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Recent Advances in the Morphology and Histochemistry of Steroid-synthesizing Cellular Sites in the Gonads of Fish

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Electron microscopy and histochemistry of the gonads of fish have revealed their steroid-synthesizing cellular sites which include mainly the theca interna (or thecal gland cells) of developing follicles, the granulosa luteal cells of postovulatory corpora lutea and interstitial gland cells of thecal and stromal origin for the ovary, and the interstitial Leydig cells and lobule boundary or Sertoli cells for the testis. These cell types show similar cytological (or ultrastructural) and histochemical characteristics related to steroid biosynthesis. Their most conspicuous common features indicative of steroid hormone biosynthesis are (i) abundant diffusely distributed sudanophilic lipids (lipoproteins) in the cytoplasm, (ii) greatly developed smooth endoplasmic reticulum, (iii) well-developed mitochondria with a complex system of cristae which are predominantly tubular, (iv) the development of abundant diffuse lipoproteins (or agranular endoplasmic reticulum), associated with various steroidogenic enzyme activities, and (v) under certain physiological situations, accumulated sudanophilic lipid droplets in the cytoplasm, which are composed of either phospholipids, or phospholipids, triglycerides, and cholesterol and/or its esters. In their ultrastructural, histochemical, and biochemical characteristics the various steroid gland cell species of the fish gonad closely resemble the corresponding cells of the mammalian gonads. Recent cytological, histochemical, and biochemical studies on the various steroid gland cell species of the fish gonad agree with each other and are compatible with their known endocrine function. But there is very little evidence for the involvement of preovulatory corpora lutea or corpora atretica or atretic yolky eggs in steroid biosynthesis.

Introduction

Steroid hormones and their biosynthetic pathways in the fish gonads have been discussed in detail (see Ozon 1972a, b, de Vlaming 1974). Divergent opinions continue to be expressed with regard to the origin, presence or absence, fate and function of steroid-producing cells in the fish gonads (Belsare 1973, Browning 1973). During the past few years, the gonads of fish have been

subjected to techniques of histochemistry and electron microscopy to determine more precisely their steroid-synthesizing cellular sites. The steroidal secretions of the fish gonad have also been analysed recently (Ozon 1972a,b). But they have not been correlated with individual cell types or with cellular morphology.

The most striking common features of steroidogenic tissue in the mammalian gonads are: (i) abundant diffuse lipids (lipoproteins) in the cytoplasm, which apparently derive from the abundant ultrastructural agranular endoplasmic reticulum; (ii) well-developed cell organelles, especially the mitochondria with a complex system of internal cristae that are predominantly tubular; (iii) the development of diffuse lipoproteins (or agranular endoplasmic reticulum), closely accompanied by the appearance of enzyme activities indicative of the biosynthesis of steroid hormones; (iv) under certain physiological situations, stored lipid droplets in the cytoplasm, which consist of either phospholipids or phospholipids, triglycerides, and cholesterol and/or its esters; and (v) the capacity to form a variety of steroids in biochemical experiments *in vitro* (Guraya 1971, 1978a). In general, the results of cytological, histochemical and biochemical studies agree well with each other and are compatible with the known endocrine function of various steroid gland cell species of the mammalian gonad. In view of these correlations, it was of great interest to make similar correlations for the steroid-secreting cells of the fish gonad as the early literature on them is contradictory and confusing.

Ovary

The growing follicles, corpora atretica, postovulatory follicles (or corpora lutea) and interstitial cells are generally believed to form the possible sites of steroidogenesis in the ovaries of fish.

Follicle

The ovarian follicle of fish ovary is known to produce steroid hormones. Biochemical studies have demonstrated that ovaries of teleost fishes can synthesize oestrogens such as oestradiol-17β, estrone, and estriol (Ozon 1972a, de Vlaming 1974). But the actual site of their biosynthesis in the follicle is still controversial (Guraya 1976a, Nagahama et al. 1976). Follicular epithelium or thecal cells or both are believed to constitute the steroid-synthesizing cellular site in the follicle, depending upon the fish species.

Follicular epithelium: Each growing oocyte is surrounded by follicle (or granulosa) cells in the form of a continuous follicular epithelium. In some cartilaginous fish, the follicle cells remain of uniform size, but in others they become polymorphic (Guraya 1976a, 1978b). Histochemical tests have revealed the presence of lipid droplets (composed of phospholipids), mitochondria, a Golgi complex, and an abundant RNA-containing basophilic substance (or ergastoplasm) (Guraya 1978b). During the later stages of follicular growth in some elasmobranchs when extensive deposition of yolk occurs, the lipid droplets form large aggregations in follicle cells and meanwhile develop triglycerides in addition to phospholipids (Guraya 1978b). It is still not known whether these lipid accumulations are related to steroidogenesis or to yolk deposition.

The electron microscope studies have revealed the presence of elements of granular endoplasmic reticulum, several free ribosomes, a Golgi complex, mitochondria, with simple cristae etc. in the fish follicle cells (Guraya 1976a, 1978b). These cellular organelles are generally considered responsible for protein and not steroid synthesis (Guraya 1971, 1978b). But Nicholls and Maple (1972) have produced ultrastructural evidence for steroid secretion in the follicle cells of preovulatory follicles in two cichlid fishes,

which show agranular endoplasmic reticulum. The latter is actually intermediate between the smooth and the granular form, as it is only partly associated with ribosomes. Contradictory observations have also been made in regard to the enzyme activities such as Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSDH), 17β-hydroxysteroid dehydrogenase, 11β-hydroxysteroid dehydrogenase (11β-HSDH), 3α-hydroxysteroid dehydrogenase (3α-HSDH), glucose-6-phosphate dehydrogenase (G-6-PDH) etc., which are indicative of steroid hormone synthesis in the follicle cells of fish ovary. They are either present or absent in the follicle cells of the developing follicle in different fish species (Lambert & van Oordt 1974, Guraya 1976a, 1978b, Saidapur & Nadkarni 1976, Nagahama et al. 1976).

Oocyte: 17β-, 3α- and 3β-HSDH activities have been demonstrated in the peripheral ooplasm of older yolk-loaded oocytes, which may be involved in the intermediary metabolism of steroids produced by the follicle wall (Guraya 1976a, 1978b). The significance of steroid metabolism in the oocytes is difficult to understand. It may be that steroids are required as building material for the oocyte, but possibly they also play a role in the breakdown of the germinal vesicle at the time of oocyte maturation.

Theca: At the previtellogenic follicle development, the thecal layer is largely composed of concentrically arranged fibroblast-like stromal cells (Guraya 1978b). It is relatively thicker in the elasmobranch ovary and is generally differentiated into theca interna and theca externa; some teleosts also show theca interna and theca externa (de Vlaming 1974, Nagahama et al. 1976). Theca interna cells hypertrophy to form large cells with abundant cytoplasm having vesicular nuclei, and develop a moderate amount of RNA, some diffuse lipoproteins, granular mitochondria having the usual lipoprotein composition and sparsely distributed lipid droplets composed of phospholipids in the dog fish ovary (Guraya 1976a, 1978b). It is very difficult to localize comparable hypertrophied thecal cells in the follicular wall of teleosts (Guraya 1978b). However, electron microscope studies have clearly revealed the presence of isolated thecal gland cells or special theca cells close to capillaries in the thecal layer which contain abundant agranular endoplasmic reticulum and mitochondria with tubular cristae (Yamamoto & Onozato 1968, Nicholls & Maple 1972, Nagahama et al. 1976). These ultrastructural observations are also in good agreement with the histochemical data for demonstrating 3β-, 17β- and 11β-HSDH and G-6-PDH activities related to steroid biosynthesis in some thecal cells (Guraya 1976a, 1978b, Saidapur & Nadkarni 1976, Nagahama et al. 1976). These correlative ultrastructural and histochemical observations strongly suggest that the special thecal cells form the main site of steroid (possibly oestrogen) production in some fish ovary. But further correlative, comparative, ultrastructural and histochemical studies are essential before the cellular site of steroid biosynthesis in the follicle of fish ovary is finally established, particularly in those fish species where hydroxysteroid dehydrogenases (especially 3β-HSDH) has been demonstrated in the follicle cells (see de Vlaming 1974).

Corpora atretica

Corpora atretica or preovulatory corpora lutea form a conspicuous feature of the fish ovary (Browning 1973, de Vlaming 1974). They are invariably formed from the atretic yolky eggs during the prespawning, spawning, and postspawning periods (Guraya et al. 1975, 1976). The follicle cells are mainly responsible for resorption of yolky egg contents by phagocytosis and thus become densely lipoid, apparently by ingesting triglyceride yolk spheres (Guraya & Kaur 1978). These atretic yolky follicles filled with

sudanophilic lipids thus resemble a post-ovulatory corpus luteum because of their glandular appearance and, while several workers have simply regarded them as degenerate eggs, others have ascribed an endocrine function to the preovulatory corpora atretica (Browning 1973, Guraya et al. 1975, 1976, Guraya 1976a). Recently, the application of a histochemical method for the demonstration of 3β-HSDH activity to the ovaries of some fish species failed to detect any enzyme activity related to steroid biosynthesis in the atretic follicle (de Vlaming 1974, Guraya 1976a). But reverse is true for some *Torpedo* species in which it is the follicle cells of atretic follicles that develop into the corpora atretica while the corpus luteum undergoes early sclerosis and is negative to tests for cholesterol and 3β-HSDH (see Chieffi & Botte 1970). The corpora atretica in *T. marmorata* appear to have a steroid-synthesizing capacity as also supported by *in vitro* incubation of isolated atretic follicles (Chieffi & Botte 1970). Now it can be stated that the corpora atretica or preovulatory corpora lutea of the piscine ovary, in general, merely consist of large yolky eggs in the process of degeneration and reabsorption, and thus do not produce any steroid hormones as also suggested by de Vlaming, 1974. Their follicle cells in some fish species may develop abortive luteinization as evidenced by the development of some enzyme activity related to steroidogenesis in early stages of atresia, which disappears as the atresia advances (Saidapur & Nadkarni 1976). The extensive atresia of yolky eggs during the prespawning period has been attributed to the lack of proper gonadotrophic stimulation for maintaining a large number of follicles in the fish ovary (see Guraya et al. 1975).

Corpora lutea (Postovulatory follicles)

Divergent views exist about the structure and function of postovulatory follicle (or corpus luteum) of fish ovary (Browning 1973). Most early workers believed that the discharged follicles in the majority of oviparous elasmobranchs and teleosts do not become reorganised to form corpora lutea but instead collapse, become pycnotic, and are rapidly resorbed (Guraya & Kaur 1978b). Contrary to these observations, some workers have reported the persistence and marked hypertrophy of follicular epithelia and formation of lipids, cholesterol and a yellow-green substance in postovulatory corpora lutea of elasmobranchs and teleosts and have attempted to compare them with true corpora lutea of mammals on a morphological basis (Chieffi & Botte 1970, Browning 1973, Guraya 1976a, Nagahama et al. 1976, Guraya & Kaur 1978a). The thecal tissue forms a distinct and a separate sheath surrounding the central lipoidal cell mass of granulosa origin (Guraya & Kaur 1978a). However, in some elasmobranchs, the thecal tissue also contributes to the luteal cell masses (see Guraya 1976a).

Guraya and Kaur (1978a) have observed that the transformation of granulosa cells in the teleost (*Cyprinus carpio*) is closely accompanied by the development of diffusely distributed lipoproteins and some lipid droplets consisting of either phospholipids or phospholipids and triglycerides, which appear to be related to steroid biosynthesis. Such lipids also occur in some thecal cells. Positive 3β-HSDH activity occurs in granulosal and some thecal cells of discharged follicles indicating that they may be involved in steroid hormone synthesis (Chieffi & Botte 1970, Guraya 1976a Nagahama et al. 1976). This suggestion is strongly supported by the ultrastructural data of Nicholls and Maple (1972) who observed that during the transformation of granulosa cell into a luteal cell type, there are formed abundant membranes of smooth reticulum and mitochondria with tubular

cristae specific to steroidogenic tissues; the diffuse lipoproteins of histochemical preparation seem to derive from membranes of smooth reticulum. Thecal elements remain separate from granulosa cells and unchanged in ultrastructure for up to 10 days. After approximately 3 days, numerous signs of degenerative processes begin to appear in the granulosa luteal cells of postovulatory follicles, indicating their transitory nature. Ultrastructurally, the follicle cells of postovulatory follicles of the goldfish (*Carassius auratus*) contain many lipid droplets and numerous Golgi elements, suggesting the possible immediate transformation of follicle cells to lutein cells during ovulation (Nagahama et al. 1976). Various degenerative changes begin to appear in the granulosa lutein cells and special theca cells about 30 hr after ovulation. The mitochondria cristae still show an atypical tubular arrangement; the lipid droplets are irregular and vary in size and density. In some follicle lutein cells, cellular organelles and inclusions coalesce to form large masses of electron-dense materials. Many lysosome-like bodies appear in the Golgi area. Guraya and Kaur (1978a) using histochemical techniques have observed that the regression of granulosa lutein cells in the ovary of *C. carpio* is closely accompanied by the storage of highly sudanophilic lipids which gradually coalesce to form masses of variable size and meanwhile develop cholesterol and/or its esters, triglycerides and very little phospholipids.

It is suggested here that in order to reach some definite conclusion about the functional significance of fish postovulatory follicles correlative *in vitro* biochemical and physiological experiments should be carried out at various stages of their evolution and involution. The ovarian tissue of pregnant *Squalus acanthias* has been found to synthesize twice as much progesterone *in vitro* as that of nonpregnant specimens (see Chieffi & Botte 1970, Guraya 1976a). The postovulatory follicles of *T. marmorata* when separated from the main ovarian mass and studied for steroidogenic activity do not secrete progesterone but can produce oestrogens since they are not luteinized, whereas the postovulatory follicles of *S. stellaris* produced progesterone and no estrogens under similar conditions (Chieffi & Botte 1970). These discrepancies in the formation of steroid hormones in *in vitro* experimental studies may be due to variable tissue composition of corpora lutea and other conditions.

Interstitial cells

Almost all previous workers using histological techniques denied the presence of interstitial cells in the fish ovary. For the first time, Guraya (1972), by applying histochemical tests to frozen sections, clearly revealed the origin, development and histochemistry of interstitial cells in the dogfish (*S. sorrakowah*) ovary. They originate by the "luteal-like" transformation of theca interna and surrounding stromal elements of atretic previtellogenic follicles. The vascularized interstitial cells forming patches in the wall of atretic follicles show sudanophilic lipid droplets consisting of triglycerides, cholesterol and/or its esters and phospholipids and diffuse lipids (lipoproteins); the latter appear to derive from the abundant agranular endoplasmic reticulum of ovarian interstitial cells (Guraya 1978a). The interstitial cells filled with sudanophilic lipids are distributed singly or in groups in the ovaries of carp (*C. carpio*) (Guraya & Kaur 1978a). Some of them appear to originate from the residual granulosa lutein cells or thecal gland cells which are dispersed in the stroma. The interstitial cells containing 3β-, 17β- and 11β-HSDH, and G-6-PDH activities have also been reported in the ovaries of some

teleosts (see Lambert & van Oordt 1974, Saidapur & Nadkarni 1976). Saidapur and Nadkarni (1976) have attributed their origin to thecal cells of atretic follicles as reported earlier by Guraya (1972). 3β-HSDH-positive interstitial cells in the teleost ovary are mainly distributed in the stroma, as well as against the follicle wall (Lambert & van Oordt 1974, Saidapur & Nadkarni 1976). The interstitial cells of juvenile rainbow trout (*Salmo gairdneri* R.) are found embedded in the mesenchymal stroma at a very early stage of ovarian development (12 weeks old fish) and show abundant vesicular or tubular smooth endoplasmic reticulum, round or oval mitochondria with tubular cristae, scattered ribosomes and a few membrane bound dense bodies (Upadhyay 1977). These features are considered typical of the active steroid producing cells (Guraya 1976a,b). The physiological significance of the interstitial cells remains doubtful as in the Swordtail they show clear cytochemical indications of steroid metabolism but in the Zebra fish they lack any G-6-PDH activity (Lambert & van Oordt 1974). The nature of steroidal secretions of ovarian interstitial cells is still to be determined.

Testis

Recent studies have indicated that the steroid-synthesizing cellular sites of fish testis are the interstitial Leydig cells, lobule boundary cells, and Sertoli cells which show conspicuous cytological and histochemical changes with the testicular cycle. However, in some fish, germinal ampullae and semen have also been associated with steroidogenesis.

Interstitial Leydig cells

Interstitial Leydig cells similar to those of the amniotic testis are distributed singly or in small groups in the interstices between the seminiferous tubules or lobules of several elasmobranchs and teleosts (Belsare 1973, de Vlaming 1974, Guraya 1976b,c). Their size and activity vary greatly in different fish species as well as with the testicular cycle of the same species. The interstitial Leydig cells originate from fibroblastlike connective tissue elements present in the interstitium, which form a single, or more rarely, a double layer encircling each seminiferous tubule (Nicholls & Graham 1972). The fibroblastlike cells show an elongated nucleus, occasional small mitochondria, and isolated vesicles of smooth membrane. The intermediate cells between the stromal cells and differentiated Leydig cells possess extensive smooth membrane and a large Golgi apparatus thus representing the developmental stages of the Leydig cell.

In some species, the steroidogenic tissue forms a glandular mass adjacent to the mesorchium which is quite distinct from the seminiferous region of the testis although squamous epithelium commonly encloses both the glandular and seminiferous regions (Colombo & Burighel 1974).

Both the interstitial Leydig cells and separate testicular gland of the fish testis possess the cytological and histochemical features of well-established steroid gland cells. They are the abundant (diffusely distributed) sudanophilic lipoproteins, agranular endoplasmic reticulum, numerous mitochondria with a complex internal structure (i.e., with tubulovesicular cristae), enzyme activity (hydroxysteroid dehydrogenases) indicative of steroidogenesis, and accumulation of cholesterol-positive lipid droplets (liposomes) and lipofuscin pigment granules under certain physiological conditions (i.e., when secretory activity is generally very low) (Colombo & Burighel 1974, de Vlaming 1974, Guraya 1976b,c). The cytoplasm of interstitial Leydig cells in the juvenile rainbow trout also shows large amounts of smooth endoplasmic reticulum, some elements of rough

endoplasmic reticulum, abundant free ribosomes and mitochondria with tubular cristae (Upadhyay 1977).

The Leydig cells of *Oryzias latipes* show endoplasmic reticulum in the form of loosely stacked, flattened cisternae coated with ribosomes (Gresik et al. 1973); it is not agranular, as is usual in steroidogenic tissue. They also show negative reactions for Sudan black B and Schultz staining, as well as for 3β-HSDH activity. But these Leydig cells show three features also possessed by the interstitial cells of other species; (1) a vesicular nucleus, (2) mitochondria with tubular cristae; and (3) lipofuscin pigment granules. This study has indicated that caution must be applied in interpretation of histochemical data. Sudanophilic lipid droplets and 3β-HSDH have also not been observed in the Leydig cells of some other teleosts (Guraya 1976b).

The interstitial Leydig cells in most species show conspicuous seasonal changes by showing accumulation and prenuptial depletion of cholesterol-positive lipids (Guraya 1976b); their depletion has been related to the active synthesis of steroid hormones which influence the development and maintenance of secondary sexual characters and reproductive behavioral activity. The disappearance of cholesterol-positive lipid droplets is also closely related to the increase in the intensity of 3β-HSDH activity. Such an increase has not been observed with the different seasons of the year in *Gobius paganellus* (Stanley et al. 1965). Coarse lipid droplets accumulate during the involution of interstitial Leydig cells. The regeneration of interstitial cells occurs during the postnuptial period of testicular activity in *Monopterus* (see Guraya 1976b).

Lobule boundary cells

The second type of arrangement of steroidogenic tissue includes the lobule boundary cells in the testes of a variety of freshwater and marine teleosts (Guraya 1976b, c). The typical interstitial distribution of endocrine cells is generally absent from fish testes that develop lobule boundary cells. However, there are some fish species whose testes appear to contain both interstitial and lobule boundary cells (de Vlaming 1974, Guraya 1976b, c). The lobule boundary cells show the cytological and histochemical characteristics of well-established steroid gland cells, which include abundant diffuse lipoproteins or agranular endoplasmic reticulum, mitochondria with tubular cristae, 3β-HSDH activity indicative of steroidogenesis, and cholesterol-positive lipid droplets under certain physiological conditions (de Vlaming 1974, Guraya 1976b,c). Their cytological and histochemical features show marked seasonal cyclic changes which are closely related to the reproductive cycle. The lobule boundary cells of some teleosts show a negative reaction for steroid dehydrogenase activity. But Gresik et al. (1973) have observed that the ultrastructural appearance of Leydig cells in the testis of *O. latipes* provides evidence of steroidogenesis in the absence of histochemical evidence. These investigators also cautioned against relying on purely histochemical means to identify Leydig cells in teleosts and emphasized the use of the electron microscope in establishing their presence.

Sertoli cells

In some teleost species, Sertoli (or sustentacular) cells appear to form the sites of steroidogenesis as evidenced by the postnuptial storage of cholesterol-positive lipid droplets in their spent lobules (Guraya 1976b). The lipid droplets disappear with spermatogentic activity, suggesting their possible conversion into steroid hormone(s). In the testes of elasmobranchs and some teleosts, Sertoli cells provide relatively much

better evidence for their involvement in steroidogenesis as indicated by their ultrastructural and steroid dehydrogenase activity (de Vlaming 1974, Guraya 1976b). Upadhyay (1977) has stated that the Sertoli cells of juvenile rainbow trout possess the basic ultrastructural features of steroidogenic cells such as the smooth endoplasmic reticulum and mitochondria with tubular cristae. But these features are not very pronounced, suggesting the possibility that Sertoli cells at this stage of testicular development are not actively involved in steroidogenesis.

Based on the intimate association of developing spermatids or their residual bodies with lobule boundary cells and their placement inner to the basement membrane of the lobule in the testes of some teleosts, several workers have recently suggested that lobule boundary cells are homologous with the Sertoli cells of higher vertebrate groups (Grier & Linton 1977, Upadhyay 1977). Some workers have suggested that in those teleosts where both Leydig and lobule boundary cells have been reported, the latter are, in fact, Sertoli cells (Upadhyay 1977). It must be determined more precisely whether the distinct lobule boundary cells and Sertoli cells are present simultaneously in the fish testis. Functionally, the Sertoli cell may be nutritive, contractile, supportive and/or a steroid producing endocrine cell (de Vlaming 1974).

Spermatozoa

Spermatozoa of several elasmobranchs have been shown to contain hydroxysteroid dehydrogenase activity (Guraya 1976b). Considerable concentrations of steroid hormones (e.g., deoxycorticosterone, progesterone, pregnenolone, androstenedione, dehydroepiandrosterone, and probably androsterone) have also been observed in the semen of *Squalus* (see Guraya 1976b). The active conversion of cholesterol or pregnenolone into progesterone is proof that the sperm of this species is able to synthesize the precursors of C_{19} steroids. It is also possible that the steroids of semen may derive from Leydig cells or lobule boundary cells which have already been shown to have the cytological and histochemical features of well-established steroid gland cells. The situation in *Squalus* appears to be exceptional since no steroids have been found in the sperms of other elasmobranchs.

Conclusions

From the discussion and integration of recent ultrastructural and histochemical data, it can be concluded that the cellular sites in the fish testis include mainly interstitial Leydig or lobule boundary cells which are known to respond to gonadotrophins (Guraya 1976b, Upadhyay 1977). However, in some fish species, Sertoli and germ cells also appear to be involved in steroid biosynthesis. The possible cellular sites of steroid biosynthesis in the fish ovary are the special thecal gland cells of maturing follicles, the granulosa lutein cells (and theca lutein cells in some fish) of postovulatory corpora lutea, and interstitial cells of thecal and stromal origin. But further application of refined methods of histochemistry, electron microscopy, biochemistry, and cell culture is still required to determine the precise functional nature of various cell species of fish gonads and their products, which may vary in nature and amount under different environmental and physiological conditions.

The various steroid-synthesizing cellular sites of fish gonad possess similar ultrastructural and histochemical features typical of well-established steroid gland cells of the gonads of mammals (see Introduction). The physiological significance of these cytological and histochemical features, especially in relation to steroid biosynthesis, has already

been discussed in detail in previous reviews (Guraya 1976a,b). The abundant diffuse lipoproteins or membranes of smooth reticulum are a source of steroidogenic enzymes and may also serve to some extent as a reservoir for the accumulation of a hormone precursor (cholesterol). The enzyme activity necessary for splitting off the cholesterol side chain resides in the mitochondria which develop complex tubular cristae. The cholesterol-positive lipid droplets represent potential precursor materials which are converted into steroid hormones when proper gonadotrophic stimulation becomes available. Similar physiological function can also be assigned to the comparable steroid-synthesizing cellular sites of the fish gonad. It can, therefore, be concluded that steroid hormones in the steroid gland cells of fish gonad are also formed as a result of interaction between diffuse lipoproteins or membranes of smooth reticulum, mitochondria, and lipid droplets, which have been clearly demonstrated in their cytoplasm. This is further supported by the fact that the enzymes that catalyze biochemical transformations during steroid biosynthesis have also been demonstrated in the gonads of different fish in both *in vivo* and *in vitro* experiments (see Ozon 1972a,b).

Morphological and histochemical data on the steroid-producing cells of the fish gonad also correlate well with the various biochemical studies that have clearly demonstrated the presence of steroids in gonadal extracts and plasma of different species of fish as well as of enzyme systems catalyzing transformation of radioactive hormone precursors into steroid hormones in *in vitro* biochemical experiments (see excellent reviews by Ozon 1972a, b, de Vlaming 1974). However, the pathways of steroid biosynthesis, and consequently the nature of the steroid hormones secreted, differ in the gonads of different fish species under different environmental and physiological conditions.

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