Postembryonic development of the ovaries of Oncopeltus fasciatus (Dallas)

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UMI®
POSTEMBYRONIC DEVELOPMENT OF THE OVARIES
OF ONGOPELTUS FASCIATUS (DALLAS)

by

James R. Wick

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Entomology

Approved:

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In Charge of Major Work

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Dean of Graduate College

Iowa State College

1954
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T11168
The present study has demonstrated certain parameters of the hypothalamus.

In order to describe some of the functional neural connections found in the hypothalamus, certain aspects of the hypothalamic organ have been studied.

Koeller (1970), and Wastek, (1969) have described the hypothalamus in the literature.


There are several important papers dealing with the hypothalamus, which need extensive coverage in the literature. The sequence of events leading to the production of the hypothalamus and trophic tissue, and second, the origin of the hypothalamus, are topics of much interest for many years.

The origin of the various structures of the hypothalamus has been a

INTRODUCTION
MATERIAL AND METHODS

Stock cultures of the milkweed bug were maintained in the laboratory in crooks where they were fed dried milkweed seeds and were provided with water. Cellucotton was placed in each container as a site for oviposition. Reproductive activity in the milkweed bug is continuous throughout the year under laboratory conditions.

The milkweed bug passes through five nymphal instars before becoming adult. In order that the chronological development of the ovarian tissues could be followed it was necessary that insects of known age be used for histological preparation. Slides were prepared from ovaries at closely spaced intervals during the nymphal and adult stages of the life cycle.

Ovaries used in this study were fixed in alcoholic Bouin's, Dietrich's and Carnoy's fixatives. Bouin's fluid proved to be the most successful although Carnoy's and Dietrich's solution provided good fixation for adult ovaries.

Due to the small size of the ovaries, it was impossible to dissect these organs in the first through third nymphal stadia. These nymphs were fixed in toto by placing them directly into the fixative; penetration of the tissues by the fixative was aided by the use of a vacuum chamber. After fixation the nymphs were dehydrated in alcohol, cleared with xylol and embedded in tissuemat (500 - 560). The blocks were trimmed and the nymphs were separated. Each nymph was trimmed so only the posterior part of the thorax and anterior segments of the abdomen remained (these parts
contain the ovaries of the early nymphs). These thoracic and abdominal segments were re-embedded in tissuemat after a short period of infiltration. Retention of only the essential parts of the nymphs for sectioning improved infiltration, reduced the amount of tissue surrounding the ovaries so they might be more easily located in the serial sections, and made sectioning easier due to the removal of many of the cuticular parts.

In the fourth and fifth instar nymphs and the adults the ovaries were exposed by vivisection before fixation. The insects were immobilized for vivisection by partially embedding them, ventral side down, in paraffin-lined Syracuse watch glasses. The insects were covered with physiological saline and the wings and tergites of the thorax and the tergites of the abdomen were removed with scissors and jeweler's forceps. Under a binocular microscope, the fat body and the alimentary canal were removed to expose the ovaries. The saline was poured off and the fixative was added. After fixation the preparations were rinsed with alcohol, which was equal in concentration to the alcoholic content of the particular fixative that was used. Dissection of the ovaries was completed under the microscope and the ovaries were excised and placed in depressions of color reaction spot plates. It was found that staining with acid fuchsin aided handling and orientation of the ovaries during the paraffin embedding process. This stain was removed during the hydration process (leading to the final staining process) by dissolving a small amount of potassium acetate in the 70 per cent alcohol.

Due to the small size and delicacy of the ovaries, it was important to avoid handling them as much as possible. After the ovaries were excised
were not part of the study sample. Strips stained with hematoxylin and eosin were used for the examination of the tropoelastic behavior. Sections were also stained with hematoxylin and eosin for the examination of the least need for detailed histological description.

Hematoxylin was the most useful for detailed histological description. Although the least need for detailed histological description,

The information given here were used to confirm the histological details.

The ribbons were fixed to sticks with the help of a gum.

After examining the blocks, sections were cut at 3, 5, and 10/μm thickness. After this, the hour to be sure that all the samples had been extracted from the open temperatures. The primary pattern was changed three or four times.

This was found that if the temperature were allowed to remain in a mixture.

In the above case, the purest water used on alcohol lamp

A hydrophilic matrix and the hydrophobic pattern were changed with a matrix of embedding agents that were transferred and placed in the spot plate depressions they were not handled until the

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OBSERVATIONS

The ovary of the milkweed bug consists of seven ovarioles. Since the ovarioles are structurally similar and their development is parallel, only one ovariole from each ovary (at each stage of development) is described. Unless otherwise indicated, all length measurements are of the entire ovariole including the terminal filament (anterior strands of the younger ovarioles) and the pedicel (posterior strands of the younger ovarioles). Width measurements were taken at the widest region of each ovariole.

First Nymphal Stadium

The ovariole of the first instar nymph (Figs. 1 and 1b) is a spindle-shaped structure averaging 100\(\mu\) in length and 25\(\mu\) in width. The ovariole consists of four tissues. Three of these, the anterior ovarian strand, the posterior ovarian strand, and the primary epithelial sheath are of mesodermal origin. The fourth tissue consists of the oogonia; these cells are set aside as primordial germ cells early in embryonic development and become surrounded by the mesodermal tissues before the embryonic period ends.

The anterior strand of the ovariole extends from the oogonia to the point where it joins the corresponding strands of the other six ovarioles to form the suspensory ligament of the ovary. In histological preparations, the cells of the anterior strand do not have visible cell
boundaries.* Anteriorly the strand is narrow and the nuclei are arranged in a single row. At its base the strand is expanded and two nuclei are arranged across its diameter. Each ovate nucleus contains a prominent nucleolus and coarse chromatin granules. No mitotic activity was seen in this tissue during the first stadium.

The posterior ovarian strand is widest near the germ cells. This region forms a cup-like depression in which the end of the germ cell mass lies. The nuclei of the broad end of the posterior strand are somewhat flattened. The cytological details of the posterior strand of the ovariole are similar to those of the anterior strand.

The primary epithelial sheath is a single layer of cells surrounding the germ cells and extending onto the anterior and posterior ovarian strands. In longitudinal sections of the ovariole the cells appear spindle-shaped with boundaries evident in most cases. Mitotic figures are occasionally encountered but most cells are in the resting stage. Each large, elliptical nucleus contains a nucleolus and coarse chromatin bodies.

In longitudinal sections of the ovarioles, the oogonia occupy an oval area which is surrounded by the mesoderm-derived tissues. These cells have distinct boundaries and are loosely arranged in the area they occupy. The nuclei are relatively large and various stages of mitosis can be seen.

*Although cell boundaries are not apparent in this tissue, the fact that these boundaries become distinct during mitotic activity indicates that cellular integrity is maintained even when the nuclei are in the resting condition.
The octaole have increased in number by cell division.

The primary epithelium sheath have increased.

The interior walls of the penultimate layer of the posterior strand.

These mucous are arranged in the interior of the penultimate layer of the posterior strand.

The number of cells in the interior of the posterior strand has increased.

The average size of an octaole by the middle of the strand is.

In the second nymphaeid stadia the ovarioles increase in length and width.

This is the change in the anterior strand of the ovariole except an average 120 to length and 20 width by the middle of the third stadia (p. 2 and 15) of the ovariole.

**Third Nymphaeid Stadia**

**Jawrise**

1. **Mucocystic nodule can be seen in the cells of both the third nymphaeid stadia.**

2. **This condition is similar to that found in the normal and overlap the exterior of the penultimate layer of the ovariole.**

3. The posterior cells of the penultimate epithelial sheath have increased in number.

4. The ovariole forms throughout the length of the posterior strand.

5. These mucous are arranged in the interior of the penultimate layer of the posterior strand. **The average size of an octaole by the middle of the strand is**.

6. The number of cells in the interior of the posterior strand has increased.

7. The ovariole forms throughout the length of the posterior strand.

8. The average size of an octaole by the middle of the strand is.

9. The ovariole forms throughout the length of the posterior strand.

**Second Nymphaeid Stadia**
the other hand there is considerable change in the posterior ovarian
strand. The cells of the posterior strand have become organized into the
prefollicular primordium and the pedicel region. The prefollicular pri-
modium is derived from the anterior end of the posterior ovarian strand;
the pedicel is derived from the posterior part of the strand. The pedicel
can be recognized by the arrangement of the cells which form a short tube;
the prefollicular primordium is solid tissue.* Mitotic activity is
conspicuous in both regions of the posterior ovarian strand.

The number of oogonia has further increased due to cell division.

As a result of growth and mitosis in the epithelial sheath this
enveloping tissue has kept pace with the increase in volume of the other
areas of the ovariole.

Fourth Nymphal Stadium

The ovariole of the nymph increases in size during the first half
of the fourth stadium (Fig. 3) until it averages 250µ in length and 50µ
in width by the middle of the stadium. Besides increase in volume, as a
result of mitotic activity and growth, marked histological changes are
apparent at this time.

The anterior ovarian strand has differentiated into a basal body and
a vesicle. The basal body is a syncytium and no mitotic activity has
been observed in this tissue during this or subsequent instars. The
basal body is separated from the germ cell region of the ovariole by a

*See footnote on page 6.
In a later part of the paper, the steps in the detachment of the germ cells (especially the prooceptoid process) are discussed. The steps in the detachment of the germ cells have been separated from the germ cell region of the germ mass, the detachment of the germ mass within the prooceptoid process. The prooceptoid process is a spherical mass of cells. In most instances the prooceptoid process is formed in a stratum of loose connective tissue, in the volume of the prooceptoid process.

According to some recent observations, the prooceptoid process is not a fluid layer. The prooceptoid process is formed in the middle of the germ mass. The prooceptoid process is formed from the mural portion of the germ mass. The mural portion of the germ mass, the transverse section, is the greatest part of the wall.
The primary ooocytes can be distinguished from oogonia at the posterior part of the gonad region have differentiated into primary

The number of oocytes continues to increase during the fourth

Boundaries and appear separated from each other.

The upper ovotestes extend from the outer end of the peduncle to the base of the body. The third and the inner ovotestes extend from the posterior end of the peduncle to the base of the body and are separated from the anterior ovotestes by a thick layer of connective tissue. These inner ovotestes are surrounded by a layer of the inner ovotestes and are separated from the inner ovotestes by a thick layer of connective tissue. The inner ovotestes extend from the apex of the ovary to the base of the peduncle. The outer ovotestes extend from the apex of the ovary to the base of the peduncle. The outer ovotestes extend from the apex of the ovary to the base of the peduncle. The inner ovotestes extend from the apex of the ovary to the base of the peduncle.

The two layers of the outer ovotestes are very abundant.

The junction of the peduncle is separated from the deep-seated germ cells.

10
early period by their characteristic prophase condition; the primary oocytes remain in prophase until after they pass out of the follicles of the adult ovariole.

Fifth Nymphal Stadium

The fifth nymphal stadium (Fig. 4) is of considerable duration (about ten days). In the latter part of the stadium the condition of the ovariole is very similar to that found in the early adult; for this reason the following description of the fifth instar ovariole applies only to the early part of the stadium. The description presented later for the young adult will also be applicable to the late fifth instar nymph.

The ovariole of the fifth instar nymph averages 525 µ in length and 90 µ in width in the early part of the fifth stadium. There are no significant changes in the histological organization of the basal body and vesicle except an increase in size and an increase in the number of cells in the vesicle due to growth and mitosis.

The prefollicular tissue increases in volume as a result of growth and the resumption of mitosis; mitotic activity continues at a rapid rate in this tissue during the fifth stadium. A mass of displaced germ cells, like those of the fourth stadium, can be seen in the prefollicular tissue in most of the histological preparations.

The pedicel increases considerably in length due to growth and abundant mitotic activity. The transverse epithelial layer separating the displaced germ cells from the lumen of the pedicel in the fourth stadium is lacking in the early fifth instar ovariole. The absence of this transverse epithelium allows the ball of displaced germ cells in
the prefollicular tissue to lie against the anterior end of the pedicel. In some preparations germ cells can be seen in the lumen of the pedicel; these are discharged oocytes (Fig. 1).

The histological organization of the outer epithelial sheath and inner envelope is similar to that described in connection with the fourth stadium.

The region of the ovariole occupied by oogonia in earlier instars has proliferated and differentiated into trophocytes and oocytes in the fifth instar. Differentiation of the trophic tissue and oocytes from the oogonia is first apparent in the late fourth instar, however it is not until the fifth stadium that three zones of trophic tissue and an extensive accumulation of oocytes can be seen. The upper portion of the trophic area is occupied by cells which have distinct boundaries. All stages of mitotic activity are present; the resting cells have a distinct nucleolus and many fine chromatin granules. These cells are Zone I trophocytes. At the posterior margin of Zone I is a narrow band of mitotically arrested cells containing coarse chromatin granules. These cells are known as "arrested cells" (Bonhag and Wick, 1953). Posterior to the "arrested cells" the nuclei move together to form small clusters and all indication of cell boundaries disappears, thus forming a true syncytium. In the middle of the syncytium is an area of cytoplasm, the trophic core. The nuclei of those clusters immediately surrounding the trophic core are largest due to nuclear fusion and are similar to those of Zone III trophic tissue of the adult. The anterior and lateral margins of the syncytium are occupied by aggregates of smaller nuclei and are like those of the Zone II trophic tissue of the adult (Bonhag and Wick, 1953). The definite stratification of trophic tissues into Zone II and Zone III
in the fifth instar is not readily apparent but this arrangement becomes quite clear in the adult.

Following the trophic tissue is the extensive area containing numerous primary ooocytes. The ooocytes are spherical in shape; they have very distinct cell boundaries and are in a characteristic prophase condition. As was noted earlier the ooocytes remain in prophase until after leaving the ovary.

Young Adult

The ovariole of the early adult (first four days) averages two millimeters (2,000\(^\times\)) in length and 150\(^\times\) in width (Fig. 5). Besides this increase in over-all dimensions of the ovariole as compared to the early fifth instar, there is a considerable change in the ratio of volumes of the different tissues (compare Figs. 4 and 5).

The basal body and vesicle of the anterior strand, seen in the early fifth nymphal instar, are differentiated into the terminal filament in the young adult. Actually this differentiation takes place in the latter part of the fifth nymphal stadium: The cavity of the vesicle is obliterated when the walls of the vesicle unite to form a syncytium and this syncytium becomes confluent with the cytoplasm of the basal body. Mitotic activity is absent in the fully differentiated terminal filament.

The pedicel in the early adult is equal in length to the rest of the ovariole (not including the terminal filament). The cells at the anterior end of the pedicel have reorganized into a transverse epithelium which separates the lower part of the prefollicular tissue from the lumen of the pedicel. Cell boundaries can be observed in histological preparations.
of the pedicel epithelium. The epithelial cells of the pedicel wall are columnar and uniform in height; the nuclei are located near the basal ends of the cells. Only an occasional mitotic figure can be found in this tissue in the early adult ovariole.

The nuclei of the outer epithelial sheath of the early adult are numerous and are arranged for the most part in three rows. Mitotic activity in the outer sheath of the early adult is rare. The inner envelope has increased in length in proportion to the increase in size of the ovariole, however it remains as a single layer of separate cells enclosed between two membranous lamellae. An occasional mitotic cell can be observed in the inner envelope of the early adult.

The anterior lanceolate region of the adult ovariole is known as the germarium; it contains the apical trophic tissue, the young oocytes, and the anterior portion of the prefollicular tissue.

The three zones of apical trophic tissue are stratified and distinct.

The cytological features of the Zone I trophocytes are similar to those described in the fifth nymphal instar. Mitotic activity continues at a high rate and the "arrested cells" are a persistent feature of Zone I. Of course the "arrested cells" are constantly replaced as they differentiate into Zone II trophic tissue.

Zone II is more clearly defined than in the early fifth nymphal instar. The Zone II trophic tissue is a syncytium lying anterior to the trophic core and posterior to the "arrested cells" of Zone I.

Zone III is the largest zone of the trophic tissue. As was described earlier, the nuclei of Zone III are larger than those of the previous
results in a tubular mass of prefollicular tissue that terminates in a bulbous enlargement. The bulbous end of the prefollicular tissue rests against the anterior end of the pedicle. This position was formerly occupied by the displaced germ cells of earlier instars. After the displaced germ cells were discharged in the fifth instar, this position was filled by the rapidly proliferating prefollicular tissue.

Seven Day Old Adult

The ovariole of the seven-day-old adult (Figs. 8, 16 and 17) varies in length from four to five millimeters (not including the terminal filament). The marked increase in the length of the mature ovariole as compared to the young adult is due to the development of the vitellarium. The vitellarium is the portion of the ovariole which follows the germarium and contains a series of oocytes arranged in a single row. The enlargement of the oocytes distends the vitellarium into a series of follicles which become progressively larger toward the posterior end of the tube (Figs. 16 and 17).

The follicular epithelium forms the wall of the vitellarium and all stages of differentiation of this trophic tissue can be seen in a single ovariole (Figs. 9-13). The prefollicular tissue is found in the posterior part of the germarium of all adults (Fig. 16), and also in the undeveloped vitellarium of the one-day-old adults (Fig. 5). Generally, prefollicular tissue is devoid of distinct cell boundaries, although a semblance of cell limits is obtained when the tissue shrinks due to certain types of histological treatment. However, as the prefollicular "cells" orient themselves about the enlarging oocytes, cellular boundaries become quite clear. This
indicates that cellular integrity is maintained in prefollicular tissue even though the cell boundaries are not optically apparent. The prefollicular tissue contains many small nuclei; each nucleus has a central nucleolus surrounded by coarse chromatin granules. Mitotic figures are occasionally found in the prefollicular tissue of seven-day-old adults, but these are more common in one-day-old adults. As each of the oocytes of the germarium enlarges and is added to the vitellarium, prefollicular "cells" become arranged around the oocytes in the form of an epithelial layer.

The nutritive cords are the cytoplasmic processes through which nutritive contributions from the apical trophic tissue pass to the growing oocytes. As new follicles are added to the vitellarium the older follicles are pushed farther from the germarium. The nutritive cords of the older follicles persist and increase in length; these cords are surrounded by the follicular epithelium and interfollicular tissue of the younger (more anterior) follicles (Figs. 16 and 19). The nutritive cords attached to the older follicles become very tenuous and are disrupted before the formation of the chorion.

In the most anterior (youngest) follicle, the relatively small oocyte is surrounded by several layers of small columnar cells which are obviously formed by enlargement and differentiation of prefollicular cells. The nuclei and nucleoli of these cells show a marked increase in size over those of the prefollicular cells. In older follicles, the columnar epithelium becomes one-layered and is characterized by a further increase in the size of the cells, the nucleus, and the nucleolus; each nucleus contains numerous, large chromatin bodies and a large, irregular nucleolus which
stains orange with Mallory's connective tissue stain (Fig. 10). Later the nucleolus divides to form two parts within a single nucleus (Fig. 11). This stage is followed by an apparent amitotic division of the nucleus to form a binucleate cell (Fig. 12). The division of the nucleus into two parts is accompanied by a change in form of the follicular cell so that the columnar type ultimately transforms into a rounded, binucleate follicular cell (Fig. 13). The final binucleate stage of the follicular cells is characteristic of the posterior follicles, but individual cells of this type may be found in the epithelium of follicles that are made up predominantly of columnar cells. Evidence for and against the amitotic explanation of the ultimate binucleate condition of the follicular epithelium has been presented in detail by Bonhag and Wick (1953).

As the prefollicular tissue of the egg tube becomes arranged about the developing oocytes to form the follicular epithelium, groups of prefollicular "cells" become trapped between the successive follicles; these are the interfollicular plugs (Fig. 18). The tissue of the interfollicular plugs does not differentiate further but always retains prefollicular characteristics; it may become compacted, however, resulting in a flattening of the nuclei. Occasionally a few mitotic figures are found in the interfollicular plugs; this is characteristic of prefollicular tissue.

The term "epithelial plug" has been applied by many authors to the mass of cells that closes off the posterior end of the vitellarium. The first epithelial plug is formed from the prefollicular tissue that intervenes between the first follicle and the anterior end of the pedicel. At the time of ovulation, the epithelial plug breaks down and the egg in the last follicle enters the pedicel. When this is accomplished, the follicle
anterior to the ruptured follicle becomes the terminal follicle and the last interfollicular plug assumes the function and position of the epithelial plug.

Ovulation is not common in the seven-day-old adults, but in some of the more precocious individuals the egg in the last follicle may have been passed. After the egg has left its follicle, the walls of the follicle collapse, producing a conspicuous structure which is generally called the "corpus luteum". This term is unfortunate because the collapsed follicle does not show a progressive development after ovulation; neither is it known to have an endocrine function as is the case in mammals. Figure 20 shows two corpora lutea soon after ovulation. The shrunken follicles contain the disorganised follicular epithelium with the cells in various stages of disintegration. The nucleoli are recognisable as orange granules in sections stained with Mallory's connective tissue stain. In the center of the corpus luteum is a mass of darkly staining particles of unknown origin. The epithelial plug has almost completely disintegrated, but the lumen of the ruptured follicle has again been cut off from the pedicle by a temporary reorganization of the transverse epithelium of the pedicle. Later the contents of the corpus luteum are discharged and the old follicle shrinks to a very inconspicuous size.

The epithelium at the upper end of the pedicle in the seven-day-old ovariole is made up of columnar cells of various heights giving an irregular appearance to the pedicle wall in longitudinal sections. The transverse epithelium described in the earlier adult is present until the first oocyte passes into the pedicle.
The outer epithelial sheath is quite thin as compared to the early adult. In the region of the ovariole where the follicles are enlarged, the inner and outer lamellae of the inner envelope are appressed and the enclosed cells are displaced; these cells come to lie in the constrictions between the follicles (Figs. 18 and 19).
second, the number of cells present at the time of harvest is determined. By
comparing the number of cells present at the time of harvest and the number
of cells present at the time of inoculation, the level of cell proliferation can be
assessed.

At the time of inoculation, the yeast cells are undercoordinated. A study of these
yeast cells at the time of inoculation has been reported by Watanabe et al. (1979)
and by Watanabe and Hara (1980). These studies have shown that the yeast
cells at the time of inoculation have not undergone development. The yeast
cells are in a larval stage. The results of these studies indicate that in
although the coordination hypothesis of the yeast cells of Oomycota was

coordinated and synchronized

that development may also be suppressed.

The studies of Watanabe et al. (1979) and Watanabe and Hara (1980) have shown
that the yeast cells at the time of inoculation have not undergone development. The
yeast cells at the same time of inoculation have been found in the data from the
developmental stage of each organism. In this study, therefore, we have followed the
in vitro procedure for the development of a yeast organism. At the time of
inoculation, the yeast cells were undercoordinated. In the previous section the
phenomena of the coordination of the yeast

DISCUSSION
considerably but further differentiation is not apparent. In the latter part of the fourth stadium a few oogonia undergo critical division resulting in oocytes and trophocytes. The trophocytes produced are relatively undifferentiated cells capable of continued mitotic division and may be considered comparable to the Zone I trophocytes of older ovarioles.

Differentiation of the trophocytes results in three zones of trophic tissue. This zonation begins at the end of the fourth instar when a few trophocytes near the center of the germarium lose their cell boundaries and the nuclei move together to form small clusters. Early in the fifth instar three zones of trophic tissue can be recognised, and in the adult ovariole these three zones become clearly stratified. The trophocytes remaining behind in Zone I continue mitotic activity; this zone may be considered the proliferative zone of trophic tissue. Zone III is occupied by fully differentiated, syncytial trophic tissue. Zone II may be described as the transitional zone of trophic tissue differentiation. As Zone III trophic tissue is utilized by the developing oocytes the volume of Zone III is maintained in dynamic equilibrium by the activity in Zones I and II.

The oocytes produced from the oogonia remain in the prophase condition. Thus we find in the early fifth nymphal instar an extensive accumulation of oocytes at the posterior end of the gonial region; these are readily distinguishable from the apical trophic tissue by their prophase condition.

Not all of the oocytes produced in the fourth and fifth nymphal instars are retained; some of these are displaced into the prhofollicular region. The various stages in displacement of these germ cells are illustrated in Figure 6: first oocytes at the posterior end of the gonial region project
into the prefollicular tissue (Fig. 6A); later, as more oocytes are added to the projection, the latter may attain the anterior end of the pedicel (Fig. 6B). Oocytes accumulate at the anterior end of the pedicel leaving a narrow bridge of oocytes united with the main gonial region. Later this connection disappears and the prefollicular tissue separates the displaced germ cells from the main mass of oocytes (Fig. 6C). In the fourth nymphal instar the displaced germ cells are separated from the lumen of the pedicel by a transverse layer of pedicel epithelium. The transverse layer is not present in the early fifth nymphal instar and displaced germ cells may be found being discharged into the pedicel (Fig. 4). In the late fifth instar and the adult, the displaced germ cells are no longer present and the area previously occupied by displaced germ cells is filled in with prefollicular tissue. The transverse epithelium at the anterior end of the pedicel is re-established, separating the contents of the ovariole from the lumen of the pedicel. There is a possibility that germ cells may be displaced and discharged more than once from each ovariole. This would help to explain the reason why there are fewer oocytes in the young adult than in the ovariole of the early fifth nymphal instar (cf. Figs. 4 and 5).

It is difficult to understand the purpose of this mechanism for discharging some of the oocytes. The fate of the displaced germ cells has not been determined, but it is assumed that they disintegrate, since serial sections of entire adult female reproductive tracts show that the germ cells do not become established in any part of the tract. Certainly they do not serve as gametes since they have not been provided with the necessary food reserves. Considering the origin of these germ cells however, one may speculate that the discharge of some of the oocytes
provides a more favorable ratio of trophocytes to oocytes. It will be remembered that each oogonium produces on one hand a trophic cell and on the other hand an oocyte. Thus if some of the oocytes are lost there may be more trophocytes available to the remaining oocytes. There is another explanation for the large amount of trophic tissue as compared to the number of oocytes in the adult ovariole. The Zone I trophocytes continually produce, by mitosis, more trophic tissue. Nevertheless the loss of surplus oocytes may constitute an accessory mechanism for providing a tremendous amount of apical nurse tissue for a relatively small number of oocytes.

In the young adult some of the oocytes increase in size and extend beneath the main mass of oocytes into the prefollicular tissue (Fig. 5). These larger oocytes are connected to the trophic region by the nutritive cords. In the older adults the oocytes become arranged in a single row and are surrounded by follicular epithelium. The prophase condition is retained as long as the oocytes are in the ovariole. Bonhag and Wick (1953) suggested that the larger size and the attachments with the trophic region by the nutritive cords were criteria for recognizing oocytes and believed that the smaller germ cells directly beneath the trophic tissue were oogonia. The present study reveals that all of the germ cells in the adult ovariole are actually oocytes and the increase in size and the attachments of the nutritive cords are only stages in their development.*

As is mentioned earlier in this paper the germ cells become recognizable as oocytes in the fourth instar following the critical division of the oogonia.

*Thus in Figures 53 and 62 (Bonhag and Wick, 1953), the germ cells labelled oogonia are actually oocytes.
It is interesting to note that the distribution of germ cells in the adult ovariole of *Oncopeltus* differs from that reported by Payne (1912) for the adult ovarioles of *Gelastocoris oculatus* Fabr. In young adults of *Oncopeltus*, the oocytes are concentrated posterior to the apical trophic tissue in the form of an irregular disc. As the oocytes grow and are added to the vitellarium they are surrounded by follicular epithelium and eventually are enclosed in a series of follicles in the vitellarium. Payne, on the other hand, finds in *Gelastocoris* that oogonia occur in the apex of the germarium and that oocytes mingle with the trophic tissue, then pass posteriorly into the vitellarium. As a result of these observations, Payne does not find a distinct stratification of nurse tissue and oocytes and concludes that the oocytes in the adult ovariole of *Gelastocoris* "undoubtedly arise by the growth of the small oogonial nuclei at the apex (of the germarium) and hence in this form there is no break in the continuity of the cells from the oogonial stage to the fully developed ova" (Payne, 1912, p. 345). It is also true in *Oncopeltus* that there is a continuity of development of the germ cells from oogonia to oocytes, but the oogonia produce the apical trophic tissue and the oocytes earlier in the postembryonic development of the ovariole. Thus in the adult ovariole the mitotic cells present at the anterior end of the germarium (Zone I trophocytes) produce trophic tissue and do not give rise to oocytes. Furthermore in the adult ovariole of *Oncopeltus* germ cells have never been found migrating through the apical nurse tissue to the posterior end of the germarium. In *Oncopeltus* as was noted earlier the oogonia produce Zone I nurse tissue and oocytes in the fourth and fifth nymphal
stadies; this process is completed before the adult stage is attained.

Troedsson (1944), in the course of her investigation of the behavior of the compound sex chromosomes in females of *Gelastocoris* and several representatives of the family Reduviidae, accepted Payne's conclusions about the distribution of germ cells in the adult ovariole of *Gelastocoris*. As the result of her investigation she made the generalization (Troedsson, 1944, p. 110) that "in the Heteropteran ovary it is possible to trace and seriate the stages of oogenesis by starting at the anterior end of the ovariole and proceeding posteriorly". The present investigation of *Oncopeltus* has demonstrated that her generalization cannot be applied to all adult ovarioles of the Heteroptera.

**Outer Epithelial Sheath and Inner Envelope**

The primary epithelial sheath increases in volume and number of cells by growth and mitotic activity during the first nymphal stadium. In the second instar the accumulated spindle-shaped cells overlap each other to the extent that the sheath approaches a two-layered condition. Multiplication of the cells in the sheath continues in the third nymphal instar and by the end of the third instar this epithelium differentiates into an outer epithelial sheath and an inner envelope of cells; the inner envelope is bounded by a pair of membranes. These tissues become more extensive during the fourth and fifth nymphal instar and keep pace with the increase in dimensions of the rest of the tissues of the ovariole. Although the sheaths retain their identity throughout the life of the adult, the outer epithelial sheath becomes very thin in the area of the
follicles and most of the cells of the inner envelope are displaced into
the constrictions between the follicles.

Pedicel, Epithelial Plugs and Follicular Epithelium

The nuclei of the posterior strand are in the resting condition in
the first instar. During the second nymphal stadium mitotic activity
occurs and the strand increases in size. At the beginning of the third
instar two regions are recognizable in the posterior strand: The anterior
region is the prefollicular primordium and the posterior region is the
pedicel. Cell division is frequent in both regions. The simple epithelium
of the pedicel increases in length throughout the remaining instars until,
in the adult, the pedicel is equal in length to the rest of the ovariole
(not including the terminal filament). Cell division was not observed in
the pedicel of the adult.

In the fourth stadium the prefollicular primordium becomes trans-
formed into prefollicular tissue. The characteristics which distinguish
prefollicular primordium from the prefollicular tissue have been presented
earlier. Although mitotic activity does not occur in the prefollicular
tissue of the fourth nymphal instar a great burst of mitosis takes place
during the fifth nymphal stadium. In the late fifth instar and early
adult a column of prefollicular tissue is produced which terminates
posteriorly in a bulbous enlargement; this enlargement represents the
space previously occupied by displaced germ cells, which has been filled
with prefollicular tissue (Fig. 5).

The steps in differentiation of prefollicular tissue into follicular
epithelium can be seen in the series of follicles which are found around
The walls of the vestibule fuse and form a symmetry that becomes continuous near the middle of the third intersept. During the fourth intersept, the caudal part of the caudal end forms the base and vestibule expands throughout the fourth intersept and during the fifth intersept.

The dorsal portion forms the floor of the vestibule as it forms the base and intersept at the second intersept. During the second and third intersept the intersept expands towards the anterior end of the third intersept.

Final activity is not present in the anterior vestibule strand during the fourth intersept.

Terminally Proliferate

during period of the adult life.

active and there is to the prostatic and prostatic epithelium throughout the posterior end of the posterior section of the mammary gland.

The epithelial phase are objects that are interconnected into the peduncle. The are.

becomes internal prostatic tissue. The internal prostatic tissue forms later.

The prostatic tissue is divided between successive zones.

when proceeds the first zone of the zone adult (range adult) terminating the pre-

not all of the prostatic tissue forms a zone of proliferation.

seven- to old adult.

prostatic proliferation development can be seen in a single cell. At the ends of the first one of the first proliferation is binucleate. All of these proliferate prostatic tissue becomes binucleate and in the oldest prostatic proliferation is surrounded by common wall.

* The youngest coelocytes are surrounded by common wall (Plate 9-17)
with the cytoplasm of the basal body. The resulting syncytium is the fully differentiated terminal filament that persists throughout the remainder of the life of the insect.

The significance of the intermediate steps in the formation of the terminal filament from the anterior ovarian strand is unknown. However some light may be shed on the problem by comparing the development of the anterior ovarian strand with the posterior ovarian strand during the first through the fourth nymphal stadia. In the first instar, the anterior and posterior strands are very similar in appearance even in regard to cytological details (Fig. 1). Although the lines of differentiation of the anterior and posterior ovarian strands diverge from each other in subsequent nymphal stadia, there is a general parallelism in their development (Figs. 3 and 4). Thus in the fourth nymphal instar, the solid mass of tissue (basal body) at the basal end of the anterior ovarian strand may be comparable to the solid mass of tissue (prefollicular tissue) at the basal end of the posterior strand; similarly, the vesicle may be comparable to the pedicel. Possibly the anterior and posterior ovarian strands retain this vague parallelism of development as the result of an earlier influence, perhaps present at the time of hatching. Thus on one hand, the hollowing out of the posterior strand eventually produces a functional pedicel while, on the other hand, the distal end of the ovarian strand forms the hollow vesicle that has no apparent function and which is only a transitory structure in the formation of the terminal filament.
Summary
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PIATE I

Fig. 1. Longitudinal section of ovariole of first instar nymph at time of hatching.

Fig. 2. Longitudinal section of ovariole of third instar nymph showing histological appearance at middle of stadium.
ANTERIOR OVARIAN STRAND

PRIMARY EPITHELIAL SHEATH

DOGONIA

PREFOLLICULAR PRIMORDIUM

POSTERIOR OVARIAN STRAND

PEDICEL
PICTURE II

Fig. 3. Longitudinal section of ovariole of fourth instar nymph showing histological appearance at middle of stadium.

Fig. 4. Longitudinal section of ovariole of fifth instar nymph showing histological appearance at beginning of stadium.
PICTURE III

Fig. 5. Longitudinal section of ovariole of young adult; only a small part of the pedicel and terminal filament is shown.

Fig. 6. Steps in the displacement of germ cells in the fourth nymphal instar.

A. Projection of germ cells into prefollicular tissue.

B. Constriction of germ cells from main germ cell mass.

C. Displaced germ cells.
Fig. 7. Histogenetic chart of the ovarian tissues.
PLATE V

Fig. 8. Sagittal section of the base of the terminal filament and the apex of the germarium of a seven-day-old adult.

Fig. 9. Prefollicular tissue.

Fig. 10. Columnar mononucleate cell from follicular epithelium.

Fig. 11. Columnar mononucleate cell with two nucleoli.

Fig. 12. Binucleate follicular cell soon after division of nucleus.

Fig. 13. Fully differentiated binucleate follicular cell.
Fig. 14. Longitudinal section of first instar nymph shortly after hatching (cf. Fig. 1); 3/4, oil immersion X1300.
PLATE VII

Fig. 15. Longitudinal section of ovary of third instar nymph showing four ovarioles (cf., Fig. 2); 3/4, oil immersion X900.
PLATE VIII

Fig. 16. Longitudinal section of anterior end of ovariole of seven-day-old adult; 6/₁₀₀, X100. FE, follicular epithelium; NC, nutritive cord; O, oocyte; PT, prefollicular tissue (cf. Fig. 5).

Fig. 17. Longitudinal section of posterior end of ovariole of seven-day-old adult; 6/₁₀₀, X100. F, follicle; P, pedicel.
Fig. 18. Longitudinal section of follicles of ovariola showing interfollicular tissue; $8^\circ$, $X 50$.

Fig. 19. Similar to Figure 18, but showing passage of nutritive cord through interfollicular tissue.
PLATE X

Fig. 20. Longitudinal sections of ovarioles showing "corpora lutea"; 8/, x160.
PLATE XI

Fig. 21. Section of ovarirole showing final step in the displacement of germ cells; oil immersion X900.