Effects of administration of testosterone on some biochemical correlates in seminal vesicle of *Heteropneustes fossilis* (Bloch) during preparatory phase: A study correlating changes in plasma testosterone level and testis activity

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In the catfish *H. fossilis*, administration of testosterone (0.25, 0.5, 1 and 2 Jlg/g body weight for 20 days) during mid-preparatory phase (March) increased plasma testosterone, gonadosomatic index, seminal vesicle-somatic index and concentrations of total proteins, fructose and hexosamines in seminal vesicle (SV) and testis in a dose-related manner. In the lowest dosage (0.25 Jlg) group, only the hexosamine and SV protein levels were significantly high. Glucose level decreased in a dose-related manner, the decrease being not significant in the 0.25 Jlg group. The results indicate that testosterone stimulates SV and testicular secretions of total proteins, hexosamines and fructose in catfish. Decrease in glucose content suggests its conversion into fructose under testosterone stimulation.

The male reproductive system of catfish is characterized by the presence of one or more pairs of seminal vesicle (SV) lobes which open to the common sperm duct. The SVs showed marked annual variations in their structure and secretory activity that can be correlated with the testis. The secretory activity of SV is stimulated by androgens. In mammals, the epididymis and accessory sex organs secrete a variety of chemical substances like proteins, fructose, hexosamines, sialic acid, etc., into the lumen which facilitate various functions such as capacitation, nutrition and transport of spermatozoa. These chemical correlates have been used as sensitive parameters to assess androgenic actions on the accessory sex glands. But in fishes, there are no studies involving the use of such sensitive markers to evaluate androgenic influences on testicular and SV metabolism except for a recent study by Singh and Joy in the catfish *Clarias batrachus*. In both *C. batrachus* and *H. fossilis*, total proteins, hexosamines and fructose showed significant annual variations with their concentrations increasing during gonadal recrudescent phase and decreasing during gonadal quiescent phase with the exception of glucose. Furthermore, the concentrations of these variables except glucose can be positively correlated with plasma testosterone titre.

Although the functional androgen responsible for the stimulation of spermatogenesis and secondary sexual characters in many teleosts including catfish is 11-ketotestosterone, testosterone was shown to stimulate spermatogenesis in hypophysectomized *Carassius auratus*, *Fundulus* and *H. fossilis*, possibly through its conversion into active androgens. Therefore, in the present study testosterone has been used to induce hyperandrogenism in the catfish *H. fossilis* to demonstrate androgenic control of SV and testicular function using biochemical parameters such as total protein, hexosamine, fructose and glucose.

**Materials and Methods**

Male *H. fossilis* (25-35 g body weight) were collected locally in Varanasi in early preparatory phase (last week of February 1997). They were acclimatized for 15 days under natural photoperiod and temperature (L:D 11.2:12.8; 18.88°C ± 6.53°C in February and L:D 11.6:12.4; 24.02°C ± 7.07°C in March) and fed goat liver during acclimatization and experiments. The acclimatized fish were divided into 6 groups of 50-55 each. Group 1 (initial control) fish were sacrificed at the start of the experiment. Fish were weighed and blood was collected by caudal puncture in heparinized tubes. The blood samples were centrifuged at 3000 rpm for 15 min at 4°C to separate plasma. Plasma was stored at -20°C for testosterone RIA. The fish were sacrificed by decapitation; testis and seminal vesicle (SV) were dissected out, weighed, and stored at -20°C for biochemical analysis. Group 2 (parallel control) fish were injected with 0.1 ml propylene glycol as vehicle.
control. Groups 3, 4, 5, and 6 were injected with testosterone (Sigma) intraperitoneally daily for 20 days in dosages of 0.25, 0.5, 1 and 2 μg/g body weight, respectively. Testosterone was dissolved in propylene glycol. After termination of the experiments, fish in all the groups were weighed, plasma collected and sacrificed by decapitation. The tissues (SV and testis) were removed quickly, weighed and stored at -20°C for biochemical analysis.

Study parameters—Seminal vesicle-somatic index (SVSI) and gonado-somatic index (GSI) were expressed as the weight of the SV and testis in g percent of body weight, respectively.

Plasma testosterone was assayed by an Equate-RIA125I testosterone diagnostic kit procedure (Binax, Portland, USA). The minimum sensitivity of the assay was 18 pg/ml. Intra-assay and inter-assay coefficients of variation were 3.33 and 5.96%, respectively.

Total protein levels in the SV and testis was determined by the method of Elson and Morgan22, as modified by Davidson23. Fructose concentration in the SV and testis was measured according to the method of Mann24. Glucose was measured by a standard glucose test reagent (acetate acid 94% and o-toluidene 6%). The details of the procedure were described previously21.

Statistical analysis—Data were expressed as means ± SEM and analyzed by a one way analysis of variance (ANOVA), followed by Newman-Keuls’ test. Differences were considered significant at \( P < 0.001 \) for ANOVA and \( P < 0.05 \) for Newman-Keuls’ test.

Results and Discussion
A comparison of initial control and vehicle-injected parallel control data show that significant temporal changes were noticed in the levels of plasma testosterone, SV proteins and glucose, and both SV and testicular hexosamines and fructose \( (P < 0.05, \) Newman-Keuls’ test). The administration of testosterone produced an overall significant effect on plasma testosterone level (Fig. 1; \( F = 12.97, P < 0.001, \) one way ANOVA). Testosterone level was significantly higher in 0.5, 1 and 2 μg groups compared to the vehicle control group \( (P < 0.05, \) Newman-Keuls’ test; for other comparisons, see Table 1). The injected dosage range was effective to induce hyperandrogenism in the fish in a dose-dependent manner. In a similar study in *C. batrachus*, testosterone administration caused elevations of both plasma testosterone and estradiol-17β levels7. In this study also, 0.25 μg steroid injection elevated plasma gonadotropin-II level (positive feedback) and other

<table>
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<th>Vehicle Testosterone dosages</th>
<th>0.25</th>
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<th>1.0</th>
<th>2.0</th>
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<td>6.50</td>
<td>7.20</td>
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<td>SVSI</td>
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<td>0.028</td>
<td>0.034</td>
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<td>GSI</td>
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<td>571.43</td>
<td>472.38</td>
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</table>

Mean values of all parameters except glucose are arranged in ascending orders and of glucose in a descending order. Groups that are not significantly different are underscored.
Fig. 1—Effect of testosterone injections on plasma level of testosterone in *H. fossilis* (mean ± SE, n=5). Comparisons with the vehicle control group are shown. *P* < 0.05; NS- not significant (one-way ANOVA, Newman-Keuls' test).

Fig. 2—Effect of testosterone treatment on seminal vesicle somatic index (SVSI) and gonadosomatic index (GSI) in *H. fossilis* (mean ± SE, n=5). Comparisons with the vehicle control group are shown. *P* < 0.05; NS- not significant (one-way ANOVA, Newman-Keuls' test).
Fig. 3—Effect of testosterone treatment on seminal vesicle (SV) and testicular concentrations of total proteins in *H. fossilis* (mean ± SE, n=5). Comparisons with the vehicle control group are shown. *P < 0.05; NS-not significant (one-way ANOVA, Newman-Keuls' test).

Fig. 4—Effect of testosterone treatment on seminal vesicle (SV) and testicular concentrations of hexosamines in *H. fossilis* (mean ± SE, n=5). Comparisons with the vehicle control group are shown. *P < 0.05 (one-way ANOVA, Newman-Keuls' test).
Fig. 5—Effect of testosterone treatment on seminal vesicle (SV) and testicular concentrations of fructose in *H. fossilis* (mean ± SE, n=5). Comparisons with the vehicle control group are shown. *P < 0.05; NS- not significant (one-way ANOVA, Newman-Keuls’ test).

Fig. 6—Effect of testosterone treatment on seminal vesicle (SV) and testicular concentrations of glucose in *H. fossilis* (mean ± SE, n=5). Comparisons with the vehicle control group are shown. *P < 0.05; NS- not significant (one-way ANOVA, Newman-Keuls’ test).
dosages decreased it (negative feedback) in a dose dependent manner. Therefore, the increase in plasma testosterone level in the 1 and 2 μg groups might be caused by the administered steroid itself. The testosterone level in 2 μg group (8.93 ng/ml plasma) was within the maximal limit of the steroid level (9.33 ng/ml) measured in plasma in prespawning phase of the annual reproductive cycle. The steroid treatment resulted in dose-dependent increases in the weight (Fig.2) of SV (SVSI, F = 28.59, P < 0.001, one way ANOVA) and testis (GSI, F = 7.96, P < 0.001, one way ANOVA). The increases were not significant in the 0.25 μg dosage group but were significantly higher in the higher dosage groups (see Table 1 for groupwise comparisons). The increase in SVSI and GSI indicate an overall stimulatory effect of the androgen treatment on these organs as has been reported in this species and in *C. batrachus*.

In testosterone treated *H. fossilis*, the concentrations of proteins (Fig. 3; SV: F = 19.07; testis: F = 12.74; P < 0.001, one way ANOVA), hexosamines (Fig. 4; SV: F = 36.72; testis: F = 29.36; P < 0.001, one way ANOVA) and fructose (Fig. 5; SV: F = 10.78; testis: F = 6.44; P < 0.001, one way ANOVA) increased in a dose-dependent manner. In the low dosage group (0.25 μg / g body weight), the changes were insignificant for fructose and testicular proteins. In the remaining dosage groups, the changes were significant (except for testicular fructose in the 0.5 μg group) and maximal in the highest dosage (2 μg) group (see Table 1 for group comparisons). Proteins, hexosamines and fructose are major components of the epididymis and accessory sex gland secretions in mammals and *C. batrachus*.

In *C. batrachus*, a dose-dependent increase of total proteins, fructose and hexosamines were reported similar to the present observations. Thus, the positive changes were insignificant for fructose and testicular proteins in the low dosage group (0.25 μg / g body weight), the changes were insignificant for fructose and testicular proteins. In the remaining dosage groups, the changes were significant (except for testicular fructose in the 0.5 μg group) and maximal in the highest dosage (2 μg) group (see Table 1 for group comparisons). Proteins, hexosamines and fructose are major components of the epididymis and accessory sex gland secretions in mammals and *C. batrachus*.

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In conclusion, the concentrations of proteins, hexosamines and fructose responded positively, and glucose negatively to hyperandrogenism induced by testosterone treatment. They can be used as sensitive markers to evaluate androgen actions in the gonads. That the SV and testicular correlates responded similarly to the steroid treatment suggests basic similarities in the regulatory mechanism of these metabolites.

Acknowledgement
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