# SPERMATOGENESIS AND OOGENESIS IN *MUSCA DOMESTICA* L.

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Allen L. French

1964

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## ABSTRACT

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Development of the testes and ovaries of the house fly, *Musca domestica* L., were studied from the third instar larva to six day old adults. Whole larvae, pupae and adult abdomens were fixed, dehydrated and embedded by a tetrahydrofuran--parlodion double embedding technique. Ten micron sections were stained with Feulgen--fast green and Pyronin Y--methyl green methods. The specificity of the stains were verified by RNAase and DNAase controls. Feulgen whole mounts of testes and ovaries were prepared from fixed tissues. Sperm motility was confirmed by phase microscopy.

It was found that spermatogonial divisions occurred during the third instar larva and early pupal period. Considerable meiotic activity took place during the pupal period, with sperm maturation being essentially completed by emergence. Oogonial divisions were observed during early pupation with the first egg chambers being formed before emergence. Females five days old possessed one mature egg and two immature egg chambers per polytrophic ovariole. Egg chambers were observed to contain fifteen nurse cells, with polyploid nuclei, and one oocyte. The nurse cell nuclei produced large amounts of RNA during vitellogenesis. Meiosis could not be observed during oocyte maturation.

# SPERMATOGENESIS AND OOGENESIS IN *MUSCA DOMESTICA* L.

By

ALLEN L. FRENCH

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1964

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## ACKNOWLEDGEMENTS

The author would like to express his gratitude and appreciation to Dr. R.A. Hoopingarner and Dr. G.B. Wilson for their guidance and counsel throughout the course of this investigation, to the Michigan Department of Agriculture for supplying the initial culture of house flies, and to my fiance for her help and inspiration.

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# INTRODUCTION

The proposal to control insect populations through the use of "chemosterilants" has many possible advantages over conventional insecticides (Smith, LaBrecque, and Borkovec, 1964). Whether the sterility is a result of lethal mutations in the zygote, failure of egg maturation, disruption of the meiotic or mitotic cycles, or failures involving some aspect of the gametogenic process in many cases remain unanswered. Recently many chemosterilants have been applied to the house fly, Musca domestica L., in the field and in the laboratory (Konecky and Mitlin, 1955; Mitlin and Baroody, 1958; LaBrecque, 1961; Kilgore and Painter, 1962; Morgan and LaBreque, 1962; Gouck and LaBrecque, 1963). The criteria used to judge the effects of the tests were egg viability, failure of ovary development (a gross difference in volume between test flies and controls), delayed oviposition, and post dosage lethality in the larva, pupa and adult. The purpose of this investigation was to establish the normal sequence of events involved in gametogenesis in Musca domestica L. in hopes that a more definitive basis could be employed in the future study of "sterilizing" compounds.

Among the criteria used to characterize normal gametogenesis were 1) the relative size of the reproductive units, 2) the stage of development, 3) the normal cytology, and 4) the functional relationships of components at varying ages during maturation from larva to adult.

## REVIEW OF THE LITERATURE

Spermatogenesis. According to Snodgrass (1935), a typical adult testis consists of sperm tubes, containing male germ cells in varying degrees of maturation, eveloped in a mesodermal, peritoneal sheath. In Apterygota and Plecoptera the sperm tubes are free from one another, while in higher insects a more compact structure is found with incomplete separation of the sperm tubes. The number of tubes vary from insect to insect in the higher forms---Diptera having but one sperm tube contained in a simple sac-like organ.

The earliest work investigated for this review on spermatogenesis in Diptera was carried out by Stevens in 1908. Stevens established the 2n chromosome number in Musca domestica L. at twelve. Since that time it has been verified by Metz (1910), Perje (1948) and White (1949). Stevens, using aceto--carmine and a squash technique, investigated chromosomal behavior during meiosis in adult flies. Of noted importance was the observation of somatic pairing in nonreproductive tissue and the absence of the usual prophase configurations found in meiosis, i.e., no observable leptotene, zygotene or tetrad formation. That somatic pairing is a common occurrence in Diptera was established by Metz (1910), as he found this to occur in eighty different Dipteran species. The absence of pachytene

pairing and chiasma formation was noted in the male Drosophila sp. by Darlington (1934). Perje (1948) found leptotene, zygotene, diplotene and diakinesis absent in Musca domestica L., the chromosomes being already paired before the beginning of meiosis.

In the literature there is little reference to spermatogenesis in Musca domestica L. However, spermatogenesis has been well studied in Culicidae. Rishikesh (1959), working with Anopheles stephensi, found the general form of the testes the same in larvae, pupae, and adults. The author describes them as being small, translucent, pale yellowish, ellipsoidal bodies with a tough, darkly pigmented outer coat surrounding an inner squamous epithelium of a syncytial nature. He found the testicular cavities divided into separate cysts, bounded by two layers of squmosal cells produced by a double infolding of the epithelium of the inner testicular wall. The cysts were found to contain spermatogonial cells, and primary and secondary spermatocytes. The transforming spermatids and mature spermatozoa were found to be held freely within the posterior parts of each testicular cavity. He noted the fourth instar larval testes contained dividing spermatogonia and early primary spermatocytes. The pupal period was observed to have germ cells at various stages of meiosis along with spermatids and spermatozoa. The newly emerged adults possessed mainly mature sperm with a few cysts of spermatogonial cells and spermatocytes.

Breland and Rieman (1961), working with Culisita inornata (Williston) , found a similar developmental sequence. They described most of the meiotic activities in this species as occurring near the end of the last larval stage with mature sperm present in pupae.

An extensive survey of spermatogenesis in Culicidae was made on three species of Aedes, two species of Orthopodomyia, as well as Culex pipens quinquefascitus Say, and Culista inornata (Williston), by Warren and Breland (1963). In general thay found that spermatogonial divisions occurred in the late fourth instar larva and sperm maturation was complete by the late pupal stage.

Oogenesis. According to Snodgrass (1935) and Patton (1963), there are two classes of ovarioles, the functional units of the ovary, panoistic and meroistic. The classification is based on the presence or absence of nutritive cells in the ovariole. Lacking nurse (trophic) cells, the panoistic type derives its nutriment from the hemolymph via the absorptive capacity of its follicular epithelium. The meroistic type, with nurse cells, is further divided into two groups: (1) those having the nurse cells alternating with the developing egg (polytrophic), and (2) those with the nurse cells restricted to the germarium of the ovariole; the latter is termed telotrophic or acrotrophic. According to Bonhag (1958), the panoistic ovariole is found in the orders Thysanura, Orthoptera, Isoptera, Odonata, Plecoptera, and Siphonoptera. The telotrophic ovarioles

are found in Hemiptera and many Coleoptera. Most holometabolous insects, plus the orders Dermaptera, Psocoptera, Anopleura and Mallophaga, have the polytrophic ovariole.

King, Rubinson, and Smith (1956) undertook an extensive investigation of oogenesis in Drosophila melanogaster. As this investigation provided a basis for further studies on other Dipteran species, a detailed review of their work is warranted. The adult ovary was found to have an average of twelve ovarioles. Each ovariole was composed of an anterior germarium of mitotically active cells, followed posteriorly by fourteen nurse cell--oocyte complexes or egg chambers. The authors assumed each egg chamber arose from a germarial cyst of sixteen cells, formed by four successive divisions of a common germarial cell. Subsequently, the germarial cyst was surrounded by follicular cells and was pinched off from the germarium, forming the first egg chamber of fifteen nurse cells and one oocyte. Further, they assumed each of the other thirteen egg chambers, or stages of development, was formed in the same manner. In stages two through four, they noted changes in the chromatin of the nurse cell nuclei, from a fine network to clumps. In stages six through nine, the authors reported that the deoxyribonucleic acid content in the nurse cell nuclei had increased. Up to stage seven, the growth rate of the nurse cells was comparable to the oocyte. At stage seven, yolk deposition began, and the oocyte increased in volume at a faster rate than the nurse cells. At stage

nine, the nurse cell complex was partitioned from the oocyte by the follicular epithelium, the oocyte was increased from one-fourth to one-half the volume of the egg chamber, and chorion deposition was begun. By stage ten, the oocyte had increased in volume to occupy one-half to three-fourths of the egg chamber. Stages eleven through fourteen were marked by the loss of the oocyte "nuclear membrane", chorion formation, and degeneration of the nurse cell complex. At stage fourteen, the egg was mature and had increased in volume over 100,000 times. Throughout the course of their investigation, no observable meiotic divisions were noted in the oocyte.

LaChance and Bruns (1963) investigated oogenesis in Cochliomyia hominivorax (Coql.). They found the ovary to consist of mitotically active germaria in pupae five-days-old. In six-day-old pupae, epithelial cells had surrounded the germaria, and the first egg chambers were completely differentiated and consisted of an outer layer of follicular epithelium surrounding fifteen nurse cells and one oocyte. Twenty-four hours following eclosion, nurse cell chromosome replication was completed, and nucleoli were identifiable in the oocytes with phase microscopy. Forty-eight-hour adults had developed second egg chambers. Endomitosis of the nurse cell nuclei in the second egg chambers was seen in seventy-two-hour adults, and the oocytes in the first egg chambers had begun to elongate. The nurse cells of the first egg chambers had degenerated by ninety-eight hours,

and the oocytes were at their maximum size. Adults of 122 hours were reported to have a mature egg ready for oviposition and two developing egg chambers per ovariole. LaChance and Leverich (1962) described the beginnings of meiotic divisions in the oocytes of three-day-old adults. The oocytes were observed to be in first prophase of meiosis. At four days the oocytes were in first metaphase and passed to first anaphase at the fifth day, remaining at this stage until oviposition occurred. Meiosis occurring before oviposition appears to be the only fundamental difference in the oogenetic process between Drosophila melanogaster and Cochliomyia hominivorax (Cqrl.). Sonneblick (1950), using D. melanogaster, found that first and second metaphase occurred subsequent to the time the eggs leave the ovarioles. Fahmy (1952) found freshly deposited eggs to be in first prophase, and in nine to ten minutes the reduction divisions were completed in Drosophila subobscura. It is interesting to note that in the Orthopteran Melanoplus differentialis freshly laid eggs are in metaphase of the first meiotic division (Swift and Klienfeld, 1953).

The only publication found on oogenesis in Musca domestica L. was by Morgan and LaBrecque (1962). Using haematoxylin and eosin and sectioned material, they described the development of the ovary from twenty-four-hour adult flies to seventy-two hours. At twenty-four hours they found each ovary to consist of sixty-two to seventy ovarioles. Each ovariole possessed two egg chambers of fifteen

nurse cells and one oocyte. Chromatin clumps were reported in the nurse cell nuclei of the first egg chambers. Nucleoli were reported in all cells of the first and second egg chambers with "dark clumps" being observed in the oocytes of the first egg chambers. At forty-eight hours the first egg chambers were elongated with about one-half the volume occupied by the oocytes. Chorion formation was completed at seventy-two hours, and they could no longer locate the "oocyte nuclei". At ninety-six hours the oocytes in the first egg chambers were completely developed, and the third egg chambers had formed. During the test period, they reported the average change in volume of an ovary to increase from 0.197 $mm^3$ at twenty-four hours to 3.097 $mm^3$ at ninety-six hours.

Since the original work in oogenesis on Drosophila melanogaster, King and other workers in the field have pursued oogenesis in this species on a more sophisticated level. Much of the work has been concerned with the morphology and function of the nurse cells. King and Devine (1958), with the aid of electron microscopy, observed passage of material through large gaps in the oocyte and nurse cell plasma membranes. This event was noted to occur just prior to and during yolk deposition. This confirmed an earlier observation by Hsu (1952), who noted the emission of granules from nurse cell nucleoli into the cytoplasm of egg chambers, and their subsequent movement into the cytoplasm of the oocytes. Using flies fed for one hour on dead yeast

containing uridine-$H^3$, King and Burnett (1959) and King and Falk (1960) noted tritium appeared first in the nurse cell nuclei. Subsequently it appeared in the nurse cell cytoplasm and ooplasm as well, but at lower concentrations. Using the same technique, but substituting orotic acid-6-$C^{14}$ for uridine and checking the results against RNAase controls, Sirlin and Jacob (1960) observed passage of RNA from the nurse cells into the ooplasm. They proposed that the RNA represented passage of preformed templates that may later be utilized in the ooplasm for synthetic processes. King and Vanoucek (1960) held similar opinions. Hsu (1953) concluded that fatty yolk granules are produced directly by the nurse cells and transported to the developing oocytes. He also maintained the opinion that proteid yolk is synthesized in the ooplasm from precursors produced in the nurse cells. None of the authors observed nucleolar emissions from the "oocyte nuclei". This is contrary to the highly active nucleoli found in the oocytes of panoistic ovarioles as reported by Bonhag (1958). Jacob and Sirlin (1959), using adenine-$C^{14}$ incorporation into DNA, estimated the degree of ploidy produced by the endomitotic processes in the nurse cell nuclei to range from 256 ploidy to 512 ploidy with the larger value being found in nurse cell nuclei closest to the developing oocyte. The authors noted in a later paper (Sirlin and Jacob, 1960), that RNA synthesis in nurse cell nuclei increases with increasing ploidy.

There appears in the literature references to either oocyte nuclei or germinal vesicles. King (1960) is of the opinion that the oocyte chromosomes extend from a Feulgen positive body( karyosome) and that they are contained in a true nuclear boundry consisting of a double membrane. The germinal vesicle viewpoint, as held by Bonhag (1958) and Mulnard (1954), considers the true nuclei of the oocytes to be the Feulgen positive bodies and not the zonation surrounding the karyosomes as reported by King.

## MATERIALS AND METHODS

House flies, Musca domestica L., of the N.A.I.D.M. (National Association of Insecticide and Disinfectant Manufacturers) strain were used in this study of gametogenesis. Freshly oviposited eggs were placed in a yeast fortified larval rearing media consisting of two parts C.S.M.A. (Chemical Specialties Manufacturing Association), one part oat hulls and two parts water. The larvae passed through three instars and pupated just below the surface of the media. The length of the pupal period was approximately five to five and one-half days at 30°C. The adults were reared at 21-22°C. and fed whole milk absorbed in a sponge. The females began oviposition at five to six days of age.

Ageing of pupae was accomplished by isolation of the newly formed pupae (before darkening and hardening began). Since the culture media was dry just below the surface (the site of pupation), darkening of the puparia began within an hour after the onset of pupation. Once the newly formed pupae were isolated, they were incubated in shell vials at 30°C. or transferred to 2000 ml. beakers. In the latter case, these pupae were raised to adults. Six samples were taken every twelve hours starting from the onset of pupation and continuing until oviposition occurred.

Whole larvae and adult abdomens were fixed in methanol,

chloroform, and proprionic acid (six: three: two) for forty-eight hours. The puparia were punctured to allow better penetration of the fixative. Tissues to be sectioned were dehydrated in two changes of tetrahydrofuran for a total of six hours. A modified double embedding technique was used (Salthouse, 1958) of one percent, two percent, and three percent solutions of parlodion in tetrahydrofuran. After a ten minute rinse in tetrahydrofuran, the flies were embedded in 56°C. tissuemat. Embedding time of not more then two hours was used, as longer exposures to the hot tissuemat produced leaching of the parlodion from the tissues. The double embedding procedure prooved to be superior to a single as it enhanced the quality of the sections by producing more rigidity in the tissues.

Sections were cut at ten microns and stained with a Schiff's reagent, according to the procedure in Stain Technology (1951), and counterstained with fast green. The tissues were hydrolized seven minutes at 60°C. with one normal hydrochloric acid, and the Feulgen reaction was carried out for forty-five minutes.

Histochemical tests for both deoxyribonucleic acid and ribonucleic acid were made on sectioned material with Pyronin Y and methyl green (Pearse, 1961). The specificity of Pyronin Y for RNA and methyl green for DNA was verified by treatment of some of the sectioned tissues with RNAase or DNAase (Pearse, 1961) prior to staining. Distilled water controls were made to parallel the times and temper-

atures required for nucleic acid extraction. Ribonucleic acid extraction occurred after treatment for four hours at 40°C. with a concentration of one mg./ml. of RNAase. Deoxyribonucleic acid extraction was accomplished after treating the sections for eighteen hours at room temperature with a concentration of 3,000 Dornase units/ml.

Feulgen whole mounts of testes and ovaries were prepared from fixed tissue. By increasing the hydrolysis time to fifteen minutes, the gonads could be stained *in situ* thereby increasing the ease of identification and removal. After staining, the gonads were dissected in twenty-five percent acetic acid and counterstained with fast green in twenty-five percent acetic acid. The whole mounts were then dehydrated in tertiary butyl alcohol and mounted in diaphane.

Unfixed pupal ovaries and testes were examined by phase contrast. This method did not prove useful for cytological examination of the developing oocytes due to the interference produced by the follicular epithelium.

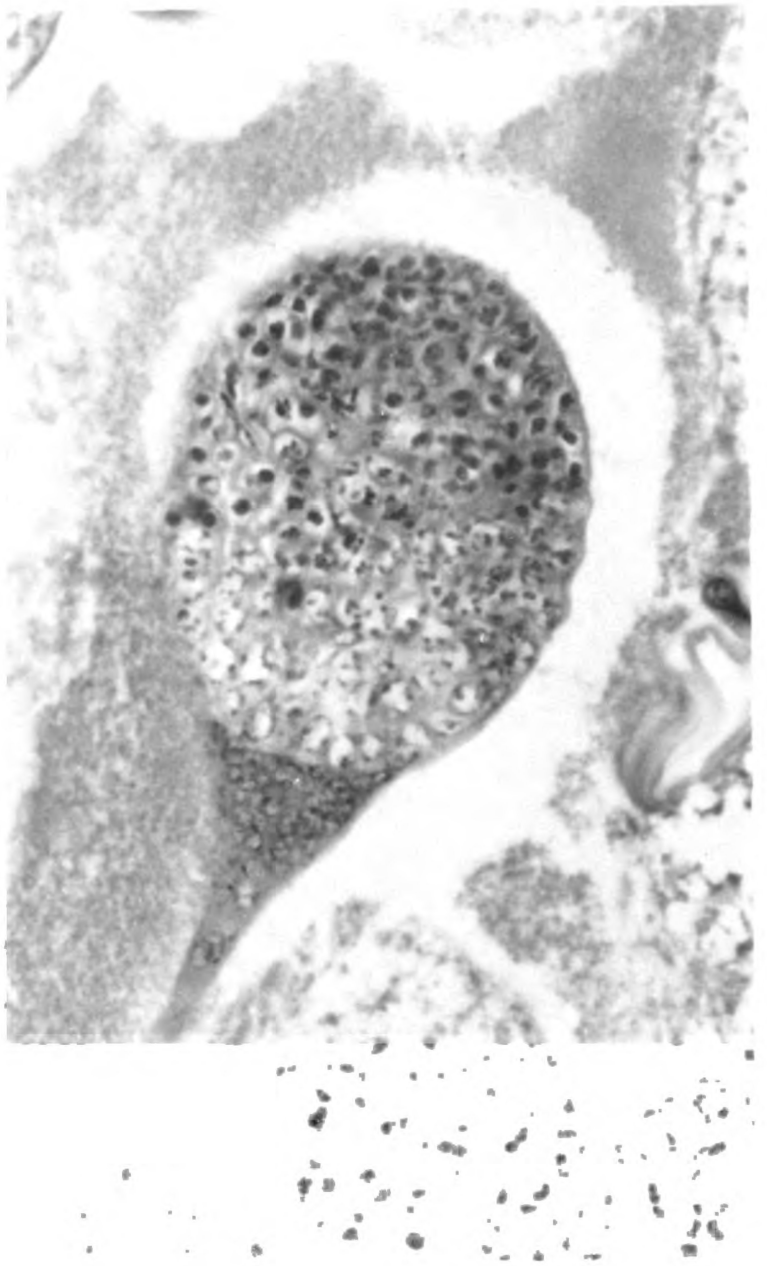
## RESULTS ON SPERMATOGENESIS

**LAST INSTAR LARVAE.** In sectioned material, the spherical testes are found in the posterior--dorsal aspect of the larvae, embedded in fat cells, and measuring about 116 microns in diameter (Fig. 1). Each testis consisted of a single testicular cavity bounded by an outer delicate squamous epithelium. The inner primary spermatogonial cells were found free from each other and mitotically active. There was no indication of cyst formation. An apical cap of somatic cells was found to be continuous with the cells of the testicular wall. Extending from each cap, the developing vas deferens was noted passing caudad into the fat cells.

**PUPAE 12-13 HOURS OLD.** Approximately one-half of the cells occupying each testicular lumen were spermatogonial (Fig. 2). All stages of meiosis were observed and were synchronous within a given cyst. Absence of the typical meiotic stage of prophase one was noted. At metaphase one pairing of homologous chromosomes was so intimate that only six were observable. In each testis, the cysts nearest the vas deferens contained spermatids. Associated with each cyst of spermatids, was found a rather large (approx. three times larger than a spermatogonial cell) somatic cell with a higher Pyronin Y affinity.

**PUPAE 1-2 DAYS OLD.** During this period the size of the

Figure 1

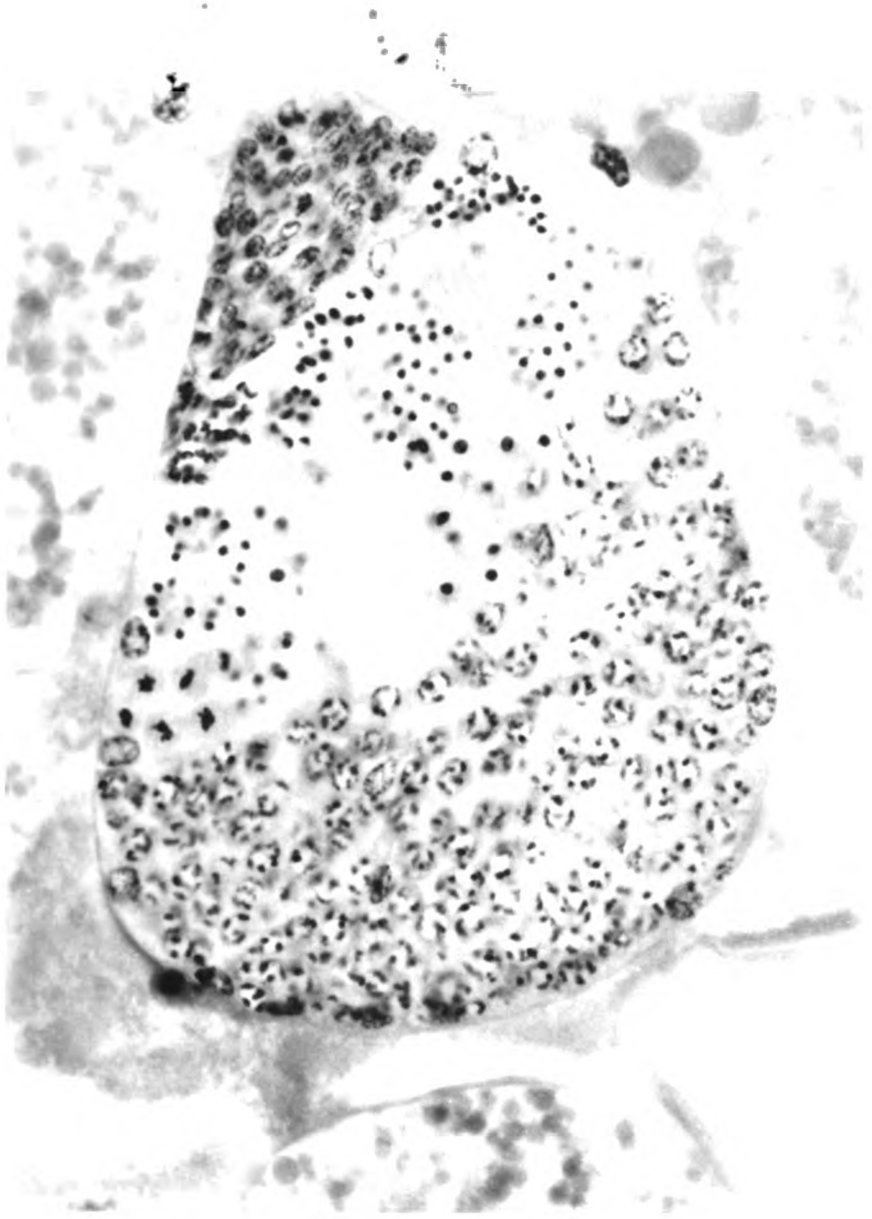


HORIZONTAL SECTION OF A LARVAL TESTIS.

Testicular lumen filled with spermatogonial cells. An apical cap of somatic cells with the vas deferens extending into the fat cells is shown.

Stain -- Feulgen and fast green. Magnification -- 540x

Figure 2



HORIZONTAL SECTION OF THE TESTIS OF A PUPA 12-HOURS OLD.

Spermatids are located in cysts nearest the vas deferens. Division figures are metaphase of the first meiotic division. Spermatogonial cells are located in the basal portion of the testis.

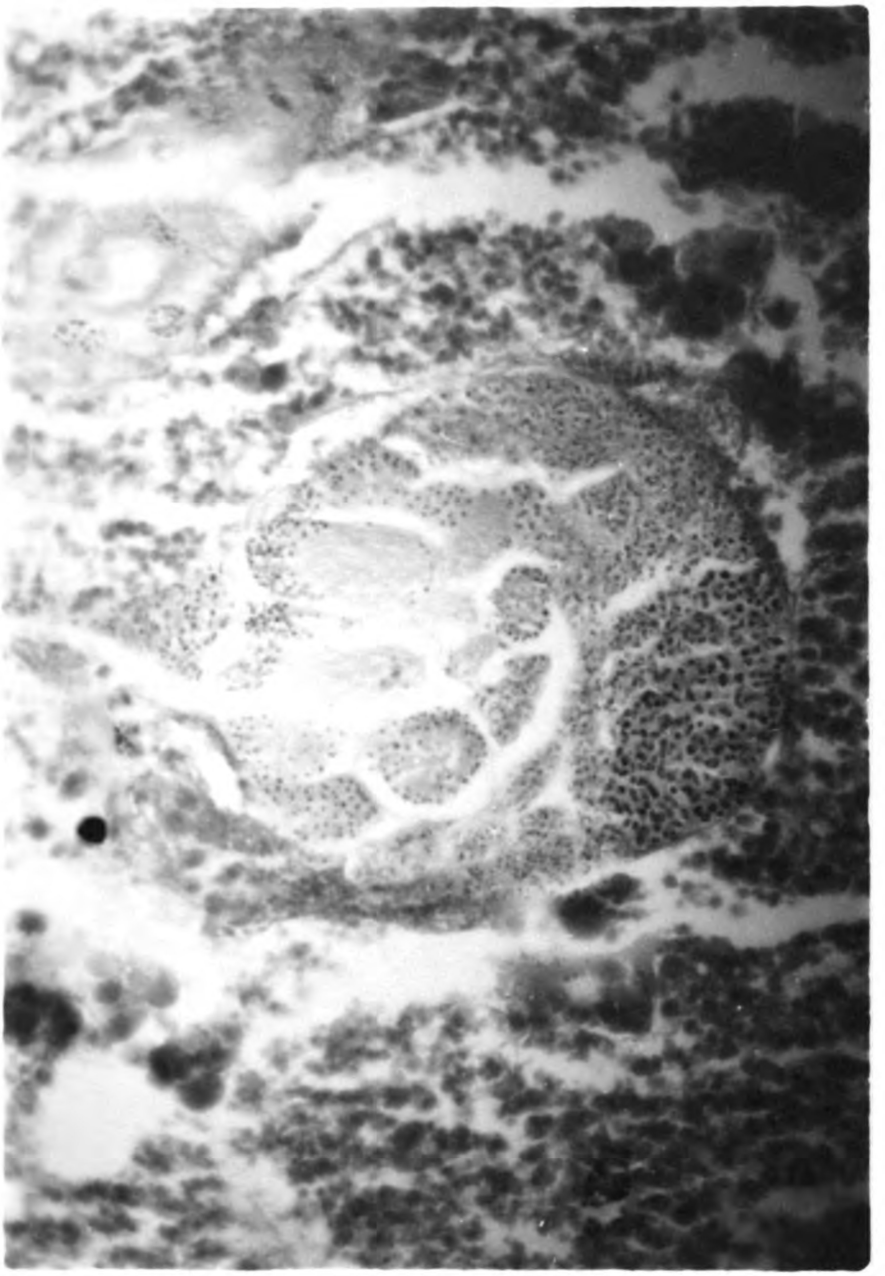
Stain -- Feulgen and fast green. Magnification -- 540x

testes continued to increase. The number of cells undergoing meiosis was at its peak. By the end of two days, one-half the volume of each testis was occupied by spermatids, one-fourth by germ cells undergoing maturation division, and the remaining one-fourth consisted of primary and secondary spermatogonia.

PUPAE 2.5-5 DAYS OLD. During this period of pupal development, a steady decline in number of spermatogonial cells and primary spermatocytes was observed (Figs. 3 & 4). Motile, mature sperm were first noted in two and one-half day pupae, and their numbers increased steadily. At five days the testicular elements consisted primarily of mature sperm arranged in pointed packets. In suitable sections nutritive cells could be seen at their apices. At five days meiosis was rarely observed. The spermatogonial region of each testis was confined to a small, crescent shaped area along the testicular wall most distal from the vas deferens (Fig. 5). Spermatids were present, but nearly all had maturated by five days. Adjacent to the epithelial wall of each testis, an outer pigmented testicular sheath had developed. Increasing numbers of epithelial cells were produced to accommodate the increasing volume of the testes.

THE ADULT TESTES. From emergence until the adults were two to three days old, meiotic activity of the germ cells was almost nil. During this period the spermatids

Figure 3

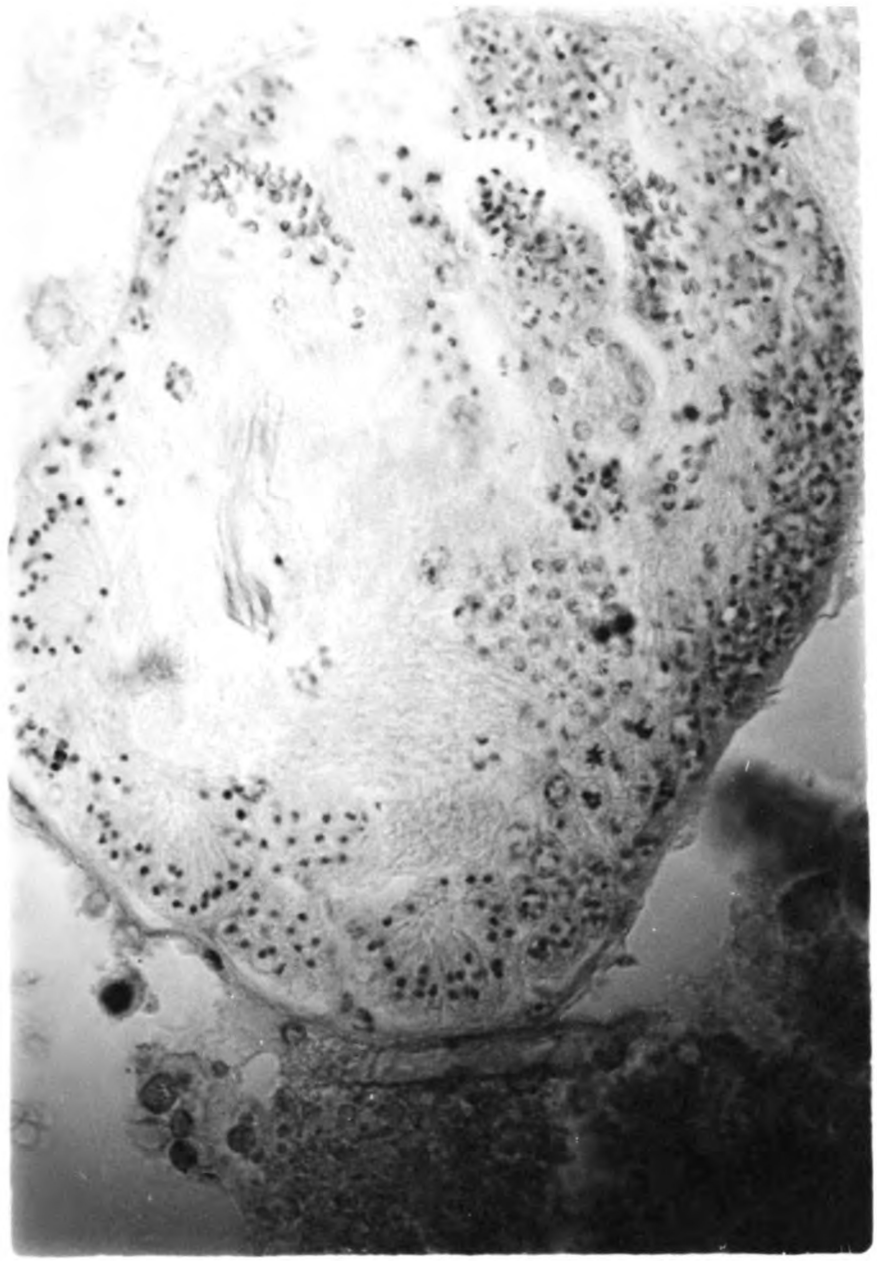


LONGITUDINAL SECTION OF THE TESTIS OF A PUPA 2.5 DAYS OLD.

Observable decrease in the number of spermatogonial cells with an increase of cysts containing spermatids.

Stain -- Feulgen and fast green. Magnification -- 265x

Figure 4



HORIZONTAL SECTION OF THE TESTIS OF A PUPA 3.5 DAYS OLD.

A cyst of mature sperm and an increase in the number of spermatidial cysts is shown.

Stain -- Feulgen and fast green. Magnification -- 540x

Figure 5

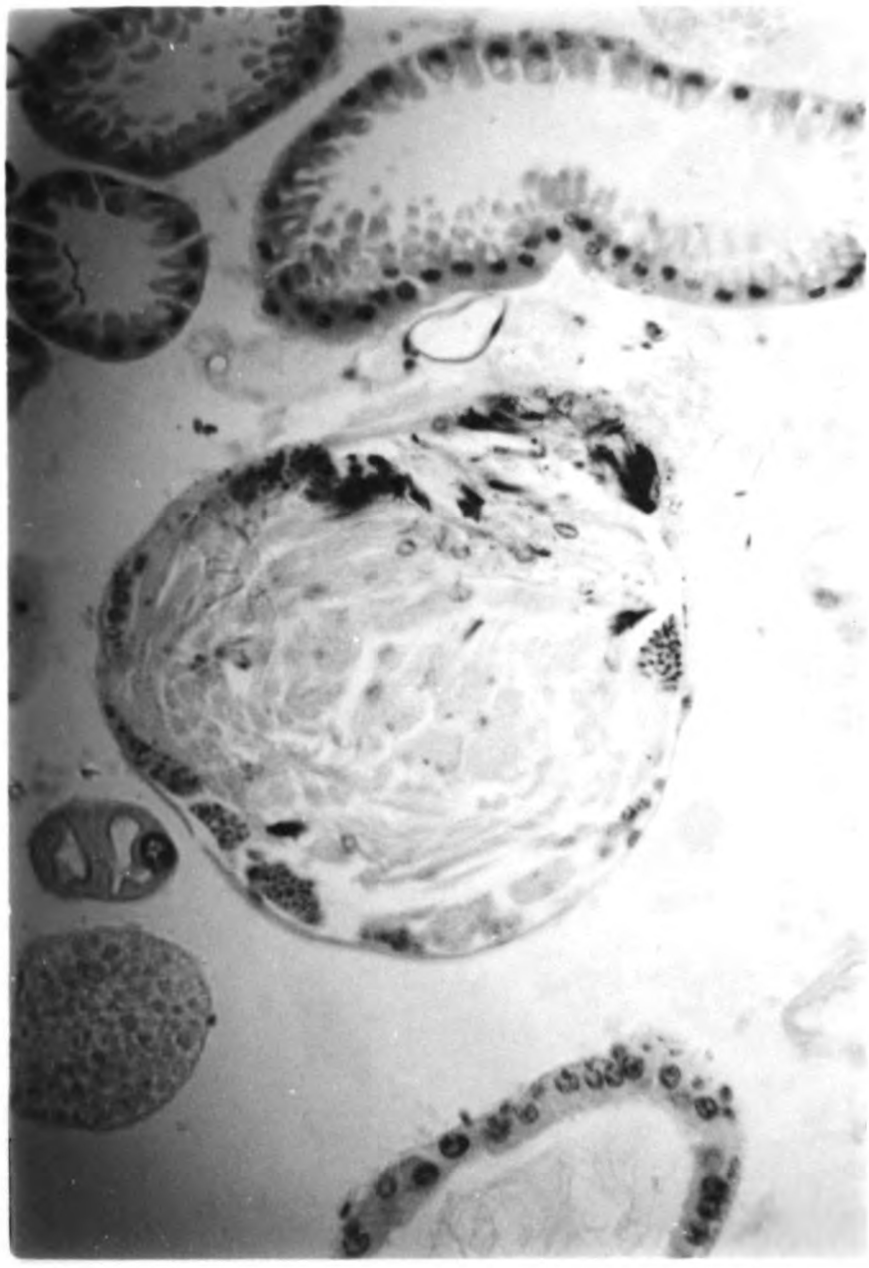
SQUASH PREPARATION OF A TESTIS FROM A PUPA 3.5 DAYS OLD.

Two cysts of spermatids are shown.

Stain -- Feulgen and fast green. Magnification -- 1100x

decreased in number. Primary spermatocytic cysts also decreased but were never totally absent. Mitotic activity of the spermatogonial regions remained at low levels. Cysts in meiotic configurations were rare (Fig. 6). Sperm observed in the vasa deferentia were no longer in packets. After two to three days of age, a marked reduction in mature sperm was noted and spermatogonial divisions began to increase. Increased meiotic activity and spermatid formation followed. At the end of the observation period, (six day adults) the process of producing mature sperm was still continuing. It is worth noting that this increase in sperm production never approached the initial maturation observed during the pupal period. Although sperm production appeared to be a continuous process, the immature and mature sperm produced during the pupal period vastly outnumbered the sperm produced during adult life. The testes reached their maximum size when the adults were two days old (approx. 300 x 350 microns), chiefly due to pupal sperm and maturation of pupal spermatids (Fig. 7). After the loss of mature sperm, presumably from copulation, the sperm titer lost was never regained, and the size of the testes averaged 250 x 300 microns.

Figure 6



LONGITUDINAL SECTION OF THE TESTIS OF A NEWLY EMERGED ADULT.

Testicular elements consist primarily of mature sperm, decreasing numbers of spermatids, and small groups of spermatogonial cells confined to the basal region of the testis.

Stain -- Feulgen and fast green. Magnification -- 265x

Figure 7

LONGITUDINAL SECTION OF THE TESTIS OF AN ADULT 2 DAYS OLD.

Observable decrease in the number of spermatidial cysts with an increase of cysts containing mature sperm.

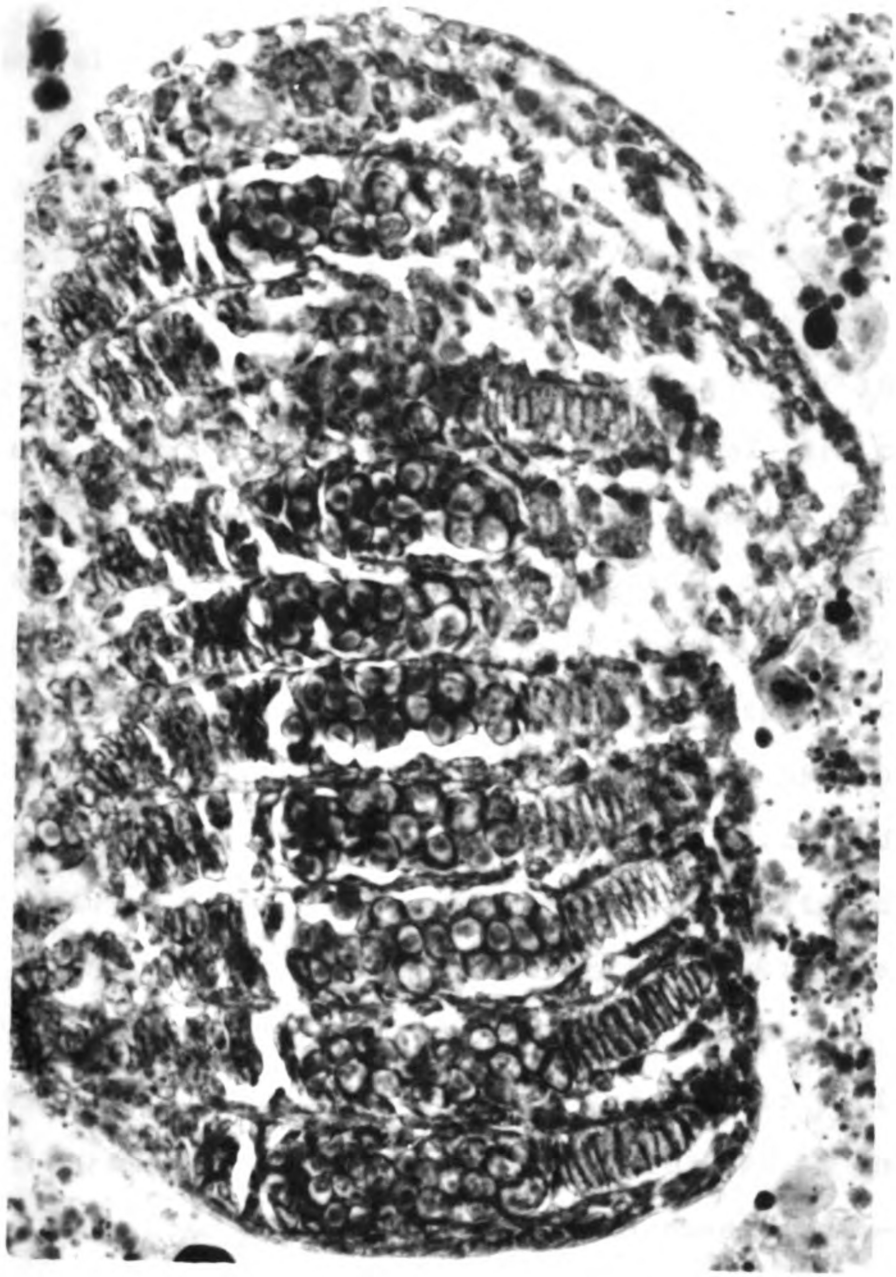
Stain -- Feulgen and fast green. Magnification -- 265x

## RESULTS ON OOGENESIS

LAST INSTAR LARVAE. As was the case with the larval testes, the primordial ovaries were found embedded in fat cells in the posterior--dorsal aspect of the larvae. Vertical sections through each larva revealed two oval bodies of cells, with diameters of sixty-four microns. The bodies were found to be composed of three cell types: 1) an outer layer of syncytial cells with oval nuclei--the ovarian sheath, 2) an inner mass of cells undergoing mitosis, and 3) a group of very flat cells with their nuclei containing a Feulgen positive sphere polarized to one end. This latter group of cells was found in the anterior--dorsal part of each pro-ovary. Their nuclei remained unchanged in forming the terminal filaments of the ovarioles, and thus proved of considerable value in locating the undeveloped ovaries in sectioned material prior to the formation of ovarioles. The interphase cells found in the medulla of each pro-ovary measured six microns, with nuclei of four microns. Each cell contained a Pyronin Y positive nucleolus.

PUPAE 0-1 DAY OLD. The gross shape of each ovary had changed to a somewhat flattened oval measuring 250 microns at the broad base and seventy-five microns at the narrowed end with a height, in vertical section, of 224 microns (Fig. 8). Some of the medullary cells were undergoing active divisions. However, most of the cells observed

Figure 8



HORIZONTAL SECTION THROUGH THE OVARY OF A PUPA 1 DAY OLD.

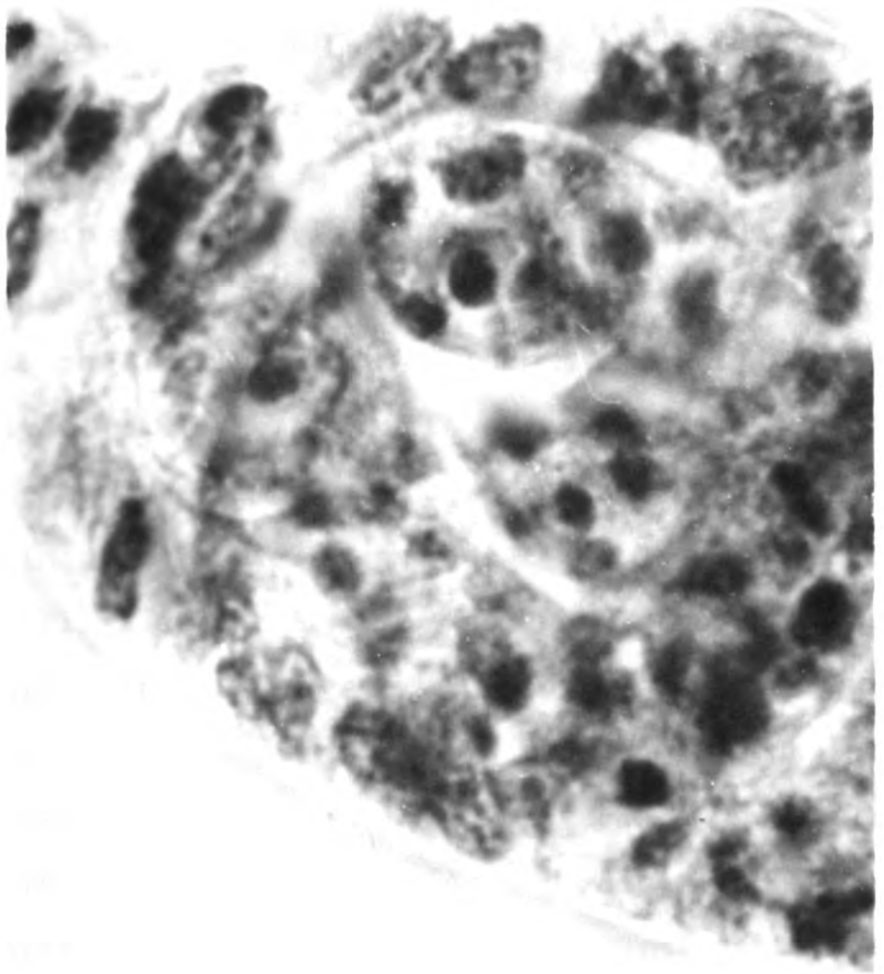
Formation of the germaria is complete, surrounded by a sheath of epithelial cells, joined below the basal cells and at the apical ends to the ovarian sheath. The terminal filaments with their flattened nuclei are located at the apical aspect of each germarium in the right hand portion of the figure.

Stain -- Pyronin Y and methyl green. Magnification -- 540x

contained either doubled, compact, Feulgen positive coils united to a common Feulgen positive chromocenter or a single, fine, continuous coil, appearing to radiate from a chromocenter and filling the nuclear volume of the cells. Pyronin Y positive nucleoli were present in those cells not showing division figures. Vertical orientation of the germ cells into germaria had begun. Each column of germ cells was surrounded by a sheath of squamous epithelial cells, syncytial in nature, joined below the basal cells and at the apical ends to the thicker ovarian sheath thereby partitioning off each ovariole. At the apical aspect of each germarium was the terminal filament consisting of a protoplasmic cylinder containing ovoid nuclei as described previously. Surrounding each germarium was a thin tunic of syncytial squamous cells which was continous with the terminal filament and the basal cells.

**PUPAE 1.5-2.5 DAYS OLD.** By the end of this period distinct chromosomes could no longer be located in the germaria. In some cases mitosis was observed in one-and one-half and two-day-pupae, but by two and one-half days all apparent mitosis had ceased. During the latter part of this period an indentation was noted in the basal region of each germarium forming a rounded projection. Epithelial cells were observed to have separated this newly formed region from the remainder of each germarium in which closely packed aggregates of cells were observed (Fig. 9). The constricted basal portions contained ger-

Figure 9



SQUASH PREPARATION OF THE BASAL PORTION OF A GERMARIUM OF A PUPA 2.5 DAYS OLD.

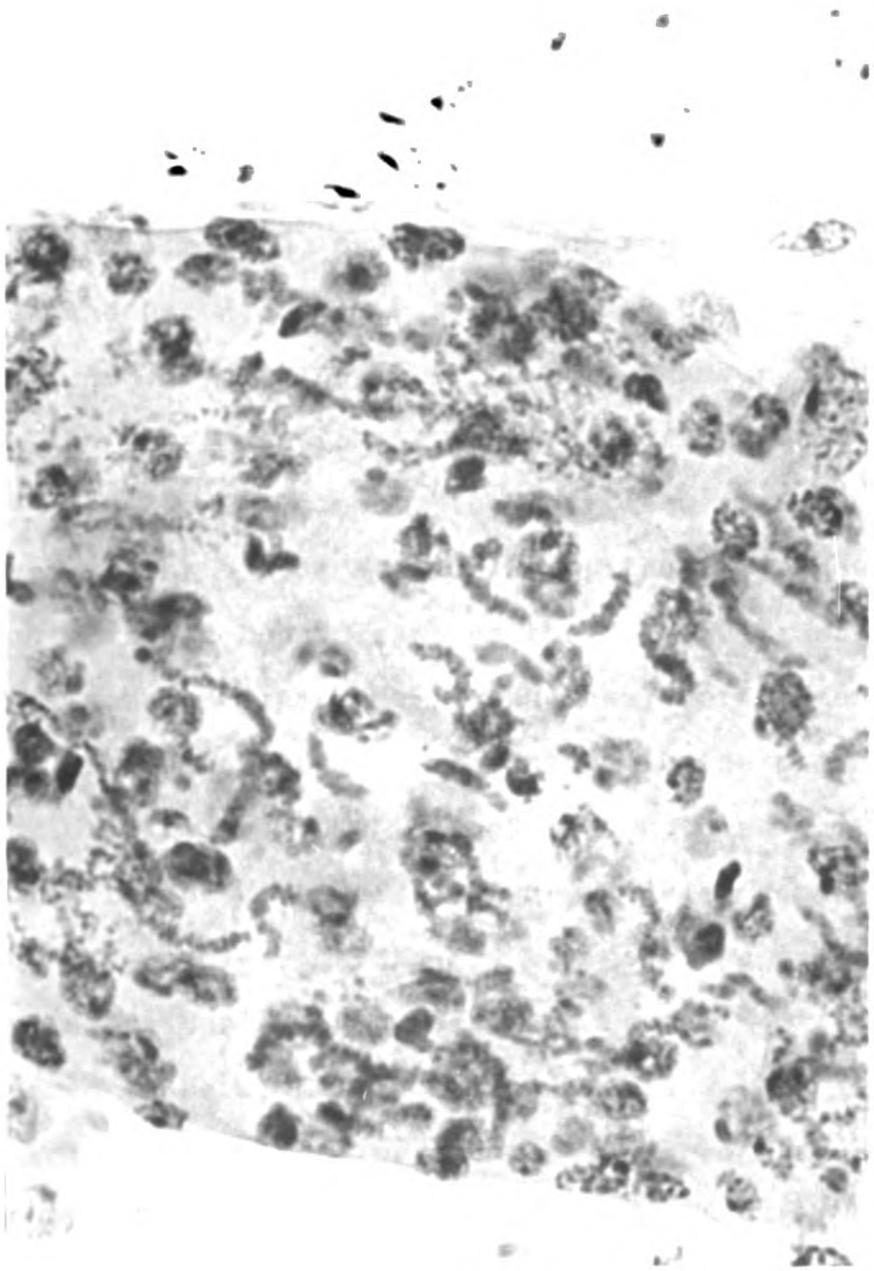
Several closely associated cells forming at least three germarial cysts.

Stain -- Feulgen and fast green. Magnification -- 2700x

marial like cells, but owing to their syncytial nature, the compactness of the structure, and the density of the surrounding epithelium, the exact number could not be counted (Fig. 10). There were at least ten present with no apparent difference in their nuclear morphology. Using the terminology of King (1956), the basal projection was the newly formed germarial egg chamber, and the aggregates of cells found apically to it, the incipient cysts. Each pro-nurse cell nuclei measured four microns and contained compactly coiled chromatin with an associated Feulgen positive chromocenter. Egg chambers averaged twenty-two microns in diameter. The average length of a germarium, terminal filament and chamber was 140 microns. The over all architecture of the ovaries at this point was generally spaceous. Developing trachea had entered through the ovarian sheaths and were distributed between the ovarioles. The general shape of the ovaries was maintained as before and measured in longitudinal section 300 microns laterally and 250 microns from the apical to the basal borders.

PUPAE 3-4.5 DAYS OLD. By the end of this period all ovaries observed possessed first egg chambers. Each germarial chamber had separated spatially from its germarium forming the first egg chamber, but remained connected by a stalk of follicular cells (prior to this, when discussing the formation of a chamber, the cells had been termed epithelial cells). The mitotically active follicular epithelium surrounding each egg chamber possessed distinct cu-

Figure 10



SQUASH PREPARATION OF A GERMARIAL CHAMBER IN A 2—DAY OLD PUPA.

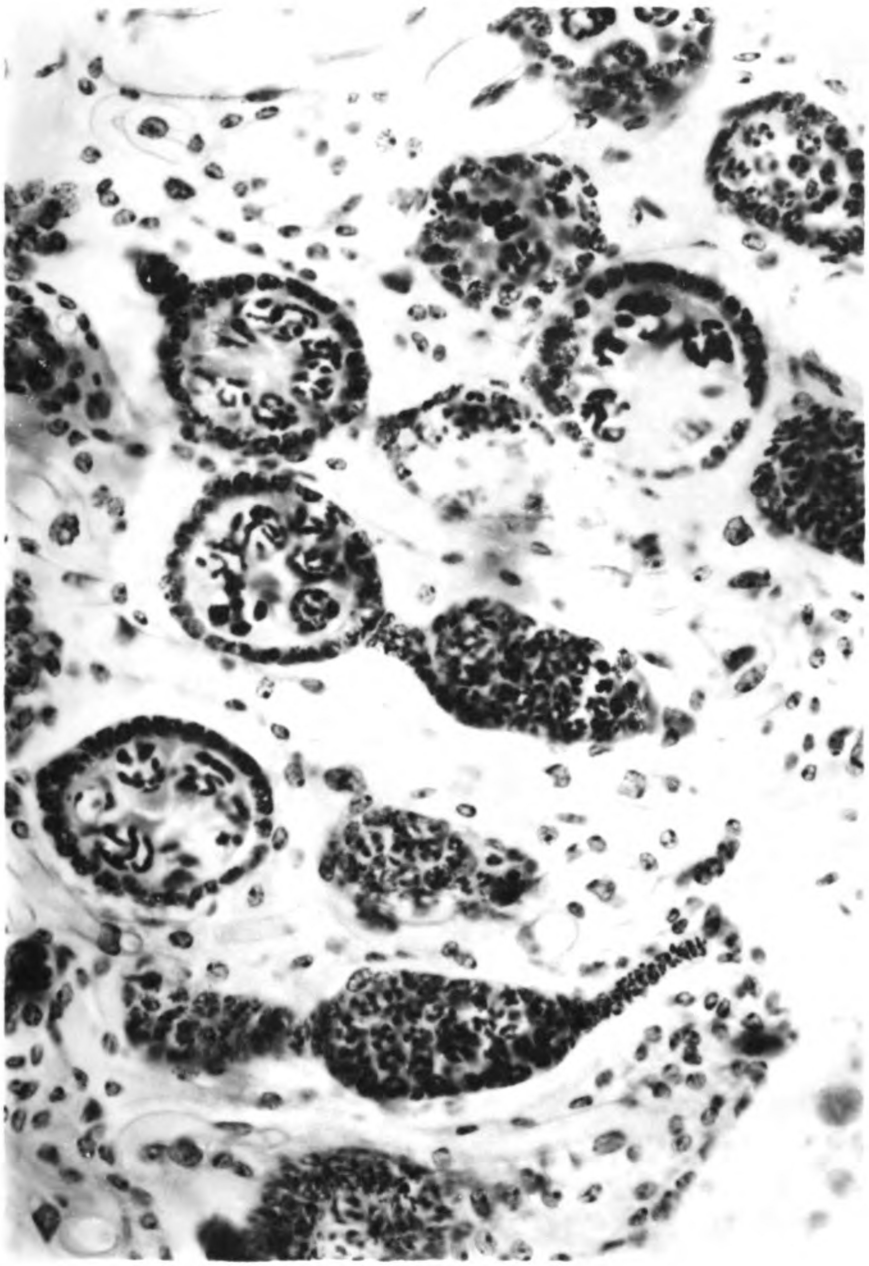
Syncytial pro--nurse cell nuclei contain compactly coiled chromatin with an associated Feulgen positive chromocenter. Smaller cells are the epithelial cells of the ovariole sheath.

Stain -- Feulgen and fast green. Magnification -- 2700x

boidal cells having a Pyronin Y positive nucleolus (Fig. 11). Each follicular stalk was observed to be continuous with the tunica of an ovariole and the follicular epithelium of a corresponding first egg chamber. Each egg chamber was spherical and had a diameter of forty microns. Fifteen nurse cell nuclei were observed in each chamber, and located in the basal portion of some chambers was the oocyte. At this point in development, the oocytes were masked in many preparations by the follicular epithelium. However, a Feulgen positive body measuring three microns could be located in some preparations in the basal portion of each chamber. Since these bodies remained with the oocytes throughout their development, they were used as a means of locating the oocytes before clear cellular demarcations occurred. The nuclear morphology of the nurse cells was not homogeneous during this period. Chromatin material was observed to be either in a single coil with a lateral measurement of one micron or clumped in aggregates about the periphery of the nuclei. The maximum diameter of the nuclei measured ten microns.

**PUPAE 5 DAYS OLD.** This stage was marked by the development of the second egg chamber in all ovaries observed (Fig. 12). Before pupation had ended, ovarian development included a first egg chamber and the formation of a second egg chamber (germarial). Each germarial chamber developed along similar patterns noted for the first egg chambers during the one and one-half- to two-and-one-half-day pupal

Figure 11

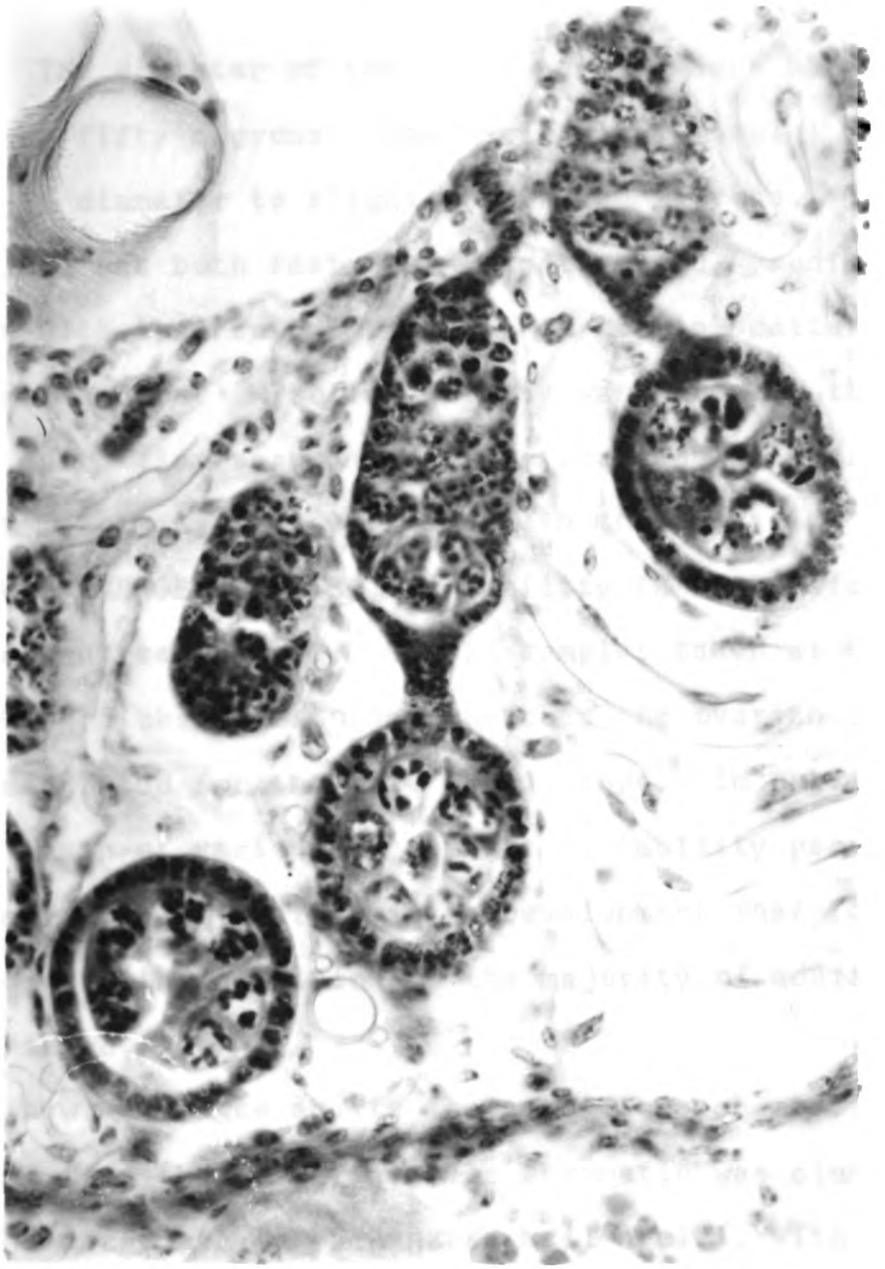


LONGITUDINAL SECTION THROUGH A PORTION OF AN OVARY OF A PUPA 4.5 DAYS OLD.

The first egg chamber remains connected to the germarium by a follicular stalk. Cuboidal follicular epithelium surrounds the first egg chamber. The chamber contains nurse cell nuclei with the chromatin a single coil or clumped about the periphery of the nuclei.

Stain -- Feulgen and fast green. Magnification -- 540x

Figure 12



LONGITUDINAL SECTION THROUGH A PORTION OF AN OVARY OF A PUPA 5 DAYS OLD.

A second egg chamber is forming at the base of the germarium in the centerally located ovariole.

Stain -- Feulgen and fast green. Magnification -- 540x

period. The diameter of the first egg chambers had increased to fifty microns. Their nurse cell nuclei had increased in diameter to slightly over ten microns. The nucleoplasm was both fast green negative and Pyronin Y negative with the Feulgen positive chromatin scattered in many small clumps. Mitotic activity was noted in the follicular epithelium surrounding the first egg chambers. No apparent mitotic activity was seen in the germaria.

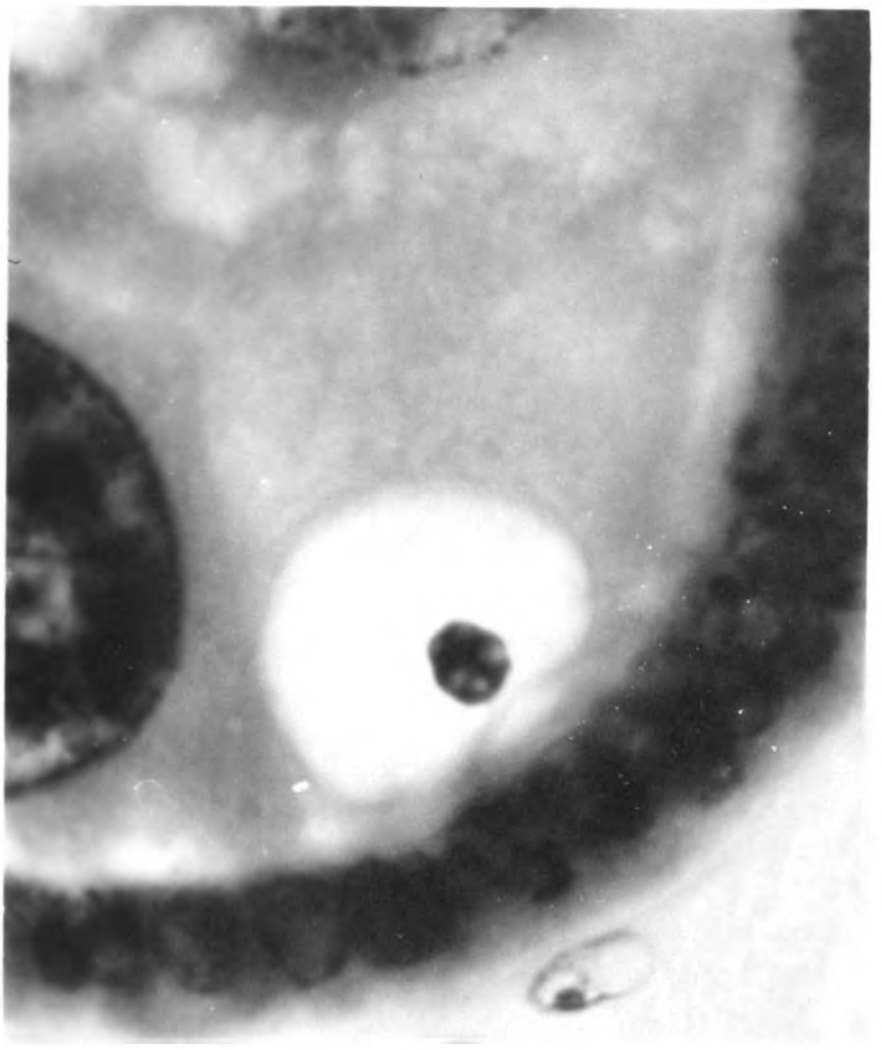
NEWLY EMERGED ADULTS. Variability in oogenesis up to this age had been slight. Of six samples taken at this age, two were observed to have reached the ovarian development described for the late pupal stage. In subsequent samples taken of various age groups variability persisted. The description of adult ovarian development that follows will describe the condition of the majority of adults that were observed.

In newly emerged adults the second egg chambers had separated from the germaria. The chromatin was clumped about the periphery in each nurse cell nuclei, with the general cytology of the chambers as a whole being similar to that observed for the early first egg chambers in the pupae. The first egg chambers had increased to an average diameter of sixty microns. Their nurse cells (diameter twenty-eight microns) contained chromatin material that had changed little from that of the five-day pupae, i.e., many small clumps scattered throughout the nuclei, connected by Feulgen positive strands. Oocytes were now quite appar-

ent, especially in sectioned material. Each oocyte occupied the basal portion of a chamber and measured twenty microns by thirty microns (Fig. 13). Borders between an oocyte and adjacent nurse cells could be observed easily. The three micron, Feulgen positive bodies previously described were each embedded in a clear matrix measuring twenty-four microns in diameter.

ADULTS 12 HOURS TO 2 DAYS OF AGE. During this period gradual enlargement of all first egg chambers occurred. Toward the end of the period, the first egg chambers became somewhat elongate, one hundred by seventy-four microns, with a basal nurse cell nuclei measuring forty microns in diameter and an anterior nuclei measuring thirty-six microns in diameter (Fig. 14). Early in the period the chromatin of the nurse cell nuclei began to spread out, became detached, and was observed to divide longitudinally without a spindle apparatus (Fig. 15). Near the end of the period the longitudinal divisions could no longer be observed and the chromatin was diffused throughout each nuclear volume. Pyronin Y positive granules were observed to be associated with the chromatin of the nurse cell nuclei, increasing in quantity toward the latter part of the period. Pyronin Y affinity was observed to be increasing steadily in the nurse cells throughout this period. There was little change in the oocytes of the first egg chambers, however a slight increase in the ooplasmic volumes was observed. The Feulgen positive bodies had not increased in size or in cytological

Figure 13

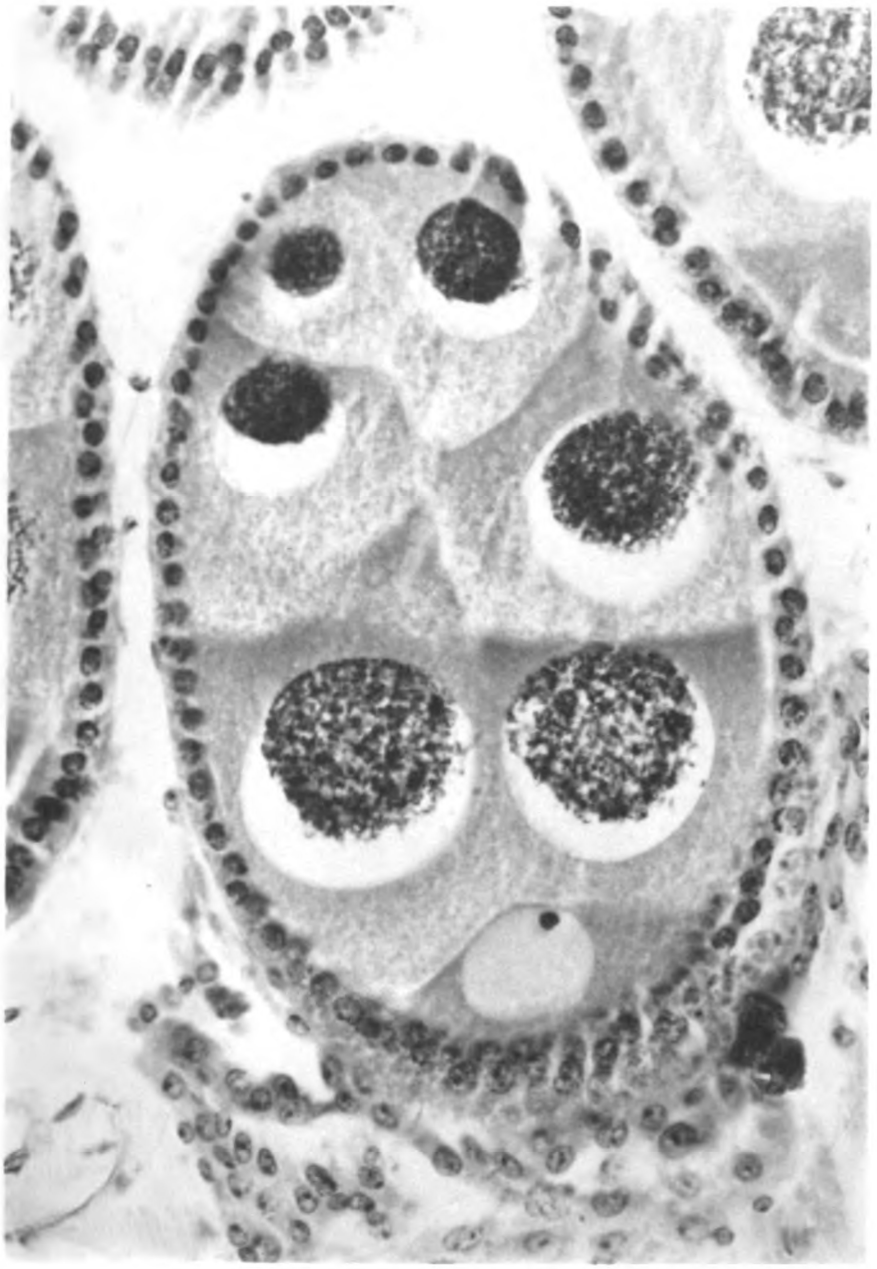


SECTION THROUGH THE FIRST EGG CHAMBER OF A NEWLY EMERGED ADULT.

Oocyte in the basal portion of the egg chamber containing a Feulgen positive body embedded in a clear matrix.

Stain -- Feulgen and fast green. Magnification -- 2700x

Figure 14

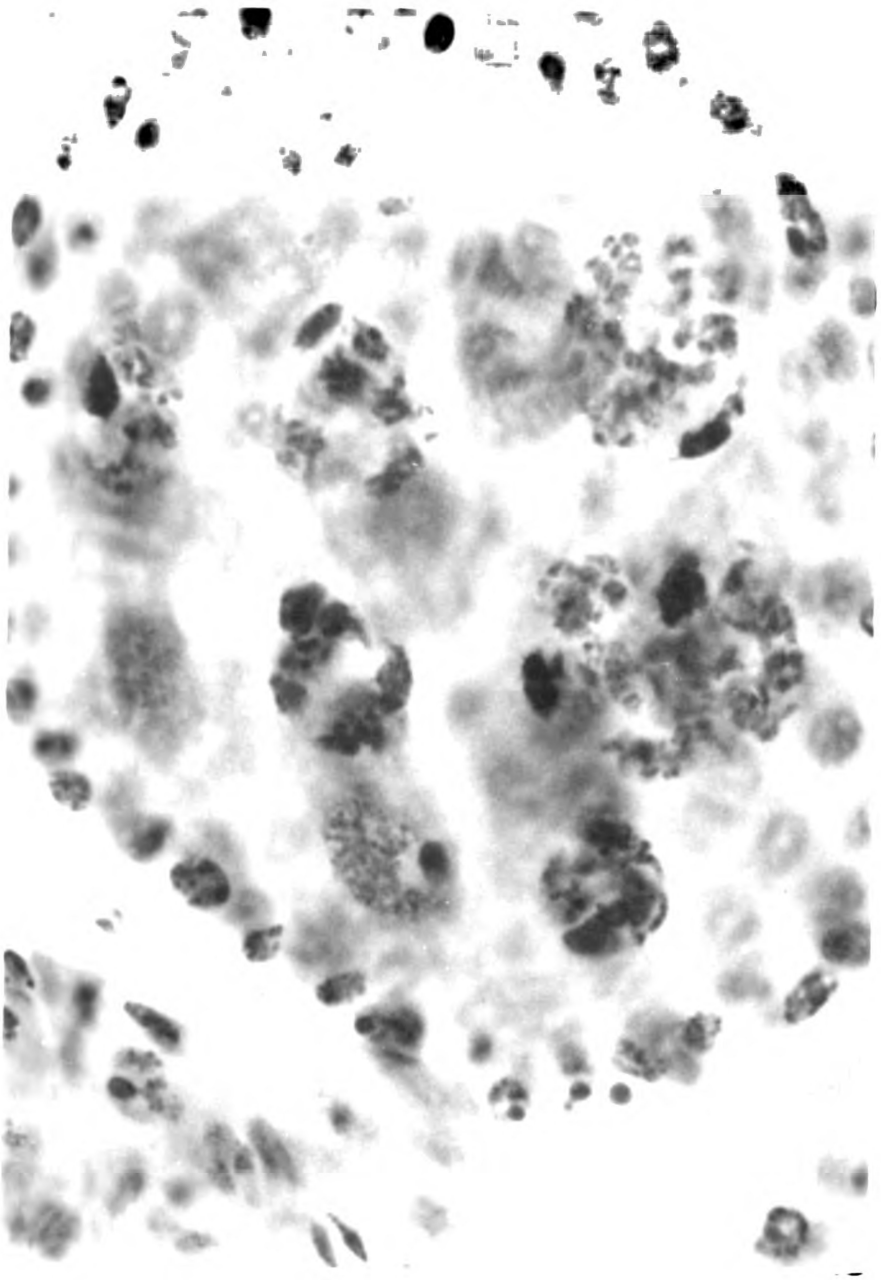


LONGITUDINAL SECTION OF THE FIRST EGG CHAMBER OF AN ADULT 1.5 DAYS OLD.

Oocyte located in the basal portion of the chamber with the nurse cell chromatin beginning to fill the nuclear volume.

Stain -- Feulgen and fast green. Magnification -- 540x

Figure 15



SQUASH PREPARATION OF THE FIRST EGG CHAMBER OF AN ADULT 12 HOURS OLD.

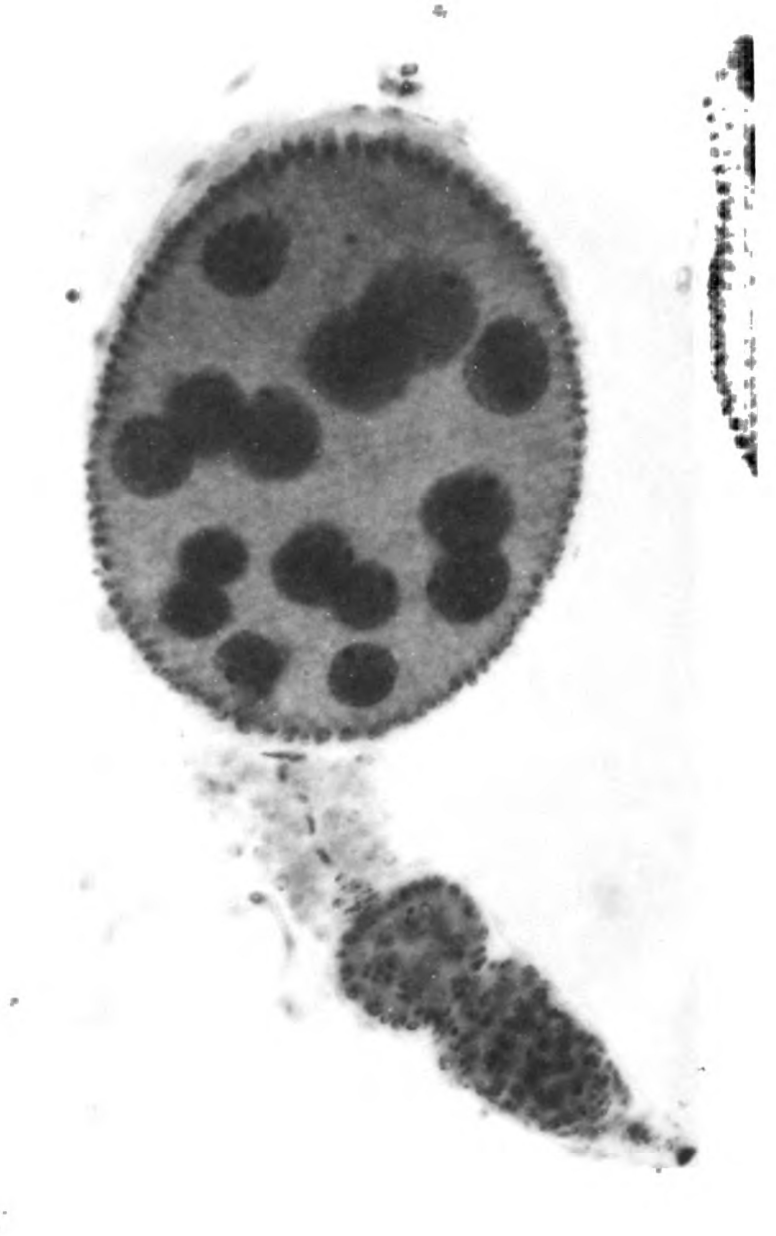
Chromatin of the nurse cell nuclei have spread out, become detached, and are undergoing longitudinal divisions without a spindle apparatus.

Stain -- Feulgen and fast green. Magnification -- 1100x

detail. Associated with each Feulgen positive body (karyosome or chromocenter) was found a Feulgen negative body of about the same size, three microns, that stained slightly with fast green and Pyronin Y. This body was not observed in all the oocytes and seemed to be dependent upon the material examined. The second egg chambers had developed to the point observed for the first chambers in the five-day pupae (Fig. 16).

ADULTS 2.5-3.5 DAYS OLD. During this period the nurse cell nuclei in the basal region of the first egg chambers reached a maximum diameter of seventy microns. The chroatin was diffuse and faintly Feulgen positive, which probably resulted from physical dilution of the DNA. Chains of fast green and Pyronin Y positive blobs were noted within, and near, each nurse cell's nuclear membrane. The expansions in these chains measured about one micron and may have resulted from the aggregation of smaller Pyronin Y positive granules that were observed in intimate association with the diffuse chromatin. The oocytes during this period had undergone remarkable increases in their volumes. An average diameter reached at three and one-half days was 225 microns with a length of 400 microns. The oocyte nuclei or germinal vesicles measured thirty microns. The karyospheres or chromocenters had decreased markedly in methyl green and Feulgen affinity producing somewhat diffuse appearances (Fig. 17). The nucleoplasm of each oocyte had increased in Pyronin Y and fast green affinity. Transverse

Figure 16

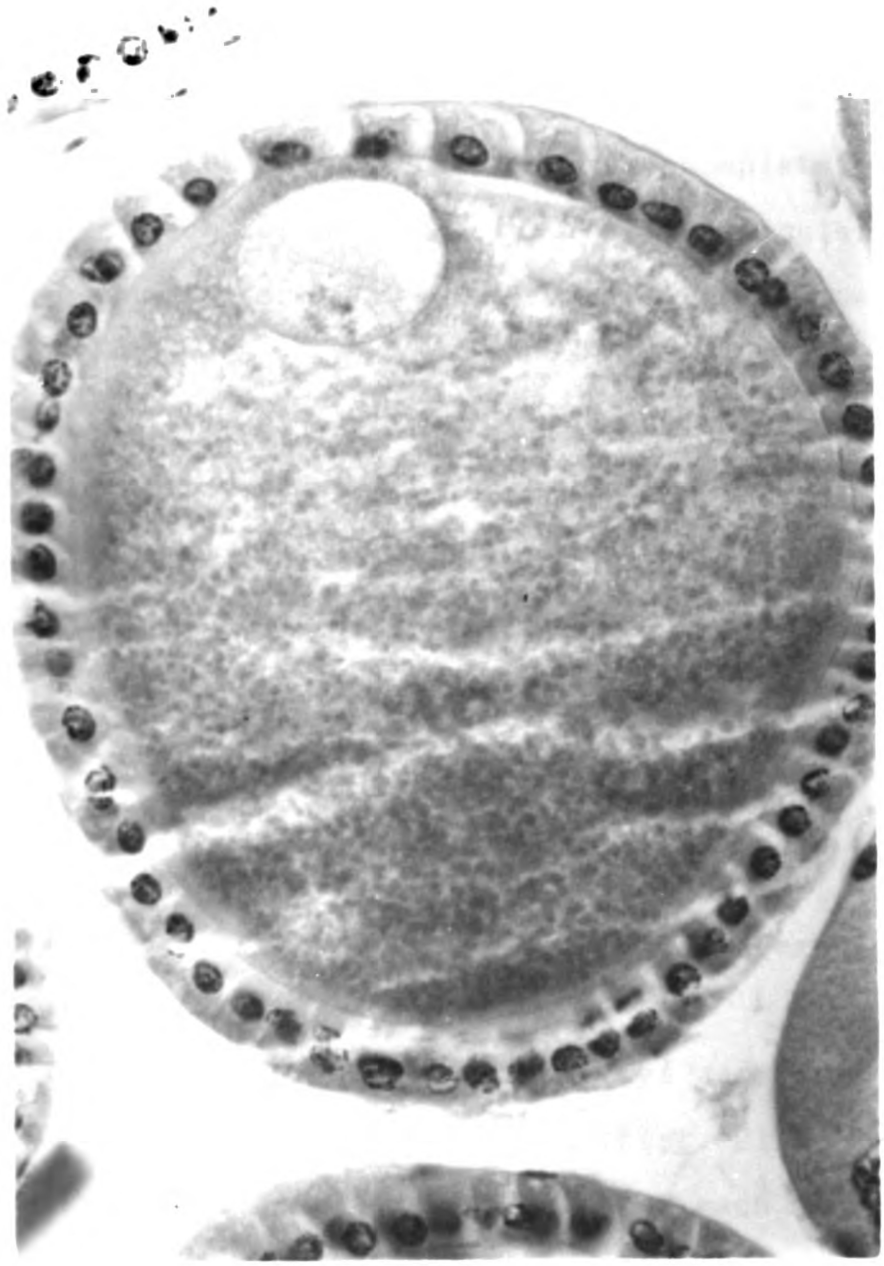


SQUASH PREPARATION OF AN OVARIOLE OF AN ADULT 1.5 DAYS OLD.

Germarium, second, and first egg chambers. The first egg chamber contains 15 nurse cell nuclei with those closest to the oocyte being larger.

Stain -- Feulgen and fast green. Magnification -- 265x

Figure 17



CROSS SECTION OF AN OOCYTE IN AN ADULT 3.5 DAYS OLD.

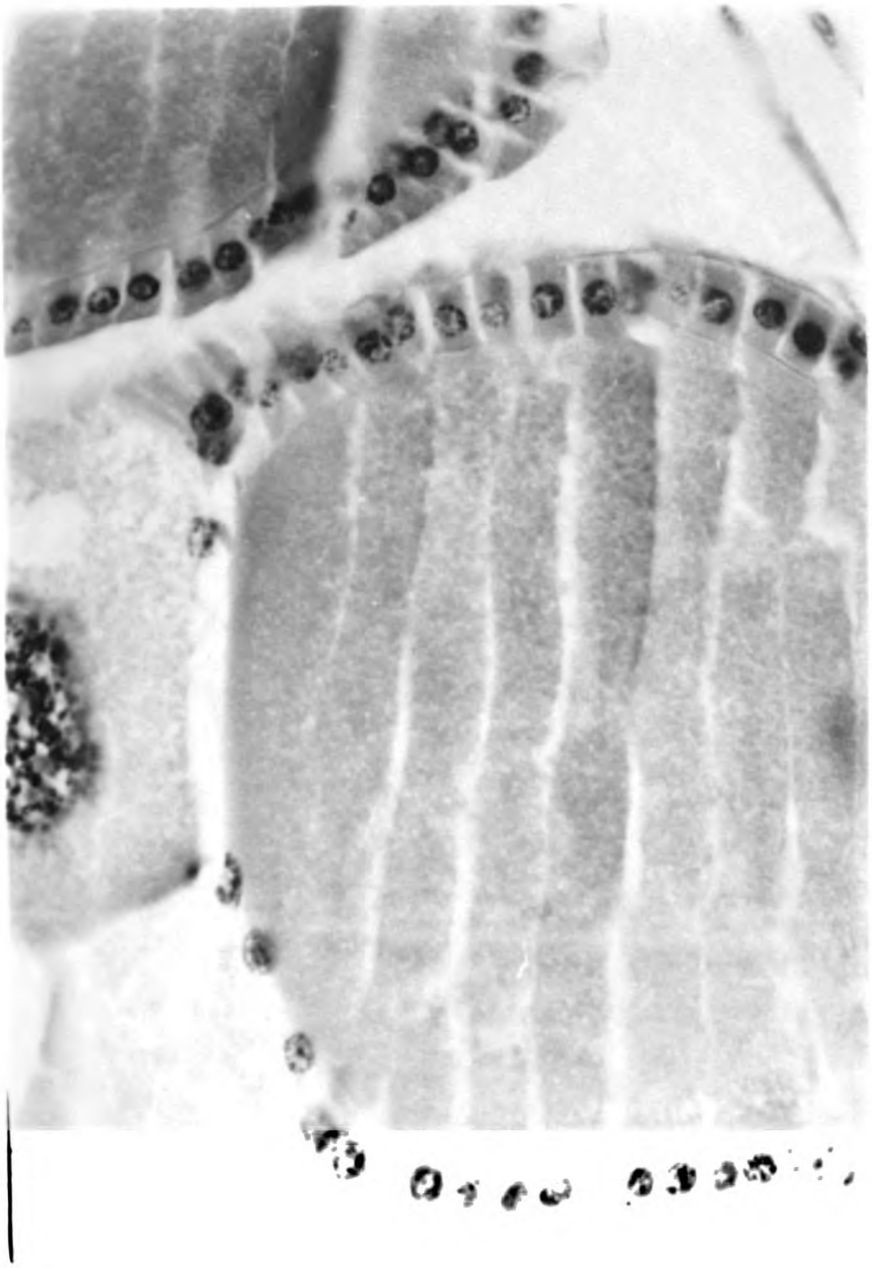
Diffuse appearance of the karyosome or chromocenter is shown.

Stain -- Feulgen and fast green. Magnification -- 540x

proliferation of the follicular epithelium between each nurse cell complex and the corresponding oocyte was observed. The epithelial partitions were incomplete toward the center of each border (Fig. 18). The follicular epithelium surrounding each nurse cell complex was squamous in nature, and that around the oocytes was observed to be columnar (Fig. 19). A single large nucleolus was observed in the nucleus of each columnar cell, but no emmision bodies were noted. The ooplasms appeared vacuolated with Pyronin Y affinity about equal to the cytoplasm of the nurse cells. The nurse cells were observed to have a greater fast green affinity than the oocytes. The second egg chambers had developed to a point comparable to the first egg chambers noted in newly emerged adults. Third egg chambers were observed to be developing in each germarium (Fig. 20).

ADULTS 4-6 DAYS OF AGE. At four days degeneration of the nurse cell complexes was observed (Figs. 21 & 22). There now existed a complete separation of each nurse cell complex from its corresponding egg by a transverse layer of follicular epithelial cells between them. Pycnosis of nurse cell nuclei was followed by loss of fast green and Pyronin Y affinity of the cytoplasm of the nurse cells. Subsequent loss of Feulgen positive material in the nurse cell nuclei and a marked decrease in the volume of the cytoplasm of the nurse cell complexes was observed. By the fifth and sixth days, degeneration was complete in

Figure 18

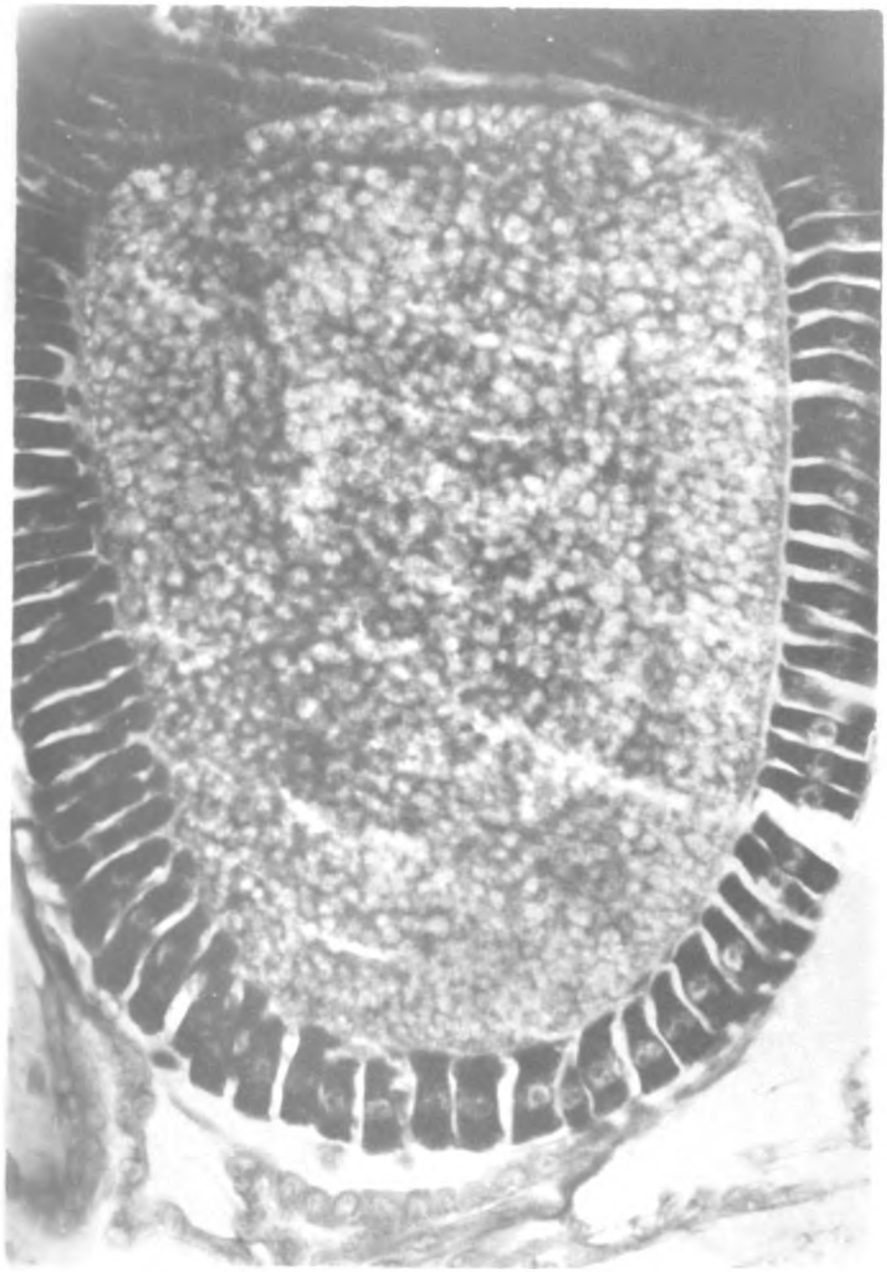


LONGITUDINAL SECTION THROUGH THE BORDER OF THE OOCYTE AND NURSE CELL COMPLEX OF AN ADULT 3.5 DAYS OLD.

An incomplete transverse layer of follicular epithelial cells between the nurse cell complex and the oocyte.

Stain -- Feulgen and fast green. Magnification -- 540x

Figure 19

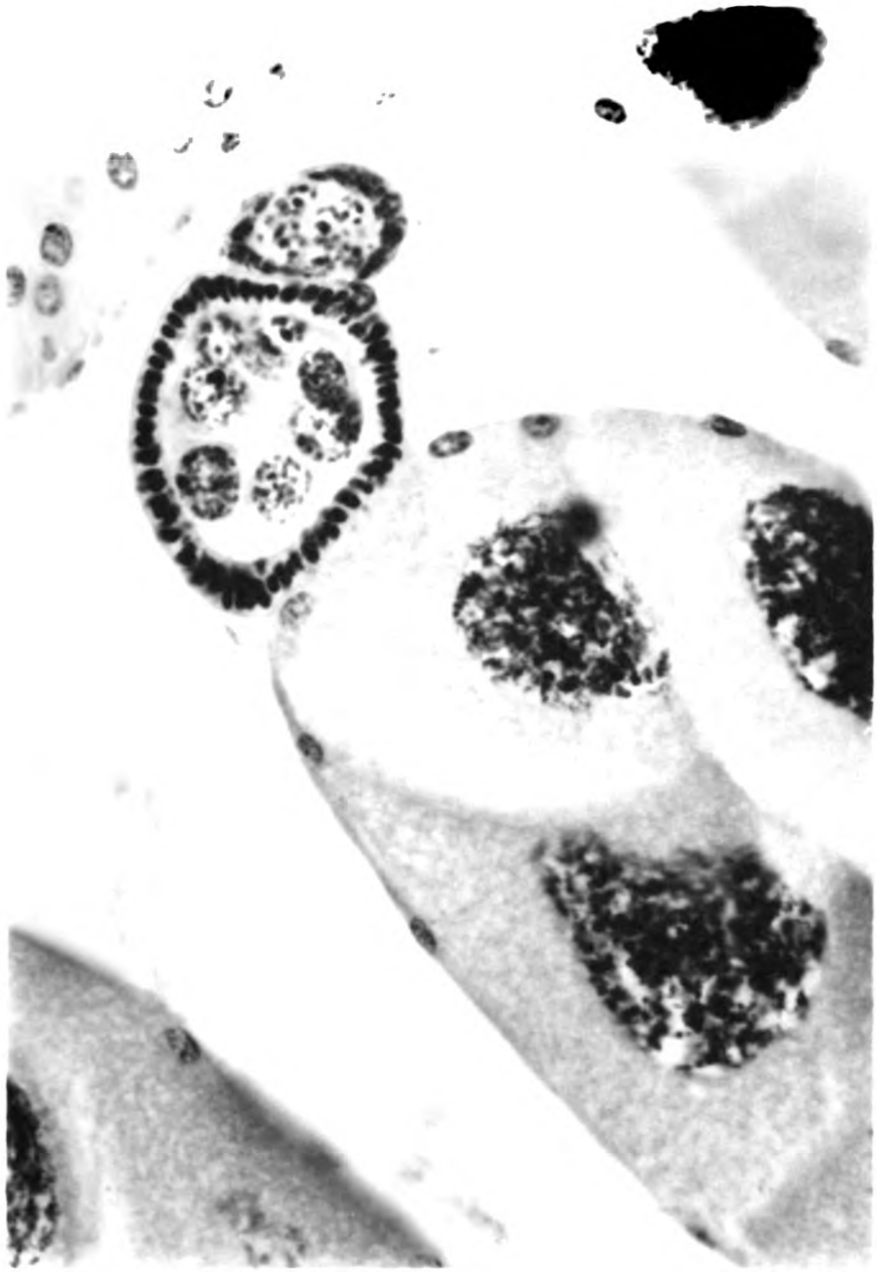


LONGITUDINAL SECTION THROUGH THE OOCYTE OF AN ADULT 3 DAYS OLD.

A strong Pyronin Y affinity was demonstrated by the columnar epithelium surrounding the oocyte prior to chorion formation.

**Stain** -- Pyronin Y and methyl green. Magnification -- 540x

Figure 20

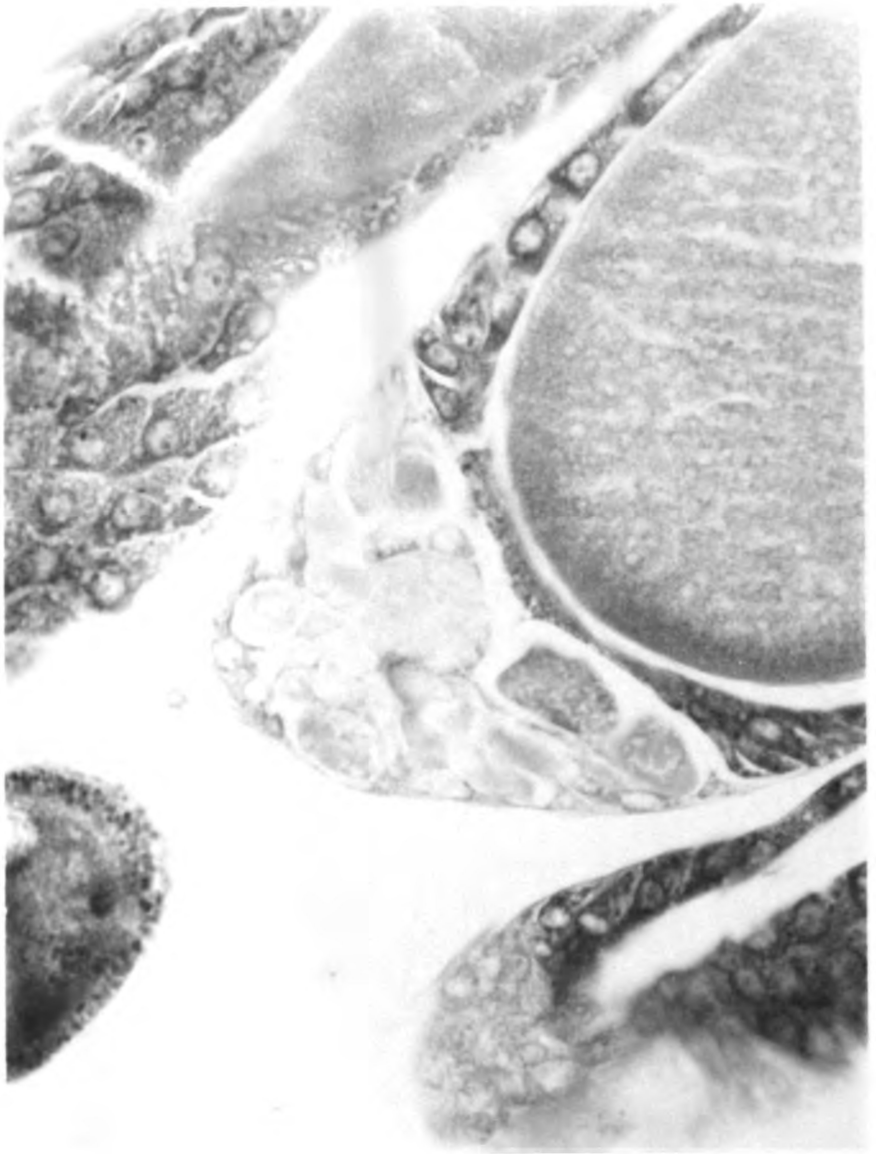


LONGITUDINAL SECTION THROUGH A PORTION OF AN OVARIOLE OF AN ADULT 3.5 DAYS OLD.

The third, second, and a portion of the nurse cell complex of the first egg chamber are shown.

Stain -- Feulgen and fast green. Magnification -- 540x

Figure 21

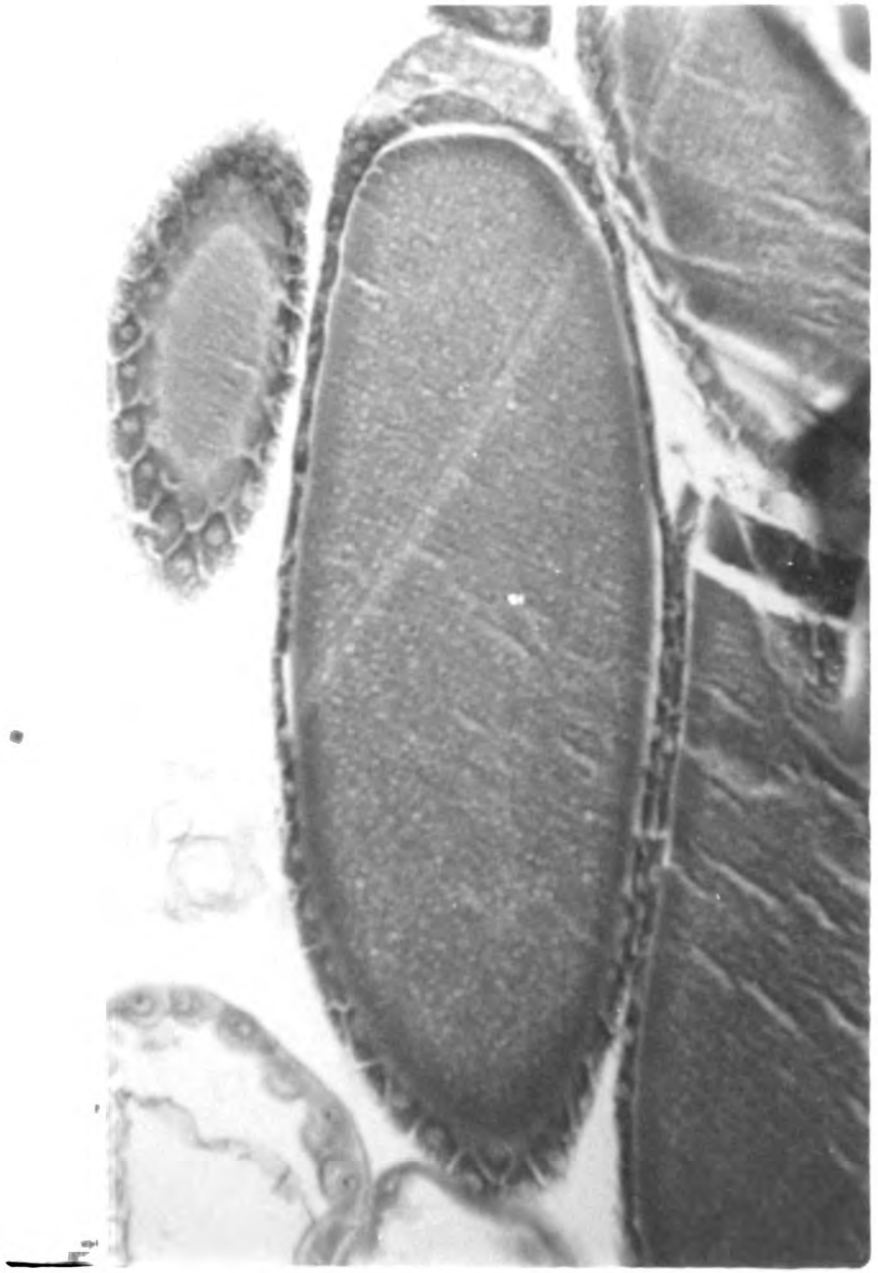


LONGITUDINAL SECTION THROUGH A PORTION OF THE FIRST EGG CHAMBER OF AN ADULT 4.5 DAYS OLD.

Degeneration of the nurse cell complex is shown.

Stain -- Feulgen and fast green. Magnification -- 1100x

Figure 22



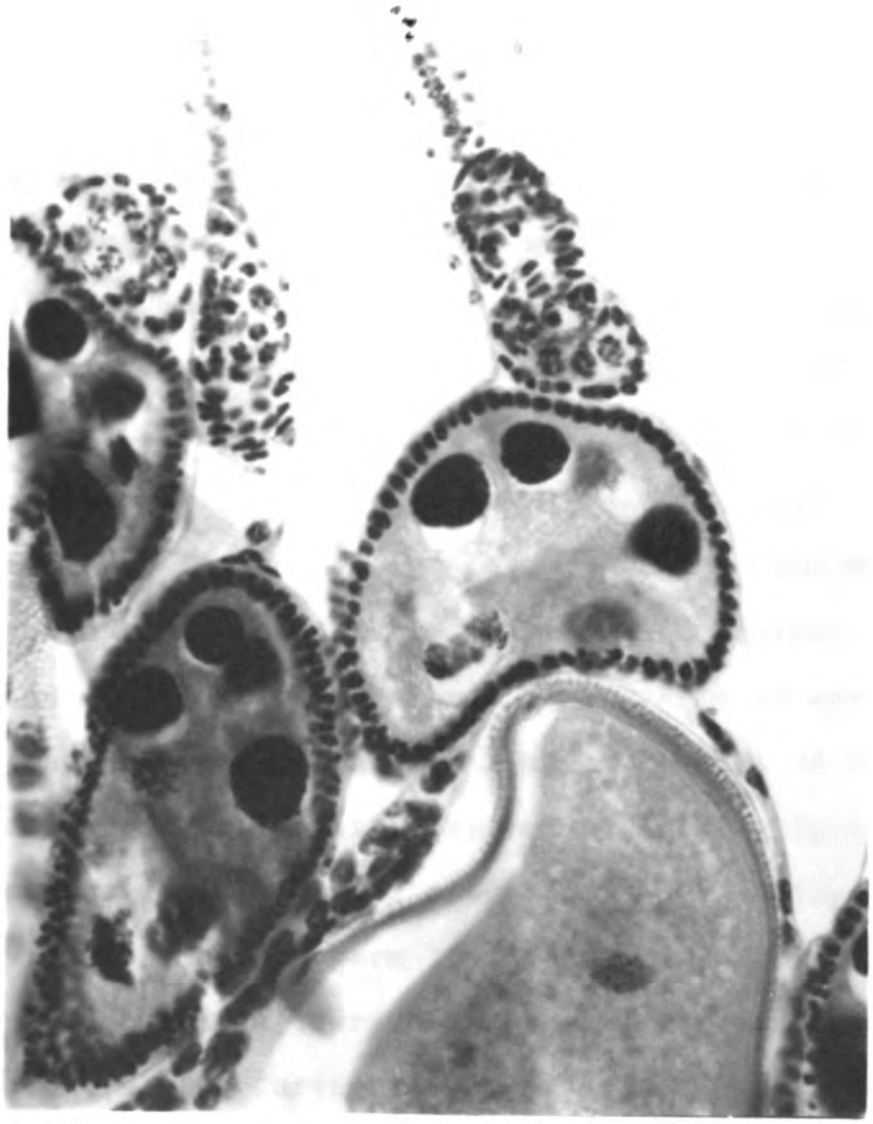
LONGITUDINAL SECTION OF A FIRST EGG CHAMBER OF AN ADULT 4.5 DAYS OF AGE.

Further degeneration of the nurse cell complex is shown.

Stain -- Pyronin Y and methyl green. Magnification -- 265x

the flies sampled. The nuclear boundry of each oocyte had also broken down. The karyosomes could still be found in some specimens. The height of the follicular epithelium surrounding each oocyte had gradually diminished, until by chorion formation its degeneration was complete (Fig. 23). An invagination of the follicular epithelium had produced a deep cleft extending over half the length of each egg with the cells persisting in mature eggs. The diameter of a mature egg, at the mid point, was 0.3 mm. with a length of about 1.2 mm. The second egg chambers had increased in size little from that observed at three and one-half days. The third egg chambers had separated from the germaria and were comparable to the early first egg chambers found in the pupae.

Figure 23



SECTION THROUGH A PORTION OF AN OVARIOLE FROM AN ADULT 5 DAYS OF AGE.

Germarium, third and second egg chambers, and a portion of a mature egg are shown. Degeneration of the follicular epithelium had preceeded chorion formation.

Stain -- Feulgen and fast green. Magnification -- 540x

## DISCUSSION OF SPERMATOGENESIS

As in Culicidae (Rishikish, 1959; Breland and Rieman, 1961; Warren and Breland, 1963), spermatogonial and the majority of reduction divisions were found to occur in the larvae and the pupae. In Musca domestica L., reduction division commenced when the pupae were twelve hours old. The absence of the usual prophase one chromosomal behavior confirmed similar results obtained by Stevens (1908), Metz (1910), Perje (1948) and White (1949). Reduction division and sperm maturation in the cysts nearest the vasa deferentia preceeded those located in the basal regions. Spermatids were first noted in twelve-hour pupae, while morphologically mature and motile sperm were noted to develop as early as two and one-half days. The testicular elements of five-day-old pupae were chiefly mature sperm. The activity of adult testes at the cellular level appeared to be a rather feeble attempt to replace the sperm lost following copulation. Few spermatogonial cells remained, however; therefore, the flies potential for further sperm production was greatly reduced. After six days of adult life, the quantity of spermatids and primary spermatocytes produced since the initial pupal differentiation had not approached that found in two-day-old pupae.

The greatest amount of meiotic activity in Musca domestica L. is restricted to their quiescent stage--the pupae. The active stages of the life cycle, i.e.,

those stages when the flies are actively feeding and liable to contact mutagenic agents, seem to be devoid of maturation divisions. Therefore, the effects observed by LaBrecque (1961) in inducing sterility by administering chemosterilants to adult males cannot be the result of disruption of the meiotic process.

## DISCUSSION OF OOGENESIS

FORMATION OF GERMARIA. The medullary cells of the pro-ovaries became oriented, lining up beneath the flattened nuclei of the terminal filaments. Subsequently, they became distinct from one another by clefts that began at the regions of the terminal filaments of each ovary and proceeded to the basal portion of the ovary. The clefts appeared to be the result of the proliferation of the apical cells of each terminal filament forming the tunica of the corresponding germarium. Surrounding each ovariole, an ovariole sheath appeared to be produced by infolding of the ovarian sheath. Eventually, the sheath separated the developing ovarioles and, in addition, served as avenues for trachial penetration. The formation of the germaria was completed in pupae one and one-half days of age.

PRODUCTION OF NURSE CELLS AND OOCYTES. During the pupal period of one and one-half to two and one-half days, the germaria had reached their maximum length, 140 microns. This was accomplished by mitotic divisions of the germ cells in larval and early pupal periods, ceasing at two and one-half days. Subsequent to this age, a decrease in the volume of the germaria was observed as the flies matured, with no apparent mitotic activity. In contrast to this, King, Rubinson and Smith (1956) reported mitotic activity in the germaria of twenty-four-hour adult Drosophila melanogaster. LaChance and Bruns (1963) noted

mitotic activity in the pupae of Cochliomyia hominivorax (Cqrl.), but made no mention of its presence in the adults. This discrepancy is not surprising when the number of ovarioles and reproductive capacities are considered. Drosophila melanogaster had, on the average, twelve ovarioles per ovary, and in the gravid female possessed fourteen successive egg chambers (King, Rubinson and Smith, 1956). In Cochliomyia hominivorax (Cqrl.) an ovary possesses 100 to 150 ovarioles and at reproductive maturity contains three egg chambers (LaChance and Bruns, 1963). Musca domestica L. contains sixty-two to seventy ovarioles per ovary, also with three egg chambers (Morgan and LaBrecque, 1962). It appears that in Musca domestica L. the potential number of nurse cells and oocytes produced by a given ovariole is determined during the pupal development. However, it is possible that oogonial divisions in the adult house fly were masked due to crowding of the adult germarial contents and the techniques used in this investigation.

FORMATION OF EGG CHAMBERS. As in Cochliomyia hominivorax (Cqrl.), formation of the first egg chambers occurred during the pupal period. In pupae one and one-half to two and one-half days, an indentation was observed in the basal region of each germarium forming a cyst of cells. In each ovariole, epithelial cells separated this newly formed region from the remainder of the germarium in which closely packed aggregates of cells could be seen. About twenty-four hours later, each chamber had separated spatially from the

corresponding germarium but remained connected by a stalk of follicular cells, thus forming the first egg chamber. The second and third egg chambers appeared to develop in like manner. This process was essentially the same as in D. melanogaster and C. hominivorax (Cqrl.). All three species possessed fifteen nurse cells and one oocyte per chamber.

MATURATION OF OOCYTES. During adult oogenesis the volume of an egg increased from $7.25 \times 10^3$ cubic microns, as found in newly emerged adults, to $8.5 \times 10^7$ cubic microns in a mature egg. This amounts to an increase in volume of over 10,000 times. King, Rubinson, and Smith (1956) reported an increase in ooplasmic volume in Drosophila melanogaster by a factor of 100,000. King's measurements were based on frozen sections while those for the house fly were based on alcoholic fixed material--notorious for shrinkage.

In D. melanogaster proteid yolk is synthesized in the ooplasm from precursors produced in the nurse cell nuclei (Hsu, 1953; King and Vanoucek, 1960; Sirlin and Jacob, 1960), the growth being dependent upon the synthetic activity of the nurse cell nuclei. A similar mechanism probably exists in the house fly, as prior to vitellogenesis, endomitosis was observed in the nurse cell nuclei. It was first noted about twelve hours after emergence, and after forty-eight hours loosely associated threads gave the appearance of a diffuse nature to the chromatin. Also synthesis of fast

green positive material reached its peak in the nurse cell nuclei during yolk deposition--two and one-half to four and one-half days. That chains of Pyronin Y positive material were composed of RNA was verified by RNAase treatment. Their fast green affinity may indicate a proteinaceous coat. Similar results were observed in the nurse cell nuclei of D. melanogaster during vitellogenesis (King, 1960). Also as in D. melanogaster, prior to yolk deposition, the follicular cells about the oocytes differentiated into columnar epithelium. During yolk formation no change in RNA concentration was observed. Decrease in height of the columnar cells occurred during chorion formation, indicating chorion formation was a function of these cells. Similar events were noted by King (1960). Throughout this investigation no meiotic divisions were observed. The behavior of the oocyte "nuclei" closely followed the pattern observed in D. melanogaster by King, Rubinson and Smith (1956). The oocytes of the adults show a Feulgen positive body embedded in a reticulate nucleoplasm. This may be a true chromocenter, i.e., an association of the heterochromatic elements of the various chromosomes. Then the reticulate appearance of the nucleoplasm was the result of chromatin material that appeared weakly stained, due to physical dilution. If this was the case, then the results of Fahmy's (1952) and Sonneblick's (1950) work on Drosophila melanogaster would also apply to Musca domestica L., i.e., metaphase of the first meiotic division occurs after ovi-

position. On the other hand, the Feulgen positive body could represent the true nucleus. The zonated area surrounding it would then be a germinal vesicle. Lastly, meiosis could have occurred during vitellogenesis as reported by LaChance and Leverich (1962) in Cochliomyia hominivorax (Cqrl.). Although a diffuse appearance of each oocytic Feulgen positive body was observed at this time in Musca domestica L., distinct chromosomes were not apparent. King's (1960) observance of a double membrane surrounding the oocytic body makes the first hypothesis seem quite attractive. Further investigation of the chromatin material of freshly oviposited eggs and electron micrographs of the oocyte nucleus would shed a great deal of light on this problem in Musca domestica L.

## SUMMARY

1. Sperm maturation was essentially completed by emergence of adult flies, with the majority of spermatogonial divisions occurring during the third instar larval and early pupal periods.

2. Meiotic activity in the testes occurred throughout adult life but was considerably less than that found in the pupae.

3. Oogonial divisions were observed during early pupation with the first egg chambers developing before adult emergence.

4. Gravid females possessed one mature egg and two immature egg chambers per polytrophic ovariole at five days of age.

5. Fifteen polyploid nurse cell nuclei produced ribonucleic acid during vitellogenesis in each egg chamber.

6. Meiosis was not observed during oocyte maturation.

7. The fundamental oogenetic processes were similar to those found in Drosophila melanogaster.

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