AN ANATOMICAL AND HISTOLOGICAL STUDY OF THE REPRODUCTIVE SYSTEM OF THE TWO SEXES OF *Eurygaster integriceps* Put.

by

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A Thesis Submitted to the Graduate Faculty
of the School of Agriculture in Partial Fulfillment of
The Requirements for the Degree of

MASTER OF SCIENCE IN AGRICULTURE

Split Major: Entomology-Horticulture

Approved:

In Charge of Major Work

Chairman, Graduate Committee

American University of Beirut

1962

Sex Organs of *E. integriceps*

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ABSTRACT

The present research work is an attempt to contribute to the knowledge of the anatomy and histology of the reproductive organs of the hemipterous insect E. integriceps. Both male and female sexual organs of this bug were fixed in Newcomer's Solution and stained in four different stains. Of the four stains used, Mellory's Tripple Stain and Delafield's Hematoxylin-Eosin gave the better results; the former being more useful for the "trophic core" in the ovarioles and "apical cell" in the testicular tubules.

The ovarioles are of the acrotrophic type, consisting of three tissue elements: the nurse cells, the pre-follicular cells, and the oocytes. The wall of the ovariole consists of an outer "peritoneal sheath", and an inner "tunica propria". In the adult insect the "oogonia" are absent from the ovarioles.

The internal structure of the testicular tubules is similar to that of many other hemipterous insects. It consists of an "apical germarium" and a series of cysts containing germ cells in various stages of development. The germarium contains the large "apical cell" and

"primary spermatogonia". The germ cell cysts following the apical germarium can be recognized into zones of secondary spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa.

ACKNOWLEDGEMENTS

It is with heartfelt and sincere wish that I take the opportunity to express my gratitude to Dr. A.S. Talhouk for his indefatigable and constant help and advice in the course of this investigation. He has been a source of ready reference especially in staining techniques, and his encouragements and moral supports are all that contributed towards the completion of this manuscript.

I am particularly grateful to my colleague Mr. N. R. Jaffri who very kindly spared his valuable time and improved the rough drawings. Thanks are also extended to Mr. Hogop for photographic reproductions; Miss. Y. Kalaidjian for her technical assistance in the use of phase-contrast microscope.

Finally I wish to extend my sincere thanks to my colleague Mr. Abdul Wahab Khan for typing this thesis, and to all those friends whose moral support and encouragement has contributed a great deal in completing this work.

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INTRODUCTION

Eurygaster integriceps Put., Hemiptera, Pentatomidae, also known as the Sunn bug in the Middle East, is a very serious pest of wheat and barley and some times oats in Syria, Turkey, Iraq, Iran, USSR (Crimea), Pakistan and Lebanon. Heavy financial losses inflicted to wheat crop by this pest aroused great interest in the study of its biology, and subsequent control both by chemicals and through its parasites (17).

However, comparatively little has been done on the anatomy of this pest. Papers by Trukhanov (18), Vodjdani (19) and Talhouk (17) mainly dealt with different phases of the anatomy of this pest, but none has given much attention to the reproductive system, especially its histology. The important part played by the reproductive system in building up heavy populations which ultimately result in incalculable damages to the agricultural economy, needs no emphasis.

The purpose of the present research is to elucidate the anatomical changes that take place during oogenesis and spermatogenesis. Since nothing seems to be published on the histology of the reproductive system of *Eurygaster integriceps*, the present work was undertaken so that this system may be better understood.

REVIEW OF LITERATURE

The first record of the modern research on the study of the histological structures of the reproductive system started in 1847, when the histology of the female reproductive organs of some beetles were studied. From then onward, as the methods were improved by the adoption of special cytological techniques, great advances have been made in this field, especially in the histochemistry and the synthesis of yolk (3).

Important studies on the morphology of the reproductive system of *Eurygaster integriceps* have been made by Trukhanov (18) and Vodjdani (19). Their studies indicated that the form and the size of the testes vary greatly according to the age and the activity of the insect. The appearance of the red pigment in the testes and the sperm ducts also becomes more prominent as the insect approaches the stage of sexual activity. In the female the length of the egg tube and the number of septa seperating one egg follicle from the other also vary with the age of the insect.

A. Female.

A good illustrative work on the anatomy and the histology of the reproductive systems in Hemiptera is given by Malouf (13) who worked on Nezara viridula which belongs to the same family (Pentatomidae) as Eurygaster integricep. According to this author, in the adult female 'stink bug', a clear distinction can be made between the "nurse cells", interstitial cells" and"oocytes". He has also reported the absence of the "oogonia" from the germarium of the adult insect. He further stated that the boundary line between the two regions of the germarium containing nurse cells, and the developing oocytes and follicle cells is very clear. At the anterior part of the germarium the nurse cells are smaller than those at the posterior parts. The nurse cells lack cell boundaries but are covered with a matrix from which are formed the nutritive channels for the nourishment of the developing oocytes. The interstitial cells like the nurse cells also do not have cell boundaries. This lack of cell boundaries in the interstitial cells has been thought to be of great advantage by Malouf in that they facilitate the easy descending of the oocyte as they grow in size.

Wieman (21) working on the germ cells of Leptinotarsa signaticollis (Coleoptera, Chrysomelidae) reported that the functional germ cells and the nurse cells of the egg tube are of primordial germ cell origin that have lost their reproductive ability, whereas the epithelial cells originate from mesodermic somites. Hegner (12) stated that the origin of the ovarian tissues is different in different species. In Hymenoptera, he described all the three ovarian tissue elements, oocytes, nurse cells and epithelial cells, to be descendants of primary germ cells. Shinji (15) also recognised the three kinds of ovarian tissue elements to be derived from the primordial germ cells in coccids. Abul-Nasr (1) is of the view that the primary cells are capable of differentiating into follicular cells and oogonial cells after they have been stimulated by certain (unknown) factors.

Bonhag and Wick (5) and Wick and Bonhag (20) recognized three zones of the apical trophic tissue of the germarium of Oncopeltus fasciatus (Heteroptera, Lygaedae). They maintained that the germarium of this species contains the "apical trophic tissue", the "oogonia", the "primary oocytes" and the anterior portion of the "prefollicular tissue". They further reported that the three zones of the apical trophic tissue are quite distinct, with the upper "zone I" containing cells with clear cell boundaries and showing all stages of mitotic activity. They called them as the undifferentiated "trophocytes". Following zone I is the region where the cells group together to form small clusters, and this they called "zone II" where differentiation takes place. The remaining posterior portion which they called "zone III", has been described to be the biggest one comprising two-third of the trophocyte region with fully differentiated trophic tissue and being characterized by the aggregation of nuclei surrounding a central portion of cytoplasm known as the "trophic core".

It has also been reported that some large migratory nuclei from zone II and giant nuclei from zone III of the trophic tissue enter into the trophic core and ultimately breakdown thus releasing the deoxyribonucleic acid (DNA) into the cytoplasm of the trophic core (20). In Oncopeltus fasciatus the primary oocytes, which are smaller in size, lie posterior to the trophic tissue and are embedded in a mass of prefollicular tissue. They are differentiated from the oogonia and are attached to the apical trophic tissue by means of cytoplasmic strands, the "nutritive cords" (5, 20).

The number of ovarioles in each ovary, as observed in many Hemiptera and Coleoptera, is seven. Each ovariole in turn is distinguished into four regions: the "terminal filament", the "germarium", the "vitellarium" and the "pedicel". The length of the vitellarium increases with the increase in the developing oocytes. Malouf (13) reported two nucleated coverings of the ovariole, an inner "tunica propria" and an outer "peritoneal sheath", in Nezara viridula (Hemiptera, Pentatomidae). He further stated that in this species as the growing oocyte increases in size and starts developing yolk, the surrounding follicular tissue invaginates and separates the two adjacent oocytes from each other. The tunica propria also folds in along with the invaginating follicular tissue, but the peritoneal sheath remains as such making the passage of the growing oocyte easier downwards. Malouf (loc. cit.) quoted Köhler as stating that the tunica propria also enters into the formation of the terminal filament in *Nepa* (Hemiptera).

Nelson (14) reported that in *Melanoplus differentialis* (Orthoptera, Acrididae) the outer peritoneal membrane is relatively thick at the posterior end, but anteriorly it becomes thin and delicate ultimately becoming continuous with the terminal filament.

B. Male.

Wieman (21) reported that in *Leptinotarsa signaticollis* (Coleoptera, Chrysomelidae) each testis is made up of seven "testicular follicles" which contain cysts of germ cells in various stages of maturation. The central lumen of the testicular follicle becomes filled with mature spermatozoa during the breeding season. He further stated that in this species there are two kinds of tissue elements found in the testicular follicles: the "germ cells", and the "epithelial cells". The epithelial cells in the adult insect enlarge between the follicles and produce a thin layer which separates the follicles from each other.

Bonhag and Wick (5) reported that in *Oncopeltus fasciatus* the seven testicular follicles of the testis are covered with a common membranous sheath known as the "tunica". Each follicle is again enclosed in a membranous wall which is devoid of cytoplasm. Beneath the tunica there is a thin layer which at its basal end forms the "vas efferens". Their histological findings revealed that the interior of each testicular follicle of this species is composed of an "apical germarium" and a series of cysts containing successive stages of germ cells. The cysts are separated from each other and from the germarium by thin membranes.

Snodgrass (16)[+] and Wigglesworth (22)[≠] recognised a similar histological structure of the testicular follicles in many insects. They further stated that the germarium of the testicular follicle consists of "spermatogonia" and an "apical cell". This apical cell has been described by them as functioning as a trophocyte in the early stages of development. Bonhag and Wick (5) preferred the term "apical complex" rather than the apical cell in view of the fact that they found it to be a multinucleate condition of a cytoplasmic mass surrounding a central cluster of nuclei.

The portion of the testicular follicle after the germarium, as stated by Snodgrass (16)[x], Bonhag and Wick (5) and Wigglesworth (22)[≠], is distinguished into zones of

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spermatogonia, spermatocytes, spermatids and spermatozoa. The zone of spermatozoa usually occupies more than the half of the testicular follicles in the adult insect. Bonhag and Wick (5) also reported that in Oncopeltus fasciatus the sperm cells in each cyst are generally in the same stage of development and that the differentiated spermatozoa at the posterior end of the testicular follicles are without cysts.

Wigglesworth (22)[+] stated that the spermatozoa at the posterior end of the testicular follicles are also enclosed in cysts but that they break through their cyst walls at the time when they are fully mature and ready to leave the testis. He further stated that even after breaking through their cyst walls, they still remain held together in bundles by a cap or rod of gelatinous material. After leaving the testis and at the time they reach the "seminal receptacle" of the female, the caps get dissolved by the action of some (unknown) enzyme.

An interesting account of the histology of the testis is given by Davis (9) who worked on Cimex lentularius (Heteroptera, Cimicidae). According to this author the various developmental series of sperm cysts observed in other insect species are lacking in this particular species. He has also reported the absence of the apical cell or the

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apical complex in the germarium of Cimex lentularius. According to him, the germarium of this species is a small mass of tissue consisting only of primary spermatogonia. The usual sequence of the germ cells in successive stages of development is different in that the primary spermatogonia leave the germarium and form clusters which then become enclosed by groups of cells. The cysts thus formed by the cell enclosures move downward as the enclosed germ cells develop and increase in size. Here also all the germ cells of a single cyst are in the same stage of development. By the time the moving cyst reaches the vas efferens, it has already become greatly enlarged containing mature spermatozoa with their heads directed towards the vas efferens.

MATERIALS AND METHODS

Sexually mature, and young adults were collected from wheat fields at the American University Farm, located in the Bekaa plain about 80 Kilometers east of Beirut. Dissections were made in May and July of both the mature and young adults, respectively. The male and female reproductive organs were fixed in Newcomer's solution for several months. The fixed material was then washed in water, dehydrated, cleared and embedded in paraffin.

Some difficulties were encountered in the beginning in embedding the tissues while following the usual technique described by Guyer (11). The tissues invariably stuck to the object while being transferred from the paraffin in the embedding oven to the pure melted histowax (m.p. 53-55°C) in the paper box. This difficulty, however, was overcome by taking the tissue from the embedding oven on a flattened surface of a warm wire, letting it dry along with the small amount of paraffin, and then separating it carefully from the carrier object. Pure melted histowax was poured into the paper box and the tissue was then placed on its surface as this was just hardening. More melted wax was poured after the tissue was properly orientated.

The entire procedure for dehydration, clearing, embedding (except for the changes mentioned above) and section cutting followed in this work was after the methods described by Guyer (loc. cit.).

The embedded material was cut in ribbons of 5 and 7 micra thick. The sections were stained in Delafield's Hematoxylin-Eosin, Hiedenheins Hematoxylin-Eosin, Harris' Hematoxylin-Eosin, and Mellory's Tripple Connective Tissue Stain (McFarlane's modification, Guyer, 11). The different stains gave variable results. Delafield's Hematoxylin-Eosin and Mellory's Tripple Connective Tissue Stain gave better results, but other stains were often used as control.

RESULTS AND DISCUSSION

I. ANATOMY

The anatomy of the reproductive system of *Eurygaster integriceps* has been studied at some length by Trukhanov (18) and Vodjdani (19).

A. Female.

The female reproductive organ of the Sunn bug consists of a pair of "ovaries", a pair of "lateral oviducts", a "common oviduct", and a "vagina" (Fig. 1). In addition to these structures, there is a pair of "accessory glands" and a sac-like "spermatheca". Each ovary consists of seven "ovarioles". Anteriorly each ovariole is distinguishable into a thread-like structure known as the "terminal filament" (Fig. 1 & 3, TF). All the seven terminal filaments of the ovarioles of each side join together to form a common thread known as the " suspensory ligament" which attaches itself to the body wall and suspends the ovary in the abdomen (Fig. 1, SL). In addition to the support of the suspensory ligament in keeping the ovaries in position, each ovariole is further supported by the tracheal system. The ovaries in this species are heavily tracheated. There is a trachea running to each ovariole, which before reaching it bifurcates, and each of the smaller trachea further ramifies into a large number of tracheoles which anastomose and in turn keep the ovarioles in proper position with respect to each other (Fig. 1, Tr).

The ovarioles are of the acrotrophic type. They consist of an apical chamber known as the "germarium" and a posterior region known as the "vitellarium" (Fig. 3, Grm). The germarium is separated from the vitellarium by a clear constriction. Such constrictions are also found separating the egg follicles from each other. In the young female there is only one, but a sexually mature adult may have as many as three constrictions. Thus in the ovariole of this species two eggs can mature at a time which coincides with the observations of Trukhanov and Mrs. Tepliakova (18) who found a number of 28 eggs laid by a single female at one time.

All the seven ovarioles open into the lateral oviducts of their sides, which are short and wide (Fig. 1, LOvd). The lateral oviducts in their turn open into the somewhat spherical and fibrous common oviduct or the "uterus" which, too, is wide enough to accommodate a large number of eggs at a time (Fig. 1, Covd). The vagina is long and thick. The chitin covering the vagina from inside is quite conspicuous and furrow-like. It opens ventrally in a longitudinal orifice of the genital chamber. Opening into the anterior part of the vagina is the sac-like spermatheca (Fig. 1, Spt). The spermatheca is distinguished into three parts: the spherical hind part, the tube-like elongated middle part, and the anterior outlet which opens into the vagina. The chitin covering the inside of the spermatheca like that of the vagina is also quite clear. The additional glands (Fig. 1, AG) are membranous and situated on the lateral sides of the vagina. As stated by Wigglesworth (22)+ and Vodjdani (19), they secrete a gelatinous liquid in the adult insect which helps in securing the eggs to the surfaces on which they are laid. In addition to these structures, there is, in Eurygaster integriceps, a pair of "chitinous plates" on the dorsal side of the posterior end of the vagina which have been termed by Trukhanov as "supports" for the vagina (Fig. 1, Sp).

B. Male.

The reproductive organ of the male consists of a pair of "testes", a pair of "sperm ducts" or "vasa deferentia", an "ejaculatory pump", an "ejaculatory bulb", an "ejaculatory canal" and the "penis". In addition there are six "accessory glands" attached to the ejaculatory pump. The testes vary greatly in their size, form and colour depending upon the activity and the age of the insect. In young adults, and during diapause, they are elongated and more or less pear-shaped in form and large in size. During this period their colour is white but as the insect becomes sexually active a red pigment appears to cover both

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the testes and the vasa deferentia. According to Trukhanov (18) this pigment is taken from the food and represents a "carotinoides" or a group of "autocianines flavones". At this stage the testes are small and somewhat spherical in shape.

Each testis is made up of seven tubules, the "testicular tubules" or the "testicular follicles" (Fig. 2, TT). In the young adult or during diapause these testicular follicles are quite conspicuous, but after the accumulation of red pigment they become indistinct. The testes are held in position in the abdomen by tracheae.

The sperm ducts or the vasa deferentia are long, covered with a red pigment, wide at the anterior part, and narrow towards the posterior end. The "seminal vesicle" which is often found as a dilated portion of the vas deferens in many insects, is not distinguishable in Eurygaster integriceps. Vodjdani (19) described the membranous sac-like widened portion of the ejaculatory canal, in which the sperm ducts open, as the seminal vesicle. Trukhanov (18) calls this structure as simply a dilated anterior portion of the ejaculatory duct. A similar structure in Cimex lectularius and Nezara viridula has been termed as ejaculatory pump and ejaculatory duct by Davis (9) and Malouf (13), respectively. In the present study this structure is considered, in agreement with Trukhanov, as a dilated anterior portion of the ejaculatory duct and it is preferred to term it as an "ejaculatory pump" (Fig. 2, EjP). The remaining portion of the ejaculatory canal, (Fig. 2, EjC), following the ejaculatory pump is narrow, but, prior to its joining the penis, it widens and forms a swollen bulb-like structure known as the "ejaculatory bulb" (Fig. 2, EjB). The penis and the posterior end of the ejaculatory canal are situated in the ninth and tenth abdominal segments of the body. The ejaculatory pump is supported by a heavy mass of fibrous muscles and cannot be easily seen unless the muscles are removed carefully.

The additional glands are six in number, four of which are very long and much convoluted in an irregular coil, whereas the remaining two are short (Fig. 2, AcGL). These glands lie as a compact mass on either side of the ejaculatory duct ventrad of the alimentary canal. They open into the anterior dilated portion of the ejaculatory duct, are transparent, and, according to Vodjdani (19), are filled with a secretion containing the spermatozoa and seminal liquid in the adult insect.

II. HISTOLOGY

A. Female.

The two regions of the egg tube of an ovariole, the "germarium" and the "vitellarium", are quite distinct in the mature adult (Fig. 3). The vitellarium is comparatively long and, during the active sexual period of the insect, there may be found two oocytes developing simultaneously. The outer "peritoneal membrane", which often gets destroyed in histological preparations, is clearly seen in Fig. 3, and seems to continue with the terminal filament.

The interior of each ovariole of the mature adult consists of three tissue elements: the "nurse cells", the "prefollicular cells", and the "oocytes". In agreement with the findings of Malouf (13) in Nezara viridula, the "oogonia" seem also to be absent from the ovarioles of the mature adults of Eurygaster integriceps. The three tissue elements just mentioned are quite apparent and can be easily distinguished from each other (Fig. 6, NrC, Oc, PfT). The nurse cells at the apical portion of the germarium are small and without cell boundaries. They do not seem to be connected to the central cytoplasmic mass as do the other cells. Posterior to the apex and about the middle of the germarium towards the periphery, some of the nurse cells have grown into giant nurse cells from which flow-lines appear to enter the central cytoplasmic mass. According to Wick *et al.*(20) the giant cells are formed by the fusion of small nurse cells and the flow-lines coming from them to the central cytoplasmic mass represent the deoxyribonucleic acid (DNA), being contributed to the developing oocytes. A little behind the apical portion of the germarium, the nurse cells are large in size and appear to aggregate in groups. These cells as well as the giant cells near the periphery are without cell boundaries. They, however, are enclosed in a cytoplasmic matrix from which are formed the cytoplasmic strands for the nourishment of the developing oocytes. Malouf (13) reported that in the adult *Nezara viridula* the nurse cells do not undergo cell division. In view of the fact that in *Eurygaster integriceps* the nurse cells posterior to the apical region of the germarium are large and multinucleate, it is felt safer not to draw any such conclusion.

The cytoplasmic mass in the center of the germarium is known as the "trophic core" (Fig. 4, TC). This core, in the histological preparations in question, became more clear only when the tissue was stained with Mellory's tripple connective tissue stain. Some of the nurse cells appear to enter into the body of the trophic core (Fig. 6). Davis (9) quoted Bonhag as stating that these cells are the cast nuclei which eventually disintegrate and release DNA into the cytoplasm of the core. The developing oocytes are connected with the trophic core by means of cytoplasmic lines known as "nutritive cord" (Fig. 4, NtC), through which they derive their nourishment.

The primary oocytes and the prefollicular cells are aggregated at the basal end of the germarium (Fig. 6, PfT, OC). The prefollicular cells are densely packed around the second developing oocyte. They are without cell boundaries and are scattered in a mass of cytoplasmic matrix. As the primary oocyte grows in size, the loose prefollicular tissue makes it easier for the oocyte to pass down into the vitellarium and then finally surrounds it and forms what is known as the "follicular epithelium" of the egg (Fig. 4 & 7, FE). At first when the leading oocyte is not yet fully developed, the epithelial layer is incomplete between the two neighbouring oocytes and the prefollicular cells can be seen loosely scattered in that area (Fig. 7). As the formation of yolk starts, the follicular epithelium completely surrounds the oocyte and thus separates the two neighbouring ones from each other (Fig. 4, a). At the time when the follicular epithelium is constricting and separating the two oocytes, some of the cells become trapped in between the walls of the follicular epithelium (Fig. 4, b). These cells have been termed as "intersticial cells" by Malouf (13). According to Davis (9) they are follicular cells that precede the developing oocyte as it passes down, and form the "epithelial plug" in the pedicel which serves as a preventive wall for the premature escape of the egg into the oviduct. As soon as the egg follicle reaches its full maturity and is ready to leave the ovariole, the follicular cells of the epithelial plug disintegrate and allow the egg to pass into the oviduct. These follicular cells are of two types and are separated from each other by a fine constriction. Those at the anterior side and posterior to the second developing oocyte (Fig. 4) are flattened cells, whereas those posterior to this part are spherical or oval. A similar arrangement is described by Davis (9) in Cimex lectularius.

The leading oocyte, which is almost completely separated from the second developing oocyte, contains many vacuoles and granules (Fig. 4). The vacuoles and granules represent the yolk, proteins and carbohydrates that are being accumulated in the egg. A greater part of this oocyte is still occupied by the cytoplasm, especially at the anterior and posterior ends. Fig. 9 shows an egg which is completely surrounded by the follicular epithelium, and which is almost filled with yolk material with no trace of cytoplasm remaining. Of particular interest in this egg follicle is the fact that the epithelial cells appear to be in a great state of change, and, some of them are larger in size than the others. This enhanced activity on the part of the epithelial cells is probably due to the fact that they are secreting yolk material for the egg. Reference to the secretion of yolk material by the follicular epithelium has often been made in the literature. According to Bonhag (3) some authors believe that the yolk-forming material in the egg is supplied by the nurse cells, whereas others consider the epithelial cells as mainly responsible for the yolk formation in the egg. Wigglesworth (22)[+] also stated that the oocytes in their later stages of development are fed through the secretions of the follicular cells, and that the follicular cells at this stage greatly enlarge and divide amitotically into two. Davis (9) reported that in <u>Cimex lectularius</u>, at the early stages of yolk deposition, the nuclei of the follicular epithelium undergo fragmentation into two equal halves. He further stated that the fragmentation, or amitosis, in the ovarian tissue of insects is not an uncommon thing to come across. Looking to the instances in which the occurrence of fragmentation has been cited in the literature, he considered it to be of general occurrence in the ovarian tissue of insects.

Each ovariole is enclosed into two nucleated membranes: an inner "tunica propria", and an outer "peritoneal sheath" (Fig. 4 and 6, PS, TP). The peritoneal sheath continues beyond the apical end of the germarium, and takes part in the formation of terminal filament. The tunica propria ends at the base of the terminal filament and does not go into its formation. The terminal filament is cut off from the germarium by a thick transverse septum.

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The tunica propria invaginates along with the invagination of the follicular epithelium to constrict and separate the neighbouring oocytes from each other when they have grown to a certain size and started developing yolk. The peritoneal sheath does not take part in the invaginating process of the follicular epithelium and tunica propria, but it stands out as a separate membrane as can be seen in Fig. 4, at the place where the separation between the two neighbouring oocytes is more or less nearing its completion.

The pedicel consists of a simple tube of epithelium covered externally by a peritoneal membrane. It joins the egg tube anteriorly and, posteriorly it opens into the lateral oviduct. The structure of the lateral oviduct is similar to that of the common oviduct. They consist internally of a convoluted epithelium resting on a basement membrane. The epithelium is surrounded by a layer of longitudinal muscles and an outer layer of circular muscles. The whole structure is covered by a peritoneal membrane.

The spermatheca (Fig. 8) consists of three parts: the spherical posterior portion, the elongated tube-like middle part, and the anterior outlet opening into the vagina (Fig. 8, Post, Mid, & Ant). Fig. 8 is a longitudinal section through the spermatheca; it shows the circular spots outside the cuticular lining in the posterior spherical part. In manipulating the fine adjustment of the microscope, the spots appear as black dots and ring-like circular spots. According to Wigglesworth (22)[+] they are glands with intracellular ducts. The intracellular ducts pass through the cuticular lining and pour their secretions into the lumen of the spherical part of the spermatheca. The middle tube-like part of the spermatheca consists of cuticular lining and fibrous muscles. The lumen of the posterior spherical part seems to be filled with some thing which may be the spermathecal secretion of the spermatozoa. The anterior outlet is composed mainly of chitin surrounded by muscle fibres.

B. Male.

The testicular tubules are enclosed in a nucleated "peritoneal sheath" which at the posterior end forms the connecting tube known as the "vas efferens" (Fig. 10, PS). Beneath the peritoneal sheath is a thin membrane covering the interior of the testicular tubule.

The internal structure of each testicular tubule is distinguished into an "apical germarium" (Fig. 10, Grm), and a series of sperm cysts containing sperm cells in successive stages of development. A peculiar thing about the testicular tubule of Eurygaster integriceps is that it is divided into a number of compartments by thin membranes

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into which can be seen a few nuclei scattered here and there. Each compartment consists of a number of sperm cysts. Each sperm cyst encloses a number of sperm cells that are at the same stage of development, but the sperm cells of different cysts in one compartment may be at different stages of development. Some times two compartments may have the cysts in which the sperm cells are at the same stage of development.

The germarium is situated at the apex of the testicular tubule and occupies a small apical portion. It consists of a mass of "primary spermatogonia" and a large cell known as the "apical cell" (Fig. 10, ApC). Bonhag et al. (5) termed the apical element of the *Oncopeltus fasciatus* as the "apical complex"; this term was coined due to the fact that they found the element in question to be composed of a mass of cytoplasm with a central cluster of nuclei. In *Eurygaster integriceps*, the apical element consists of a large central cell surrounded by a number of primary spermatogonia. The primary spermatogonia that are in close contact with the central large cell appear to be connected to it by protoplasmic strands. These contain large granules that seem to be originating from the apical cell. The apical cell probably performs a trophic function. Snodgrass (16)+ quoted Grünberg and Zick as claiming that the apical cell derives its nourishment from the

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spermatogonia in the immediate vicinity, by first dissolving and then absorbing them. From this statement he concluded that the apical cell is spermatogonial nurse cell, which dissolves and absorbs some of the spermatogonia in its immediate neighbourhood; also it nourishes some other spermatogonia by the nutritive material that it has thus accumulated in its cytoplasm, through the cytoplasmic strands.

The germarium is followed by the zones of growth, maturation and transformation. Unlike the primary spermatogonia in the germarium, the secondary spermatogonia and the following stages of germ cell development are all encysted except the posterior portion where differenciated spermatozoa are found. The cysts of the germ cells following the secondary spermatogonia are divided into zones of primary and secondary spermatocytes, and the spermatids in various stages of development. The remaining posterior portion of the testicular tubule contains the cyst-free spermatozoa. At this stage of the adult life, the heads of the spermatozoa are directed towards the apex of the testicular tubule.+ This indicates that during this period the spermatozoa are not fully mature and, therefore, not ready to be discharged into the vasa deferentia.

+ Fig. 10 represents a longitudinal section of the adult testicular tubule taken during the diapause period.

During the sexually active period of the adult insect, a greater part of the testicular tubule becomes filled with mature spermatozoa. Fig. 11 represents a cross section of the whole testis of a sexually active adult through the middle portion in which all the seven testicular tubules are filled with mature spermatozoa. It can be seen that the spermatozoa at this stage of the insect life have small heads, long tails and a some what curved body as against the ones with big heads and small tails shown in the posterior part of the testicular tubule in Fig. 10.

The vasa deferentia consists of a thick outer muscularis (Fig. 12, Mcl) surrounding the wall of the duct. Fig. 12 represents the cross section of the vas deferens of a sexually active adult, and, therefore, the muscularis is completely covered with the red pigment which,in Fig. 12, appears as a thick red covering surrounding the wall of the duct. The wall of the vasa deferentia consists of a layer of simple epithelium (Fig. 12, Ept) surrounding the lumen which seems to be filled with spermatozoa (Fig. 12, Spz).

The ejaculatory pump (Fig. 13) is mostly made up of muscles running in all directions which seems to be a continuation of the muscularis of the vasa deferentia that thickens at this portion. Beneath the muscular covering is the layer of simple epithelium (Fig. 13, Ept).

The accessory glands consist of a layer of simple epithelium surrounded by a muscular layer which is also a continuation of the muscular layer of the ejaculatory pump (Fig. 14, Mcl, Ept).

SUMMARY AND CONCLUSIONS

Eurygaster integriceps Put. is a well known serious pest of wheat and barley in Iran, Iraq, Lebanon, Pakistan, Syria, Turkey and some parts of USSR.

The present work is an attempt towards the better understanding of the anatomy and histology of the reproductive systems of the adults of both sexes.

The tissues were fixed in Newcomer's Solution for some time. Among the various stains used, Mellory's Tripple Connective Tissue Stain and Delafield's Hematoxylin-Eosin gave better results. Mellory's Tripple Stain was especially useful for the trophic core and nutritive cords in female and the apical cell in the male. Some difficulties and their solutions regarding embedding are also described.

The testes and the vasa deferentia are covered with a red pigment during the breeding and the sexually active period of the insect. The accessory glands are six in number, transparent, and attached to the anterior dilated part of the ejaculatory duct. Each testis consists of seven tubules.

In the sexually immature adult female ovary, there is one septum, but a mature female may have as many as three

septa separating different egg follicles. The spermatheca is distinguished into three parts: a posterior spherical portion, a middle tube-like elongated part, and the anterior outlet opening into the mouth of vagina. The vagina is supported by a chitinous plate on either side.

The interior of each testicular tubule consists of an apical germarium and a series of cysts in compartments containing sperm cells in successive stages of development. The germarium consists of an apical cell surrounded by the primary spermatogonia. The remaining part of the testicular tubule following the germarium can be recognized into zones of secondary spermatogonia, primary and secondary spermatocytes, spermatids in various stages of development, and the differentiated spermatozoa. The wall of the testicular tubule consists of a nucleated peritoneal sheath, which at its posterior end forms the vas efferens. Beneath the peritoneal sheath is a thin membrane covering the interior of the testicular tubule. The vasa deferentia and the ejaculatory duct consist of a simple spithelium surrounded by a thick muscularis.

The wall of each ovariole consists of two nucleated membranes: an outer peritoneal sheath, and an inner tunica propria. The ovarioles are of the acrotrophic type, consisting of an apical trophic tissue and a posterior portion of developing egg follicles. There are three tissue elements found in the trophic tissue: the nurse cells, the prefollicular cells and the young oocytes. The oogonia are absent in the ovarioles of the adult insect. The developing oocytes are fed through the nutritive cords from the trophic core located in the middle of the trophic tissue. As the growing oocytes increase in size and develop yolk, the surrounding follicular epithelium invaginates and separates the neighbouring oocytes from each other. Some of the prefollicular cells precede the developing oocyte as it descends towards the posterior part of the vitellarium, and forms a plug known as the epithelial plug in order to prevent the premature escape of the oocyte into the lateral oviduct. At the time when the egg is fully developed, this plug automatically disintegrates and thus clears the passage of the egg into the lateral oviduct. The lateral and common oviducts are similar in structure. They consist of an inner epithelium resting on a basement membrane and surrounded by a layer of longitudinal muscles and an outer layer of circular muscles. The whole being covered by a peritoneal sheath.

The anatomy of the male reproductive organs is more or less the same as found in other hemiptera. The seminal vesicle is absent.

The anatomy of the female reproductive organs is also similar to many other hemipterous insects. There are seven ovarioles in each ovary.

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APPENDIX

EXPLANATION OF ABBREVIATIONS

Abbreviation	Explanation	Abbreviation	Explanation
AcGl	= Accessory gland (male)	Oc	= Oocyte
AG	= " " (female)	Ovr	= Ovariole
ApC	= Apical cell	P	= Penis
C	= Canal	Pd	= Pedicel
Ch	= Chitin	Pft	= Prefollicular tissue
COvd	= Common oviduct	PS	= Peritoneal sheath
Cyt	= Cytoplasm	SL	= Suspensory ligament
Cyt S	= Cytoplasmic strands	SP	= Supporting plate
EjB	= Ejaculatory bulb	Spd	= Spermatids
EjC	= Ejaculatory canal	Spg	= Spermatogonia
EjP	= Ejaculatory pump	Spt	= Spermatheca
FE	= Follicular epithelium	Spz	= Spermatozoa
Gl	= Gland	TC	= Trophic core
GNrC	= Giant nurse cell	TF	= Terminal filament
Grm	= Germarium	TP	= Tunica propria
GV	= Germinal vesicle	Tr	= Trachea
LOvd	= Lateral oviduct	TT	= Testicular tubule
Mcl	= Muscularis	Vg	= Vagina
NrC	= Nurse cell	VD	= Vas deferens
NtC	= Nutritive cord	Y	= Yolk

Fig. 1. Dorsal view of the ventral abdominal wall and female reproductive organs.

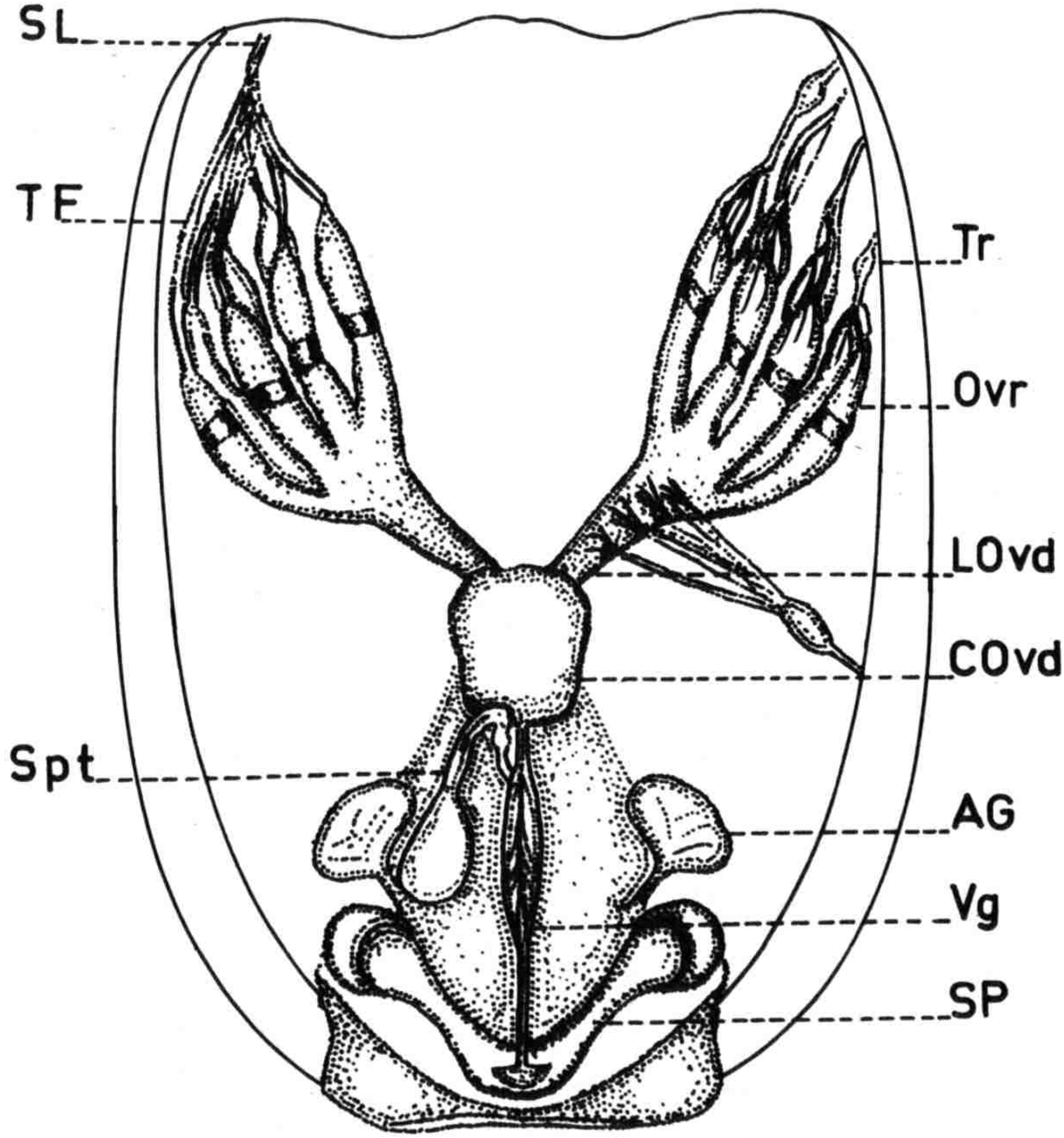

FIG.1

Fig. 2. Dorsal view of the ventral abdominal
wall and male reproductive organs.

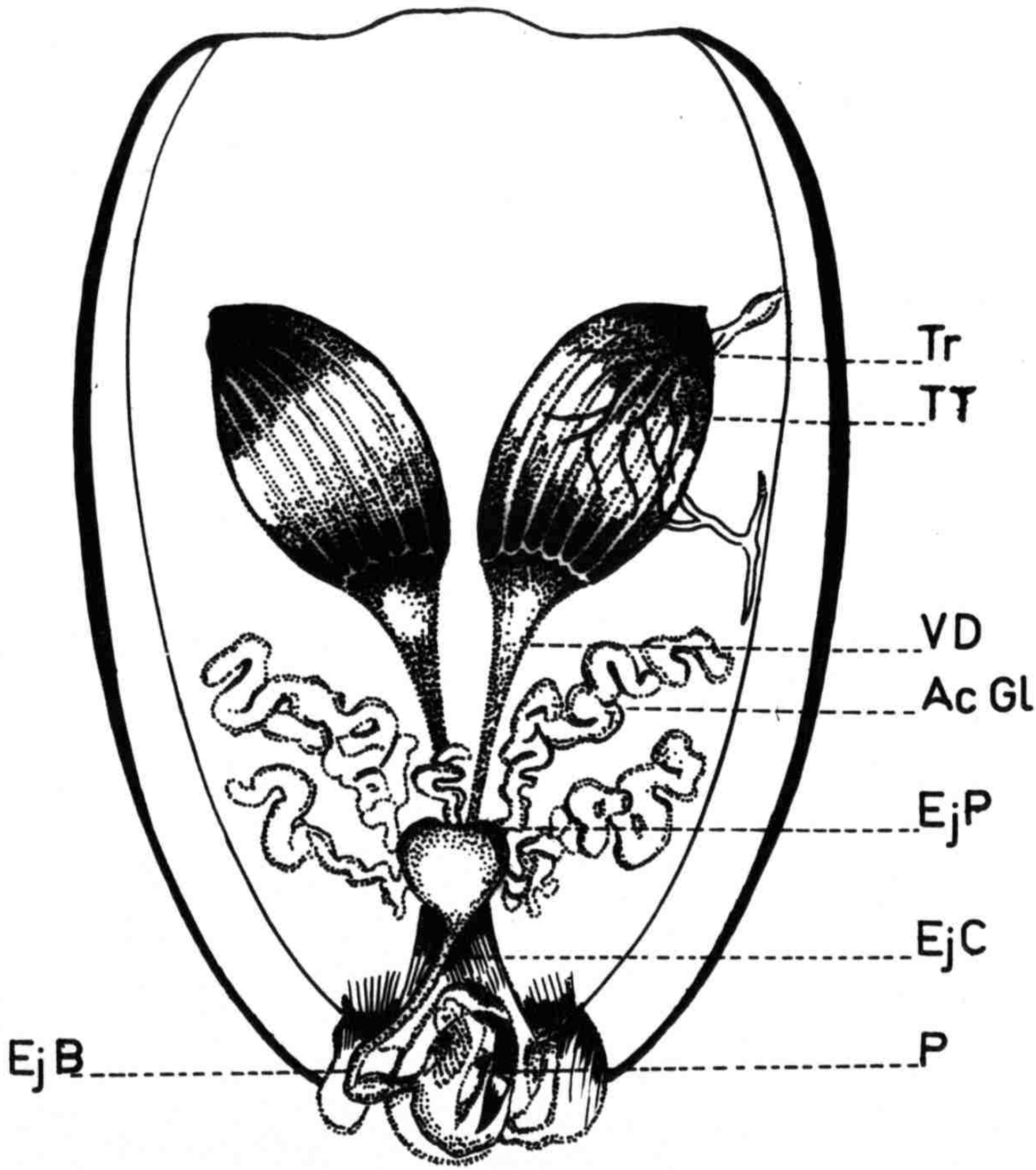

FIG. 2

Fig. 3. Ovariole of a sexually mature female.

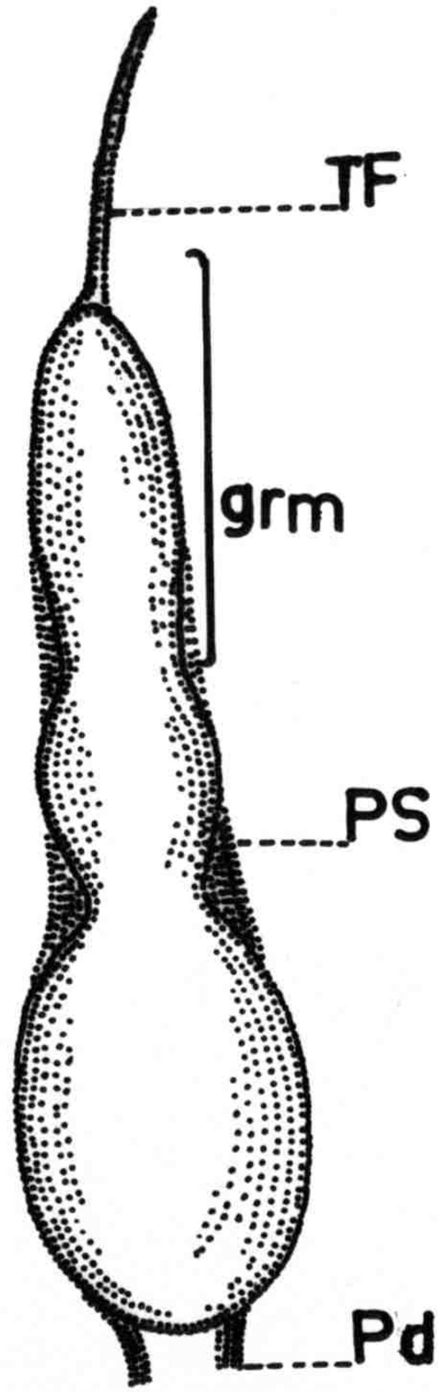

FIG.3

Fig. 4. Longitudinal section of the anterior
part of an ovariole.

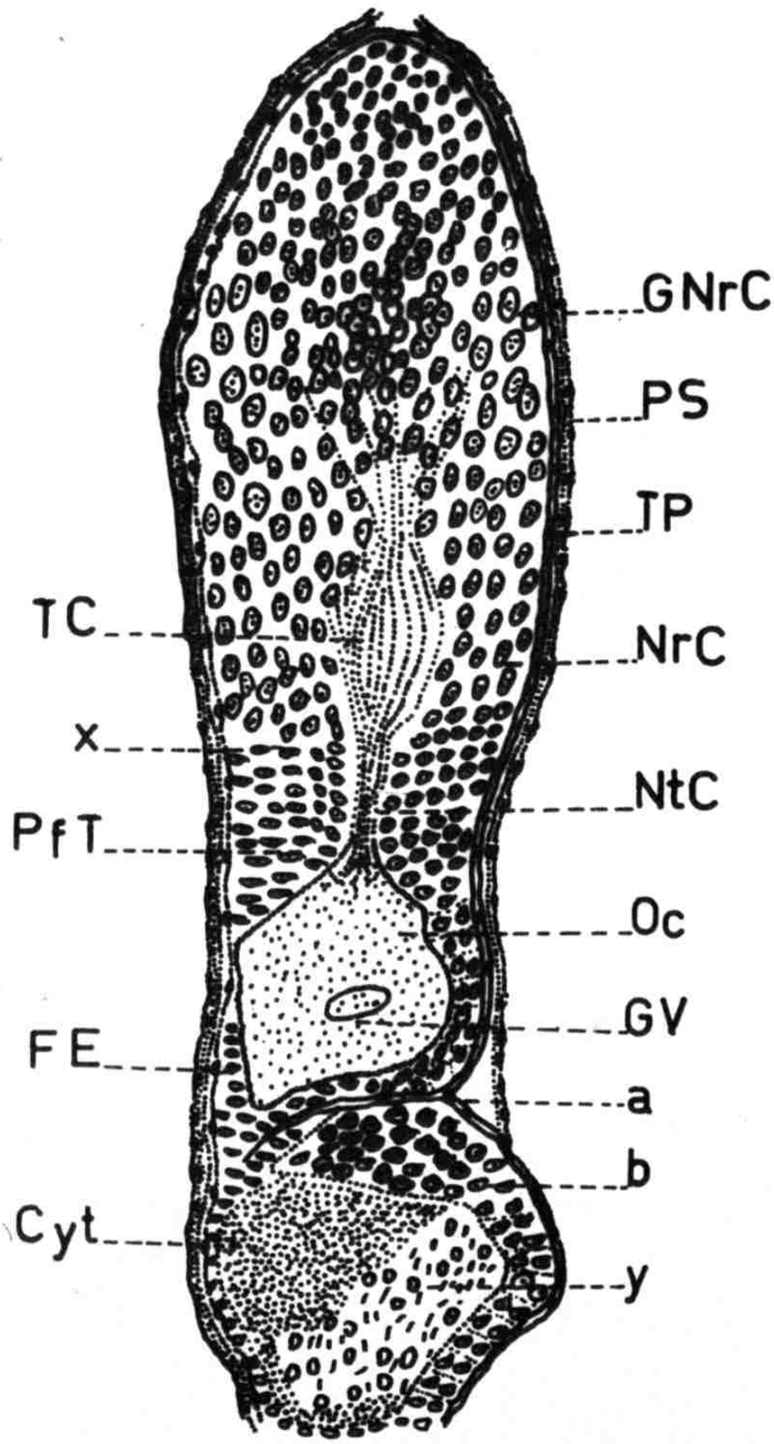

FIG.4

Fig. 5. Nurse cells and nutritive channels,
highly magnified.

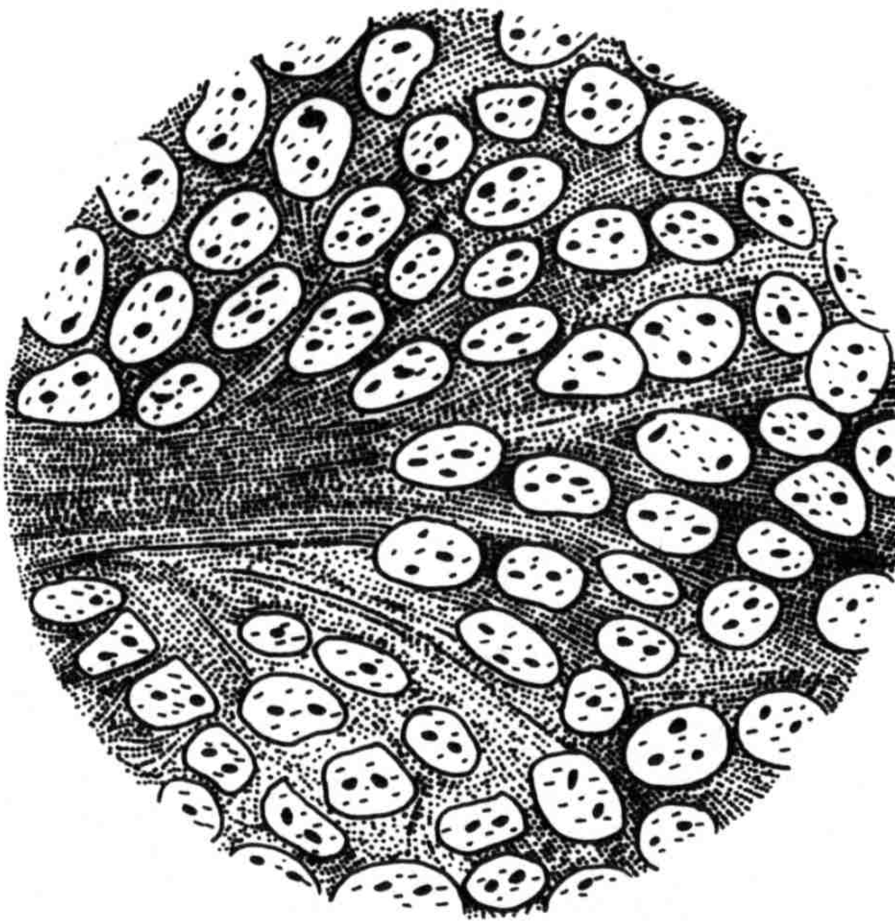

FIG.5

Fig. 6. Nurse cells, prefollicular cells. Oocyte, and nutritive channels at region x (Fig.4) highly magnified.

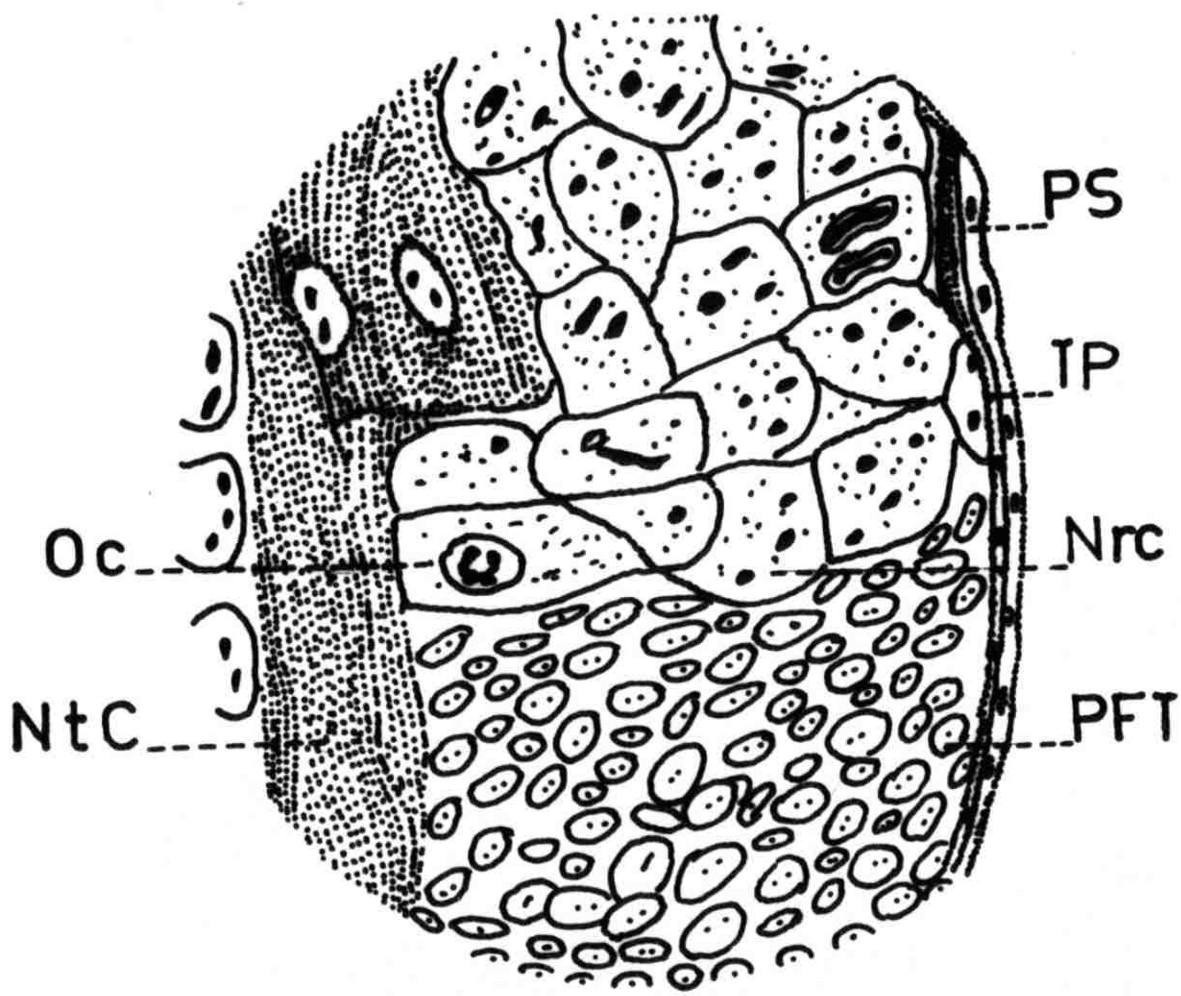

FIG.6

Fig. 7. Prefollicular cells in between the two developing oocytes before the separation by invaginating follicular epithelium.

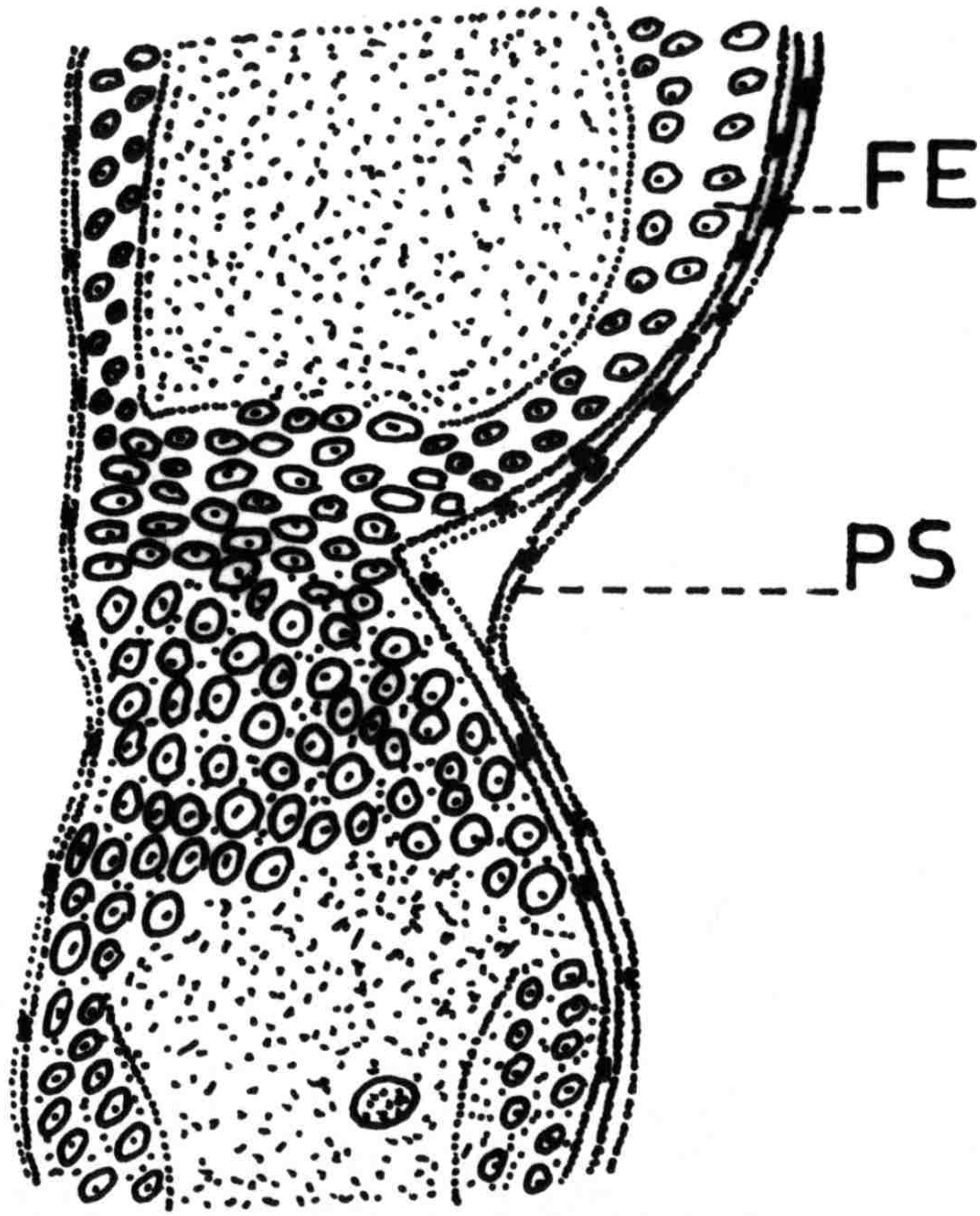

FIG.7

Fig. 8. Longitudinal section of spermatheca.

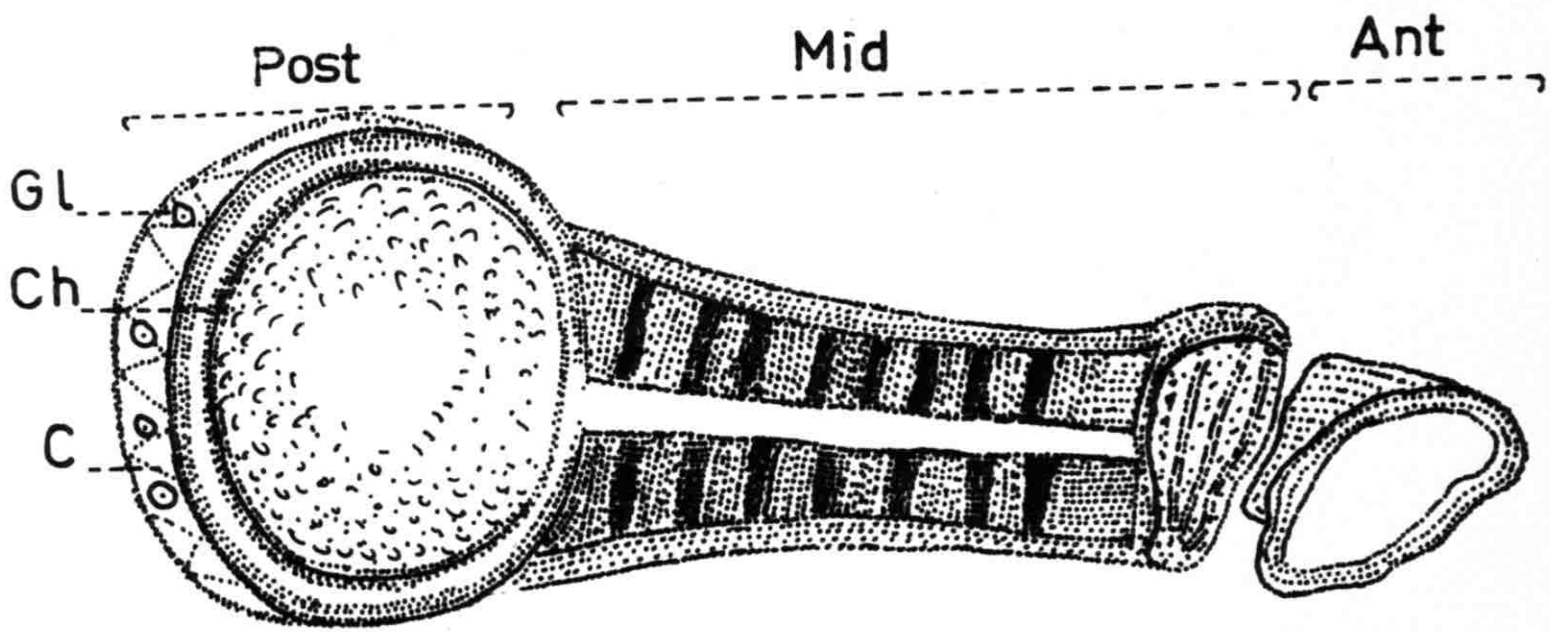

FIG.8

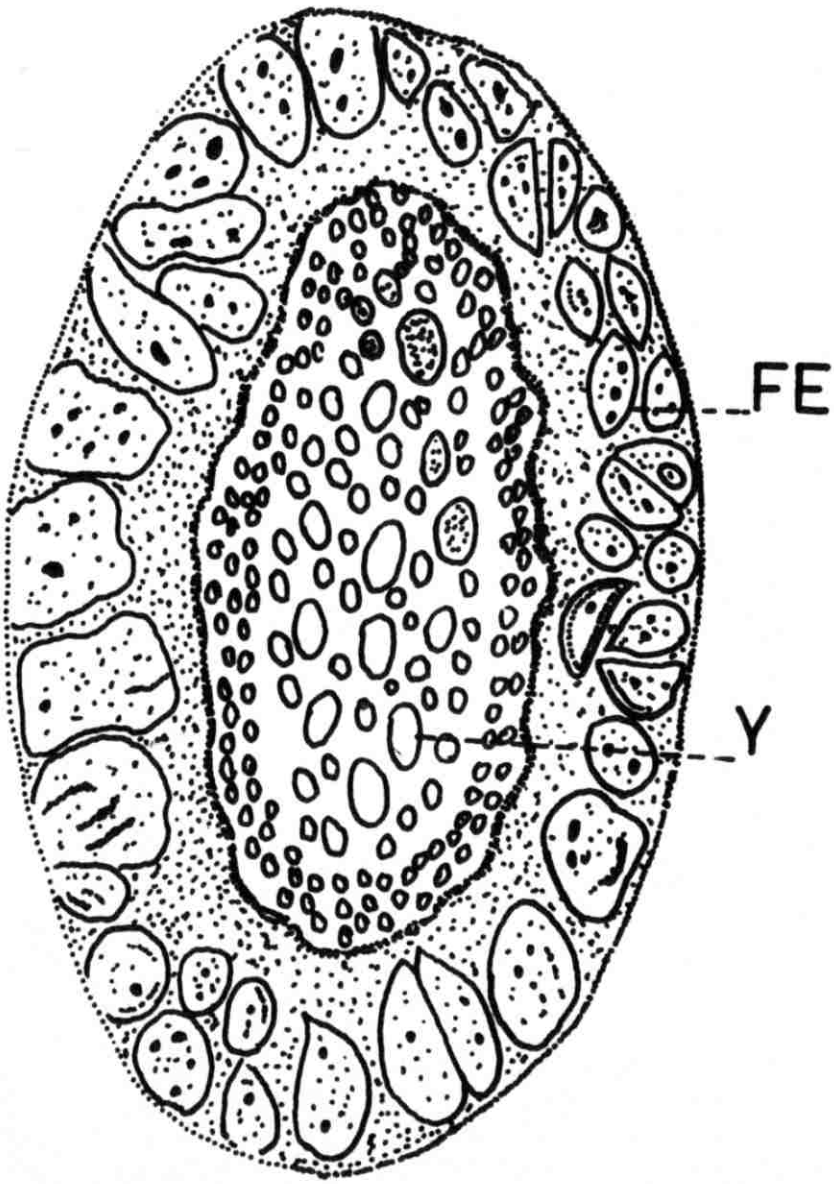

FIG.9

Fig. 10. Longitudinal section of a testicular tubule.

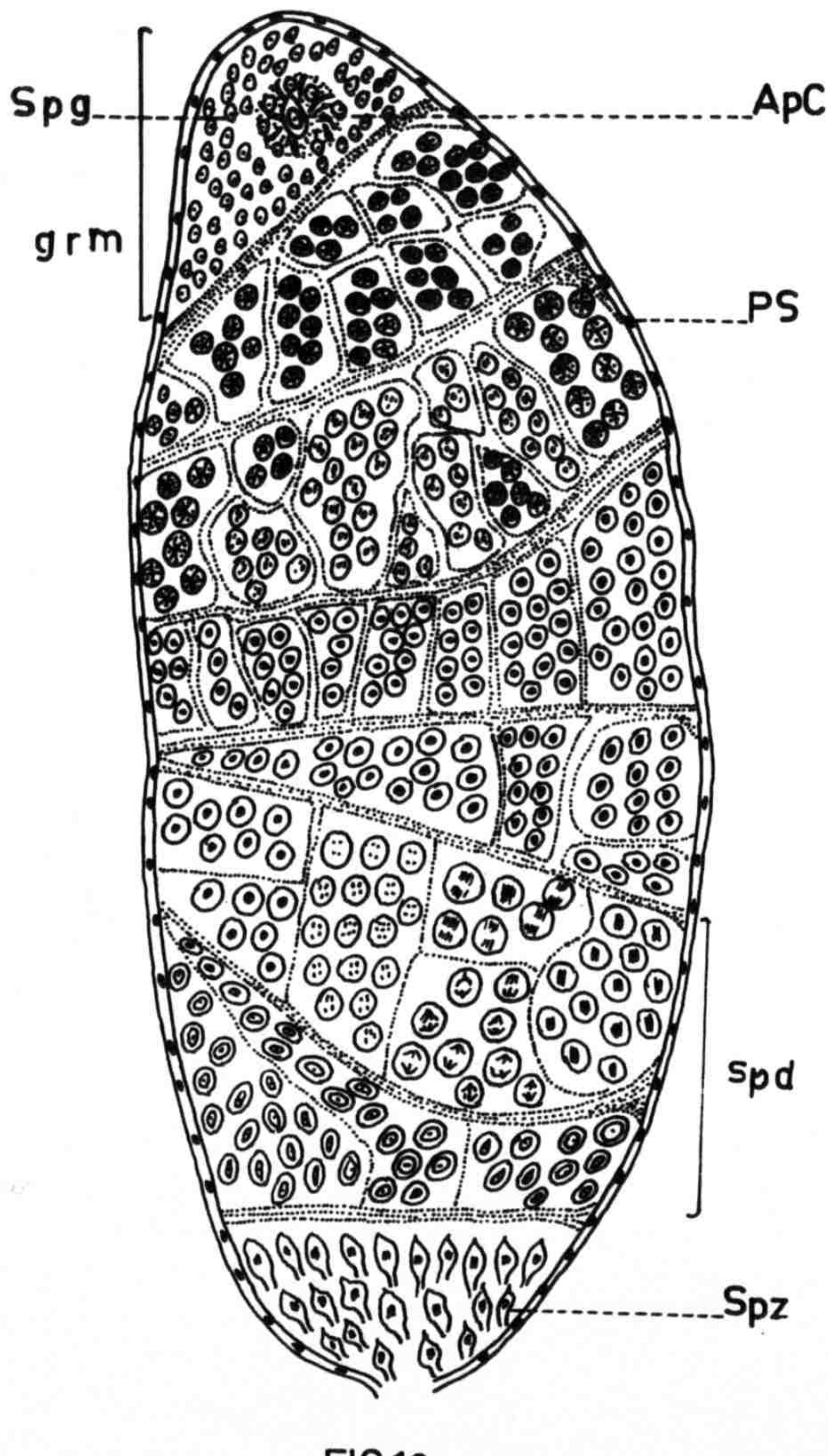

FIG.10

Fig. 11. Cross section of a whole testis showing all the seven testicular tubules of a sexually active male.

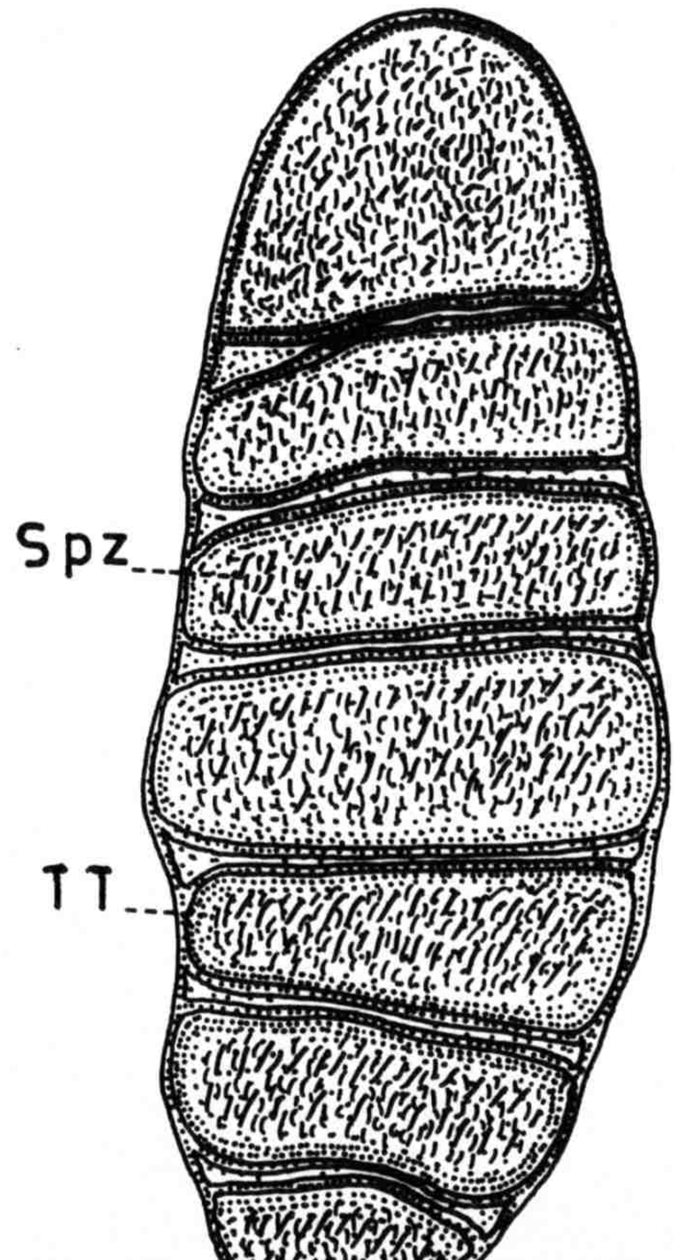

FIG.11

Fig. 12. Cross section of the sperm duct.

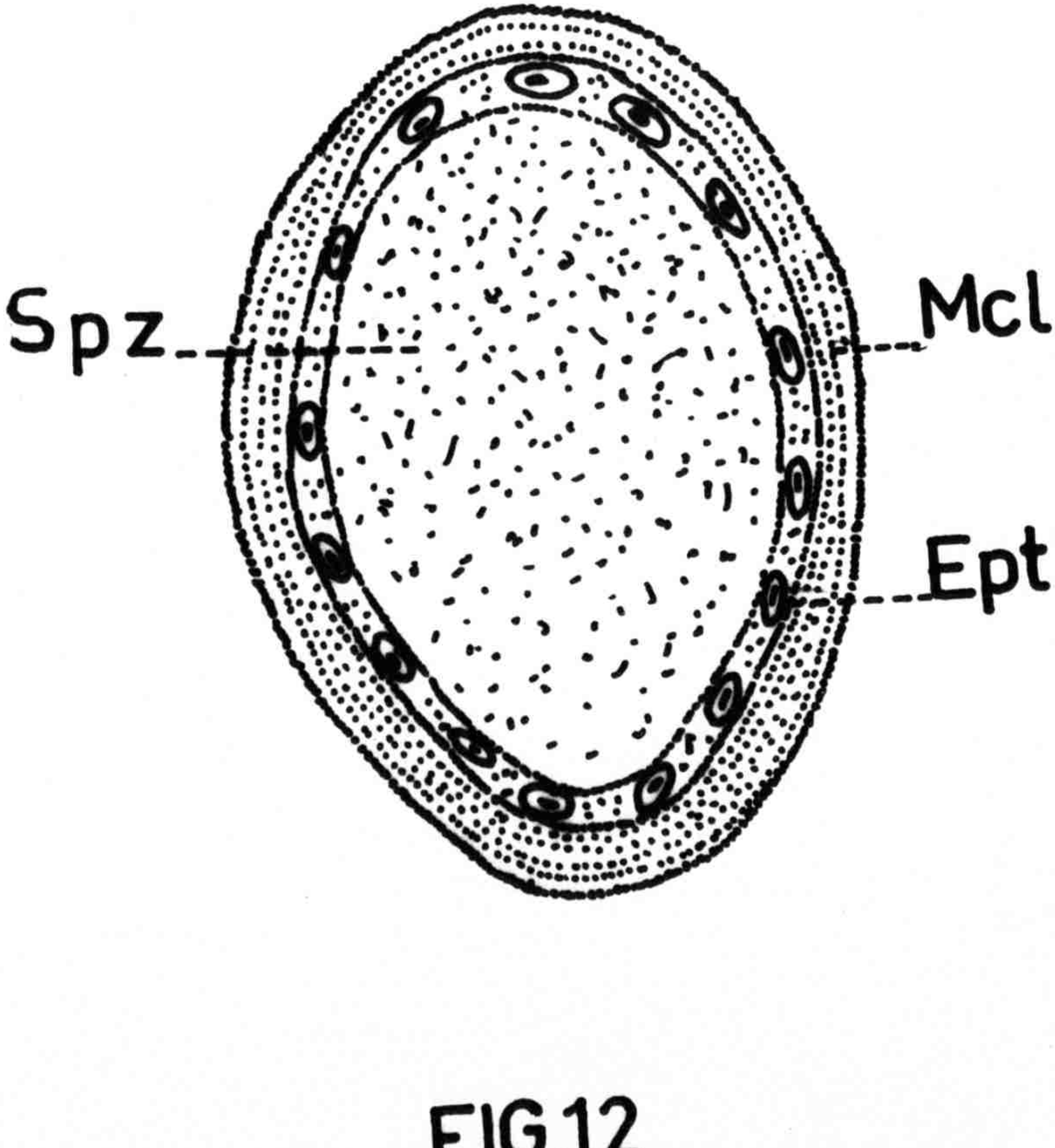

FIG.12

Fig. 13. Cross section of the ejaculatory pump.

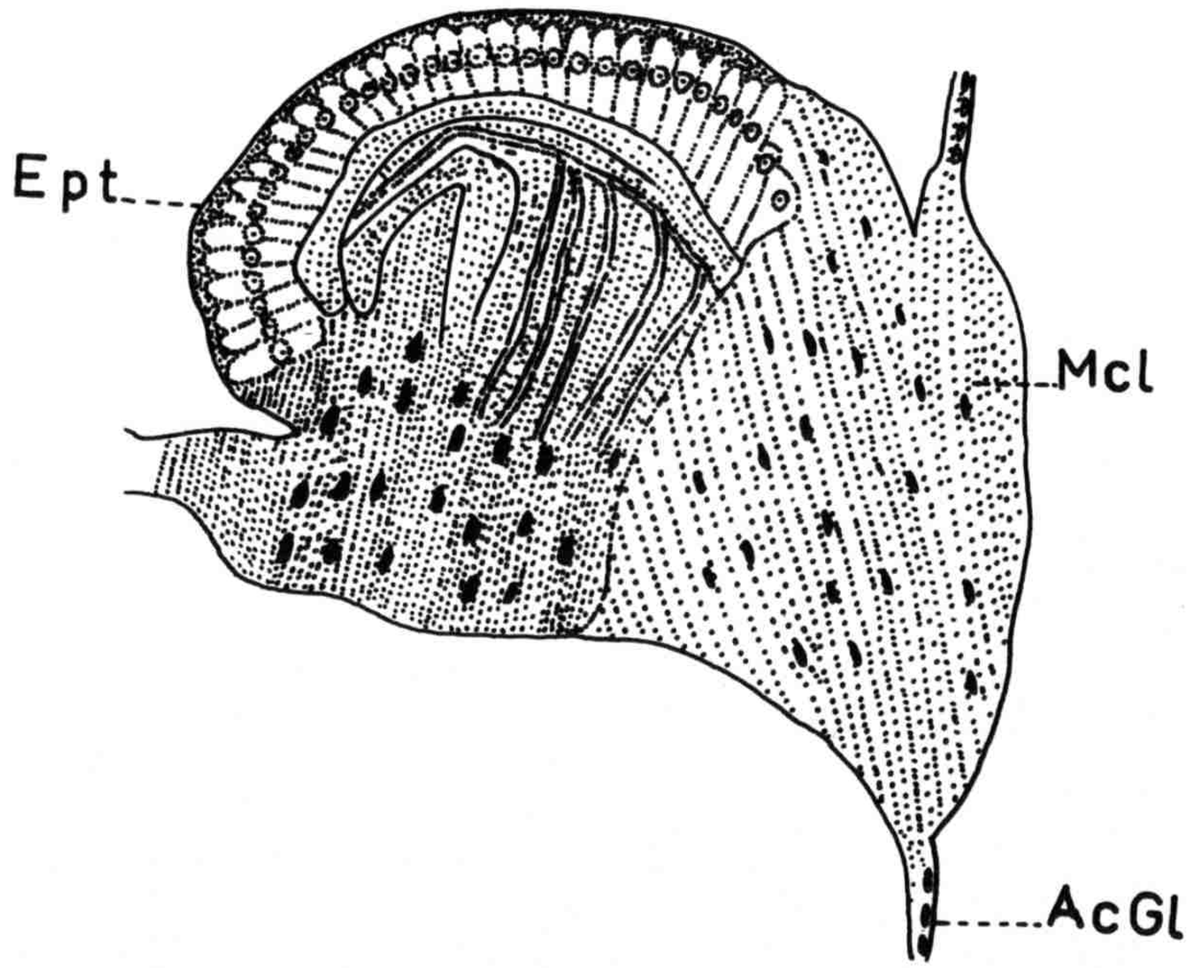

FIG.13

Fig. 14. A portion of the cross section of male
accessory gland. Highly magnified.

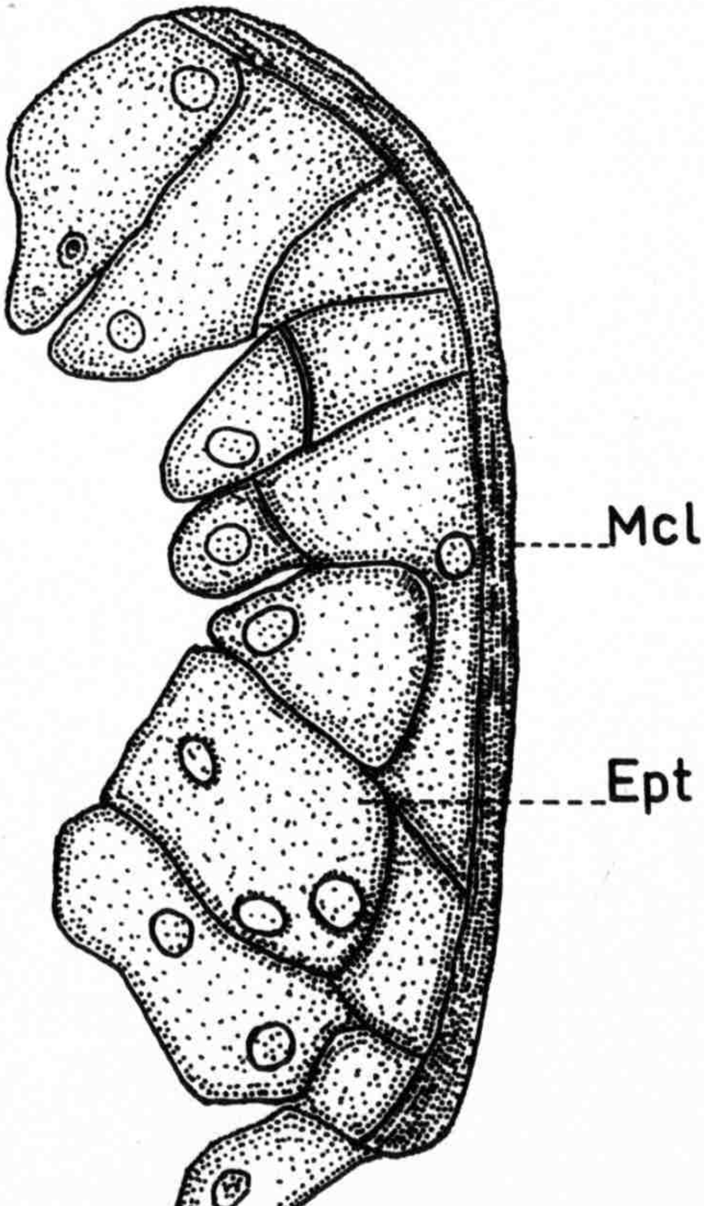

FIG.14