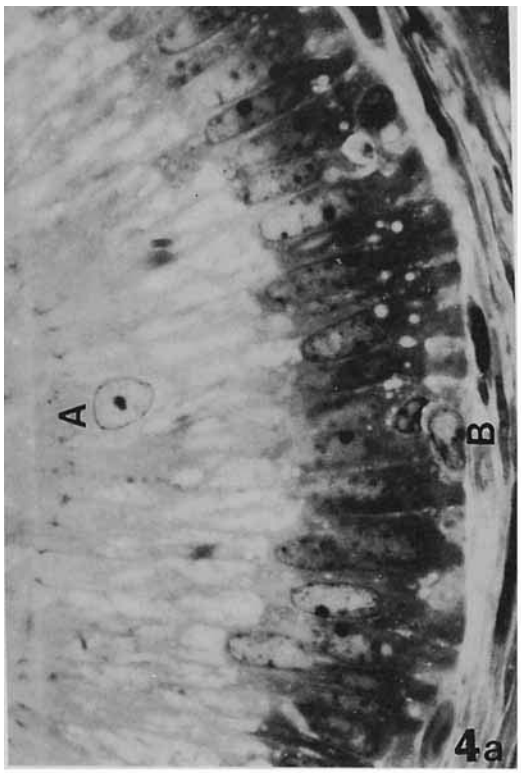


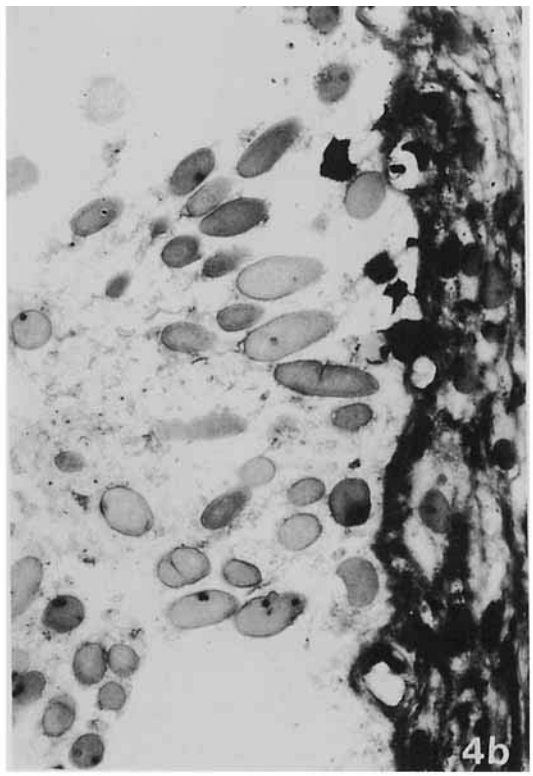
Fig. 2a. Histology of the seminiferous tubule of normal rhesus monkey showing Sertoli cells, germ cells in various stages of development and spermatozoa in the lumen. ×1000.

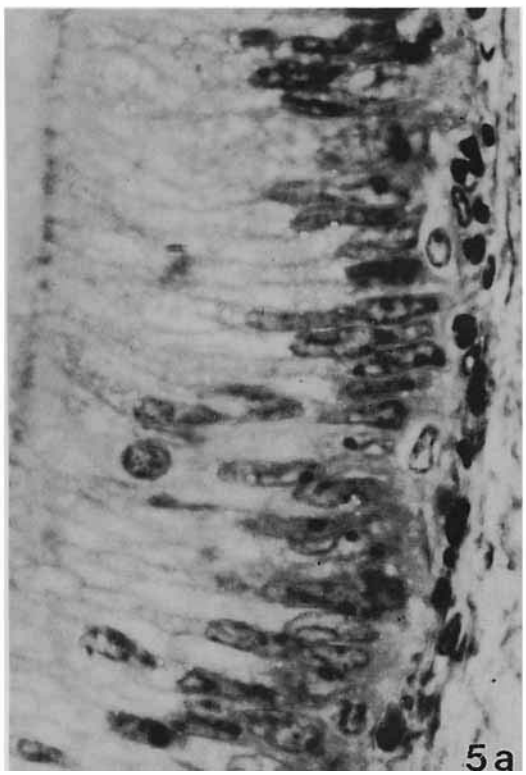
Fig. 2b. Histology of the seminiferous tubule of monkey treated with cyproterone acetate (1 mg/kg body weight/day for 70 days). Note that only spermatogonia and Sertoli cells are resting on the basal lamina. Spermatocytes and spermatids are exfoliated into the lumen. × 1000.

Fig. 3a. Histology of the epithelium of the efferent duct from normal monkey. Both ciliated (C) and non-ciliated cells are visible. × 1000.

Fig. 3b. Histology of the efferent duct epithelium of monkey treated with cyproterone acetate. Note the degeneration of both ciliated and non-ciliated cells. Cilia (C) is retained by one of the cells. × 1000.







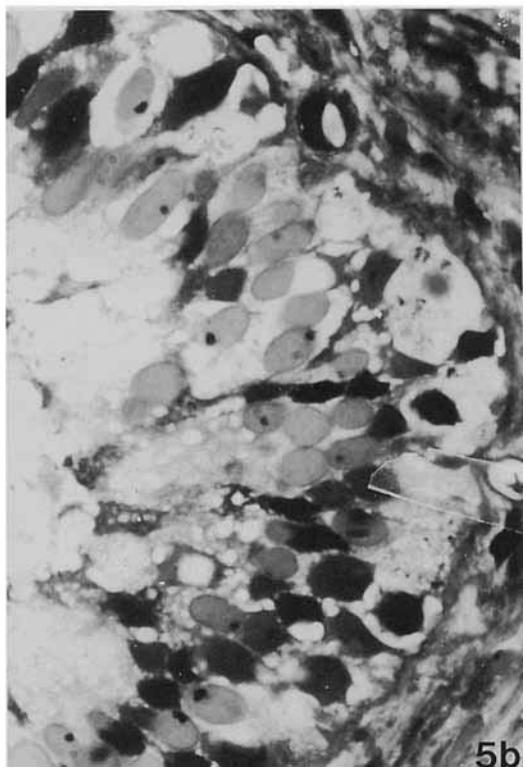
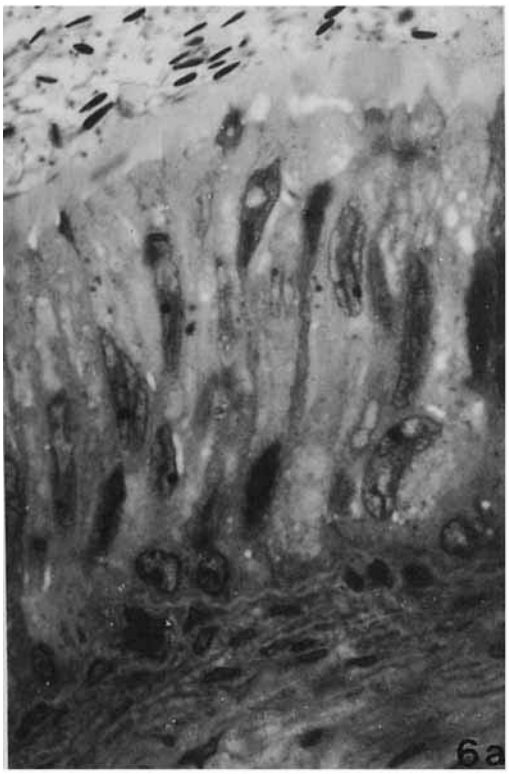


Fig. 4a. Histology of the initial segment of the epididymis from normal monkey showing the tall columnar principal cells with the supranuclear cytoplasm showing vacuolation. A basal cell (B) and apical cell (A) are also visible. × 1000.

Fig. 4b. Histology of the initial segment of monkey treated with cyproterone acetate. The epithelium is almost completely lost and only the nuclei are visible. The connective tissue stains darkly. × 1000.

Fig. 5a. The histology of the pseudostratified epithelium of caput epididymidis of a normal monkey. Apical and basal cells are clearly visible. × 1000.

Fig. 5b. Histology of the caput epididymidis from monkey treated with cyproterone acetate showing degeneration of the epithelium. The cytoplasm is vacuolated in infra- and supra-nuclear areas. Connective tissue stains darkly. × 1000.



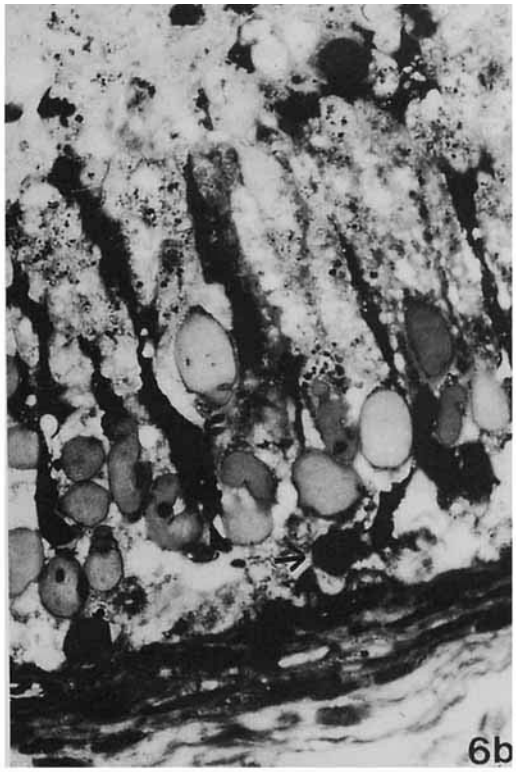
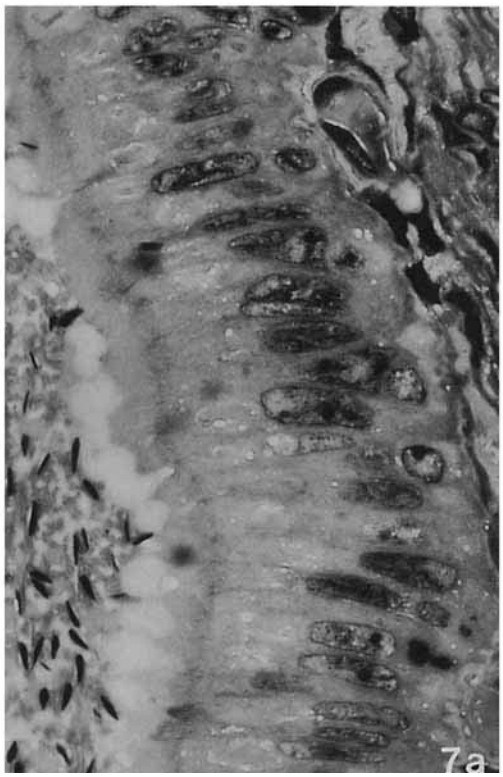


Fig. 6a. The histology of the pseudostratified epithelium of corpus epididymidis of normal monkey. The nuclei are infolded. Spermatozoa are seen in the lumen. × 1000.

Fig. 6b. High power magnification of epithelium of corpus epididymidis of monkey treated with cyproterone acetate. The cytoplasm of the cells show extensive vacuolation. The basal cells (arrow) stains darkly. Decrease in nuclear infoldings is seen. × 1000.



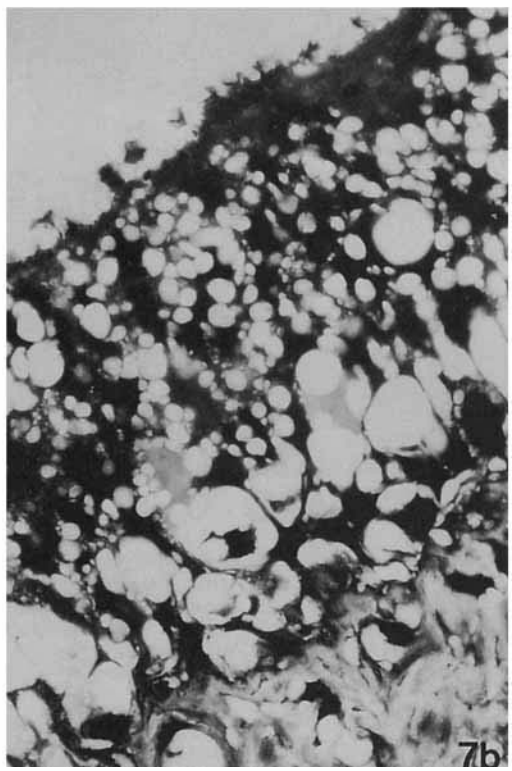


Fig. 7a. Histology of the epithelium of cauda epididymidis of normal monkey. The height of the epithelium and stereocilia have decreased. Basal cells are clearly visible. The lumen contains large number of spermatozoa. × 1000.

Fig. 7b. The histology of the cauda epididymal epithelium from monkey treated with cyproterone acetate showing varying degrees of degeneration of the epithelium. × 1000.

TABLE 1. Morphometric parameters of the epididymis in control and CPA-treated monkeys

Parameter (μm)	Groups	Epididymis			
		Initial segment	Caput	Corpus	Cauda
Epithelial height	Control	96.1 ± 9.8	76.5 ± 3.6	52.9 ± 9.8	33.3 ± 12.8
	CPA treated	NM	45.1 ± 7.8	35.3 ± 4.9	22.6 ± 2.9
Tubule diameter	Control	210.5 ± 7.0	231.6 ± 21.1	287.7 ± 21.0	364.9 ± 35.1
	CPA treated	196.5 ± 7.0	210.5 ± 14.0	217.5 ± 28.1	235.1 ± 35.0
Lumen diameter	Control	86.6 ± 3.8	111.0 ± 5.7	165.6 ± 7.9	327.4 ± 22.6
	CPA treated	NM	97.8 ± 15.4	109.5 ± 11.3	124.2 ± 82.8
Intertubular connective tissue diameter	Control	13.5 ± 2.1	15.4 ± 1.9	28.9 ± 3.1	59.8 ± 3.9
	CPA treated	27.0 ± 1.7	38.8 ± 3.9	47.4 ± 2.4	137.8 ± 19.3

NM = could not be measured.

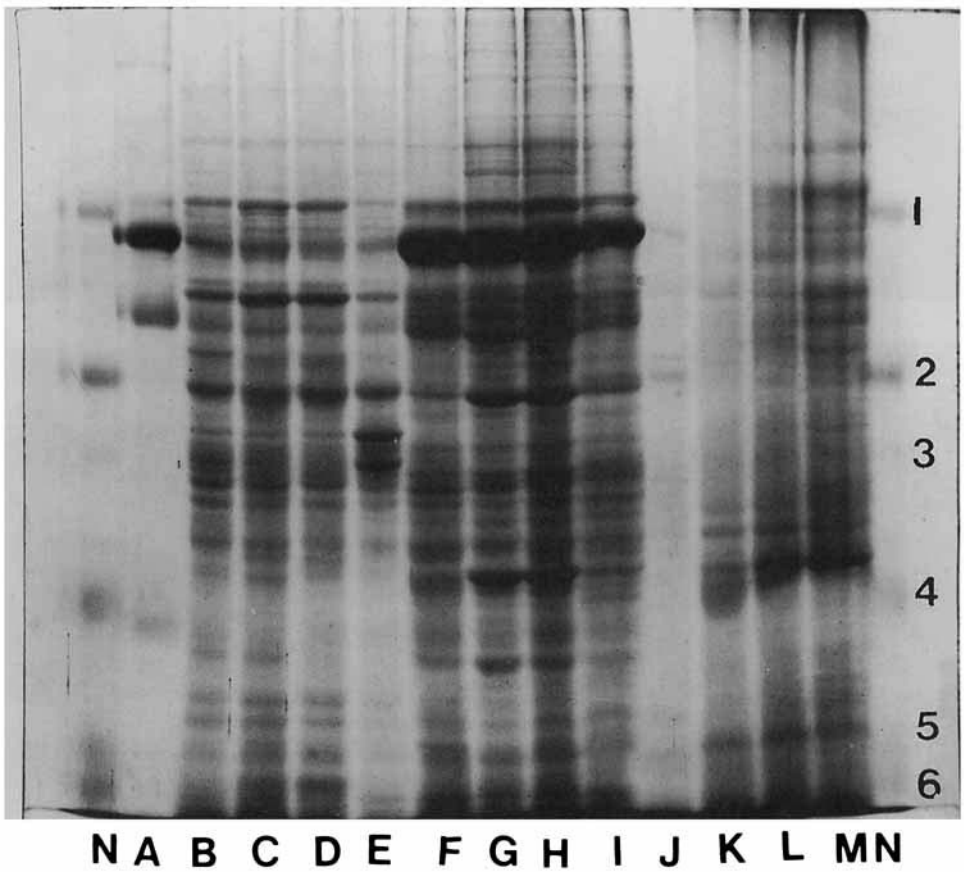


Fig. 8. Polypeptide pattern of cytosol, luminal fluid and spermatozoa from epididymis of monkey treated with cyproterone acetate (1mg/kg body weight/day for 70 days). A, Serum; B, Initial segment cytosol; C, Caput epididymidal cytosol; D, Corpus epididymidal cytosol; E, Cauda epididymidal cytosol; F, Initial segment luminal fluid; G, Caput epididymidal luminal fluid; H, Corpus epididymidal luminal fluid; I, Cauda epididymidal luminal fluid; J, Initial segment spermatozoa; K, Caput epididymidal spermatozoa; L, Corpus epididymidal spermatozoa; M, Cauda epididymidal spermatozoa; N, Molecular weight markers; 1) Lysozyme = 14.3 Kdal; 2) B-Lactalbumin = 18.4 Kdal; 3) Trypsinogen = 24.0 Kdal; 4) Pepsin = 34.2 Kdal; 5) Egg albumin = 45.0 Kdal; 6) Albumin bovine plasma = 66.0 Kdal.

sidered for this analysis. The changes in sperm associated polypeptides were classified as: (1) acquisition, (2) elimination, and (3) modification.

Acquisition

In CPA-treated animals, 19 polypeptides are acquired by spermatozoa during epididymal transit as compared to only 12 in controls. Of these, five (MW 16.5, 26.0, 26.5, 49.5, and 77.0 Kd) are acquired in caput, four (MW 19.8, 44.5, 53.0, and 70.0 Kd) in corpus, and 10 (MW 14.0, 15.0, 21.5, 25.5, 29.0, 33.5, 37.5, 38.5, 40.0, and 43.5 Kd) in cauda epididymides. Polypeptides of MW 26.5, 38.5, and 44.5 Kd appear to be mainly of testicular origin; additionally, polypeptides of MW 26.5 and 38.5 Kd are also present in cauda epididymidal cytosol. Polypeptide of MW 29.0 Kd is

TABLE 2. Effect of cyproterone acetate on the polypeptide pattern of cytosol, luminal fluid and spermatozoa from monkey epididymis[1]

Approximate molecular weight(Kd)	Cytosol[2]				Luminal fluid				Spermatozoa				Remarks[3]
	IS	CT	CR	CD	IS	CT	CR	CD	IS	CT	CR	CD	
13.5	−	−	−	−	−	−	−	−	−	+	+	+	VII
14.0	+	+	+	−	+	+	+	−	−	−	−	+	III
14.5	+	+	+	+	+	+	+	+	+	−	−	−	IV
15.0	+	+	+	+	+	+	+	+	−	−	−	+	III
15.5	−	−	−	−	−	−	−	−	−	−	−	+	VII
16.5	+	+	+	+	+	+	+	+	−	+	+	+	I
17.5	−	−	−	−	+	−	−	−	+	−	−	−	IV
19.8	+	+	+	+	+	+	+	+	−	−	+	+	II
21.5	+	+	+	+	+	+	+	−	−	−	−	+	III
25.5	+	+	+	−	+	+	+	−	−	−	−	+	III
26.0	+	+	+	+	+	+	+	+	−	+	+	+	I
26.5	−	−	−	+	+	+	+	+	−	+	+	+	I
29.0	+	−	−	−	−	+	+	−	−	−	−	+	III
30.0	−	−	−	−	−	−	−	+	−	−	−	+	VII,VI
33.0	+	+	+	+	+	+	+	+	+	−	−	−	IV
33.5	+	+	+	+	+	+	+	+	−	−	−	+	III
35.5	−	−	−	−	−	−	−	−	−	−	−	+	VII
37.5	+	+	+	+	−	−	−	+	−	−	−	+	III
38.5	−	−	−	+	+	+	+	+	−	−	−	+	III
40.0	+	+	+	+	−	−	−	+	−	−	−	+	III
43.5	+	+	+	+	+	+	+	+	−	−	−	+	III
44.5	−	−	−	−	+	+	+	+	−	−	+	+	II
46.5	−	−	−	−	+	+	+	+	+	−	−	−	IV
49.5	−	+	+	+	−	+	+	−	−	+	+	+	I
50.0	−	−	−	−	+	+	+	+	+	−	−	−	IV
53.0	+	+	+	+	−	−	−	−	−	−	+	+	II
54.0	−	−	−	−	+	+	+	+	+	−	−	−	IV
69.0	−	−	−	−	−	−	−	+	−	−	−	+	VII,VI
70.0	+	+	+	+	−	+	+	+	−	−	+	+	II
77.0	+	+	+	−	−	+	+	−	−	+	−	−	I,V

[1]Only those polypeptides that show sperm associated changes are given.
[2]IS = initial segment; CT = caput epididymidis; CR = corpus epididymidis; CD = cauda epididymidis.
[3]I = acquired in CT; II = acquired in CR; III = acquired in CD;IV = shed in CT; V = shed in CR; VI = shed in CD; VII = surface modification.
+ present, − absent.

present in the initial segment only while those of MW 14.0, 25.5, and 77.0 Kd are present in cytosol of all regions except the cauda epididymidis. Polypeptide of MW 49.5 Kd is absent only in the initial segment. The cytosol of all regions of epididymis contains 15.0, 16.5, 19.8, 21.5, 26.0, 33.5, 37.5, 40.0, 43.5, 53.0, and 70.0 Kd polypeptides.

Elimination

Only nine polypeptides are lost by spermatozoa in treated monkeys as compared to 14 in controls. Of these, six are lost in the caput (MW 14.5, 17.5, 33.0, 46.5, 50.0, and 54.0 Kd), one (MW 77.0 Kd) in corpus, and two (MW 30.0 and 69.0 Kd) in the cauda epididymides. The polypeptides of MW 17.5, 46.5, 50.0, and 54.0 Kd are testicular in origin. Two polypeptides (14.5 and 33.0 Kd) are present in the cytosol of all regions, whereas 77.0 Kd polypeptide is contributed by all regions except the cauda epididymidis. Polypeptides of MW 30.0 and 69.0 Kd appear only in the caudal spermatozoa, perhaps due to modification and or partial shedding into the lumen of the cauda epididymidis.

Modification

Polypeptides lost from spermatozoa at specific regions of the epididymis without simultaneously appearing in luminal fluid are considered to be due to the modification of preexisting proteins.

One polypeptide (MW 13.5 Kd) appeared in the caput and four (MW 15.5, 30.0, 35.5, and 69.0 Kd) in the cauda epididymidal spermatozoa of treated animals. Two polypeptides (MW 49.0 and 50.5 Kd) were absent from the corpus and cauda epididymidal spermatozoa of control monkeys.

Appearance of New Polypeptides

Treatment with CPA also resulted in the appearance of 14 new polypeptides in epididymal cytosol and luminal fluid. These are: 14.5 14.8, 15.0, 16.0, 16.5, 30.5, 31.5, 86.0 and 102.0 Kd polypeptides in all four regions; 14.0 and 77.0 Kd polypeptides in initial segment, caput, and corpus epididymides; 110.0 and 115.0 Kd polypeptides in caput, corpus, and cauda epididymides; and 26.5 Kd polypeptide only in cauda epididymidis. Among these, 16.5 and 77.0 Kd polypeptides are acquired by spermatozoa in the caput and 14.0 and 15.0 Kd polypeptides in the cauda epididymides.

Protein Profile of Epididymal Tubules in Organ Culture

The pattern of polypeptides in cytosol of the caput and cauda epididymidal tubules prior to culture, on day 4 of culture in the presence of androgen and 72 hours

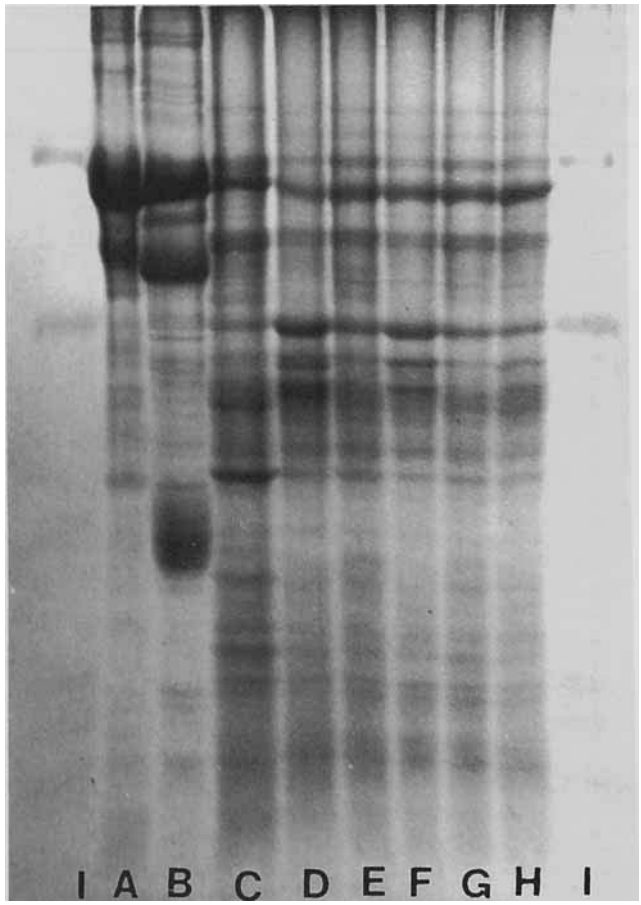


Fig. 9. Polypeptide pattern of cytosol from monkey epididymal tubules in organ culture. A, Serum; B, Medium; C, Caput epididymidal tubule prior to culture; D, Cauda epididymidal tubule prior to culture; E, Caput epididymidal tubule after four days in culture. The medium contained only DHT; F, Cauda epididymidal after four days in culture. The medium contained only DHT; G, Caput epididymidal tubule after four days in culture. The medium contained DHT and cyproterone acetate (100 μM); H, Cauda epididymidal tubule after four days.

after addition of 100 μM CPA into the medium (Fig. 9), was analyzed to record significant changes (Table 3). No major changes were observed in the protein profile of caput and cauda epididymidal tubular cytosol even after 4 days in organ culture, indicating the viability of the culture system. Three caput (MW 11.5, 54.5, and 63.0 Kd) and six cauda (MW 37.5, 41.0, 42.5, 44.0, 52.0, and 56.0 Kd) specific polypeptides were observed in the cytosol of tubules taken prior to and after 4 days in organ culture. The remaining polypeptides were common to both regions.

The protein pattern obtained in in vitro studies could not be compared with that obtained in vivo as the cytosol obtained after culture was from tubules containing luminal fluid and spermatozoa.

Effect of CPA (100 μM) on the Protein Profile of Caput and Cauda Epididymidal Tubules in Vitro

CPA decreased the total number of polypeptides in the cytosol of both caput and cauda epididymidal tubules (caput: from 48 to 42; cauda: from 51 to 44). One caput specific polypeptide (MW 63.0 Kd) and two cauda specific polypeptides (MW 37.5 and 41.0 Kd) were not observed after treatment. Additionally, five polypeptides (MW 20.5, 26.5, 31.5, 51.0, and 57.0 Kd), present in both caput and cauda epididymides in control tubules, were not observed after treatment in either of the regions.

DISCUSSION

Cyproterone acetate at a dose of 1 mg/kg for 70 days induced adverse effects on testes, efferent ducts, and epididymis. The seminiferous tubular epithelium in the testis of CPA-treated animals contained only spermatogonia and Sertoli cells due to exfoliation of other types of germ cells into the lumen. These deleterious effects could be a result of inhibition of local effects of androgen on the testis due to decreased androgen production and or competitive inhibition of androgens at the receptor level. Adverse effects of CPA on testes were reported earlier in primates (Michael et al., 1972; Petry et al., 1972; Morse et al., 1973; Koch et al., 1976; Neumann and Schenk, 1976; Roy et al., 1976; Moltz et al., 1978; Roy and Chatterjee, 1979). The presence of metachromatically staining lipids in the Sertoli cell cytoplasm after CPA treatment could be due to quiescent spermatogenesis as reported for normal monkeys during the summer months (Sehgal et al., 1986).

The alterations in histological parameters of different epididymal regions of the normal adult monkey described in this study viz. proximo-distal increase in diameter of tubules and their lumen and intertubular connective tissue, decrease in height of epithelium, infoldings of principal cell nuclei in corpus and cauda epididymides, and presence of intraepithelial crypts in the cauda epididymidis corroborate earlier reports in monkey (Ramos and Dym, 1977; Alsum and Hunter, 1978; Prakash, 1980) and in other species (Rajalakshmi et al., 1976; Dyson and Orgebin-Crist, 1973; Dinakar et al., 1977; Koch et al., 1976; and Roy et al., 1976; Prakash et al., 1979; Rastogi et al., 1979). The decrease in serum testosterone levels (14.04 ± 1.25 nmol/L in control vs. 3.93 ± 1.37 nmol/L in treated animals) shows that these degenerative changes may be a consequence of androgen deprivation. The relative absence of nuclear infoldings in the principal cells of corpus and cauda epididymides may indicate decreased metabolic activity due to androgen deprivation since these infoldings in caput and cauda epididymidal epithelia in normal monkeys were related to high metabolic activity of these regions (Ramos and Dym, 1977).

The changes in the polypeptide profile following CPA treatment include: (1) decrease in total number of polypeptides in spermatozoa, luminal fluid, and cytosol of all regions, (2) acquisition of larger number of polypeptides by spermatozoa, and (3) decrease in the number of polypeptides lost. These alterations in polypeptide profile of spermatozoa may be due to inhibition of androgen mediated functions of epididymis by CPA.

In CPA-treated animals, new polypeptides appear in cytosol and luminal fluid, of which four were acquired by spermatozoa. Synthesis of new proteins for lysosomal activity has been reported in rats following treatment with CPA (Rajalakshmi and Prasad, 1975) and in experimentally induced cryptorchidism (Rajalakshmi and Prasad, 1974). It is known that in-

TABLE 3. Effect of cyproterone acetate on the polypeptide pattern of epididymal tubules in culture

Approximate molecular weight(Kdal)	CT-0[1]	CD-DHT[2]	CT-CA[3]	CD-0[1]	CD-DHT	CD-CA	Remarks[4]
11.5	+	+	+	−	−	−	I
20.5	+	+	−	+	+	−	V
26.5	+	+	−	+	+	−	V
31.5	+	+	−	+	+	−	V
37.5	−	−	−	+	+	−	II,IV
41.0	−	−	−	+	+	−	II,IV
42.5	−	−	−	+	+	+	II
44.0	−	−	−	+	+	+	II
51.0	+	+	−	+	+	−	V
52.0	−	−	−	+	+	+	II
54.5	+	+	+	−	−	−	I
56.0	−	−	−	+	+	+	II
57.0	+	+	−	+	+	−	V
63.0	+	+	−	−	−	−	I,III

[1]CT = caput epididymidis; CD = cauda epididymidis; 0 = day 0 (before begining of culture).
[2]DHT = day 4 of culture (with only 5 α-dihydrotestosterone in the medium).
[3]CA = day 4 of culture (72 hours after addition of 100 μM cyproterone acetate to the medium.
[4]I = CT specific polypeptide; II = CD specific polypeptide; III = CT specific polypeptide not observed after the addition of cyproterone acetate; IV = CD specific polypeptide not observed after the addition of cyproterone acetate; V = not observed either in CT or CD after the addition of cyproterone acetate.
+ present; − absent.

creased synthesis of lysosomal proteins occurs as a necessary prerequisite for tissue involution and degeneration during amphibian tail metamorphosis (Tata, 1966) and in ventral prostate of rats following estrogen administration (Bashirelahi et al., 1969). The appearance of new proteins in cytosol and the acquisition of some of these by spermatozoa in CPA-treated animals may indicate the occurrence of involutionary changes due to androgen deprivation.

Analysis of the protein profile of epididymal tubules maintained in organ culture for 4 days shows no significant change compared to tubules taken prior to culture indicating the viability of culture. The protein pattern of epididymal tubules in culture is androgen dependent since eight polypeptides were absent in cultures exposed to CPA.

The results of the present study indicate that low doses of CPA administered to adult male rhesus monkeys resulted in spermatogenic arrest, degeneration in epididymal morphology, alteration in polypeptide profile of spermatozoa, and the appearance of new polypeptides of possible lysosomal origin in epididymal fluid and cytosol. Cyproterone is tolerated well even at doses ranging from 100–200 mg/day with no adverse effects on carbohydrate and lipid metabolism, adrenocortical, and liver functions (Rausch-Stroomann et al., 1970). Calcium and phosphate balance in men exposed to CPA were normal (Neumann and Schenk, 1975). The limitation of using CPA alone for male contraception is related to lowered serum testosterone levels and consequent effects on libido and sexual function. Use of CPA in combination with a long-acting androgen ester at doses that maintain sexual function without stimulating spermatogenic activity would be more acceptable for male contraception.

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LITERATURE CITED

Alsum, D.J., and A.G. Hunter 1978 Regional histology and histochemistry of the ductus epididymis in the rhesus monkey (Macaca mulatta). Biol. Reprod., *19:* 1063–2069.

Arslan, M., M.Z. Haider, and M.H. Qazi 1986 Characterization and androgen dependence of specific proteins in the epididymis of adult rhesus monkey (Macaca mulatta). Arch. Androl., *16:* 67–74.

Bashirelahi, N., G.J. Chander, and C.A. Villee 1969 Effect of dihydrotestosterone on the synthesis of nucleic acid and ATP in prostatic nuclei. Biochem. Biophys. Res. Commun., *37:* 976–981.

Belsey, M.A., R. Eliasson, A.J. Gallegos, K.S. Moghissi, C.A. Paulsen, and M.R.N. Prasad 1980 Laboratory Manual for the Examination of Human Semen and Semen-cervical Mucus Interaction. Press Concern, Singpore.

Cunliffe, W.J., S. Shuster, and A.S. Cassels Smith 1969 Effect of topical cyproterone acetate on sebum secretion in patients with acne. Br. J. Derm., *81:* 200–201.

Davies, T.S. 1974 Cyproterone acetate for male hypersexuality. J. Int. Med. Res., *2:* 159–163.

Dinakar, N., R. Arora-Dinakar, P. Kumar, C.L. Talesra, and M.R.N. Prasad 1977 Histochemical localization of dehydrogenases in different regions of the epididymis of the adult rhesus monkey (Macaca mulatta). Ind. J. Exp. Biol., *15:* 865–869.

Dyson, A.L.M.B., and M.C.-Orgebin-Crist 1973 Effect of hypophysectomy, castration and androgen replacement upon the fertilizing ability of rat epididymal spermatozoa. Endocrinology, *93:* 391–402.

Eddy, E.M., R.B. Vernon, L.H. Mueller, A.C. Mahnel, and B.A. Fenderson 1985 Immunodissection of sperm surface modifications during epididymal maturation. Am. J. Anat., *174:* 225–237.

Haider, M.Z., M.H. Qazi, A. Khanum, and M. Arslan 1983 Effect of testosterone on epididymal proteins in castrated rhesus monkeys. Am. J. Primatol., *4:* 73–80.

Hammerstein, J., and B. Cupceancu 1969 Treatment of hirsuitism with cyproterone acetate. Dt. med. Wschr., *16:* 829–834.

Kaur, J., P.R. Ramakrishnan, and M. Rajalakshmi 1991 *In vitro* organ culture of rhesus monkey epididymal tubules. Contraception, *43:* 295–305.

Koch, U.J., F. Lorenz, K. Danehl, R. Ericsson, S.H. Hasan, D.V. Keyserlingk, K. Lubke, M. Mehring, A. Rommler, U. Schwartz, and J. Hammerstein 1976 Continuous oral low-dosage cyproterone acetate for fertility regulation in the male? A trend analysis in 15 volunteers. Contraception, *14:* 117–135.

Laemlli, U.K. 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.), *43:* 345–348.

Mastroianni, L. Jr. and W.A. Jr. Manson 1963 Collection of monkey semen by electroejaculation. Proc. Soc. Exp. Biol. Med., *112:* 1025–1027.

Michael, R.P., T. M. Plant and M.I. Wilson 1972 Preliminary studies on the effects of cyproterone acetate on sexual activity and testicular function in adult male rhesus monkeys (Macaca mulatta). Adv. Biol. Sci., *10:* 197–208.

Moltz, L., A. Rommler, U. Schwartz and J. Hammerstein 1978 Effect of cyproterone acetate (CPA) on pituitary gonadotrophin release and on androgen secretion before and after LH-RH double stimulation of testis in men. Int. J. Androl., Suppl., *2:* 14–18.

Morse, H.C., D.R. Leach, M.J. Rowley, and C.G. Heller 1973 Effect of cyproterone acetate on sperm concentration, seminal fluid volume, testicular cytology and levels of plasma and urinary ICSH, FSH and testosterone in normal men. J. Reprod. Fertil., *32:*365–378.

Neumann, F., and B. Schenk 1976 New antiandrogens and their mode of action. J. Reprod. Fertil., Suppl., *24:* 129–149.

Petry, A., J. Mauss, J.G. Rausch-Stroomann, and A. Vermeulen 1972 Reversible inhibition of spermatogenesis in men. Horm. Metab. Res., *4:* 386–387.

Prakash, A. 1980 Light and electron microscopic studies on the excurrent ducts of the male reproductive system in the adult rhesus monkey. Ph.D. thesis, Delhi University.

Prakash, A., M.R.N. Prasad, and T.C.A. Kumar 1979 Light microscopic features of the rete testis, the vas efferens, the epididyimis and the vas deferens in the adult rhesus monkey. J. Biosci., *1:* 185–205.

Prasad, M.R.N. and M. Rajalakshmi 1977 Recent advances in the control of male reproductive functions. In: Int. Review Physiol. Reproductive Physiology II, Vol. 13. R.O. Greep, ed. University Park Press, Baltimore, pp. 153–199.

Rajalakshmi, M., and M.R.N. Prasad 1974 Changes in the epididymis of adult rats following experimentally induced cryptorchidism. Andrologia, *6:* 293–302.

Rajalakshmi, M., and M.R.N. Prasad 1975 Action of cyproterone acetate on the accessory organs of reproduction in pre-pubertal and sexually mature rats. Fertil. Steril., *2:* 137–143.

Rajalakshmi, M., R. Arora, T.K. Bose, N. Dinakar, G. Gupta, T.N.R.V. Thampan, M.R.N. Prasad, T.C.A. Kumar, and N.R. Moudgal 1976 Physiology of the epididymis and induction of functional sterility in the male. J. Reprod. Fertil. Suppl., *24:* 71–94.

Rajalakshmi, M., A. Sehgal, J.S. Pruthi, and T.C.A. Kumar 1983 Steroid metabolism by monkey and human spermatozoa. Steroids, *41:* 587–595.

Rajalakshmi, M., and P.R. Ramakrishnan 1989 Pharmacokinetics and pharmacodynamics of a new long-acting androgen ester: Maintenance of physiological levels of androgens for 4 months after a single injection. Contraception, *40:* 399–412.

Ratnasooriya, W.D. 1982 Effects of drugs on the epididymis. J. Sci. Ind. Res., *41:* 455–461.

Ramos, A.S., and M. Dym 1977 Fine structure of the monkey epididymis. Am. J. Anat., *149:* 501–532.

Rastogi, R.K., M. Milone, M. DiMeglio, M.F. Caliendo, and G. Chieffi 1979 A critical appraisal of the effects of the antiandrogen, cyproterone acetate, on the epididymis in connection with fertility control of the male. J. Steroid Biochem., *11:* 101–105.

Rausch-Stroomann, J.G., R. Petry, V. Hocevar, J. Mauss and J.H. Senge 1970 Influence of an antiandrogen (cyproterone) on the gonadotrophic function of the pituitary gland, on the gonad and on metabolism in normal men. Acta Endocrinol. Kbh., *63:* 595–608.

Robaire, B., and L. Hermo 1988 Efferent ducts, epididymis and vas deferens: Structure, functions and their regulation. In: The Physiology of Reproduction, Vol. 1. E. Knobil and J.D. Neill, eds. Raven Press, New York, pp. 999–1080.

Roy, S., and S. Chatterjee 1979 Studies with cyproterone acetate for male contraception. J. Steroid Biochem., *11:* 675–680.

Roy, S., S. Chatterjee, M.R.N. Prasad, A.K. Poddar, and D.C. Pandey 1976 Effects of cyproterone acetate on reproductive functions in normal human males. Contraception, *14:* 403–420.

Sehgal, A., G.F.X. David, A.K. Dubey, J.S. Pruthi, and T.C.A. Kumar 1986 Circardian and circannual changes in the testicular function of adult rhesus monkeys. J. Biosci., *10:*443–457.

Tata, J.R. 1966 Requirement for RNA and protein synthesis for induced regression of the tadpole tail in organ culture. Dev. Biol., *13:* 77–94.

Young, L.G., B.T. Hinton, and K.G. Gould 1985 Surface changes in chimpanzee sperm during epididymal transit. Biol. Reprod., *32:* 399–412.