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THE STRUCTURE AND DEVELOPMENT OF THE SEMINIFEROUS FOLLICLE IN *SCYLIORHINUS CANICULUS* AND *TORPEDO MARMORATA* (ELASMOBRANCHII)

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Summary. A light microscopic and phase-contrast study of the developing seminiferous follicles in two elasmobranch species was made. After an initial period of independent mitotic divisions, each spermatogonium becomes entirely surrounded by a cytoplasmic extension of a single follicle cell (Sertoli cell). These bicellular units may be termed spermatocysts. This relationship remains through six succeeding germ cell generations, usually resulting in a group of 64 isogenous spermatids in each spermatocyst. Thus, all substances exchanged between the blood stream and the germ cells must pass through the body of the follicle cell. As the spermatids transform into spermatozoa, they are drawn into a compact bundle and are finally released from the follicle cell. Germ cell development is closely synchronous within each spermatocyst and spermatocyst development is synchronous within each follicle. Germ cell and Sertoli cell lines appear to be entirely separate. Intercellular cytoplasmic connections are retained among large groups of daughter germ cells. It is suggested that these cytoplasmic bridges as well as spermatocyst formation serve in promoting equality of physiological opportunity throughout the large germ-cell mass.

Introduction

A review of the literature on elasmobranch testicular structure reveals varied interpretations as to the origin and identification of cell types and their morphological relationships (for a good review of the older literature see MATTHEWS, 1950). Specifically, there has been much difference of opinion as to whether Sertoli cells arise from or give rise to the germ cells or are a completely separate line. The question of Sertoli cell migration within the seminiferous follicles or ampullae has also been a matter of dispute and has given rise to some of the erroneous interpretations encountered in the literature. There has also been a conflict of opinion as to the existence of a Sertoli cell syncytium or a Sertoli cell-germ cell syncytium. Even in the most recent studies (MATTHEWS, 1950; FRATINI, 1953) there are numerous points of difference concerning these questions. The present study was undertaken to attempt a clarification of the morphological relationships between cell types and the developmental events taking place within the adult elasmobranch testis.

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Material and Methods

Specimens of the genera *Scyliorhinus* and *Torpedo* were collected with the assistance of the staff of the Zoological Station of Naples, Italy. This paper is based primarily upon investigations on *S. caniculus* and *T. marmorata*, but some observations were made on *S. stellaris* and *T. torpedo*. Some quantitative data are given for *Mustelus mustelus*, *Raja rhina* and *Hydrolagus colliei*, the last two species having been collected at the University of Washington Marine Laboratories, Friday Harbor, Washington.

For routine histological work, Bouin's fluid, Zenker-formol and Stieve's fluid were used as fixatives. Stieve's fluid proved to be the most useful. Paraffin sections were stained in Weigert's iron haematoxylin or Masson's trichrome stain. Fresh tissues of *Scyliorhinus* and *Torpedo* were studied under the phase-contrast microscope, either in simple squash preparations or after treatment for 30 to 45 min in a 1% trypsin solution at 37° C followed by very slight pressure on the coverslip.

Observations

Light Microscopy

In the testes of elasmobranch fishes the seminiferous elements are formed into spherical follicles or "ampullae" (Fig. 1) situated at the terminae of a highly branched collecting ductule system. These seminiferous follicles originate from fixed germinal sites on the lateral or dorso-lateral aspect of the testis. Follicular genesis appears to be continuous, and the newly formed follicles move steadily away from the germinal sites as they grow and mature, followed closely by successively younger elements. All germ cells within a given follicle are in the same stage of development. As the follicles approach the ventral and ventro-medial areas of the testis, they contain nearly mature spermatids attached to large supporting cells. When spermatid development is completed, an opening is effected into the attached terminal branch of the collecting ductule system and the spermatozoa are expelled from the follicle. The latter, along with the retained supporting cells, then undergoes a period of degeneration and resorption. Thus in a single transverse section of the testis, one can study the complete range of events from follicular genesis through all the stages of cellular division, growth and differentiation, to the final expulsion of the germinal products and dissolution of the somatic elements. These various stages may be recognized in rows roughly concentric with the germinal zone as indicated in Fig. 1.

Large (18 μ diamter), spherical primary spermatogonia are located beneath the coelomic epithelium in the germinal zone. These cells are grouped into clusters or cords embedded in a dense matrix of stromal cells and collagenous connective tissue fibers. The spermatogonia are closely surrounded by smaller cells that are similar to the somatic cells of the "germinal" epithelium. Only occasional germ cells in this area are seen in mitosis.

Deep to this germinal cord region, the germinal elements become segregated into small spherical units containing one or two spermatogonia surrounded by several epithelial cells (Fig. 2). These spherical units each constitute a newly formed seminiferous follicle. Each follicle retains attachments to a terminal branch of the collecting ductule system, which is a solid cord of cells near the point of follicular attachment. This ductule is carried along with the follicle as the latter grows and matures, but does not form an open connection between follicle and collecting ductule system until the spermatids are nearly mature. Two distinct cell types comprise the contents of the follicles: the spermatogonia, and the smaller surround-

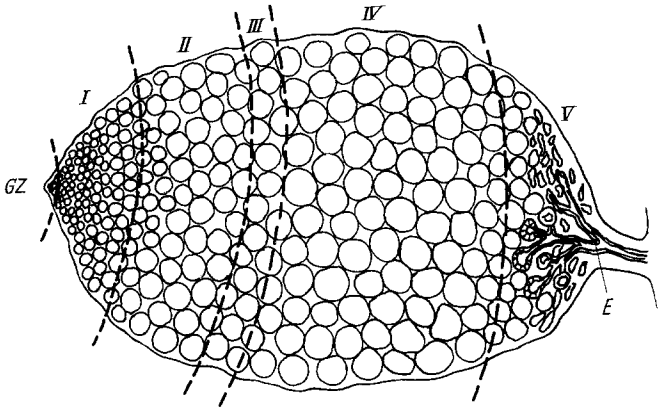

Fig. 1. A diagram to indicate the zonation of the testis of *S. caniculus* as seen in tranverse section. At the left the lateral area of the testis contains the germinal zone (*GZ*) from which seminiferous follicles are continuously formed. Zones *I*—*V* indicate areas in which follicles of progressively later development are found. *I* spermatogonia; *II* primary spermatocytes; *III* secondary spermatocytes; *IV* spermatids; *V* zone of sperm release and follicular degeneration. *E* efferent ductule

ing follicle cells which become the supporting or sustentacular cells. These may be considered homologous with the Sertoli cells of mammals. No cell types intermediate between these two were distinguished in the follicles.

The follicle cells and spermatogonia undergo repeated mitotic divisions and a lumen gradually appears in the center of the enlarging follicle. At first the two cell types line the interior of the limiting membrane in no apparent order (Fig. 2), but soon the cells segregate into two concentric single layers, the Sertoli elements around the central lumen and the spermatogonia at the limiting membrane (Fig. 3). The germ cell nuclei retain their spherical configuration, while the Sertoli nuclei are oblong or even conical in shape with their longer axes conforming to the lines of follicular radius. In many cases, the outer or peripheral end of the Sertoli nucleus is indented as a result of pressure from the spermatogonium lying adjacent to it. From this stage on, no mitotic figures are seen among the Sertoli cells.

It is estimated, on the basis of cell volume compared with follicular volume, that when proliferation of Sertoli cells ceases (two-layered follicle in *Scyliorhinus*, Fig. 3), there are 480—500 Sertoli cells in *Scyliorhinus* but only 230—260 Sertoli cells in comparable follicles of *Torpedo*. The number of spermatogonia in each follicle corresponds closely to the number of Sertoli cells. In mature follicles the number of Sertoli cells is also about 250 and 500 in *Torpedo* and *Scyliorhinus* respectively, substantiating the observation that the full complement of Sertoli cells is reached at the earlier stage. In this two-layered stage in *Scyliorhinus* and in the comparable stage in *Torpedo*, the follicles are about 100 μ in diameter, the germ cells about 12 μ in diameter, while the height of the epithelium is about 30 μ in *Scyliorhinus* and only 20 μ in *Torpedo* (compare Figs. 3 and 4). Thus in follicles of the same diameter containing germ cells of comparable stage and size, *Torpedo* has only about one-half the total number of cells. The crowding of a greater number of cells in follicles of *Scyliorhinus* results in an epithelium of greater height in which the elongate Sertoli cell nuclei are apparently obliged to occupy the more central

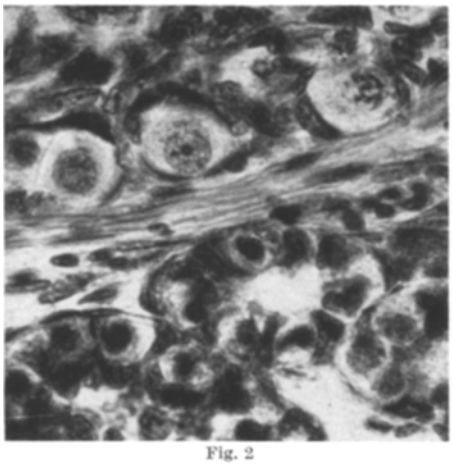

Fig. 2

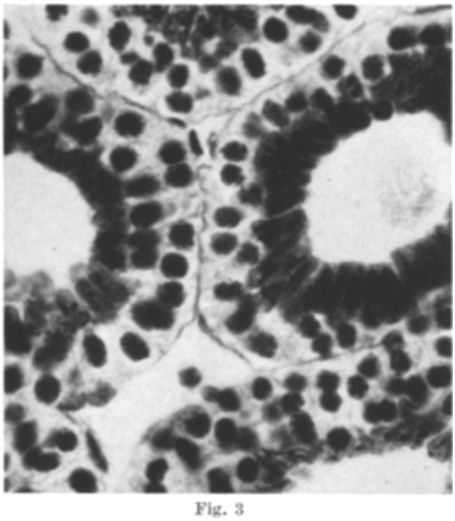

Fig. 3

Fig. 2. A section from the early spermatogonial zone showing the earliest follicle stage (upper) consisting of a single spermatogonium surrounded by a thin layer of follicle cells. In the lower portion of the figure are follicles of later development which contain a central lumen surrounded by a layer of intermixed spermatogonia (larger, rounded cells) and follicle (Sertoli) cells. Spermatocyst formation has not yet occurred. × 800

Fig. 3. The follicle at the right is in the early spermatocyst stage with the Sertoli nuclei pressed toward the central lumen and the spermatogonia lying peripherad. This stage yields spermatocysts containing single spermatogonia (Figs. 9, 10). The other follicles shown are slightly later in development and each spermatocyst contains two or four spermatogonia as in Fig. 11. × 300

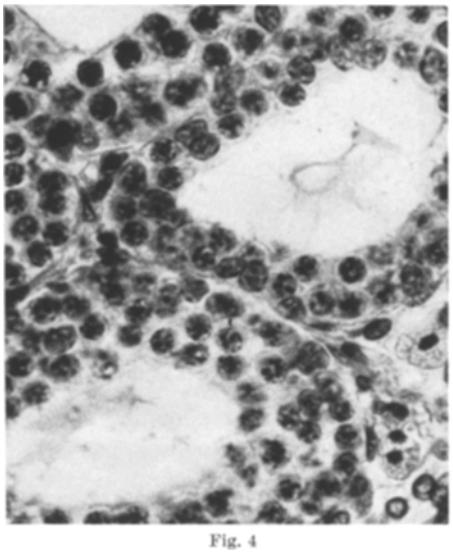

Fig. 4

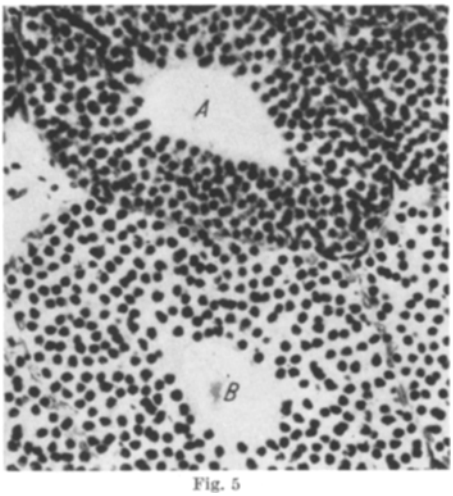

Fig. 5

Fig. 4. Early spermatogonial follicles of *T. marmorata* showing tendency for the Sertoli nuclei to remain near the periphery. Note the larger lumen in these follicles as compared with follicles of similar size and germ cell stages in *Scyliorhinus* (Fig. 3). × 300

Fig. 5. Follicles of *S. caniculus* in the spermatogonia — primary spermatocyte transition area. Follicle *A* is in the final spermatogonial stage while follicle *B* contains early primary spermatocytes. The increase in follicle size from *A* to *B* is due to the swelling of the germ cells, not to increase in cell number. × 120

region (Fig. 3). In comparable stages in *Torpedo* the germ cells and Sertoli cells occupy various positions relative to each other along the follicle wall (Fig. 4).

As development proceeds, spermatogonia continue to divide. Daughter cells grow in size between divisions so that the follicle as a whole continues to enlarge, but the cells do not attain the same volume as the mother cell. Thus, although the follicle steadily increases in diameter spermatogonia gradually decrease in size from a maximum diameter of about 18 μ in the germinal cord region to a diameter of about 10 μ just before they enter the primary spermatocyte stage (Fig. 5, follicle A). During this period of mitotic division the germ cells transform from the larger "primary" spermatogonia with light-staining cytoplasm and finely granular chromatin (Fig. 2) to the smaller "secondary" spermatogonia with coarse, dark-staining chromatin in large flakes or patches (Fig. 3). These flakes of chromatin are especially evident in *Torpedo* (Fig. 4). One or two very large nucleoli also characterize the spermatogonial nuclei in both species. Thus the primary and secondary spermatogonia described here appear similar in their behavior and cytological detail to spermatogonia "A" and "B" described in rodent testis (see review by ROOSEN-RUNGE, 1962).

As the germ cells in *Scyliorhinus* multiply, they push the Sertoli elements centrad, so that the lumen becomes almost occluded. When the germ cells have formed a layer five to seven cells deep, the more centrally located Sertoli nuclei begin to move toward the periphery of the follicle. This migration evidently takes place rather rapidly, since only occasional follicles are seen in which the Sertoli nuclei lie somewhere between the central and peripheral areas. MATTHEWS (1950) has illustrated this migration very well in *Cetorhinus*. The Sertoli nuclei take up positions just beneath the basement membrane with the long axes of their nuclei now parallel to it. Migration of the Sertoli nuclei occurs toward the end of the spermatogonial period in *Scyliorhinus*.

In *Torpedo* this remarkable migration is much less evident. Some Sertoli nuclei occupy positions bordering the follicular lumen, but others remain next to the basement membrane. By the time two or three layers of germ cells have developed, all the Sertoli nuclei are seen at the periphery, considerably in advance of the end of the speramtogonial division period (Fig. 4).

At the end of the spermatogonial stage, the follicles increase sharply in size from about 150 μ to 225 μ diameter (in *Scyliorhinus*). This increase appears to be due to increase in size of the germ cells (Fig. 5, follicle B). In histological preparations, the nuclei may appear contracted at first, but toward the end of the primary spermatocyte period they are large and contain very distinct, elongate synaptic chromosomes.

The secondary spermatocyte stage is apparently short (though distinct nuclei are reconstituted) because relatively few follicles are seen in this stage compared with those containing primary spermatocytes or spermatids.

Early in the spermatid stage, and sometimes starting with the secondary spermatocyte stage, expanding fluid spaces develop among the germ cells. The follicle as a whole appears to enlarge mainly by this means, even though the germ cells do not grow noticeably between the maturation divisions. Although the boundaries of the Sertoli cells are always difficult to distinguish, some preparations show that each Sertoli cell has formed a single large pocket containing a group of spermatids. Sertoli cell cytoplasm appears to form a complete wall around the cavity (outlined in Fig. 6), and the early spermatids form a single layer oriented

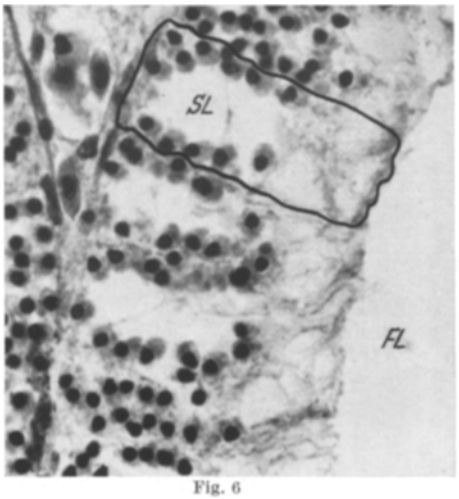

Fig. 6

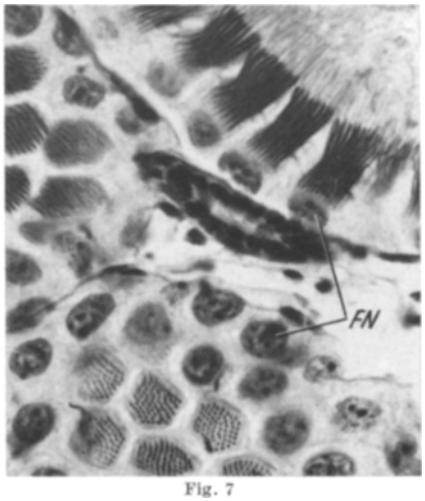

Fig. 7

Fig. 6. A section through a follicle in the early spermatid stage. A single spermatocyst is outlined at top. The spermatids attach to the inner surface of the follicle cell nearer the periphery, while their tails extend centrad toward the follicular lumen (*FL*). Spermatocyst lumen, *SL*. × 200

Fig. 7. Sections of follicles in later spermatid stages in which bundles of late spermatids are seen in longitudinal section above and transverse section below (note inner plasma membrane of follicle cell around each bundle). The outer plasma membranes of follicle cells are indistinct. A portion of one of the efferent tubules is seen in the center. Follicle cell nuclei (*FN*) are indicated. × 200

so that their anterior poles are attached to the inner Sertoli wall, and their tails project into the "cyst" lumen. There still remains a follicular lumen bounded by the central ends of the cysts (Fig. 6). Within these "spermatocysts" (a single Sertoli cell together with its enclosed complement of germ cells) the spermatids form a cup-shaped layer with the open end directed centrally toward the follicular lumen (Fig. 6).

With further development, the spermatids elongate and are gradually formed into loose bundles directed toward the Sertoli nucleus. Toward the end of their transformation period they have become very elongate and are grouped into tight bundles (Figs. 7, 17). The spermatid heads are all directed peripherad toward the Sertoli nuclei and their tails protrude into the central lumen of the follicle. As the bundles of spermatids become more compact, the follicle as a whole decreases in size from a maximum diameter (in *Scyliorhinus*) of about 350 μ to approximately 240 μ at the time of sperm release.

In the most mature follicles (nearest the ventral and medial sides of the testis), an opening is formed through the follicle wall into the collecting ductule system and bundles of spermatozoa pull away from their respective Sertoli cells and flow into the collecting ductules (Fig. 8). The follicle then gradually contracts until the vacuolate Sertoli cells form a solid mass in its interior. Stages in the gradual degeneration and resorption of the follicles and their contents can be followed until they are no longer distinguishable in the stromal and hemopoietic tissue lying along the ventral side of the testis (Figs. 8, 17).

If one examines sections in which the bundles are cut transversely (Fig. 7), it is often possible to make accurate counts of the number of sperm in each bundle. Table 1 tabulates sperm bundle counts for several species including the two under present discussion.

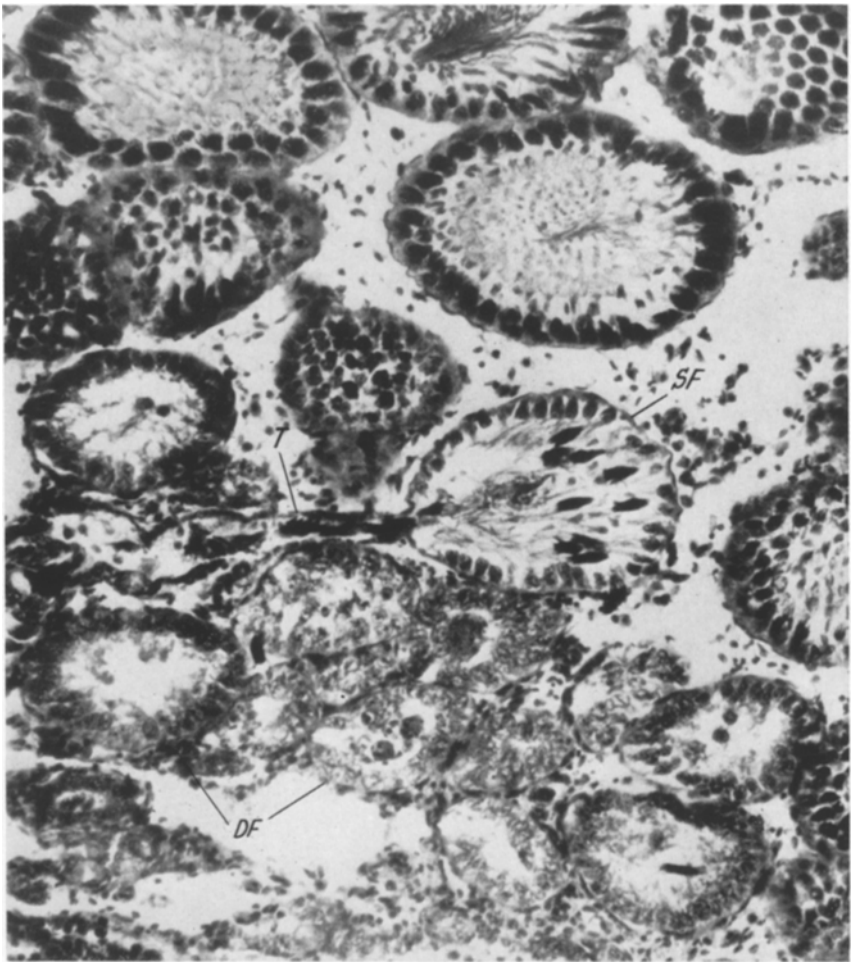

Fig. 8. A section through late follicles in the more medial part of the testis. At the top are numerous follicles containing bundles of late spermatids. In the center is a follicle (*SF*) releasing its spermatids into the now patent branch of the efferent ductule system (*T*). In the lower portions of the section are degenerating follicles (*DF*) containing the retained follicle cells and occasional unshed spermatids. × 135

Table

Species	Number of bundles counted	Number of bundles with 64 spermatids	Other counts
Scyliorhinus caniculus	25	20	63, 70, 62, 63, 62
Scyliorhinus stellaris	25	17	32, 37, 48, 50, 57, 58, 61, 63
Torpedo marmorata	14	3	20, 49, 59, 60, 62, 63, 65, 65, 66, 73, 84
Raja rhina	22	13	62, 63, 63, 65, 65, 65, 65, 65, 79
Mustelus mustelus	10	9	67
Hydrolagus colliei	25	24	63

It may be seen that a high percentage of bundles contain just 64 spermatids and that when the number differs from 64 it is usually only by two or three. The

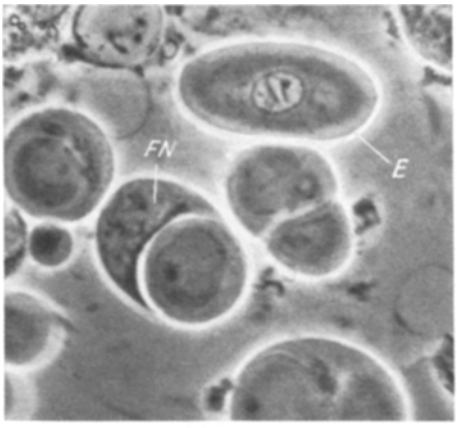

Fig. 9

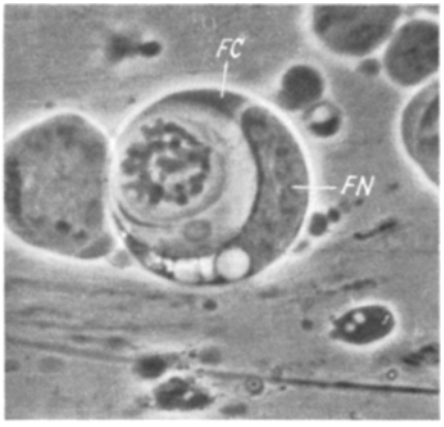

Fig. 10

Fig. 9. A phase-contrast photomicrograph of a newly formed spermatocyst containing a single spermatogonium. Follicle cell nucleus, *FN*. An erythrocyte (*E*) lies at upper right. × 1100

Fig. 10. A spermatocyst from the same region as that in Fig. 9 but undergoing mitosis. *FN*, follicle cell nucleus; *FC* follicle cell cytoplasmic sheath enclosing the spermatogonium. × 1100

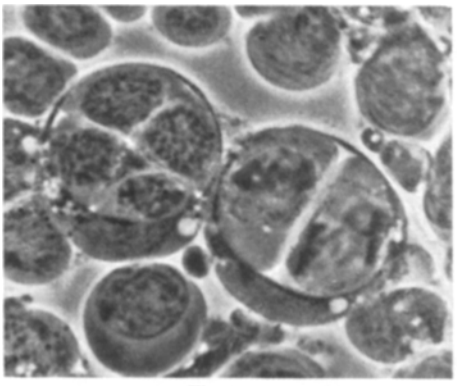

Fig. 11

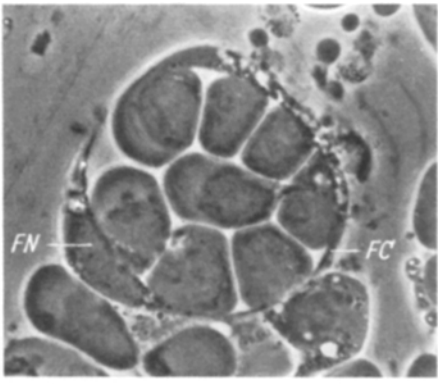

Fig. 12

Fig. 11. Spermatocysts containing two spermatogonia undergoing mitosis (right) and four spermatogonia (left). × 1100

Fig. 12. A spermatocyst containing 8 spermatogonia. *FN* follicle cell nucleus; *FC* follicle cell wall. × 835

counts for *Torpedo* differ more widely than those of the other species examined, but most of the counts here also center around 64. It is much more difficult to obtain accurate sperm counts for this species since the sperm bundles are not so compact and good transverse sections of them are rare. By multiplying the estimated number of Sertoli cells in mature follicles (500 in *S. caniculus* and 250 in *T. marmorata*) by 64, it is calculated that about 32,000 spermatozoa are produced per follicle in the former and about 16,000 spermatozoa per follicle in the latter.

Phase-Contrast microscopy

If small pieces of tissue from the germinal zone are subjected to trypsin digestion and gently pressed with a coverslip, the cellular associations (cords, early follicles and collecting tubules) are, for the most part, broken up. Spindle-shaped

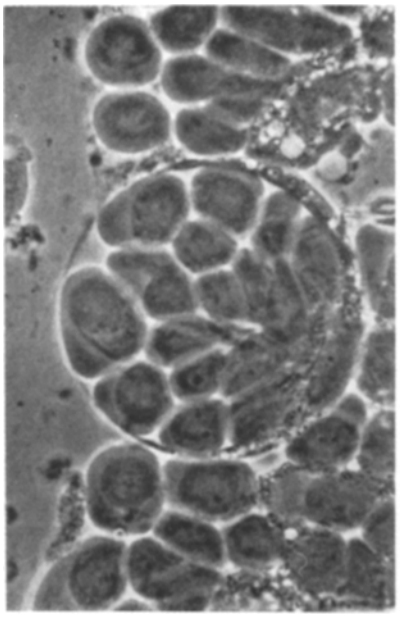

Fig. 13. A spermatocyst containing 16 late spermatogonia. × 835

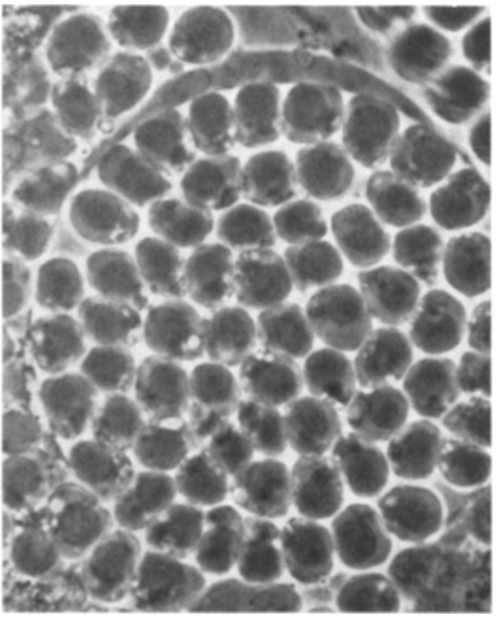

Fig. 14. A spermatocyst containing 64 early spermatids. × 570

connective tissue cells, blood cells, primary and secondary spermatogonia, and Sertoli cell precursors may be recognized. Because of abundance of connective tissue fibers in this region, dissociation of cells is more difficult than in regions farther from the germinal site, but when the germinal cords and the earliest follicles have been dissociated, the component cells separate as individuals.

In the zone of the two-layered follicles (Sertoli cells internal, spermatogonia external, Fig. 3), many units are observed in which a single spermatogonium is entirely surrounded by the cytoplasm of a single Sertoli cell (Figs. 9, 10, 17). In some instances of gentle dissociation, eight or ten such units are seen close together. These are presumably derived from the same follicle. It is only in this zone that such units, containing but a single spermatogonium, are found and it appears that in such follicles all or most of the cells are associated in these two-celled units. In each preparation many individual cells of both types are also seen, but especially so if coverslip pressure has been greater. They may represent associations that have been broken apart by dissociation procedures or possibly associations in which the Sertoli cell has not yet completely engulfed the spermatogonium. Usually, in trypsin preparations, a slight space is seen between the germ cell and the surrounding Sertoli cell (Fig. 9), indicating that the germ cell is not within the Sertoli cytoplasm proper, but lies in a pocket lined by a portion of the Sertoli plasma membrane. These two-celled associations are termed spermatocysts and represent the first stage of such units.

Tissue taken from the region in which histological sections demonstrate two or three layers of germ cells exhibits associations in which a single Sertoli cell engulfs 2, 4 or 8 germ cells (Figs. 11, 12, 17). The spermatogonia are nearly always

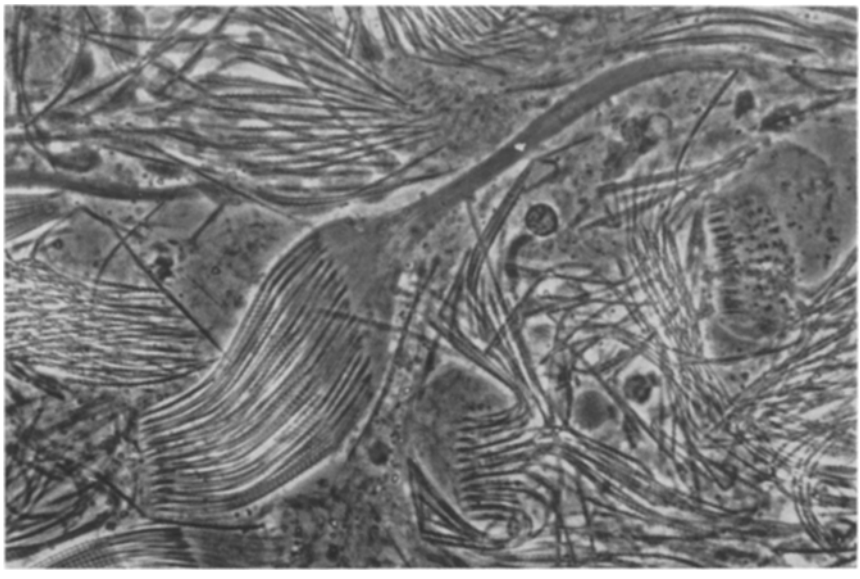

Fig. 15. Phase-contrast micrograph of a late spermatid bundle still apparently retained within the inner follicle-cell plasma membrane although the outer follicle-cell plasma membrane has been broken. × 570

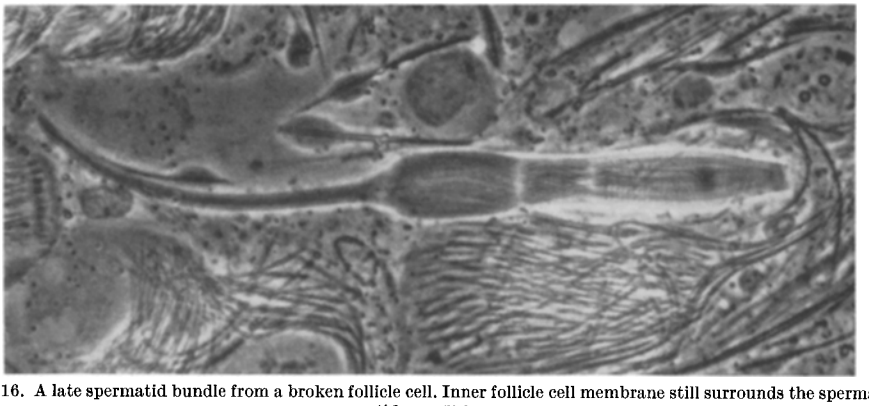

Fig. 16. A late spermatid bundle from a broken follicle cell. Inner follicle cell membrane still surrounds the spermatids. × 570

of equal size and of the same nuclear stage. Here again, many adjacent spermatocysts are observed to contain the same number of germ cells and again they are assumed to be derived from one follicle.

In the midregion of the testis, where follicles contain late spermatogonia and early primary spermatocytes (Fig. 5), each spermatocyst contains a maximum of 16 germ cells (Fig. 13). Thus, in each region of the testis one may predict and observe a maximum number of germ cells per spermatocyst of 1, 2, 4, 8, 16, etc., in

Fig. 17. A diagram to indicate the developmental history of a single spermatocyst. Development proceeds from lower right (*I*) around to the upper right (*VIII*). I indicates a newly formed seminiferous follicle as also shown in Fig. 2. The segment marked *II* indicates the period of mitotic proliferation of spermatogonia and of follicle cells as separate individuals. *III* marks the period in which spermatogonial mitoses continue after engulfment by a follicle cell. The arabic numeral at the inner end of each spermatocyst indicates the number of germ cells contained within. No. 1 corresponds to the formation of a spermatocyst by engulfment of a single spermatogonium by a single follicle

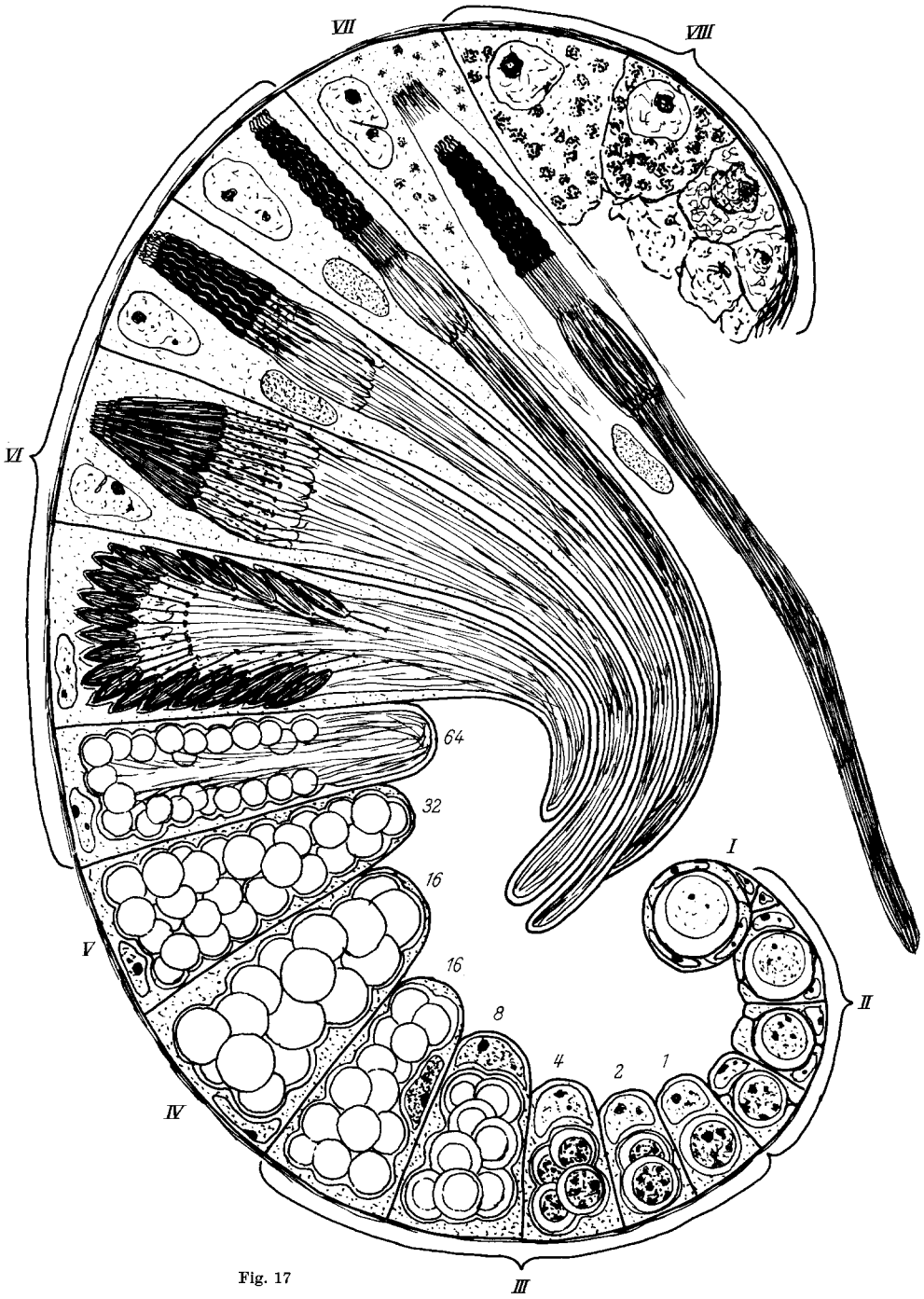

Fig. 17

cell. The next developmental stages of a spermatocyst after proliferation of 16 spermatogonia involves their transformation into 16 primary spermatocytes (*IV*), 32 secondary spermatocytes (*V*) and 64 spermatids (*VI*). Stages in spermiogenesis (*VI*) are followed by spermiation (*VII*) and progressive degeneration of the retained somatic elements of the spent follicle (*VIII*). It should be remembered that an actual follicle contains many spermatocysts all in the same stage of development

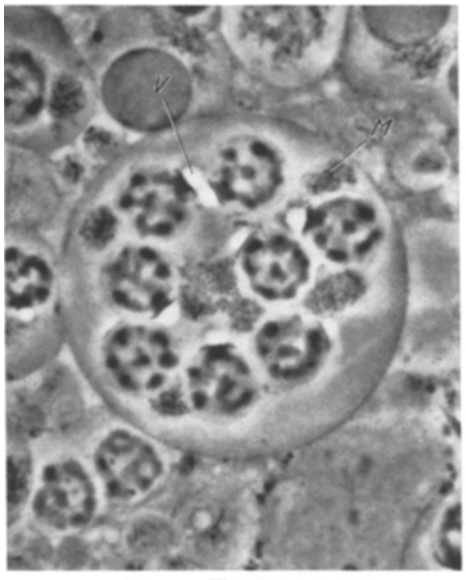

Fig. 18

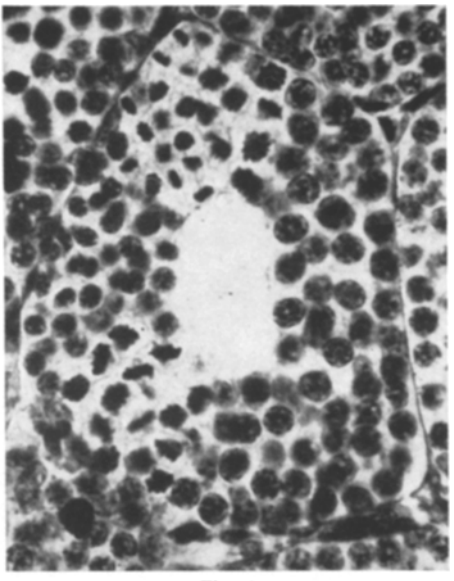

Fig. 19

Fig. 18. A phase-contrast micrograph of an 8-nucleate early spermatid. This type of multinucleate cell apparently forms by coalescence of a group of spermatids all inter-connected by cytoplasmic bridges. The small clear vesicles (*V*) are early stages in acrosome development. *M* mitochondria. × 1500

Fig. 19. A section of a spermatogonial follicle indicating the typical wave of mitotic divisions which spread from spermatocyst to spermatocyst around the follicle. The cells at the left have divided and reconstituted their nuclei, those at the top and lower left are in mitosis while those at the right have not yet undergone this particular division stage. Slight asynchrony of development usually becomes evident only in such division periods. × 330

direct relation to the distance from the germinal zone. In preparations in which the separation of spermatocysts has been gentle, the great majority of cysts remain intact and contain the expected numbers of germ cells.

A maximum number of 32 secondary spermatocytes and 64 spermatids (Fig. 14) are found in each cyst in tissues taken from regions still further removed from the sites of follicular origin. The 64 spermatids then undergo transformation into a bundle of 64 spermatozoa. Successive stages in this transformation are seen in Figs. 15, 16, 17. The transforming spermatids of each bundle adhere closely to one another, still bound by the inner cyst membrane. A distinct membrane is difficult to discern in fresh preparations, although it may be seen readily in suitably sectioned material (Fig. 7).

Mitoses were never observed in Sertoli cells associated with germ cells in spermatocysts. Neither histological sections nor dissociated preparations give any indication that Sertoli nuclei divide after the cell has surrounded a spermatogonium.

In trypsin preparations multinucleate germ cells occur very frequently at all stages of spermatogenesis. In the earliest follicles occasional 2, 3 or 4-nucleate spermatogonia are seen, while in later follicles cells with higher numbers of nuclei are present. The highest number of nuclei counted in one cell (secondary spermatocyte stage) was 19. Intercellular bridges are also observed between germ cells. Coalescence of groups of conjoined isogenous cells during the dissociation procedures results in large multinucleate cells such as that in Fig. 18.

Recent observations with the electron microscope on other elasmobranch species (STANLEY, unpublished) have confirmed the observations made by light microscopy, namely: the non-syncytial nature of the follicle (Sertoli) cells, the envelopment of germ cells by single follicle cells to form spermatocysts, and the presence of cytoplasmic bridges between germ cells.

Synchrony of development is a marked feature in the germ cells of elasmobranchs. It is usually complete within any one spermatocyst. On rare occasions intact spermatocysts may be observed to contain an odd number of germ cells of two different sizes, suggesting that some cells occasionally divide later or earlier than the others. Often, the cells within one particular spermatocyst divide before cells in neighboring cysts. In most cases divisions radiate from the slightly advanced cysts in a wave which passes around the follicle. The last cells to divide are usually those in the cyst directly opposite the one whose germ cells were first to divide (Fig. 19).

Discussion

The findings of this study confirm, in essential features, the interpretations of LA VALATTE ST. GEORGE (1878) and JENSEN (1883) that the follicle (Sertoli) cells form cysts around groups of germ cells. The former author, however, thought that the cysts are each formed by concrescence of several follicle cells. The present observations indicate that in its early stages the seminiferous follicle contains a complement of spermatogonia and follicle cells as separate entities. After a period of mitotic divisions in both cell types, *each single* follicle cell envelops one spermatogonium to form a new sub-unit, the spermatocyst. This unit maintains its integrity through six succeeding germ cell generations, usually producing 64 spermatids within each spermatocyst as noted by SWAEN and MASQUELIN (1883).

There appears to be no syncytium of Sertoli cells and germ cells as maintained by numerous earlier investigators (SEMPER, 1875; BALBIANI, 1879; HERMANN, 1882; SANFELICE, 1888; SABATIER, 1895; 1896, STEPHAN, 1902). The belief in a germ cell-Sertoli cell syncytium led many investigators to conclude that Sertoli cells or nuclei give rise to the germ cells or germ cell nuclei or *vice versa*. In 1950 MATTHEWS published a comprehensive description of the reproductive systems in the basking shark *Cetorrhinus maximus*. He interpreted the cells bordering the follicle lumen (in the two-layered follicle) as spermatogonia. Divisions of these luminal cells supposedly give origin peripherally to "primary sprematocytes" whose own divisions then result in secondary spermatocytes. After a time the "spermatogonia" migrate to the follicle periphery and transform into Sertoli cells. The illustration given as evidence of luminal cell division does not show such division. The "spermatogonia" cited by MATTHEWS appear to be Sertoli cells and the "primary spermatocytes" are actually spermatogonia.

Other investigators interpreted the germ cells and Sertoli cells as essentially separate lines (LA VALETTE ST. GEORGE, 1887; SWAEN and MASQUELIN, 1883) while FRATINI (1953) thought it possible that the two cell types may stem from common ancestors. My own observations all point to a complete separation of germ cell and Sertoli cell lines. The earliest germ cells seen in the adult testis are all distinct from the surrounding follicle cell precursors. No intermediate stages are seen. After engulfment of a secondary spermatogonium, follicle cells cease to

divide, so that the ultimate bundles of 64 spermatids stem solely from divisions of a single engulfed spermatogonium.

Much of the confusion of cell types has resulted from a failure of many workers to recognize the migration of the Sertoli elements. The migration of Sertoli "cells" was affirmed by SWAEN and MASQUELIN (1883), MATTHEWS (1950) and FRATINI (1953). It is clear from the present study that Sertoli cells as such do not migrate, only their nuclei do so. This interesting phenomenon might be presumed to have functional significance, but it is here suggested that the early migration of the Sertoli cell nucleus to the central region bordering the follicle lumen and its subsequent migration from the lumen to the follicle periphery may be of mechanical significance only. As shown by the present study, the follicle cell nuclei migrate centrad just after the follicle cells have each engulfed a single spermatogonium to form the spermatocyst. This event changes the unit structure of the follicle from a mixture of individual cells randomly oriented to a structure containing a number of bicellular units. Each spermatocyst tends to be approximately pear-shaped with a larger pole (germ cell) and a smaller pole (Sertoli cell nucleus) as depicted in Fig. 17. The follicle is spherical and units having a pear-shape would find their most economical arrangement with their larger, basal ends peripherad against the limiting membrane and their smaller apices directed centrad nearest the follicular lumen. This orderly arrangement is probably aided by the growth of the enclosed germ cell antecedent to the next division (Figs. 9, 10).

The fact that central migration of the follicle cell nuclei occurs feebly or not at all in *Torpedo* even though spermatocysts are formed in the same way as in *Scyliorhinus*, is evidence against a functional interpretation. There appears to be no essential difference in the structure or development of the seminiferous follicles in *Scyliorhinus* and *Torpedo* except the apparent degree of crowding of spermatocysts within the follicle. Comparing the seminiferous follicles of the two forms at the point of spermatocyst formation, they are of approximately equal size and contain cells of comparable size. However, the follicle in *Torpedo* possesses many fewer cells. This gives more space per spermatocyst and reduces the tendency for the Sertoli nuclei to be pushed toward the center of the follicle. The greater crowding of spermatocysts in the follicles of *Scyliorhinus* forces the Sertoli nuclei (smaller pole of the spermatocyst) to a more central position and results in spermatocysts which are longer in the radial axes of the follicle.

The secondary migration of the Sertoli cell nuclei to the periphery of the follicle occurs toward the end of the period of spermatogonial divisions, prior to the end of the period of spermatogonial divisions, as they transform into primary spermatocytes. During the period of mitotic proliferation of the spermatogonia, the Sertoli nuclei are pressed centrad so that the lumen is nearly obliterated. Also, the size of individual spermatogonia is steadily decreased. It seems possible that increased pressure on the Sertoli nucleus, the breaking up of the original engulfed spermatogonium, and the reduction in size of individual spermatogonia may all reach a critical point at which time the bulkier mass of the Sertoli nucleus is forced away from the central position to take up its final position just beneath the basement membrane (Fig. 17).

The non-germinal cells within the seminiferous follicles of elasmobranch fishes are probably homologous with the Sertoli cells of mammalian seminiferous tubules.

Germ cells develop in synchronous clones in close physical relationship to the Sertoli cells. In both groups the early spermatogonia appear to be less closely associated with the Sertoli cytoplasm than do later stages; the closer association of the Sertoli cytoplasm with the germ cells becomes more evident as the number of germ cells increases and as they are moved further from the blood supply.

Characteristic and fundamental differences between mammalian and elasmobranch testes include the fact that germ cells of only a single developmental stage are associated with a Sertoli cell at any one time in the Elasmobranchii, while each mammalian Sertoli cell may enfold three or four germ cell generations at once. While in the elasmobranchs the Sertoli cells form a complete cyst about each developing germ cell clone, in mammals the Sertoli cytoplasm is highly branched, each germ cell being in close proximity to a Sertoli cell cytoplasmic process. Finally, the mammalian Sertoli cell may function for many successive germ cell generations, but its counterpart in elasmobranchs degenerates after its involvement with a single germ cell clone.

Functional aspects of follicular structure may only be inferred from this purely morphological study. From the earliest stages the follicle cells interpose themselves between the germ cells and the capillaries of the testicular stroma. No capillaries invade the follicle itself. In the earliest stages the follicle cells form an epithelium covering the germ cords and early follicles. Later each individual follicle cell surrounds a spermatogonium and maintains this configuration in subsequent germ cell generations. Thus, everything exchanged between the blood stream and the germ cells must pass through the body of the follicle cell. Whether the follicle cell synthesizes nutritive substances or merely serves as a transporter, its position and form suggest that it functions in bringing nutritive materials quickly to the central regions of the follicle. This would seem to be a logical function of spermatocyst formation.

As the number of germ cells within each spermatocyst increases, it appears that many of the cells more centrally located lose contact with the follicle cell cytoplasm. This might place these central cells at a disadvantage in comparison with the cells more peripherally located. However, it is seen that cytoplasmic bridges are maintained between groups of germ cells. FAWCETT, ITO and SLAUTTERBACK (1959) have noted the existence of intercellular bridges among spermatocytes in various animal groups and also among other types of synchronously developing cells. These authors postulate that the bridges form the physical pathways for substances causing or controlling synchronous development. Placing a slightly different emphasis on this concept, I suggest that cytoplasmic bridges are maintained as a mechanism for more rapid and equitable distribution of nutritive substances among clonal cells and for more rapid and equitable clearance of the waste products of metabolism. In this interpretation, based upon morphological relationships, both the subdivision of the total germ cell mass by spermatocyst formation and the retention of cytoplasmic bridges function to promote *equality of physiological opportunity* among the developing germ cells. This equality of opportunity might in itself be sufficient to ensure synchronous development of the clone, although the observations of a wave of divisions passing from one spermatocyst around the follicle suggests the diffusion of a specific (possibly humoral) stimulus, at least at that time.

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