Postembryonic development of the reproductive system of the European corn borer, *Ostrinia nubilalis* (Hübner)

by

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

Department: Zoology and Entomology
Major: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1973

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INTRODUCTION

Ostrinia nubilalis (Hübner), the European corn borer, was first introduced near Boston, Massachusetts. It has since spread throughout the corn producing regions of the ccuntry and is now one of the most destructive pests of corn in the United States. In spite of extensive research, many aspects of its basic biology remain unknown and unexplored. The first thorough account of the internal morphology of the larva was published as recently as 1966, and the first detailed description of the adult reproductive system only appeared in 1967. Research into various aspects of the reproductive biology, morphology and histology of this pest can benefit from a knowledge of the organogenesis and morphogenesis of its reproductive system. Therefore this study was undertaken to describe the normal development of the reproductive system of O. nubilalis and to compare its development with that of other Lepidoptera.

LITERATURE REVIEW

The literature on the insect reproductive system is extensive. This review is limited to the research on Lepidoptera. It is addressed primarily to those works which deal with the morphology and development of the internal genitalia. Anatomical studies, *per se*, are included only if, in my opinion, a significant contribution to our knowledge of lepidopteran morphology or development is made. Purely descriptive studies of external genitalia are excluded, as are embryologically orientated papers.

Although studies of the morphology of insects date back to the first naturalists, proper investigation of the internal organs could not begin until the invention of the microscope. Thus we find the earliest descriptions of the reproductive system of Lepidoptera are the classic works of Malpighi (1669) and Swammerdam (1738). Then, in 1762, Lyonet published a remarkable monograph on the larva of *Cossus cossus* (published as *Cossus ligniperda*). Among the organs discussed and illustrated are a pair of "*Corps reniformes*" found dorsally in the fifth abdominal segment. Although his description is somewhat confused, Lyonet correctly deduced these to be the larval gonads. I believe this work also marks the discovery of imaginal discs, although their significance was not realized until Weismann (1864) described their role in the postembryonic development of Diptera.

The first major contribution regarding reproductive system development is that of Herold (1815). His study of the metamorphosis of Pieris brassicae (published as Papilio brassicae) also included accounts of the musculature, digestive system and nervous system. Herold's work is the foundation for subsequent studies. He describes the growth of the gonads; the fusion of the paired larval testes into a single organ; and the genesis of the internal genital tract from rudiments in the larva.

The earliest histological study I have seen is that of Meyer (1849). Working with several different species, he compiled a fairly accurate picture of the histology of the gonads. He also described oöcyte and follicular growth and spermiogenesis. His observations were elaborated upon by Bessels (1867), but little else appears on the lepidopteran reproductive system until a series of papers by Cholodkovsky (1880, 1884, 1885). The last paper may be the first description of the monotrysian type reproductive system.

Cholodkovsky (1880, 1884) separated the testes of adult Lepidoptera into four types. Type 1, found in the Hepialidae, is the assumed primitive condition. It is characterized by two separate testes, each with four completely separated tubules or follicles. In Type 2, the "larval" type, the testes are separate but their follicles are enclosed within a common epithelium. Cholodkovsky de-

scribed this form in Bombyx mori, but it also occurs in some Tineidae, Lasiocampidae and Papilionidae. In the third type, the "pupal" type, the testes are fused and enclosed in a common epithelium, but they are medially constricted so that their paired nature remains evident. This is found in some Tineidae, Papilionidae, Geometridae and Lycanidae. Type 4 is the "imaginal" type and occurs in the majority of Lepidoptera. In this case the testes appear as a single structure, the medial constriction being absent. The follicles are usually spirally coiled around the longitudinal axis of the organ, and the vasa deferentia are usually crossed.

By the end of the ninteenth century, microtechnique had progressed far enough to encourage the first in-depth studies of morphogenesis. Thus we find that the works of Jackson (1890) on Nymphalis io (published as Vanessa io) and Verson and Bisson (1896a, 1896b) on Bombyx mori utilize serial sections. These are classic studies and details of them are cited throughout this paper. They were soon followed by the important contributicns of Petersen (1900, 1904), Stitz (1901, 1902), Gross (1903), Zander (1903, 1904) and Zick (1911). Between 1915 and 1929, Kuznetsov published the introduction to his monumental work on Lepidoptera in which he reviewed and summarized the previous literature. Usually ignored, no doubt largely because of its Russian language,

parts of it have recently been translated into English (Kuznetsov, 1967).

The 1920's and 1930's saw a flurry of activity, comparatively speaking. Weidner (1934) and Musgrave (1937) made important histological studies, as did Machida (1926). Norris (1932, 1933), Hewer (1934) and Ômura (1936, 1938a, 1938b) studied various aspects of the structure and operation of the reproductive system and Mehta (1933) and Dodson (1937) studied its development. Mehta's paper is based primarily on Pieris rapae. Dodson's work on Zygaena did not identify the species.

Details of the most recent developmental studies are discussed later. The most important of these are the works of Florin (1945), Ammann (1954) and Brunold (1957). Together they have given an especially thorough account of development in Solenobia triquetrella (Psychidae). Additional observations were made by Srivastava and Srivastava (1959a, 1959b) upon Leucinodes orbonalis (Pyraustidae). Joubert (1964a, 1964b, 1965) reported on the development and physiology of the reproductive system of Sitotroga cerealella (Gelechiidae) and of the pyralids Cadra cautella and Plodia interpunctella (Joubert, 1967, 1969). The study of Choristoneura murinana (Tortricidae) by Wittig (1960) is the latest publication in the specific area of this study.

MATERIALS AND METHODS

All specimens used in this study were obtained from the continuously reared stock at the USDA European Corn Borer Laboratory, Ankeny, Iowa. Freshly hatched larvae, no more than three generations from the wild, were placed on corn leaf diet (Guthrie et al., 1965b) in individual three dram shell vials. They were reared at 26.7 C and 75% relative humidity in continuous light. Under these conditions the pupal stage is reached in about 15 days and the first adults emerge about six days later. Each of the five larval stadia is approximately three days long.

Determination of Instar Age

A new stadium of an insect begins prior to the shedding of the cuticle of the previous instar and not with the shedding of the cuticle as is generally accepted (Hinton, 1968). "Apolysis" (Jenkin and Hinton, 1966), the freeing of the hypodermis from the old exoskeleton, marks the beginning of the new stadium. However, in the European corn borer I found no consistent externally visible indication of either the onset or completion of apolysis. Furthermore, it is apparent from sectioned material that apolysis does not occur uniformly throughout the organism, but that the dorsal hypodermis becomes free of the cuticle before the ventral hypodermis does; and, in at least the fifth larval and the

pupal stages, the posterior abdominal segments are freed well before the anterior segments. At what point, then, can one say a new corn borer stadium begins? Unable to satisfactorily answer this question, I separated the stadia in the traditional manner by using ecdysis to mark the end of one stadium and the beginning of the next. Consequently, "pupa," "fifth instar," etc. refer to the insect while still within the respective cuticle and "pupation" refers to the larval-pupal ecdysis.

Because of variations in growth rate of individual larvae, chronological age alone is a poor estimator of physiological development. Therefore larvae were fixed on each of 15 successive days and the instar was determined prior to dissection or embedding by head capsule measurements. Thus the chronological age and the instar were known for each specimen. Larvae were further categorized within stadia as "early," "mid," or "late" instars on the basis of the extent of apolysis. I made no attempt to distinguish pharate stages, but it is obvious that a "late fourth instar," for example, could easily be a pharate "fifth instar."

Pupal ages are stated in hours after pupation, determined as follows. Once pupae had begun to appear in a stock, each individual vial was examined every 15 minutes. Vials containing an insect that had started or completed the larval-pupal ecdysis were marked with the date and time.

Pupal age is dated from this point, that is, within 15 minutes of pupation. Specimens were fixed at pupation ("0-hour pupae") and at 6-hour intervals for the first 36 hours afterwards; at 12-hour intervals for the next 60 hours; and at 24-hour intervals until the adults began to emerge.

Gross Morphological Technique

"Macroscopic" and "gross" are used herein when referring to any structure large enough to be studied with a dissecting microscope at magnifications of 40X or less. This also applies to operative procedures, i.e., "gross" dissections that can be carried out at these magnifications. Contrarily, any structure small enough to require greater magnification is considered "microscopic". Such material was serially sectioned for study with the compound microscope at magnifications of 100X or above.

Fixation and dissection

Larvae and pupae intended for gross morphological study were fixed in the formalin-acetic acid-chloral hydrate solution of Chauthani and Callahan (1966). The specimens were stored in fresh fixative. The procedure used was the same as described under "Microtechnique" for the preparation of specimens for sectioning.

Living specimens were dissected in a saline solution and fixed specimens were dissected in water. Measurements were made with an ocular micrometer fitted to the dissecting mi-

croscope. There were no evident differences between living and fixed organs and all measurements reported herein are taken from fixed materials. The first instar was never dissected, and most attempts at dissecting the second instar were unsatisfactory. Consequently, no gross measurements were taken and descriptions of these two instars are based entirely on sectioned specimens.

An aqueous solution of methylene blue or aniline blue was used to differentiate the internal organs. Methylene blue stains less intensely, but it is more selective and so was generally preferred. Some dissections were made of larvae and pupae fixed in Bouin's fluid. This fixative makes the tissues brittle, but this was alleviated somewhat by leaving the specimens in water overnight.

Microtechnique

Fixation

Larvae to be sectioned were dropped into a jar of hot (50 C to 55 C) alcoholic Bouin's fixative (Davenport, 1960). Then, with fourth and fifth instars, several prolegs were cut off to facilitate fluid exchange. The first three instars are so small that removal of the prolegs was not necessary. The larvae, in the fixative, were placed in a vacuum desiccator and the vacuum was slowly brought to 25 cm Hg. This step was carefully controlled for too great a reduction of pressure either caused the fixative to boil or distorted

the specimen. After 24 hours the vacuum was slowly reduced and the specimens were transferred to 70% ethanol for storage. The alcohol was changed at irregular intervals throughout the storage period. Pupae were fixed by the same two procedures. After being in the fixative 10 or 15 minutes the cremaster was cut off and the abdomen was severed at the second or third segment.

Infiltration and embedding

The cuticle presents a tremendous obstacle to sectioning insects. Although the literature is replete with sectioning techniques, there is no truly satisfactory method. Several procedures were tried in addition to many variations of the common ethanol-xylene technique. The best results, considering both quality of sections and simplicity of procedure, were obtained from two ethanol-toluene techniques. Larvae and excised organs were dehydrated in ethanol, cleared in toluene, infiltrated, and embedded in paraffin. Pupae, and frequently large larvae, were dehydrated in ethanol and double-infiltrated with celloidin and paraffin. The following schedules were arrived at by trial and error. Both procedures are flexible and, except for the clearing and infiltration steps, can be modified almost at will. Clearing or infiltration for less than the minimal time stated produced inferior sections; longer times, especially for large specimens, are advised. Prolonging the time in these steps

did not adversely affect the quality of the sections and, for convenience, specimens were routinely left overnight in the first toluene and first paraffin baths.

Procedure for larvae and excised organs Specimens fixed and stored as previously described were dehydrated in ethanol and cleared for two hours in each of three changes of toluene. After clearing, the specimens were transferred to melted Paraplast® (Fisher Scientific Co., melting point 58 C) in a vacuum oven and kept at 60 C and 37.5 cm Hg overnight. Two additional baths of two hours each followed. The specimens were embedded in fresh paraffin in small aluminum foil boats as described by Echols (1955).

Procedure for pupae (or large larvae) The procedure was the same through the clearing step. After toluene, the specimens were placed for one hour in each of two changes of ether-absolute ethanol (1:1) from which they were transferred to 2% Parlodion® (Mallinckrodt Chemical Works) in ether-ethanol for three days. They were then rinsed well in ether-ethanol and the celloidin was hardened in chloroform for one hour. Three changes of toluene (minimum of two hours each) were followed by Paraplast infiltration and embedding as before.

Sectioning

Sections were cut on a Spencer rotary microtome at 6 μ, 8 μ, or 10 μ depending upon the size of the specimen. Trans-

verse and sagittal serial sections were made and some specimens were cut in the frontal plane. Continuous ribbons through entire larval and pupal abdomens were usually obtained. The ribbons were floated on 2% formalin on slides previously cleaned in alcohol and smeared with a gelatin affixative (Haupt, 1930). The sections were flattened on a warming tray (45 C) and the excess formalin removed. After covering the slides with filter paper soaked in distilled water, the sections were then pressed firmly to the slide by rolling over the filter paper with a small glass jar. The slides were dried overnight on the warming tray and stored in dustproof boxes.

Staining

All sections were washed for 10 minutes in Lenoir's solution (Gray, 1964) and rinsed in running water before being stained regressively with Ehrlich's hematoxylin (Humason, 1967). Most were counterstained with eosin (Luna, 1968) or an eosin-orange G mixture (Humason, 1967) and mounted in Permount® (Fisher Scientific Co.)

Whole mounts

Glycerine jelly whole mounts were prepared of excised organs using the procedure outlined by Galigher and Kozloff (1964). Whole mounts were made of both stained and unstained specimens. To stain, excised organs were brought to 70% ethanol to which a drop of Ehrlich's hematoxylin had been

added. Destaining, if necessary, was by visual inspection in 0.5% hydrochloric acid in 70% ethanol; blueing was in 0.5% ammonium hydroxide in 70% ethanol. All procedures were carried out in a depression slide under the dissecting microscope.

Stained and unstained specimens were also mounted in Permount. After dehydration in absolute ethanol, the material was cleared in toluene and Permount was gradually added to the last toluene bath until the mixture approached the consistency of fresh Permount. The specimen was then mounted in fresh medium on an alcohol-cleaned slide.

Illustration Techniques

The photomicrographs were taken with a Leitz Orthoplan® microscope equipped for bright field and phase microscopy and fitted with a 35 mm camera. The drawings were made from photomicrographs or with a microprojector (Bausch and Lomb Tri-Simplex®) having 10X and 43X objectives. Some were made freehand with a dissecting microscope fitted with a grid micrometer, and others are freehand reconstructions from serial sections. It is stated in the legends which method was used for a given figure.

RESULTS

Female Reproductive System: Morphology

The morphology of the internal reproductive system of the adult female European corn borer has been described briefly by Vukasović (1947) and in detail by Drecktrah and Brindley (1967). Brief descriptions of the larval reproductive systems have also been published (Larsson, 1929; Drecktrah et al., 1966). Both the larval and adult systems conform to the basic morphological pattern of other Lepidoptera as described by numerous researchers. I have diagrammatically represented these systems in Figs. 1 and 6 in order to summarize the previous studies and to serve as a point of departure for the research presented here.

Larva

Three distinct components of the reproductive system can be recognized in the female larva. They are (1) the ovaries, (2) the genital cords, anlagen of the lateral oviducts, and (3) paired imaginal discs, the anlagen of the ectodermal parts of the definitive system.

The small ovaries are located dorsally in the fifth abdominal segment, ventrolaterad of the dorsal blood vessel. They are almost completely surrounded by fat body and are very difficult to see, which may explain why some otherwise thorough accounts of larval morphology (Peterson, 1912;

Teotia and Pathak, 1957) either give inadequate descriptions of the reproductive organs or ignore them altogether. Each ovary consists of four digitate ovarioles enclosed within a peritoneal sheath or ovarian sac. The ovarioles of each ovary converge posteriorly and are confluent with a slender strand, the genital cord. Each genital cord (Fig. 1) extends caudolaterally to the seventh abdominal segment where it passes dorsad to the visceral trachea, turns ventrad, and follows the ventral segmental trachea toward the midline. After passing below the ventral musculature, the cord separates from the trachea and terminates blindly on the hypodermis near the posterior margin of Segment 7. The two cords may meet in the midline. Although often referred to as the "lateral oviducts" (Dodson, 1937; Joubert, 1964a, 1969; Drecktrah et al., 1966) the genital cords are thin, solid, lumenless strands, quite threadlike in appearance. Herold (1815) actually called them "Fäden," but "Genitalstrãng" has gained wide acceptance (Verson and Bisson, 1896b; Sato, 1932; Ammann, 1954; Wittig, 1960). It is from this word that I have taken the term "genital cord" which I use until the time they assume their tubular form to become the definitive lateral oviducts. This usage is a moot point, but it contributes to a more precise description of their morphogenetic state.

The anlagen of the ectodermal portions of the reproductive system are variously referred to as imaginal discs, embryonic discs, genital discs, genital buds or genital primordia. They are paired invaginated thickenings of the ventral hypodermis of the eighth and ninth segments. I simply designate them Discs 8 and Discs 9. Using Heinrich's (1919) setal map of the European corn borer they are located between Setae VII and VIII of their respective segments (Fig. 2). There is no external evidence of their presence, and they cannot be seen in living larvae without opening the abdomen and removing the ventral musculature. Occasionally they can be seen through the cuticle of preserved larvae.

Pupa

Mosher (1919); Heinrich (1919); Buligan (1929); and Caffrey and Worthly (1927) have all described the pupa of the European corn borer, and the pupae used in this study did not vary significantly from their descriptions. Length of the female pupa (based on 40 specimens) is 14.4 mm to 16.2 mm, mean 15.3 mm; width is 3.4 mm to 3.8 mm, mean 3.6 mm. Length of the male (based on 40 specimens) is 13.6 mm to 15.3 mm with a mean of 14.4 mm; width is 3.0 mm to 3.5 mm, mean 3.3 mm. Pupal weights were not recorded but Lewis, Mutchmor, and Lynch (1971) report weights of approximately 80 mg and 110 mg for the male and female, respectively.

The difference in the size of the two sexes is more obvious than the foregoing figures would indicate. The male is more tapered and slender, so the female appears much more robust. Thus the sexes can often be distinguished on the basis of size and shape alone. However, the best way to determine pupal sex is by the position of the genital openings (Figs. 4 and 5) which appear as small, dark, mesoventral grooves in the cuticle. In the male (Fig. 5), Segment 8 is ringlike and the groove does not reach its anterior margin. But in the female (Fig. 4), Segments 8 and 9 are highly emarginate ventrally and the genital opening reaches from the anterior margin of Segment 9 to the posterior margin of Segment 7. Furthermore, the male genital opening is caudad of the eighth abdominal spiracle, whereas the female opening is cephalad of it. Caffrey and Worthly (1927) incorrectly position the respective openings caudad and cephalad of the seventh spiracle.

Adult

The European corn borer, like most Lepidoptera, has a ditrysian reproductive system (Fig. 6) with two external openings, one being for copulation and the other for oviposition. The copulatory opening, the ostium bursae, is on the eighth segment. It opens into a slightly twisted duct which leads cephalad to a terminal pouch, the corpus bursae. These three structures, the ostium bursae, the ductus bursae

and the corpus bursae, compose the lepidopteran bursa copulatrix. The corpus bursae lies mediodorsally in approximately the fourth segment. Its position may vary a segment or so either cephalad or caudad. A short thick duct, ending in a relatively thin-walled sac, extends caudad from the posteriodorsal margin of the corpus bursae. Drecktrah and Brindley (1967) named this the "bursal gland" and stated that no similiar structure had been reported in any other species. Mutuura and Munroe (1970) have since revised the genus Ostrinia. They illustrated the corpus bursae of specimens representing 17 of the 20 species in the genus. Within each species illustrated, at least one subspecies has a structure similar to the bursal gland of the European corn borer.

The oviporus opens into a short tube that is often called the vagina (Kuznetsov, 1967; Norris, 1932; Swart, 1966; Drecktrah and Brindley, 1967). This tube is a derivative of the ninth segment and therefore is not homologous to the vagina of other insects. However, the term is so deeply embedded in the literature that I use it in preference to coining a new term and adding to the confusion. For a concise discussion of the insect vagina and its homologues see Snodgrass (1935). In the context of this study I shall define the vagina as that part of the median egg passageway posterior to the origin of the seminal duct and spermatheca.

From the dorsal surface of the vagina, near the oviporus, the short accessory gland duct leads to the accessory gland reservoir. Just anterior to the accessory gland duct are two lateral swellings, the "vaginal pouches" (Drecktrah and Brindley, 1967). The accessory glands, each about 20 mm long and 0.1 mm wide, extend from the dorsolateral margins of the reservoir. They leave the reservoir in a caudad direction and then turn cephalad. In the vicinity of the corpus bursae they again reverse directions and finally terminate near their origin.

Petersen (1900) defined the lepidopteran vestibulum as the part of the egg passageway that receives the spermathecal and seminal ducts. This is the sense in which Norris (1932), Weidner (1934), Ammann (1954), Kéler (1963), Callahan and Chapin (1960) and Swart (1966) all use the term. I will follow their usage and also show that the vestibulum is derived from the eighth segment, and its homologue in other insects is the "vagina" of Snodgrass (1933, 1935). It is imperative that one realize that Peterson's "vestibulum" is not the same as that of Snodgrass. Snodgrass (1933, 1935) uses the term for a secondary external cavity above the seventh sternum of insects in which the seventh sternum extends beyond the eighth. Additionally, my concept of the lepidopteran vagina and vestibulum automatically restricts the usage of "median oviduct" or "common oviduct" to that

part of the egg passage anterior to the vestibulum. This is contrary to many authors who use these terms to indicate the entire common egg passage.

Drecktrah and Brindley (1967) use "vestibulum" in a totally different context; they use it to designate an abrupt swelling at the base of the spermathecal duct where it joins the median egg passage. Eidmann (1929) and Khalifa (1950) also use the term this way. Although absent in representative Pieridae (Kuznetsov, 1967) and Noctuidae (Callahan and Chapin, 1960; Callahan and Cascio, 1963), a similar enlargement has been reported in many species. Some authors have illustrated it without labelling it (Crawford, 1971; Tedders and Osburn, 1970). Others have used descriptive words such as "tubercle" (Joubert, 1964b) or "bulla" (Fatzinger, 1970). But the most widely used designation is "infundibulum" (Musgrave, 1937; Srivastava, 1960a; Swart, 1966; Outram, 1971). I use this term instead of Drecktrah's and Brindley's appellation because I accept Petersen's usage of "vestibulum."

The spermatheca proper of the European corn borer is a bilobed organ consisting of an elongated lobe, the utriculus, and its mesad dilation, the lagena. Distally, the utriculus joins the very long spermathecal gland via a short thin duct. The utriculus and lagena fuse proximally to form the tightly coiled spermathecal duct which opens into the abruptly ex-

panded infundibulum. The spermathecal gland is about 40 mm long and usually makes two loops through the hemocoel before ending in a bifrucation near the accessory gland reservoir.

The 1.5 mm long seminal duct leaves the vestibulum from its left ventrolateral side and makes a cephalic loop before entering a ventral dilation of the ductus bursae. The median oviduct is continuous with the anterior end of the vestibulum. Both it and the lateral oviducts are about 1 mm long. The four ovarioles of each ovary are 30 mm to 35 mm long. They double back upon themselves two or three times, ending dorsally in the third or fourth segment. Except for the distal 0.3 mm to 0.5 mm, the ovarioles are separated from each other by the fat body, but their distal ends are bound together. There is no terminal filament.

Female Reproductive System: Development

I will describe the development of the female reproductive system from the time of hatching to the point at which the definitive organs can first be clearly recognized. Thus, this study deals primarily with organogenesis and disregards the growth of the organs once they appear. Neither is a detailed study of histogenesis attempted. The hematoxylin and eosin technique used for most of the work is not selective enough for critical histological observations. The histology reported here is selected to emphasize morphological uniformity or change at a given developmental

stage, and is not intended to be a complete description.

Larva

First stadium Serial sections of larvae fixed within one hour of hatching reveal a small patch of tissue on each side of the dorsal blood vessel in the fifth abdominal segment (Figs. 7,8). These are the gonads, but at this stage the sex cannot be determined. The young gonads consist of a few cells enclosed within a thin peritoneal sheath. The boundaries of the sheath cells are not distinct, but their elongate nuclei are 4 μ long and 2 μ wide. The germ cells number from four to eight and may be 6 μ or 7 μ in diameter. They have large (5 μ), rounded, granular nuclei and basophilic cytoplasm. The entire gonad is about 25 μ long and 10 μ in diameter. Its general structure and appearance do not differ significantly from the young gonad of other Lepidoptera (Machida, 1926; Sato, 1932; Lautenschlager, 1932; Wittig, 1960). In light of the many reports of recognizable gonads in first instar lepidopterans, it is noteworthy that Joubert (1964a, 1965, 1967, 1969) found no sign of the gonads in either sex of S. cerealella, C. cautella or P. interpunctella prior to the third stadium.

The sexes can be distinguished a day after hatching by the presence in the male of the primordium of the ectodermal portions of the reproductive system. This is an unpaired invagination of the midline near the hind margin of the ninth

segment (Fig. 3, Herold's organ). No such invagination is present in the female. The ectodermal primordia of the female, paired invaginations of both the eighth and ninth segments, do not appear until the third stadium. The gonads themselves, however, do not yet show significant differences.

Usually the anterior parts of the genital cords can be found by the second day of larval life. Each cord consists of a single row of cells leading caudad from the gonad. They are very thin (2 μ to 3 μ) and, consequently, so difficult to trace that in no first instar could their posterior terminations be found. Possibly they are incomplete and do not reach the venter as they do in later stages. Joubert (1964a, 1969) reported this to be the case in the Angoumois grain moth, the almond moth, and the Indian meal moth. He found the genital cords are not completed in these insects until the pupal stage. On the other hand, both Ammann (1954) and Wittig (1960), respectively, traced the completed genital cords of first instar larvae of S. triquetrella and C. murinana to the venter of Segment 7.

Although more easily seen in second instar larvae (Fig. 9), the anterior end of the genital cord sometimes appears slightly clavate, resembling the "Ausführgang" of Ammann (1954) and the "proximale Endmasse" of Wittig (1960). It lies adjacent to a cluster of small undifferentiated cells now visible at the base of the gonad. Wittig (1960) de-

scribed similar cells as the "halbkreis förmiger Mittelteil," but Lautenschlager (1932) and Ammann (1954) simply referred to apparently homologous cells as an undifferentiated cell mass. In sagittal section these cells are spindle shaped, about 6 μ long, and have small oval nuclei. This cell mass is the primordium of the pedicels and the calyx.

Second stadium During the second stadium the ovarioles almost double in size, attaining a respective length and diameter of 65 μ and 40 μ. The genital cords attain a 6 μ diameter and their attachment to the ventral hypodermis is established. Each cord ends blindly laterad of the midline. By midstadium the germ cells are grouped into four poorly defined clusters within the peritoneal sheath or ovarian sac (Fig. 10). The clusters are quite distinct in the third stadium (Fig. 12). The germ cells multiply, and a few small cells resembling the sheath cells form a loose stroma between the germ cells and the ovarian sac.

Third stadium The third instar is large enough to be dissected easily. The gonads lie so close to the dorsum that they are best revealed by making an incision in the ventral midline and removing the gut. Staining is necessary to differentiate the ovaries and genital cords from the dorsal fat body (Fig. 19). (However, the testes can usually be seen without a stain.) The stained genital cords can be traced from the ovaries to their point of attachment to the

hypodermis.

The undifferentiated cell mass produces four short branches. These are the future pedicels (Fig. 11) which, distally, are continuous with the young ovarioles. The proximal common base of the pedicels, the future calyx, is continuous with the genital cord. The cells of the calyx and pedicels are smaller than those of the genital cord. However, their oval nuclei stain more intensely than the nuclei of the genital cord cells. At midstadium an ovariole and its pedicel are about 80 μ long and they may become as much as 130 μ long before the third ecdysis. As the stadium progresses the innermost stromal cells, those adjacent to the germ cells, become flattened and begin to form a simple epithelium around the germ cells. This layer fully differentiates during the fourth stadium (Fig. 24).

The most significant event in the third stadium is the appearance of the imaginal discs of the ectodermal portions of the female reproductive system. Their location has already been given. Discs 8 (Fig. 13) appear a little earlier than Discs 9 (Fig. 14), and the anterior pair is slightly larger throughout the course of development. In transverse section one of the pair in Segment 8 measures 40 μ wide and 15 μ thick, which is about three times the thickness of the hypodermis. A small cluster of undifferentiated cells (at the arrows, Figs. 13-18) sits atop each disc. Wittig (1960)

described similiar cells associated with the genital discs of C. murinana.

The genital discs are independent of the mesodermal portions of the reproductive system, the ovaries and genital cords. The genital cords extend no farther posteriad than the seventh segment, and there is no connection between them and the imaginal discs other than the hypodermis itself. Furthermore, no connection between the mesodermal and ectodermal parts of the system is established until the fifth stadium.

Fourth stadium No new structures appear in the fourth stadium, but growth of those previously formed proceeds rapidly. The ovarioles (Figs. 20, 21) usually reach a diameter of 35 μ to 40 μ and a length of 175 μ to 200 μ, but the longest fourth instar ovariole measured was 250 μ. An ovary (Fig. 24) consists of four groups of germ cells, each surrounded by an inner epithelium. Adjacent inner epithelia are separated by the stroma, which forms a loose reticulum of varying thickness. Germ cells, inner epithelia, and stroma are all enclosed within the outer epithelium. Some small cells, possibly young follicle cells, are scattered among the larger germ cells. They are not very numerous and are concentrated toward the pedicel. Machida (1926) identified follicle cells within the ovary of freshly hatched silkworm larvae, but Wittig (1960) indicated they did not

differentiate in C. murinana until the fourth stadium.

The genital cords grow to a diameter of 7 μ to 9 μ by midstadium and by the end of the stadium their diameter (12 μ to 14 μ) is nearly twice that seen in third instar larvae. The two genital cords may become joined by a transverse strand of cells that lies adjacent to the ventral hypodermis. There is no indication of a hypodermal origin of this connection. As it appears identical to the cords, I assume it results from the mesial growth of the genital cords themselves. The time of formation of this transverse connection is variable; it is often not established until near the middle of the fifth stadium.

The imaginal discs enlarge considerably. Figures 15 and 16 are typical transverse sections through Discs 8 and Discs 9, respectively, of a late fourth instar. As previously noted, Discs 8 develop somewhat earlier than do Discs 9, and toward the end of the stadium both pairs begin to invaginate. Although the hypodermal invaginations become quite deep, the cuticle is not similarly folded and the discs are not readily seen except in sections. Rarely was I able to see the discs through the cuticle, although Umeya (1927) reports the discs of the silkworm are easily seen through its cuticle "with the naked eye." However, the discs of B. mori are much larger than those of O. nubilalis.

The mass of previously undifferentiated cells associated with the surface of each disc gives rise to a long thin strand which can be traced to one of the nerves from the eighth abdominal ganglion. No attempt was made to determine the exact relationship of this developing nerve to the rest of the nervous system, but Wittig's (1960) description of what is doubtlessly its homologue in Choristoneura fits quite well.

Fifth stadium Ovarian growth is conspicuous in the fifth stadium (Figs. 22, 23). About midstadium, the innermost stromal cells once again become flattened. They soon differentiate as another sheath, the middle epithelium, around the individual ovarioles (Fig. 25). Three epithelia are now associated with each ovary. The outer epithelium encloses the entire ovary, and a middle and inner epithelium constitute the walls of the ovarioles. This three-layered condition is very transitory, however, for even as the middle epithelium is forming, the outer epithelium begins to degenerate (Fig. 26). Elongating rapidly, the ovarioles rupture the weakened outer epithelium late in the fifth stadium. This observation agrees with that of Srivastava and Srivastava (1959b) on L. orbonalis, but Machida (1926) and Dutkowski (1969), respectively, report the ovarian sac of B. mori and Galleria mellonella is not ruptured until well into pupal life.

When the ovarioles penetrate the outer epithelium, the middle and inner epithelia are not broken; they remain intact and grow with the ovarioles. The former middle epithelium is now the outer wall of the ovariole (Fig. 27). Except for their upper ends, the ovarioles are free of each other until fusing at the calyx. The upper ends of the ovarioles, approximately the length of the germarium, remain bound together within the remnants of the outer epithelium. This area soon becomes invaded by tissue elements which form a dense mass that adheres tightly to the ovarioles. This mass was not studied further, but Joubert (1969) described similar cells which he designated as the "histogenetic sheath."

A few follicles begin to develop in the lower portion of the ovarioles. At this point they are poorly formed, but their typical polytrophic structure is evident. At about the time the ovarioles penetrate the outer epithelium the pedicels develop lumina. The lumina first appear just below the lowermost follicle. The follicle is separated from the pedicellar lumen by a plug of tissue consisting of the upper end of the pedicel and some interfollicular tissue. This epithelial plug persists until just before ovulation. By the end of the fifth stadium the calyxes also have lumina, but the genital cords are still solid.

The fifth stadium, especially the latter part, is a period of very rapid differentiation and growth of the

ectodermal parts of the reproductive system. In short, Discs 8 and Discs 9 enlarge, and each pair fuses in the midline. An additional imaginal disc appears in the seventh segment, and the three anlagen move close together as the posterior abdominal segments telescope into the seventh segment. Differentiation of the definitive organs begins, and at pupation the following structures, or their rudiments, can be seen: median oviduct, bursa copulatrix, spermatheca, vagina, and accessory glands. Their formation is described in detail below.

The imaginal discs of an early fifth instar are shown diagrammatically in Fig. 31a; in transverse section in Figs. 17 and 18; and in sagittal section in Fig. 28. The condition of the discs at midstadium is illustrated by Figs. 29 and 30. The discs are slightly depressed in the center and the depressions are occupied by the terminus of the developing nerve described in the fourth instar.

With the loosening of the larval cuticle the discs begin to migrate toward the midline. It was not determined exactly how this migration is brought about, although it is accompanied by a partial breakdown of the midventral hypodermis. The end result of this movement is the fusion of the two pairs of lateral discs into two unpaired medial rudiments. The fused discs of Segment 8, hereafter referred to as the <u>vestibular rudiment</u> (Figs. 31b, 32), will give rise

to the vestibulum, bursa copulatrix, spermatheca, seminal duct, and a portion of the median oviduct. The rudiment in the ninth segment, hereafter the vaginal rudiment (Figs. 31b, 33), results from the coalescence of Discs 9; it will produce the vagina and the accessory glands.

From the dorsal aspect the vestibular rudiment is a subspherical growth on the ventral hypodermis, 0.25 mm to 0.35 mm in diameter. In transverse section it resembles an inward fold which forms a small groove in the hypodermis. This vestibular groove is extended both anteriorly and posteriorly by the progressive folding of the hypodermis. As it elongates posteriorly it meets a similiar groove, the vaginal groove, formed beneath the vaginal rudiment. The vestibular groove becomes continuous anteriorly with the oviducal groove which is formed from an imaginal disc in Segment 7. Disc 7 and the oviducal groove will be described later.

As soon as the vestibular groove elongates, its margins fuse together. Thus a small tube is formed just beneath the hypodermis. The central part of the vestibular groove remains open, and the tube grows anteriorly and posteriorly from this opening. The anterior growth becomes the median oviduct and the posterior portion becomes the vestibulum. The central opening will become the ostium bursae. Concurrent with the closure of the vestibular groove, the

anterodorsaland posterodorsal portions of the vestibular rudiment enlarge and elongate (Fig. 31c). The former enlargement is the anlage of the bursa copulatrix; the latter is the anlage of the spermathecal complex, that is, the infundibulum, spermathecal duct, spermatheca proper, and the spermathecal gland. In O. nubilalis the rudiments of the bursa and spermatheca appear simultaneously, although in one specimen the bursal anlage developed a little before the spermathecal primordium. Brunold (1957) observed the reverse sequence in S. triquetrella in which the posterior spermathecal rudiment normally appears first and then the bursal rudiment differentiates.

The first radical difference in the developmental pattern of Discs 8 and Discs 9 occurs at the time Discs 9 coalesce to form the vaginal rudiment. Although intimately fused anteriorly, Discs 9 do not quite join posteriorly so that, from the dorsal aspect, the young vaginal rudiment resembles a very fat letter Y (Figs. 31b, c). The stem of the Y is directed anteriad and is open ventrally as the vaginal groove. The two arms of the Y are directed posteriad. They are at first open ventrally, but they close quickly to form two short blunt tubes. These are the rudiments of the accessory glands. Their short common base, the future accessory gland duct, is ccntinuous with the stem of the Y, i.e., the dorsal wall of the vaginal groove. The

vaginal groove closes by the fusion of its margins in the same manner as did the vestibular groove. The most posterior part of the groove remains open to become the oviporus. The fusion process starts near the origin of the accessory gland duct and proceeds anteriorly. As it does so, the hypodermis is progressively folded, extending the groove anteriad, and, as already mentioned, it eventually becomes continuous with the posterior extension of the vestibular groove.

The median oviduct is derived partly from the vestibular rudiment and partly from an imaginal disc in the seventh segment. The latter, Disc 7 (Figs. 31b, 34), appears late in the fifth stadium and, unlike Discs 8 and Discs 9, is apparently unpaired. Ammann (1954), however, reported that in S. triquetrella this disc is originally paired, and I may not have observed a specimen in the proper morphogenetic state to show its bilateral origin. First seen about the time the vestibular groove begins to close, Disc 7 is a broad thickening of the hypodermis just posterior to the transverse connection of the genital cords. Almost immediately it flexes inward and once again a groove is formed, this one being the oviducal groove (Fig. 35). Compared to the vestibular and vaginal grooves, the oviducal groove is very short, broad and shallow. The anterior margin of the infolded disc forms a solid tongue of tissue adjacent to the genital cords' transverse connection, and the oviducal groove

begins just behind this solid fold. With the growth and folding of Disc 7, the genital cords are lifted above the hypodermis while the oviducal groove elongates posteriorly (Fig 31c). The oviducal groove becomes continuous with the anterior part of the vestibular groove and, with the complete closure of the two, the median oviduct is established (Fig. 31d). Most of these changes occur late in the fifth stadium, and the condition of the reproductive system at pupation is shown in Figs. 36 and 37.

Pupa

Although pupae of many ages were studied, female reproductive system development can be described in five stages represented by pupae 0, 18, 36, 48, and 72 hours old. The development of an organ is not followed all the way to the adult condition but to the point where the organ becomes a clearly differentiated entity, and subsequent growth is not described.

There is considerable variation in the degree of development within each of the forementioned pupal stages. Some of this is simply variation in individual growth rates; some of it is doubtlessly due to the crude method of defining the point at which the larva becomes a pupa. Therefore the illustrations, unless stated otherwise, show the appearance of the least developed specimens within each pupal stage; the most developed specimens grade into the next stage.

Moreover, little significance should be given to an organ's exact position, and especially not to that of its distal parts, even though its site of origin is constant. The pupal stadium is characterized by rapid histo- and organogenesis accompanied by much movement of the abdomen, and mechanical stresses alone may account for many of the spatial rearrangements that take place.

0-hour pupa During the quiescent prepupal stage, Segments 8 and 9 (and 10) telescope into Segment 7. As a result of this shortening of the abdomen, together with the growth of the rudiments, the ectodermal parts of the female reproductive system appear at pupation as in Figs. 36 and 37. At one time the rudiments form a more or less continuous ventral groove. A corresponding furrow, reaching from the rear of Segment 7 to the front of Segment 9, is formed in the pupal cuticle. The eighth and ninth pupal sterna are so emarginate that the furrow is only about 0.2 mm long, but its position, and the appearance of the emarginate sterna, are the characters used to identify the sex of the pupa.

The dorsoanterior portion of the vestibular rudiment is elongated into a somewhat laterally flattened tube which is slightly swollen at the apex. This swelling is the future corpus bursae. The dorsoposterior portion of the vestibular rudiment has produced the primordium of the spermathecal complex. It is 0.18 mm to 0.25 mm long and has a diameter of

70 μ to 80 μ. The vestibular rudiment is joined anteroventrally by the almost completed median oviduct which is 0.3 mm to 0.4 mm long and 60 μ to 100 μ wide. Completion of the median oviduct occurs very near pupation for in half the 0-hour pupae, and in all 6-hour pupae, the oviducal groove and the anterior part of the vestibular groove were closed ventrally.

The vaginal groove is continuous with the posterior portion of the vestibular groove, but complete ventral closure usually does not occur until six hours after pupation. Thus at pupation the vaginal rudiment and vestibular rudiments share a common external opening (dotted outline, Figs. 36, 37). From the posterodorsal surface of the vaginal groove a short (80 μ) duct leads to the paired accessory glands which are now 0.1 mm to 0.2 mm long and 80 μ wide.

Histologically, the ectodermally derived parts of the reproductive system are composed of a continuous epithelium 20 μ to 40 μ thick. This epithelium consists of columnar cells with oval or round nuclei, 5 μ in diameter, located basally in the cells. The cytoplasm is very basophilic and stains intensely with hematoxylin. The basement membrane and the cell boundaries are very indistinct. A very low and poorly defined brush border can be found throughout the system. Most of the system is covered by an external sheath of rather undifferentiated cells with small oval or round

nuclei 2 μ to 3 μ in diameter. This presumptive muscle tissue is thickest around the vestibulum and vagina. There the muscle sheath is 10 μ to 20 μ thick (three to six layers of cells), but around the median oviduct and the proximal half of the accessory gland rudiment it is only one cell thick. The basal half of the spermathecal rudiment is covered with a thick sheath, but there is no such tissue around the distal half of either the spermathecal or accessory gland rudiments.

18-hour pupa During the 18 hours following pupation no new structures differentiate, but several refinements are produced in those already present. A well-developed brush border (3 μ to 5 μ high) is the most striking histological feature of the 18-hour pupa. It uniformly lines the lumen of all the ectodermally derived organs. At 48 hours it is irregular and only about half as high as at 18 hours; it is not seen at all in the 72-hour pupa. The epithelium itself varies from 20 μ to 40 μ in height. It is tallest in the area of the vestibulum and gradually becomes lower distally; it remains very basophilic.

As previously indicated, the ventral groove is closed six hours after pupation and the definitive ditrysian openings are established. The vestibulum is now reduced to a small area shared by the median oviduct, ductus bursae, spermatheca, and vagina. The axes of the median egg passage

and ductus bursae (dctted lines, Fig. 38) cross in the vestibulum and will not be separated for another day. The ductus bursae is 0.3 mm to 0.4 mm long and the corpus bursae, which may be rounded or flattened, is 0.2 mm to 0.3 mm in diameter. The vagina leaves the vestibulum posteriorly and there is nothing to indicate exactly where the derivatives of the eighth and ninth segments join.

About six hours after pupation, a distal bifurcation of the spermathecal rudiment (Fig. 38e) is formed. Of five 6-hour pupae examined, three had very short bifurcations and two had none; all 12-hour spermathecae were bifurcated, and at 18 hours the branches were 0.10 mm to 0.12 mm long and about 50 μ in diameter. The remainder of the rudiment is 0.50 mm to 0.65 mm long.

Except for a three- or four-fold increase in length, the accessory gland rudiments are little changed. Their short common duct arises from the dorsum of the vagina a short distance distinctly anteriad of the oviporus, as opposed to its originally posteriad position. The proximal half of the accessory gland rudiment in both 12-hour and 18-hour pupae is oriented transversely to the long axis of the abdomen. This portion will become the accessory gland reservoir, although at this time the only distinction between the proximal and distal halves is the absence of the external muscle sheath on the distal portion, and this can be seen only in sectioned

material (Fig. 38f).

The median oviduct leaves the vestibulum anteriorly. Its length is more variable than that of the other organs, but it may be more subject to mechanical stretching during specimen preparation because of its position. It is 0.35 mm to 0.60 mm long and its diameter varies from 50 μ to 80 μ, being widest near the vestibulum. It is composed of a simple columnar epithelium 20 μ to 27 μ high.

With the formation of their lumina, the genital cords are converted directly into the lateral oviducts. About 12 hours after pupation, lumina develop at both ends of the genital cords, that is, at the calyxes and the junction with the median oviduct. The lumina quickly grow toward the middle of the cords and 18 hours after pupation the definitive lateral oviducts are established. Brunold (1957) also noted that the lumina are formed at both ends of the cords. The lumina are not continuous with the lumen of the median oviduct, however, for they are still separated by a solid mass of cells which persists to the 96-hour stage. The genital cords were progressively shortened as the ovarioles and median oviduct lengthened. Thus the newly formed lateral oviducts are only 0.6 mm to 0.7 mm long. They consist of a simple low columnar epithelium 10 μ to 18 μ high surrounding a lumen 12 μ to 18 μ wide.

The ovarioles are 2.1 mm to 2.5 mm long. They have a fairly uniform diameter of 60 μ for most of their length, but the upper 0.40 mm to 0.60 mm tapers slightly. This tapered portion lies within the remains of the ovarian sac and roughly delineates the extent of the germarium. Developing follicles can be seen in sectioned ovarioles, but they are not evident in whole mounts.

The pedicels are about 0.20 mm long. Their walls consist of a low simple columnar epithelium which is continuous with the epithelium of the lateral oviducts. The lumina of the pedicels are continuous with those of the lateral oviducts, but they are still separated from the lumina of the ovarioles by the epithelial plug. A very thin simple sheath surrounding the lateral oviducts continues around each pedicel. In the vicinity of the epithelial plug it becomes three or four cells thick and then abruptly splits into two distinct simple layers, the middle and inner epithelia previously described. Both are only 4 μ thick and have elliptical nuclei 2 μ wide by 4 μ long.

Drecktrah (1966) described a single syncytial sheath surrounding each ovariole of the adult European corn borer, and this appears to be the usual condition in adult Lepidoptera, as well as insects in general (Snodgrass, 1935; Bonhag, 1958; Davey, 1965). But the developing ovary of the gypsy moth has three sheaths (Sato, 1932) similar to those I

have described here. Machida (1926) also reported three ovariole coverings in the silkworm. He followed the development of the ovarioles through the pupal stadium and found that the outer covering degenerates immediately after being ruptured by the growing ovarioles. The inner epithelium breaks down late in pupal life to leave only one external covering, the original middle sheath. Machida further states that the cells of the former inner sheath then aggregate in the constrictions between the follicles. Such cell aggregations appear in the illustrations of many authors, including Drecktrah (1966), but neither their origin nor their significance is discussed.

The fate of the inner sheath in O. nubilalis is as Machida (1926) described it in B. mori. In the 96-hour pupa (Fig. 39) it appears thinner except in the constrictions between the follicles. At 120 hours (Figs. 40, 41), which is very near emergence, the inner sheath is fragmentary and the ovariole of the newly emerged adult is bounded by a single peritoneum, the direct derivative of the original middle layer of the ovarian sac.

Between the 36-hour and 48-hour pupal stages the ovarioles begin a period of rapid elongation. Their diameter does not increase significantly but their length almost triples, and the 48-hour ovary is doubled back upon itself so that a transverse section of the abdomen usually cuts each

ovariole three times. No further reference will be made to the development of the ovary. Oogenesis and vitellogenesis are considered outside the scope of this study, and no morphogenetic changes other than those already described take place prior to emergence.

36-hour pupa Figure 43 illustrates the reproductive system of a 36-hour-old female pupa. The corpus bursae has a posterodorsal evagination that may be as much as 0.2 mm long. This is the rudiment of the bursal gland. Its epithelium consists of highly basophilic columnar cells 15 μ high. The wall of the corpus bursae itself is thinner dorsally (8 μ) than ventrally (27 μ). The ductus bursae is longer, but otherwise appears unchanged. The definitive parts of the spermathecal complex are now barely evident. The spermathecal duct leads from the infundibulum to a slight dilation, the rudiment of the utriculus and lagena. Both the infundibulum and spermathecal duct are well muscled, but the utricular-lagenal dilation (the definitive spermatheca proper) has only a thin sheath, and even this is lacking on the spermathecal gland.

The spermathecal gland is 3 mm to 4 mm long and so delicate and transparent that it is very difficult to follow its winding path. It is so similar to the accessory glands, in both gross appearance and histology, that they are easily confused. The accessory glands, however, are about half as

long. They now follow the same path they do in the adult. Upon leaving the reservoirs they turn caudad, ventrad, and then cephalad. They then follow a more or less direct course cephalad at a level between the gut and the median oviduct, usually reaching the corpus bursae before reversing their paths. Their reservoirs are proximal dilations joined at their posterior margins to form the accessory gland duct. The accessory gland duct is well muscled, but the reservoirs have only a thin sheath and no muscle is found on the glands themselves. The walls of the reservoirs consist of a simple layer of cuboidal epithelium that contrasts sharply with the columnar cells of the accessory glands and the accessory gland duct.

The vagina is about 0.4 mm long and is slightly flattened for a short distance anterior to the accessory gland duct. Its epithelium is now distinctly acidophilic and the oval nuclei tend to be near the center of the cells. The epithelium is histologically uniform for the length of the vagina, but the flattened part of the tube is possibly the first indication of the formation of the vaginal pouches.

The vestibulum (Fig. 44a) is much smaller. Its walls have begun to grow inward, gradually separating the ductus bursae from the median egg passage. The separation is never entirely completed, however, and the small connection that remains between the two tubes will become the seminal duct.

The seminal duct is formed in a relatively short time; there was no indication of it in any pupa less than 36 hours old, but it was present in all 48 hour old specimens.

As the ovarioles lengthen the lateral oviducts shorten so that at 36 hours they are 0.45 mm to 0.55 mm long. The diameter of the lateral oviducts has doubled, and the epithelium has lost the extreme basophilia which characterized it before. The cells near the junction of the lateral and median oviducts contain many highly basophilic globules. These globules are most numerous in the cells of the wall separating the oviducts, but they are seen for almost 80 μ on either side of this partition. The appearance and disappearance of the globules appears associated with the breakdown of the wall.

48-hour pupa The seminal duct (Fig. 44b) is now a small, distinct tube connecting the right ventrolateral side of the ductus bursae with the left ventrolateral side of the vestibulum just posterior to the infundibulum. Its junction with the ductus bursae is slightly anterior to its attachment to the vestibulum. Its position and size make it difficult to measure accurately, but in the 48-hour pupa it is no more than 0.8 mm long and 60 μ in diameter. The seminal duct is composed of a simple layer of low columnar cells covered by a thin muscle sheath continuous with the muscle of the vestibulum and the ductus bursae.

The corpus bursae is 0.5 mm to 0.6 mm in diameter. It is usually spherical, but in some specimens it is dorsoventrally flattened. The frequency of this flattened condition increases with pupal age, and all 96-hour pupae had a flattened corpus bursae. The corpus bursae remains flattened until the adult mates and it is then distended by the spermatophore.

The epithelium of the corpus bursae, like that seen in the 36-hour pupa, consists of cuboidal cells dorsally and tall columnar cells ventrally. The cytoplasm is slightly less basophilic than before and there is a low brush border. Ventrally, the epithelium is thrown into a medial longitudinal fold (Fig. 45) which protrudes into the lumen. The fold averages 260 μ long by 80 μ high. It is more or less spindle shaped and has a maximal width of 80 μ. The appearance of this fold marks the onset of the formation of the internal sclerite of the corpus bursae, the signum. Many cytoplasmic protrusions give the surface of the fold a very irregular appearance. A fine granular eosinophilic material appears to stream from the protrusions. The 48-hour pupa seems to be the stage of maximal production of this material. A similar substance is seen throughout much of the reproductive system, but the streaming effect was not seen in other organs, and it may be an artifact.

Although not obvious upon gross examination, the vaginal pouches are beginning to differentiate. Transverse sections of the vagina anteriad of the accessory gland duct reveal a pair of small dorsolateral evaginations which will soon grow a short distance anteriad. The evaginations are now histologically similar to the remainder of the vagina, but in the 72-hour pupa (Fig. 46) the cuboidal cells and round nuclei of the pouches contrast sharply with the columnar cells and oval nuclei of the vaginal wall.

The utriculus and lagena of the spermatheca (Fig. 44b) are each about 0.1 mm at their widest pcint, and there is no significant histological difference between the two. Each is composed of a thin (18 μ) epithelium of cuboidal cells with round nuclei and, in sharp contrast to the spermathecal duct, both have a very thin external muscle sheath. This combination of low epithelium, thin muscle, and large lumen makes the walls of the spermatheca appear thinner than they actually are. The utriculus continues distally as a slightly constricted duct which leads to the somewhat wider spermathecal gland. In dissected specimens the duct is barely distinguishable from the spermathecal gland, although sectioning reveals the thin muscle of the utriculus continues around the duct but is absent from the gland.

72-hour pupa The last two definitive structures to appear are a U-shaped sclerite of the ductus bursae and a

small spiral sclerite of the spermathecal duct. Both of these are evident in the 72-hour pupa.

Drecktrah and Brindley (1967) described an internal sleevelike sclerite of the ductus bursae near the ostium. The first indication of its differentiation is the flattening of the lower part of the ductus in the 72-hour pupa (Fig. 47). The dorsal wall then becomes further depressed, and the lateral margins of the ductus reflect mesially. In the area of the dorsal depression the epithelium is columnar, 20 μ to 25 μ high. The nuclei are apical, that is, toward the lumen. The lateral and ventral walls are about a third as high, being of very low columnar or cuboidal cells. As the depression and reflection of the lower ductus continue, the lumen assumes a narrow U shape and becomes lined with a thick cuticle while the epithelium becomes uniformly cuboidal. Simultaneously, where the ductus bursae receives the seminal duct, it develops a ventral dilation and, in so doing, the bursa copulatrix is completed.

The spermathecal duct of a 72-hour pupa may have a single loose coil, and at 96 hours it has the several tight coils characteristic of the adult. The vestibulum is slightly twisted so that the infundibulum, which in previous stages was on its right side, now appears on its left.

Drecktrah and Brindley (1967) described a small heavily sclerotized sublumen which traces a spiral path around the

periphery of the major lumen of the spermathecal canal. A similar canal has been noted in many species, and it may be a universal character of the lepidopteran spermathecal duct. It has been given a variety of names such as "Befruchtungskanal" (Weidner, 1934); "spiral fertilization canal" (Callahan and Cascio, 1963); "microduct" (Joubert, 1964b, 1969); and "subsidiary lumen" (Swart, 1966). In any case, there is no sign of it in 48 hour old corn borer pupae. But at 72 hours it appears as a very small groove in the epithelium of one side of the spermathecal duct. It is not yet fully formed and can not be seen in whole mounts, but its distinctly spiral path can be easily traced in serial sections. Thus, 72 hours after pupation, all parts of the reproductive system of the imago are present, and the system is considered complete within the limits of this study.

Male Reproductive System: Morphology

Larva

Brief descriptions of the larval reproductive system of the male European corn borer have been given by Larsson (1929) and Drecktrah et al. (1966). Additional observations, principally on the testes, have been made by Parker and Thompson (1927); Crowell (1929); Cloutier and Beck (1963); and Chaudhury and Raun (1966). However, none of these papers is primarily concerned with the larval reproductive system itself, and no detailed study of it or

its development has previously been made.

As in the female, the male larval reproductive system consists of the gonads, the genital cords, and the anlage of the ectodermal portions of the definitive system. But there are striking differences in the appearance of the systems of the two sexes, the most conspicuous being the relative size of the gonads. The testes of a mature larva (Fig. 48) are paired reniform bodies located on either side of the dorsal blood vessel in the fifth abdominal segment. In contrast to the small hidden ovaries, they are mostly free of the fat body and, being relatively large (1.6 mm by 0.70 mm), are easily seen upon opening the abdomen.

Each testis, as in most Lepidoptera, consists of four conical chambers within a common covering. The chambers are variously referred to as the "testicular tubes" (Ruckes, 1919); "sperm tubes" (Snodgrass, 1935); "lobuli testi" (Ômura, 1936); or even "bladders" (Joubert, 1965, 1967). Currently the most widely used designations appear to be "follicle" or "testicular follicle" (Srivastava, 1960b; Wittig, 1960; Virkki, 1963; Holt and North, 1970; Swart, 1966; Shen and Berryman, 1967; Chen and Graves, 1970; and Outram, 1970). The four testicular follicles, faintly visible externally, converge mesially and meet at the origin of the genital cord.

The epithelium of the follicle has also been designated by a variety of terms. Ruckes (1919) called it the "testicular tube coat" while Omura (1936) used "capsula lobuli." Snodgrass (1935) preferred "epithelial sheath," whereas Sato (1932) and Wittig (1960), respectively, favored "innere Hulle" and "innere Schicht." I refer to the part of the follicular epithelium on the periphery of the follicle as the inner (of the testis) epithelium, and its "internal" portions between follicles as the follicular septa. However, this distinction is only for purposes of orientation for the septa are simply continuations of the inner epithelium.

The nomenclature of the outer covering of the testis is equally diverse. Snodgrass (1935) calls it the "peritoneal sheath"; Davey (1965) refers to it as a "connective tissue capsule." Ômura (1936) used the term "membra communis" while Ruckes (1919) called the outer layer the "capsular coat." Cholodkovsky (1880) refers to the covering simply as the "äussere Kapsel," but noting that it is only a covering and not an intimate part of the testis itself, he said that it is analagous to the scrotum of vertebrates. In a later publication (Cholodkovsky, 1884) he actually refers to the covering as the "scrotum" and that designation is still widely, but not universally, used (Norris, 1932; Callahan, 1958; Srivastava, 1960b; Swart, 1966; and Outram, 1970). In keeping with my use of inner epithelium for the wall of the

follicle, I designate the scrotum as the outer epithelium.

In the mature larva, the inner and outer epithelia are about the same thickness (14 μ to 20 μ) but contrast sharply in appearance. The outer epithelium contains many elements that readily stain with eosin or orange G, whereas the inner epithelium has comparatively few acidophilic inclusions. Tracheae enter the outer epithelium and ramify through the inner epithelium and the septa. Cholodkovsky (1884) stated that the tracheae do not penetrate the inner epithelium to enter the cavities of the follicles, but Ruckes (1919) disagreed. However, in this study no tracheae were ever seen within the follicular lumina. Ruckes has also been disputed by Musgrave (1937) and Swart (1966). The only thing seen inside the follicles of O. nubilalis are numerous spermatogonia and spermatocysts in various stages of development. The younger spermatocysts are found on the periphery of the follicle; the older spermatocysts are located centrally (Fig. 68).

The genital cords are slightly expanded where they emerge from the "hilum" (Ruckes, 1919) on the ventromesial surface of the testes. Each cord passes more or less diagonally across the posterior half of its testis (Figs. 49, 50) and proceeds caudolaterally to the eighth abdominal segment where it passes between the dorsal and visceral tracheae of the eighth spiracle and turns ventrad. Each then follows

a branch of the ventral trachea to the ectodermal primordium located in Segment 9. The paths of the male genital cords are quite different from those of the female which end blindly on Sternum 7 (compare Figs. 1 and 3).

The male genital cords are lumenless for almost all of larval life, but the lumina begin to develop late in the fifth stadium. Transverse sections of the cords near the testes or near the ectodermal primordium reveal a small lumen surrounded by low columnar or pyriform cells with round nuclei, but the remainder of the cord is solid. At this stage they are similar to the genital cords of the female.

The most remarkable difference in the two sexes is the nature of the ectodermal primordia. In the female these are paired imaginal discs located laterally in Segments 8 and 9 and an unpaired medial disc in Segment 7. But the ectodermal anlage of the male is an unpaired median invagination of Segment 9; Segments 7 and 8 are not involved at all. In gross dissections the genital cords seem to terminate on the anterolateral margin of a small pearshaped structure (Fig. 3, Herold's organ) attached below the rectum to the hind part of the ninth abdominal sternum. Drecktrah et al. (1966) called this structure the "genital pouch," and the cavity it encloses is the "genital cavity" of many authors. It is 0.25 mm to 0.35 mm long, and its maximal width is about the same as its length. It tapers posteriorly, often rather

abruptly, to a very small stalk attached to the hypodermis. It lies in the midline between the ventral musculature, and its position relative to Setae VII and VIII (Heinrich, 1919) is indicated in Fig. 3.

Although formed as an invagination of Sternum 9, there is no external evidence of the primordium. It could usually be seen through the cuticle of fixed specimens and its presence was often used to distinguish the sexes, but it could not be seen in living larvae. Stewart et al. (1970) used the presence of a dark spot on the midventral surface of Segment 9 to distinguish between male and female larvae of both the tobacco and tomato hornworm. They do not associate this spot with any internal structure, but from their description, I think the spot is actually the point of invagination of the male ectodermal anlage.

Pupa

All pertinent details of the external morphology of the male pupa are included in the description of the female.

Adult

Drecktrah and Brindley (1967) have given a thorough description of adult reproductive system morphology, and their terminology is used here. The male system is diagrammed in Fig. 51. It occupies most of the hemocoel posterior to the third abdominal segment. In the newly emerged moth the

testes are fused into a single ovoid structure about 1 mm wide and not quite as long which lies in the dorsal portion of Segment 5. Two ducts extend posteriad from the testis. These are the vasa deferentia, of which Drecktrah and Brindley recognized three distinct parts. The "upper vas deferens" is 2 mm long and 0.3 mm wide, tapering slightly before abruptly expanding to form the "seminal vesicle." The upper vasa deferentia cross immediately after leaving the testes, the apparent right duct being dorsad. Each duct continues below the seminal vesicle as the "lower vas deferens" which is 3 mm long and 0.1 mm in diameter.

After reversing directions, the lower vasa deferentia join the paired parts of the primary ejaculatory duct, the "paired ejaculatory ducts" of many authors. The paired ejaculatory ducts are continuous proximally with the unpaired part of the primary ejaculatory duct; they are continuous distally with the accessory glands. A small constriction delimits the paired ducts from the accessory glands. The latter are about 5 mm long and end in thin transparent "terminal sacs" 1 mm or 2 mm long. The distal halves of the accessory glands, excluding the terminal sacs, are fused together, but their lumina are separate.

The unpaired primary ejaculatory duct is continuous with the coiled and twisted "cuticular ejaculatory duct" which leads to the aedeagus. The cuticular and unpaired primary

ejaculatory ducts together are often referred to as the "common ejaculatory duct." In the European corn borer, the common ejaculatory duct is 50 mm to 60 mm long, but only 0.2 mm to 0.3 mm wide.

Male Reproductive System: Development

As with the female, I will describe the development of the male reproductive system from hatching only to the appearance of the definitive organs.

Larva

First stadium I could not distinguish between the male and female gonads of first instar larvae, and the description given in the discussion of the female system will suffice. It is possible however, to identify the sex by the presence, in the male, of a small tubular invagination near the hind margin of the ninth abdominal segment. This invagination (Fig. 52) occurs in the midline, just anterior to the point of attachment of the extrinsic muscles of the rectum. It can be found in larvae only one day old and may even be present at hatching.

Such a structure is clearly illustrated by Lyonet (1762, Pl. IV, Fig. 5) in the mature larva of C. cossus, but he does not seem to associate it with the reproductive system. Herold (1815) described it in P. rapae, and apparently he was the first to recognize its significance. Although the German

researchers usually refer to it as "Heroldsches organ" (Verson and Bisson, 1896a; Meisenheimer, 1909; Heberdey, 1931; Florin, 1945; Wittig, 1960), no single term is in general use in English language publications. For example, Mehta (1933) and Srivastava and Srivastava (1959a) give it no name, but only say it encloses the genital cavity. Drecktrah et al. (1966) call it the "genital pouch." Joubert (1965) describes a "claviform body" in S. cerelella but later (Joubert, 1967) refers to the homologous structure in C. cautella and P. interpunctella as the "primary nodule." In recognition of Herold's classic contribution to our knowledge of lepidopteran development, I prefer to use the term Herold's organ.

In first instar larvae (Fig. 52) Herold's organ is 30 μ to 50 μ long and 8 μ to 16 μ in diameter, depending upon the age of the larva. It is a more or less clubshaped, anteriorly directed invagination of the hypodermis. Its wall consists of a single layer of cells which are columnar at the blind distal end but very low elsewhere. The genital cords are attached to the distal end of Herold's organ. I was unable, however, to trace their entire path to the testes. They are so thin (2 μ to 3 μ in diameter) that I invariably lost them somewhere in the eighth or ninth segment when they become confounded with the tracheae.

Mehta (1933) reports that in P. rapae the genital cords (Mehta's "vasa deferentia") terminate ventrolaterally on Segment 8 and do not join the ectodermally derived organs until near the end of larval life. Furthermore, he implies that this is also the case in B. mori, Hepialus lupulinus, and Earias fabia. Rakshpal (1944) found the "vasa deferentia" of Galleria mellonella and Achroia grisella do not even appear until the last larval instar, and then they terminate on Segment 7. He maintains that in these insects the mesodermal and ectodermal derivatives first meet in the pupal stadium. Srivastava and Srivastava (1959a) agree that the genital cords and ectodermal ducts first meet in the pupal stage, but they concluded the genital cords of L. orbonalis terminate on Segment 6 during larval life. On the other hand, Verson and Bisson (1896a); Florin (1945); and Wittig (1960), respectively, found the genital cords reach from the testes to Herold's organ in the earliest larval instar of B. mori, S. triquetrella, and C. murinana.

Second stadium Although the gonads of the two sexes are superficially similar, they can be distinguished in the second stadium by a combination of characters. First, four clusters of germ cells marking the differentiation of the follicles are present in the testis of the youngest second instar. Such clusters do not appear in the ovary before the middle of the stadium. Second, the inner epithelium of the

testis begins to differentiate early in the second stadium while the inner epithelium of the ovary does not differentiate until the middle of the third stadium. Third, the testes grow faster than the ovaries and the resulting size differential is visible in second instar larvae. The faster growth of the testes continues through larval life, as illustrated by Figs. 48-50. And, fourth, about midstadium the so-called apical cell appears, albeit indistinctly, in the apex of each testicular follicle. This presumably nutritive cell, also known as Verson's cell, is characteristic of the male gonad. Except for the presence of Verson's cell, these differences are slight and almost subjective in intrepretation. Nevertheless, taken together they allow one to identify the sex of a larval gonad quite early in the second stadium.

Except for growth, the genital cords and Herold's organ (Figs. 53, 54) change very little during the second stadium. By the end of the stadium the cords are large enough for their entire path to be traced from serial sections. Their lower ends, adjacent to the distal end of Herold's organ, are slightly enlarged. These small bulblike swellings are the "Terminalampulle" of Verson and Bisson (1896a); the "ampullae" of Parker and Thompson (1927); and the "distalen Endanschwellungen" of Florin (1945). As yet, the genital cords, including the ampullae, are without lumina. Herold's

organ now measures 50 μ to 55 μ long and is about 35 μ wide at its upper end.

Third stadium On opening third instar larvae along the ventral midline and removing the gut, the testes are seen as miniatures of those of mature larvae. The smallest testis observed was 0.15 mm long; the largest was 0.40 mm long. The mean testicular length (10 specimens) was 0.28 mm. There is little to distinguish the outer epithelium, i.e., the scrotum, from the inner follicular epithelium. Both are about 3 μ thick, stain lightly, and appear to form a fibrous network with many intercellular spaces. The follicular septa consist of a very thin monolayer. There is a group of cells at the base of each follicle distinguishable from the cells of the follicular epithelium by their small size and basophilic cytoplasm. They will eventually form the vasa efferentia, the small ducts that lead from the follicles to the vas deferens.

The spermatogonia multiply rapidly and gather into the small groups known as spermatocysts. Verson's cell (Fig. 56) is more easily seen than in second instar larvae. Its pink cytoplasm contrasts sharply with the purplish germ cells, but its nucleus is no more distinct than before. Verson's cell persists for at least 48 hours after pupation for it was found in each of six 48-hour old pupae sectioned, but could not be found in the testes of the 72-hour old pupa. I made

no special study of either the apical cell or spermatogenesis. Both have been examined in numerous Lepidoptera, and spermatogenesis in O. nubilalis, specifically, has been studied by Parker and Thompson (1927); Cloutier and Beck (1963); Guthrie et al. (1965a); and Chaudhury and Raun (1966).

With a little difficulty the genital cords can be followed from the testes to Herold's organ in a young third instar; they can be followed quite easily late in the stadium. The cords remain solid and may attain a diameter of 10 μ to 12 μ. In transverse section they appear composed of five to eight cells with round or ovoid nuclei. The ampullae, at the lower ends of the genital cords, have a diameter of 14 μ to 18 μ and may develop very small lumina just prior to the third larval ecdysis.

Herold's organ (Figs. 59, 63) attains a length of 70 μ to 80 μ. Its greatest diameter is 40 μ to 50 μ and it tapers to 20 μ or 25 μ posteriorly. Its distal wall is about twice as thick as it is elsewhere, but there is little other differentiation.

Fourth stadium During the fourth stadium the testes (Figs. 57, 58) grow rapidly, more than doubling in size. They may measure 0.85 mm or more in length and 0.35 mm to 0.50 mm in diameter, although they are usually smaller. Spermatogonia and spermatocysts fill the follicles. Other

than an obvious thickening of the septa, there is little change in the follicular epithelium. For the most part, the inner and outer epithelia are still not sharply distinguishable. But late in the stadium the outer epithelium seems better defined and it begins to acquire some eosinophilic inclusions. However, these differences are so slight that they produce more of a subjective impression than a measurable variance. The vasa efferentia resemble small covered funnels. Their lumina are quite small and do not extend into the vas deferens which is still lumenless.

About midway through the fourth stadium there is a proliferation of the cells of the distal and lateral walls of Herold's organ (Figs. 60, 61). This produces two folds or lobes which project into the genital chamber (Figs. 62, 65). Other workers have made similar observations, although the formation of the lobes may occur during either earlier or later stadia depending upon the species. Verson and Bisson (1896a); Mehta (1933); Florin (1945); and Srivastava and Srivastava (1959a), respectively, called these lobes the "Keimwülsten," the "penis lobes," the "primären Anlage," and the "primary genital lobes." Concurrent with the formation of these lobes, the terminal ampullae of the genital cords rapidly enlarge. By the end of the fourth stadium Herold's organ and the ampullae appear as in Fig. 65.

Fifth stadium The testes of young fifth instar larvae (Fig. 68) measure 0.80 mm to 1.05 mm long and 0.35 mm to 0.45 mm in diameter. They are quite reniform for most of this stadium. Spermatocysts can be seen through the nearly transparent epithelia. Histologically, the testes of young fifth instars closely resemble those of fourth instars, but soon the two epithelial layers become markedly different. The cells of the outer epithelium enlarge, attaining a diameter of about 15 μ to 20 μ. They acquire so many eosinophilic inclusions (Fig. 69) that the scrotum stains a bright red. These inclusions persist until about 120 hours after pupation. The inner epithelium does not change so dramatically. It appears somewhat more compact but remains a fibrous reticulum, and it lacks the eosinophilic inclusions seen in the outer epithelium.

The low columnar cells composing the vasa efferentia (Fig. 70) are strongly basophilic and their deep blue cytoplasm and small size (10 μ to 15 μ high) contrast sharply with the cells of the two epithelial layers. The vasa efferentia become hollowed out by mid-stadium and the lumina thus formed gradually extend into the genital cords. At the same time the terminal ampullae are growing, and their lumina penetrate the genital cords from their lower ends. The solid genital cords are thus converted directly into the vasa deferentia by the progressive formation of the lumina from

both ends towards the middle, just as occurred in the formation of the oviducts in the female. But the conversion of the male genital cords into the vasa deferentia begins earlier (fifth stadium) and is completed earlier (6-hour pupa) than the comparable conversion of the female genital cords into the lateral oviducts. In the female the lumina do not penetrate the genital cords until about 12 hours after pupation, and the oviducts are completed in the 18-hour pupa.

The testes of late fifth instar larvae are 1.40 mm to 1.70 mm long and 0.60 mm to 0.85 mm in diameter. As they increase in size the two testes come closer and closer together, finally fusing into a single body. The fused testes are 1.5 mm to 1.6 mm across and about 0.80 mm thick. The two vasa deferentia are the only external evidence of their paired nature, although each individual testis is easily distinguished in sections. The vas deferens attains a diameter of 16 μ to 18 μ and its lumen, when first completed, is only about 2 μ in diameter.

During the fifth stadium Herold's organ undergoes dramatic development and the parts of the definitive system appear. In young fifth instars, Herold's organ (Fig. 66) is 0.15 mm to 0.20 mm long and about as wide at its upper (distal) end. The primary genital lobes, which differentiated from the distal and lateral walls during the fourth stadium, soon become incompletely divided by a

vertical cleft that forms near their base. By midstadium (Fig. 67) four lobes are seen within the genital cavity. The larger proximal lobes will become the valvae; the smaller distal lobes will produce the aedeagus. As the aedeageal lobes fully differentiate, the epithelium at their base secondarily invaginates to produce the primary gonopore. This invagination deepens, becoming the rudiment of the cuticular ejaculatory duct. Shortly after their formation the aedeageal lobes fuse in the midline, dorsally and then ventrally, to form the tubular aedeagus. When they fuse they enclose the gonopore and the cuticular ejaculatory duct.

Simultaneously with the formation of the aedeagal and valvular lobes, the ampullae become flattened against the anterior ends of Herold's organ (Fig. 66). The genital cords, now hollow near their lower ends, arise near the middle of the ampullae, dividing them into anterior and posterior halves. The unpaired primary ejaculatory duct will develop from the parts of the ampullae most posterior to the vasa deferentia. The accessory glands will develop from the most anterior parts of the ampullae, that is, the portions in contact with each other at the anterior midline. The middle part of the ampullae, in the vicinity of the genital cords, becomes the paired portion of the primary ejaculatory duct.

Essentially similar findings are reported by Florin (1945) and Joubert (1965, 1967). The "primary ejaculatory

duct", as used here, is equivalent to Florin's "unpaarig Vas deferens" and Joubert's "mesospermatic duct." My usage of "cuticular ejaculatory duct" is equivalent to the "ductus ejaculatorius" in both their terminologies. Florin derives the accessory glands and primary ejaculatory duct from the "distalen Endanschwellungen" of the genital cords; Joubert derives them from the "primary mesodermal mass." Both of these are equivalent to the terminal "ampullae" as used here.

Mehta (1933) and Srivastava and Srivastava (1959a) disagree with this analysis, however. They derive the primary ejaculatory duct (Mehta's "ductus ejaculatorius duplex" and the "paired ejaculatory duct" of Srivastava and Srivastava) from Hercld's organ. They say that the hypoderm at the distal end of the genital cavity (neither uses the term "Herold's organ") proliferates, is hollowed out, and forms two ampullae. These ampullae subsequently divide to form the accessory glands and primary ejaculatory duct. The actual morphogenetic processes are essentially the same as described by Florin, Joubert and myself, but we three assign a mesodermal origin to the ampullae. By deriving them from the genital cavity, Mehta and Srivastava and Srivastava assume an ectodermal origin.

As the fifth stadium comes to a close the genital cavity is greatly reduced as Segments 8, 9, and 10 telescope into Segment 7. With this reduction of the genital cavity, the

valves are gradually forced to the outside and eventually (24-hour pupa) come to lie on Sternite 9.

Pupa

Morphogenesis of the male reproductive system during the pupal stadium can be summarized by comparing the 0-, 24-, 48-, and 72-hour old pupal stages. As with the female, organ development is followed only until the definitive parts become clearly recognizable and growth thereafter is not studied in detail.

Histologically, the developing male system is monotonously uniform. With the exception of the testes, the organs are composed of a columnar epithelium which originated from one of two sources, these being the ectodermal Herold's organ or the mesodermal vasa differentia and their terminal ampullae. The ectodermal and mesodermal portions are continuous even though their lumina are separated by a septum. Both parts are highly basophilic; the cell borders are not well defined; and few vacuoles or inclusions are seen. The brush border so extensively developed in the female is only slightly developed in the male. Only the testicular epithelia display any striking changes in histology. However, histogenesis was not the primary concern here and other techniques, especially histochemical ones, would probably reveal significant events not seen with hematoxylin and eosin.

A very thin, possibly monolayered, nucleated sheath covers the surface of the developing mesodermal organs. Its nuclei are small and either oval or quite flattened. The exact nature of this sheath is uncertain for no striations or other distinguishing features were seen. It appears to be continuous with the well-developed muscular layer surrounding the lower portions of the common ejaculatory duct. Drecktrah (1966) observed this sheath in the adult European corn borer, but he, too, was unable to characterize it.

There is considerable confusion in the literature concerning the outer limiting sheath of the male reproductive system. Stitz (1901) found a nucleated sheath, similar to the one described here, around the ducts of the several microlepidoptera he examined. He called it the "tunica propria," but he used the same term for the inner testicular epithelium, and these two layers are certainly not the same. He also found that except for the common ejaculatory duct, the male organs are devoid of musculature. This is in direct opposition to the findings of Ruckes (1919). In a study based primarily on Saturniidae, Ruckes concluded that the outer sheath is muscle. But according to Musgrave (1937), the outer sheath in the Mediterranean flour moth is a thick basement membrane.

More recently, Swart (1966) reported that only a non-cellular basement membrane covers all the mesodermally

derived organs of the false codling moth (Olethreutidae). Outram (1970) found the same structures in the spruce budworm (Tortricidae) to be covered by either a thin layer of circular muscle or by a thin layer of circular muscle over an inner layer of longitudinal muscle. Furthermore, he found a "thin double layer of connective tissue" encloses the entire cuticular ejaculatory duct.

The corn earworm (Noctuidae) seems to lie between these two histological extremes. Callahan and Cascio (1963) found striated muscle around the cuticular ejaculatory duct, but the remainder of the organs, except for the accessory glands, are enveloped by a non-striated sheath. Both circular and longitudinal layers surround the paired ejaculatory ducts and the lower third of the primary ejaculatory duct, but around the vas deferens, seminal vesicle, and the upper two-thirds of the primary ejaculatory duct, only the circular layer is present. All these areas are reported to be highly contractile, a characteristic not usually ascribed to epithelial cells or basement membrances and, for lack of a better term, Callahan and Cascio designated the layer as "smooth circular muscle." The accessory gland lacks this muscle sheath and is bounded only by a non-cellular basement membrane which is a thin layer between the so-called smooth circular muscle and the epithelium of all the other organs. From these conflicting descriptions it is evident that the

true composition of the outer covering of the male reproductive ducts remains undetermined.

0-hour pupa Figures 71 and 72 show the state of development of the male reproductive organs at pupation. The paired larval testes are now fused, and in gross dissections the ectodermal and mesodermal ducts appear continuous, with nothing to indicate they indeed have different origins.

The developing aedeagus is easily seen. It lies between the valves which are now partially everted from the genital chamber. Their eversion is completed by the 24-hour stage, and thereafter they appear as ventrolateral appendages of the ninth segment (Figs. 79, 80). The aedeagus is continuous with a short, slightly twisted duct. Distally this duct separates into two distinct ducts which almost immediately converge and adhere tightly together. Even in this early stage, where the total length of these developing ducts is less than a millimeter, the basic plan of the definitive system can be recognized. It is obvious that the two separate ducts are the future paired ejaculatory ducts and their distal approximated portions will become the accessory glands. The remainder, from the paired ducts to the aedeagus, is the future common ejaculatory duct.

The common ejaculatory duct is actually three ducts so intimately fused that superficially they appear as one. The proximal half becomes the cuticular ejaculatory duct; the

distal half becomes the primary ejaculatory duct. The median walls separating the two component ducts of the primary ejaculatory duct are easily seen when the duct is cut transversely (Fig. 73d). Each duct has a diameter of 40 μ to 60 μ; their walls consist of columnar cells 16 μ to 30 μ high. Near the paired ejaculatory ducts the primary duct has an ovoid cross-section 70 μ by 150 μ. It becomes smaller and less ovoid farther down, and at its lower (proximal) end it is invested with a comparatively thick layer of muscle.

The cuticular part of the common ejaculatory duct is 0.15 mm to 0.25 mm long and 60 μ to 80 μ wide. It is quite straight at this stage. Although the cuticular and primary portions appear continuous, their three lumina (one cuticular, two primary) end blindly and do not communicate with each other (Figs. 72, 73e). Furthermore, the partition separating the two parts remains intact throughout pupal life.

There is no demarcation in either gross appearance or histology between the paired ejaculatory ducts and the accessory glands. Together the paired ducts and accessory glands are 0.30 mm to 0.40 mm long. Each is 60 μ to 70 μ in diameter with an epithelium 16 μ to 20 μ high. Near its middle, each paired duct is joined by a much smaller duct, the lower part of the vas deferens, which after proceeding posteriorly a short distance, turns dorsoanteriorly and then

leads directly to the fused testes in the fifth abdominal segment. Although the vas deferens has a nearly uniform diameter (21 μ to 27 μ) for most of its length, it is up to twice as wide in the vicinity of the dorsoanterior loop. This is a gradual change in diameter rather than an abrupt dilation. The seminal vesicle will eventually be derived from this enlarged area. At pupation the enlargement is located at the level of the intersegmental membrane between Segments 8 and 9, but it will be carried anteriad into the sixth abdominal segment as the common ejaculatory duct elongates. This translocation also involves a lengthening of the prospective lower vas deferens and a shortening of the prospective upper vas deferens, the portions of the duct on either side of the enlargement. The vas deferens, sens. lat., now has a continuous lumen, 6 μ to 9 μ wide, which opens into the paired ejaculatory duct at one end and the vasa efferentia at the other.

In the 6-hour pupa the fused testes begin the morphogenetic movements that bring them to their final form. They rotate around the longitudinal axis so that the follicles of the left testis are twisted dorsad and toward the right side; the follicles of the right testis are twisted ventrad and toward the left. Therefore, in the adult the apparent right vas deferens is the morphological left and vice versa. Eventually the follicles come to be arranged

whorl-like around each other. Concurrent with the twisting of the follicles, the points of origin of the vasa deferentia shift from near the center of the ventral side of the gonad to its posterior margin. The fused testes thicken dorsoventrally and by the middle of the pupal stadium they have the spheroid shape characteristic of the adult gonad. Although this description is not as detailed as that made by Chase and Gilliland (1972) in their study of testicular development in the tobacco budworm, it agrees in essence with their findings.

24-hour pupa Comparatively few changes occur to the male reproductive system between pupation and the 24-hour pupal stage. Of these, the most significant are the eversion of the valves and aedeagus from the genital chamber and the rotation of the testicular follicles around the longitudinal axis of the fused testes. The ducts have grown, of course, with the ectodermal portions elongating at a faster rate than the mesodermally derived portions. Macroscopically, the system appears as in Fig. 74. Although the valves of the 6-hour pupa are only about half way out of the genital chamber, by 24 hours they are completely everted and are seen as ventrolateral appendages of the ninth segment. The aedeagus is a straight tube, about 0.3 mm long, with walls of columnar epithelial cells 11 μ to 14 μ high. The endophallus, also straight at this stage, is nearly 100 μ in

diameter near its posterior end (the secondary gonopore), tapering to about 50 μ at the base of the aedeagus. Longitudinal muscles and tracheae occupy much of the space between the endophallus and the aedeagus. There is no evident histological difference between the endophallus and the remainder of the cuticular ejaculatory duct. The endophallus is recognized simply as the part which lies within the aedeagus. The point at which the cuticular ejaculatory duct becomes the endophallus is the primary gonopore. The cuticular duct is also about 0.3 mm long, 50 μ to 60 μ in diameter, and its epithelium is 15 μ to 25 μ thick.

Figure 75 is a schematic sagittal section of the terminal parts of the male ducts 24 hours after pupation. The distal third of the aedeagus, approximately, lies free between the valves. The remainder lies within the abdomen. The diaphragma, the membrane that closes the posterior end of the abdomen, is folded conelike around the aedeagus. This fold is the annellus and its innermost layer is the manica. The manica is attached to the aedeagus in a narrow area called the "zone" (Klots, 1956), but it continues beyond the zone as a distinct epithelium surrounding the aedeagus. The manica and aedeagal epithelium then fuse at the base of the aedeagus on the latter's ventral side. From this point the two epithelia are continuous. In transverse section (Fig. 76) they form a sort of membranous double arch over the

cuticular ejaculatory duct, the manica being the outer arch. I refer to the inner arch as the aedeagal membrane to distinquish it from the epithelium of the aedeagus proper.

Also at the base of the aedeagus the longitudinal muscles of the endophallus become rearranged into transverse bands. These bands reach from one side of the aedeagal membrane to the other, passing below the cuticular ejaculatory duct. Thus the cuticular ejaculatory duct is free to stretch and follow the endophallus when it is everted (Callahan, 1958; Callahan and Cascio, 1963).

After continuing along the cuticular ejaculatory duct for most of its length, the arches become progressively shorter, covering less and less of the duct. Simultaneously, the transverse muscle becomes progressively more circular in its arrangement. Near the upper end of the cuticular ejaculatory duct the arches terminate and the duct emerges surrounded by a thick layer of circular muscle. Within 40 μ to 80 μ of the end of the arches the circular muscle becomes much reduced, the cuticular ejaculatory duct ends and the primary ejaculatory duct begins.

At this stage the morphology of the cuticular ejaculatory duct and the epithelial arches appear somewhat at variance with Drecktrah's (1966) description of the adult condition. He found the cuticular ejaculatory duct surrounded by a "non-sclerotized cuticular tube" (italics

mine) continuous with the aedeagus. This "tube" is in turn surrounded by a "thin membrane with a few very flat nuclei." The muscles are arranged transversely between the sides of the inner non-sclerotized tube. Outram (1970) makes essentially the same interpretation of the structure of the cuticular ejaculatory duct (his "cuticular simplex") of the spruce budworm, except he labels the thin outer membrane the "connective tissue sheath." I have not examined this insect, but it is likely the connective tissue sheath is homologous to the outer epithelial arch which, in turn, is a continuation of the manica. Likewise, Drecktrah's (1966) "non-sclerotized cuticular tube" is a continuation of the aedeagal epithelium, and this must surely be Outram's "outer cuticular tube"; his "inner cuticular duct" is the cuticular ejaculatory duct as I use the term.

Neither Drecktrah (1966) nor Outram (1970) interpret the two epithelia as continuous and arch-like as I do. Examination of later pupal stages and newly emerged adult European corn borers explains Drecktrah's interpretation and, presumably, Outram's also. The cuticular ejaculatory duct becomes twisted and coiled, and the two epithelial arches become very thin, the outer one (the manica) extremely so. They are very close together, often touching, and the attachment of the muscles to the inner arch further confuses the picture. In transverse sections of pupae older than 96 hours (Fig. 78) it

is almost impossible to follow the course of the epithelia; only in a few sections was I able to ascertain that they indeed formed "arches" rather than a "tube within a tube."

From the descriptions given by Musgrave (1937), Callahan and Cascio (1963), and Swart (1966), respectively, of the Mediterranean flour moth, the corn earworm, and the false codling moth, it would appear that the manica does not extend beyond the aedeagus. These authors report the cuticular ejaculatory duct is surrounded by the inner aedeagal membrane only (my term, not theirs). However, their observations are based entirely on the adult structure and the picture in these insects is probably as confused as it is in the European corn borer. To my knowledge, Zander (1903, 1904) is the only other author to interpret the structure of the cuticular ejaculatory duct as I do.

48-hour pupa. Between 24 and 48 hours after pupation the common ejaculatory duct, paired ejaculatory ducts, and accessory glands approximately double in length, but there are no really significant changes in their histology. The testes lie in Segment 5 with the tangled mass of ducts occupying Segment 6. The vasa deferentia are crossed as a result of the rotation of the follicles described earlier. The upper vas deferens (1.3 mm to 1.6 mm long) has a diameter of 80 μ to 100 μ. In some specimens a slight constriction separates the upper vas deferens from the tapered seminal

vesicle. The lower vas deferens has a diameter of 25 μ to 35 μ. In sections, the upper and lower vas deferens and the seminal vesicle are indistinquishable except for the smaller diameter of the lower vas deferens. All three consist of a basophilic columnar epithelium 11 μ to 20 μ high, the lesser measurements being from the lower vas deferens.

There is still no obvious distinction between the paired ejaculatory ducts and the accessory glands, although a marked constriction now delineates the terminal sacs of the latter organs. The combined length of the paired ejaculatory ducts and accessory glands (from the primary ejaculatory duct to the terminal sacs) is 1.0 mm to 1.2 mm. The paired ejaculatory ducts and the lower part of the accessory glands are 65 μ to 75 μ in diameter. The accessory glands taper toward the constriction, and in the area of maximal constriction each gland is only about 30 μ in diameter. Here the epithelium is 14 μ high, so the lumen is considerably reduced. Beyond the constriction the terminal sacs are 60 μ wide, with walls 18 μ to 22 μ thick. The terminal sacs are about 0.3 mm long and histologically similiar to the accessory glands and the paired ejaculatory ducts.

The primary portion of the common ejaculatory duct has reached a length of 3 mm or 4 mm, its path forming two broad loops through the hemocoel. As stated previously, it is actually two ducts fused together (Fig. 83a) and enclosed

within a thin common sheath, presumably muscle. Its oval cross-section becomes more circular toward the lower end, and a section through the cuticular ejaculatory duct (Fig. 83c) is quite circular. The cuticular duct is also more heavily muscled.

The cuticular ejaculatory duct is no longer a straight tube. Near its upper end it is thrown into a distinct loop (Fig. 77), the future "distal coil" of Drecktrah and Brindley (1967). Their "proximal coil" does not form until the 72-hour stage. Between the base of the aedeagus and the distal coil, the cuticular ejaculatory duct is also rotated about its long axis, approximately a quarter turn to the right. It is difficult to measure the duct accurately; five specimens measured from 1.1 mm to 1.5 mm, averaging 1.2 mm. The upper end of the cuticular ejaculatory duct is about 60 μ in diameter, circular in cross-section, and with walls about 16 μ high. The circular muscle sheath consists of several layers, totaling 15 μ to 20 μ in thickness. Within the distal coil, and for a short distance below it, the epithelium decreases to a height of 8 μ to 10 μ and the duct is flattened. As it approaches the aedeagus the duct again becomes circular in cross-section. Its epithelium increases to a height of 11 μ to 14 μ and remains so throughout the endophallus.

The manica and the aedeagal membrane are thinner than previously seen. Near the aedeagus the manica may be as much as 14 μ thick, but it becomes thinner and thinner distally; where it surrounds the distal coil it is only 2 μ or 3 μ thick. At a given level the aedeagal membrane appears slightly thicker than the manica.

The aedeagus is now 0.5 mm long and the endophallus is beginning to twist within it. This is evidenced not so much from the endophallus itself as from its muscle which is seen to follow a spiral path around the endophallus. The wall of the aedeagus is 8 μ to 11 μ thick except for a much thicker (25 μ to 30 μ) ventral area near the secondary gonopore. This thickening (Fig. 79), distinctly enlarged at 72 hours (Fig. 80), is the first indication of the formation of a Y-shaped sclerite found on the underside of the aedeagus in the adult.

Some other sclerites of the external genitalia are also beginning to form. There are characteristic thickenings (Fig. 79) on the medial surfaces of the valves which are the early stages of the differentiation of the claspers. However, the cornutus of the endophallus is not identifiable until the 72-hour stage; it is completely formed at 96 hours.

72-hour pupa The outer epithelium of the fused testes is now one to four cells thick, totaling 15 μ to 40 μ. The cells are large and contain so many eosinophilic inclu-

sions that their borders and nuclei are partially obscured. The nuclei are rounded and about 5 μ in diameter. The cells of the inner epithelium, on the other hand, are very flat and have oval nuclei 10 μ long and 3 μ in diameter. The nuclei lie parallel to the surface of the gonad. The inner epithelium lacks the eosinophilic inclusions seen in the scrotum.

The contrast between the two layers is now at its maximum, but the outer epithelium soon begins to lose its eosinophilia and by 120 hours (Fig. 81) only a few of the inclusions are seen. With their loss the cells of the outer epithelium become flattened and by the 144-hour stage (Fig. 82) the two epithelia are similar in appearance. Both are 3 μ to 6 μ thick and have a finely granulated "fibrous" cytoplasm which closely resembles the condition of the adult gonad.

Upon opening the abdomen of the 72-hour pupa, one immediately notes the extensive elongation of the reproductive ducts. The cuticular ejaculatory duct has developed the "proximal coil" described by Drecktrah and Brindley (1967) and is now so twisted that accurate measurement of its length is nearly impossible. Except for its increased length and the presence of the proximal coil, it is not much different from the 48 hour stage.

On the other hand, the primary ejaculatory duct exhibits the most striking development evidenced in this stage. It is now 13 mm to 15 mm long and follows a tortuous path throughout the abdomen. But this four- or five-fold increase in length since the 48-hour stage is insignificant compared to the change that occurs inside the duct. The septum separating the lumina of the two ducts composing the primary ejaculatory duct breaks down to produce a single tube. The disappearence of the septum is comparatively sudden. It appeared normal at 60 hours, but was absent at 96 hours. Of four 72-hour pupae sectioned, the septum was intact in one (Fig. 84), absent (Fig. 86) in two, and in the fourth (Fig. 85) only traces of it remained. This abrupt disappearence may indicate the septum breaks down simultaneously along the entire duct rather than progressively as seen in the formation of the lumen of the vas deferens. Although the small sample precludes a definite conclusion on this point, it is clear that the wall separating the primary and cuticular portions of the ejaculatory duct does not break down. This double partition, consisting of the lower end of the primary ejaculatory duct and the upper end of the cuticular ejaculatory duct, persists until the adult emerges. It was intact in all three 144-hour pupae sectioned (Fig. 88) but was absent in one day old adults. No attempt was made to establish the precise time of its disappearance.

The derivation of the definitive single primary ejaculatory duct from originally paired ducts is similarly described by Florin (1945) in S. triquetrella and by Joubert (1965, 1967) in S. cerealella, C. cautella and P. interpunctella. Although their terminologies differ from each other and from that used here, it is clear that our basic findings agree.

The paired ejaculatory ducts and accessory glands are completed at 72 hours. The paired ejaculatory ducts are 1 mm to 1.5 mm long and 70 μ to 95 μ in diameter. They taper distally, and the constriction which delimits them from the accessory glands is now visible. Although the epithelial cells of the paired ejaculatory ducts are somewhat taller (19 μ to 27 μ) than those of the accessory glands (15 μ to 20 μ) there is little else, histologically, to distinguish the two. The accessory glands themselves are 3 mm to 3.5 mm long, including their terminal sacs. Proximally, i.e., toward the paired ejaculatory ducts, the accessory glands are 60 μ to 70 μ in diameter. But for most of their length they are only 40 μ to 50 μ across while their terminal sacs have a diameter of 60 μ to 70 μ. The constriction between the accessory glands and the terminal sacs is now very marked. Its diameter is only 25 μ and the epithelium, which is 8 μ to 11 μ high, nearly closes the lumen.

For the first time the seminal vesicles can be easily distinquished histoloqically from the upper and lower vas deferens (Fiq. 87). Althouqh the seminal vesicle and upper vas deferens have about the same diameter (100 μ to 120 μ), the seminal vesicle can be recoqnized by the combined characteristics of a very tall columnar epithelium (40 μ to 45 μ) and a small lumen. The upper vas deferens has a larger lumen and lower epithelium (30 μ to 35 μ), whereas the lower vas deferens is only 35 μ to 40 μ in diameter and has a very small lumen within walls just 14 μ to 16 μ thick.

Every definitive orqan of the male reproductive system can now be identified and, althouqh marked histological chanqes occur throughout the remainder of pupal life, the male reproductive system is considered complete within the stated limits of this study.

DISCUSSION

The rather scanty literature on the subject of the development of the reproductive system in Lepidoptera is, for the most part, harmonious. The inconsistencies that exist are usually reconcilable if one makes allowances for the different nomenclature used by the various authors, and is very careful in homologizing structures described by one worker with those of another. Furthermore, some investigators have begun their study too late in the insect's life; the organs are already formed in the earliest stage examined and their origins are consequently obscured. Subsequent statements made concerning those origins may therefore be questionable.

Herold (1815) found two oval bodies on the eighth and ninth sterna of female P. brassicae larvae. Although his description is somewhat confused, the oval bodies clearly represent fused vestibular and vaginal rudiments such as I described. He also showed how the rudiments of the bursa copulatrix (Herold's "Saamenbehälter"), the spermatheca (Herold's "einhörnige Absonderungsorgan"), and the accessory glands (his "zweyhörnige Absonderungsorgan") arise from these oval bodies during the prepupal stage. But all of his observations were based upon gross dissections which led to two notable deficiencies in his classic work; he apparently failed to recognize the originally paired nature of the oval bodies, and he did nothing toward explaining the origin of

the two genital openings. The latter he knew to exist for he described and illustrated them in his account of the reproductive system of the adult.

Jackson (1890) is possibly the most quoted of the early workers, and he is certainly the one most quoted in English language publications. I believe his work on N. io is the first explanation of the origin of the genital apertures. From serial sections he elucidated the formation of the ostium bursae and oviporus from persistant openings beneath the vestibular and vaginal rudiments (Jackson's "anterior hypodermal vesicle" and "posterior hypodermal vesicle," respectively). He described in detail the development of the "azygous oviduct," by which he means the entire median egg passage, and he also experienced some difficulty interpreting the formation of its anterior end. He notes it is confused with the hypodermis near the genital cords and, in Jackson's words, "my own impression is that there is an ingrowth from the hypodermis of cells which subsequently arrange themselves in the form of a tube; in other words the invagination of cells is at first solid (italics mine)." I am confident that these events in N. io are comparable to those I ascribe to Disc 7 in O. nubilalis.

Jackson shows the bursa and spermatheca are derived from the anterior hypodermal vesicle. But as he worked only with pupae and mature larvae, he also failed to see the paired

imaginal discs. Nevertheless, just from the appearance of the vesicles he deduced that each may have originally been paired.

To my knowledge, the earliest description of Discs 8 and Discs 9 is that of Verson and Bisson (1896b). Using B. mori, they trace the origin and later fusion of these "Imaginalscheiben." They confirm Jackson in deriving the bursa copulatrix and spermatheca from the anterior rudiment and the accessory glands from the posterior one; and they agree that Segments 7, 8 and 9 all contribute to the formation of the median egg passage. Furthermore, they also find the ostium bursae results from the incomplete closure of the vestibular groove (their "erste Genitalfurche"), and the oviporus results from the incomplete closure of the vaginal groove (their "zweite Genitalfurche").

Ammann (1954) and Brunold (1957) have together given one of the most thorough accounts of female reproductive system organogenesis in any lepidopteran. My findings substantiate theirs remarkably well. Ammann and I both derive the bursa and spermatheca from the respective anterior and posterior portions of the rudiment in Segment 8. We also agree that the accessory glands and the posterior part of the egg passage arise from Segment 9. Furthermore, Segments 7, 8 and 9 all contribute to the median egg passage which is formed by the closure of a ventral groove. The groove itself is the

result of the coalescence of ventrally open ectodermal rudiments in each of the three segments. Brunold and I visualize the definitive genital openings as arising on the eighth and ninth segments. They temporarily share a common opening, the ventral groove, and are finally established when the groove closes.

Dodson (1937) examined only the pupa in her study of Zygaena, the earliest stage examined being comparable to the condition of O. nubilalis at pupation. She did not, of course, observe the origin of the rudiments, but she concedes the median egg passage (Dodson's "common oviduct") may have once been an open groove. She also derives the seminal duct from the wall of that part of the egg passage shared by the oviduct and bursa by constricting this area into a narrow tube connecting the two ducts. This is exactly what I observed in the European corn borer.

The studies by Joubert (1964b, 1969) leave as many questions unanswered as they answer. He apparently sectioned only the gonad bearing segments, for the first sign he found of the development of the ectodermally derived organs was in the prepupa. In the earlier paper this was in the form of a small "claviform nodule" close to the ventral base of the rectum in Segment 8. This growth is obviously what I have represented as the fused vestibular and vaginal rudiments, but the anlagen of the bursa, spermatheca, and accessory

glands are already present. Joubert makes no reference to the pertinent literature on the subject, and although he goes to great lengths to discover the earliest possible differentiation of the gonads, he seems unconcerned that the ectodermal rudiments make their appearance full-blown, as it were.

In his 1969 paper Joubert describes, in the prepupa, a small linear apodeme occupying the position of the claviform nodule. The nodule appears later, in the middle of the apodeme. I believe the apodeme to be what I have described as the fold in the pupal cuticle that marks the genital opening, i.e., the ventral groove.

An exception to the general accord of the pertinent literature is the study of L. orbonalis by Srivastava and Srivastava (1959b). Their conclusions are at variance with those summarized here on several points, the most important being the nature of the very origin of the ectodermal anlagen. The genital invaginations, according to Srivastava and Srivastava, are unpaired median invaginations of the eighth and ninth sterna, the "uterine rudiment" and the "spermathecal rudiment," respectively. What then of the imaginal discs? Srivastava and Srivastava report that late in larval life a pair of hypodermal swellings appear near the posterior margins of both the eighth and ninth sterna. The swellings increase in length and become invaginated to form

paired external grooves. Their photomicrographs of transverse sections of these swellings are almost identical to my Figs. 29 and 30 which are transverse sections of the imaginal discs. But Srivastava and Srivastava found that the paired grooves on the eighth and ninth sterna "disappear" toward the end of the prepupal stage. Furthermore, they go on to say that ". . .no connection whatsoever has been observed between them and the genital invaginations."

They agree that the ostium bursae and oviporus are the persistent external apertures of the two genital invaginations, and Srivastava and Srivastava also derive the bursa and accessory glands from the respective anterior and posterior invaginations. But, in marked contrast to the other researchers, they derive the spermatheca from the anterior end of the posterior rudiment, that is, from Segment 9.

Can organogenesis in L. orbonalis be so different from that of other Lepidoptera? Too few species have been thoroughly studied to answer this question. But if there is no difference, who is in error? I know of no work that supports Srivastava and Srivastava, but Umeya (1927) offers considerable substantiation of the conclusions of Verson and Bisson (1896b) concerning the role of the imaginal discs in B. mori. The results he obtained by extirpating and transplanting the discs leave little doubt as to the

derivation of the median oviduct, the bursa, and the spermatheca from Discs 8; the vagina and accessory glands clearly originate from Discs 9.

Another disputed point, although less significant, concerns the origin of the lateral oviducts. Jackson (1890), Verson and Bisson (1896b), Ammann (1954), and I all consider them to be mesodermal in origin. However, Srivastava and Srivastava (1959b) and Joubert (1964a, 1969) concluded they are of mixed origin. In the insects they studied, the anterior part of the lateral oviducts is mesodermal; the posterior part is ectodermal, being derived from the anterior end of the median oviduct. The two portions do not join until the pupal stadium.

Both views may be correct. The essential difference lies in the anteriad extent of the development of the oviducal rudiment. If it reaches the genital cords as it does in the European corn borer, then no ectodermal lateral oviducts are required. If it does not, as Srivastava and Srivastava (1959b) and Joubert (1964a, 1969) have found, then there must either be a posteriad extension of the mesodermal cords, or an anteriad growth from the oviducal rudiment, i.e., ectodermal rudiments of the posterior part of the lateral oviducts.

The most important discrepancy in the literature of male reproductive system development involves the determination of

the origin of the vas deferens.

There are two schools of thought on this issue. In one, the genital cords are variously reported to terminate in the larva on Segment 6, 7 or 8, and they later join ectodermal rudiments from Segment 9. The definitive vas deferens, therefore, is thought to be of mixed origin. The opposing school reports the genital cords reach Herold's organ in Segment 9, and the conclusion reached is that the vas deferens is entirely mesodermal in origin.

If the only question was the origin of the vas deferens the two viewpoints could be reconciled with the same sort of rationale previously used to explain the different views concerning the origin of the lateral oviducts. But, much more is involved here. It is significant that those who find the cords reach Herold's organ (Verson and Bisson, 1896a; Florin, 1945; Joubert, 1965; Wittig, 1960; and myself) derive the accessory glands, paired ejaculataory ducts, and part of the common ejaculatory duct from enlargements of the lower ends of the genital cords. That is to say, these organs are assigned a mesodermal origin.

On the contrary, those who find the genital cords fail to reach Herold's organ in the young larva (Mehta, 1933; Rakshpal, 1944; Srivastava and Srivastava, 1959a) conclude the genital cords are of mixed mesodermal and ectodermal origin and the organs mentioned are derived from the

ectodermal portion.

Although Rakshpal's (1944) treatment of the development of the internal genital organs is too superficial to be convincing, Mehta (1933) and Srivastava and Srivastava (1959a) are more thorough. Nevertheless, it is impossible to reconcile the two viewpoints without reexamining the species studied, and Srivastava and Srivastava have apparently made at least one serious error which casts doubt upon their conclusions. They say the ampullae split longitudinally to form two pairs of ducts. The inner pair represent the paired ejaculatory ducts; the outer pair represent the accessory glands. They illustrate this stage with their Fig. f, Pl. 16, but the structures they label as accessory glands are surely the vasa deferentia. They repeat this labelling in Fig. d, Pl. 17 which is very similar to my Fig. 73e. My figure is a single section from a complete series through the developing system. The series shows that the organs under discussion are unquestionably the vasa deferentia. I am convinced that Srivastava and Srivastava have confused the developing accessory glands and vasa deferentia. In so doing they have failed to explain the origin of either.

SUMMARY

The gonads of Ostrinia nubilalis are present at hatching, but they are not sexually distinct until the second stadium.

The ovaries remain small and hidden in the fat body throughout larval life. Each consists of four ovarioles enclosed within a common epithelium, the ovarian sac. Within the ovarian sac the individual ovarioles are bounded by two additional epithelial layers. At pupation the ovarioles elongate and rupture the ovarian sac. The inner epithelium degenerates late in pupal life leaving the ovariole of the adult surrounded by one wall, the original middle epithelium.

The primordia of the lateral oviducts, the lumenless genital cords, are present in the first instar but they may not be complete. They can be followed from the ovaries to their termination on the sternum of Segment 7 in second instar larvae. The lumina develop soon after pupation, thus establishing the definitive lateral oviducts. However, their lumina remain separated from the lumen of the median oviduct until late in the pupal stadium when the wall between them breaks down.

A pair of imaginal discs appears on the ventral hypodermis of Segments 8 and 9 during the third stadium. They enlarge and begin to invaginate during the fourth stadium. In the fifth stadium invagination is completed, and

each pair fuses into a single median rudiment, the vestibular rudiment in Segment 8 and the vaginal rudiment in Segment 9. Late in the fifth stadium an unpaired disc appears mesially in Segment 7. It forms the oviducal rudiment which becomes the anterior end of the median oviduct.

Near the end of larval life the three rudiments fuse and, for a brief time, form a medial longitudinal groove in the hypodermis of the developing pupa. This ventral groove persists in the pupal cuticle as the genital opening, the form and location of which is used to identify the pupal sex.

The vestibular rudiment gives rise to the bursa copulatrix, spermatheca, the posterior part of the median oviduct, and the vestibulum. The vaginal rudiment gives rise to the accessory glands, the vagina, and the oviporus.

The vestibulum is defined as the area of the median egg passage which receives the spermathecal and seminal ducts. It also receives the median oviduct anteriorly and the vagina posteriorly. The seminal duct is actually derived from the vestibulum by constriction of its walls.

Unlike the ovaries, the testes are not buried in the fat body and are easily seen. Each consists of four follicles within a common covering. At first the follicular epithelium and the outer epithelium are similar, but in the fifth stadium the outer epithelium acquires many acidophilic inclusions. These are lost late in pupal life and the two layers

are similar in the adult.

At pupation the paired larval testes fuse to become the single organ found in the adult. The fused testes twist about the longitudinal axis causing the vasa deferentia to cross. The left testes is twisted dorsad and to the right so that the apparent right vas deferens of the adult is the morphological left.

Herold's organ, the primordium of the ectodermal parts of the male reproductive system, is present in one day old larvae. It is a mesial invagination of the ventral hypodermis at the hind margin of Segment 9. In second instar larvae the genital cords can be traced from the testes to the distal end of Herold's organ where they terminate as small ampullae.

During the fourth stadium the lateral walls of Herold's organ give rise to a pair of primary genital lobes which, in the fifth stadium, are subdivided into the posterior valvular lobes and the anterior aedeagal lobes. The aedeagal lobes fuse and the cavity they enclose becomes the endophallus and the cuticular part of the common ejaculatory duct.

The mesodermal ampullae split to form the accessory glands anteriorly and the primary portion of the common ejaculatory duct posteriorly. The latter originates as a double duct. The upper part forms the paired ejaculatory ducts, but the remainder is fused so that it superficially

appears as a single duct. Not until the middle of the pupal stadium does the wall between the two ducts break down to form the unpaired primary ejaculatory duct of the adult. However, the partition separating the lumina of the primary and cuticular ejaculatory ducts persists until emergence of the adult.

Two thin simple epithelia form "arches" over the cuticular ejaculatory duct. These epithelia are continuations of the manica (outer arch) and the aedeagal epithelium (inner arch). Beneath the ejaculatory duct, bands of muscle reach from one side of the inner arch to the other. By the middle of the pupal stage the epithelia are so thin and close together, and the duct so tortuous, that the epithelia are hardly recognized as archlike and are easily interpreted as forming two concentric tubes around the cuticular ejaculatory duct.

Although the basic morphology of the reproductive system of both sexes is clear soon after pupation, the definitive organs are not completely differentiated until the middle of the pupal stadium.

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ACKNOWLEDGEMENTS

I wish to express my gratitude to my major professor, Dr. Tom A. Brindley, for his understanding and patient support throughout the long course of this study.

I am also indebted to the other members of my graduate committee, Dr. Willard F. Hollander, Dr. Robert E. Lewis, Dr. John A. Mutchmor, and Dr. Oscar E. Tauber, for their advice and critical comment on the preparation of the manuscript.

Special recognition is due the late Dr. Jean L. Laffoon. He was both professor and friend. I often sought his help and advice, and he gave both generously.

APPENDIX: FIGURES

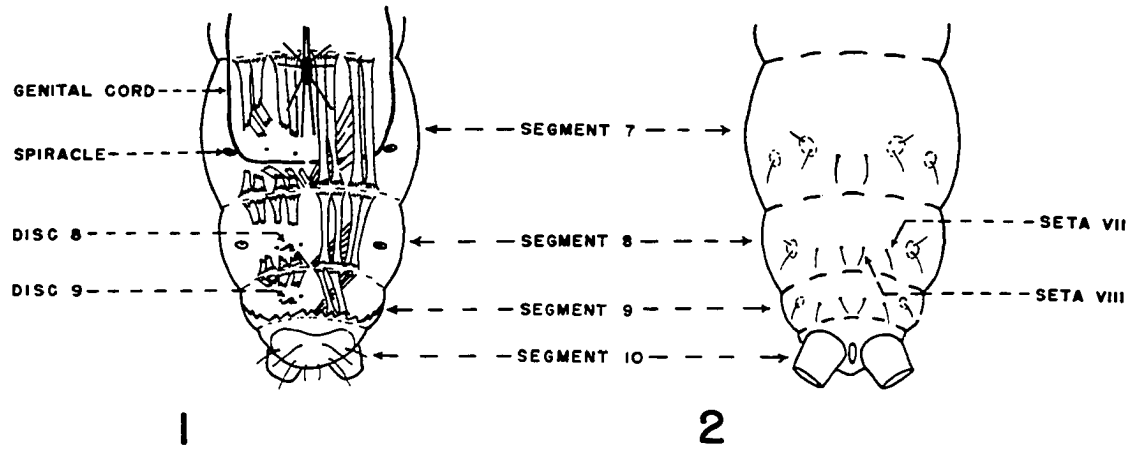
Figure 1. Female larva, abdominal segments 7-10. Diagrammatic internal view of the lateral and ventral surfaces. The position of the last abdominal ganglion is shown, but the gut, tracheae and lateral musculature are omitted. The dots on either side of Discs 8 and 9 indicate the positions of Setae VII and VIII.

Figure 2. Larva, abdominal segments 7-10. Diagrammatic external ventral view.

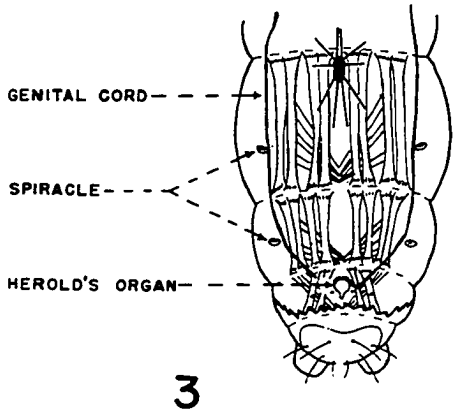
Figure 3. Male larva, abdominal segments 7-10. Diagrammatic internal view of the lateral and ventral surfaces. The position of the last abdominal ganglion is shown, but the gut, tracheae and lateral musculature are omitted. The dots on either side of Herold's organ indicate the position of Setae VII and VIII.

Figure 4. Female pupa, abdominal segments 6-10. Ventral view.

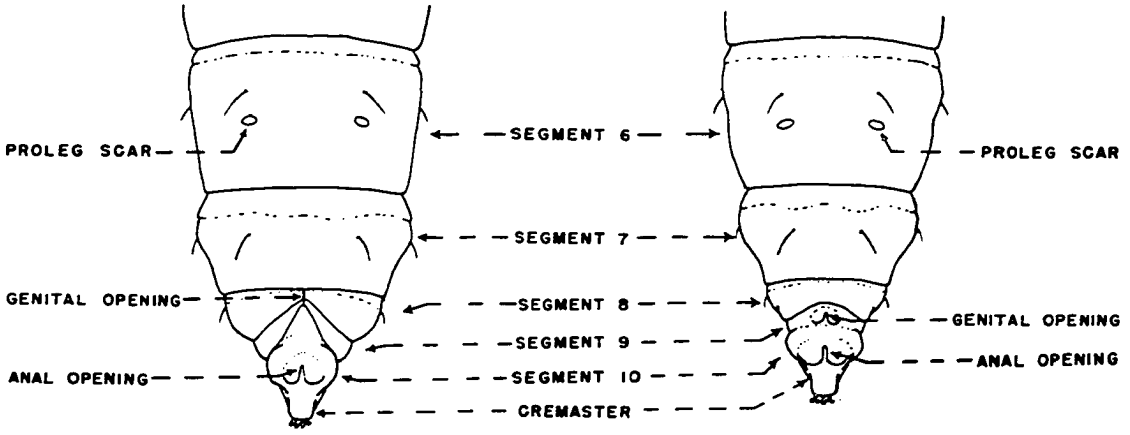
Figure 5. Male pupa, abdominal segments 6-10. Ventral view.


GENITAL CORD
SPIRACLE
DISC 8
DISC 9
SEGMENT 7
SEGMENT 8
SEGMENT 9
SEGMENT 10
SETA VII
SETA VIII

1 2

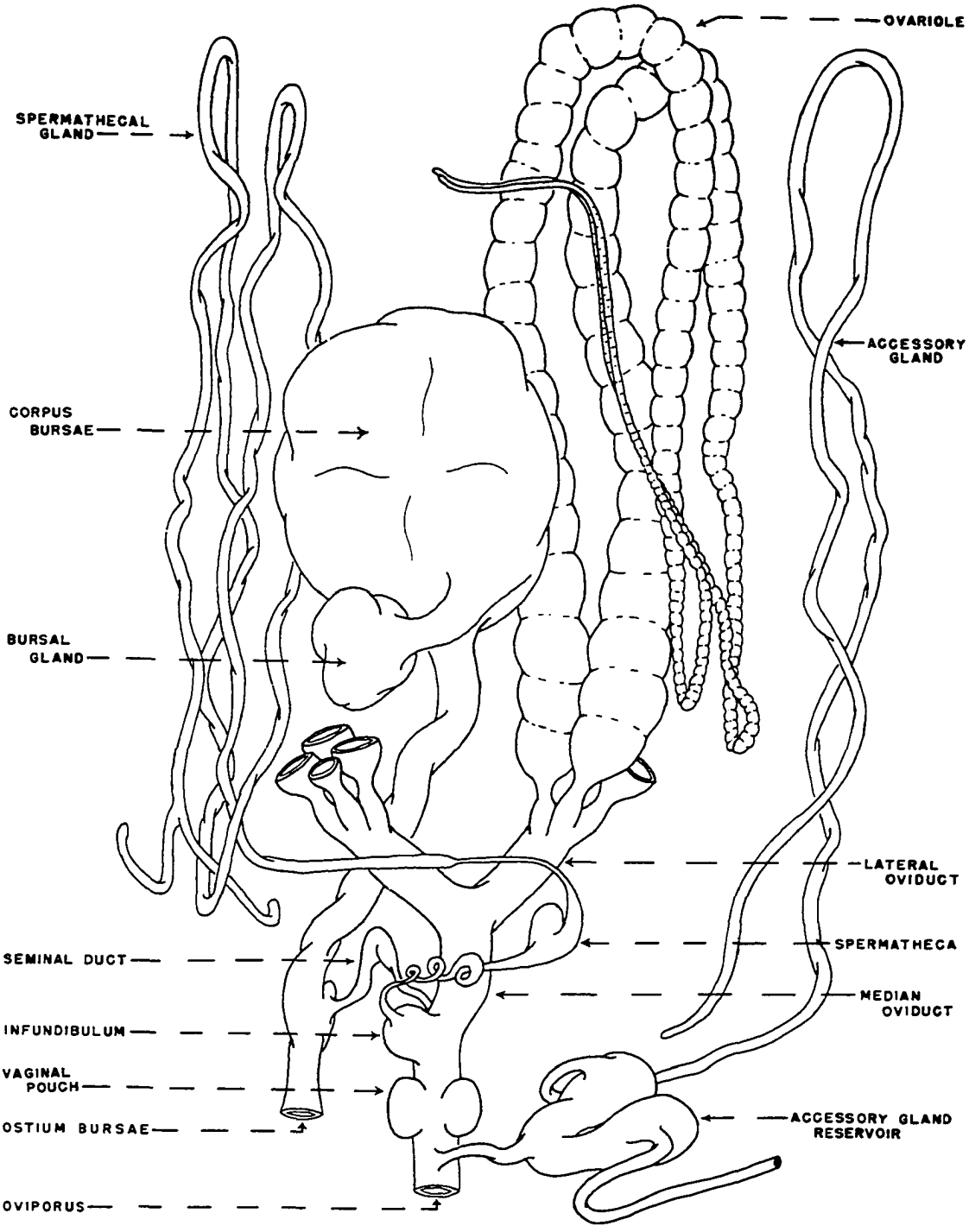

GENITAL CORD
SPIRACLE
HEROLD'S ORGAN

3


PROLEG SCAR
GENITAL OPENING
ANAL OPENING
SEGMENT 6
SEGMENT 7
SEGMENT 8
SEGMENT 9
SEGMENT 10
CREMASTER
PROLEG SCAR
GENITAL OPENING
ANAL OPENING

4 5

Figure 6. Adult female reproductive system. Diagrammatic dorsal view with the organs shown spread and arranged for visibility. The bursa copulatrix and accessory gland reservoir, respectively, are shown to the right and left of their usual midline positions. One accessory gland and one loop of the spermathecal gland are usually found on each side of the abdomen. Only two of the eight ovarioles are shown in their entirety.


OVARIOLE
SPERMATHECAL GLAND
ACCESSORY GLAND
CORPUS BURSAE
BURSAL GLAND
LATERAL OVIDUCT
SPERMATHECA
SEMINAL DUCT
MEDIAN OVIDUCT
INFUNDIBULUM
VAGINAL POUCH
ACCESSORY GLAND RESERVOIR
OSTIUM BURSAE
OVIPORUS

6

Figure 7. Gonads, early in the 1st stadium. Transverse section.

Figure 8. Gonad, early in the 1st stadium. Sagittal section.

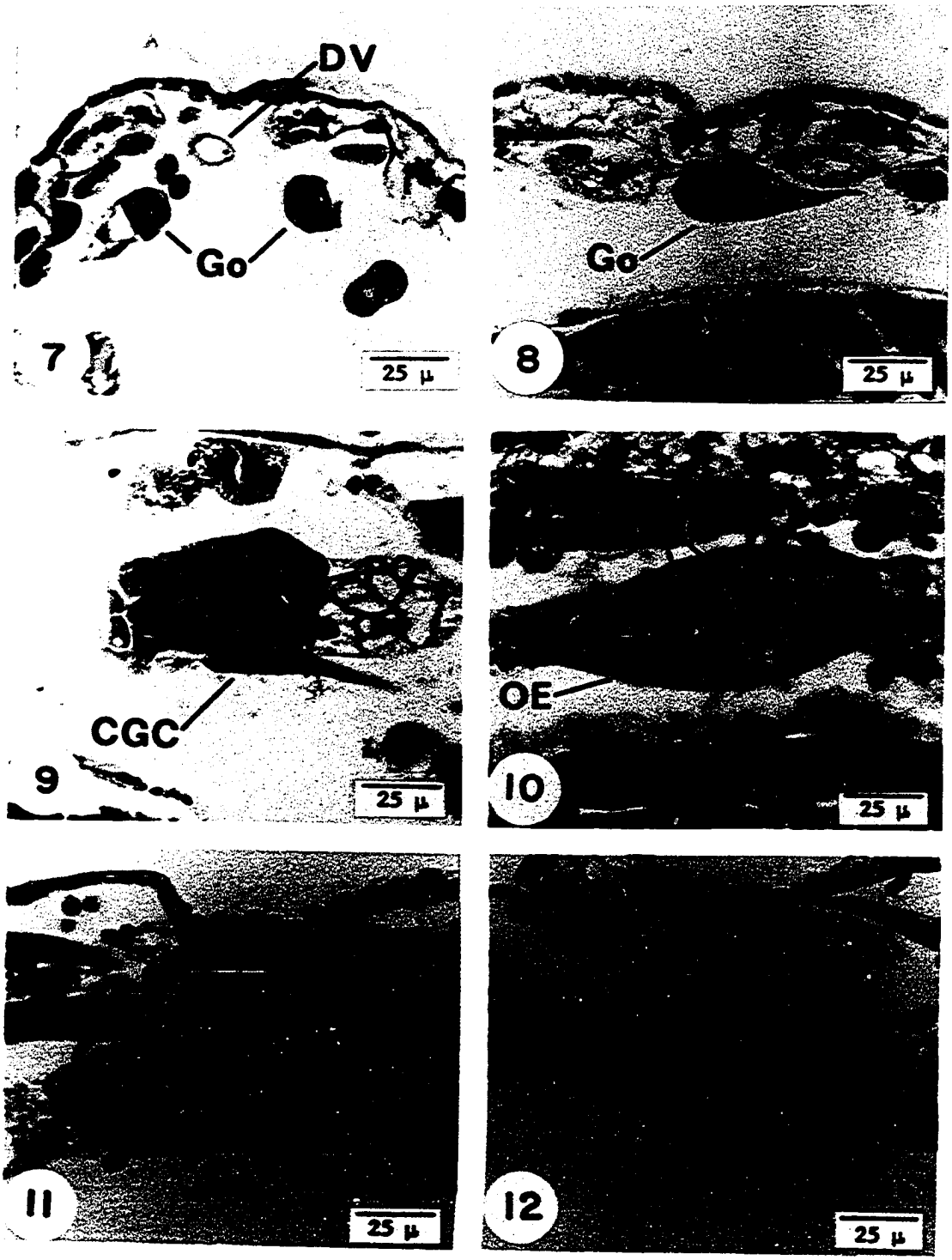
Figure 9. Ovary, early in the 2nd stadium. Sagittal section.

Figure 10. Ovary, early in the 2nd stadium. Transverse section.

Figure 11. Ovary, in the middle of the 3rd stadium. Sagittal section.

Figure 12. Ovary, in the middle of the 3rd stadium. Transverse section.

Lettering. C, calyx; CGC, clavate end of the genital cord; DV, dorsal vessel Go, gonad; OE, outer epithelium; Ov, ovariole; PC, primordium of the calyx (and pedicels); Ped, pedicel; St, stroma


DV
Go
7
25 µ
Go
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25 µ
CGC
9
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OE
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11
25 µ
12
25 µ

Figure 13. Disc 8, in the middle of the 3rd stadium. Transverse section.

Figure 14. Disc 9, in the middle of the 3rd stadium. Transverse section.

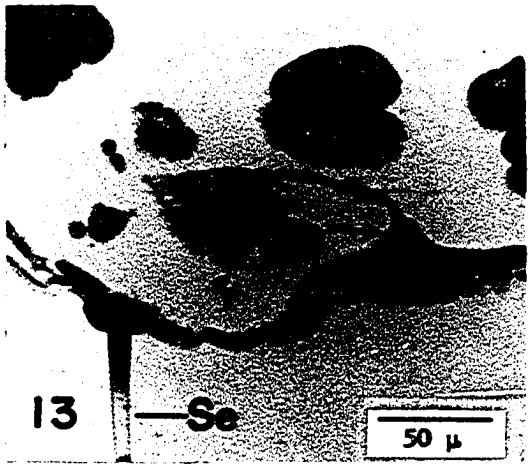
Figure 15. Disc 8, late in the 4th stadium. Transverse section.

Figure 16. Disc 9, late in the 4th stadium. Transverse section.

Figure 17. Disc 8, early in the 5th stadium. Transverse section.

Figure 18. Disc 9, early in the 5th stadium. Transverse section.

Lettering. Cu, cuticle; FB, fat body; Hyp, hypodermis; Mu, muscle; Se, Seta VII. The unlabelled arrows indicate the mass of undifferentiated cells found on top of each disc.



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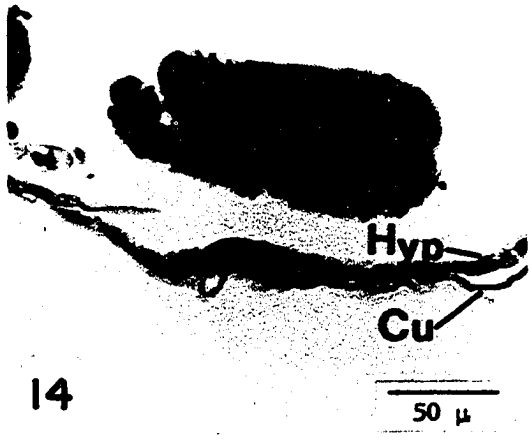

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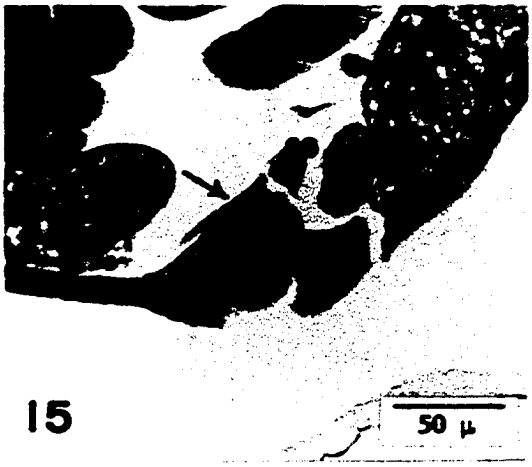

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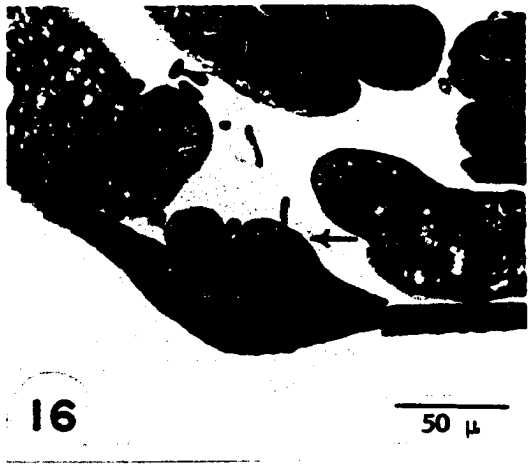

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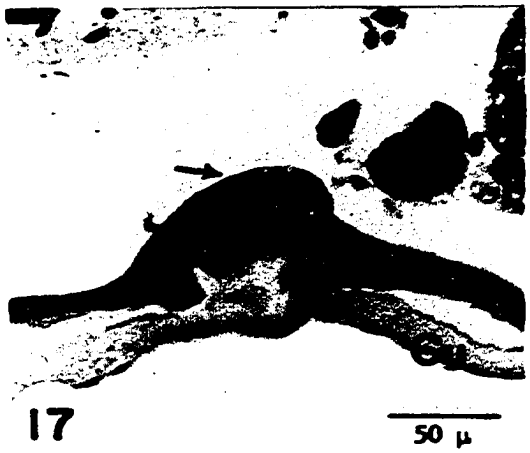

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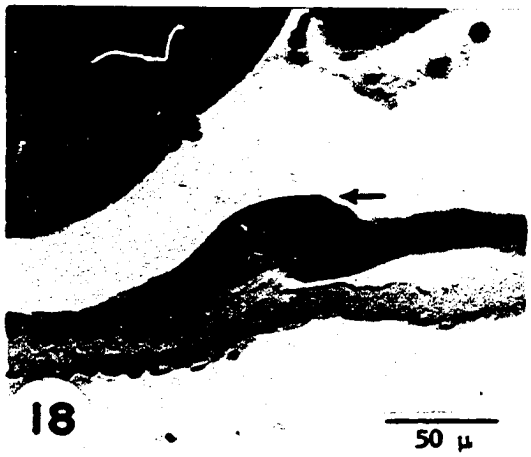


18

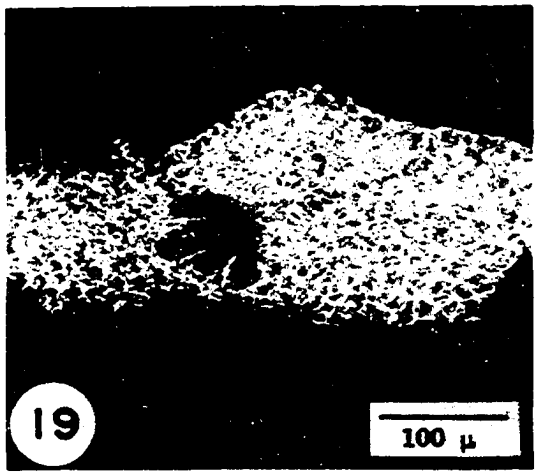
Figure 19. Ovary, early in the 3rd stadium. Glycerine jelly whole mount, hematoxylin stain.

Figure 20. Ovary, early in the 4th stadium. Glycerine jelly whole mount, hematoxylin stain.

Figure 21. Ovary, late in the 4th stadium. Glycerine jelly whole mount, unstained.

Figure 22. Ovary, early in the 5th stadium. Glycerine jelly whole mount, unstained.

Figure 23. Ovary, late in the 5th stadium. Glycerine jelly whole mount, unstained.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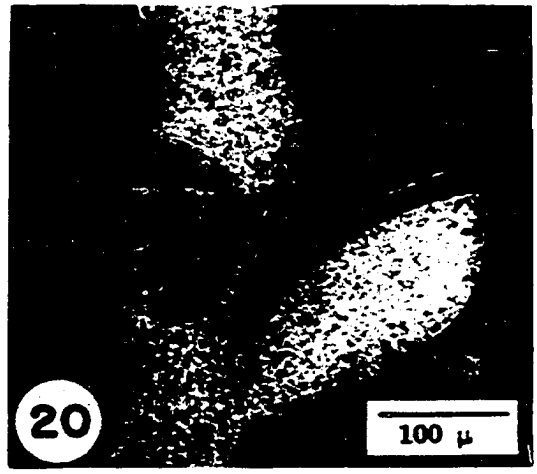
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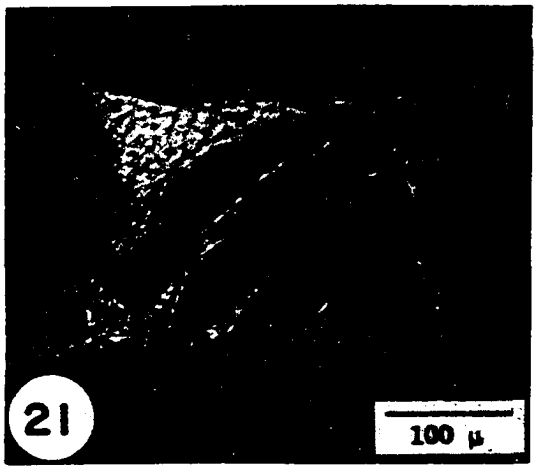
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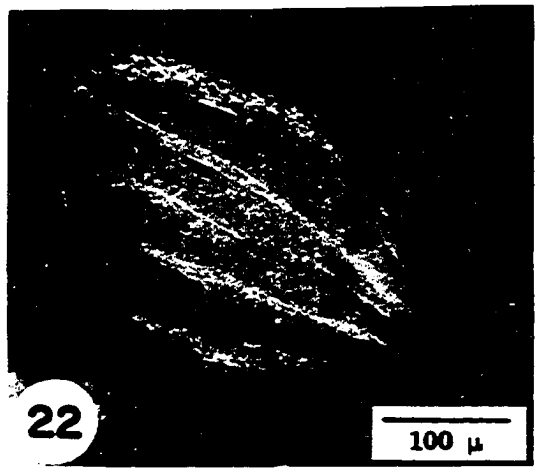
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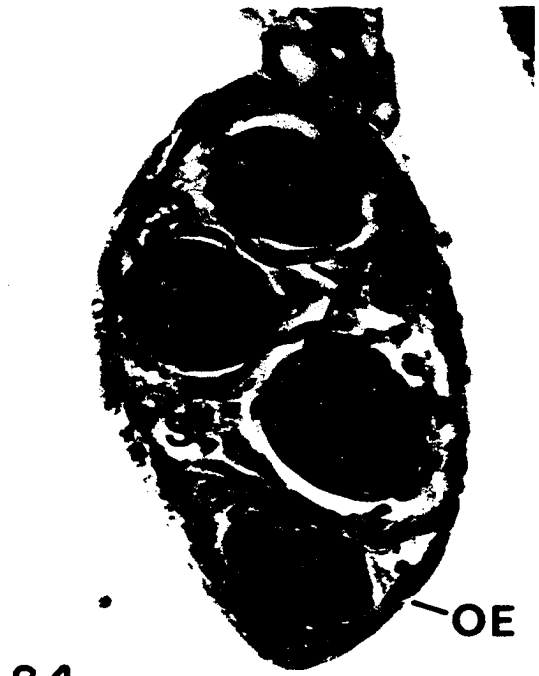
Figure 24. Ovary, in the middle of the 4th stadium. Transverse section. The inner epithelium is differentiating.

Figure 25. Ovary, in the middle of the 5th stadium. Transverse section. The inner epithelium is established and the middle epithelium is differentiating.

Figure 26. Ovary, late in the 5th stadium. Longitudinal section. The inner, middle and outer epithelia are distinct, but the outer epithelium is degenerating.

Figure 27. Ovary of a 6-hour-old pupa. Transverse section. Each ovariole is surrounded by the inner and middle (now the outer) epithelia. The original outer epithelium is absent.

Lettering. IE, ME, OE, inner, middle, and outer epithelium, respectively; St, stroma



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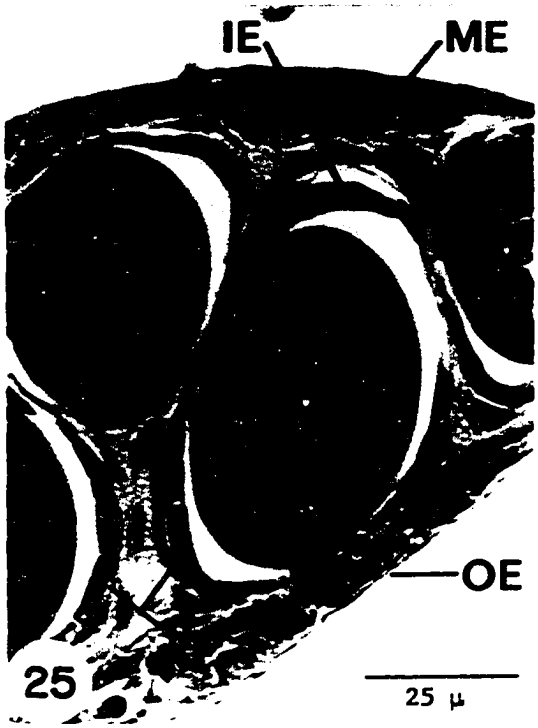

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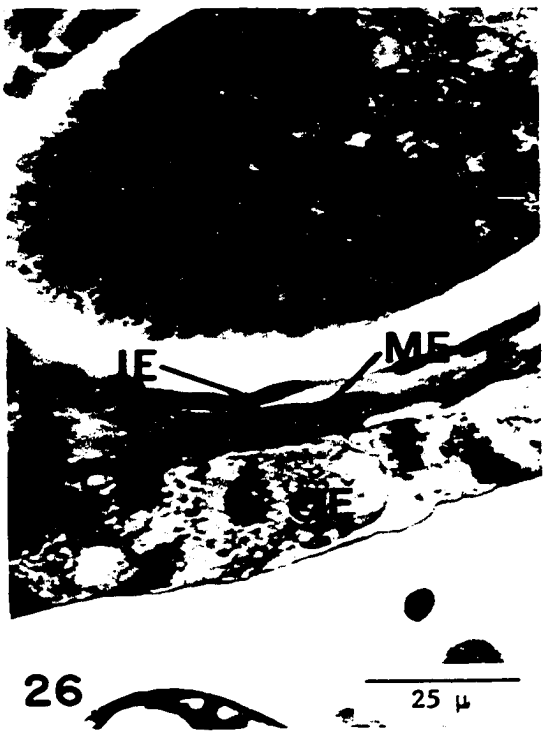

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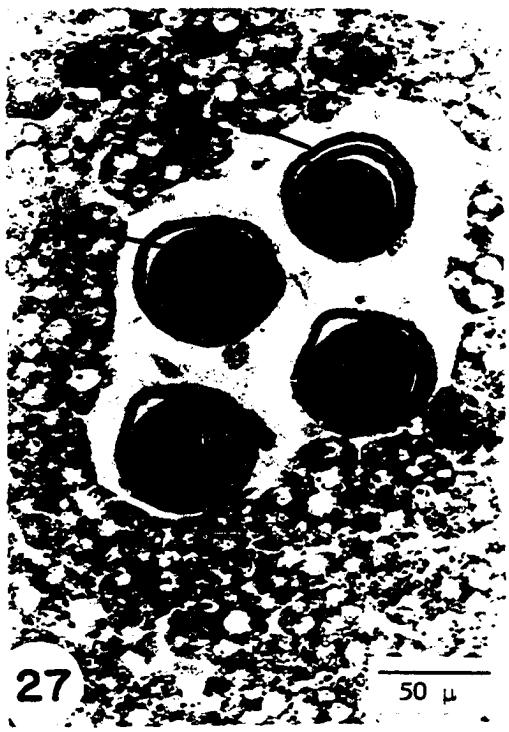


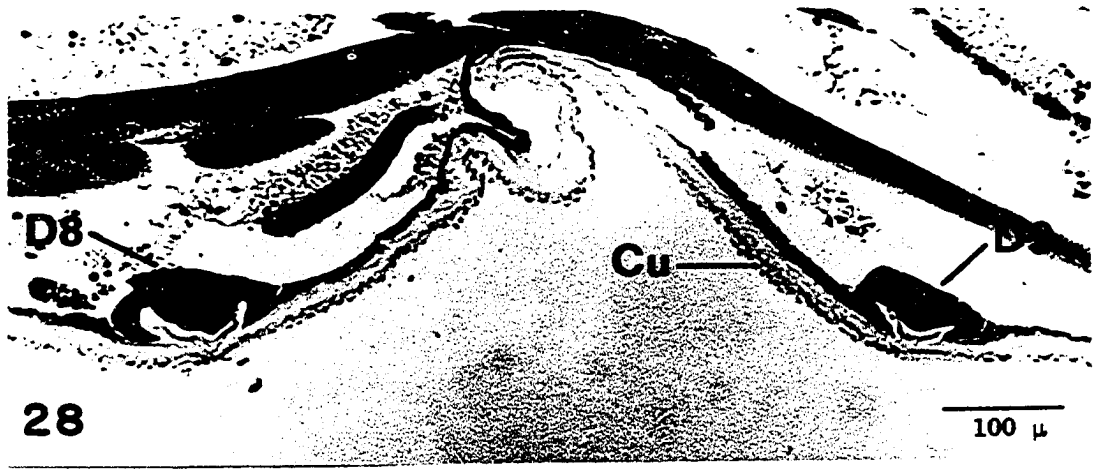
27

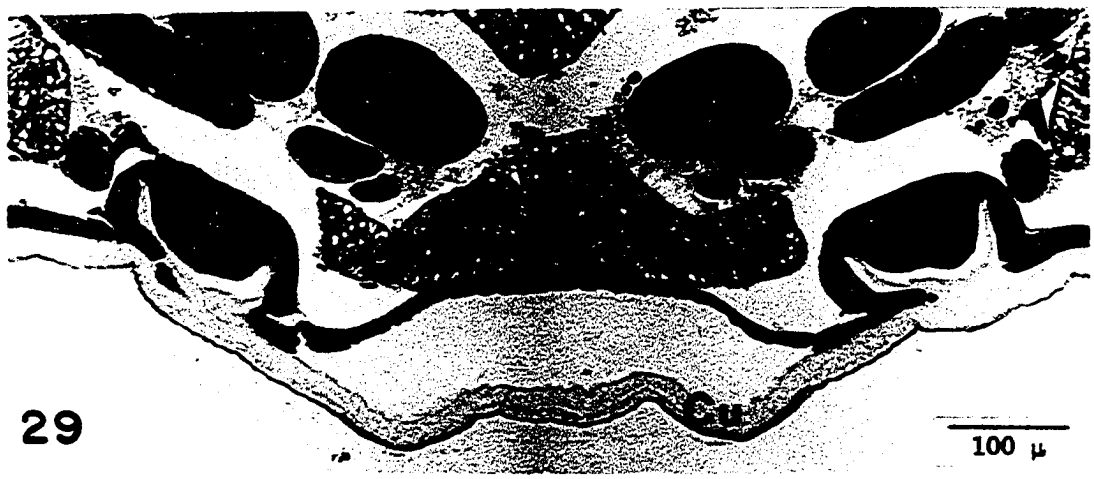
Figure 28. Discs 8 and 9, early in the 5th stadium. Sagittal section. Phase contrast.

Figure 29. Discs 8, in the middle of the 5th stadium. Transverse section.

Figure 30. Discs 9, in the middle of the 5th stadium. Transverse section.

Lettering. Cu, cuticle; D8, Disc 8; D9, Disc 9; FB, fat body; Mu, muscle; Se, Seta VIII


D8
Cu
28
100 µ


Cu
29
100 µ

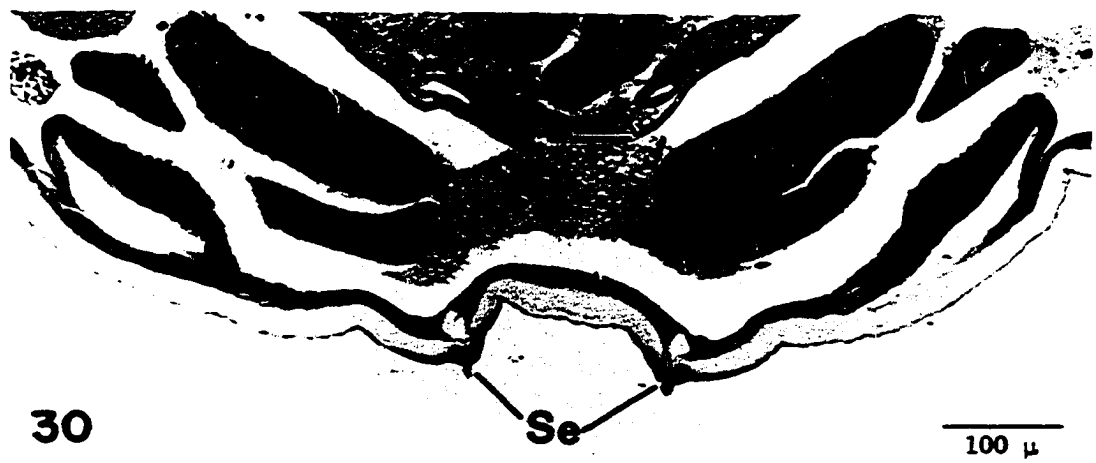
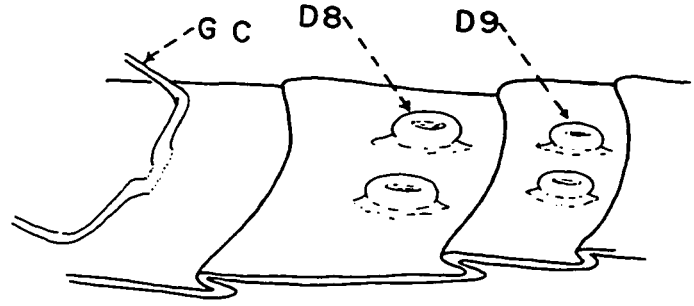
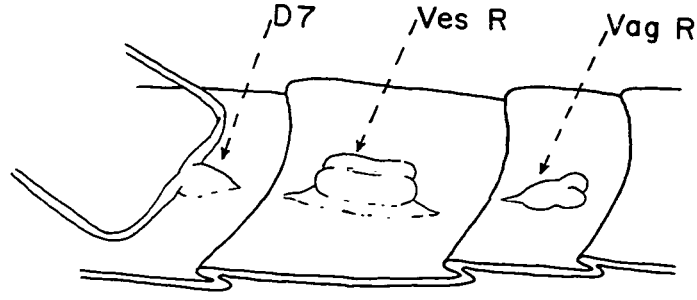

Se
30
100 µ

Figure 31. Morphogenesis of the ectodermal portions of the female reproductive system during the 5th stadium. At the onset of the stadium (a), paired imaginal discs lie on the ventral hypodermis of Segments 8 and 9. The paired discs fuse, and a median unpaired disc appears in Segment 7 (b). As the abdomen shortens prior to the larval-pupal ecdysis, the rudiments in the three segments approximate (c) and form a continuous system at pupation (d).

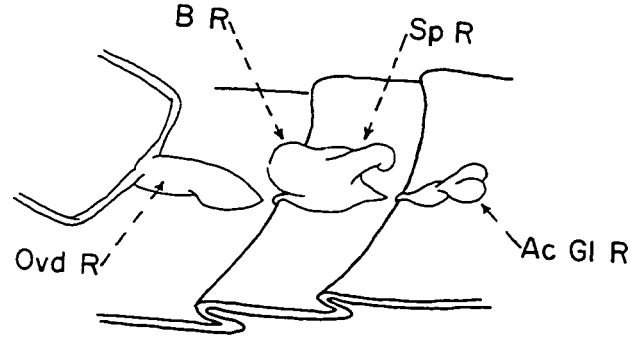
Lettering. AcGlR, accessory gland rudiment; BR, bursal rudiment; CB, corpus bursae; D7, D8, D9, Discs 7, 8, 9, respectively; GC, genital cord; OvdR, oviducal rudiment; SpR, spermathecal rudiment; VagR, vaginal rudiment; Ves, vestibulum; VesR, vestibular rudiment



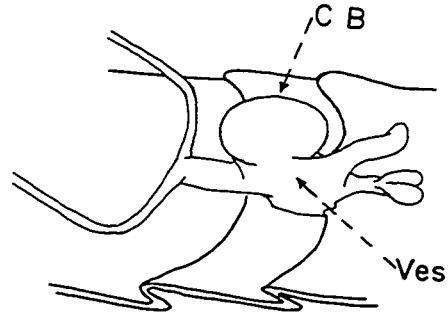
a



b



c



d

31

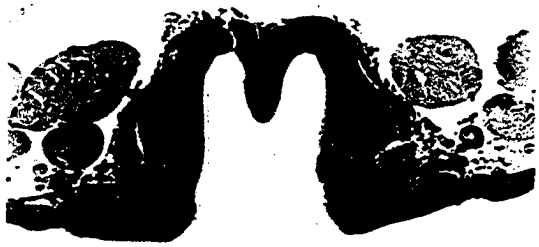
Figure 32. Vestibular rudiment, late in the 5th stadium. Transverse section of an isolated rudiment, cuticle removed, showing the fusion of Discs 8.

Figure 33. Vaginal rudiment, late in the 5th stadium. Transverse section of an isolated rudiment, cuticle removed, showing the partial fusion of Discs 9.

Figure 34. Disc 7, late in the 5th stadium. A near sagittal section through an intact abdomen, cuticle removed. The section is slightly oblique to the longitudinal axis of the abdomen.

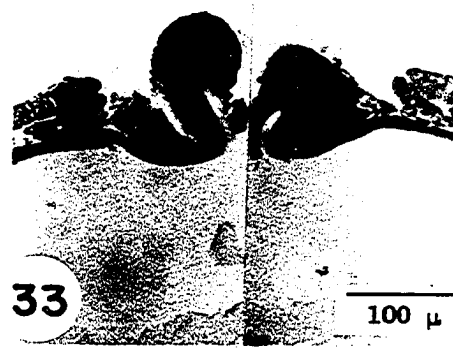
Figure 35. Oviducal groove, late in the 5th stadium. Transverse section of an abdomen after removal of the gut, fat body, and most of the cuticle. The section, through the intersegmental area, is slightly oblique to the vertical axis of the abdomen, and shows the posteriad extension of the oviducal groove into the anterior part of Segment 8. Sections anteriad to this, in Segment 7, show the groove closed ventrally to form the anterior part of the median oviduct. Sections posteriad to this show the depth of the groove progressively reduced, and it does not reach the vestibular rudiment.

Lettering. D7, Disc 7; GC, genital cord; OvdG, oviducal groove; S7, Sternum 7; S8, Sternum 8



32

100 µ

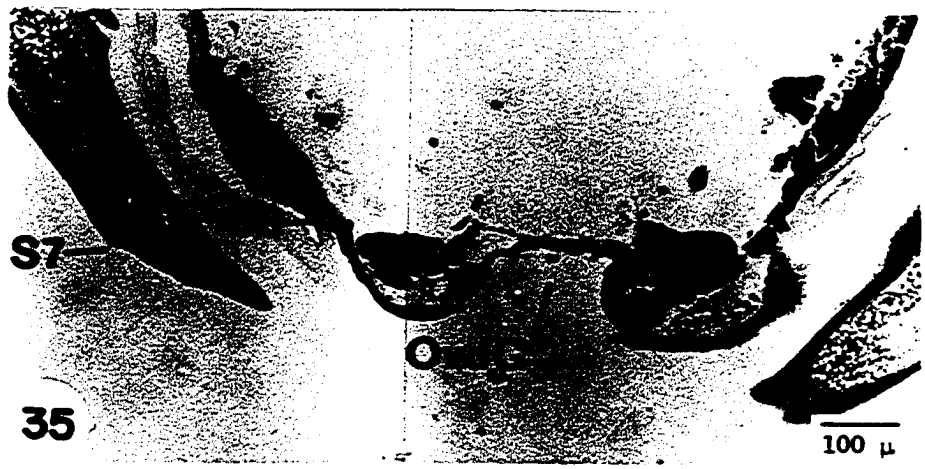


33

100 µ

34

50 µ



S7

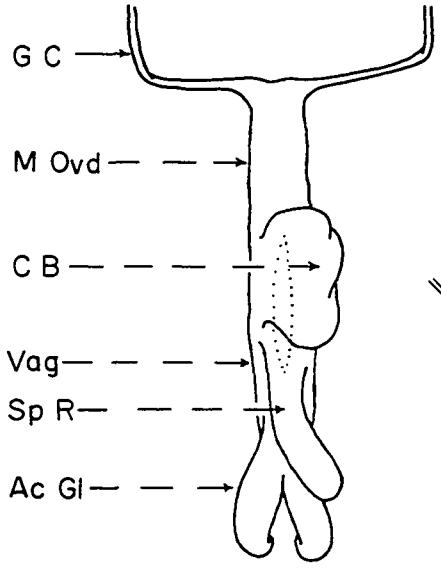
35

100 µ

Figure 36. Female reproductive system at pupation. Dorsal view, ovaries excluded. Reconstructed from serial sections. The extent of the ventral groove is indicated by the dotted outline.

Figure 37. Female reproductive system at pupation. Lateral view, ovaries excluded. Reconstructed from serial sections. The ventral groove is delineated by the dotted outline. Lines a-g indicate the approximate plane of the corresponding transverse sections a-g. The outlines of the sections were traced from microprojected images. The cuticle has been omitted.

Lettering. AcGl, accessory gland; AcGlD, accessory gland duct; CB, corpus bursae; GC, genital cord; Hyp, hypodermis; MOvd, median oviduct; Mu, muscle; OvdG, oviducal groove; SpR, spermathecal rudiment; Vag, vagina; VagG, vaginal groove; Ves, vestibulum; VesG, vestibular groove.



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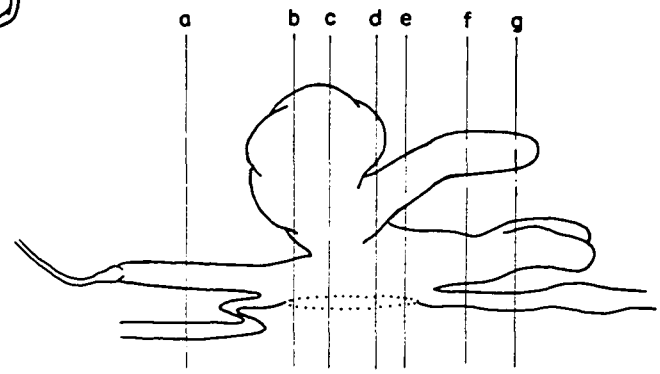

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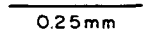
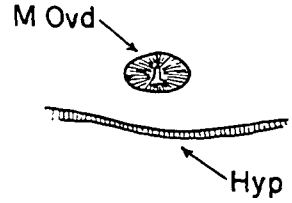
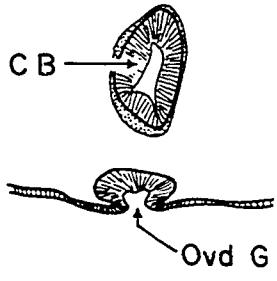
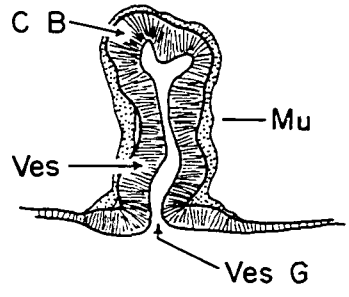




a



b



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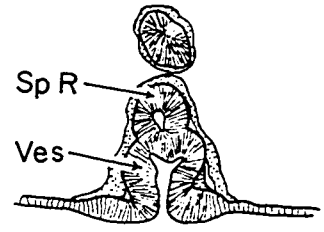

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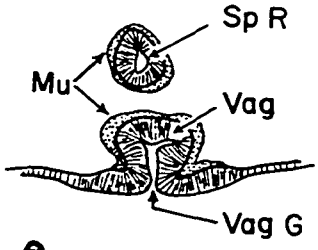
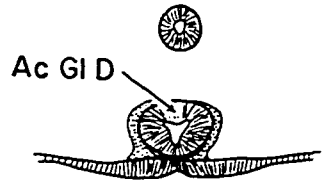
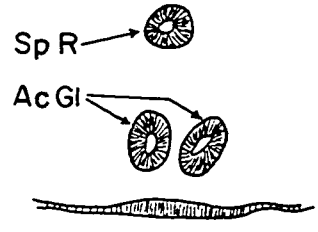


e



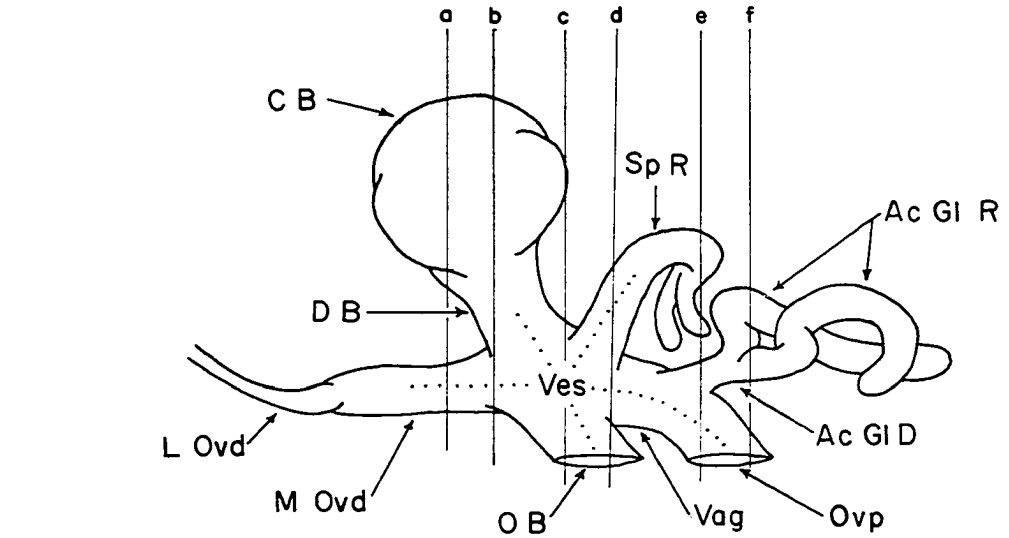
f



g

Figure 38. Female reproductive system, 18-hour-old pupa. Lateral view, ovaries excluded. Reconstructed from serial sections. Lines a-f indicate the approximate plane of the respective sections a-f. The outlines of the sections were traced from microprojected images. The cuticle is omitted.

Lettering. AcGlR, accessory gland rudiment; AcGlD, accessory gland duct; CB, corpus bursae; DB, ductus bursae; Hyp, hypodermis; LOvd, lateral oviduct; MOvd, median oviduct; Mu, muscle; OB, ostium bursae; Ovp, oviporus; SpR, spermathecal rudiment; Vag, vagina; Ves, vestibulum


a
b
c
d
e
f
C B
Sp R
Ac Gl R
D B
Ves
Ac Gl D
L Ovd
M Ovd
O B
Vag
Ovp

38

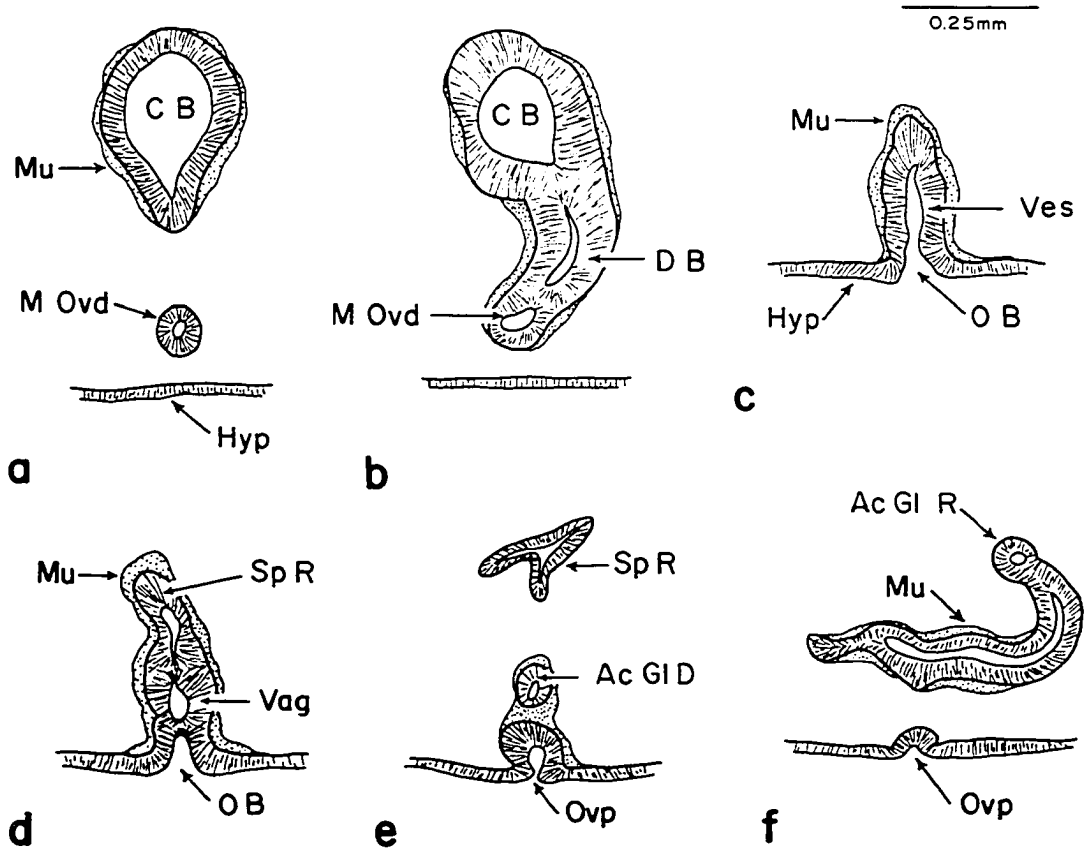

0.25mm
C B
Mu
M Ovd
Hyp
a
C B
D B
M Ovd
b
Mu
Ves
Hyp
O B
c
Mu
Sp R
Vag
O B
d
Sp R
Ac Gl D
Ovp
e
Ac Gl R
Mu
Ovp
f

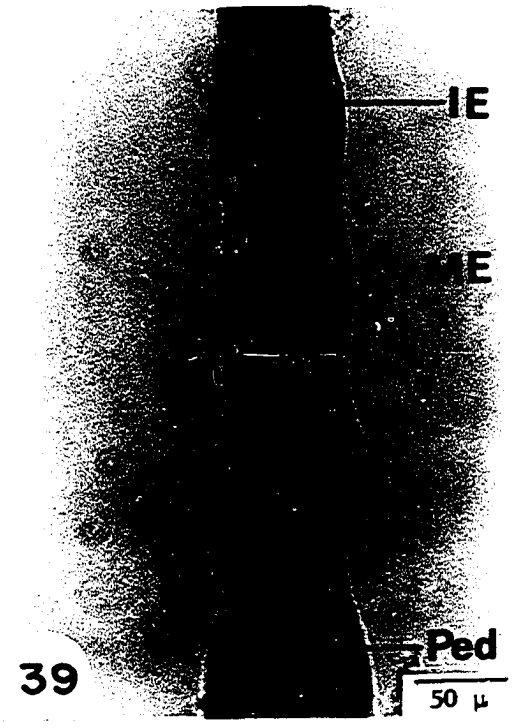
Figure 39. Ovariole of a 96-hour-old pupa. Permount whole mount, hematoxylin stain.

Figure 40. Ovariole of a 120-hour-old pupa. Longitudinal section through the upper part of the ovariole. The inner epithelium appears intact.

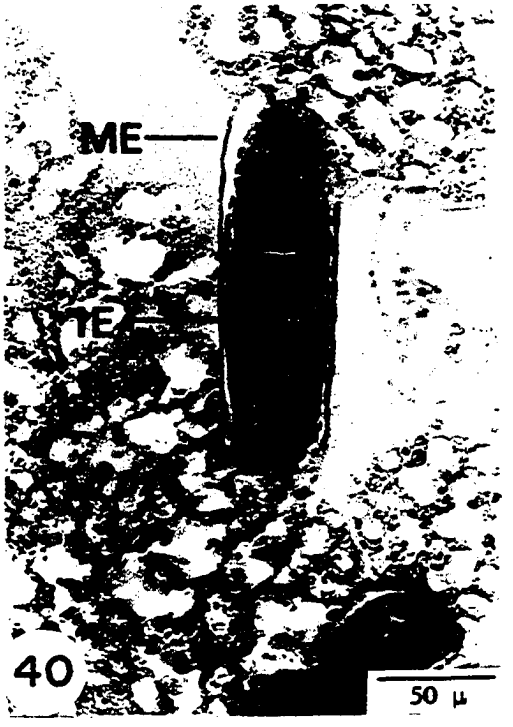
Figure 41. Ovariole of a 120-hour-old pupa. Longitudinal section through the lower part of the ovariole. The inner epithelium has degenerated, leaving only clusters of cells (at the unlabelled arrows) between the follicles.

Figure 42. Ovariole of a 144-hour-old pupa. Longitudinal section through an ovariole at the pedicel. The "epithelial plug" still separates the lumen of the pedicel from that of the ovariole.

Lettering. EP, epithelial plug; FC, follicle cell; GV, germinal vessicle; IE, inner epithelium; ME, middle (now the outermost) epithelium; NC, nurse cell; Oo, oocyte; Ped, pedicel



39



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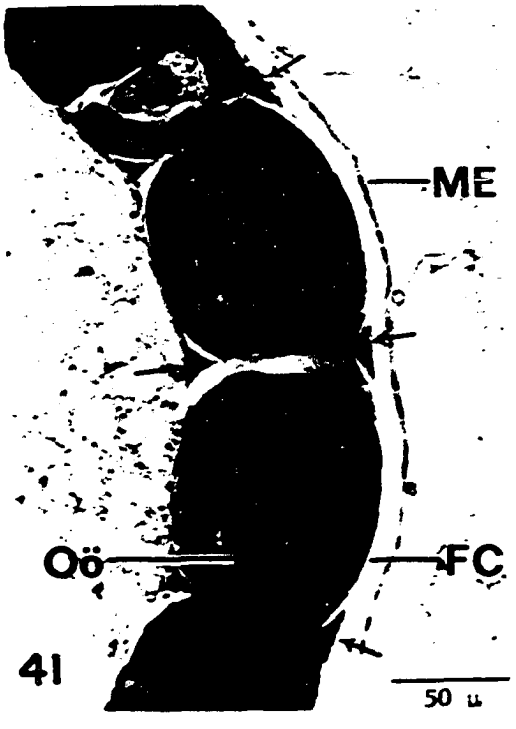
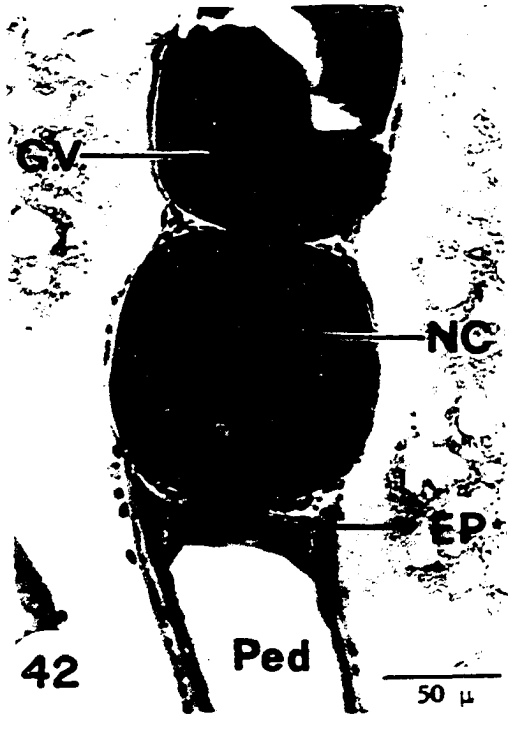


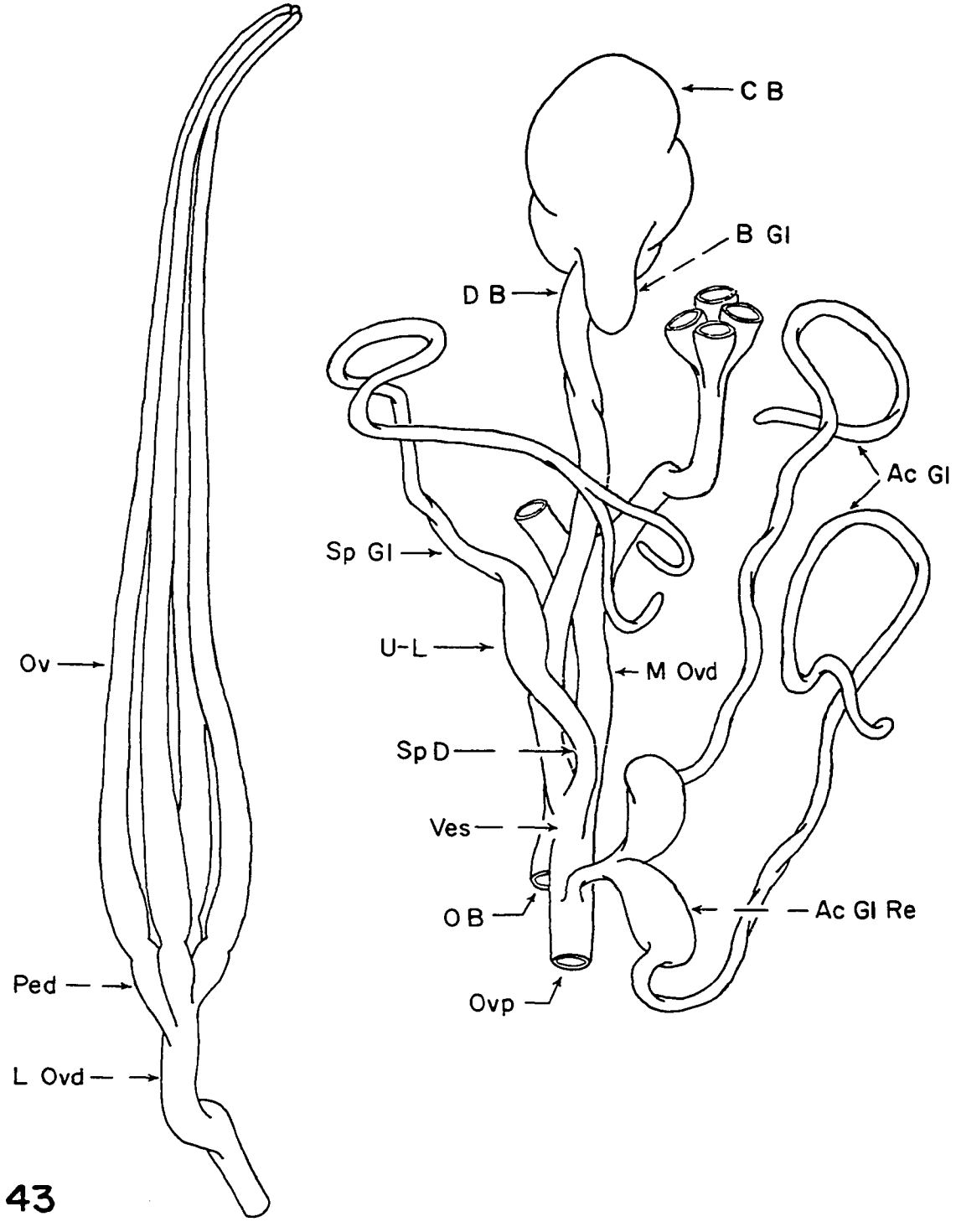
41



42

Figure 43. Female reproductive system in a 36-hour-old pupa. Diagrammatic dorsal view with the organs spread. The right ovary is omitted.

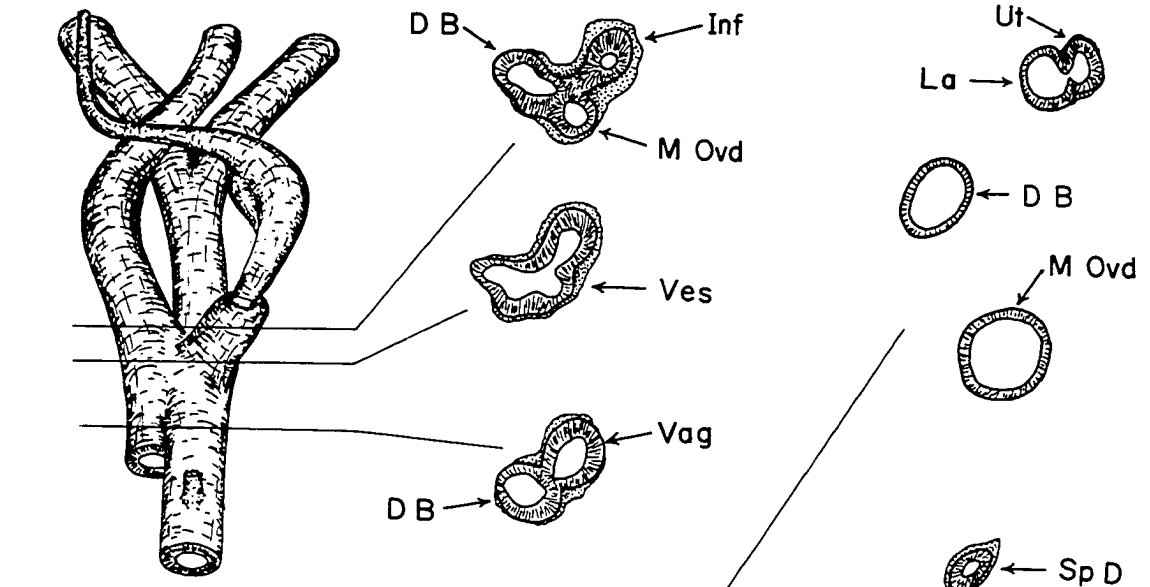
Lettering. AcGl, accessory gland; AcGlRe, accessory gland reservoir; BGl, bursal gland; CB, corpus bursae; DB, ductus bursae; LOvd, lateral oviduct; MOvd, median oviduct; OB, ostium bursae; Ov, ovariole; Ovp, oviporus; Ped, pedicel; SpD, spermathecal duct; SpGl, spermathecal gland; U-L, utriculus-lagena dilation of the spermathecal rudiment; Ves, vestibulum



43

Figure 44. Derivation of the seminal duct from the vestibulum. Dorsal view of the lower parts of the reproductive tract of a 36-hour-old pupa (a) and a 48-hour-old pupa (b), reconstructed from serial sections. The ducts are shown slightly spread. The thin lines indicate the approximate plane of the corresponding transverse sections. The outlines of the sections were traced from microprojected images.

Lettering. DB, ductus bursae; Inf, infundibulum; La, lagena; MOvd, median oviduct; SeD, seminal duct; SpD, spermathecal duct; Ut, utriculus, Vag, vagina; Ves, vestibulum


D B
Inf
M Ovd
Ves
Vag
D B

44a

Ut
La
D B
M Ovd
Sp D
D B
M Ovd
Se D
Inf
D B
M Ovd
Se D
Ves
D B
Vag
D B
0.20 mm

44b

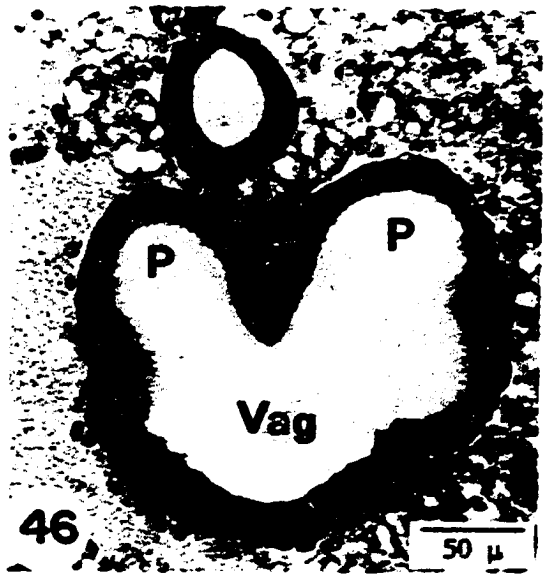
Figure 45. The origin of the signum. Transverse section of part of the ventral wall of the corpus bursae of a 48-hour-old pupa. Phase contrast. A fine granular material seems to stream from protrusions of an internal ridge on the ventral wall of the corpus bursae. The ridge first appears in in the 48-hour-old pupa. Between 96 and 120 hours its surface sclerotizes to form the signum.

Figure 46. Vaginal pouches in a 72-hour-old pupa. Transverse section. The accessory gland duct is dorsad to the vagina.

Figure 47. The origin of the U-shaped sclerite. Transverse section of the ductus bursae of a 72-hour-old pupa showing the beginning of the depression of its dorsal surface. The inner surface of the depression sclerotizes, forming the U-shaped sclerite.

Lettering. CB, corpus bursae (ventral wall); Mu, muscle; P, vaginal pouch; S8, Sternum 8; Vag, vagina


CB
45
25 µ


P
P
Vag
46
50 µ

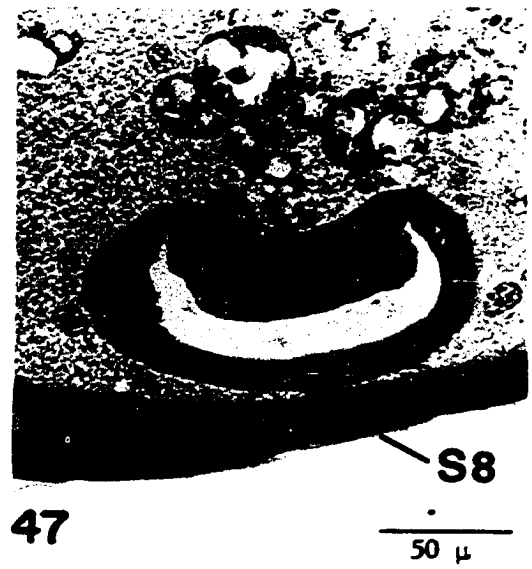
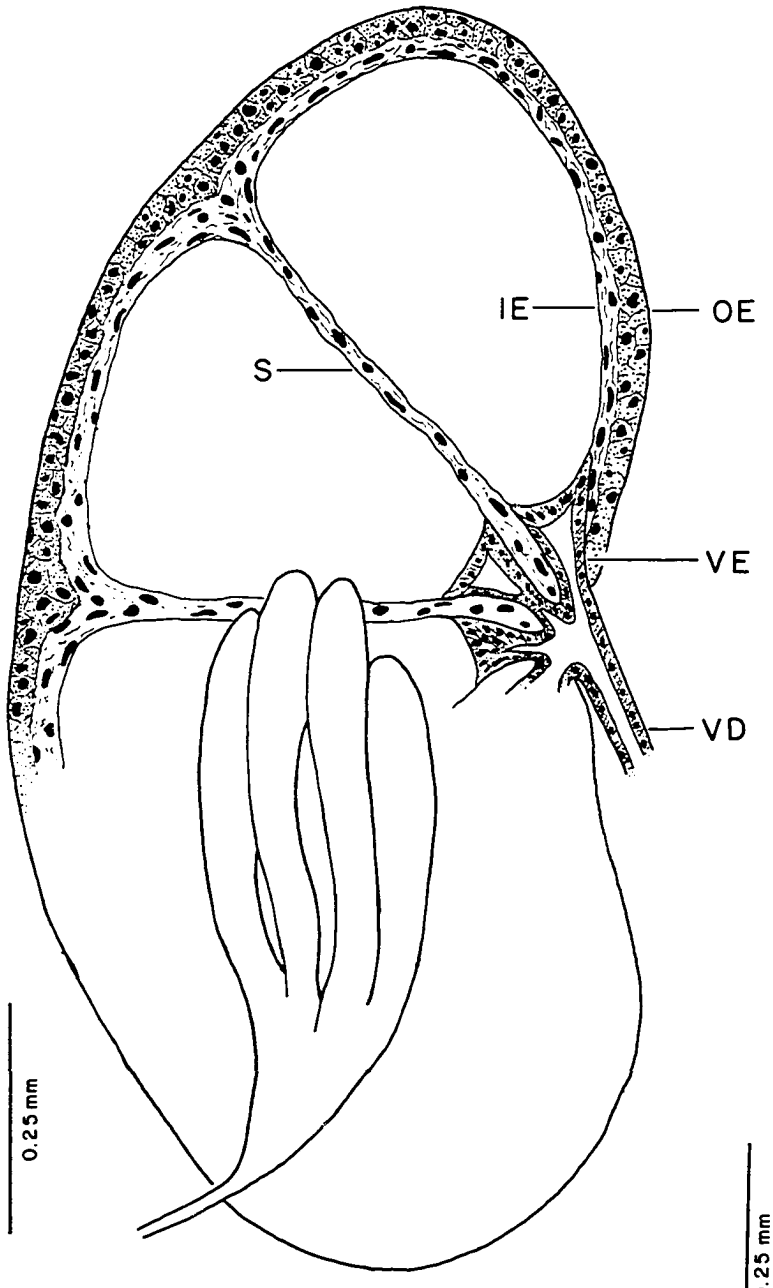

S8
47
50 µ

Figure 48. Testis and ovary, late in the 5th stadium. Outlines of organ whole mounts were traced from microprojected images and superimposed for comparison. The histology of the testis is highly stylized.

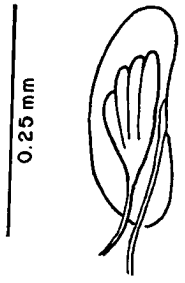
Figure 49. Testis and ovary, in the middle of the 4th stadium. Superimposed outlines of microprojected whole mounts.

Figure 50. Testis and ovary, in the middle of the 3rd stadium. Superimposed outlines of microprojected whole mounts.

Lettering. GC, genital cord; H, hilum; IE, inner epithelium; OE, outer epithelium; S, septum; VD, vas deferens; VE, vas efferens



48



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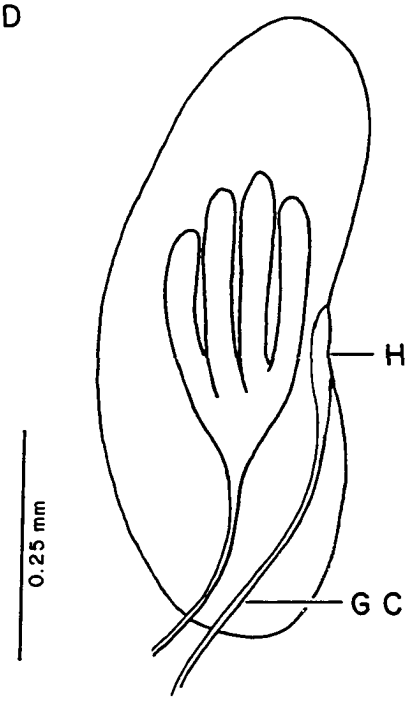
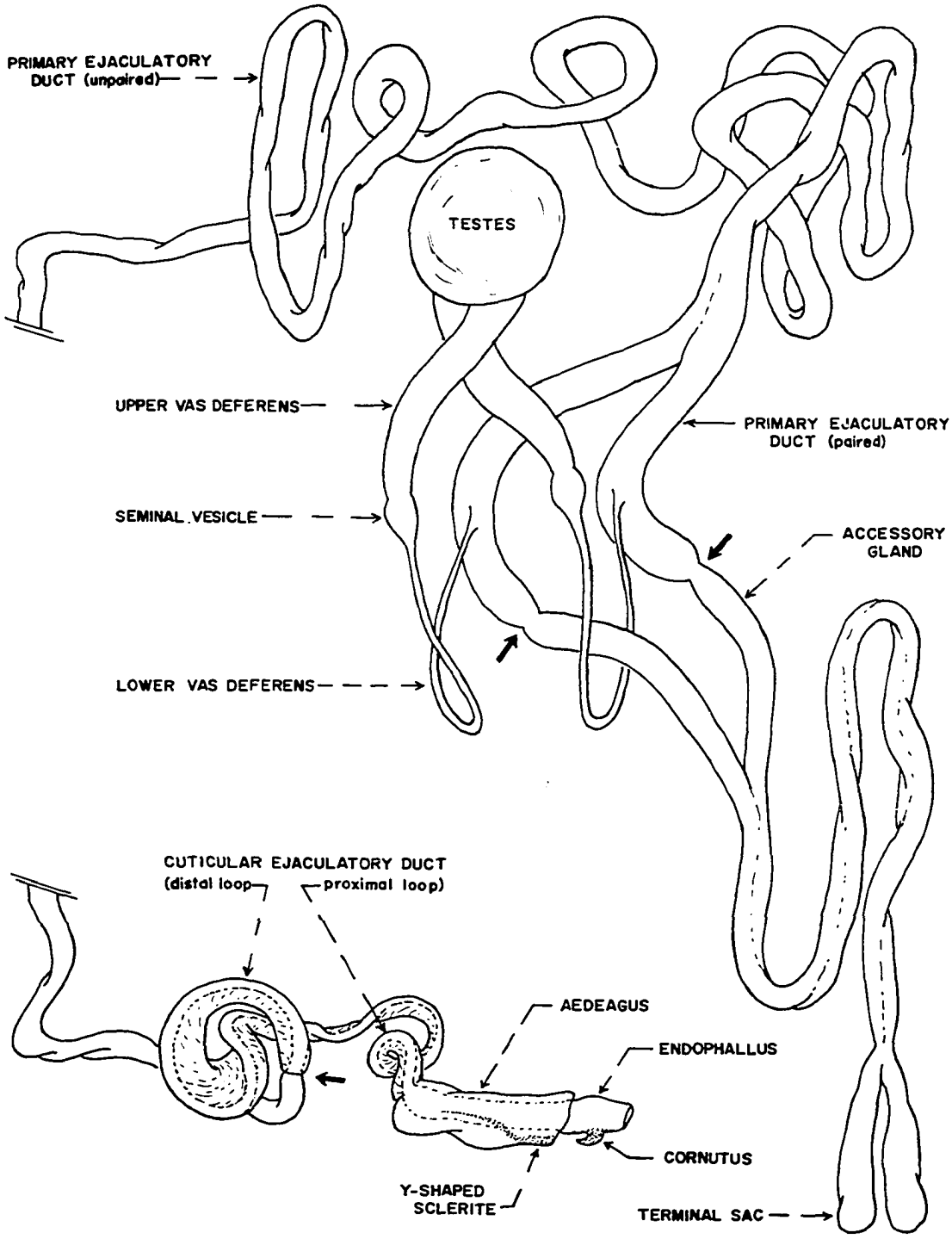


49

Figure 51. Adult male reproductive system. Diagrammatic. The organs are shown spread and arranged for visibility. The aedeagus and cuticular ejaculatory duct are shown from the left lateral aspect; the remainder of the system is shown from the dorsal aspect. The primary ejaculatory duct is shortened; it is cut at the double lines, and the great intervening length is omitted. The heavy unlabelled arrows indicate the junctions of the accessory glands and paired ejaculatory ducts and the primary and cuticular ejaculatory ducts.


PRIMARY EJACULATORY DUCT (unpaired)
TESTES
UPPER VAS DEFERENS
PRIMARY EJACULATORY DUCT (paired)
SEMINAL VESICLE
ACCESSORY GLAND
LOWER VAS DEFERENS
CUTICULAR EJACULATORY DUCT
(distal loop
proximal loop)
AEDEAGUS
ENDOPHALLUS
CORNUTUS
Y-SHAPED SCLERITE
TERMINAL SAC

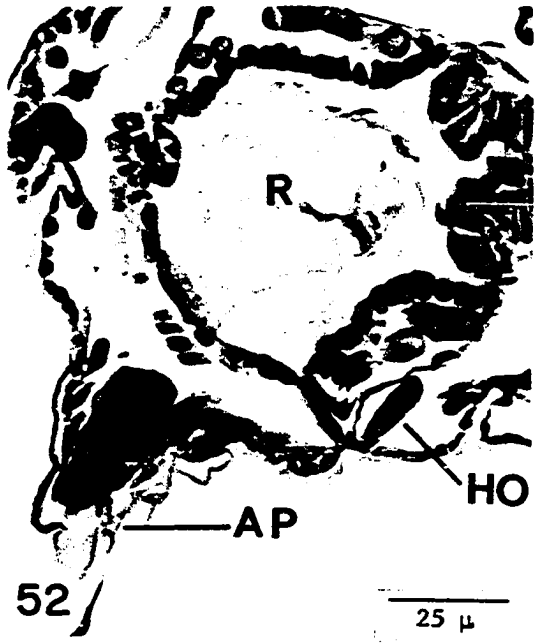
51

Figure 52. Herold's organ, in the middle of the 1st stadium. Sagittal section of Segments 9 and 10, slightly oblique to the longitudinal axis.

Figure 53. Herold's organ, early in the 2nd stadium. Sagittal section of Segment 9.

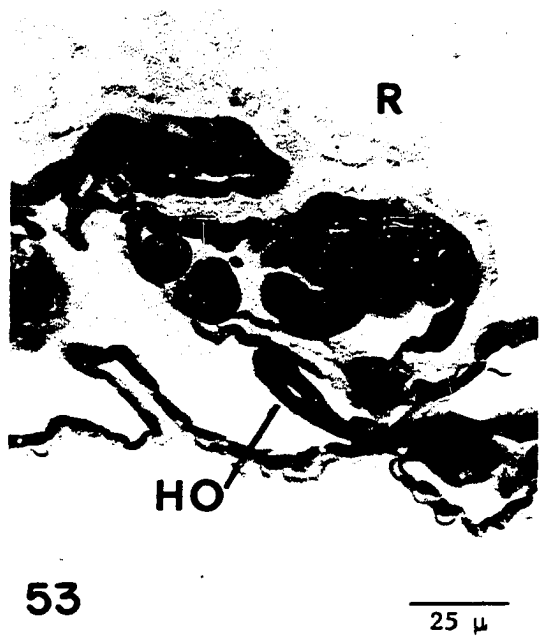
Figure 54. Herold's organ, in the middle of the 2nd stadium. Transverse section of Segment 9 through the anterior end of Herold's organ.

Lettering. AP, anal proleg; GC, genital cord; HO, Herold's organ; MT, Malpighian tubule; R, rectum



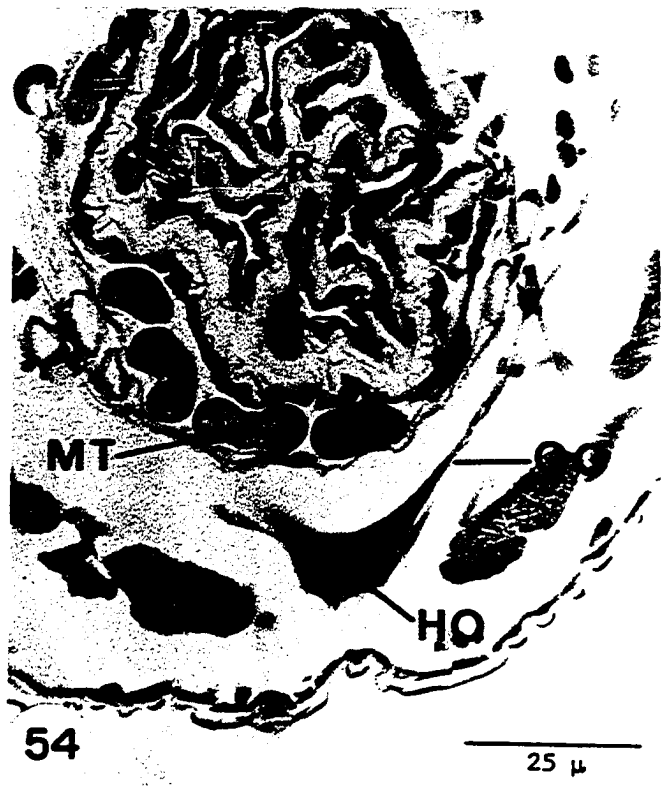
52

25 μ



53

25 μ



54

25 μ

Figure 55. Testis, in the middle of the 3rd stadium. Sagittal section.

Figure 56. Testicular follicle, in the middle of the 3rd stadium. Transverse section through adjacent follicles.

Figure 57. Testis, in the middle of the 4th stadium. Permount whole mount, hematoxylin stain. Phase contrast.

Figure 58. Testicular follicle, in the middle of the 4th stadium. Frontal section.

Lettering. GC, genital cord; S, septum; Spc, spermatocyst; T, trachea; VC, Verson's cell; VE, vas efferens


50 µ
55


VC
25 µ
56


100 µ
57

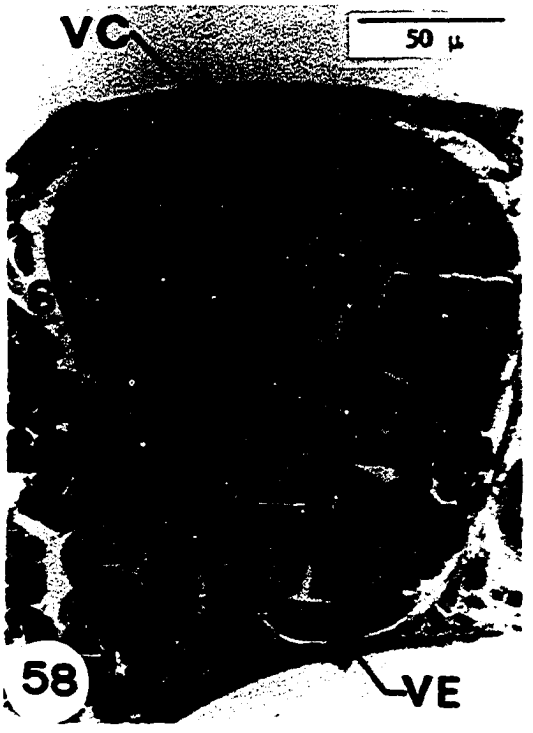
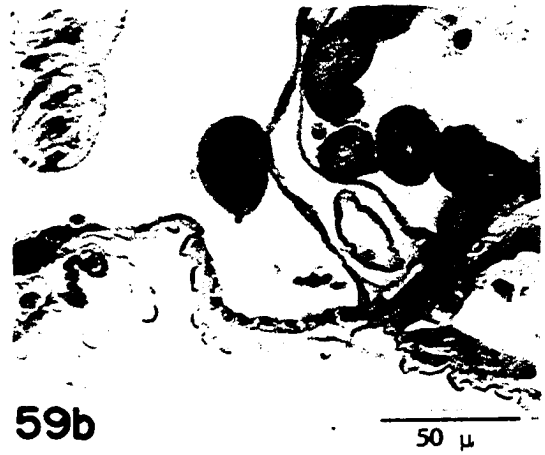

VC
50 µ
58
VE

Figure 59. Herold's organ, in the middle of the 3rd stadium. Fig. 59a, a sagittal section; Fig. 59b, a parasagittal section through the anteriolateral wall and the contiguous ampulla, which at this stage is lumenless.

Figure 60. Herold's organ, in the middle of the 4th stadium. Fig. 60a, a parasagittal section showing the thickening of the anterolateral wall during the formation of the primary genital lobes; Fig. 60b, a parasagittal section through the anteriolateral wall and the contiguous ampulla, which at this stage has a well developed lumen.

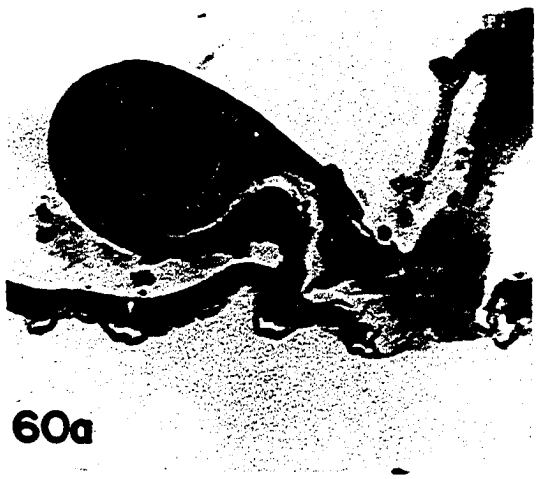
Figure 61. Herold's organ, in the middle of the 4th stadium. Fig. 61a, a transverse section showing the anterior end of Herold's organ and the contiguous ampullae; Fig. 61b, a transverse section, oblique to the vertical axis, through the posterior part of Herold's organ.

59a

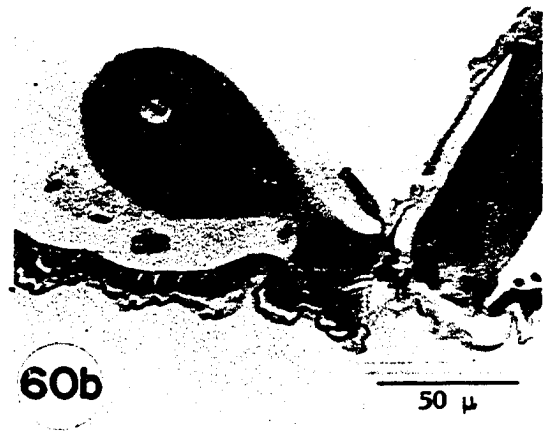


59b

50 μ



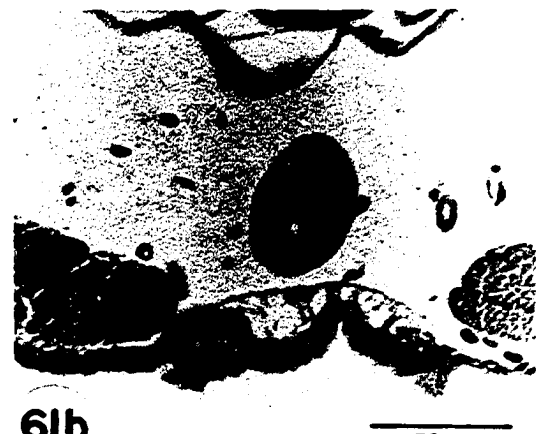
60a



60b

50 μ

61a



61b

50 μ

Figure 62. Herold's organ, late in the 4th stadium. Serial transverse sections of an intact abdomen, cuticle removed, near the anterior (a), middle (b), and posterior (c) of the ampullae; near the middle (d) and posterior (e) of the primary genital lobes; at the point of invagination (f) of Sternum 9. Compare to Fig. 65.

Lettering. Am, ampulla; GC, genital cord; PGL, primary genital lobe.

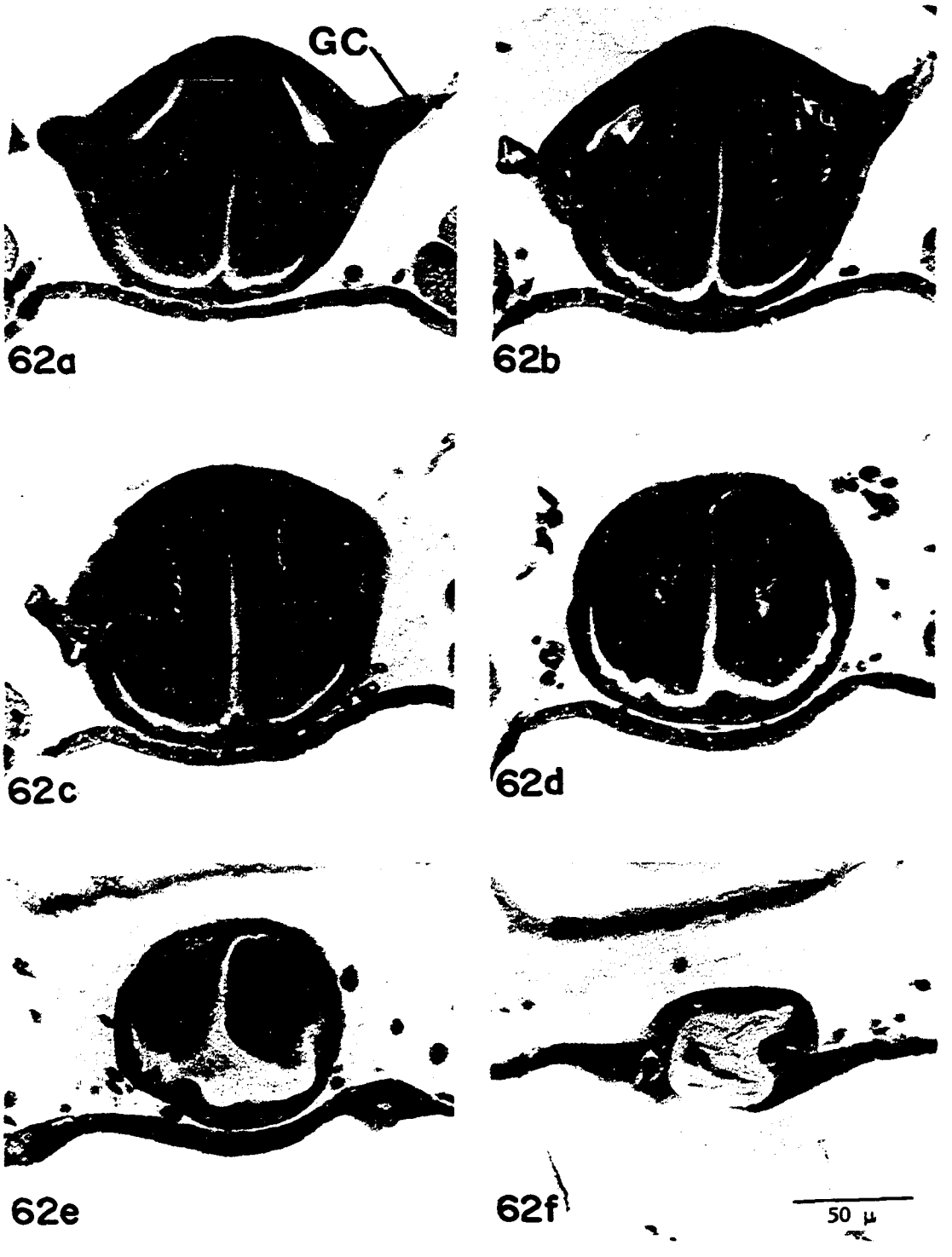

GC
62a
62b
62c
62d
62e
62f
50 µ

Figure 63. Herold's organ and ampullae, in the middle of the 3rd stadium. Permount whole mount, hematoxylin stain. Phase contrast. In this preparation Herold's organ was left attached to the hypodermis, through which Setae VIII (at the arrows) can be seen.

Figure 64. Herold's organ and ampullae, early in the 4th stadium. Permount whole mount, hematoxylin stain. Small lumina can be seen in the ampullae.

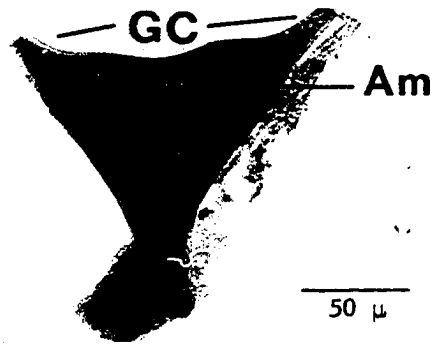
Figure 65. Herold's organ and ampullae, late in the 4th stadium. Permount whole mount, hematoxylin stain. The letters a-f indicate the approximate plane of the correspondingly lettered sections in Fig. 62.

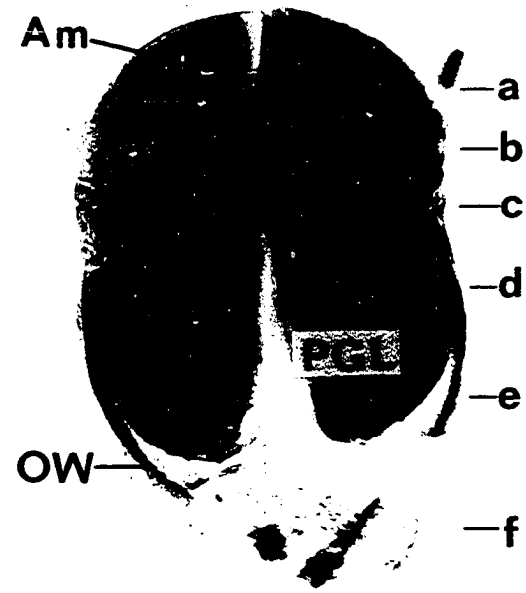
Figure 66. Herold's organ and ampullae, early in the 5th stadium. Permount whole mount, hematoxylin stain.

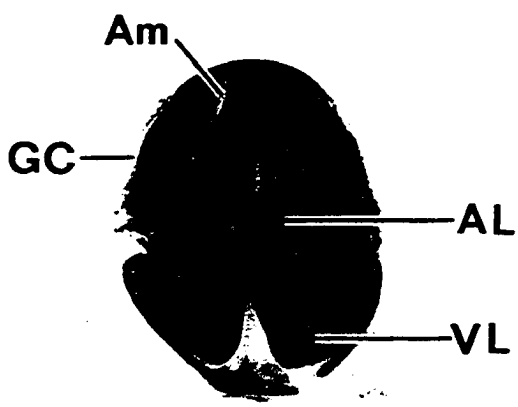
Figure 67. Herold's organ and ampullae, in the middle of the 5th stadium. Permount whole mount, hematoxylin stain.

Lettering. AL, aedeagal lobe; Am, ampulla; GC, genital cord; OW, outer wall of Herold's organ; PGL, primary genital lobe; T, trachea; VL, valvular lobe


T
63
50 µ


GC
Am
64
50 µ


Am
a
b
c
d
PGL
e
OW
f
65
50 µ


Am
GC
AL
VL
66
100 µ

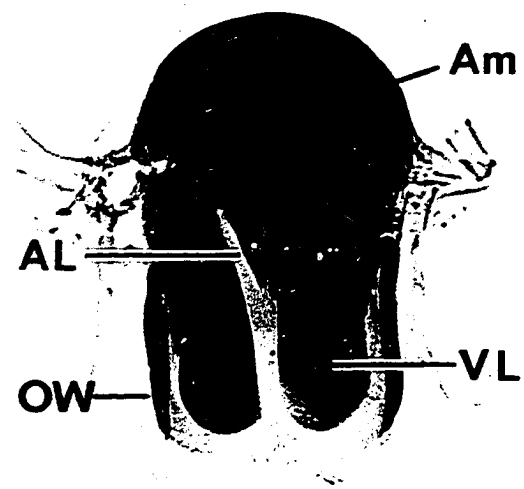

Am
AL
VL
OW
67
100 µ

Figure 68. Testis, early in the 5th stadium. Frontal section.

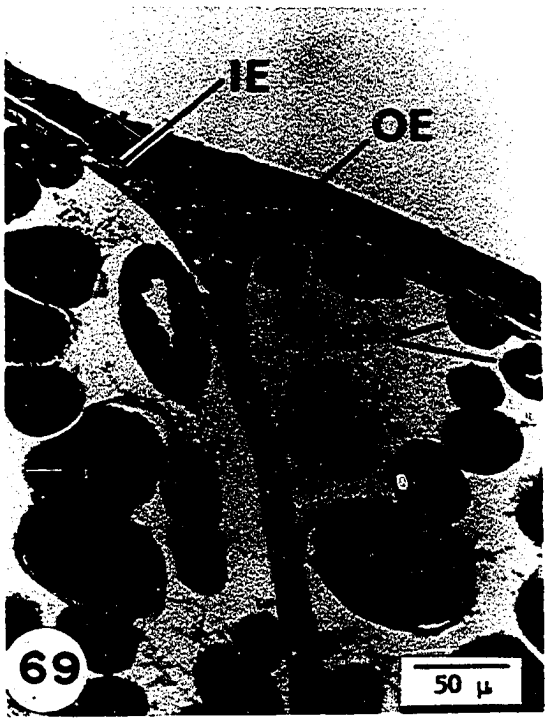
Figure 69. Testicular epithelia, in the middle of the 5th stadium. A frontal section of a testis showing the numerous eosinophilic inclusions in the outer epithelium.

Figure 70. Vasa efferentia, in the middle of the 5th stadium. Frontal section.

Lettering. IE, inner epithelium; OE, outer epithelium; S, septum; SpB, sperm bundles; Spc, spermatocysts; VE, vas efferens

68

100 µ



70

50 µ

Figure 71. Fused testes, at pupation. Transverse section.

Figure 72. Male reproductive system at pupation, testes excluded. Sagittal section, slightly oblique. Lines b, d, f and h indicate the approximate plane of the correspondingly lettered sections in Fig. 73.

Lettering. A, anus; Ae, aedeagus; CED, cuticular ejaculatory duct; En, endophallus; IE, inner epithelium; LVD, lower vas deferens; Ma, manica; Mu, muscle; OE, outer epithelium; PED, primary ejaculatory duct; S, septum; UVD, upper vas deferens; V, valve; VE, vas efferens


71
100 µ

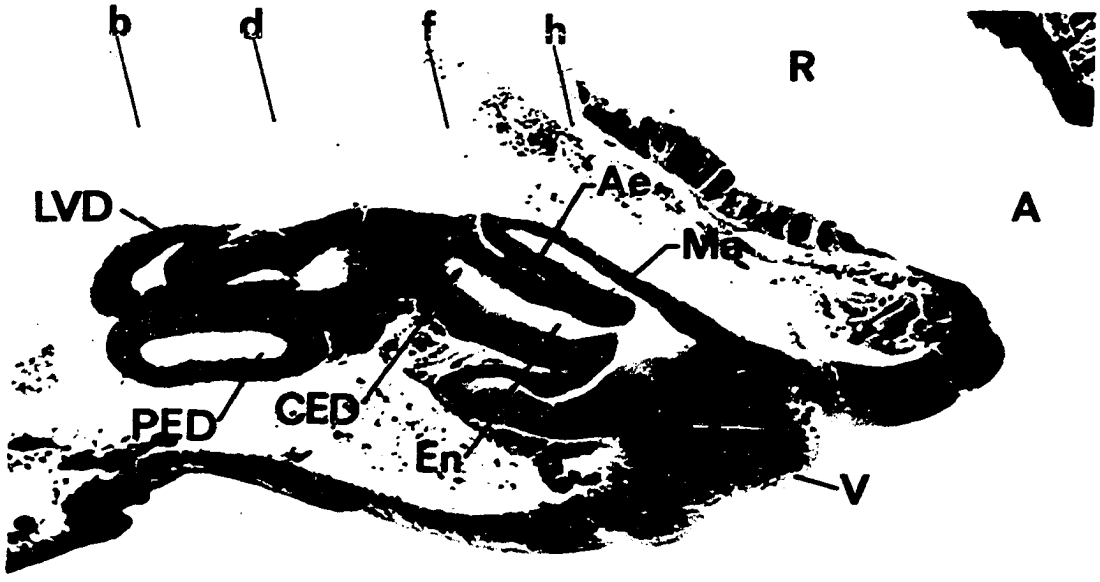

b
d
f
h
R
A
LVD
Ae
Me
PED
CED
En
V
72
100 µ

Figure 73. Male reproductive system at pupation, excluding the testes. Serial transverse sections, anterior (a) to posterior (i). Compare to Fig. 72.

Lettering. AcGl, accessory gland; Ae, aedeagus; CED, cuticular ejaculatory duct; En, endophallus; LVD, lower vas deferens; Ma, manica; PED, primary ejaculatory duct, unpaired part; PED^2, primary ejaculatory duct, paired part; UVD, upper vas deferens; V, valvular lobe

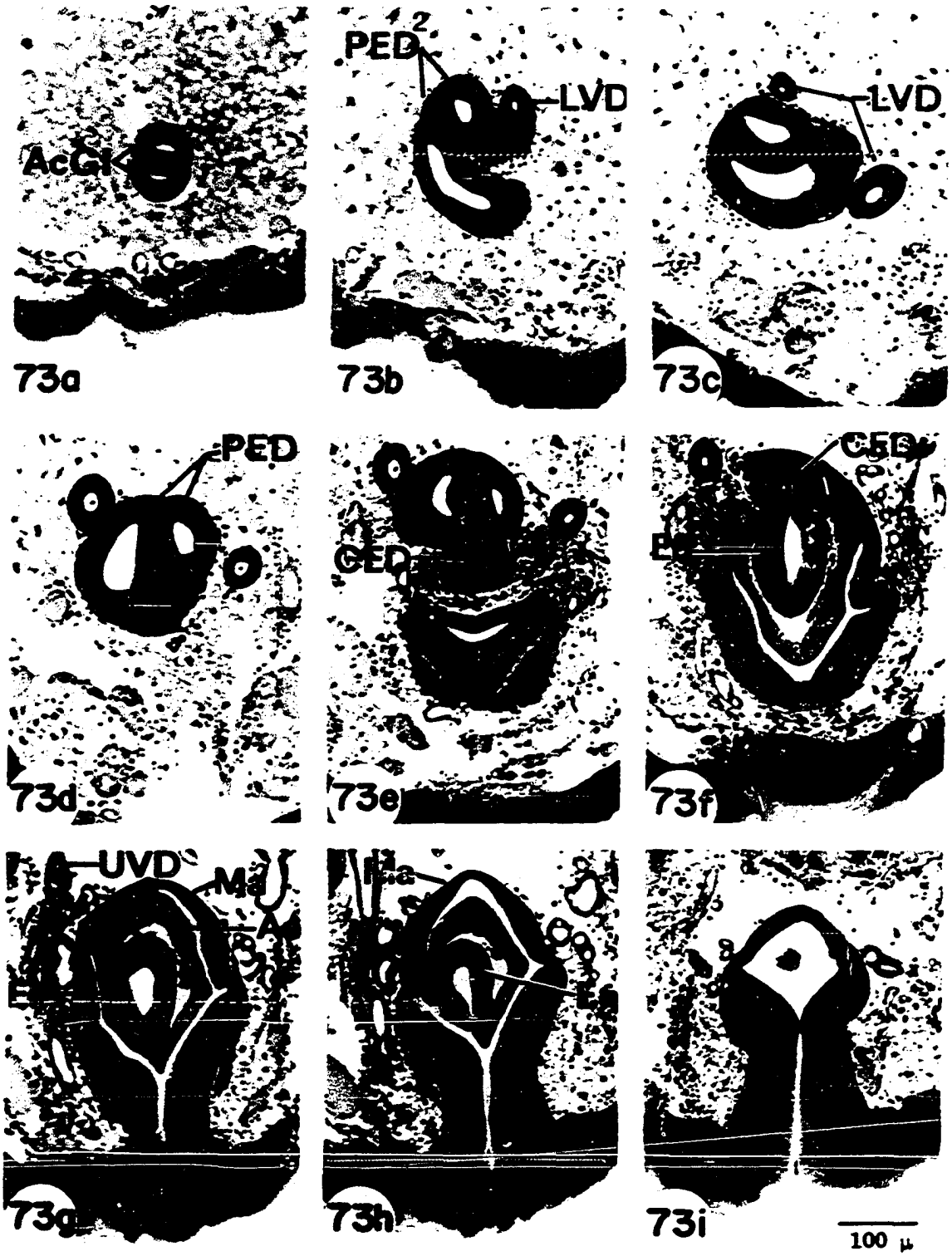
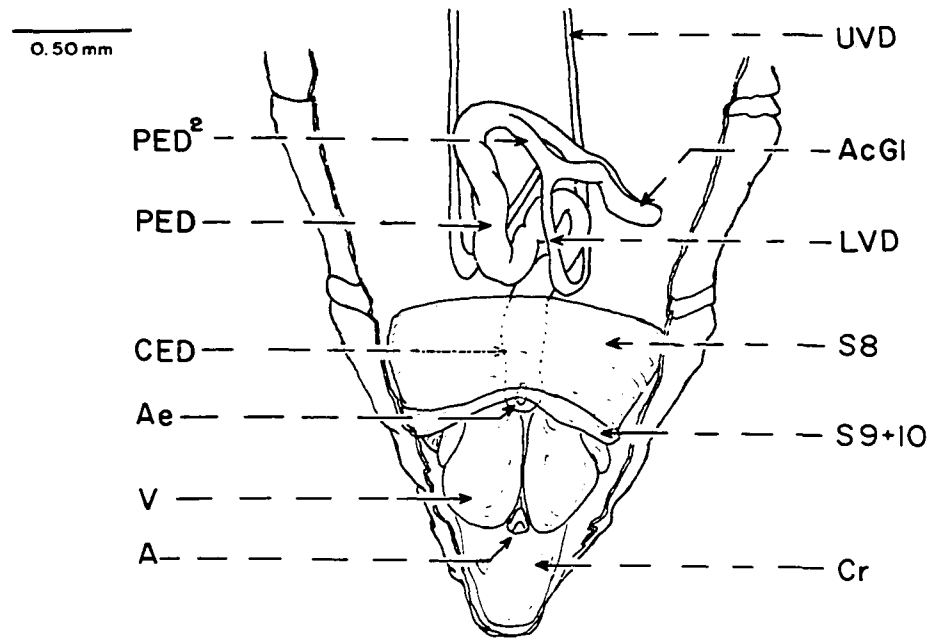

AcGl
73a
PED
LVD
73b
LVD
73c
PED
73d
CED
73e
CED
73f
UVD
73g
73h
73i
100 µ

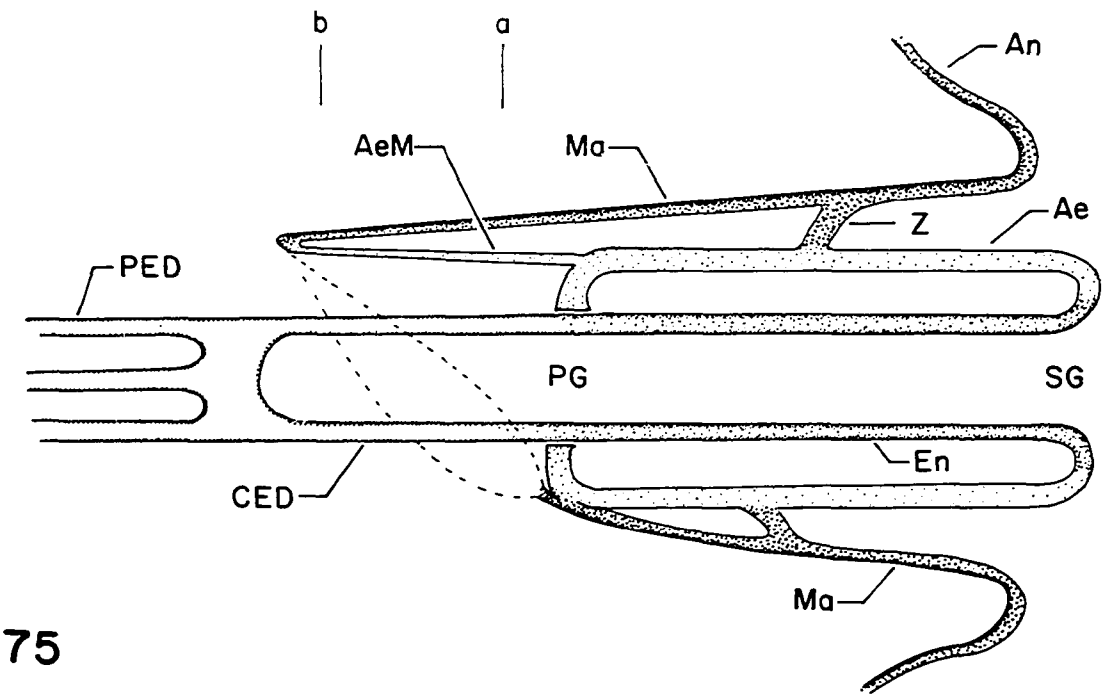
Figure 74. Ventral view of the reproductive system, excluding the testes, of a 24-hour-old pupa. Camera lucida drawing.

Figure 75. Schematic sagittal section of the terminalia of a 24-hour-old pupa. The arrows indicate the approximate plane of the correspondingly lettered sections in Fig. 76.

Lettering. A, anus; AcGl, accessory gland; Ae, aedeagus; AeM, aedeagal membrane; An, anellus; CED, cuticular ejaculatory duct; Cr, cremaster; En, endophallus; LVD, lower vas deferens; Mn, manica; PED, primary ejaculatory duct, unpaired part; PED^2, primary ejaculatory duct, paired part; PG, primary gonopore; SG, secondary gonopore; S8, Sternum 8; S9, Sternum 9+10; UVD, upper vas deferens; Z, zone


0.50mm
UVD
PED²
AcGl
PED
LVD
CED
S8
Ae
S9+10
V
A
Cr

74


b
a
An
AeM
Ma
Z
Ae
PED
PG
SG
En
CED
Ma

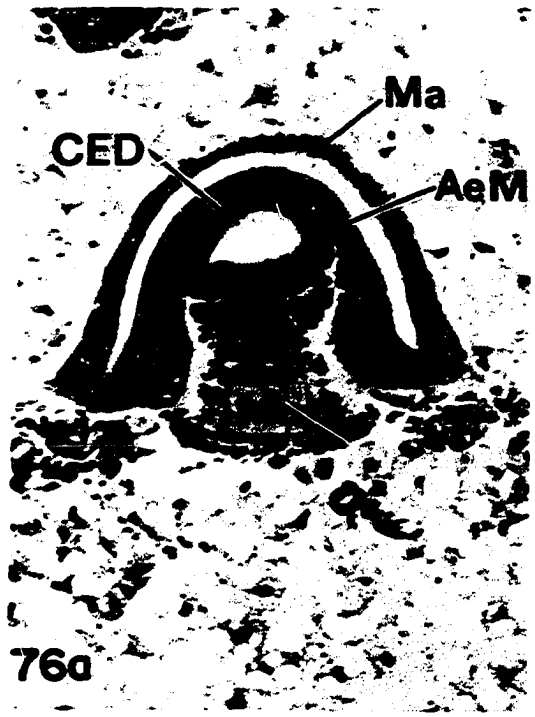
75

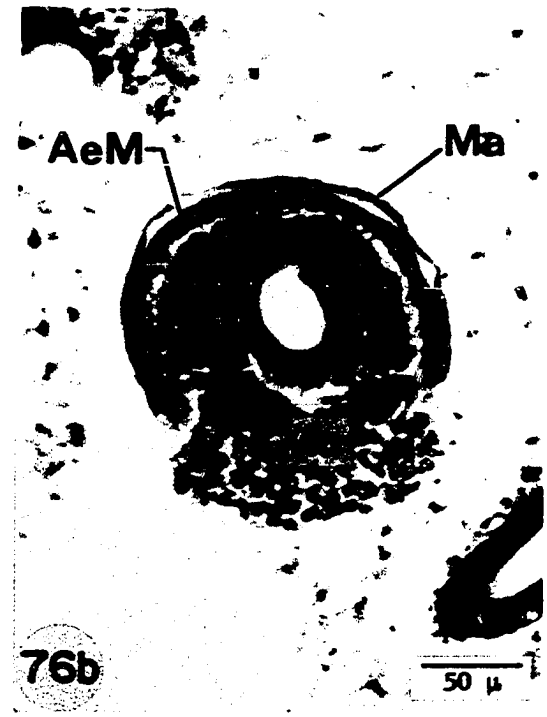
Figure 76. Cuticular ejaculatory duct of a 24-hour-old pupa. Transverse sections near the base of the aedeagus (a) and near its junction with the primary ejaculatory duct (b). The manica is much thicker near the aedeagus.

Figure 77. Distal loop of the cuticular ejaculatory duct of a 48-hour-old pupa. Longitudinal section.

Figure 78. Cuticular ejaculatory duct of a 144-hour-old pupa. Transverse section. The manica (at the arrows) is extremely thin and difficult to see, and in most sections from this stage it is even more indistinct.

Lettering. AeM, aedeagal membrane; CED, cuticular ejaculatory duct; Ma, manica; Mu, muscle


Ma
CED
AeM
76a


AeM
Ma
76b
50 µ


Ma
AeM
77
50 µ


78
50 µ

Figure 79. Aedeagus and valvae of a 48-hour-old pupa. Transverse section. In both Figs. 79 and 80, Segments 8 and 9+10 are telescoped into Segment 7 which is not shown. The arrows indicate hypodermal thickenings where sclerites of the valvae and aedeagus will be formed.

Figure 80. Aedeagus and valvae of a 72-hour-old pupa. Transverse section.

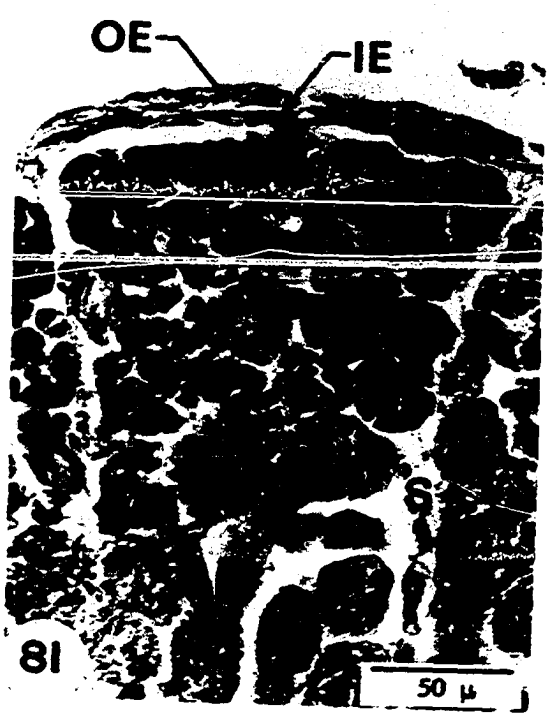
Figure 81. Testicular epithelia of a 120-hour-old pupa. Transverse section. The outer epithelium has lost most of the eosinophilic inclusions. Compare to Fig. 69.

Figure 82. Testicular follicles of a 144-hour-old pupa. Transverse section. The outer and inner epithelia are very thin and similar in appearance.

Lettering. Ae, aedeagus; En, endophallus; G, gut; IE, inner epithelium; OE, outer epithelium; S, septum; Spc, spermatocysts; SpB, sperm bundles; S8, Sternum 8; T9, Tergum 9+10; V, valvae; Y, Y-shaped sclerite (site of)


G
Gr
79
100 µ


G
Ae
Gr
X
80
100 µ


OE
IE
G
81
50 µ

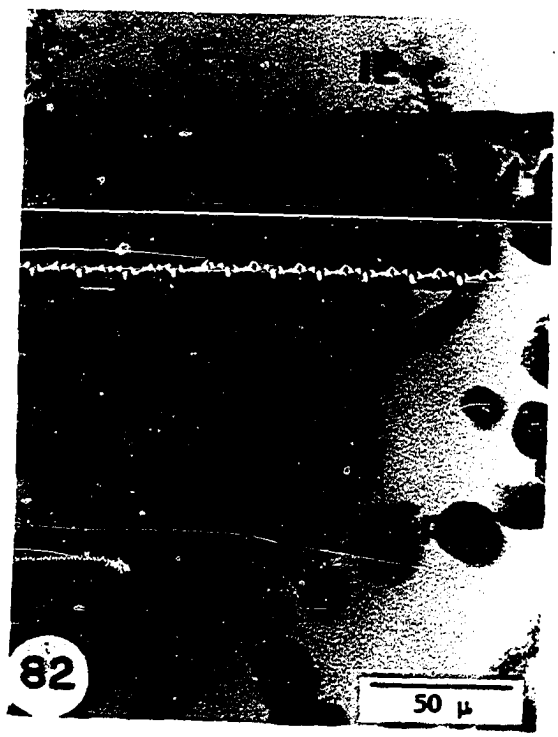

82
50 µ

Figure 83. Primary and cuticular ejaculatory ducts of a 48-hour-old pupa. Transverse sections. The primary ejaculatory duct (a) is a double duct separated from the cuticular ejaculatory duct (c) by a thick wall (b).

Figure 84. Primary ejaculatory duct of a 72-hour-old pupa. Transverse section. Although the septum is thin, it completely separates the lumina of the two component ducts.

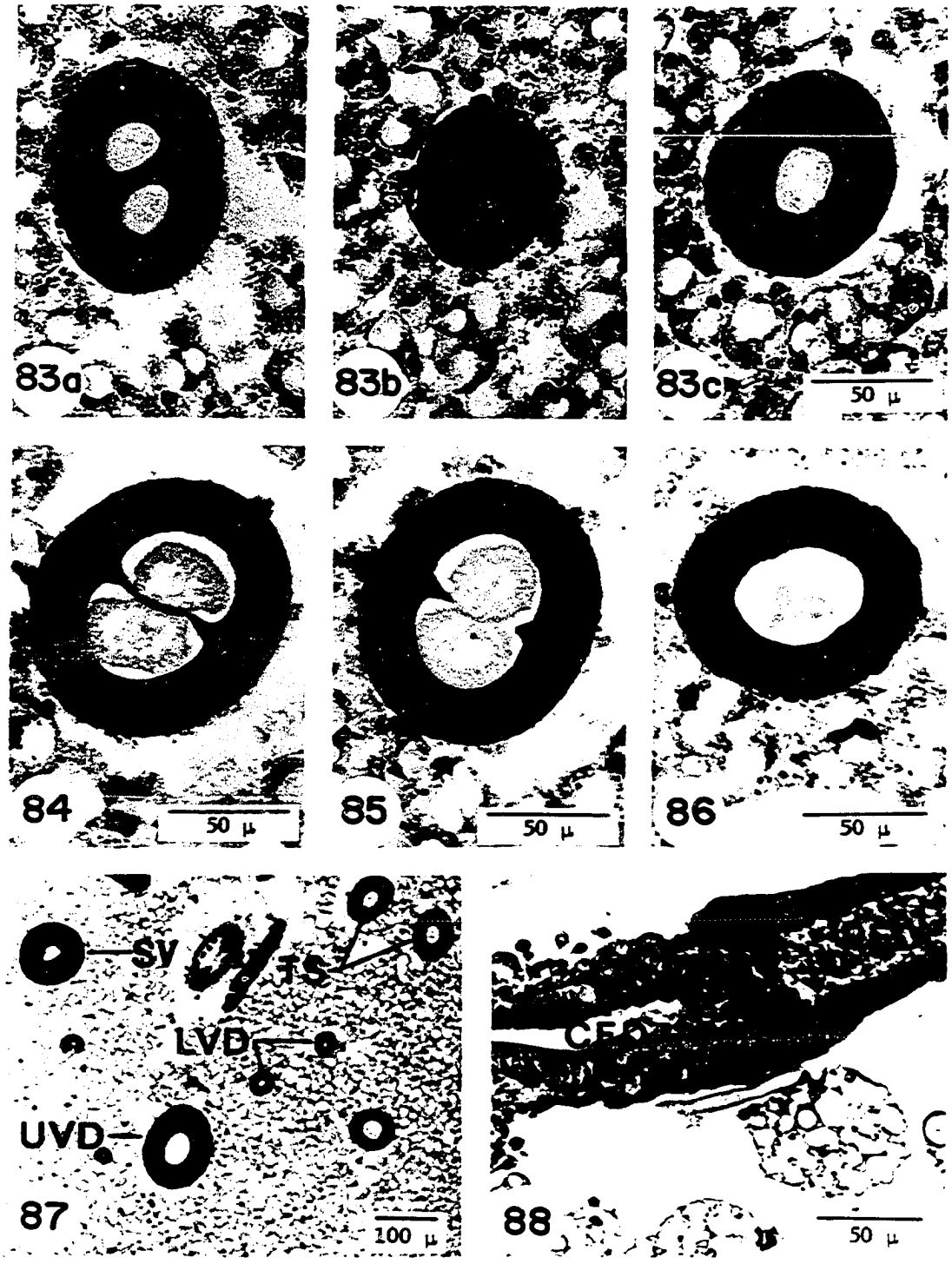
Figure 85. Primary ejaculatory duct of a 72-hour-old pupa. Transverse section. Only remnants of the septum remain.

Figure 86. Primary ejaculatory duct of a 72-hour-old pupa. Transverse section. No trace of the septum remains.

Figure 87. Transverse section of a portion of an intact abdomen of a 72-hour-old pupa.

Figure 88. Junction of the primary and cuticular ejaculatory ducts of a 144-hour-old pupa. Longitudinal section.

Lettering. CED, cuticular ejaculatory duct; LVD, lower vas deferens; PED, primary ejaculatory duct; SV, seminal vesicle; TS, terminal sacs of the accessory glands; UVD, upper vas deferens.


83a
83b
83c
50 μ
84
50 μ
85
50 μ
86
50 μ
Sv
FS
LVD
UVD
87
100 μ
CF
88
50 μ