EFFECTS OF CYPROTERONE ACETATE ON THE TESTICULAR FUNCTION OF BAT (*RHINOPOMA KINNEARI* WROUGHTON)

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Summary : 1-Cyproterone acetate administration (0.2 *mg*/day/animal for 25 days) caused widespread testicular necrosis. The lumen of the epididymides were devoid of spermatozoa. The RNA, protein, sialic acid and phosphatase enzyme activity of the testes were reduced. Serum transaminase enzyme activity was slightly changed. Haemoglobin, hematocrit, blood sugar, and blood urea levels were in the normal range. Regressed Leydig cell tissue and decreased production of RNA and sialic acid in the testes could be due to the antiandrogenic action produced by cyproterone acetate.

Key words : cyproterone acetate
growth of accessory sex organs
inhibition spermatogenesis
antiandrogenicity

Cyproterone acetate (CA) has aroused much interest due to its antiandrogenic properties (9). It causes a marked atrophy of the accessory sex glands and suppression of spermatogenesis.

CA has not been used for its antiandrogenic properties in a hibernating mammal. It was thought worthwhile to study the long-term effects of CA on the testicular function of a common rat-tailed bat (*Rhinopoma kinneari* Wroughton). The present investgiation is a part of the phased programme to study testicular function after chronic administration of a large number of anti-spermatogenic compounds.

MATERIALS AND METHODS

The bats (*Rhinopoma kinneari* Wroughton) were caught from the surroundings of Amber-Fort in the month of June-July, 1977. Healthy adult sexually mature males were housed in wire cages in groups of 20 each. They were acclimatized in the laboratory for at least 7 days at 23±1°C. CA was dissolved in a mixture of benzyl-benzoate and castor oil (1:10). Bats were injected with CA (ip) daily in doses of 0.2 *mg*/day/animal for a period of 25 days. The control animals received an equal amount of vehicle alone.

All animals were killed by rapid decapitation 24 hr after the last injection. Final body weight was recorded. Testes, epididymides, seminal vesicles, adrenal and thyroid glands were dissected free of fat and weighted on a torsion balance. Right testes and epididymides were fixed in Bouin's fluid. Six μm paraffin sections were prepared and stained with haematoxylin and eosin. Left testes and epididymides were frozen for determination of total protein, RNA, sialic acid, alkaline and acid phosphatase activities (2, 5, 8, 10).

Plasma was separated from the blood obtained directly from the heart. Hepatic function was followed with determination of serum glutamic oxaloacetic transaminase (SGOT) and serum

glutamic pyruvic transaminase (SGPT) (7). Haemoglobin, hematocrit, blood-urea and blood sugar levels were also determined.

One hundred seminiferous tubules appearing circular in section were traced with camera lucida at X80. Two perpendicular diameters of each tracing were measured, averaged and expressed in terms of mean tubular diameter. Student's 't' test was applied in comparing means. The measurements of the diameter of the 100 Leydig cell nuclei were carried out on four sections from each testicle with camera-lucida drawings at X800.

RESULTS

Adult males receiving 0.2 *mg* of CA for a period of 25 days showed a significant reduction in the weights of testes, epididymides, seminal vesicles and thyroid glands (Table I).

The testes were small, flaccid and somewhat oedematous. Histological preparations of the testes showed a complete inhibition of spermiogenesis; sperms and spermatids were absent (Fig. 1 and 2). The spermatogonia and Sertoli cells appeared unaffected. Spermatocytes showed degenera-

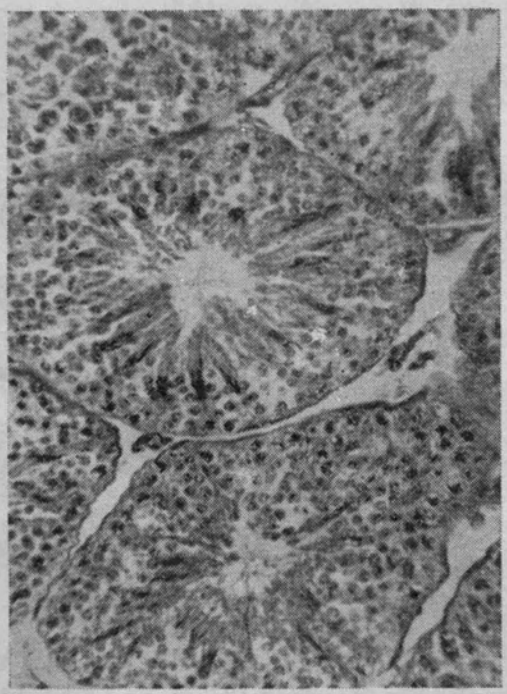

Fig. 1: Section of a testis from a control bat. The seminiferous tubules show full spermatogenesis. X100 HE

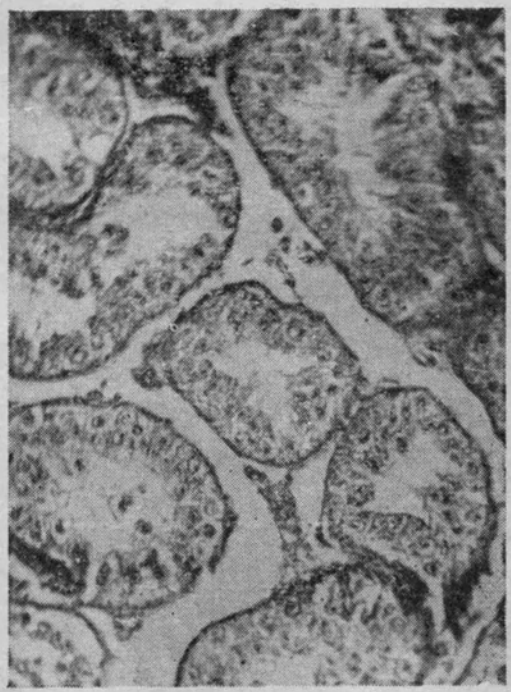

Fig. 2: Section of a testis after cyproterone acetate treatment (0.2 *mg*/day for 25 days). Note the degenerative changes and the absence of spermatozoa, X100 HE.

Table I: Effects of cyproterone treatment on body and tissue weights and certain biochemical parameters in bats.

Treatment	*Mean body wt. g±S.E.M.*	*Mean weights of tissue±S.E.M. (mg/100 g body wt.)*					*Protein, RNA & Sialic acid contents of testis (μg/mg tissue±S.E.M.)*		*Mean phosphatase enzyme activity ±S.E.M.*	
		testes	*epididymides*	*seminal vesicle*	*adrenal*	*thyroid*			*alkaline*	*acid*
Control (20)	32±5	183±13	163±17	62.2±9.3	16.5±3.7	14.6±2.7	215±21	3.2±0.5	6.6±0.5	6.7±0.3	2.3±0.5
Cyproterone acetate (20)	21±7	112±9†	89.5±11†	44.8±5.7†	17.1±1.5†	9.8±2.1†	107±9†	1.2±0.3†	2.7±0.3†	4.5±0.2†	1.2±0.3†

†$P<0.01$ compared with controls

Figures in parentheses represent the number of animals examined.

Biochemical estimations: mean of six determinations ±S.E.M.

*μg phosphorus/hour/*mg* of tissue.

tive changes. Cytoplasmic vacuolization, nuclear shrinkage, and pycnosis were evident in Leydig cells (mean nuclear diameter CA: 7.3±0.2 μ; control: 10.5 ± 0.2 μ).

There was regression of the epithelium of epididymides. The lumen of the epididymides and vas deferens were devoid of spermatozoa.

Biochemical changes :

Total RNA, protein and sialic acid contents of the testes were significantly reduced in CA-treated bats as compared with those in controls ($P<0.01$, Table I).

The alkaline and acid phosphatase enzyme activities were reduced in the testes of bat after cyproterone acetate treatment (Table I).

Blood sugar and blood urea levels were not affected by CA (blood sugar: CA 57.8±3; control 62.5±5 *mg*/100 *ml*; blood urea: CA 30.5±2.7; control 35.6±3.5 *mg*/100 *ml*).

Hepatic functions and liver biopsy :

SGOT activity was moderately increased (CA: 179±29; control 73±13) whereas SGPT activity was not affected (CA: 37±9; control: 31±5). The histopathological examination of the liver taken at biopsy did not show any damage. The architecture was microscopically normal.

Hematological studies :

Red cells (4.40 x 10^6/mm^3), haemoglobin (12.2 *g*/100 *ml*), packed cell volume (31.5%) and leucocytes (7500/mm^3) were in the normal range.

DISCUSSION

Chronic administration of CA (0.2 *mg*) caused complete inhibition of spermatogenesis at the level of primary spermatocytes. The levels of RNA, protein and sialic acid were low. This is probably due to inhibition of spermatogensis. Jager *et al.* (4) reported a fall in nucleic acid metabolism after 5 daily injections of CA.

In men CA treatment results in severe Leydig cell suppression (6) and a marked decrease of plasma testosterone levels (3). Regressed Leydig cell tissue and decreased production of RNA and sialic acid in the testes observed in the present study could be due to the antiandrogenic action produced by CA.

The function of alkaline/acid phosphatase in the metabolism of the testis and accessory sex glands is undetermined. A possible association of alkaline phosphatase with the lipid metabolism in the uterus has been suggested (1). This may also be true for accessory sex glands in males.

Decreased phosphatase enzyme activity in the testis of CA-treated bats may be due to functional impairment of spermatogenesis.

Our data weigh in favour of normal functioning of liver as revealed by transaminase (SGPT) activity and histopathological examination.

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REFERENCES

1. Atkinson, W.B. and H. Elftman. Mobilization of alkaline phosphatase in the uterus of the mouse by oestrogen. *Endocrinology*, **40** : 30-36, 1947.
2. Fiske, C.H. and Y. Subbarow. The colorimetric determination of phosphorus. *J. Biol. Chem.*, **66** : 375-400, 1925.
3. Geller, J., G. Vazakas, B. Fruchtman, H. Newman, K. Nakao and A. Leh. The effect of cyproterone acetate on advanced carcinoma of the prostate. *Surgery Gynec. Obstet.*, **127** : 749-758, 1968.
4. Jager, E., K.O. Mosebach, H.P. Bluementhal and A. Scheuer. The influence of cyproterone acetate on the uptake of testosterone and on the DNA-RNA amount in liver, prostate and seminal vesicles of immature male rats *in vivo*. *Acta Endocr.* (*Kbh.*), *Suppl.*, **138** : 43 (abst.), 1969.
5. Lowry, O.H., M.J. Rosebrough, A.L. Farr and R.J. Randall. Protein measurement with Folin-phenol reagent. *J. Biol. Chem.*, **193** : 265-275, 1951.
6. Markewitz, M., R.J. Veenema, B. Fingerhut, D. Nehme-Haily and S.C. Sommers. Cyproterone acetate (SH 714) effect on histology and nucleic acid synthesis in the testes of patients with prostatic carcinoma. *Invest. Urol.*, **6** : 638-643, 1969.
7. Mohun, A.F. and I.J.Y. Cook. Simple method for measuring serum levels of the glutamic oxalacetic and glutamic pyruvic transaminases in routine laboratories. *J. Clin. Path.*, **10** : 394-399, 1957.
8. Munro, H.N. and A. Fleck. The determination of nucleic acids. In: Methods of Biochemical Analysis, Vol., 14, p. 113, ed. D. Glick, New York: Interscience, 1966.
9. Neumann, F., R. Van Berswordt-Wallrabe, W. Elger, H. Steinbeck, J.D. Hann and M. Kramer. Aspects of androgen dependent events as studied by antiandrogens. *Recent Prog. Horm. Res.*, **26** : 337-358, 1970.
10. Warren, L. The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.*, **234** : 1971-1975, 1959.