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Annual Correlative Changes in Some Biochemical Contents of Seminal Vesicle and Testis in the Catfish *Clarias batrachus* (L)

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ABSTRACT—The seminal vesicle (SV) and testis of *Clarias batrachus* (L) exhibit significant annual variations in SVSI and GSI, and in the levels of total proteins, fructose, hexosamines and sialic acid with low values in resting phase and high levels in spawning phase. Histologically, the SV is composed of numerous lobules lined by epithelial cells enclosing a lumen and an interstitial matrix containing interstitial cells (homologous to Leydig cells) and fibroblasts. In the preparatory phase, the SV undergoes extensive growth and proliferation of the lobules (April) with numerous buds of differentiating lobules. With the progress of secretory activity, the epithelial cells which were tall and columnar initially become cubical and squamous. The secretions filled the lumen resulting in distension and enlargement of the follicles and spermatozoa appear in the lumina. After voiding the contents, the SV lobules collapse and degenerate. New lobules are formed from the interstitium by proliferation. They showed transient secretory activity and remained inactive during the resting phase. The increases in total proteins, fructose, hexosamines and sialic acid levels in the SV are directly correlated with the histological changes and secretory activity. The levels of phospholipids and free fatty acids which showed significant variations are low during the spawning phase. The decline in the concentrations of esterified and free cholesterol (EC and FC) in both the testis and SV during the breeding season suggests their utilization as precursors in the synthesis of steroid hormones. The two, however, maintained an inverse relation during the non-breeding season with EC levels high and FC levels low. Scattered spermatogenic cysts in different stages of development are present in the SV epithelium. The similarities in the annual distribution of various biochemical correlates, and the presence of interstitial (Leydig) cells and scattered spermatogenic cysts suggest that the SV has a common origin with the testis and is specialized for secreting a glyco-lipoprotein-rich substance for temporary storage of the sperm.

INTRODUCTION

Seminal vesicles (SV) are accessory reproductive structures present in some teleosts, notably in catfishes, gobies and blennies (Sundararaj, 1958; Nayyar and Sundararaj, 1970; Van den Hurk *et al*., 1987; Fishelson *et al*., 1994; Lehri, 1967; Rastogi, 1969; Asahina *et al*., 1989; Lahnsteiner *et al*., 1990). Seasonal changes in the weight and histology of the SV in correlation with the testicular cycle were reported in catfishes, such as *Heteropneustes fossilis* (Bloch), *Clarias batrachus* (L) and *Mystus tengara* (Hamilton) (Sundararaj, 1958; Lehri, 1967; Rastogi, 1969; Nayyar and Sundararaj, 1970). Qualitative histochemical studies show that the SV secretes acid-mucopolysaccharides, mucoproteins and phospholipids (Nayyar and Sundararaj, 1970; Van den Hurk *et al*., 1987). The presence of spermatozoa in the SV of catfishes (Rastogi, 1969; Van den Hurk *et al*., 1987; Lahnsteiner *et al*., 1990; Senthilkumaran and Joy, 1993) indicates that it is a site for storage of spermatozoa in the nutritive carbohydrate-protein-phospholipid-rich secretion. The SV, like the testis, was reported to be steroidogenic (Van den Hurk *et al*., 1987; Lahnsteiner *et al*., 1990; Singh and Joy, 1998) and produce a variety of steroids and steroid glucuronides (Schoonen and Lambert, 1986; Resink *et al*., 1987). The steroid glucuronides of SV origin were reported to have a pheromonal role in reproduction (Van den Hurk and Resink, 1992). In blenniid fish, the testicular glands are involved in the differentiation and nutrition of spermatids, secretion of sialomucins which enhance the viscosity of seminal fluid and agglutination of spermatozoa, thereby enhancing fertilization, and in storage of lipids and phospholipids during the interspawning period (Lahnsteiner *et al*., 1990; Lahnsteiner and Patzner, 1990; Ota *et al*., 1996).

Previous research investigators have focussed on changes in gross morphology, weight, histology, epithelial cell height, lobular/luminal volume, *etc*., as study parameters for assessing the SV function. There is a paucity of information on quantitative chemical analysis of SV constituents. This is in stark contrast to the numerous chemical analysis studies

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available on the epididymis and accessory sex glands in mammals (Price and Williams-Ashman, 1961; Mann and Lutwak-Mann, 1981). The mammalian epididymis and accessory sex glands synthesize various constituents of seminal plasma, such as proteins, fructose, hexosamines, citric acid, sialic acid, phospholipids, etc. In a few teleosts, the chemical composition of semen (seminal fluid and spermatozoa) has been described (Kruger *et al*., 1984; Linhart *et al*., 1991; Billard *et al*., 1995; Kara *et al*., 1996). Senthilkumaran and Joy (1993) reported the presence of sialic acid in the SV of *H. fossilis* which showed cyclic changes during the annual reproductive cycle. Furthermore, they have shown that the secretion of sialic acid is androgen-dependent. This apart, there are no studies describing a quantitative chemical analysis of the SV constituents in teleosts.

The objective of the present study was to investigate annual changes in histology and concentration of total proteins, fructose, hexosamines, sialic acid, and various classes of lipids in the SV in relation to the testicular cycle.

MATERIALS AND METHODS

Adult *Clarias batrachus* (60–65 g) were collected from local fish markets in Varanasi in the middle of each month beginning from 1989 for one complete year. Thirty male fish were used in each month for sampling. They were maintained in the laboratory in flow-through aquaria. After two days, fish were weighed and sacrificed by decapitation and dissected out to expose the male reproductive system. Testis and SV were fixed in Bouin's fluid for histology, weighed and stored at –20°C for estimation of various biochemical constituents.

Study Parameters

Whole mount preparation

The male reproductive system (July) was carefully dissected out *in toto* and photographed.

Gravimetric measurement

Weights of the testis and SV were expressed in 100 g body weight of fish to give, respectively, gonado-somatic index (GSI) and seminal vesicle- somatic index (SVSI).

Histology

The SV and testis were fixed in Bouin's fluid for 24 hr and paraffin sections were taken at 7.5 μm in transverse or horizontal planes and stained with Ehrlich's hematoxylin-eosin. Since histology of the testis of this species was described previously, it is not described in the results.

Estimations of Biochemical Correlates

The following biochemical correlates were studied:

(i) Total Proteins

Protein contents of SV and testis were determined by the method of Lowry *et al.* (1951). Tissues were weighed, homogenized in 1 ml triple distilled water (TDW) and centrifuged at 4000 rpm for 20 min. The supernatant (0.1 ml) was taken in a test tube and the volume was made up to 1.0 ml with TDW and subsequently 5.0 ml of alkaline copper reagent (prepared at the time of estimation by mixing 50 ml reagent A with 1 ml reagent B [Reagent A: sodium carbonate (2%), 2 g sodium carbonate in 100 ml 0.1 N sodium hydroxide (w/v); Reagent B: copper sulphate (0.5%) –5.0 mg copper sulphate was dissolved in 1.0 ml 1.35% sodium-potassium tartarate (w/v)]. The contents were mixed well and allowed to stand at room temperature for 10 min. 0.5 ml of 1.0 N Folin-Ciocalteu's reagent was then added and mixed well. After 20 min, optical density (OD) of the blue color developed was read at 750 nm against a reagent blank in a Systronics spectrophotometer. Protein contents were determined from a standard curve using bovine serum albumin standard.

(ii) Fructose

Fructose content was estimated by the method of Mann (1964). Tissues (SV and testis) were homogenized in 4.0 ml 80% ethanol and centrifuged at 5000 rpm for 10 min. To the supernatant, 0.5 ml 5% zinc sulphate and 0.5 ml 0.3 N barium hydroxide were added. Tubes were allowed to cool for 2 hr and centrifuged at 5000 rpm. The supernatant was transferred to another test tube. The fructose content in the supernatant was estimated in duplicate. 0.2 ml supernatant was taken in a test tube and the volume was made up to 2.0 ml with TDW. To this, 2.0 ml 0.1% alcoholic resorcinol was added and shaken properly. Six ml 30% hydrochloric acid was added. The mixture was incubated for 10 min at 80–90°C and cooled. Optical density of the brown color developed was recorded at 490 nm against the water blank in a spectrophotometer and the content determined from a standard curve using fructose as a standard.

(iii) Hexosamines

Hexosamine content was estimated according to the procedure of Elson and Morgan (1933), as modified by Davidson (1966). Tissues (SV and testis) were minced properly and transferred to test tubes. They were hydrolysed with 2.0 ml 3 N hydrochloric acid for 4 hr at 100°C. The tubes were then cooled and neutralized with 1.0 ml of 6 N sodium hydroxide. To 1.0 ml of the samples, 1.0 ml freshly prepared acetyl acetone reagent (prepared by adding 1.0 ml of redistilled acetyl acetone to 50 ml 0.5 N sodium carbonate solution) was added, closed with a lid and kept in a boiling water bath for 45 min. The tubes were cooled and then 4.0 ml 95% ethanol was added and mixed well. This was followed by addition of 1.0 ml freshly prepared Ehrlich's reagent (prepared by adding 0.4 g of p-dimethyl amino benzaldehyde to the solvent mixture of 15.0 ml methanol and 15.0 ml concentrated hydrochloric acid). Tubes were allowed to stand at room temperature for 1.0 hr. Optical density of the pink color developed was recorded at 530 nm against the blank in a spectrophotometer and the content determined from a standard curve using a mixture of glucosamine and galactosamine as standard.

(iv) Sialic acid (n-acetyl neuraminic acid)

Sialic acid contents of the tissues were determined by the method of Warren (1959). The tissues were washed with 1:1 chloroform-methanol mixture to remove fat and 50 mg tissues were homogenized in 2 ml TDW. One ml 1% phosphotungstic acid was added to precipitate protein. The precipitate formed was washed twice with 1 ml 5% trichloroacetic acid and dried in a desiccator containing sodium hydroxide pellets for 12 hr. The tubes were then taken out from the desiccator and the precipitate in each tube was hydrolysed with 1 ml 0.1 N sulphuric acid for 1 hr at 80°C. The hydrolysed sample was centrifuged at 1000×g for 10 min and to 0.3 ml supernatant, 0.1 ml sodium metaperiodate (0.2 M, 2.13 g sodium metaperiodate was dissolved in 100 ml 9 M phosphoric acid) was added. The tubes were shaken well and allowed to stand at room temperature for 20 min. One ml 10% sodium arsenite solution (prepared by adding 10 g sodium arsenite in 100 ml 0.5 M sodium sulphate) was then added to each tube and the tubes were shaken well until the yellow- brown color disappeared. (0.5 M sodium sulphate was prepared by adding 7.102 g sodium sulphate in 100 ml of 0.1 N sulphuric acid w/v). Three ml of 0.6% thiobarbituric acid solution (prepared by adding 600 mg of thiobarbituric acid in 100 ml 0.5 M sodium sulphate and warmed) was then added. The tubes were covered with glass marbles and kept in a vigorously boiling water bath for 15 min. The tubes were then placed in cold water bath for 5 min. To this, 4.3 ml acidified butanol was added, the tubes were shaken vigorously in order to extract the pink color into

the organic phase and then centrifuged for 3 min at 1000×g. The upper organic phase was transferred to a cuvette and optical density was determined at 532 and 562 nm against a reagent blank in a spectrophotometer. Sialic acid in the tissues was calculated using Warren's formula II (Warren, 1959), as follows:
μmoles of N-acetyl neuraminic acid (sialic acid) = 0.138×OD at 562 nm – 0.009×OD at 532 nm. The sialic acid concentration was expressed as μ moles N-acetyl neuraminic acid/100 mg tissue weight.

(v) Lipids

Total lipid (TL) contents of SV and testis were estimated by the procedure of Folch *et al.* (1957). The tissue was quickly extirpated washed and blotted in 0.6% NaCl. About 40-50 mg of the tissue was homogenized in 20 volume of chloroform and methanol mixture (2:1, v/v). The suspension was left at room temperature for 2 hr and filtered. An equal volume of 0.6% NaCl was added to the pooled filtrate. After mixing thoroughly, it was transferred to a separating funnel and left in dark for overnight. Later, the lower organic phase was separated and evaporated at 60°C in an oven. The residue was weighed for total lipids.

Separation and estimation of different classes of lipids by thin layer chromatography (TLC)

Phospholipids (PL), free fatty acids (FFA), esterified cholesterol (EC), and free cholesterol (FC) were separated using the double solvent system of Freeman and West (1966). Silica gel G-coated (500 μm thickness) glass plates (20 × 20 cm) were activated at 110°C for 45 min after air- drying. A known amount of the chloroform and methanol (1:1 v/v)- reconstituted lipid extract was loaded on the activated plates by a microsyringe. The plates were left at room temperature for drying and were kept in a saturated tank containing solvent system I (diethylether : benzene : ethanol : acetic acid = 40 : 50 : 2 : 0.2, v/v). When the solvent reached 12 cm from the origin, it was taken out and dried in an oven at 60°C to remove acetic acid. After brief cooling at room temperature, the plates were placed in a second tank containing solvent system II (diethylether : hexane = 6 : 94, v/v) and allowed to run 16 cm from the origin. Later it was dried at room temperature and exposed to iodine vapour for 5 min to visualize the spots. The spots were scraped and quantitated by the procedure of Marzo *et al.* (1971). The scraped lipid fractions along with silica gel were taken separately in test tubes containing 5 ml concentrated sulphuric acid for 30 min incubation at 180°C. The test tubes were immediately cooled in cold water and subsequently in ice bath. The samples were centrifuged at 700 g for 5 min and absorbance of the supernatant was read at 375 nm in a Systronics spectrophotometer. For preparation of standard curves, stock solutions of phospholipid (L-α-phosphatidylcholine); free fatty acid (palmitic acid); free cholesterol (cholesterol oleate); and esterified cholesterol were prepared in chloroform (1.0 mg/ml). Graded concentrations of stock solutions, such as 25, 50, 100, 200, and 250 μg were loaded in the activated TLC plates and processed like the samples. After repeated loading, Rf values were calculated and were found to be 0.00, 0.27, 0.44 and 0.90, respectively, for PL, FFA, FC and EC. A linear relationship was also obtained between the concentrations tested and absorbance read for each lipid. The tissue content of the various lipids was calculated from the following formula :
Lipid (PL, FFA, FC and EC) concentration = (OD of unknown/OD of known concentration) x Standard concentration. The lipid contents were expressed in μg/100 mg tissue weight.

Statistical Analysis

All data were expressed as means±SEM (standard error of mean). The data were analyzed by one-way analysis of variance (ANOVA), followed by Newman-Keuls' multiple range test ($p < 0.05$) for comparison of group means.

RESULTS

Annual changes in the morphology of the reproductive system

The reproductive system of male *C. batrachus* consists of a pair of elongated testis which unite posteriorly to form a common sperm duct that opens out ventrally through a genital pore. The inner margins of the testes are smooth while the outer ones are frilled and deeply indented to form incomplete lobes, especially in the breeding season. On either side of the sperm duct arise 5 to 8 pairs of lobes which are filled with secretions in the spawning phase (Fig. 1). The SV is composed of numerous lobules embedded in the connective tissue stroma. It showed marked histological changes during the reproductive cycle which are described under the following headings :

Resting phase—The period extends from November to January. The SV is small and consists of a few differentiated lobules and several cords of cells that are not organized into lobules (Fig. 2).

Preparative phase (February-April)—The SV increases further in size and the lobules are differentiated and lined by tall columnar epithelia. The interstitium is thick and contains numerous round or spherical cells, spindle-shaped fibroblasts and blood capillaries. The lumina of the lobules are empty or scant secretions may be seen. In the late preparatory phase (April), the increase in size of the SV is matched by both proliferation and growth of the lobules (Figs. 3, 4). Numerous young proliferating buds can be seen throughout the SV (Fig. 3). During the formation of a new lobule, fibroblasts appear to play an active role. They are hypertrophied and move to the proliferating sites and secrete a matrix layer. The epithelial cells adjacent to it divide and crowd together (Fig. 4). As the matrix elongates, these cells are arranged forming single-layered epithelia on either side. Proliferating buds grow from other sites and meet forming new lobules. The epithelial cells of the fully formed lobules start secretory activity, secretions appear first at the apical (mucosal) part of the cells as apocrine-like processes and are shed subsequently into the lumen. The interstitium is thick, extensive and highly vascularized. The interstitial cells are large and spherical with prominent nuclei and nucleoli (Figs. 2, 4). These cells are surrounded by blood capillaries.

Prespawning phase (May-June)—The epithelium becomes further tall (Fig. 9) and secretory activity is intensified further. The secretion is stainable with alcian blue and periodic acid-Schiff (figure not shown). As the secretory activity progresses, the cells change their shape from columnar to cubical and finally to flat (squamous) condition.

Spawning phase (July-August)–The lobules are laden with the secretory material and the epithelium becomes flat (Fig. 5). Spermatozoa are present in the lumen. In some lobules,

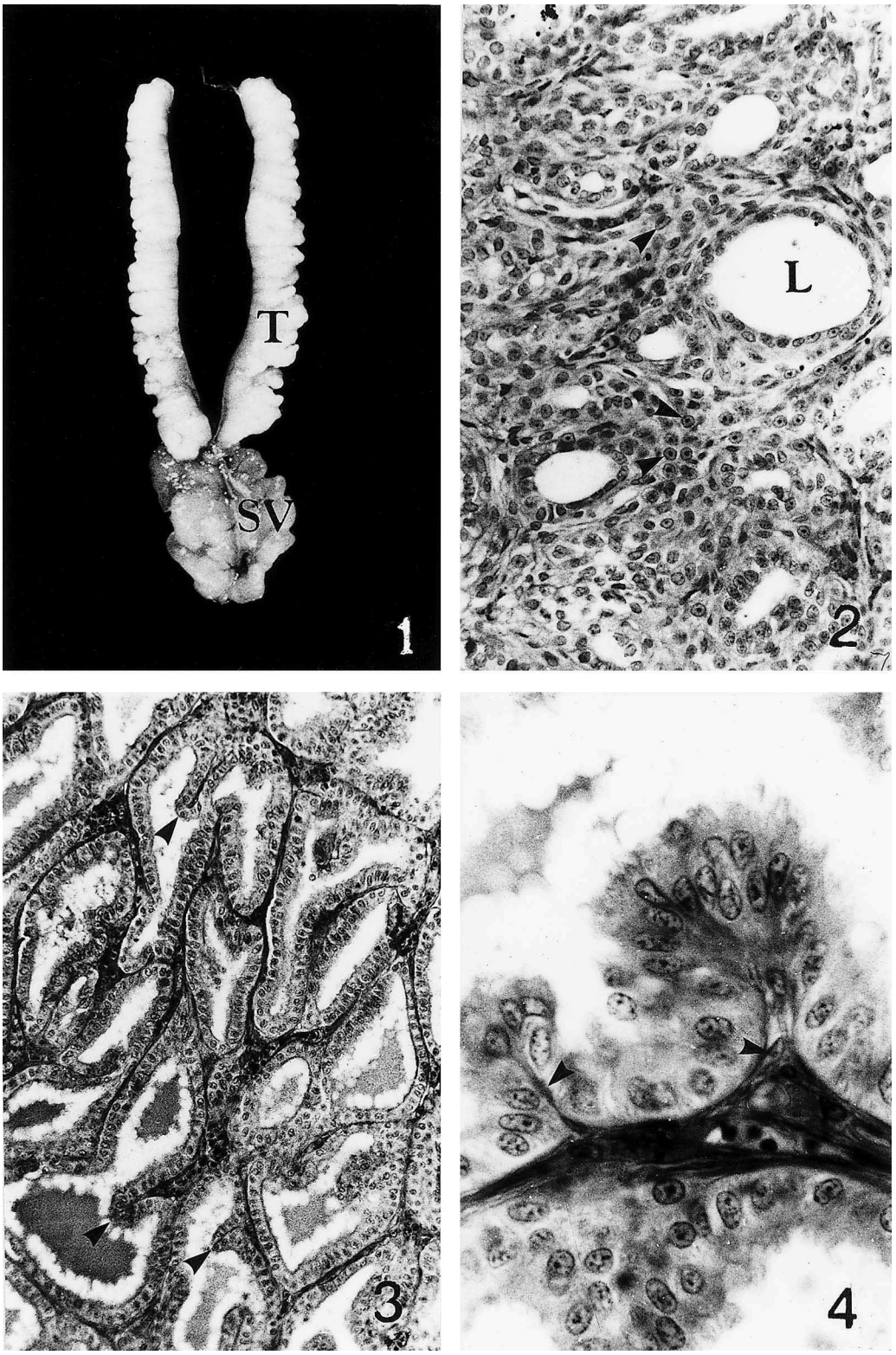

Fig. 1. A whole-mount preparation of the male reproductive system of *C. batrachus* in spawning phase (July) . ×2.4
Fig. 2. A transverse section (TS) of the SV in January (resting phase) showing a few differentiated lubules and several undifferentiated cell cords. Arrow-heads show interstitial cells. L-Lumen, Ehrlich's Hematoxylin-Eosin. ×240
Fig. 3. A horizontal section of the SV in late preparatory phase (April) showing proliferation buds (arrow-heads). Note the tall and columnar epithelium and the highly vascularized interstitium. ×240
Fig. 4. A horizontal section of the SV in April showing proliferation buds. The epithelial cells divide mitotically and crowd together for a while. Note fibroblasts (arrow-heads). ×925

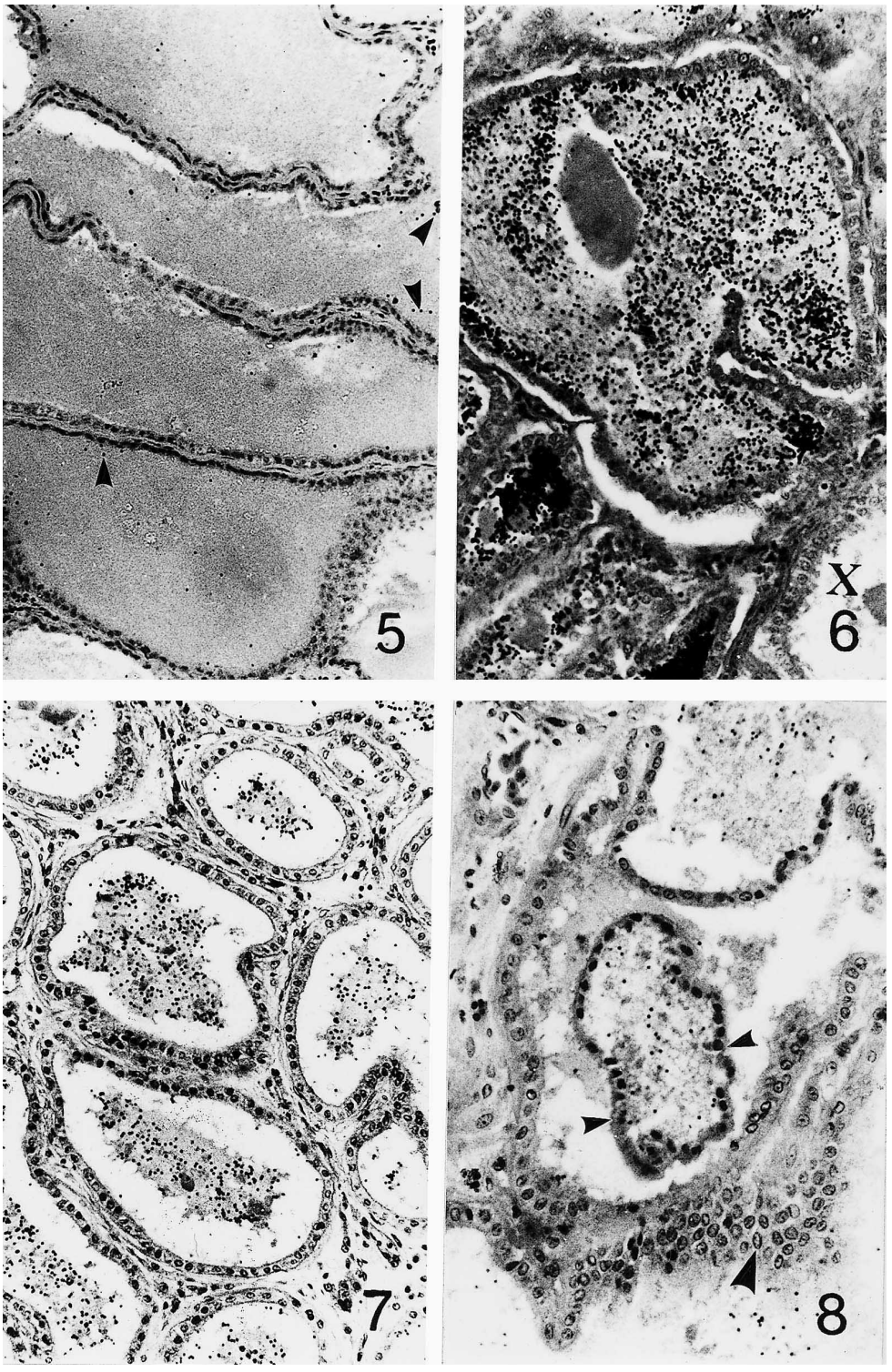

Fig. 5. A TS of the SV of a spawning phase (July) fish showing secretion- filled lobules containing sparse amount of spermatozoa (arrow-heads). ×240

Fig. 6. A TS of the SV of another fish in spawning phase (July) showing spermatozoa embedded in the secretion. Note some lobules are voided (X) and the epithelium is thick. ×260

Fig. 7. A TS of the SV of a postspawning phase (September) fish showing decreased size of the lobules with pycnotic epithelial cells. The lumen contains small amounts of secretory material with spermatozoa embedded in it. The interstitium is thick with inactive interstitial cells and fibroblasts. ×236

Fig. 8. A TS of the SV of a postspawning phase (September) fish showing extensive degenerative and regenerative processes. The old epithelia with pycnotic cells were collapsed and exfoliated into the empty lumen (small arrow-heads). Note the proliferation of new epithelial cells (large arrow-head) which form new epithelia. The newly formed epithelial cells show transient secretory activity. ×370

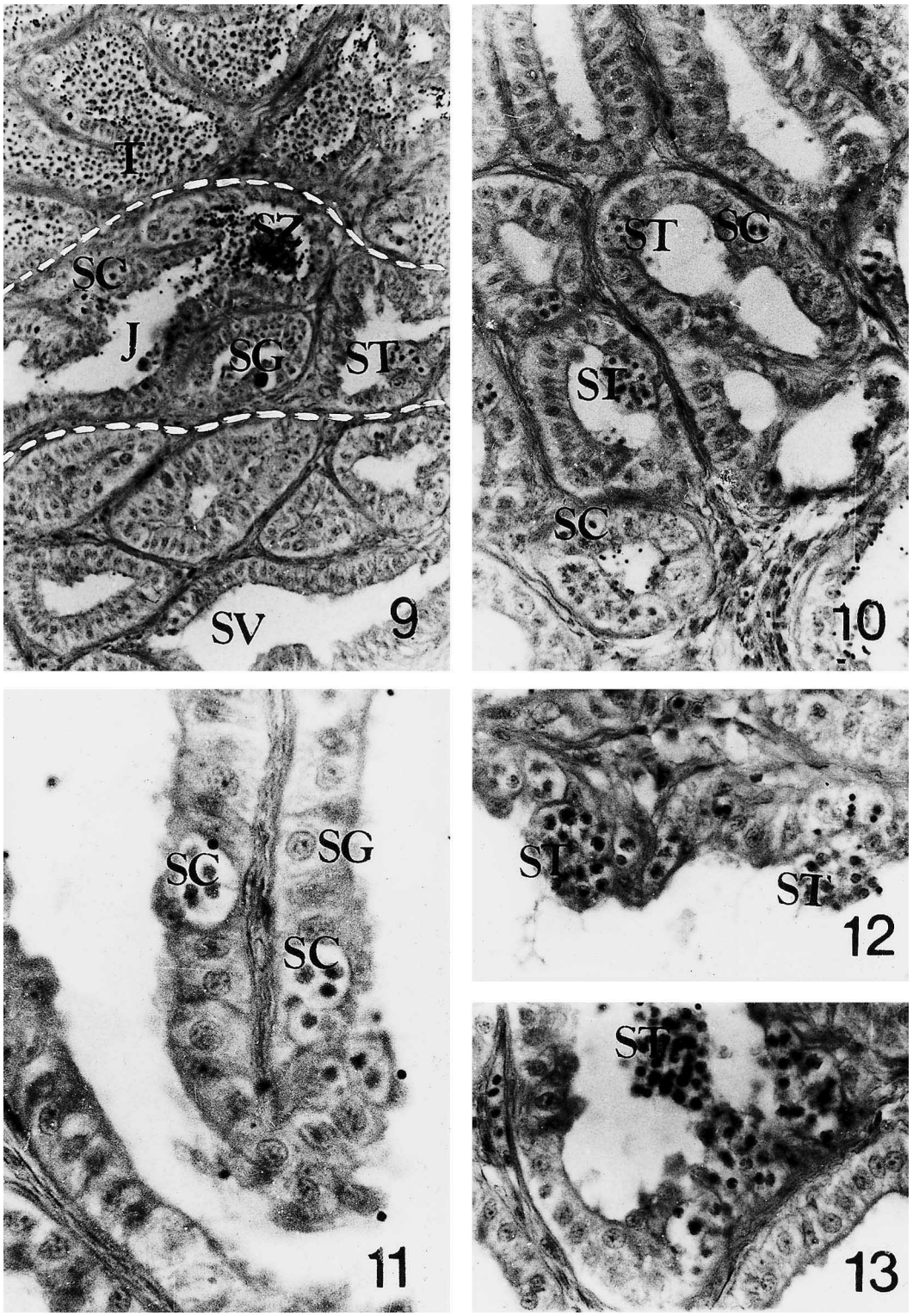

Fig. 9. A horizontal section through the junction between the testis (T) and SV (J between dotted lines) of an early prespawning fish (May). The SV lobules show several spermatogonial cysts containing spermatogonia (SG), spermatocytes (SC), spermatids (ST), and spermatozoa (SZ) in the lumen. ×240

Fig. 10. A horizontal section of the SV of a late prespawning fish (June) showing a large number of spermatogenic cysts containing spermatogonia (SG), spermatocytes (SC) and spermatids (ST) in the SV lobules. ×370

Figs. 11–13. Horizontal sections of the SV of a late prespawning fish (June) showing spermatogenic cysts in different stages of development. Abbreviations as in Fig. 9 and 10. ×925, 720, 720

the lumen is gradually emptied and the epithelium becomes irregular and wavy (Figs. 5, 6).

Postspawning phase (September-October)—The lobules are voided with sparse amount of secretory material in the lumen (Fig. 7). The epithelial cells are pycnotic, detached from the interstitium, collapsed, and exfoliated into the lumen (Fig. 8). Side by side with the degenerative changes, repair process also sets in. New epithelial cells are proliferated apparently from the interstitium and are organized to form new epithelia which surround the collapsed old epithelia in the lumen (Fig. 8). The new epithelial cells showed signs of transient secretory activity as evident from the presence of secretions inside the lumen. As the quiescent phase progresses, the lobules become smaller in size and dormant till the next breeding reason.

Occurrence of spermatogenic cysts in the SV—In the SV epithelium, numerous spermatogenic cysts in different stages of development were found (Figs. 9, 10). They are very common in the lobules at the junction between the testis and SV (Fig. 9) and are also scattered in other parts of the SV (Figs. 10, 11, 12, 13). The cysts contribute to the sperm content of the SV.

Annual changes in the SVSI and GSI (Fig. 14)—The SVSI and GSI showed significant and parallel annual variations ($F=75.045$, $p < 0.001$ and $F=89.138$, $p < 0.001$, respectively; one- way ANOVA) with peak values in July (early spawning phase). Newman-Keuls' analysis showed that the SVSI values in June and July were not significantly different from each other but were significantly different ($p < 0.05$) from all other values. The GSI values in May, June, and July were significantly different among them and also from all other values except those in May and August.

Annual changes in total proteins (Fig. 15A)—The levels of total proteins showed significant annual variations (SV : $F= 28.35$; $p < 0.001$; testis : $F=5.57$; $p < 0.001$; one-way ANOVA), with high levels in prespawning-spawning phases (May-July) and declining after spawning. Newman-Keuls' analysis showed that the SV protein values in May, June and July were not significantly different among them but were significantly different from all other values. The testicular protein values in June and July were not significantly different from those in April and May but were different from all other months.

Annual changes in fructose (Fig. 15B)—The fructose levels showed significant annual variations both in the SV ($F= 119.34$, $p < 0.001$) and testis ($F=73.69$, $p < 0.001$). The levels were not detectable in January (resting phase) and appeared in February (early preparatory phase). The concentrations increased steadily and reached the peak levels in July (early spawning phase) and declined from late spawning phase (August) onwards. Newman-Keuls' analysis showed that the SV values in July were significantly higher than all other values. The May and June values were not significantly different from each other but were significantly different from all other values. The testicular fructose level in July was significantly higher than all other values. The values in April, May and June did not vary significantly. The fructose concentrations in May and August also did not vary significantly.

Annual changes in hexosamines (Fig. 15C)—The hexosamine concentrations in the SV and testis showed significant annual variations (SV : $F = 51.10$; $p < 0.001$; testis : $F =$

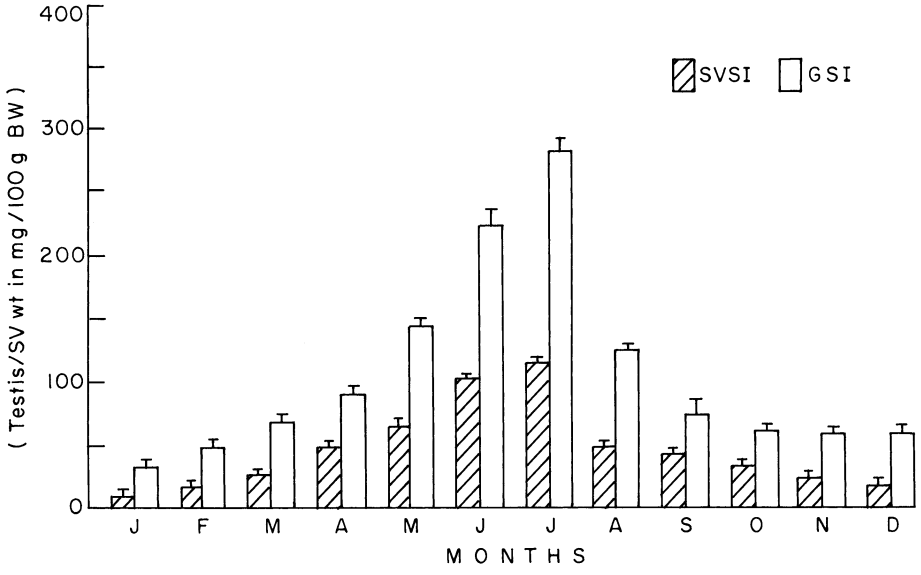

Fig. 14. Annual variations in seminal vesicle-somatic index (SVSI) and gonadosomatic index (GSI) of *C. batrachus* (means±SEM; n=5). One-way ANOVA; Newman-Keuls'test (see text for details).

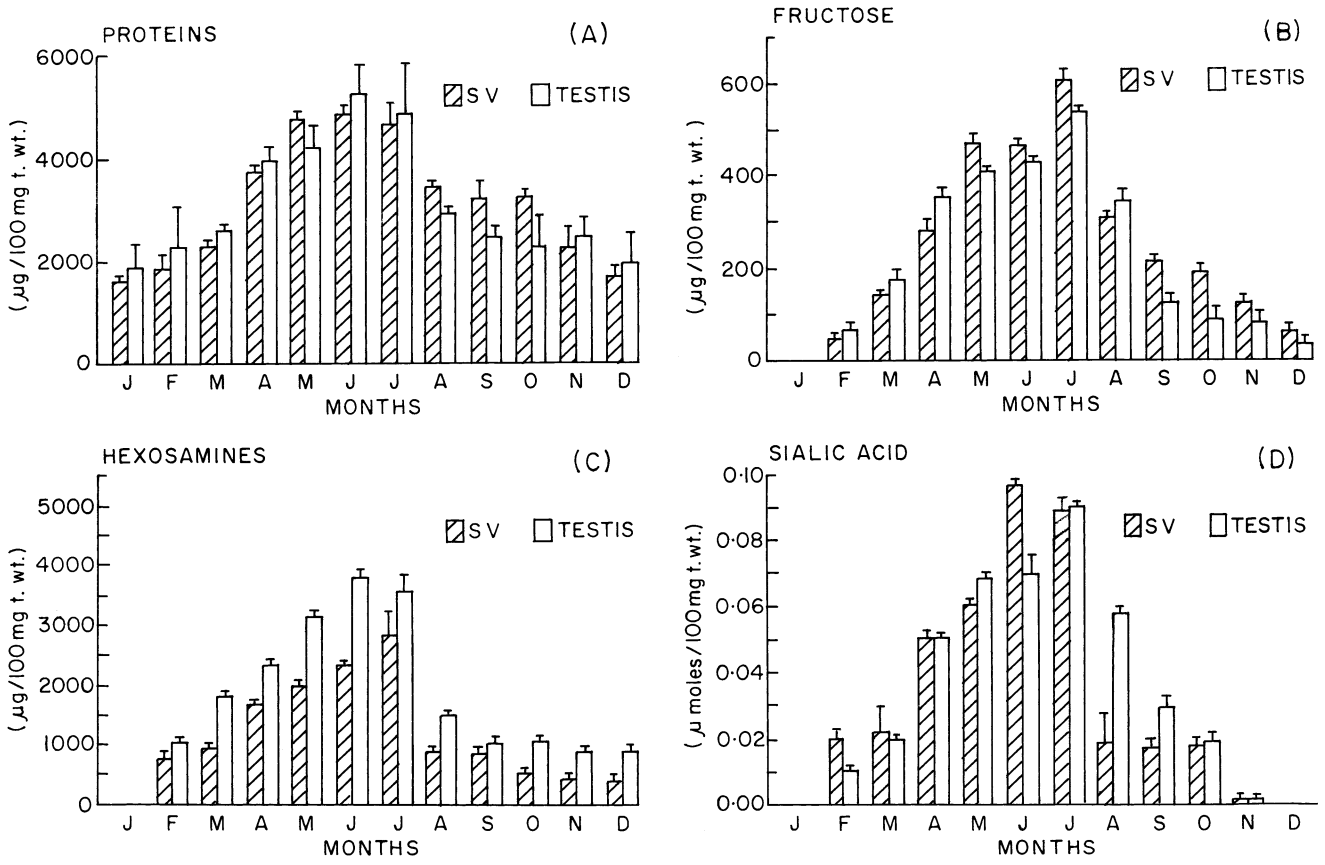

Fig. 15A–D. Annual variations in total protein (A), fructose (B), hexosamines (C), and sialic acid (D) levels in the seminal vesicle and testis of *C. batrachus* (means±SEM; n=5). One-way ANOVA; Newman-Keuls' test (see text for details).

84.18; $p < 0.001$). The level was not detectable in January (resting phase). In the testis, the levels increased steeply from the preparatory phase (February-April) to reach the peak values in June-July and declined after spawning. The hexosamine contents of the SV increased gradually till March, shot up in April, and then increased gradually to reach the peak level in July. Newman- Keuls' analysis showed that the hexosamine content of the SV in July was significantly higher than all other values. In the testis, the June-July levels did not vary significantly but were significantly higher than all other values except the June and May values.

Annual changes in sialic acid (Fig. 15D)—The sialic acid content showed significant variations both in the SV ($F = 49.81$; $p < 0.001$) and testis ($F = 86.74$; $p < 0.001$). The levels increased during the preparatory-prespawning phases to reach the peak value in June (prespawning phase) in the SV and in July (spawning phase) in the testis. The levels decreased after spawning and were undetected in December and January. Newman- Keuls' analysis showed that the SV values in June-July did not differ significantly but was significantly higher than all other values. In the testis, the July value was significantly higher than all other values.

Annual changes in phospholipids (PL) (Fig. 16A)—Significant annual variations were noticed in the PL content of the SV ($F = 6.42$; $p < 0.001$) and testis ($F = 15.50$; $p < 0.001$). There was a gradual increase in the phospholipid content during the recrudescent phase with high values in May (prespawning phase) and a gradual reduction subsequently to low values in November. The values increased again in December-January. In the SV, the mean values of phospholipid were higher than that of the testis except in June, July, August, and December. Newman-Keuls' analysis showed that in the SV, the phospholipid levels were significantly different in the following months : March *vs.* October, November and December, April vs. August, September, October, November and December, and May *vs* July, through December. In the testis, the peak value in May was significantly higher than all other values except that in June. The levels did not vary significantly (September to November).

Annual variations in free fatty acids (FFA)—The FFA levels varied annually with low values in May ($F=13.41$; $p < 0.001$) in the testis and in May-June ($F=7.61$; $p < 0.001$) in the SV (Fig. 16B). In the testis, the FFA content increased to high values during July-November but declined again in December. In contrast, the FFA levels were high in the SV during early and mid-preparatory phase (January- March). Newman-

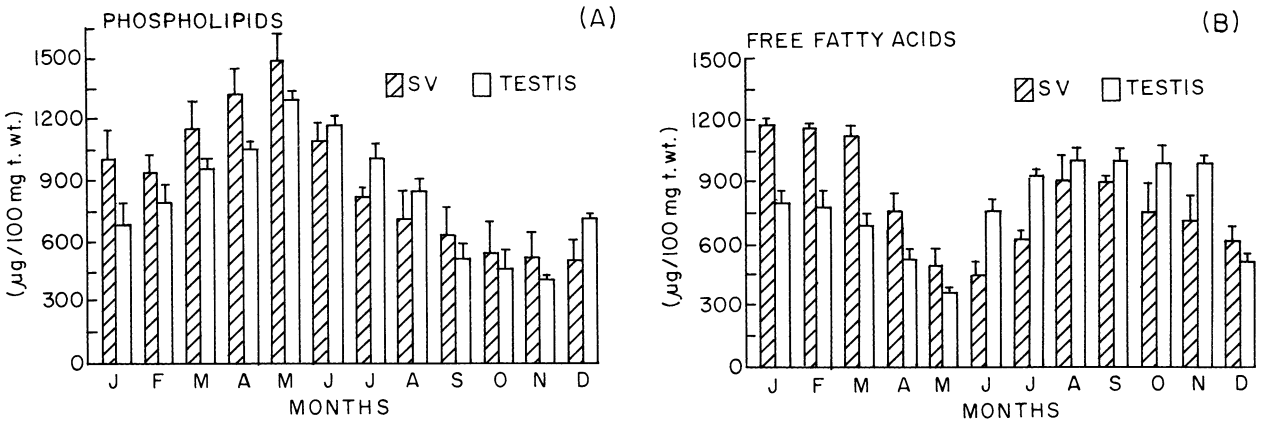

Fig. 16A, B. Annual variations in phospholipid (A) and free fatty acid (B) levels in the seminal vesicle and testis of *C. batrachus* (means ±SEM; n=5). One- way ANOVA; Newman- Keuls' test (see text for details).

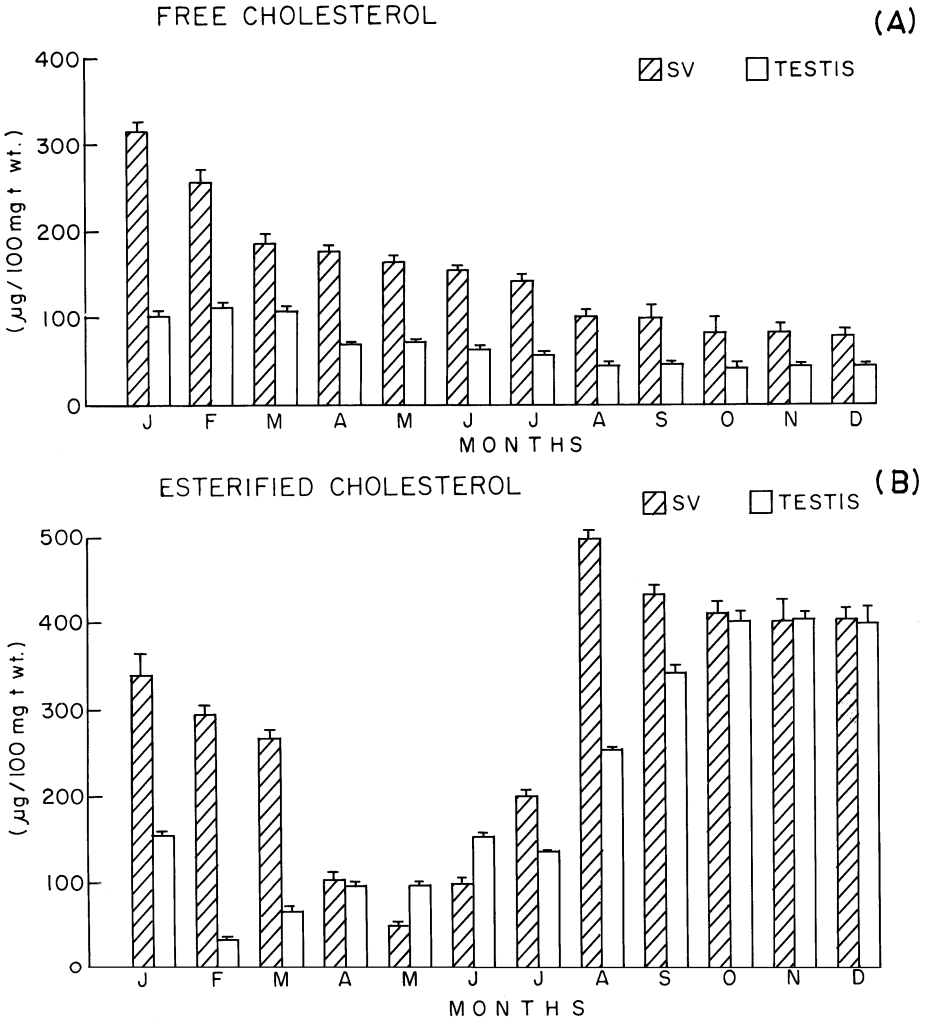

Fig. 17A, B. Annual variations in free cholesterol (A) and esterified cholesterol (B) levels in the seminal vesicle and testis of *C. batrachus* (Means±SEM; n=5). One-way ANOVA; Newman - Keuls' test (see text for details).

Keuls' analysis showed that the FFA contents of the SV in January, February and March did not vary significantly but were significantly higher than those in May, June, July, October, November, and December. In the testis, the FFA content in May was significantly lower than that in January through March, and June, through November. The levels in August, through November were not significantly different from those of January and February except February *vs* October values.

Annual variations in free cholesterol (FC)—The FC levels showed significant annual variations both in the SV ($F=103.28$, $p < 0.001$) and testis ($F=81.40$; $p < 0.001$) with values higher in the former (Fig. 17A). The highest level was noticed in January (resting phase) and declined with the progress of the recrudescent phase to low values in the postspawning phase. The FC levels in January and February were significantly higher than all other values (Newman-Keuls' test). The FC value in March did not differ significantly with those in April, through July. Likewise, the values during August-December also did not vary significantly. In the testis, the FC levels in January, through March did not vary significantly but were higher than all other values (Newman-Keuls' analysis).

Annual variations in esterified cholesterol (EC)—The EC levels showed significant seasonal variations both in the SV ($F=553.88$; $p < 0.001$) and testis ($F=573.31$, $p < 0.001$) (Fig. 17B). In the SV, the peak EC level was observed in August (late spawning phase) and decreased gradually during the postspawning-resting-preparatory phases (September, through December and January, through April) to give the lowest value in May. Newman- Keuls' analysis showed that in the SV, the EC levels were not significantly different from each other except those in April and June, and September to December. In the testis, the level was lowest in February (early preparatory phase) and increased to high levels during September-December. Newman-Keuls' analysis showed that the EC levels were significantly different in all months except the values of January *vs.* June and July, April *vs.* May, June *vs.* July, and October, through December.

DISCUSSION

Annual histological changes in the SV

The SV showed significant histological changes during the annual reproductive cycle which could be correlated with the annual testicular changes. During the late preparatory (April) and prespawning (May) phases, the SV showed two important histological events viz., (1) an extensive proliferation of the lobules, and (2) secretory activity which accounted for the significant increases in the weight and size of the SV. The large number of villi-like proliferating buds in various stages of lobule formation indicated extensive prolifera-tive activity in the SV. The fibroblasts seem to play an import-ant role in this process as they are hypertrophied and conspicuous. In the catfish, the proliferative and secretory activity in the SV coincided with the rise in the blood level of testosterone which suggests that testosterone may stimulate these processes (Singh and Joy, 1997). Secretory activity of the epithelial cells occurred concurrently with the proliferative activity which continued and intensified till the end of the prespawning phase (June). The secretions were stored in the lumen resulting in gradual distension of the lobules to the maximum extent. During this period, the epithelial cells, which were originally tall and columnar, became progressively reduced in size to assume cubical and finally flat and squamous forms. It is not clear whether the change in the morphology of the cells was due to (1) progressive shedding of the apical part of the cells into the lumen as secretory material (apocrine activity), or (2) to intraluminal pressure of the stored secretory material on the cells to become flat (Rasto-gi, 1969; Van den Hurk *et al.*, 1987). After spawning, the SV showed extensive degenerative and regenerative changes after the discharge of secretions, such as collapse of the follicles, pycnosis of the epithelial cell nuclei, exfoliation of the epithelia into the lumen, phagocytosis and resorption. Such break down of the epithelial cells were also described in the SV of *M. tengara* (Rastogi, 1969). The atrophied epithelial cells were simultaneously replaced with new ones which seem to have proliferated from the interstitium. The new epithelial cells were tall and columnar and showed transient secretory activity before complete quiescence. Degenerative changes and cell renewal have been also reported in the seminal vesicle of mammals (Brande (Ed.) 1974; Mann and Lutwak-Mann, 1981; Bollack and Clavert (Ed.) 1985). In *C. batrachus*, Sperm storage was found as has been reported in other catfishes (Rastogi, 1969; Van den Hurk *et al.*, 1987; Senthilkumaran and Joy, 1993; Fishelson *et al.*, 1994).

Annual changes in biochemical correlates

The present data show that the concentrations of proteins, fructose, hexosamines, sialic acid and various lipid moieties exhibited significant and similar annual variations in the SV and testis which followed closely the changes in histology and weights (GSI and SVSI) of the organs and circulating levels of gonadotropin and testosterone (Singh and Joy, 1997). The secretions of proteins, fructose, hexosamines and sialic acid in mammalian male accessory sex organs are androgen- dependent (Mann and Lutwak-Mann, 1981; Luke and Coffey, 1994). Their levels are significantly higher during the prespawning- spawning phase as a result of increased secretory activity of the SV and secretory/spermatogenic activity of the testis. Total proteins were demonstrated in the semen (seminal fluid and spermatozoa) of a few teleosts and exhibited both seasonal and species variations (Kruger *et al.*, 1984; Billard *et al.*, 1995; Kara *et al.*, 1996). The fish semen proteins have been suggested to have an osmotic role (Kruger *et al.*, 1984). Fructose is the chief seminal sugar in most mammals (Mann and Lutwak-Mann, 1981; Luke and Coffey, 1994). In the seminal fluid, concentrations of fructose, glucose and galactose showed great species variations (Kruger *et al.*, 1984; Lahnsteiner *et al.*, 1994). Furthermore, the sugar concentrations also varied seasonally. While studying annual variations

in fructose and glucose concentrations in the SV and testis of *H. fossilis*, fructose was found to be the main sugar in the recrudescent phase and glucose in the resting phase (unpublished data). In *C. batrachus*, fructose was not measurable in the resting phase (January) which coincided with the absence of testosterone in the blood (Singh and Joy, 1997) and its concentration increased with maturity. The sugar may be an important source of energy for spermatozoan motility.

Hexosamines or six-carbon amino sugars are characteristic constituents of accessory male organ secretions in mammals. In the catfish, the concentration of hexosamines in the SV and testis which showed significant seasonal variations increased during the recrudescent phase and decreased after spawning. Apparently, they constitute a prominent component of the secretions of the SV and testis. Sialic acid is a complex class of amino sugar and a major component of the male sex accessory secretion. In mammals, it is secreted by the testis and accessory reproductive organs (Prasad and Rajalakshmi, 1977; Mann and Lutwak-Mann, 1981; Setchell *et al*., 1994). In the catfish, sialic acid occurs mostly in bound form, as in mammals and had to be hydrolyzed with acid before the measurement. In a previous study, Senthilkumaran and Joy (1993) demonstrated seasonal variations in sialic acid in the SV of *H. fossilis* peaking in July. In *C. batrachus*, it was detected in all months except in January (resting phase). The functional significance of sialic acid in sperm physiology is not clearly understood. In blenniid fish, sialomucins have been reported to increase the viscosity of the seminal fluid and the agglutination of spermatozoa (Lahnsteiner *et al*., 1990).

Lipids play an important role in reproduction of fishes; EC and FC serves as precursors of gonadal steroids: FFA and PL serve as energy source, and PL as structural component of developing germ cells (Mukherjee and Bhattacharya, 1982; Kirubagaran and Joy, 1992). The present data on annual changes in testicular lipids compared well with previous observations of Kirubagaran and Joy (1992). Furthermore, it was shown that the uptake of P^{32} into phosphoprotein fraction of the testis increased with the progress of spermatogenic activity (Kirubagaran and Joy, 1992). The decreased FFA content in the prespawning phase (May-June) in the SV and testis might be due to its utilization for energy requirement. The increase in the PL content during the recrudescent phase may be due to spermatogenic activity in the testis and storage of phospholipid-containing secretory material and sperm in the SV. In teleost gonads, CE and FC exist in dynamic equilibrium with each other and exhibit seasonal variations (Mukherjee and Bhattacharya, 1982; Kirubagaran and Joy, 1992). FC, hydrolysed from EC is the immediate precursor of gonadal steroids. The decrease in FC during the recrudescent phase may be, therefore, due to its increased conversion into steroid precursors both in the testis and SV and its increased levels in the resting phase (January) may be due to their increased production and decreased utilization in steroid biosynthesis resulting in accumulation. The decrease in EC levels during the early part of recrudescence may be due to their increased hydrolysis into FC. In the spawning-postspawning phase, its levels increased perhaps due to decreased hydrolysis and utilization.

Significance of spermatogenic cysts in the SV

An interesting observation of the present study is the demonstration of several spermatogenic cysts in various stages of development in the lobule epithelium of the SV and, in particular, in the transition zone between the testis and SV. Occurrence of such spermatogenic cysts was also reported by Sundararaj (1958) in the transition zone of the testis and SV in *H. fossilis* and in the basal portions of the SV of *C. gariepinus* (Fishelson *et al*., 1994). In *C. batrachus*, the cysts were very active in the postspawning phase when the lobules were empty. The presence of spermatogenic cysts in the SV strongly indicates that both the SV and testis might have differentiated from a common analage, as has been discussed by Fishelson *et al*., (1994). Therefore, the male gonad of *C. batrachus* can be divided into an anterior spermatogenic part (testis) and a posterior secretory part (SV). The seminiferous or secretory tubules (lobules) open into the efferent lateral sperm ducts which are co-terminus with the testes. Posteriorly the lateral ducts fuse to form a common sperm duct. Similar annual patterns of various biochemical correlates (total proteins, fructose, hexosamines, sialic acid and different classes of lipids) in testis and SV and steroidogenic nature of the organs (Singh and Joy, 1998) further underscore their common origin.

The modification of a part of the gonadal tissue (analage) as SV for secretion of a storage and nutritive medium for sperm is advantageous to the catfish in two ways. The small body weight to testis ratio in the catfish (only 0.3% in spawning phase), compared to a high ratio in salmonids or cyprinids (over 1%) indicates small size of the testis and small amount of milt production. The development of the SV with voluminous secretion of seminal plasma may help in the efficient use of the small amount of sperm in fertilization. Secondly, catfishes live in habitats (ponds, streams, rivulets, paddy fields, etc.) where water recedes or dries in summer, but survive in moist mud-burrows with their air breathing habit. With the arrival of the monsoon rain, the habitat is flooded again and the fish wriggle out and spawn. The SV ensures temporary storage of sperm during this period to achieve reproduction.

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