For the determination of the exact concentration of oestradiol-17β, oestrone and androstenedione in bovine follicular fluid, our results show that it is necessary to withdraw the samples immediately after slaughter. These findings may well have an impact on experimental data on the steroidogenesis obtained in bovine ovarian follicles cultured in vitro.

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Effect of cyproterone acetate on the testis and epididymis of the lizard, *Psammophilus dorsalis* (Gray)

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Summary. The effect of cyproterone acetate (CPA) on the testis and epididymis of the lizard, *Psammophilus dorsalis* has been studied. Treatment with CPA affects spermatogenesis and steroid metabolism in the testis. It also causes regression of the epididymis and a decrease in steroidogenic enzyme activity.

Several drugs which suppress spermatogenesis, sperm maturation or sperm transport through accessory ducts have been investigated in a large number of laboratory mammals including man[2]. Cyproterone acetate (6-chloro-17-acetoxy 1α,2α-methylene-4,6-pregna-diene-3,20 dione) (CPA) is one such drug, known more as a potent antiandrogen which interferes with the actions of androgens and estrogens at their target tissues[3,4]. Recent biochemical studies[5] on female rats, mice and gerbils suggests the estrogenic nature of CPA. In the rhesus monkey and hamster CPA selectively alters the epididymal function[6,7]. There are only a few studies using CPA in lower vertebrates and one single report of its use in reptiles. In the male lizard, *Lacerta sicula* it causes regression of the epididymis, cloacal glands and femoral pores[8]. The present study reports the effect of CPA on the histophysiology of the testis and epididymis of the lizard, *Psammophilus dorsalis.*

Materials and methods. Sexually mature male lizards weighing 35-45 g were collected in and around Mysore city. Lizards were maintained in the laboratory under the same conditions of light and temperature as those to which they are normally exposed in their natural habitat. Lizards thus acclimatized to laboratory conditions were used for this experiment. The lizards were randomly divided into groups A, B and C each consisting of 5 animals, and were housed in clean glass cages. They were fed on cockroaches and water was given ad libitum. Group C was injected s.c. with 250 µg CPA in 0.1 ml olive oil on alternate days for a period of 28 days. Group B was injected with the vehicle only and group A served as non-treated controls. All lizards were weighed and killed by decapitation 24 h after the last injection. The testes and epididymes from all the groups were dissected out and the weights were recorded. 1 testis and 1 epididymis from each group was immediately frozen at -20 °C and 16 µm thick sections were cut in a cryostat maintained at -20 °C. Air-dried cryostat sections were incubated in a serological water bath at 37 °C for 1 h in appropriate incubation media containing different substrates, co-factors and tetrazolium salt. Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH) and 17β-hydroxysteroid dehydrogenase (17β-HSDH) were localized according to the method of Baillie et al.[9], by using pregnenolone

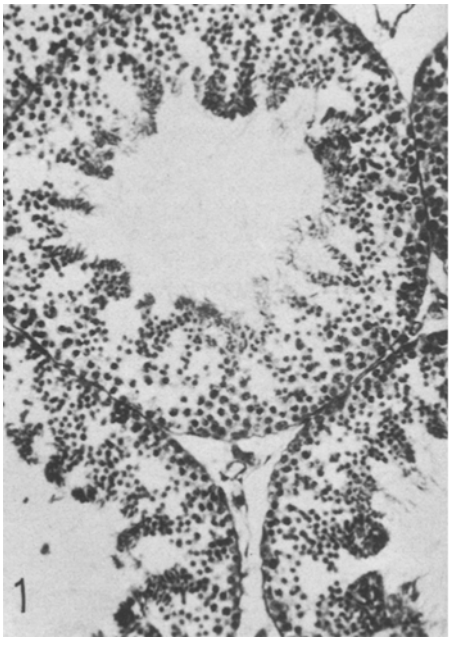


Fig. 1. Testis of olive oil-treated lizard showing active spermatogenesis. Haematoxylin-eosin. × 166.

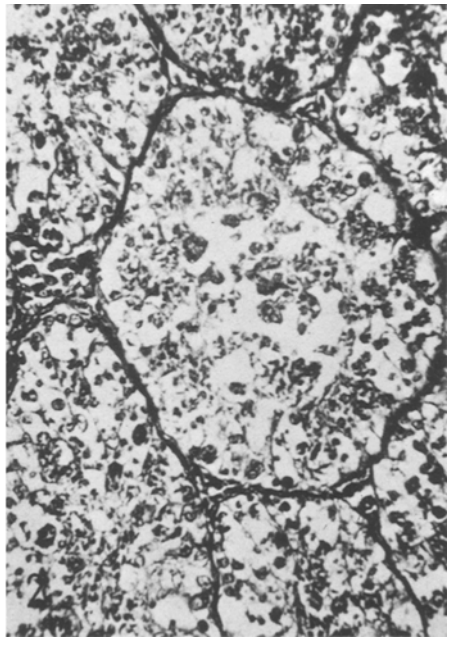


Fig. 2. Testis of CPA-treated lizard showing disorganized germ cells, Haematoxylin-eosin. × 166.

and dehydroepiandrosterone to localize Δ^5-3β-HSDH, and estrodiol-17β and testosterone to localize 17β-HSDH. Parallel cryostat sections were incubated for glucose-6-phosphate dehydrogenase (G-6-PDH) and $NADH_2$ diaphorase as described by Altman[10]. Lipids were localized using the sudan black B method of Pearse[11]. The pattern of distribution and intensity of enzyme activities was visually quantified in the testis and epididymis of both treated and control animals. Samples of testis and epididymis of all groups were fixed in hollande-bouin. Paraffin sections were cut at 6 µm thick and stained in haematoxylin-eosin for histological observation.

Results. Administration of CPA for 28 days brings about a significant loss in weight of the testes and epididymes compared to the olive-oil treated controls (table). There is a reduction in diameter of the seminiferous tubules and the lumen of the seminiferous tubules shows disorganized germ cells with cellular debris in the CPA treated lizards. There is a reduction in size of the interstitial cells and their nuclei are pycnotic when compared with those of the normal animals (figures 1 and 2). Similarly, the epididymis of treated animals also shows a reduction in tubular diameter and epithelial cell height. The lumen is completely devoid of spermatozoa (figure 6).

There is a reduction in the activity of both Δ^5-3β-HSDH (figure 4) and 17β-HSDH in the interstitium and the seminiferous epithelium as well as in the epididymal epithelium (figure 8). G-6-PDH and $NADH_2$ diaphorase also show a similar reduction in activity. There is an accumulation of sudanophilic lipids in the interstitium and seminiferous epithelium.

Discussion. CPA is an antiandrogenic compound which partially inhibits spermatogenesis and sperm maturation in the epididymis[12] and inhibits all androgen-dependent processes of differentiation[4]. It is known to cause vaginal keratinization and an increase in the quantity of uterine protein, glycogen and sialic acid in ovariectomized rats, mice and gerbils[5]. In rats it causes the regression of the epididymis and affects the motility of sperm without in any

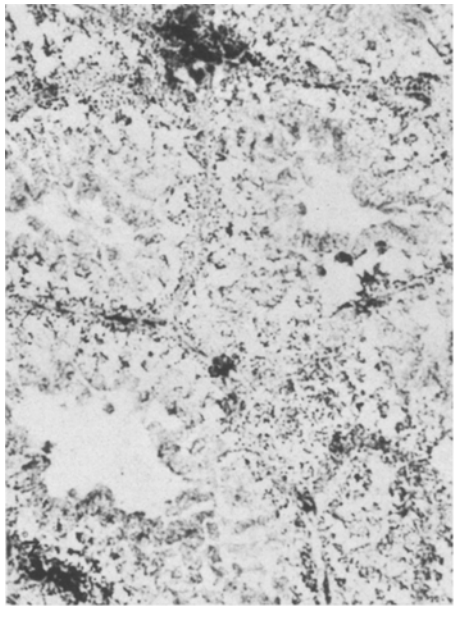


Fig. 3. Strong Δ^5-3β-HSDH (DHA as substrate) activity in the testis of olive oil-treated lizard. × 67.

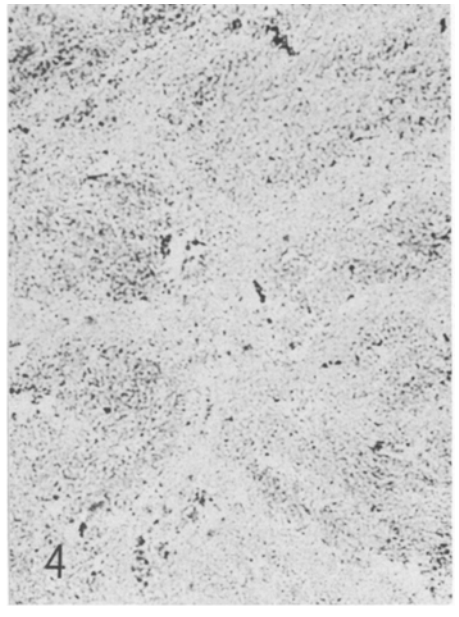


Fig. 4. Testis of CPA-treated lizard showing marked reduction in Δ^5-3β-HSDH activity. × 67.

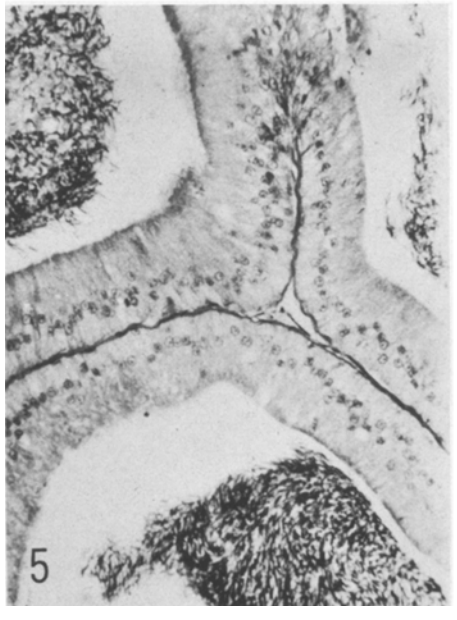


Fig. 5. Epididymis of olive oil-treated lizard showing thick columnar epithelium. Haematoxylin-eosin. × 166.

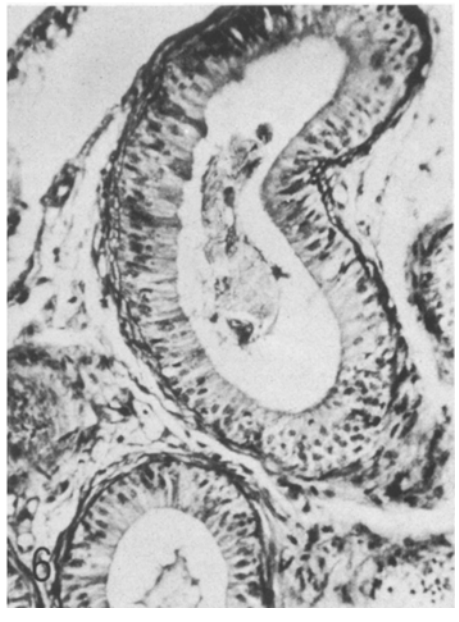
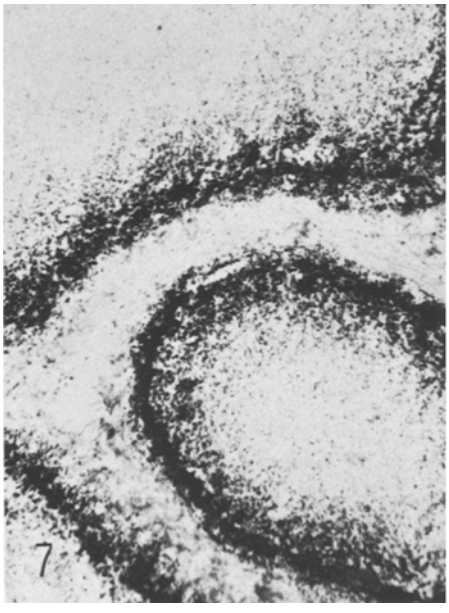


Fig. 6. Epididymis of CPA-treated lizard showing regression of tubules and absence of spermatozoa. Haematoxylin-eosin. × 166.

Effect of CPA on the testes and epididymes of the lizard, *Psammophilus dorsalis*

Treatment	n	Weight (mg/100 g b.wt.) Testes	Epididymes	Diameter (μm) Seminiferous tubules	Interstitial cell nuclei	Epididymal tubules	Height (μm) Epididymal epithelium
A Non-treated controls	(5)	1192.9±48.9	214.3± 7.6	218.4±7.8	4.56±0.07	251.6±5.6	58.0±1.8
B Olive oil-treated controls	(5)	1096.6±30.5*	186.0±16.9*	220.1±5.8	4.52±0.05*	240.3±3.8	56.1±1.2*
C CPA-treated	(5)	500.2±63.4**	82.6± 5.0**	154.4±6.9**	3.99±0.04**	130.5±5.0**	32.8±0.8**

* NS (A versus B). ** $p < 0.001$ (B versus C). n, number of animals used.



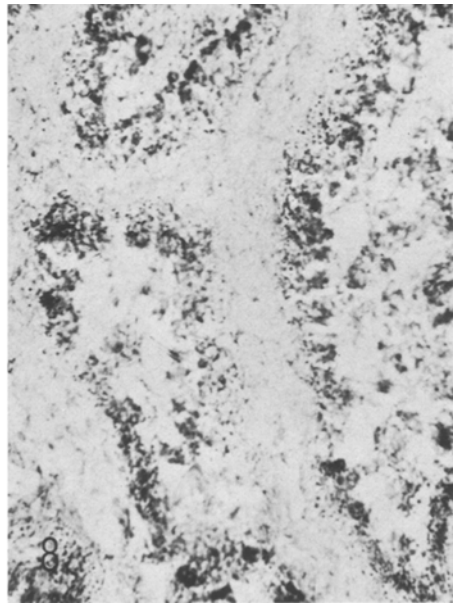


Fig. 7. Strong Δ^5-3β-HSDH (DHA as substrate) activity in the epididymis of olive oil-treated lizard. × 166.

Fig. 8. CPA-treated lizard epididymal epithelium showing weak Δ^5-3β-HSDH activity. × 166.

way affecting the testis weight or the remaining accessory duct systems[6,7]. Even in non-mammalian vertebrates CPA is said to have effects similar to those in mammals; it affects the androgen-dependent thumb pads in frogs, seminal vesicles in fish and epididymis, and cloacal glands and femoral pores in lizards[8]. The manner in which CPA causes these changes is reported to be due to its tendency to compete with gonadal steroids for receptor molecules in the target tissues, thus affecting the utilization of these steroids without interfering with steroid biosynthesis[13]. The present study indicates that CPA has a deleterious effect on the testis and steroid metabolism, as reflected in the degenerative changes in the seminiferous tubules and reduction in the activities of steroidogenic enzymes. Accumulation of sudanophilic lipids in the interstitium and seminiferous epithelium indirectly indicates interference with steroid metabolism. This is also evident from the regression of epididymis; an androgen-dependent accessory organ. Lizard epididymis has been shown to be androgen-dependent; castration reduces the activities of hydroxysteroid dehydrogenases, hydrolytic enzymes and other oxidative enzymes and testosterone administration restores the normal physiology of the epididymis[14]. The regression of the epididymis and reduction in the steroidogenic activity following CPA treatment is very similar to the castration effect and confirms the decreased androgenic activity.

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