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The effect of cyproterone acetate on spermatogenesis and thumb pads of the skipper frog, *Rana cyanophlyctis* (Schn.)[1]

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Summary. The administration of cyproterone acetate (CPA) to adult male frogs of the species *R. cyanophlyctis* caused a significant reduction in the number of cell nests with primary spermatogonia (stage 0) per seminiferous tubule cross section, and a marked reduction in the height of the thumb pad epidermis and mucous glands. It is concluded that the development and/or proliferation of primary spermatogonia in *R. cyanophlyctis* are androgen dependent.

The antiandrogenic effects of cyproterone acetate (CPA) are well known in mammalian species[2], while comparative studies on lower vertebrates are limited to a few species only[3-7]. Similarly, the role of androgens in spermatogenesis is well established for mammals[8], while for amphibians the picture is at present rather confusing[9,10]. The objective of the present work was to elucidate the involvement of androgens, if any, in the spermatogenesis of the frog, *Rana cyanophlyctis*, by using CPA, which is known to bind the androgen receptor sites. In addition, the effect of CPA on Leydig cell morphology and histochemistry, and on the thumb pads (androgen dependent structure) has been investigated.

Adult male frogs *(R. cyanophlyctis)*, obtained from the surrounding areas of Dharwad in August, were used. The 1st group (8 specimens) received 0.1 ml amphibian Ringer solution and served as controls. The 2nd and 3rd groups (10 in each group) were injected with 0.1 ml amphibian Ringer solution containing 250 µg and 500 µg CPA respectively. Injections were given i.m. on alternate days for 26 days (13 injections) and animals were autopsied 24 h after the last injection. The relative weights were recorded and representative pieces of testes and thumb pads were fixed in Bouin's fluid for histological and histometric studies[11]. The remaining pieces of testes were used for the histochemical assay of the enzymes Δ^5-3β-hydroxysteroid dehydrogenase (3β-

Table 1. Effect of cyproterone acetate on the testis of *R. cyanophlyctis*

Group	Average testis weight (mg/100 g b. wt) weight ± SE	Average diameter (µm)[a] Testis	Testis tubule	Leydig cell nuclear diameter	Leydig cell Δ^5-3β-HSDH activity[b]	G-6-PDH activity[b]
Control	185 ± 25	1700 ± 18	251 ± 9	5.2 ± 0.2	+ + +	+ + + +
Treated with	189 ± 24	1630 ± 25	241 ± 6	5.0 ± 0.03	+ + +	+ + + +
250 µg CPA	NS	NS	NS	NS		
Treated with	180 ± 17	1681 ± 23	241 ± 7	5.0 ± 0.1	+ + +	+ + + +
500 µg CPA	NS	NS	NS	NS		

[a] Values ± SE; NS, nonsignificant. [b] Intensity of reaction is visually graded.

Table 2. Effect of cyproterone acetate on spermatogenesis in *R. cyanophlyctis*

Group	Number of cell nests/seminiferous tubule cross section (± SE) Stage 0	Stage I	Stage II	Stage III	Stage IV	Stage V
Control	5.38 ± 0.05	3.71 ± 0.19	2.61 ± 0.07	1.63 ± 0.12	1.3 ± 0.05	1.35 ± 0.04
Treated with	3.21 ± 0.02	3.56 ± 0.04	2.08 ± 0.16	1.80 ± 0.09	1.64 ± 0.13	1.48 ± 0.05
250 µg CPA	$p < 0.001$	NS	NS	NS	NS	NS
Treated with	3.51 ± 0.1	3.37 ± 0.11	2.49 ± 0.52	1.34 ± 0.07	1.52 ± 0.1	1.25 ± 0.06
500 µg CPA	$p < 0.001$	NS	NS	NS	NS	NS

p-Values calculated by Student's t-test between control and experimental groups. NS, nonsignificant.

HSDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) as described earlier[12]. The spermatogenetic stages were identified as follows. Stage 0: primary spermatogonia; stage I: secondary spermatogonia, less than 10 cells in a cell nest; stage II: secondary spermatogonia, more than 10 cells in a cell nest; stage III: primary spermatocytes; stage IV: secondary spermatocytes; stage V: spermatids.

It is evident from table 1 that the average testis weight, testis diameter, tubule diameter and Leydig cell nuclear diameter were not affected by the short-term treatment with both 250 µg and 500 µg CPA. However, the quantitative analysis of the spermatogenetic stages (table 2) reveals that the number of cell nests of primary spermatogonia (stage 0) per seminiferous tubule cross section decreased significantly ($p < 0.001$) in the experimental groups. Thus, the development and/or proliferation of primary spermatogonia seems to be androgen dependent in *R. cyanophlyctis*. The other cell nests (stage I-V) were unaffected indicating, albeit indirectly, that androgens play a role in the early stages of spermatogenesis rather than the later stages in this species. In *Rana esculenta*[5,7] and *R. temporaria*[6] CPA blocked the formation of spermatids. However in the former species[5] spermatogonial multiplication was also partially affected. In the latter species[6], the author's conclusion that earlier stages of spermatogenesis are not affected is not supported by the quantitative and statistical analysis of the cell nest counts. To the best of our knowledge, the present work is the first report that shows the involvement of androgens at a very early stage of spermatogenesis in Amphibia.

In *R. temporaria* a regression of Leydig cells following CPA treatment has been reported[6]. However, in the present work on *R. cyanophlyctis* the Leydig cell nuclear diameter, 3β-HSDH and G-6-PDH enzyme activities remained relatively unaffected (table 1) possibly due to the short-term treatment with CPA. The androgen dependent thumb pads regressed significantly ($p < 0.001$, table 3) due to CPA treatment as reported in *R. esculenta*[2].

Table 3. Effect of cyproterone acetate on the thumb pad of *R. cyanophlyctis*

Group	Average height (µm ± SE) Epidermis	Glandular epithelium
Control	88 ± 3	23 ± 0.6
Treated with 250 µg CPA	67 ± 2 $p < 0.001$	15 ± 0.2 $p < 0.001$
Treated with 500 µg CPA	69 ± 2 $p < 0.001$	15 ± 0.7 $p < 0.001$

p-Values calculated by Student's t-test between control and experimental groups.

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Selective fungitoxicity of cyclohexylamine and related aromatic amines

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Summary. Cyclohexylamine, 10^{-1} mole/l, selectively inhibited the growth of various fungi in culture. *Pythium ultimum* was highly sensitive; *Fusarium oxysporum* was more tolerant. Phenylethylamine was toxic to these 2 fungi at lower concentrations than cyclohexylamine and hydroxiphenylethylamine.

The toxicity of cyclohexylamine (CHA) to mammals is well known, and the possible mutagenicity of neutralized CHA for human chromosomes has been widely discussed[2,3]. The phytotoxicity, and an apparently selective fungitoxicity, of volatile CHA have been demonstrated[4]. This study reports the effects of neutralized CHA and the closely related 2-phenylethylamine (PEA) and 2-(p-hydroxiphenyl)ethylamine [tyramine] (PEA-OH) on the mycelial growth of fungi in pure culture.

Material and methods. Single strains of thirteen species of fungi were selected from our collection. Solutions of CHA, PEA, or PEA-OH (analytical grade), neutralized or slightly acidified to pH 6.8 with 1 mole HCl/l, were sterilized by filtration and aseptically added to an autoclaved, concentrated culture medium. Final composition of this medium was: sucrose, 10 g; vitamin-free casamino acids (Difco), 2 g; yeast extract (Difco), 1 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; $FeSO_4 \cdot 7\ H_2O$, 0.01 g; oligoelements[5], 1 ml; KH_2PO_4/K_2HPO_4 buffer (pH 6.8-7.0), 0.05 mole/l; adjusted to 1 l with distilled water. 50-ml portions of the final growth medium were distributed into 200-ml Erlenmeyer flasks. Inoculation was made with 0.1 ml of a suspension of conidia (10^5 spores/ml). For non-conidial species, inoculations were made with a disc (diameter 6 mm) obtained from a young culture on water agar (for *Pythium*) or water agar supplemented with 0.5% Difco malt extract. Cultures were kept at 25 °C in the dark and orbitally agitated (125 rpm). Dry weights of triplicate cultures were determined as