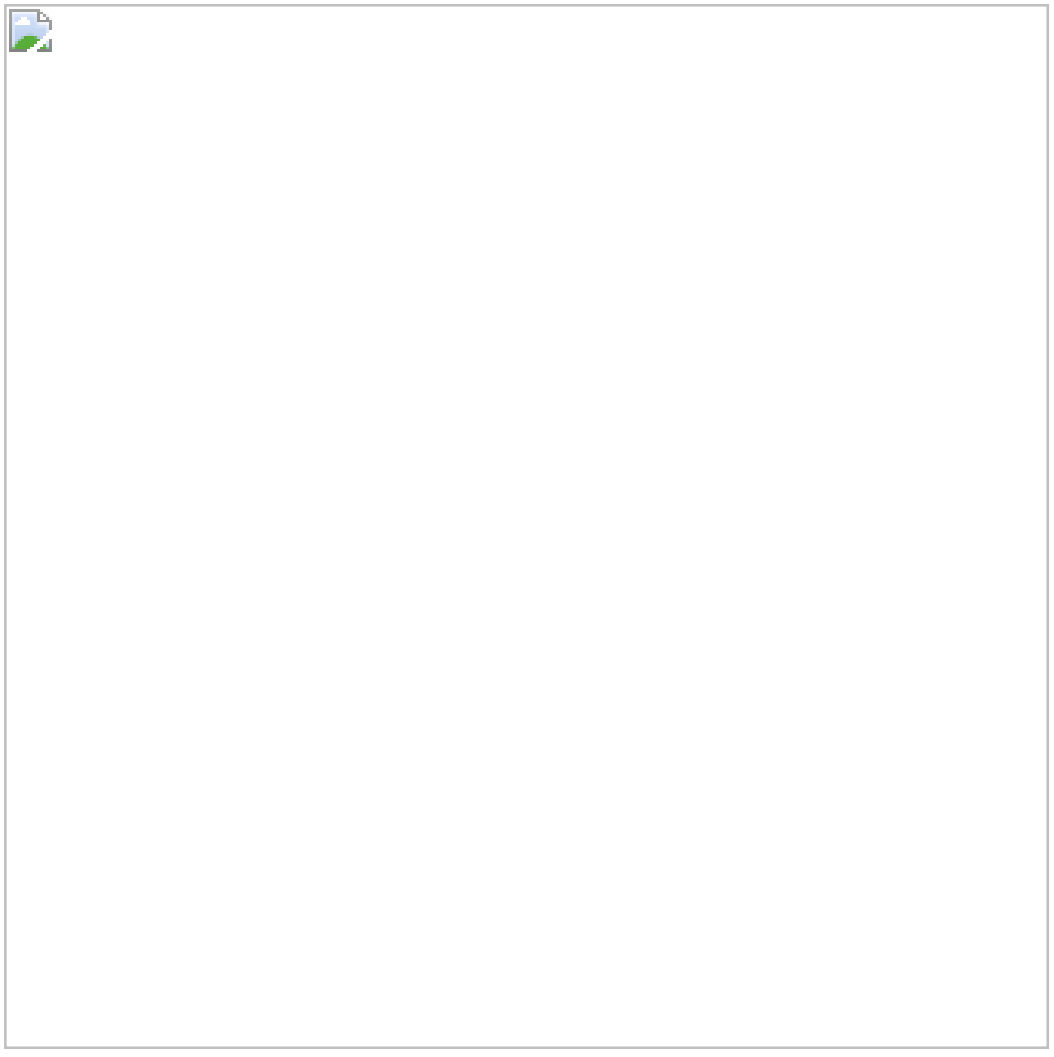

A Comparative Study on Histochemical Distribution of Some Enzymes Related to Steroid and Glucuronide Synthesis in Seminal Vesicle and Testis of the Catfish, Clarias batrachus

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A Comparative Study on Histochemical Distribution of Some Enzymes Related to Steroid and Glucuronide Synthesis in Seminal Vesicle and Testis of the Catfish, *Clarias batrachus*

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ABSTRACT—In both seminal vesicle (SV) and testis of the catfish *Clarias batrachus*, a large number of interstitial cells are distributed, singly or in groups close to blood capillaries in the interstices of the secretory lobules and seminiferous tubules, respectively. They showed strong reactions for 3β-HSD, G-6-PD, NADH diaphorase and UDPGD activities. Both the germinal epithelium and SV epithelium showed moderate to strong reaction for UDPGD activity. These results strongly indicate that the SV is steroidogenic and capable of steroid glucuronidation, like the testis. It is suggested that the interstitial cells of the testis and SV are homologous having a common embryonic origin from the genital ridge.

INTRODUCTION

In teleosts, as in other vertebrates, testicular interstitial (Leydig) cells, which are distributed usually in the interstices of seminiferous tubules singly or in clusters are the main site of production of androgens (Lofts and Bern, 1972; Guraya, 1976; Fostier *et al*., 1983; Nagahama, 1983; Lofts, 1987). In addition, lobule boundary cells or Sertoli cells have also been implicated in steroidogenesis in some teleosts (references as above). The steroidogenic cells are characterized by the presence of lipids which serve as the precursor of steroid hormones, and enzymes of steroid hydroxylations, such as Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD) which catalyzes the conversion of Δ^5-hydroxysteroids, such as pregnenolone and dehydroepiandrosterone, to Δ^4-ketosteroids, such as progesterone and androstenedione, glucose-6-phosphate dehydrogenase (G-6-PD), a key enzyme of the pentose-phosphate pathway which supplies NADPH (TPNH) for steroid hydroxylations, diaphorases (both NAD and NADP), flavoproteins which link the NAD or NADP-linked dehydrogenases to the electron transfer system and oxidize the reduced NAD or NADP for their reuse in steroid hydroxylations (Baillie *et al*., 1966; Kime, 1987). The localization of these enzymes provides a highly reliable procedure to identify the steroidogenic potential of a given tissue (Lofts and Bern, 1972). The teleost testes are unique in that they can convert androgens into water-soluble glucuronides (Ozon, 1972; Kime, 1980, 1987; Fostier *et al*., 1983). During glucuronidation, uridine diphosphoglucose dehydrogenase (UDPGD) catalyzes the conversion of UDP-glucose into UDP-glucuronic acid which supplies the glucuronyl group to different hydroxylated compounds, such as steroids (Dutton, 1980). The presence of the enzyme is taken as a histochemical index of glucuronidation (Resink *et al*., 1987; Van den Hurk *et al*., 1987a, b).

Accessory sex organs in the form of seminal vesicles (SV), testicular glands and testicular blind pouches are present in catfishes (Siluriformes), and gobies and blennies (Perciformes). Anatomical studies have shown that the SV lobules are embedded in a connective tissue stroma which contains interstitial cells, fibroblasts, blood capillaries, and nerve elements (Van den Hurk *et al*., 1987a; Fishelson *et al*., 1994) as in the testicular glands of blennies (Seiwald and Patzner, 1989; Lahnsteiner *et al*., 1990). In previous studies, 3β-HSD activity was reported in SV interstitial cells of *Heteropneustes fossilis* (Nayyar and Sundararaj, 1969) and *Clarias gariepinus* (Van den Hurk *et al*., 1987a, b) and testicular glands of blennies (Lahnsteiner *et al*., 1990). In contrast, in urohaze-goby, the enzyme activity was reported in the SV epithelial cells (Asahina *et al*., 1989). In *C. gariepinus*, the SV epithelial cells showed UDPGD and G-6-PD activities (Van den Hurk *et al*., 1987a, b). Biochemical studies in this species further showed that the SV is capable of both steroid biosynthesis and glucuronide formation (Schoonen and Lambert, 1986,1987). In view of these information, a histochemical study was conducted to demonstrate the steroidogenic and glucuronidation sites in the SV of the catfish *C. batrachus* and the results were compared with that of the testis.

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MATERIALS AND METHODS

Collection and maintenance of fish

Male adult *Clarias batrachus* weighing 60 ± 5 g were collected from local fish markets in and around Varanasi in resting (January), late preparatory (April), early prespawning (July) and postspawning (October) phases. They were maintained in the laboratory under natural conditions and fed goat liver daily.

Tissue processing

For demonstrating the spermatogenetic stages, testes of resting (January), late preparatory (April) and late prespawning (June) and postspawning (October) fish were fixed in Bouin's fluid. In the late preparatory phase, pieces of the SV were also fixed in Bouin's fluid. Paraffin sections (7.5 μm) were stained with Ehrlich's hematoxylin-eosin. For histoenzymology, SVs and testes of late preparatory fish were dissected out and quickly frozen. Fresh cryostat sections (10-15 μm) were taken in the transverse plane and mounted on clean slides and air-dried. The sections were utilized for localization of various enzymes.

a. Δ^5, 3β-hydroxysteroid dehydrogenase (3β-HSD) activity was localized according to the method of Baillie *et al*. (1966) using dehydroepiandrosterone (DHA; Sigma) as the substrate. The medium was prepared fresh at the time of incubation as follows: 10 mg nicotinamide adenine dinucleotide (NAD), 10 mg ethylene diamine tetraacetic acid disodium salt (EDTA), and 10 mg nitroblue tetrazolium (NBT) were dissolved in 8.0 ml 0.2 M phosphate buffer (pH 7.4). DHA was dissolved separately in 2.0 ml dimethyl formamide (DMF). Both the solutions were then mixed and used for the incubation. Sections were incubated at 37°C for 20 min. As a control, some sections were incubated in a substrate-free medium for checking the specificity of the reaction.

b. Reduced nicotinamide adenine dinucleotide (NADH) diaphorase: The diaphorase activity was localized according to the method of Baillie *et al*. (1966) using NADH (Sigma) as the substrate. The incubation medium was prepared fresh at the time of incubation as follows: 5 mg NBT and 5 mg NADH were dissolved in 10 ml 0.2 M phosphate buffer (pH 7.4). Cryostat sections were incubated at 37°C for 5 min. As a control, a few sections were incubated in a substrate-free medium.

c. Glucose-6-phosphate dehydrogenase (G-6-PD): G-6-PD activity was localized by the method of Cohen (1959), using G-6-P (Sigma) as the substrate. The medium was prepared at the time of incubation as follows: 5 mg NADP (NAD phosphate), 5 mg NBT, 5 mg EDTA, 10 mg G-6-P were dissolved in 10 ml 0.2 M phosphate buffer (pH 7.4). Frozen sections were incubated at 37°C for 20 min. As a control, a few sections were incubated in a substrate-free medium.

d. Uridine diphosphoglucose dehydrogenase (UDPGD): UDPGD activity was localized by the method of Jacobsen and Jφrgensen (1973). The incubation medium was prepared by dissolving 0.82 mM uridine-5'-diphosphoglucose sodium salt (Sigma), 2.15 mM EDTA, 0.24 mM NBT, 0.75 mM NAD and 5% polyvinyl alcohol in 0.02 M Tris- HCl buffer, pH 8.3. The incubation was carried out for 1 hr at 30°C. As a control, sections were also incubated in a substrate-free medium.

After the incubations, sections were fixed in 15% formol-saline for 15 min. They were washed in tap water, rinsed in distilled water and mounted in glycerine-jelly.

RESULTS

Spermatogenetic cycle

In *C. batrachus*, the testis and SV show correlated seasonal activity. The testicular cycle can be divided into 5 phases.

Resting or quiescent phase: During this phase (November- January), the testis is thin and filament-like (Fig. 1). Histologically, it consists of several small undifferentiated seminiferous cords containing spermatogonia and spermatogonial mother cells embedded within thick interstitium. Interstitial cells are embedded in the connective tissue.

Preparatory phase: In this phase (February-April), the size of the testis increases progressively (Figs. 2 and 4). The seminiferous tubules increase in size with a reduction in the size of the interstitium. Spermatogenic cysts were seen in different stages of meiosis with numerous division figures. The lumen is filled with spermatids and spermatozoa. Sertoli cells are clearly visible (Fig. 4).

Prespawning phase: In this phase (May-June), the wave of spermatogenesis continues and reaches the peak. The tubules are greatly distended and the testis is enlarged. This stage is characterized by the abundance of spermatozoa in the lumen (Fig. 3). The germinal epithelium contains several cysts of actively dividing spermatocytes. The interstitial cells are numerous and active.

Spawning phase: In this phase (July-August), the testis becomes totally opaque, fragile and attains maximum size. The interstitial cells are active and numerous. The lumen is filled with spermatozoa or empty due to release of the sperm.

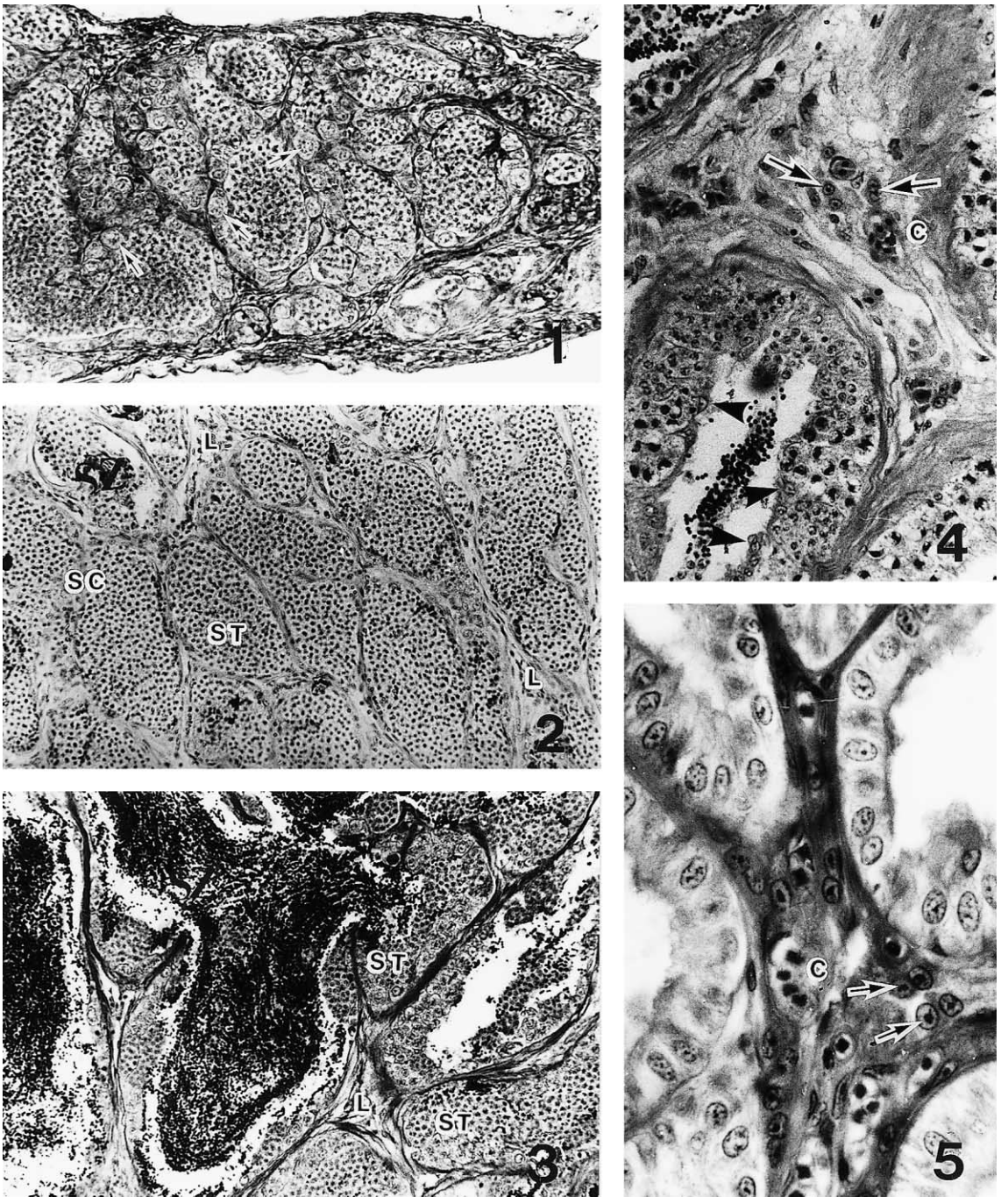
Postspawning phase: In this phase (September-October), the testis is almost empty and spent. The germinal epithelium shows residual spermatogenic activity, giving rise to a second crop of spermatozoa.

In the SV, interstitial cells are present in groups or singly between the lobules and in the periphery close to blood capillaries (Fig. 5). They are round or oval in shape and are comparable in distribution and morphology to the Leydig cells of the testis.

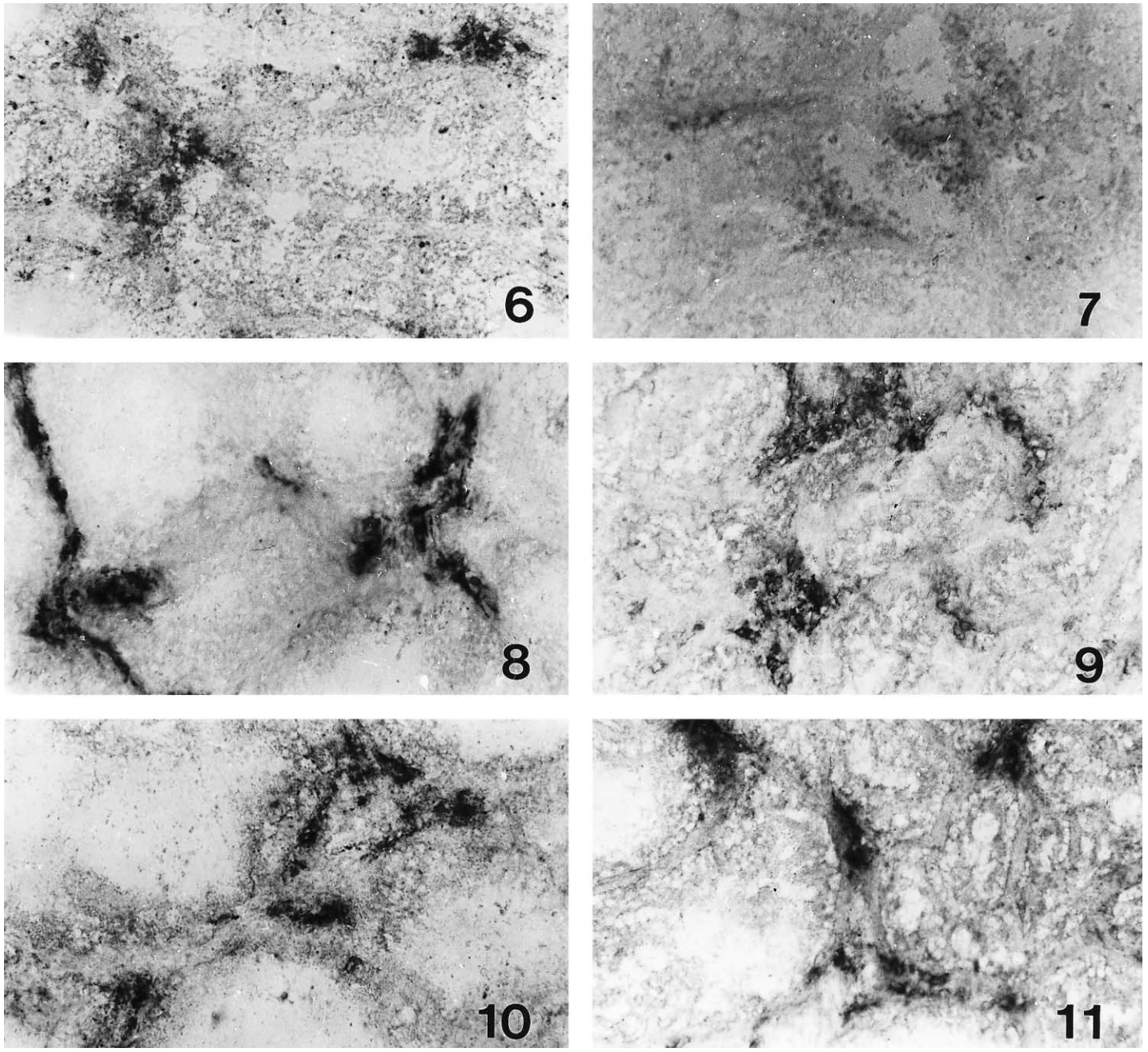
Localization of 3β-HSD, G-6-PD, NADH diaphorase, and UDPGD activities: In both testis and SV, the interstitial cells showed strong reaction for 3β-HSD (Figs. 6 and 7), G-6-PD (Figs. 8 and 9), and NADH diaphorase (Figs. 10 and 11) activities, as indicated by purple-blue diformazan deposits. The interstitial cells of both testis and SV (Figs. 12-14) showed strong UDPGD activity. The germinal epithelium of the testis and the secretory epithelium of the SV also showed moderate to strong enzyme activity. In the testis, the Sertoli cells seemed to be strongly reactive than the germ cells (Fig. 13). In control sections, which were incubated without the respective substrates, diformazan deposits were not formed indicating the specific nature of the reactions.

DISCUSSION

In the present study, the interstitial (Leydig) cells of the testis showed strong 3β-HSD activity, as has been reported by Kirubagaran and Joy (1988). In several teleosts, the Leydig cells are the main sites of steroidogenesis: *Tilapia mossambica* (Yaron, 1966); *Cymatogaster aggregata* (Wiebe, 1969); *Carassius auratus* (Yamazaki and Donaldson, 1969); *Poecilia reticulata* and *Oryzias latipes* (Takahashi and Iwasaki, 1973a, b); *Salmo gairdneri* (Van den Hurk *et al*., 1978; reviews:



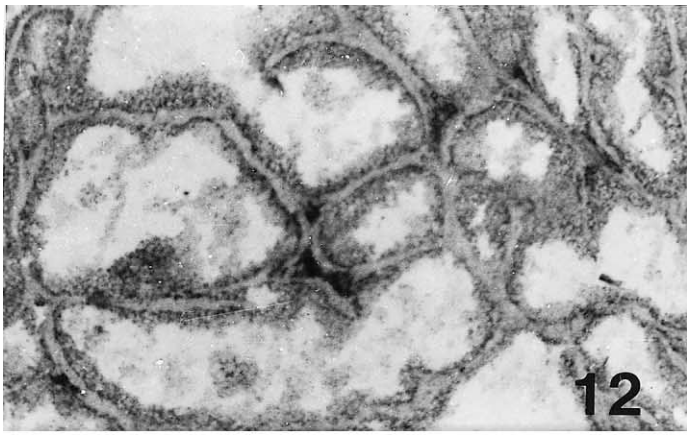
Figs. 1-5. Histology of the testis of *Clarias batrachus*. Spermatocytes (SC), Spermatids (ST). Leydig cells (L). Ehrlich's hematoxylin - eosin.
Fig. 1. A transverse section (TS) of the testis in the resting phase (January). Note a large number of spermatogonia (thick arrows) and differentiating seminiferous cords. Interstitium is thick. × 240.
Fig. 2. A TS of the testis in early preparatory phase (March) showing well developed and distended seminiferous tubules with spermatocytes and numerous spermatids and a few spermatozoa. × 240.
Fig. 3. A TS of the testis in late prespawning phase (June). Note the tubules filled with spermatozoa. Some tubules still contain spermatids. × 240.
Fig. 4. A TS of the testis of *C. batrachus* in late preparatory phase (April) showing interstitial (Leydig) cells (arrows), Sertoli cells (arrow-heads) and blood capillaries (C). × 384.
Fig. 5. A TS of the seminal vesicle of *C. batrachus* in late preparatory phase (April) showing interstitial cells (thick arrows) and blood capillaries (C). × 925.

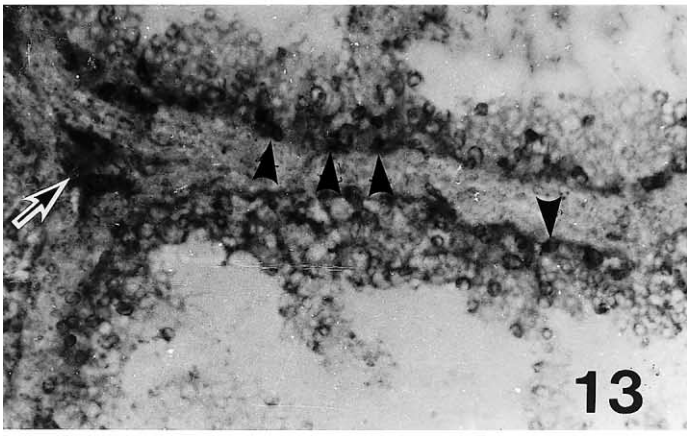


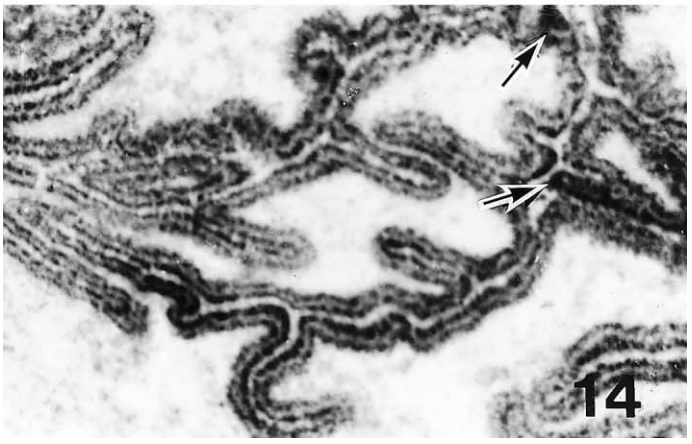
Figs. 6 and 7. Frozen sections of the testis and SV of *C. batrachus* in late preparatory phase (April) showing purple-blue diformazan deposits in the interstitial cells of the testis (Fig. 6) and SV (Fig. 7) indicating strong 3β-HSD activity. × 240.

Figs. 8 and 9. Frozen sections of the testis and SV of *C. batrachus* in late preparatory phase (April) showing intense G-6-PD activity in the interstitial cells of the testis (Fig. 8) and SV (Fig. 9). × 240.

Figs. 10 and 11. Frozen sections of the testis and SV of *C. batrachus* in late preparatory phase (April) showing NADH diaphorase activity in the interstitial cells of the testis (Fig. 10) and SV (Fig. 11). × 240.







Figs. 12 and 13. Frozen sections of the testis of *C. batrachus* in late preparatory phase (April) showing strong UDPGD activity in the interstitial cells (arrows) of the testis. Note strong reaction in the germinal epithelium, most likely in Sertoli cells (arrow-heads). × 80; × 384.
Fig. 14. Frozen section of the SV of *C. batrachus* in late preparatory phase (April) showing UDPGD activity in the SV epithelium and interstitial cells (arrows). × 240.

Fostier *et al*., 1983; Nagahama, 1983). The interstitial cells showed strong reaction for G-6-PD and NADH diaphorase activities, providing additional histochemical evidence for steroidogenic activity. The teleost testis is an important site of steroid glucuronidation (Colombo *et al*., 1977; Kime, 1980; Resink *et al*., 1987; Van den Hurk *et al*., 1987c). It has been reported that the presence of UDPGD activity indicates sites of steroid glucuronide synthesis (Van den Hurk *et al*., 1987a, b). These authors showed that the interstitial cells showed weak enzyme activity in the testis of the African catfish. In contrast, a strong enzyme activity was reported in the interstitial cells of the testis in *C. batrachus*. Furthermore, the germinal epithelium showed moderate enzyme activity with Sertoli cells perhaps showing intense activity. Thus, the combined presence of UDPGD and 3β-HSD activities in the interstitial and Sertoli cells suggests glucuronidation of steroids in these

sites. The strong reaction of UDPGD activity in *C. batrachus* may be due to the fact that they were collected from the wild. Van den Hurk *et al*. (1987b) observed that the enzyme activity was weak in captive fish which was also supported by subsequent biochemical studies (Schoonen and Lambert, 1987; Schoonen *et al*., 1987a). The moderate to strong UDPGD activity noticed in the germinal epithelium suggests perhaps incorporation of UDP-glucuronic acid into polysaccharides or glucuronidation of nonsteroidal compounds, as has been suggested in vitellogenic follicles (Van den Hurk *et al*., 1987b).

The interstitial cells of the SV of *C. gariepinus* and testicular glands of blennies show ultrastructural and histoenzymological characteristics of steroid-producing cells and are homologised to the Leydig cells (Van den Hurk *et al*., 1987a; Lahnsteiner *et al*., 1990). In *C. batrachus* they showed intense reaction for 3β-HSD, G-6-PD, NADH diaphorase and UDPGD activities. The presence of 3β-HSD activity was reported in the SV of *H. fossilis* (Nayyar and Sundararaj, 1969), *C. gariepinus* (Van den Hurk *et al*., 1987a, b), and blennies (Lahnsteiner *et al*., 1990). In contrast, Asahina *et al*. (1989) reported 3β-HSD activity only in the epithelial cells of the SV of urohaze-goby. Van den Hurk *et al*. (1987a) reported G-6-PD activity in the epithelial cells of the SV along with other enzymes, the activity being proportionate to the height of the epithelium. It was weak in squamous cells indicating decreased cellular activity. In *C. batrachus*, UDPGD activity was noticed both in the interstitial and epithelial cells. In *C. gariepinus*, the epithelial cells exhibited strong enzyme activity (Van den Hurk *et al*., 1987a, b). According to these workers, the interstitial cells of the SV of specimens obtained from wild only showed the enzyme activity. The occurrence of UDPGD along with 3β-HSD activity in the interstitial cells of the SV suggests that these cells synthesize steroid glucuronides. In testicular glands of *Gobius niger*, both 3β-HSD and UDPGD activities were localized in the same cells (Seiwald and Patzner, 1989). Apparently, steroid glucuronides are not produced in the testicular glands of blennies (Lahnstenier *et al*., 1990). The presence of UDPGD activity in the epithelial cells suggests two possibilities: (1) the steroids are taken up from the interstitial cells, converted into steroid glucuronides, and then released into the lumen, (2) incorporation of UDP-glucuronic acid into polysaccharides or nonsteroidal compounds secreted by the epithelium (Van den Hurk *et al*., 1987a, b). Biochemical studies have shown that the SV of *C. gariepinus* produces a large number of steroids and steroid glucuronides (Schoonen and Lambert, 1987). Studies of Schoonen and Lambert (1987) and Schoonen *et al*. (1987a, b) have shown that the SV seems to be more important in steroid glucuronide production, judging from its greater capacity (than testis) to synthesize them and from the greater diversity of glucuronides formed in it. They opined that, in those species in which the SV is present, the functions normally performed by the testis are divided between them; the steroid glucuronides being synthesized mostly in the SV. The present histochemical localization of UDPGD activity both in the testis and SV suggests that steroid glucuronide formation may occur in both the structures but quantitative differences, if any, in glucuronide formation can be known only by biochemical analysis. The steroid glucuronides of the SV origin are implicated in pheromonal roles (Stacey *et al*., 1987; Van Weerd, 1990; Stacey and Sorensen, 1991; Van den Hurk and Resink, 1992). In the African catfish, steroid glucuronides of the SV serve as olfactory stimuli to stimulate ovulation (Resink *et al*., 1989).

A comparison of the distribution of the different enzymes in the interstitial cells of the SV and testis shows that both structures are steroidogenic and are capable of steroid glucuronidation, indicating a common origin from the gential ridge. The homology between the two structures is further supported by other evidences. They are: (i) correlative changes in histology and some biochemical constituents (proteins, fructose, hexosamines, sialic acid), (ii) presence of scattered spermatogenic cysts in different stages of development in the SV and SV-testis interphase, (iii) extirpation of testicular component (hemicastration) leads to hyperactivity of the SV in terms of increase in size, testosterone production and secretory activity of the epithelium, and (iv) similarities in androgen and cyproterone acetate responses (Rai, 1996). The anterior part of the gonad is differentiated for a genital function (testis) and the posterior part for a secretory function with some residual spermatogenic activity (SV). Both discharge their contents through the efferent duct systems - the testis into the lateral sperm ducts and SV into the common sperm duct-to the exterior. Apart from the secretory function (both exocrine and endocrine), the SV is used for temporary sperm storage which has adaptive value under extreme drought conditions. The fish survives in moist mud burrows and sperm is protected in the SV for ensuing spawning.

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