THE UNIVERSITY OF MANITOBA

Structural and Physiological Aspects of 5th instar Ovarian Development in *Rhodnius prolixus* (Insecta: Hemiptera)

by

Douglas A. Lutz

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements for the Degree

of Master of Science

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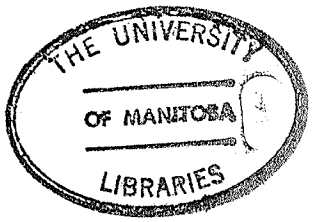

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ABSTRACT

Differentiation events accompanying the larval-adult ovarian transformation in Rhodnius prolixus can be divided into three phases: proliferative phase (unfed-8 days post feed), early differentiation phase (9 DPF-15 DPF) and late differentiation phase (16 DPF-moult at 21 DPF). Ovarioles remain morphologically larval until feeding initiates development. The unfed ovariole contains germ cells surrounding a central trophic core region with the "germarial lumen" occupying the basal region of the tropharium immediately above the prefollicular tissue. Utilizing various light and electron microscopical techniques, coupled with autoradiography, the timing and morphological details of germ cell differentiation as well as mesodermal and structural differentiation were determined. Mitosis of germ cells during the proliferative phase results in a progressive increase in tropharial size with no differentiation of tissues. Regional specialization within the ovariole marks the beginning of the early differentiation phase. A zone of oocytes is established at the base of the tropharium with nuclei containing synaptonemal complexes and condensing chromosomes. Nurse cell differentiation is characterized by nucleolar elaboration and nucleo-cytoplasmic transport, the cytoplasm becoming rich in ribosomes.

Autoradiographic results suggest that physiological nurse cell - oocyte divergence occurs concurrently with morphological divergence. Prefollicular tissue is divided into apical and basal zones with apical zone differentiation occurring during early and late differentiation phases. Intercellular bridges, derived from incomplete cytokinesis, interconnect nurse cells,

oocytes, and connect nurse cells to oocytes. Larval trophic core consists of a tortuous arrangement of cell processes. Massive membrane fusions occur in the core during the late differentiation phase, transforming this area to its adult structure. Birefringent core and cords are visible by 18 DPF. During the late differentiation phase, oocytes initiate previtellogenic growth and accumulate material from tropharium restructuring. Results from ovarian transplant experiments indicate that the presence of ecdysone initiates germ cell differentiation. An absence of JH in the 5th instar is necessary for normal differentiation of normal mesodermal components and adult ovarian structure.

INTRODUCTION

Cells interact in a variety of ways such as aggregation and adhesion, contact inhibition, electrical communication, passage of molecules or ions between cells and hormonal interactions (Deuchar, 1975). These interactions, which may be either unidirectional or reciprocal, cause a structural and/or functional change in the cells involved. Research on cell interactions, although undertaken since the first description of cells, has increased in the past 20 years. Recent advances in biochemical and microscopical technology have allowed workers from a variety of scientific disciplines to examine, in far greater detail than before, the diversities and complexities of interacting cell systems. Some researchers have utilized fully differentiated adult tissues in their investigations while others have used established tissue culture cell lines. Still others have investigated a variety of cell interactions in numerous developmental processes. Information from these latter studies has added to the understanding not only of the structural and chemical nature of the interaction but also of the dynamic nature of these interactions and their relationship with many developmental processes.

The growth of an oocyte involves a complex series of events during which the oocyte is intimately associated with one or more types of accessory cells. Oocytes within vertebrate ovaries develop in conjunction with a follicular epithelium of

varying layers. In some invertebrate groups, developing oocytes associate with two cell types: follicle cells and nurse cells. This type of cell interaction is especially well developed in insects possessing the meroistic ovary type (Telfer, 1975).

The degree of complexity and specialization of these cell interactions is truly remarkable and has provided a unique opportunity for analysis of basic developmental problems. Insights into the development of oocytes, storage of various RNA's, physiological controls and numerous other aspects have been obtained by the experimental exploitation of the meroistic system (see Davidson, 1976).

Of the two cell types that interact with the oocyte, nurse cells provide the developing oocytes with a significant portion of their structural and metabolic machinery. Mesodermally derived follicle cells enveloping the egg are involved in regulation of materials passing to the egg, in a nutritive function, and in secondary egg coat formation (Longo and Anderson, 1974). Both nurse cells and oocytes originate from the same stem cell line and retain, until late oogenesis, a syncytial association via intercellular bridges through which the nurse cell passes synthetic products to the oocyte (Telfer, 1975).

Two structurally different ovarian classes exist within the meroistic category: polytrophic and telotrophic types. Polytrophic ovarioles, the ovariole being the functional unit of the ovary, consist of two regions: a germarium region

containing oogonia and forming ovarian follicles, and a vitellarium containing completed ovarian follicles at later stages of development. Each follicle, surrounded by a follicular epithelium, consists of a fixed number of nurse cells (which varies with species) interconnected and connected to a single oocyte by intercellular bridges (Bonhag, 1958). Telotrophic ovarioles consist of a lanceolate germarium containing apical nurse cells surrounding a central trophic core region. Oocytes and prefollicular tissue occupy the basal region of the germarium and of the vitellarium. Oocytes are connected to the trophic core by cytoplasmic projections, the nutritive or trophic cords (Bonhag, 1958). Although structural differences distinguish these two ovarian types, they have certain fundamental similarities in their cell interactions. While the focus of this study is the telotrophic system, background information available on the polytrophic system can possibly provide clues to assist interpretation of the nurse cell and follicle cell - oocyte interactions in the telotrophic ovary. Parallels may exist in germ cell proliferation, oocyte determination, intercellular bridge formation, follicle formation and other ovarian developmental processes. For background information on a morphologically simpler system, the following section reviews the polytrophic situation.

Polytrophic Ovarian Development.

Structure and function of the polytrophic - meroistic ovary have been extensively explored. Many of these studies

have focussed on the events of follicle development from an oogonium to the initiation of previtellogenesis, often concentrating on determination of the precise geometric arrangement of the nurse cell - oocyte complex and on morphological and physiological events involved in nurse cell - oocyte divergence.

Brown and King (1964) and later Koch and King (1966), indicate that in Drosophila, oogonia, restricted to the apex of the germarium, serve as a stem cell population. Mitosis produces two daughter cells, one of which remains at the apex of the germarium as an oogonium. The other daughter cell, or cystoblast leaves this region and commences a series of four mitoses producing a 16 cystocyte unit. Cystocytes remain interconnected by intercellular bridges formed by incomplete cytokinesis. Differentiation of the 16 cystocytes results in 15 nurse cells and one oocyte.

The precise geometric arrangement of the nurse cell - oocyte complex is known for four cases: Macrothylacia (Hirschler, 1955) and Hyalophora (King and Aggarwal, 1965) each containing eight cell units, and Dytiscus (Giardina, 1901) and Drosophila (Koch and King, 1966) each containing 16 cell units. Physiological evidence indicates that there are two phases of cystocyte complex development. The first phase is synchronous mitosis within all cystocytes (Chandley, 1966). Following completion of mitosis, the asynchronous endomitotic phase commences, accompanying nurse cell - oocyte divergence (King and Burnett, 1959; Urbani, 1970). Asynchronous endomitotic

chromosome replications, occurring within nurse cell nuclei, are related to their role in copious RNA production.

As a result of morphological observations, early investigators attributed a nutritive function to nurse cells (Wilson, 1925). More recent molecular biological evidence confirmed some of these interpretations. Using autoradiography, Bier (1963) clearly demonstrated that polytrophic nurse cells function primarily to produce RNA for subsequent transport to the oocyte. At least two variations exist in the timing of this bulk transport within polytrophic systems. Bier (1963) reported that most RNA accumulates within nurse cell cytoplasm until the final stages of follicular oocyte growth in Musca. This stored RNA, along with the nurse cell cytoplasm, is injected into the oocyte at the last phase of follicle growth. In Hyalophora, nurse cell - produced RNA is transported to the oocyte constantly during its growth, and terminal injection of nurse cell cytoplasm plays only a minor role (Pollack and Telfer, 1969). In addition to RNA, ribosomes and proteins are transported to the oocyte. Mahowald and Strassheim (1970) have reported the transport of centrioles from Drosophila nurse cells. Many authors have suggested from static observations that nurse cell produce and export mitochondria to the oocyte, although appropriate labelling and tracing experiments have yet to be done (Telfer, 1975).

Although the asynchrony of nurse cell chromosome endomitotic replications accompanies nurse cell - oocyte divergence, this

does not signify a causal relationship. Various polytrophic ovary investigators have speculated on the cause or causes of nurse cell - oocyte divergence. Giardina (1901) proposed that the extra-chromosomal chromatin body of Dytiscus oocytes was the oocyte determinant since, through four mitotic divisions, it resided in the cytoplasm of the cystocyte which ultimately became the oocyte. However, examination of other polytrophic systems by Giardina and others revealed that the extra-chromosomal chromatin body was unique to Dytiscus and one other genus. Hirschler (1955) studying Macrothylacia postulated trophic mechanism for oocyte differentiation. Since the oocyte formed from one of the two cells with three bridges, it was one of the best fed cystocytes. Hirschler's theory provides no explanation of the way in which the polarity of nutrient flow would be established. Woodruff and Telfer's (1973) demonstration, in cecropia, of an electrical gradient establishing directional nurse cell - oocyte flow suggests that a re-examination of Hirschler's theory is required.

The most widely accepted theory of oocyte determination in polytrophic systems is that of Koch et al. (1967). They suggested that cortical cytoplasm associated with the first formed intercellular bridge is responsible for oocyte determination. Supporting evidence was the observation that the nurse cell adjacent to the oocyte and possessing four intercellular bridges forms synaptonemal complexes. Synaptonemal

complex formation indicates that this nurse cell is involved in a program of oocyte development. This model is consistent with all other polytrophic systems investigated, in that the oocyte determinant is always associated somehow with the first-formed intercellular bridge (Telfer, 1975). Of the two cells containing synaptonemal complexes, the first to interact with prefollicular tissue became the oocyte. Subsequently, synaptonemal complexes within the other cell nucleus break down and a nurse cell pattern of differentiation ensues (Koch et al., 1967).

Extensive genetic knowledge has permitted King and co-workers to analyze differentiation events within the Drosophila ovary (Koch and King, 1964; Smith and King, 1968; Klug, Bodenstein and King, 1968; King and Hewlett, 1970; Klug et al., 1970; King, 1972; Johnson and King, 1972). The recessive mutations, fused (fu, X - 59.5) and female sterile (fes, 2 - 5), produce ovarian tumors causing sterility. Findings indicated that fu and fes gene products may stabilize intercellular bridges. In addition, the authors concluded that cystocytes are programmed to enter nurse cell differentiation pathway upon completion of mitosis. Studies of the crossover suppressor of Gowen (c (3) G, 3 - 58) revealed that formation of synaptonemal complexes is required for meiotic crossing-over while the normal C (3) G gene probably codes for an essential component of zygosomes, the protein component of the complex.

Several mutations affecting nurse cell nuclear function have also been identified. Studies of these mutants provided insights into the necessary series of events for normal nurse cell nuclear function.

Despite extensive research on hormonal control of morphogenesis, few studies have directly investigated hormonal control of oogonial transformation and early ovarial differentiation. King (1975), working on the control of polytrophic ovarian development, summarized the work of earlier German investigators, integrating their results with his findings. This compilation indicated that a pulse of ecdysone from the prepupal prothoracic gland programs the germ cells to undergo incomplete cytokinesis. The phenomenon of division with incomplete cytokinesis is presumably transmitted to daughter cells since it continues into the adult after prothoracic gland degeneration. Since only oogonia in the apical germarium undergo complete proliferative mitoses, King postulated an interaction of terminal filament cells and oogonia permitting completion of cytokinesis. Daughter cells removed from the terminal influence follow the incomplete cytokinetic pathway.

Telotrophic Structure and Function

With the information from the differentiation of the relatively simple cystocyte unit with its small cell number, one can now analyze more clearly the architecturally more

complex telotrophic situation. Telotrophic ovaries, as described earlier, are found in the polyphagous Coleoptera and the Heteroptera. Polyphagous coleopteran ovaries, in contrast to heteropteran ovaries, lack the central trophic core region within the tropharium. Buning (1972) proposed that this difference is sufficient to postulate independent origin of telotrophic structure within these two groups. However, while noting this important difference, I will, for convenience and discussional purposes, consider both ovariole types together.

A number of studies of adult ovarian structure and vitellogenesis has provided understanding, at both light and electron microscopic levels, of adult structure and of cell interactions involved in egg production (Bonhag and Wick, 1953; Bonhag, 1955a and b; Eschenberg and Dunlap, 1966; Patchen and Davey, 1968; Brunt, 1971; Huebner and Anderson, 1972a,b,c; Ullmann, 1973; Schreiner, 1977a and b). Histochemical, biochemical and autoradiographic techniques have elucidated nurse cell function in telotrophic systems (Bonhag, 1955a and b; Anderson and Beams, 1956; Vanderberg, 1963; Zinsmeister and Davenport, 1971; Mays, 1972; Ullmann, 1973; Cave, 1975). As in polytrophic ovaries, telotrophic nurse cells produce RNA for transport to the oocyte. Endomitotic polyploidization of the entire nurse cell genome, rather than amplification of the ribosomal RNA genes, enhances synthesis of RNA (Cave, 1975). Controversy still surrounds the transport of DNA to the oocyte suggested by Vanderberg (1963) and refuted by Zinsmeister and Davenport (1971). Although protein synthesis can be demonstrated

in the trophic core and cords, the sequential transport of newly synthesized proteins from nurse cells to oocytes is not likely (Zinsmeister and Davenport, 1971). Transport of RNA through the core and cords is essentially unidirectional (Macgregor and Stebbings, 1970). Mays (1972) suggested slow and fast transport rates within the cords reminiscent of axoplasmic flow in neurons (Kreutzberg, 1969). The bulk of RNA, likely the ribosomal component, is transported slowly, while a smaller fraction of transported RNA, thought to be m-RNA, utilizes a faster route. In hemipteran ovaries, the transport route, via the trophic core and cords, contains longitudinally oriented microtubules (Hamon and Folliot, 1969; Brunt, 1971; Huebner and Anderson, 1970; Macgregor and Stebbings, 1970). The microtubules' role in structural maintenance and transport has been verified (Huebner and Anderson, 1970; Macgregor and Stebbings, 1970) although their contribution to motive force generation is yet unclear. Hyams and Stebbings (1977) have experimented on possible transport mechanisms.

While adult structure and function have received much attention, the larval development of telotrophic structure and various cell interrelationships within the ovariole have not been investigated as extensively, and much confusion yet exists about some aspects (polyphage Coleoptera - Buning, 1972, 1978; Ullmann, 1973; Kloc and Matuszewski, 1977. Heteroptera - Wick and Bonhag, 1955; Masner, 1968; Case, 1970; Huebner and Anderson,

1972c; Choi and Nagl, 1976, 1977). This confusion stems from limitations of technique, for example light microscope resolution as compared to the much greater resolution of electron microscopy, and from interpretational problems arising from presentation of weak data leading to deceptive conclusions. Failure to correlate changes to a developmental time frame is a limiting factor in many of these studies. The need for establishment of a reproducible time frame for accurate comparisons was recognized by Buning (1978).

A survey of literature reveals a need for integrated examination of telotrophic ovarian development. This approach would aid both the understanding of sibling relationship ontogeny and the clearer understanding of adult structure and function. In addition, more complete information would provide the basis for both further polytrophic - telotrophic comparisons and further study in cell-to-cell interactions during development in a system which has a unique and exploitable architectural organization.

The objective of this study, then, is to apply an integrated approach to the investigation of 5th instar ovarian development in the reduviid hemipteran, Rhodnius prolixus. Rhodnius was chosen because of the availability of detailed information on both its adult ovarian structure (Patchen and Davey, 1968; Huebner and Anderson, 1972 a,b,c,) and its reproductive physiology (Wigglesworth, 1948; Davey, 1967; Pratt and Davey, 1972 a,b,c,; Huebner and Davey, 1973). Some information is available on the ultrastructure of 5th instar ovarian nurse cells, oocytes,

prefollicular cells, trophic core and cell interactions (Huebner and Anderson, 1972 a,b,c). Although data from these studies indicated that fundamental differentiation events, as well as changes in cell interactions, occur during this stage, details of timing and progression of differentiation events were not determined. Developmental events for each instar and egg production in the adult are initiated by a blood meal, allowing reproducible timing of events related to feeding times. This investigation was restricted to the 5th instar as the major larval-adult ovarian transformation events take place then. The specific objectives of this study were four-fold:

1. To establish a developmental framework of the ovary during the 5th instar.
2. To investigate nurse cell - oocyte morphological divergence.
3. To study the various cell interactions within the ovary which accompany the larval-adult transformation and to determine the sequence and duration of each.
4. To investigate endocrine aspects of ovarian differentiation.

In order to accomplish these goals, I have utilized observations on live preparations, light and electron microscopical techniques coupled with autoradiography on fixed specimens, and ovarian transplant techniques.

MATERIALS AND METHODS

Rearing and Handling

Animals were kept in darkness in a Controlled Environments Inc. incubator at 27°C with 70% R.H.. Wide mouth jars with wire mesh lids were used to house approximately 20 adults or an equivalent number of instars. Within each jar, a piece of fluted filter paper served for urine absorption and as an egg laying substrate. The colony was separated into breeding stock, female virgins and experimentals. Males, upon reaching the 5th instar, were separated from females. The breeding colony was fed periodically upon the shaven bellies of rabbits. Instars and experimentals were fed as required.

Dissection Technique

Ovaries were removed from animals with a combination of jeweller's forceps, iris scissors, microscalpels, insect pins and microhooks (made from tungsten wire by electrolytic etching, Hubel, 1957). All dissections were performed using a Wild M3 or M5 dissecting microscope. Insects were immobilized by removing their legs and pinning them through the thorax in a petri dish lined with Sylgard* (Dow Corning Corp.). After removing the dorsal cuticle covering the abdomen, the animal was bathed in either Grace's Insect Culture Medium (Grand Island Biological Supply Co.), Yeager's saline or Roach Heart Ringers minus dextran (Lockwood, 1961) for live observations.

* Dow Corning Corporation, Midland, Mich. U.S.A 48640

Live Observations

To supplement fixed and sectioned material, stages of 5th instar ovarian development were studied in live tissue using interference and polarizing microscopy. Examination of ovaries by interference microscopy was performed at two day intervals from the unfed state to 8 DPF (days post feed) and from 16 DPF until the adult moult (21 DPF). During the interval 8 DPF to 16 DPF, ovaries were examined daily. Ovaries taken at daily intervals from 16 DPF until the adult moult were used in the polarizing microscopy investigations. The ovaries were placed within a small drop of medium on a clean microscope slide. Vaseline applied to the corners of the coverslip prevented uncontrolled flattening and/or rupture of the preparation by supporting the coverslip during viewing. A Zeiss Photomicroscope II equipped with Nomarski differential interference optics and with a polarizer and analyzer was used in these investigations.

Fixation and Embedding

Ovaries were fixed and embedded at daily intervals during the instar from the unfed state to the adult moult (21 DPF). Animals were dissected and ovaries were removed in fixative. Two fixatives were used in this study: a modified Karnovsky's (Karnovsky, 1965) containing 3% glutaraldehyde, 1% paraformaldehyde and 0.05% $CaCl_2$ in 0.1 M sodium cacodylate buffer pH 7.2 and a microtubule stabilizing fixative (Luftig et al., 1977)

containing 5% glutaraldehyde, 1 mM $MgSO_4$, 1 mM GTP, 2 mM ethylene glycol bis (β - aminoethyl ether) - N - N' - tetracetic acid (EGTA) in 100 mM PIPES buffer pH 6.9. Tissues were fixed for 1 - 1½ hours. After fixation, ovaries were washed in 0.1 M sodium cacodylate buffer pH 7.2 for at least 1 hour and were subsequently post-fixed for 1 hour in 1% OsO_4 in sodium phosphate or sodium cacodylate buffer pH 7.2. Following the publication by Maupin-Szamier and Pollard (1978) describing the effects of prolonged OsO_4 exposure on pure actin filament networks, post-fixation time was reduced to ½ hour. Fixation, wash and post-fixation was carried out at $4^{o}C$.

Rapid dehydration in a graded series of ethyl alcohols (70%, 80%, 95%, 100%) at $-20^{o}C$ followed post-fixation in OsO_4. Tissues remained in each change of alcohol a maximum of 30 seconds until reaching 100% ethyl alcohol. Then vials containing the tissues were allowed to warm to room temperature. The 100% ethyl alcohol was changed a minimum of 4 times, ½ hour each change. A mixture of 1 part 100% ethyl alcohol: 1 part propylene oxide replaced the final alcohol change and was followed by two changes of 100% propylene oxide, 15 minutes per change.

After propylene oxide, ovaries were placed in an infiltrating solution of Epon - Araldite (by volume, 2.5 parts Epon 812: 2 parts Araldite 502: 6 parts DDSA, Anderson and Ellis, 1965) containing between 2% and 4% DMP-30 plus an equal volume of propylene oxide. Infiltration proceeded for at least 24 hours

before embedding. Tissues were embedded in flat moulds and blocks were cured at 60°C for at least 1 day.

Light Microscopy

Semi-thin (1 μm) epoxy sections were cut with a Sorvall Porter Blum MT2B ultramicrotome using glass knives made with an LKB Knifemaker type 7801B. Sections were collected from the boat using a wire loop, transferred and heat-fixed to a clean glass slide. Tissue was stained with a mixture of 1% toluidine blue in 1% sodium borate solution by heating stain covering the sections to steaming. Slides were rinsed in running water and air dried. Coverslips were mounted using either immersion oil or Histoclad* (Clay Adams). Micrographs were taken using a Zeiss Photomicroscope II.

Transmission Electron Microscopy

Silver - silver-grey sections were cut with glass knives using either a Sorvall Porter Blum MT2B or a Reichert UM1 ultramicrotome. Sections were collected on naked copper grids with mesh sizes ranging from 150 to 400. Grids were stained in alcoholic uranyl acetate, washed with glass distilled water and double stained in lead citrate for 1½ minutes (modified Venable and Coggeshall, 1965). Grids were examined in AEI 6B or AEI 801 electron microscopes at an accelerating voltage of 60 kV.

* Clay Adams, Parsippany, New Jersey, U.S.A. 07054

Scanning Electron Microscopy

Tissues for SEM were processed using the Sorvall Critical Point Drying System. Briefly, this procedure involved the following steps. Whole reproductive tracts left *in situ* were fixed and processed as described in the fixation section until the final stages when acetone was substituted for propylene oxide. After the last acetone step, samples were placed in the Sorvall CPD pressure chamber completely filled with acetone. Following a cooling step, the replacement of acetone with liquid CO_2 was begun by alternating, at least 4 times, an inlet (of CO_2) - exhaust (of acetone) step with an equilibration step. Once the acetone was completely replaced by liquid CO_2 in the tissue, the temperature and pressure of the chamber was raised to a pressure of 1500 - 2000 psi and left at this point for 5 minutes. Subsequently, the exhaust valve was opened and the pressure within the chamber was reduced at a rate of approximately 2-3 psi/sec. At its critical pressure of 1070 psi, the liquid CO_2 passes into the gas phase encountering no interface, leaving a dry sample with minimal distortion. Dried tissues were stored in a desiccated bell jar. Tissues were mounted on stubs with double sided tape and silver dag. Stubs were coated with gold in a Balzers sputter coater and were viewed with Cambridge Stereoscan SEM. Micrographs of selected areas and at appropriate magnifications were taken by Bert Luit as directed.

Autoradiography

In order to study activity of ovarian tissues during early development, 5th instar females at stages unfed, 2,4,6,8,10, 12 days post-feed were injected with a tritium-labelled RNA precursor. Five animals at each stage were injected into a leg with 2 μCi/bug 5-H^3- uridine (113.6 mCi/mg).* Controls were injected with saline. Animals were fixed 2 hours post injection and tissues were processed for light microscopy as described in the fixation section.

Slides with unstained sections were dip coated in total darkness with undiluted Kodak NTB-2 emulsion. Once dry, they were stored at 4°C in light tight boxes containing $CaCl_2$ as a desiccant. Slides were allowed to expose emulsion for 10 days prior to development. Emulsions were developed at 17°C in total darkness using Kodak Dektol developer 1:2 dilution for 2 minutes. Slides were then rinsed in distilled water and fixed in a 30% solution of sodium thiosulfate for 8 minutes. Following fix, the slides were washed in running water for 15 minutes and then allowed to dry in a dust free area (Rogers, 1973). Dark-field micrographs of unstained sections were taken using a Leitz Dialux equipped with a dark-field condenser and a Nikon Automatic Microflex AFX photomicrography system.

Transplant Experiments

Ovarian transplant techniques were employed in order to gain insights into the endocrine control of ovarian development. Stages of recipient animals were chosen according to the unique

*New England Nuclear, Boston, Mass.

hormonal milieu present within the hemolymph of the animal after feeding: 5th instars contain ecdysone, allatectomized adult females contain little if any of the two hormones, virgin adult females contain juvenile hormone and 4th instars contain both ecdysone and JH (Wigglesworth, 1972). The following transplants were performed:

1 5th instar ovaries into unfed 5th instar females (control)
2 5th instar ovaries into allatectomized virgin adult females
3 5th instar ovaries into virgin adult females
4 5th instar ovaries into 4th instars

Using aseptic instruments, ovaries were removed under Grace's insect culture medium from donor females. Terminal filaments, oviduct and ovarian tracheoles were cut close to the ovary without damaging ovarian sheaths. Immobilized recipient was cleaned with 70% ethanol and just prior to the transfer of the ovaries, a small incision was made using a microscalpel between the 4th and 5th abdominal tergites. With a Spemann pipet, donor's ovaries were transferred with minimal culture medium through this incision into the recipient's abdominal cavity. Terga were then replaced. To facilitate wound closure, lost hemolymph was removed from the dorsal cuticle with a small piece of filter paper. Recipients were kept for 3-7 days before feeding to allow cuticle repair. In one portion of these experiments, corpora allata were removed from virgin adult females. Allatectomized bugs fed three days post surgery were isolated so that operation success could be determined for each individual bug. If after 14 days a female

did not produce an egg, the allatectomy was a success. Females producing one or more eggs were rejected for use in transplant experiments. Allatectomized females were used as recipients in the transplant experiment 38 days after the removal of their corpora allata.

Both normal and allatectomized adult recipients were left for 21 days post feed before both donor and recipient ovaries were excised and processed as described in the fixation and embedding section. Fixation of donor and recipient ovaries removed from the former 4th and 5th instar recipients was performed immediately following their moult to their respective succeeding stages. Observations were carried out on live preparations of both recipients and donor ovaries from all of the transplant experiments.

Photographic Methods

Light micrographs and scanning electron micrographs were taken with Kodak Panatomic-X 135 film. The film was developed in either Kodak Microdol-X 1:3 dilution or Acufine (Acufine Inc.) according to manufacturer's specifications. The film was processed using standard photographic procedures. Transmission electron micrographs were taken on either Kodak Electron Microscope Film 4489 or its replacement, Kodak Electron Microscope Image Film 4463. They were developed in Kodak D-19 1:2 dilution and routinely processed. Prints of micrographs for thesis plates were made using a Beseler enlarger and

Ilfoprint paper with an Ilfoprint stabilization processor. Stabilized prints were fixed, washed and finally glossed.

RESULTS

General Aspects - Overview

Prior to the adult moult, Rhodnius prolixus has five larval instars; each moult triggered by a blood meal. During the 5th instar, a complex series of growth and differentiative events transforms the larval ovary into a functional adult ovary. Gross comparison of the reproductive tracts of 5th and adult bugs, in the unfed condition, demonstrates the magnitude of the growth and differentiation (Fig. 1-4). The adult tract with its large, well tracheated ovary and thick lateral oviduct (Fig. 2,4) is contrasted with the smaller, sparsely tracheated ovary and extremely thin lateral oviducts of the larval tract (Fig. 3). The larval - adult transformation occurs within 21 days. A series of ovaries removed at two day intervals after feeding demonstrates that the ovary increases steadily in size throughout the instar (Fig. 5). These gross obvious changes reflect differentiation events at the cellular level within each of the seven ovarioles comprising the ovary.

Developmental Phases - General Aspects and Timing

Developmental events within the 5th instar ovariole during larval - adult transformation are divisible into three phases: proliferative (unfed - 8 days post feed or DPF), early differentiation phase (9 DPF - 15 DPF), and late differentiation phase (16 DPF - moult 21 DPF). The unfed ovariole, at its "resting" state, contains germ cells surrounding a central trophic core region with the "germarial

lumen" occupying the basal region of the tropharium immediately above the prefollicular tissue (Fig. 6,7). A tunica propria and connective tissue sheath envelop the entire unit. During the proliferative phase, mitosis within the tropharium (Fig. 8) increases cell number and size of the ovariole without any detectable morphological differentiation of germ tissue (Fig. 9).

The appearance of regional specialization within the tropharium marks the beginning of the early differentiation phase (Fig. 10, 11). Cytoplasm of cells in the tropharium's basal region stains less intensely than does apical cell cytoplasm. In addition, basal cell nuclei do not contain the larger nucleolus of germ cells in the apical region. Midway through this phase, and thus through the instar, nurse cell - oocyte divergence is well-established and is reflected by striking differences in nuclear morphology (Fig. 12, 13). A zone of oocytes becomes clearly defined. Zonation of nurse cells begins in the latter part of the early differentiation phase and persists into the adult.

In the late differentiation phase, nurse cells at the base of the tropharium are increasingly basophilic and one sees organization of groups of nuclei (Fig. 14, 15). The nucleoli in some of these cells are condensed. Prefollicular cells invade the oocyte region, reorganization of the trophic core occurs, and the trophic cords, extensions from the core to the oocytes are obvious (Fig. 16, 17). Several oocytes,initiate

previtellogenic growth in preparation for the adult. This brief outline of the series of events involved in larval-adult ovarian transformation provides a framework for specific examinations of the cellular changes, cell-to-cell associations, and architectural modifications.

Detailed Cellular Features

Germarial Lumen

An obvious feature of the early 5th instar ovariole is the "germarial lumen" (Fig. 18-21). The germarial lumen, a structure distinct from the trophic core, occupies the region that will later contain oocytes. Light microscopy reveals this region as a lumen filled with many small bodies (Fig. 18, 19), which are basophilic (Fig. 20). With electron microscopy, the lumen appears filled with flocculent material and electron dense bodies in various stages of breakdown (Fig. 21). After feeding, the lumen compacts as growth obliterates it (Fig. 20) and is not visible by 4 DPF.

Early Germ Cells

A prominent nucleus with elaborate nucleolus characterizes the early germ cell (Fig. 22, 23). The cytoplasm of these cells contains numerous free ribosomes, usually a single strand of rough endoplasmic reticulum, mitochondria, and a few Golgi complexes. Mitotic figures occasionally exist within unfed

ovarioles (Fig. 24). After feeding, there is an increase in mitotic activity with frequent mitotic figures evident in the tropharium (Fig. 25, 26). Ultrastructurally, chromosomes occur at the median of the cell at metaphase (Fig. 28). Vesicles of RER outline the mitotic spindle while other cell organelles such as mitochondria are peripherally located. During division, the cytoplasm is less electron dense, perhaps due to a decreased number of free ribosomes. Higher magnification of the centriole region reveals profiles of spindle microtubules (Fig. 28a). At early telophase (Fig. 20), nucleolar structure reforms within the condensed chromatin. The alignment of membrane vesicles adjacent to the chromatin indicates initiation of nuclear envelope reformation. The chromatin, during later nuclear envelope reformation, disperses and the cytoplasm begins to resume its usual structure (Fig. 30). Although, in Figure 31, the germ cell has completed nuclear envelope reformation and general cytoplasmic reorganization, it still contains the mitotic spindle remnants. These remnants are a normal feature of these cells related to intercellular bridge formation to be discussed in a subsequent section. Groups of cells which apparently divide synchronously occur frequently in developing ovarioles (Fig. 27).

Nurse Cell Differentiation

The most striking feature of nurse cell differentiation is the nuclear morphological change relating to increased

nuclear activity. Nucleoli progress from the distinctive, somewhat dispersed germ cell nucleolus (Fig. 32), through compaction and enlargement (Fig. 33, 34), to the single, tightly compacted large nucleolus characteristic of the active nurse cell (Fig. 35, 36). Although nucleolar appearance within a given area of the tropharium may vary considerably (Fig. 34), nucleolar morphologies within the nurse cell nuclear clusters are similar. Increased nuclear synthetic activity results in nucleo-cytoplasmic transport (Fig. 35 - 37). Material is transferred from the nucleus through nuclear pores into the cytoplasm. This material becomes more electron dense upon entering the cytoplasm, indicating a possible physical or chemical change. Once within the cytoplasm, mitochondria intimately associate with it (Fig. 40, 41). Although nuclear pore density varies, there is a general increase from that of early germ cells (Fig. 38) to that of differentiated stages (Fig. 39). This increase reflects the increasing activity of the nurse cell nucleus in nucleo-cytoplasmic transfer. The activity of the nurse cell nucleus is signified by autoradiographic results. Unfed and proliferative phase germ cell nuclei incorporate H^3 - uridine (Fig. 42 - 45). Incorporation of label continues until after nurse cell differentiation when grains are found over nurse cell nuclei (Fig. 46, 47). The trophic core area in both these early stages remains unlabelled.

Nurse cell cytoplasmic elements are similar to early germ cells in both constituents and organization (Fig. 48 - 50).

Numerous free ribosomes contribute to the cytoplasm's basophilia. Mitochondria occur with the single element of RER often associated with Golgi complexes (Fig. 50, 52). Occasionally observed are membrane vesicle profiles interspersed between two lamellae of RER (Fig. 51). Nuage dense material, organized near the nuclear envelope, is characteristic (Fig. 52, 55). A pair of centrioles associated with a fuzzy material exists within the nurse cell cytoplasm (Fig. 53). As nurse cells differentiate, their cytoplasm becomes restructured and isolation bodies occur within the cytoplasm (Fig. 54 - 56). These membrane-bound packages contain mitochondria, vesicles of RER, Golgi complexes, free ribosomes and ground substance. Occasionally, one finds within nurse cell cytoplasm, fibrous elements either free (Fig. 60, 61) or in association with both a membrane element in a juxtanuclear position (Fig. 67, 59) and the plasma membrane (Fig. 58).

Oocyte Differentiation

Cells which ultimately become oocytes first appear morphologically different from nurse cells in the early differentiation phase (9 DPF). Differences in nuclear appearance, as well as a lighter cytoplasm, distinguish cells in the tropharium's basal region from the differentiating apical nurse cells. Autoradiographic evidence from this stage supports the observation of morphological differentiation (Fig. 63). Incorporation of H^3 - uridine is less in these

"pre-oocytes" than in nurse cells. Cytoplasm of pre-oocytes is less electron dense than that of surrounding nurse cells and many smaller micronucleoli replace the elaborate germ cell/nurse cell nucleolus (Fig. 64, 65). In contrast to the smooth nurse cell nuclear envelope, the outline of the pre-oocyte nuclear envelope is jagged. At a slightly later stage, further nuclear differentiation is evident even at the light microscope level (Fig. 66). Strands of condensing chromosomes can be seen within the nuclei of the oocytes as they enter the first meiotic prophase. This continued morphological differentiation is indicated by autoradiographic evidence (Fig. 67). The level of H^3 - uridine incorporated into the nuclei of the oocyte region is negligible compared to that incorporated by nurse cells in which nucleolar activity is high. Condensing chromatin is clearly seen, and characteristic synaptonemal complexes are verified with electron microscopy (Fig. 68, 69). The complex has the typical sub-structure of two lateral elements and one central element. During the early differentiation phase, chromosomes, observable in the light microscope, closely associate with the nuclear envelope (Fig. 71). Electron microscopy demonstrates that by this stage, the synaptonemal complexes have attached to the nuclear envelope (Fig. 70, 72).

The oocyte nucleus is eccentrically situated within the ooplasm whose organization has become distinct from that of nurse cell cytoplasm (Fig. 73 - 75). Some areas of the

ooplasm are relatively free of organelles in contrast to other organelle-rich areas. The ooplasm contains mitochondria, membrane-bound vesicles, dense bodies as well as free ribosomes, although ribosomal density is significantly less than in nurse cells. As within nurse cells, nuage material is present within oocytes although it is not restricted to a peri-nuclear position. Rough endoplasmic reticulum exists as an anastomosing, branching network along with a pair of centrioles within the ooplasm (Fig. 75, 77). Golgi complexes and Golgi vesicles are abundant within the ooplasm of some cells (Fig. 78, 79). Ooplasm density varies with the oocyte's size and stage of development (Fig. 76).

Prefollicular Area

Mesodermally derived prefollicular tissue occupying the region between the tropharium and pedicel consists of morphologically distinct apical and basal zones. Both basal cells and the flattened, cylindrical apical cells have peripherally located nuclei but the cytoplasm of basal cells is much less basophilic than that of apical cells (Fig. 81, 82). Autoradiographic data demonstrate that from the unfed state until at least the time of oocyte - nurse cell divergence, apical zone nuclei incorporate H^3 - uridine, whereas basal cell nuclei incorporate minimal precursor (Fig. 83). As electron microscopy indicates, apical prefollicular cells of an unfed animal are cigar-shaped with oval, often lobed nuclei,

containing patches of chromatin adjacent to the inner nuclear envelope. The cytoplasm characteristically contains a prominent lipid droplet surrounded by much glycogen. Also contained within the cytoplasm are mitochondria, RER, free ribosomes, Golgi complexes, and microtubules which are oriented parallel to the long axis of the cell (Fig. 84).

During the proliferative phase of ovarian development and continuing into the early differentiation phase, apical prefollicular cells undergo mitosis (Fig. 85). By the beginning of the early differentiation phase, prefollicular cells adjacent to the oocyte region have differentiated. This differentiation involves loss of the larval cell shape, and of the lipid droplet with associated glycogen. Differentiated cells are spindle shaped with an ovoid nucleus. Distended vesicles of RER are prominent cytoplasmic constituents (Fig. 86). Cells posterior to the differentiated area retain their larval cell shape and cytoplasmic features (Fig. 87). Prefollicular cells immediately adjacent to oocytes form intimate oocyte associations, presumably through cell surface specializations such as gap junctions (Fig. 88, 89).

During the late differentiation phase, oocytes grow into the prefollicular region while prefollicular cells invade the oocyte region to associate with developing oocytes (Fig. 90). These reciprocal associations will ultimately result in the formation of an early ovarian follicle. Inner sheath cells, continuous with prefollicular cells, also associate with oocytes

at the edge of the tropharium (Fig. 91). Prefollicular cells envelop the trophic cords near the oocytes (Fig. 92). Prefollicular cell - oocyte interaction in follicle formation is initially simple but, as Figure 93 demonstrates, can become very complex upon oocyte enlargement during previtellogenic growth.

Sheaths

The ovarian sheaths covering ovarioles are mesodermal in origin and are separable into inner and outer sheaths. As the ovariole enlarges, the sheaths, through mitotic activity, also enlarge (inset, plate 20). Inner sheath cells, continuous with prefollicular tissue, line the entire tropharium residing between the nurse cells and the non-cellular tunica propria. These sheath cells, while cytologically different from nurse cells and oocytes, are similar to differentiated prefollicular cells, being flat, elongated cells with oval nuclei and central nucleoli (Fig. 94, 95). The cytoplasm consists of vesicles of RER which are often distended, mitochondria, and a few free ribosomes. The outer sheath contains a number of cell types including myoepithelial cells and tracheole cells. Cells that commonly lie immediately outside the tunica propria are elongate and contain oval nuclei, mitochondria, Golgi complexes, free ribosomes and some vesicles of RER (Fig. 95, 96). These cells are separated from the rest of the outer sheath by a thin lamina (Fig. 96). Occasionally there exists, on the outside surface of the tunica propria, a cell which is different from

the cell type described immediately above. This different cell characteristically contains within its cytoplasm, organized stacks of RER. Presumably this variety of morphologies reflects the various connective tissue, tracheole and muscle elements comprising the complex sheaths.

Having now established the timing, ultrastructural and functional features of the various cellular components, one can return to the question of cell interaction. As noted previously, the germ cells proliferate by mitosis and daughter cells eventually differentiate. The following results focus specifically on the cell interactions.

Cell Interactions - Nurse Cell - Nurse Cell Interaction

As mentioned previously, groups of nurse cells associate, and co-ordinate activities via intercellular bridges formed by incomplete cytokinesis during mitosis. Figures 98 and 99 show two daughter cells that have completed nuclear division and nuclear envelope formation but whose cytoplasm still contains spindle microtubules and mid-body. Lateral edges of the membrane surrounding the mid-body exhibit electron dense thickenings characteristic of intercellular bridges. Eventually the spindle microtubules disappear and the bridge as seen in Figures 100 and 101 results. The dense material in the middle of the bridge is presumably the mid-body remnant. Cytoplasm in the bridge vicinity contains numerous membrane vesicles and a few microtubular profiles.

Bridges are observed in thick sections, although confirmation by electron microscopy is necessary (Fig. 102). The dense material within an early bridge eventually disappears, although the bridge retains its lateral densities (Fig. 103, 104). Membrane vesicles subsequently fill the cytoplasm within the bridge. Often sections show more than one bridge impinging upon a single nurse cell suggesting the existence of clones of interconnected nurse cells (Fig. 105, 106). The position of the bridges varies greatly from being situated on opposite sides of the cell (Fig. 105) to being situated adjacent to one another (Fig. 106). Figure 106 shows a bridge system in which a fusomal-like material and membrane vesicles exclude free ribosomes and other cytoplasmic components from the bridge region.

Oocyte - Oocyte Interaction

Not only nurse cells, but also oocytes are interconnected by intercellular bridges. Thick sections imply these relationships (Fig. 107, 108) while ultrastructural results confirm the existence of oocyte - oocyte bridges. Figure 109, coupled with information from several adjacent sections, shows a system with six oocytes joined by a complex of intercellular bridges and cytoplasmic extensions. Golgi complexes, mitochondria, as well as a variety of membrane vesicles and multivesicular bodies occur in the intercellular network (Fig. 109, 110, 113). Figures 111 and 113 provide examples of other oocyte - oocyte bridge systems in which two identifiable oocytes are joined to a third cell. There is great diversity

in the type of geometric organization of these bridge systems.

Trophic Core

The trophic core exists throughout the 5th instar, undergoing major restructuring during the larval-adult transformation. Branches from all regions of the tropharium feed into the central core area (Fig. 114). At the start of the instar, and during both proliferative and early differentiation phases, the core consists of a tortuous arrangement of cell processes filled with ribosomes, mitochondria, Golgi complexes, microtubules, and breakdown elements such as lamellar bodies, multivesicular bodies and dense bodies (Fig. 115, 116). A centriole was found once within the core region (Fig. 120). Cytoplasmic extensions from nurse cells enter the core (Fig. 118). Nurse cells also have bridges with processes that lead into the core (Fig. 115, 117) and many bridge profiles are observed in the core itself (Fig. 116). Bridge systems which can be complex are found within the peripheral core (Fig. 119).

During the late differentiation phase, massive membrane fusions and elaborate restructuring occurs. These changes are evident in thick sections (Fig. 121) although ultrastructural examination dramatically reveals the restructuring (Fig. 122 - 124). Components found within the cell processes at earlier stages are still present. The core at late differentiation phase consists of large areas free of membrane (Fig. 122, 123). Although membrane fusions have taken place, bridge profiles

remain within the lateral membranes of fused channels of the transforming core (Fig. 124).

As membrane fusions occur within the core, they concurrently take place between basally located nurse cell compartments. Light microscopic examination of this area reveals clusters of nurse cell nuclei with no indication of intervening membrane (Fig. 125). Electron microscopic examinations confirm this evidence as groups of active nuclei within the region exist in common cytoplasm (Fig. 126, 127). Association with other groups of nuclei or with the core is maintained through intercellular bridges (Fig. 128).

Nurse Cell - Oocyte Interaction: The Trophic Cords

Nurse cells and oocytes which are daughter cells of a common stem cell connect through intercellular bridge systems. However, because of their geometric separation and interaction within the tortuous core, the likelihood of having both nurse cells and connected oocytes present in the same plane of section is remote.

Oocytes, on first appearance during the early differentiation phase, retain their connections to the trophic core via cell bridges which become the trophic cords of the adult (Fig. 129, 130). The trophic cords arise from the elongation of intercellular bridges connecting sister nurse cells with oocytes. A bridge profile in an extension is shown in Figure 132, the section from an area in which trophic cords are present.

These cords elongate and maintain their core connection as oocytes invade prefollicular tissue (Fig. 131 - 133). Microtubules are present within the cords (Fig. 134). The connection of both small and large oocytes to the core persists throughout the remainder of the instar and into the adult (Fig. 140, 141).

The maintenance of core-oocyte connections is necessary for adult function. Nurse cells transport material via the trophic core and cords into the oocytes. Transport of material into oocytes contributing to previtellogenic growth was observed before the adult moult in the final days of the instar. The nurse cell nucleolar and nuclear structure developed and began functioning earlier, presumably in preparation for this previtellogenic growth prior to the adult moult. The terminal oocyte (Fig. 135) and smaller oocytes (Fig. 136) contain material similar to that present within the trophic core (Fig. 137 - 139). In these figures, the appearance of the oocyte nucleus, organelles and cytoplasm suggests that these oocytes are not autolytic but rather are merely incorporating breakdown material from the trophic core.

Several days before adult moult, the core and cords are weakly birefringent in polarizing microscopy (Fig. 142), becoming strongly birefringent a few days post moult (Fig. 143). Birefringence probably is due to the production and organization of microtubules which will exist within the core and cords of the adult (Huebner and Anderson, 1970). Microtubular

organization is the final step in the production of a functional adult structure.

Control of Differentiation

Aspects of the endocrine control of ovarian differentiation were investigated using ovarian transplant techniques. Table 1 summarizes the transplant data. As a control to investigate the possibility of abnormal differentiation due to technique, unfed 5th instar ovaries were transplanted into unfed 5th instars. Donor ovaries differentiated normally (Fig. 144, 145). Normal oocyte - nurse cell differentiations, prefollicular differentiation and invasions, normal follicle production, core fusions, nurse cell fusions, and trophic cord development occurred in the donor ovary. Recipient ovary had also fully differentiated and was normal in appearance.

To test whether an absence of juvenile hormone alone could trigger ovarian differentiation, unfed 5th instar ovaries were transplanted into allatectomized adults. Donor ovaries remained undifferentiated, appearing similar to normal unfed 5th instars (Fig. 147, 148). Mitosis within the ovary was observed. Recipient ovaries were normal and no effect attributable to the transplant was observed (Fig. 146).

The possibility of initiation of ovarian differentiation by JH was tested by transplanting unfed 5th ovaries into virgin adult females which were subsequently fed. Donor ovaries

remained undifferentiated (Fig. 149, 150). Recipients appeared normal and unaffected by the operation.

Could moulting, and thus ecdysone, even in the presence of JH, initiate ovarian development? To test this hypothesis, 5th instar ovaries were transplanted into unfed 4th instars. Although donor ovaries exhibited germ cell differentiation resulting in oocytes and nurse cells, their structure was disorganized and remained larval in appearance. The germarial lumen was still present and the core and prefollicular tissue remained larval (Fig. 151, 152). Functional nurse cells had differentiated with both condensed nucleoli (Fig. 153) and nucleo - cytoplasmic transport (Fig. 154). In addition, some nurse cell - nurse cell fusions had occurred (Fig. 153). Differentiated oocytes had normal nuclear and cytoplasmic characteristics: synaptonemal complexes and micronucleoli in the nucleus, cytoplasm less dense than that of nurse cells, Golgi complexes, RER and mitochondria were present (Fig. 155, 156).

DISCUSSION

Prior to discussing differentiation events occurring in the 5th instar ovary, it is useful to review structural and cellular characteristics of the adult ovariole as determined by Huebner and Anderson (1972 a,b,c). Consideration of developmental aspects will be more clearly understood within the context of the final structure.

The tropharium consists of a central core region surrounded by nurse cells. The nurse cells are divided into three zones. Zone I, in which mitosis is common, consists of many small cells with nuclei containing dispersed chromatin or peripheral chromatin patches. Nurse cells of Zones II and III are morphologically distinct from those of Zone I due to their functional development. The transition of cells from Zone I to Zone III is signified by nuclear enlargement and elaboration of the prominent basophilic nucleolus. Nucleo-cytoplasmic transport with a concurrent nuclear pore increase is manifested. Cell membranes between adjacent nurse cells have fused, contributing to the syncytial nature of the tissue.

Previtellogenic oocytes are characterized by synapsing chromosomes. A reticular nucleolus associated with a chromosome is situated at one end of the nucleus. The ooplasm contains numerous, small Golgi complexes, one pair of centrioles, abundant ribosomes and an anastomosing network of endoplasmic reticulum. Mitochondria organize in closely packed clusters which disassociate prior to vitellogenic growth. The oolemma

of small oocytes, in close association with follicle cells, is smooth, becoming more irregular once growth commences.

Prefollicular tissue consists of a meshwork of small, spindle-shaped cells whose cytoplasm contains little of the glycogen or lipid characteristic of earlier instars. This mesh surrounds young oocytes, eventually forming a columnar epithelium that possesses a microtubular cytoskeleton and an elaboration of usual cell organelles.

Cytoplasmic extensions from the nurse cells are continuous with the central trophic core region and are filled with many microtubules, interspersed with mitochondria and ribosomes. These streams fuse centrally in the core, creating an expanse of cytoplasm with microtubules organized into various directional bundles. Nurse cell nuclei are excluded from the core region. Microtubule-containing cords interconnect oocytes with rore. A syncytial association exists in the adult ovariole between the oocytes and nurse cells through the core.

With this outline of adult ovariole structure, the ontogeny of the highly specialized cell types, their structural inter-relationships and possible endocrine controls can be discussed. The following discussion consists of developmental analysis of the differentiation, from undifferentiated larval structures, of the structures specific to the adult. For clarity and convenience, the consideration is chronological, beginning with discussion of the early, undifferentiated situation, then covering the ultrastructural aspects of specific cell differentiations,

subsequently returning to the ontogeny of the cell inter-relationships, and concluding with endocrine control of ovarian differentiation.

Fifth Instar Ovarian Development

Germarial Lumen

The germarial lumen, previously undetected or unreported, is a normal feature of the early 5th instar ovary, and is present from the 1st instar onwards, presumably developing within the embryo (Huebner, in preparation). Ultrastructure of the luminal contents supports the interpretation that material accumulates as a by-product of ovariole restructuring. After development initiated by feeding, this region compacts and regresses. Presumably autolytic action recycles breakdown products for further developmental events. The loss of the germarial lumen constitutes the first phase in larval-adult ovarian transformation.

Despite differences in structure and organization, the closest counterpart to the germarial lumen in Rhodnius exists in the ovaries of aphids (Elliot et al., 1975). In the aphid germarium, rather than the internal borders of the germ cells meeting, each cell terminates in a brush border which protrudes into a central cavity that contains particles of cell debris. Elliot called this cavity the germarial lumen and proposed for it a role in the nutrition of the oocytes. However, further histochemical and cytological work is required before functions

can be attributed to the germarial lumen in Rhodnius. Although perhaps coincidental, it is interesting that this lumen occupies the same region of the ovariole later occupied by oocytes.

Nurse Cell Differentiation

Undifferentiated germ cells are morphologically similar during the proliferative phase of development. Subsequently, dramatic changes in nuclear morphology reflect the entrance of many of these germ cells into a nurse cell differentiation program. General nuclear changes at the light microscope level were noted for Oncopeltus (Wick and Bonhag, 1955) and for Rhodnius (Case, 1970). The sequence of nuclear modification in Drosophila nurse cell differentiation was elegantly described by Dapples and King (1970). Events of normal nurse cell turnover within the adult ovariole of another hemipteran, Gerris (Eschenberg and Dunlap, 1966), are similar to those observed in germ cell - nurse cell transformation in 5th instar Rhodnius. An ultrastructural description of nurse cell turnover within the adult was given by Huebner and Anderson (1972c). Both cytoplasmic and, more importantly, nuclear changes relate to the increase in nuclear activity of the nurse cell as it assumes its adult role of providing the oocyte with RNA and ribosomes for use in early embryogenesis.

The increase in nuclear size accompanying nurse cell differentiation was first thought to result from nuclear fusion

(Bonhag, 1955a; Wick and Bonhag, 1955; Vanderberg, 1963), a postulation which, however, subsequent investigations failed to confirm. That nurse cell nuclear fusions were not observed in Rhodnius 5th instar ovariole further supports the current theory that nuclear volume increases due to endopolyploidization (Eschenberg and Dunlap, 1966; Dapples and King, 1970; Huebner and Anderson, 1972c).

Nucleolar morphology reflects the activity of the nurse cell nucleus. As nuclear involvement in RNA production increases, the nucleolus becomes dense and compacted. Nucleolar changes due to increased RNA synthetic activity were described in in vitro rat liver nucleoli treated with polyamines by Gfeller et al. (1972). Adult nurse cell nucleolar changes during their progression from Zone I to Zone III were described by Huebner and Anderson (1972c). Autoradiographic analysis of the 5th instar contributes to morphological evidence of nurse cell nuclear activity as germ cell nuclei and differentiated nurse cell nuclei incorporated H^3 - uridine. Extrusion of nuclear material into the cytoplasm accompanies this increased activity. Nucleo - cytoplasmic transport, first described in adult Rhodnius nurse cells by Anderson and Beams (1956), has subsequently been confirmed in all nurse cells studied. Surprisingly, this transport is clearly evident by the beginning of the early differentiation phase (9 DPF) in the 5th instar ovariole. This phenomenon continues in the basal tropharial region (Zone II and Zone III) throughout the remainder of the

instar and into the adult. Thus, we observe a function of nurse cell nuclei, formerly attributed to the adult ovariole, already present in the early 5th instar ovariole. Diffuse material inside the nucleus changes density, condensing once it passes to the cytoplasm. This alteration may indicate a physical or chemical change in the material because of the cytoplasmic milieu. Mitochondria associate with the nuclear extrusions as in the adult (Huebner and Anderson, 1972c). Evidence indicates that, in various species, this material is RNA (Anderson and Beams, 1956; Bier, 1967; Choi and Nagl, 1977). Although in the early 5th instar, the production and transport of RNA to nurse cell cytoplasm has been demonstrated, the ultimate fate of the RNA is unknown. Likely, it is eventually transported to developing oocytes, although the timing of such postulated transport is unknown. The presence, within the trophic core region, of dense material closely resembling the nurse cell RNA bodies suggests that, even before core restructuring, some early transport occurs. Pulse labelling experiments must be performed to verify early RNA transport. The apparent nuclear pore density increase during nurse cell nuclear differentiation is related to nucleo-cytoplasmic transport. Further studies employing freeze - fracture techniques should provide the quantitative parameters of this increase. Nucleo-cytoplasmic transport of RNA only occurs over a small portion of the nuclear surface at any particular time. The RNA's final position in the cytoplasm is variable

with respect to the trophic core. There is no explanation of the observation, suggesting a focus for further investigation into nucleo - cytoplasmic transport phenomena.

Nurse cell DNA release for export to oocytes has been reported (Schrader and Leuchtenberger, 1952; Bonhag, 1955a; Vanderberg, 1963; Luzzato and Urbani, 1969). Intact nurse cell nuclei are excluded from the trophic core region throughout 5th instar supporting the proposal of Anderson and Beams (1956) that, in Rhodnius, DNA material is released into the trophic core by nuclear breakdown.

During early differentiation, nurse cell cytoplasm closely resembles that of germ cells. The small amount of rough endoplasmic reticulum found in these cells is usually closely associated with a Golgi complex. One may speculate that products of this system are exported from the cell. Increased ribosomal number can be related to the trophic function of these cells (Huebner and Anderson, 1970; Macgregor and Stebbings, 1970). The presence of many mitochondria within the cytoplasm corresponds to earlier observations proposing a nurse cell source for some oocyte mitochondria (Wilson, 1925; Huebner and Anderson, 1970; Macgregor and Stebbings, 1970; Urbani, 1970).

Significance of the fibrous elements occasionally seen either in cytoplasmic membrane association or free in the cytoplasm is uncertain. Although speculation on their origin is difficult, their membrane association at one time, and free cytoplasmic location at another, may indicate membrane

involvement in their assembly. Structures of similar appearance were reported, in mammalian oocytes, by Szollosi (1971), and Calarco and Szollosi (1973) and were often either associated with ribosome aggregates or contiguous with endoplasmic reticulum. Szollosi postulated that the filamentous elements were what had classically been called "dark yolk". Since Rhodnius nurse cells perform a trophic function, these elements may be a minor form of yolk transported to the oocytes.

During nurse cell differentiation, restructuring of cytoplasmic constituents occurs. Organelles or cytoplasmic regions no longer required are often packaged in membrane-bound isolation bodies. Isolation bodies are a general feature of reorganizing insect tissues (Locke and Collins, 1965; Locke and McMahon, 1971) and the precision with which the segregation membrane compartmentalizes organelles or cytoplasmic areas is remarkable. The presence of membrane-bound elements in the trophic core similar to nurse cell cytoplasmic isolation bodies, suggests that these elements are released into the core during lysosomal breakdown and restructuring of the ovariole.

Oocyte Differentiation

As within nurse cells, specific transformation of nuclear morphology of certain basally located germ cells reflects their entrance into an oocyte differentiation program. Morphologically distinct oocytes are evident at the commencement of the early differentiation phase (9 DPF). Autoradiographic data demonstrate that H^3 - uridine incorporation is already less

in the oocytes than in the nurse cells. Thus, differentiation of the oocytes' physiology occurs concomitantly with morphological differentiation. Previous investigations reported that in other hemipteran telotrophic ovaries, early oocyte differentiation occurs in the 4th instar stage (Wick and Bonhag, 1955; Choi and Nagl, 1976). However, the limited resolution of light microscopy available to Wick and Bonhag (1955), as well as the ambiguous choice of criteria for the distinction of early oocytes established by Choi and Nagl (1976), makes suspect these interpretations. In order to relate their findings to this study, autoradiographic analyses of ovarian H^3 - uridine incorporation conjoined with careful electron microscopical examination utilizing established criteria for oocyte distinction must be performed on these species. Until detailed re-examination of these other systems resolves this discrepancy in results within hemipteran telotrophic ovaries, it is likely the data presented here reflect the hemipteran situation.

Further oocyte differentiation resulted in an even greater difference in H^3 - uridine incorporation between oocyte nuclei and differentiating nurse cell nuclei with minimal grains present over the oocyte region and many grains over nurse cell nuclei. Clearly, one of the earliest manifestations of oocyte differentiation is the inactivity of the oocyte nucleus while nurse cell nuclei assume the RNA production function. Thus, even during the earliest stages of nurse cell - oocyte divergence, these cells conform to the general correlation in

the adult proposed by Huebner and Anderson (1972b): "..... the degree of oocyte function is inversely related to the presence and activity of nurse cell nuclei."

Morphological oocyte nuclear changes reflect the RNA synthetic inactivity of the nucleus. Appearance of condensing chromosome strands followed by assembly of synaptonemal complexes, morphologically verify the oocyte's initiation of meiosis. Synaptonemal complexes have been reported in a wide variety of meiotic cells (see review Moses, 1968). Oocytes enter the first meiotic prophase, there arresting until fertilization, as is typical of insects generally (Wigglesworth, 1972). In some polytrophic ovaries, synaptonemal complexes appear in oocytes and in some or all nurse cells (Roth and Porter, 1964; Koch and King, 1969). All germ cells within the pupal telotrophic ovary of the beetle, Bruchidius, enter meiosis and form synaptonemal complexes (Buning, 1972). However, no synaptonemal complexes were observed in developing nurse cell nuclei of Rhodnius. In contrast to the presence of single complexes in the adult (Huebner and Anderson, 1972b), 5th instar oocytes occasionally had double complexes. Multiple complexes have been reported in various insect groups (Moses, 1968). King (1970) has suggested that these complexes have an important role in meiotic crossing over. In Rhodnius, their formation during early differentiation perhaps suggests that crossing over occurs before the adult stage. This may be important since all the oocytes are established in meiotic arrest by the early adult.

Gradual restructuring of the ooplasm occurs, eventually resulting in ooplasm closely resembling the adult pre-vitellogenic situation described by Huebner and Anderson (1972b). An anastomosing system of endoplasmic reticulum replaces the single element of early germ cells, and mitochondrial numbers increase. Mitochondrial increase results from replication within the ooplasm and transport from nutritive tissue (Huebner and Anderson, 1972b; Macgregor and Stebbings, 1970). Mitochondrial clusters, characteristic of Rhodnius (Huebner and Anderson, 1972b) and Drosophila (Koch and King, 1969) oocytes, have also elaborated during pre-adult stadium. Instar oocytes contain more Golgi complexes than do nurse cells, and evidence suggests a nurse cell origin for some of the oocyte Golgi (Lutz and Huebner, 1978).

Prefollicular Differentiation

The morphological features of the early prefollicular cells correspond with the earlier description by Huebner and Anderson (1972a). Prefollicular tissue differentiation events described here coincide temporally with the appearance and subsequent differentiation of the oocytes, which strongly suggests an interplay of these cells during normal differentiation. Induction of early follicle formation does not depend on hormonal influence, but rather, on the growing oocyte (Masner, 1968). Since only oocyte-associated prefollicular cells lose their larval characteristics while basal cells retain them, a gradient-related oocyte cue is perhaps operating.

Prefollicular - oocyte interactions are mediated, at least in part, by cell surface specializations, particularly gap junctions. Although the gap junctions observed in this study were not verified with supporting lanthanum tracing or freeze-fracture analysis, other studies of follicle cell - oocyte interactions in adult Rhodnius (Huebner and Anderson, 1972a; Huebner, 1977), in cecropia (Woodruff, 1979) and in mammals (Anderson and Albertini, 1976) indicate that gap junctions are a common form of follicle cell - oocyte interaction in the animal kingdom. Lanthanum tracing and freeze-fracture analysis of various early and late development stages would provide a greater understanding of the involvement and modulations of the cell surface during prefollicular-oocyte interactions. The ultimate fate of the basal prefollicular region is unknown, although present evidence suggests an involvement in follicular tissue plug formation.

Inner Sheath

The function(s) of the inner sheath are poorly understood. Results from this study indicate that inner sheath cells in the region of the oocytes are involved in primary follicle formation at the periphery of the ovariole. Thus, inner sheath cells and differentiated prefollicular cells may be more than morphologically similar. The inner sheath cells may function in regulating material entering or leaving the tropharium. Experiments utilizing peroxidase and other extra-cellular tracers

at various developmental stages should confirm of disprove this hypothesis.

We have now considered the distinctive cell differentiation features of Rhodnius related generally to insect ovaries. As evident from the results, these cells are involved in structurally and temporally complex reciprocal interactions during the acquisition of their other cytoplasmic and nuclear constituents. The next section focuses on these developmentally significant cell interactions.

Nurse Cell - Nurse Cell Interactions

As described fully in polytrophic ovaries (see review Telfer, 1975), and in telotrophic ovaries by Buning (1972) and Huebner and Anderson (1972c), nurse cells associate by intercellular bridges. However, Buning (1972) reported that, in the beetle ovary, these structures were transitory and that, following metamorphosis, cell fusions within the tropharium resulted in a more intimate nurse cell association and a loss of bridges. Intercellular bridges, present within the 5th instar tropharium and retained in the adult, arise by incomplete cytokinesis during mitosis rather than by some secondary association (Fawcett et al., 1959; Telfer, 1975). Rhodnius bridge morphologies are similar to those in other systems (Telfer, 1975). Interconnecting bridge systems provide a route into the trophic core, and eventually into the oocyte, for nurse cell products.

Fawcett et al. (1959) associated intercellular bridges with synchronous function in a variety of tissues. Thus mitotic synchrony within nurse cell groups may be attributable to an intercellular bridge association between these cells, although the presence of intercellular bridges alone does not preclude asynchrony of associated cells. Endomitotic divisions of nurse cell chromatin and nurse cell - oocyte divergence occurs even though these cells remain interconnected. The variety of orientations of intercellular bridges between groups of nurse cells suggests that bridges, once formed, move along a dynamic cell surface. This suggestion is contrary to the theories of Hirschler (1955) and Koch et al. (1967) that intercellular bridges, after formation, are fixed on the cell surface. However, intercellular bridge mobility corresponds with earlier observations in polytrophic ovaries in which intercellular bridge movement is critical to an explanation of rosette formation (Telfer, 1975).

The syncytial arrangement of nurse cells is dependent primarily upon the formation and maintenance of intercellular bridges. A secondary aspect of the syncytial organization is the fusion of lateral membranes between individual nurse cells in Zones II and II of the tropharium. This results in groups of nurse cell nuclei occupying a common cytoplasm as was also observed in adult ovaries (Buning, 1972; Huebner and Anderson, 1972c). Thus, the syncytial nature of nutritive tissue, classically studied with light microscopy, is established ultrastructurally by two distinct cellular events.

An unanswered question is whether or not Zone I nurse cells, existing in syncytial units, connect to other nurse cell units in more advanced zones. These cells may represent a population of undifferentiated germ cells whose daughter cells replenish, by fusion, degenerating Zone III nurse cells. This process is a normal feature of adult oogenesis.

Oocyte - Oocyte Interactions

Not only nurse cells, but also groups of oocytes are interconnected by intercellular bridge systems. This is the first report of such a developmental association in insects, demonstrating that in Rhodnius, oocytes exist in a syncytial arrangement in early determination. A number of daughter cells arising from the same stem oogonium become oocytes. The occurrence of oocyte intercellular bridge systems has yet to be described in other hemipteran ovaries. However, it appears that beetle telotrophic ovaries do not share this characteristic with Rhodnius (Buning, 1978). If oocyte interconnections are found in hemipterans generally, a fundamental difference would exist between hemipteran telotrophic ovaries and both polytrophic ovaries and beetle telotrophic ovaries.

The close geometric association of oocytes is observable only during the early differentiation phase. Restructuring (discussed below), involving prefollicular cell invasion and growth, results in retention, in the adult, of a reorganized, elongated interconnecting system. Procion fluorescent dye tracing experiments on adult ovarioles verify that oocytes

interconnect via attenuated trophic cords (Huebner, 1978).

During the late differentiation phase, these associations become extended and tangled, thus rendering improbable direct observation of continuities in single, or even adjacent, sections. The orientation of bridges within the network suggests that oocyte bridges, like nurse cell bridges, can move along the cell surface. The extent of these systems has not been fully determined, although serial section analysis of early differentiation may provide an answer. Occurrence of numerous Golgi complexes within the intercellular bridge systems suggests an early nurse cell - oocyte Golgi transport (Lutz and Huebner, 1978).

Oocyte intercellular bridge systems are involved in the formation of trophic cords and in the co-ordination of oocyte growth. In Rhodnius, only the terminal oocyte in each ovariole is vitellogenic. Thus, by cytoplasmic communication through the common core, neighbouring previtellogenic oocytes are inhibited from initiating vitellogenesis. In polytrophic ovaries, in which each oocyte associates in discrete units with nurse cells, successive units of growing oocytes intercommunicate despite the absence of cytoplasmic confluency. Adjacent follicles in cecropia moth ovaries are electrically coupled and fluorescent dye moves readily between these follicles (Woodruff, 1979). This evidence reflects the presence of an interfollicular communication pathway in polytrophic ovaries. Thus, although the unique cytoplasmic interconnections of Rhodnius oocytes are now well-established, other electrically-mediated coupling

through associated mesodermal tissue may also exist.

Nurse Cell - Oocyte Interactions: The trophic core and cords.

It is well-established that polytrophic nurse cells and oocytes evolve from a common stem cell ancestor and remain interconnected via intercellular bridges until the end of vitellogenesis. In telotrophic ovaries, the origin of nurse cells and oocytes from common germ cell ancestors is equally well-established. However, the maintenance of nurse cell - oocyte interconnections throughout oocyte development has been questioned (Telfer, 1975). The trophic core is an area of cell-to-cell interactions. Nurse cell processes enter the core, and intercellular bridge profiles within the core suggest that nurse cell - nurse cell and nurse cell - oocyte interaction do indeed occur there.

The core, present from 1st instar to the adult (Huebner, in preparation), retains its larval morphology until late 5th instar. In earlier instars, inner sheath cell processes enter the trophic core. Thus, there is in addition to germ tissue, a mesodermal component of the core. Inner sheath cell processes do not extend into the 5th instar core. Apparently, inner sheath connections to the core are severed or retracted before the 5th instar. Massive membrane fusions and major restructuring of the core occurs in the late differentiation phase. Fusion of the tortuous membrane arrangement characteristic of the larval core results in vast areas of cytoplasm containing few intervening membranes. This area is subsequently filled with

numerous organized microtubules characteristic of the adult core (Huebner and Anderson, 1970).

Trophic cords presumably originate by elongation of intercellular bridges interconnecting nurse cells and oocytes. The presence of bridges interconnecting Rhodnius oocytes indicates that trophic cords are also formed, in part, from the elongation of oocyte bridges. The presence of bridge profiles on the lateral edges of early trophic cords provides supportive evidence of an intercellular bridge origin for the trophic cords. In addition, Buning (1978) recently presented strong data for the bridge origin of trophic cords.

From the early differentiation phase into the adult, oocytes connect into the basal region of the trophic core where they associate with nurse cells. Mays (1972) reported, without supporting evidence, that newly eclosed adult Pyrrhocorris are not connected to the trophic core, suggesting a secondary fusion. Buning (1978) provided clear evidence that in beetle telotrophic ovaries, nurse cell - oocyte association commences during early differentiation, and is maintained into the adult. Similarly, Rhodnius nurse cell - oocyte associations arise during early stages of ovarian development and are maintained through the instar into the adult. Because the beetle telotrophic ovary does not possess a trophic core, Buning could demonstrate a direct nurse cell - oocyte connection. The tortuous arrangement of cell processes within the core in Rhodnius precludes the probability of direct observation with conventional techniques of nurse cell - oocyte interconnections.

However, intracellular tracer techniques may prove useful.

The transport to developing late 5th instar oocytes of material arising from trophic core restructuring and from Zone III nurse cells presumably contributes to previtellogenic oocyte growth. Thus, some transport of materials to oocytes occurs before development of the elaborate adult microtubular arrays. Microtubules probably function both as cytoarchitectural supports and as a sieving and directional system (see for discussion Hyams and Stebbings, 1977). Polarizing transport within cecropia ovarian follicles was linked to the existence of an electrical gradient within the follicle (Woodruff and Telfer, 1973, 1974; Woodruff, 1979). The existence of similar phenomena in telotrophic ovaries requires further investigation.

Endocrine Control of Ovarian Differentiation

Much information is available on endocrine control of adult ovarian function (see reviews Englemann, 1970; de Wilde and de Loof, 1973; Gilbert and King, 1973). However,little information exists on control of germ cell differentiation and ovarian development. Developmental events are related to feeding, as indicated by previous physiological evidence that a response to feeding in the neuro-endocrine system triggers cell growth and differentiation in the instars (Wigglesworth, 1934, 1948). The influence of altered hormonal milieu created by the various ovarian transplantation experiments, permitted

correlation of differentiative events and endocrine alteration. The transplant technique is valuable in two respects: first, a natural tissue environment with endogenous hormone is present and secondly, the presumed hormonal titers are physiological.

Results indicated that ecdysone triggers both germ cell differentiation and larval-adult structural transformation in the ovariole, a conclusion reached also through experiments with polytrophic ovarian systems (King, 1975). The allatectomizing of 4th instar Rhodnius, followed by a blood meal, resulted in the formation of differentiated ovaries in a pharate adult (Huebner, 1978). Again, it appears that ecdysone alone is responsible for ovarian differentiation.

By electrocoagulation of specific parts of the neuro-endocrine system of Panstrongylus megistrus, a blood-sucking reduviid, Furtado (1976a,b; 1977a,b) provided insights into endocrine control of germ cell differentiation and ovarian development. He found the A cells of the pars intercerebralis were required for a critical mitotic period between 2 DPF and 5 DPF. The induction of both meiosis, which began at 9 DPF, and moulting events was controlled by the A^1 cells of the PI. The A^1 cells act through the prothoracic gland, an intermediary between brain and ovary. Furtado's results further support the hypothesis that ecdysone triggers germ cell differentiation and ovarian development.

The absence of juvenile hormone in normally developing 5th instars apparently is necessary for mesodermal and cytoarchitectural differentiation of the ovariole since, in 5th to 4th transplants, mesodermal elements and structural features of the donor ovary remain larval. Juvenile hormone modulation of ecdysone action in tropharium development was also suggested by Furtado (1977b). The occurrence in the 5th to 4th transplant experiments of germ cell differentiation in the presence of JH while other ovarian characteristics remain larval is confusing. A possible explanation is that, while the level of JH at the implant in the 4th instar is insufficient to inhibit ecdysone-initiated germ cell differentiation, it was sufficient to inhibit mesodermal and structural differentiation. Topical applications of JH to allatectomized 4th instars prevented occurrence of differentiation events so that ovaries remained larval (Huebner, 1978). This result supports the hypothesis that the level of JH at the transplant was too low to inhibit germ cell differentiation since, in larger doses, JH did inhibit germ cell differentiation as well as structural differentiation. Since in Rhodnius, little data are available on stage specific hormone titers or on time frame of tissue-hormone responsiveness, the 5th to 4th transplant results are at present unresolvable.

An alternative hypothesis of 5th to 4th transplant results is that the time frame of implanted 5th ovary responsiveness was asynchronous with the hormone titer changes in the 4th instar.

For example, let us assume that ecdysone, triggered by the blood meal of the 4th instar recipient, can be modulated over X number of days. If, after feeding, 4th instar JH titer declines relatively quickly, its modulating effect on germ tissue ecdysone response may be withdrawn early enough to allow germ cell differentiation. Mesodermal tissue, on the other hand, may need a shorter duration in which to respond to JH, and thus JH titers in the 4th instar may be able to act long enough to inhibit mesodermal differentiation.

An experiment to test these hypotheses would involve the topical application of JH to the 4th instar recipient containing the transplanted 5th instar ovaries as well as study of hormone titers and timing of tissue responsiveness. Investigation utilizing culture methods and synthetic hormones will provide further insights into the endocrine control in insects of both germ cell and ovarian differentiation.

In conclusion, with the use of various light and electron microscopical techniques, coupled with autoradiography and ovarian transplant experiments, some structural and physiological aspects of 5th instar ovarian development in Rhodnius prolixus have been determined. Findings of this study have established the developmental framework of the ovary during the 5th instar. Morphological divergence of nurse cells and oocytes has been investigated. In addition, temporal and morphological features of the various cell interactions which accompany larval-adult ovarian transformation have been described. Finally, some aspects of the endocrine control of ovarian differentiation were

explored using ovarian transplant techniques. It is hoped that the findings of this study will provide the focus for further investigations into the morphological details of telotrophic ovarian development in insects. Also, these findings provide specific information about a developing system whose unique structural features could be exploited in investigations of fundamental questions in such fields as cell determination and cell interactions.

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Table 1

Summary of Ovarian Transplant Data

Stages					
Donor	Recipient	JH	Ecdysone	Germ Cell Differentiation	Structural Differentiation
5th	5th	-	+	+	+
5th	-CA Adult*	-	-	-	-
5th	Virgin Adult	+	-	-	-
5th	4th	+	+	+	-

+ - presence of

- - absence of

JH and ecdysone columns refer to the presence or absence of the endogenous hormones within the transplant recipient. The germ cell differentiation column indicates whether or not nurse cells and oocytes have differentiated in the transplanted donor ovary. The structural differentiation column indicates whether or not adult structural features and organization have differentiated such as trophic core differentiation and prefollicular cell differentiation.

* allatectomized adult

KEY TO FIGURES

AP - apical prefollicular

B - intercellular bridge

BP - basal prefollicular

C - centriole

CH - chromatin

CM - cytoplasmic membrane

DB - dense body

F - fibrous body

FOL - follicle cells

G - Golgi complex

GC - germ cell

GL - germarial lumen

GLY - glycogen

IS - inner sheath

L - lipid

LB - lamellar body

M - mitochondria

MB - mid-body

MF - mitotic figure

MN - micronucleoli

MT - microtubule

MVB - multivesicular body

N - nucleus

NC - nurse cell

NE - nuclear envelope

NP - nuclear pore

NU - nuage material

NUC - nucleolus

O - oocyte

OD - oviduct

OS - outer sheath

OV - ovary

P - prefollicular cells

PED - pedicel

PM - plasma membrane

RER - rough endoplasmic reticulum

S - segregation body

SC - synaptonemal complex

T - trophic core

TC - trophic cord

TF - terminal filament

TP - tunica propria

TR - tracheole

V - membrane vesicles

Z3 - Zone III of nurse cells

Plate 1

Gross anatomy and timing

Figure 1. This micrograph illustrates the life cycle of Rhodnius prolixus with five larval instars and the adult female. X1.8.

Figure 2 and 4. Adult female tracts showing well tracheated ovaries attached anteriorly by terminal filaments and posteriorly by thick lateral oviducts. X15, X15.

Figure 3. Scanning electron micrograph of 5th instar reproductive tract showing small ovary with thin terminal filament and lateral oviduct. X240.

Figure 5. This composite illustrates ovarian growth during the 5th instar. Ovaries were taken at two day intervals after feeding, starting with unfed (upper left) and finishing with 20 DPF (bottom right). X110.

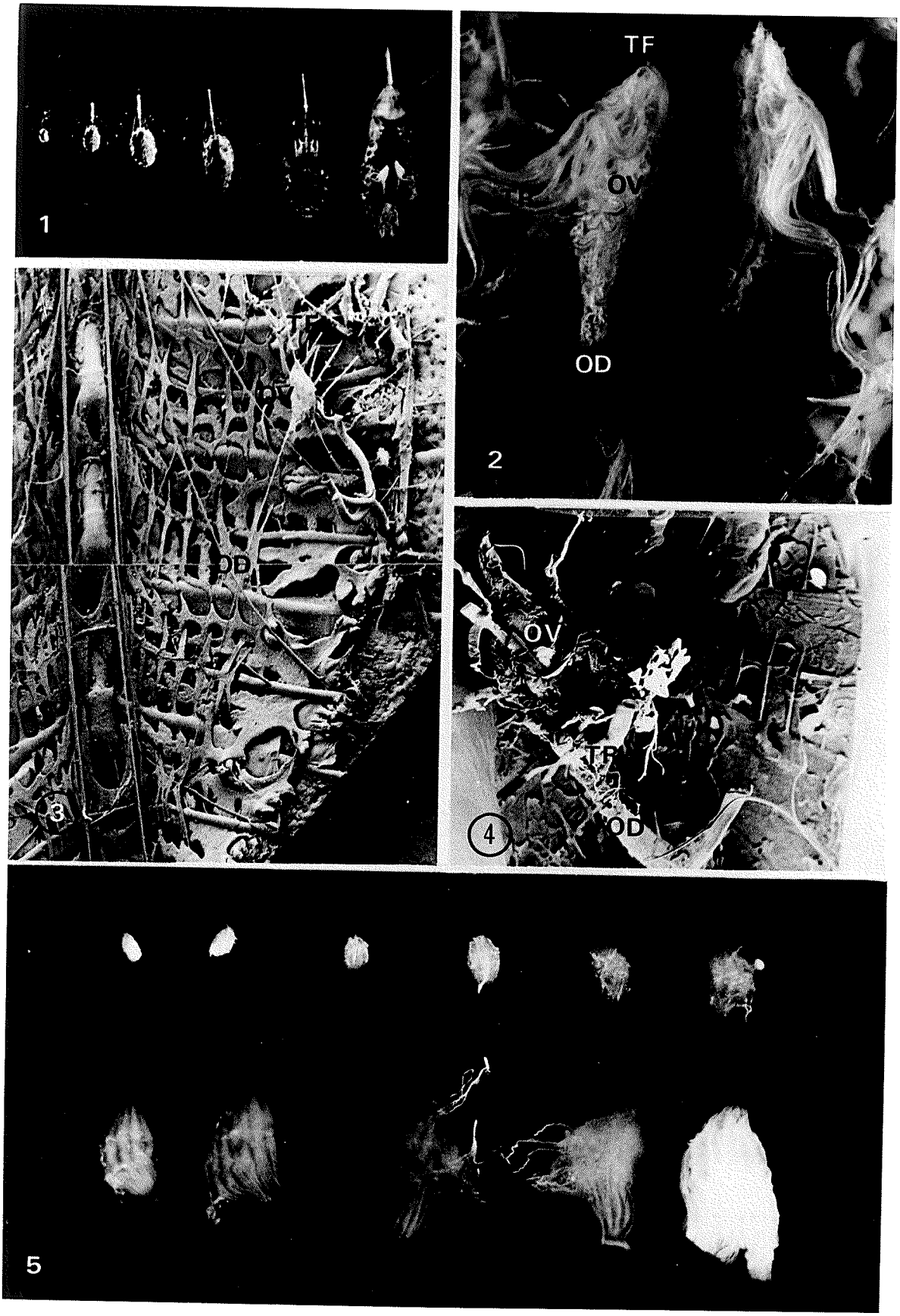
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Plate 2

Gross Cellular Changes

Proliferative and early differentiation phase

Figure 6 to 9. Proliferative phase ovarioles. Micrographs of an unfed ovariole taken with Nomarski interference of a live preparation (6), and of a thick section (7) show germ cells surrounding trophic core region. Note germarial lumen, prefollicular tissue, tunica propria (arrows) and outer sheath. Mitosis after feeding increases size and cell number of the tropharium but no morphological differentiation occurs. X1400, X1870, X1780, X1000.

Figure 10 and 11. Appearance, in both live (10) and sectioned material (11), of a distinctive basal region in the tropharium marks the start of the early differentiation phase. Basal nuclei lose prominent nucleolus while cytoplasm stains less intensely than apical cells. X460, X1280.

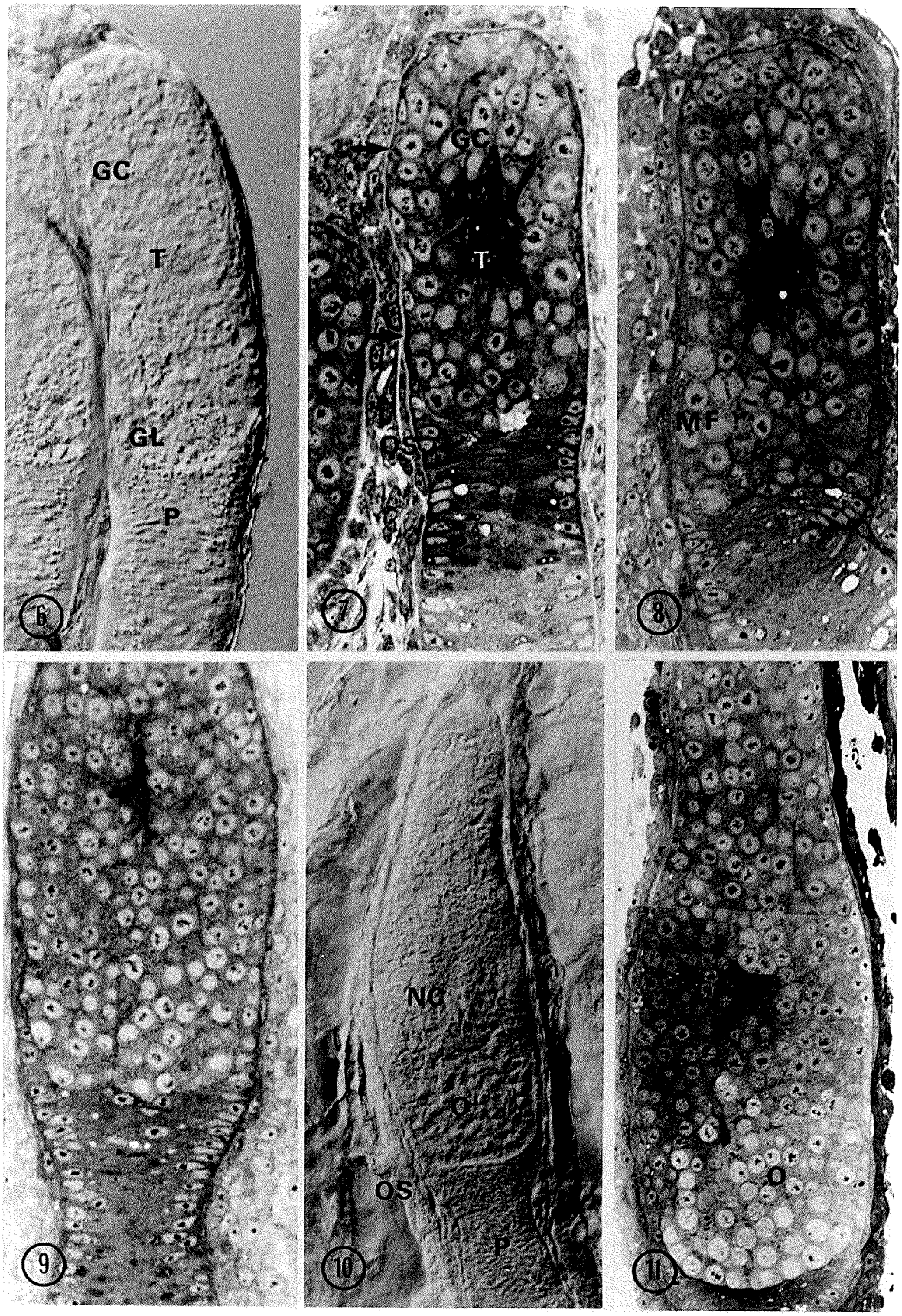
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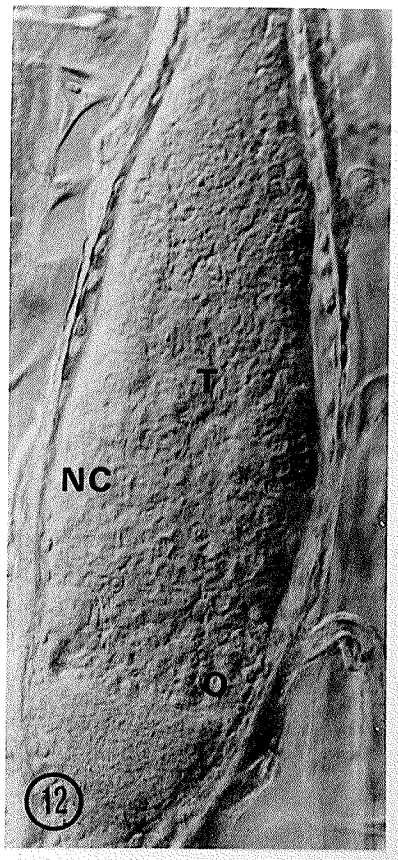
Plate 3

Gross Cellular Changes

Early and late differentiation phases

Figure 12 and 13. Further early differentiation events result in a clearly defined basal region of oocytes observable in both live (12) and sectioned material (13). Zonation of nurse cells begins with Zone III nurse cell cytoplasm becoming increasingly basophilic. X500, X1200.

Figure 14 to 17. Nurse cell nuclear groups in Zone III during the late differentiation phase can be observed in live (15) and sectioned material (14). Light micrographs (16, 17) illustrate the final reorganization events in the larval-adult ovarian transformation. Prefollicular tissue invades the oocyte region, reorganization of the trophic core occurs and oocytes initiate previtellogenic growth remaining connected to the trophic core via trophic cords. X1940, X1920, X650, X1740.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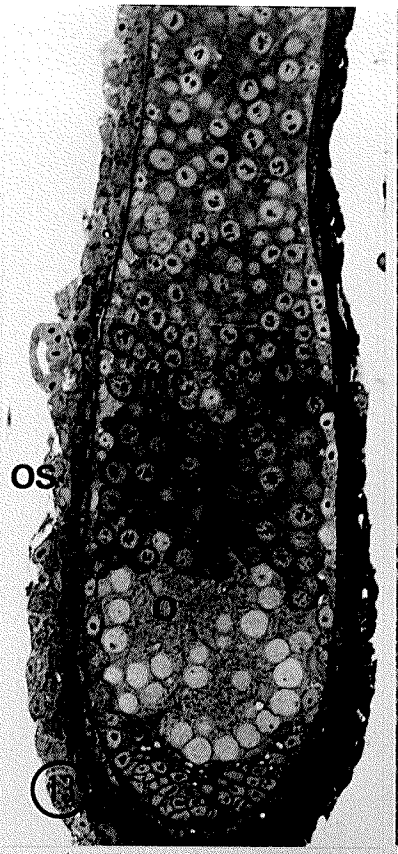
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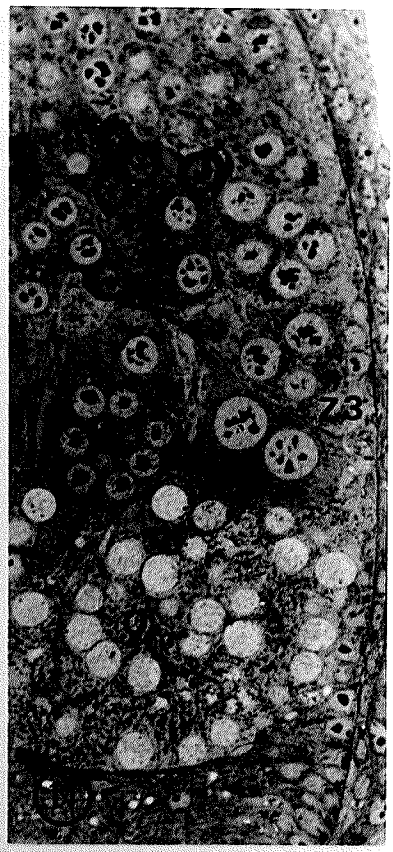
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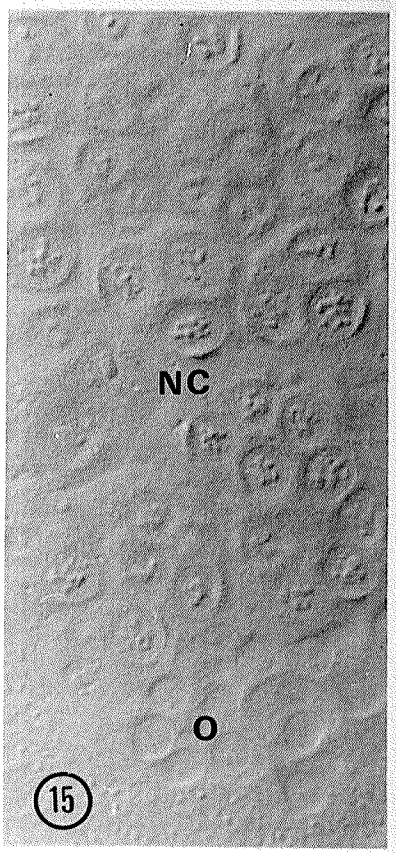
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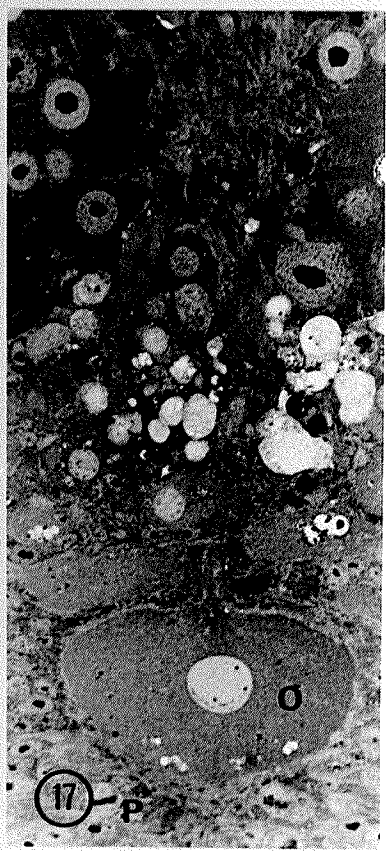
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Plate 4

Germarial Lumen and Early Germ Cells

Figure 18 and 19. These Nomarski interference micrographs of live unfed ovarioles show the germarial lumen. Note the difference between the germarial lumen and the trophic core. X1850, X2464.

Figure 20. This light micrograph demonstrates the basophilic nature of the small particles of the germarial lumen. X1240.

Figure 21. This electron micrograph reveals the ultrastructure of the lumen and dense bodies of the germarial lumen as well as the ultrastructure of the adjacent germ cells. X1270.

Figure 22 and 23. These electron micrographs (and light micrograph inset) illustrate early germ cell features. Note prominent nucleus with elaborate nucleolus situated in a cytoplasm containing numerous ribosomes, a strand of RER, mitochondria and a few Golgi complexes. X6580, X11090, X1280.

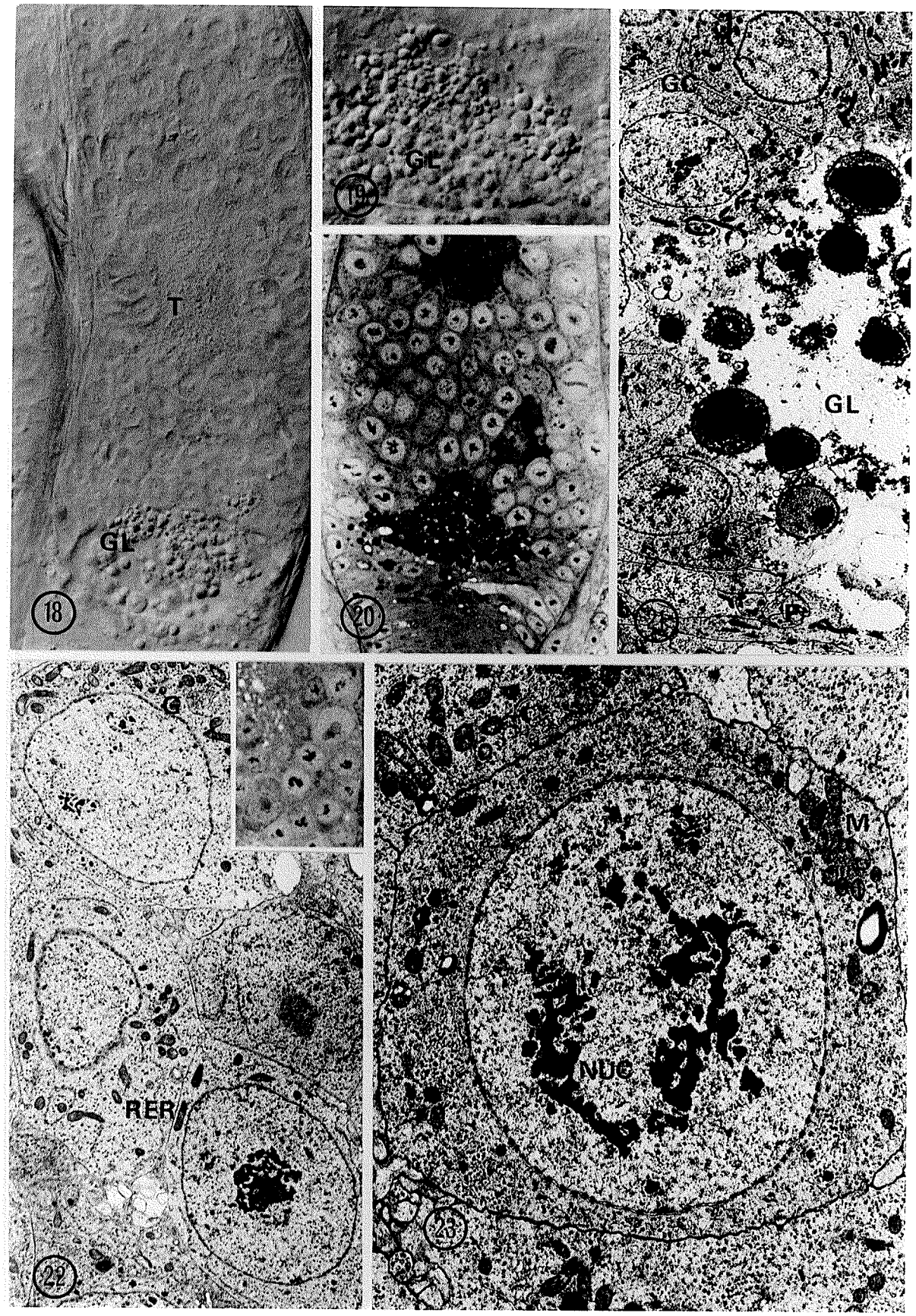
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Plate 5

Proliferative phase

Mitosis and growth

Figure 24 to 26. Mitotic germ and nurse cells in the tropharium are shown in these micrographs of live (25) and fixed material (24, 26). X1246, X1850, X2080.

Figure 27. An area in which a group of cells are dividing in synchrony (arrow) is shown in this micrograph. X1060.

Figure 28. A metaphase nurse cell is illustrated in this micrograph. Note the exclusion of most organelles from the spindle region which is outlined by vesicles of RER. A higher magnification of the centriole region (28a) reveals a few spindle microtubules and vesicles of RER. X9060, X18000.

Figure 29. The cell illustrated here is at late anaphase or early telophase. Note nucleolar structure in chromatin (arrows) and the alignment of membrane vesicles during early phases of nuclear membrane formation. X7580.

Figure 30. This micrograph shows a later stage of nuclear envelope formation. The chromatin has begun to disperse at this stage. X8520.

Figure 31. Microtubules from the mitotic spindle persist in the cytoplasm of the daughter cells as revealed in this micrograph. This phenomena is related to intercellular bridge formation. X11260.

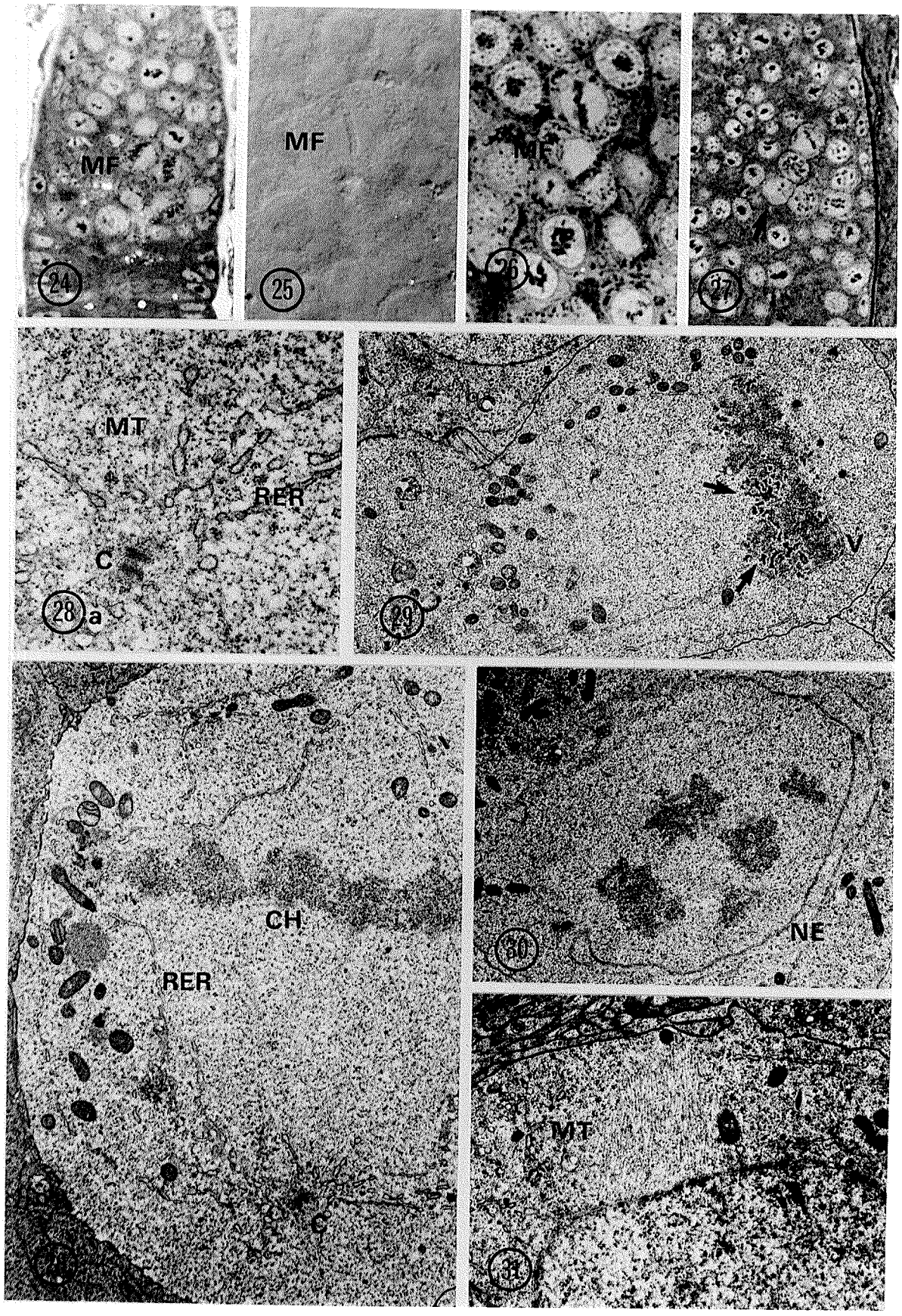
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Plate 6

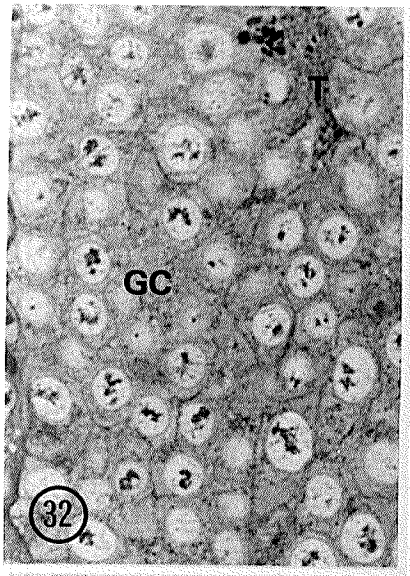
Nurse cells

Nuclear differentiation

Figure 32. As seen in figures 22 and 23, germ cell nucleoli are initially somewhat dispersed. X1800.

Figure 33 and 34. As differentiation proceeds, the nucleoli of early nurse cells becomes more compact as shown in these micrographs. X11630, X1410.

Figure 35 36. These micrographs illustrate that a fully differentiated functional nurse cell characteristically contains a tight compact nucleolus. Note nucleo - cytoplasmic transport (arrows) at light and electron microscopic level. X1330, X16730.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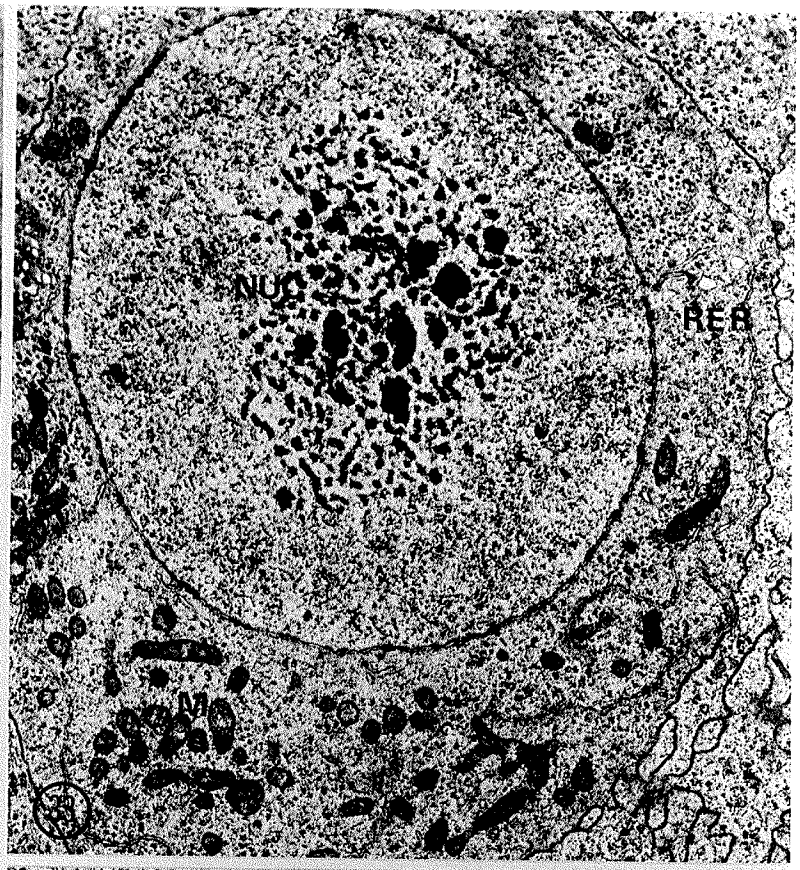
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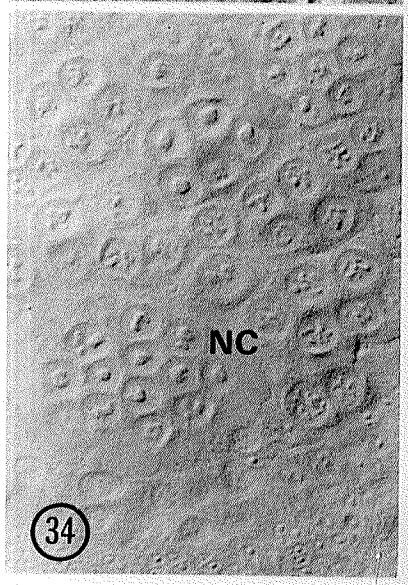
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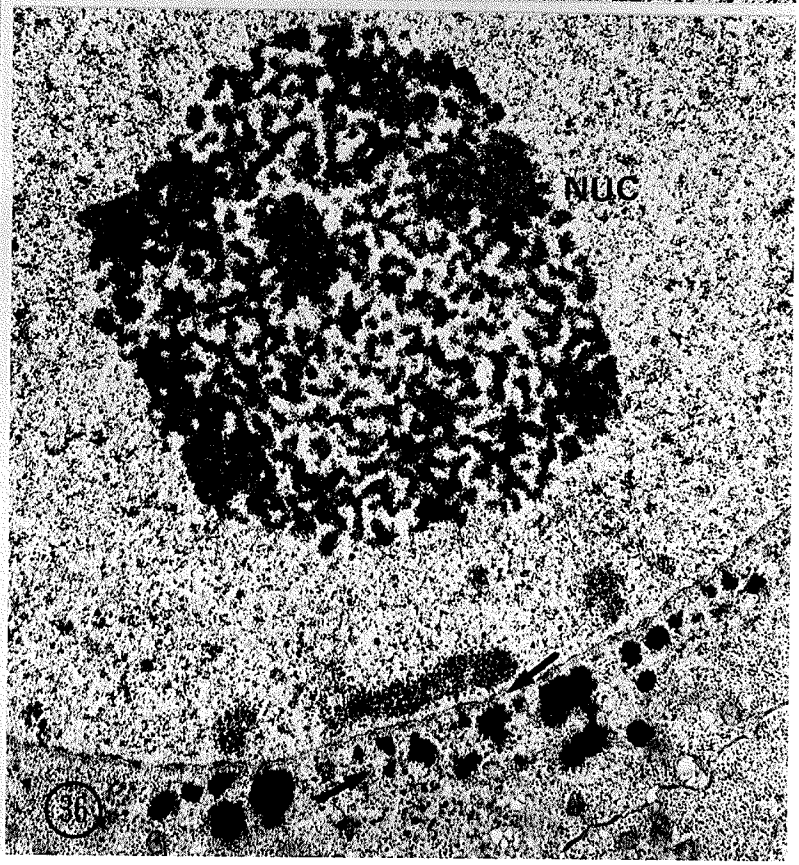
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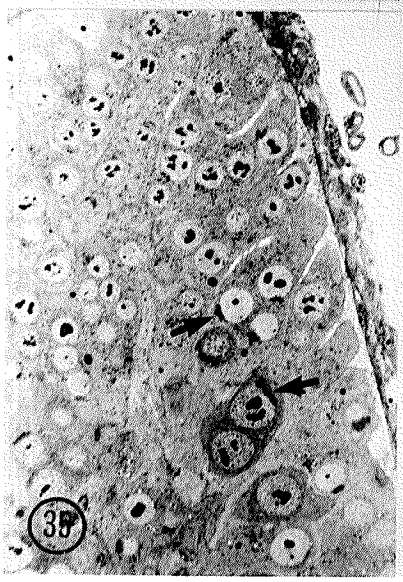
35

Plate 7

Nurse cells

Nucleo - cytoplasmic transport

Figure 37. The electron micrograph of a functioning nurse cell nucleus shows nucleo - cytoplasmic transport of material through the nuclear pores. X13150.

Figure 38. The early germ cell nuclear envelope has a relatively low pore density as illustrated here. X28400.

Figure 39. This micrograph shows the increased pore density of a differentiated nurse cell. X11250.

Figure 40 and 41. These micrographs show the association of mitochondria with nuclear extrusions. Note the change in density of the extrusion when it enters the cytoplasm. X16150, X34000.


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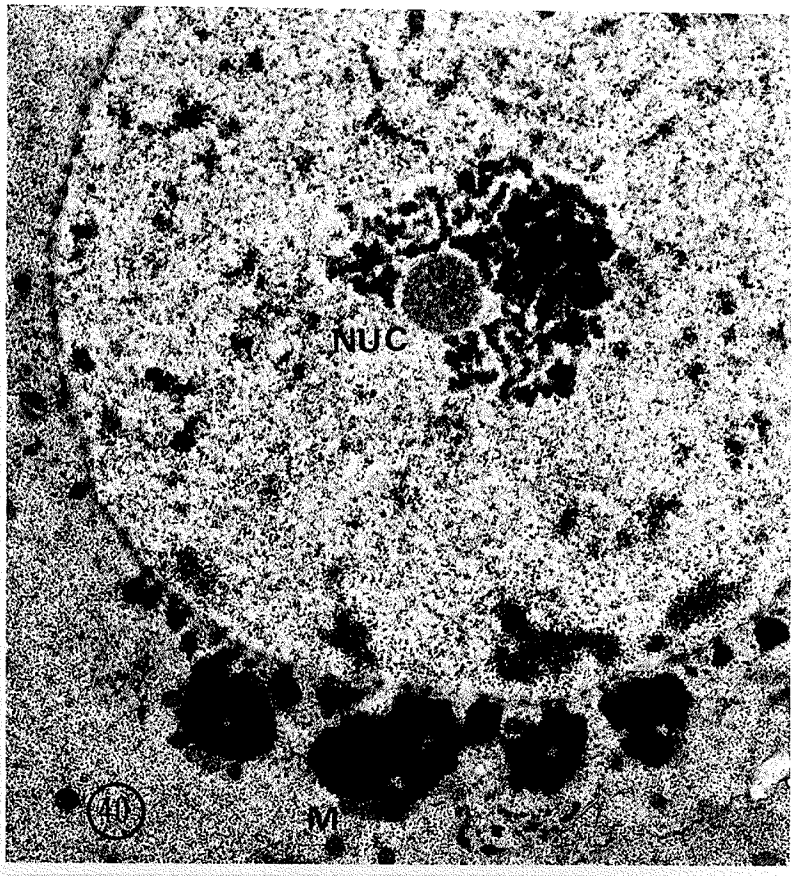
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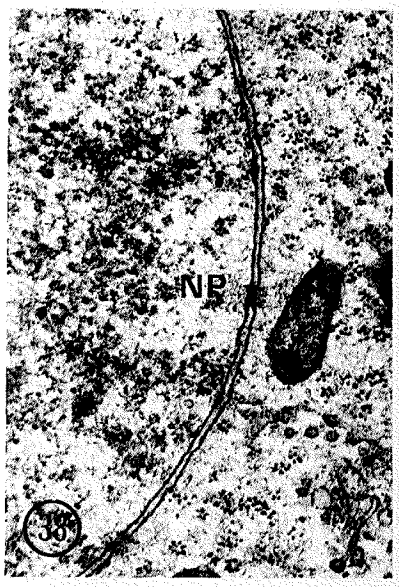
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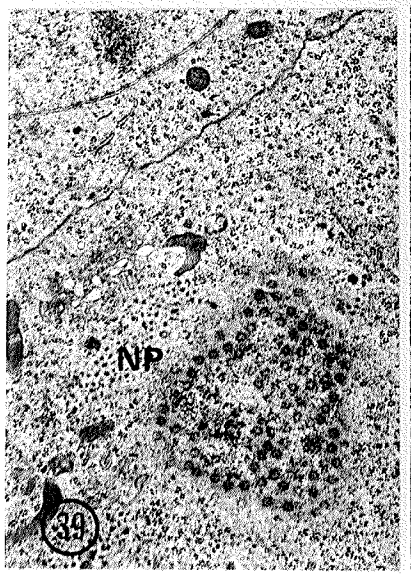
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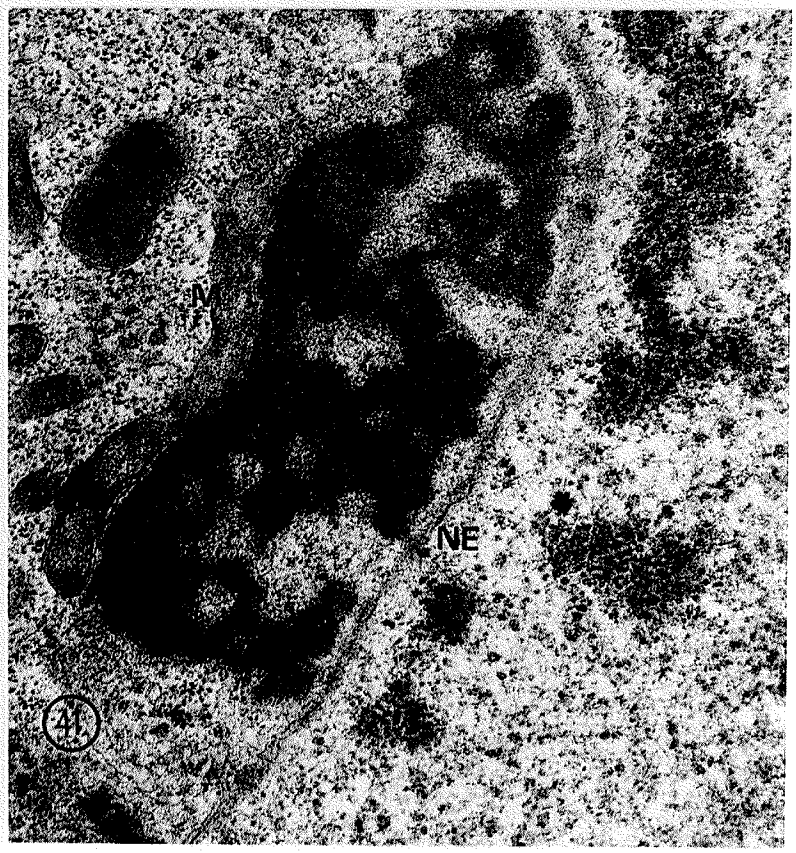
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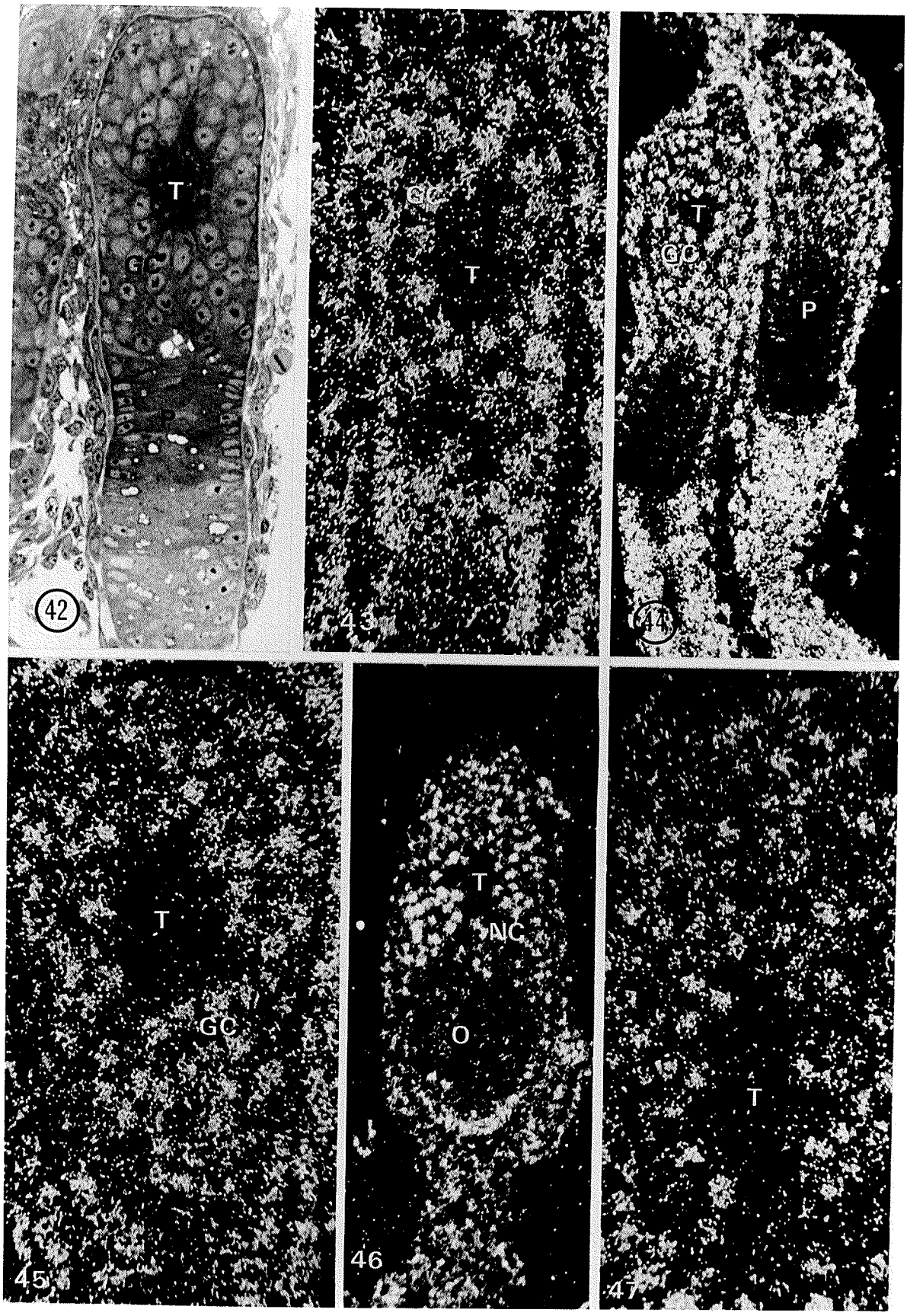
Plate 8

Nurse cell nuclear activity

Figure 42. This is a micrograph of a toluidine blue stained section of an unfed ovariole placed here for orientation purposes. X1730.

Figure 43 to 45. Dark-field autoradiographs of H^3 - uridine labelled early germ cells. X2300, X1000, X1890.

Figure 46 and 47. H^3 - uridine incorporation occurs in differentiated nurse cells shown here. Note that core does not incorporate label during this or earlier stages. X990, X1710.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45
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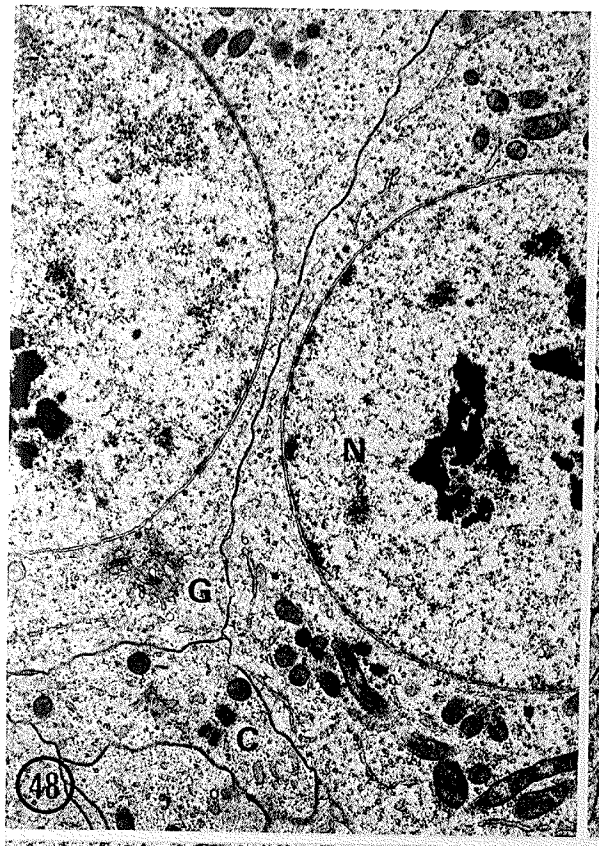
Plate 9

Nurse Cells - Cytoplasmic features

Figure 48 to 50. These micrographs reveal the cytoplasmic organization and general constituents of differentiated nurse cells. X3000, X11540, X7690.

Figure 51. This micrograph illustrates a type of endoplasmic reticulum organization occasionally seen in nurse cell cytoplasm. Membrane vesicles are interspersed between two lamellae of RER. X31180.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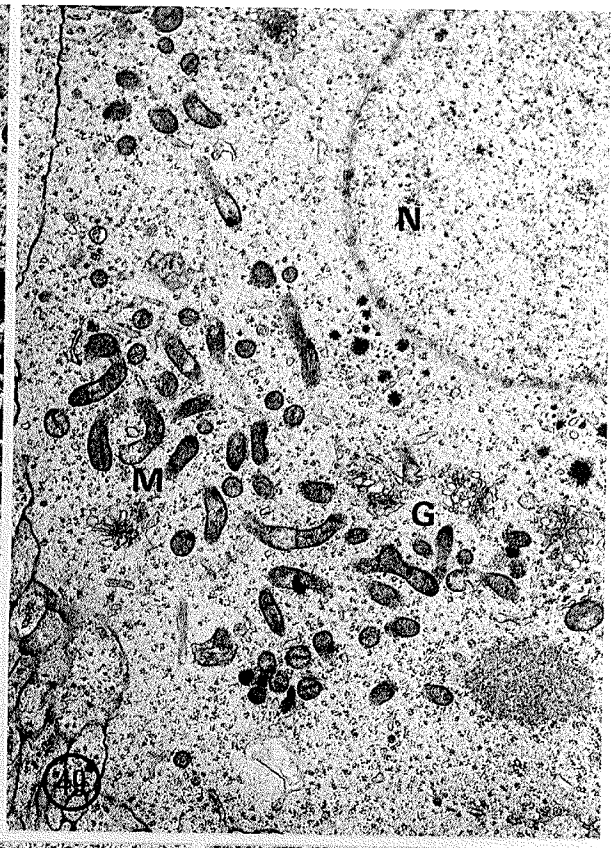
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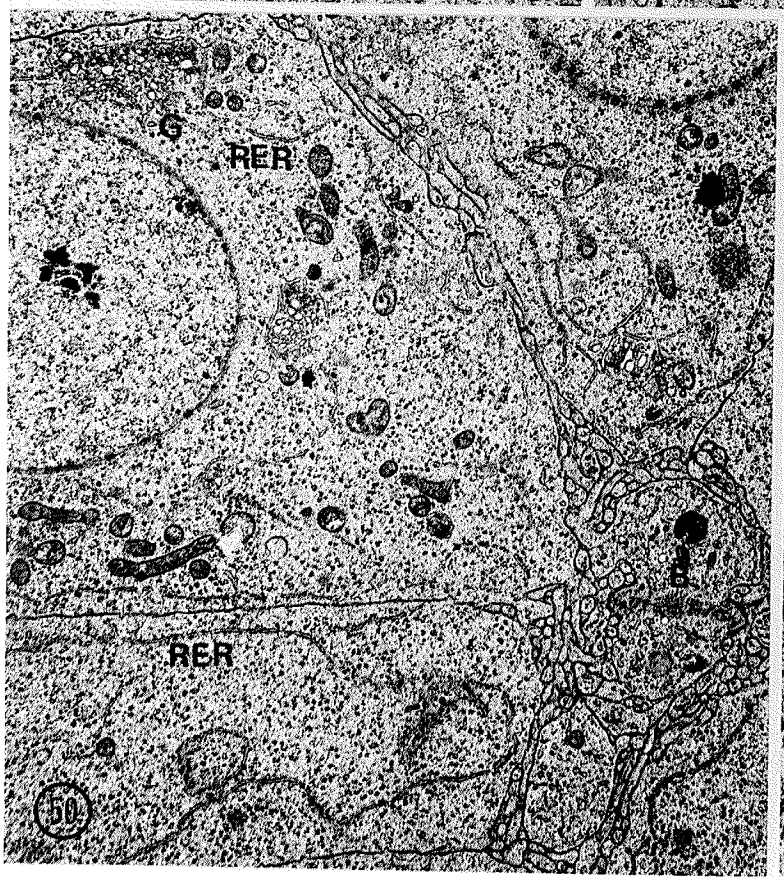
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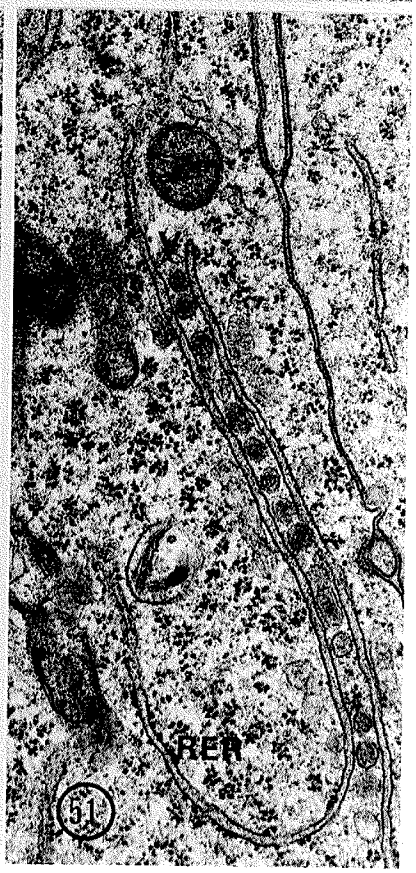

RER
51

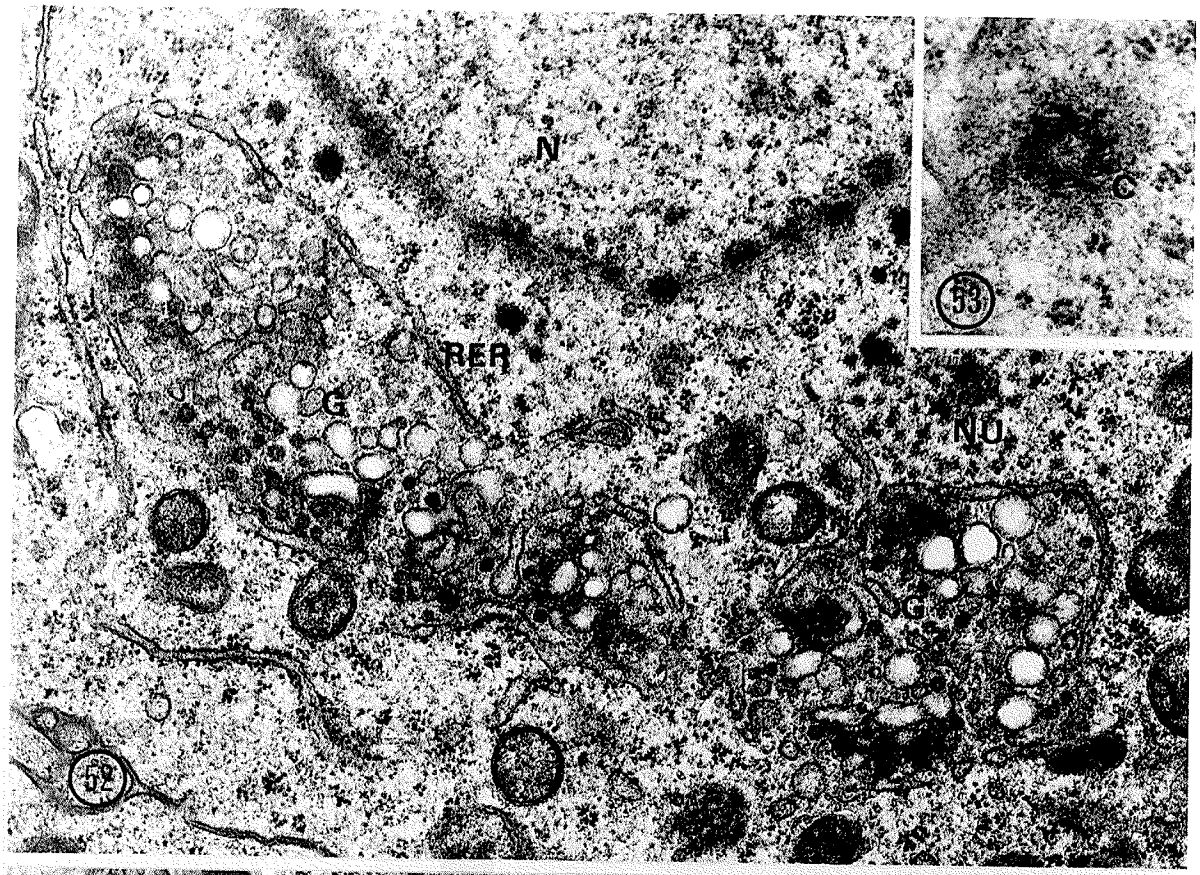
Plate 10

Nurse Cells - Cytoplasmic features

Figure 52. The association of RER with Golgi complexes often found in nurse cell cytoplasm is shown in this micrograph. X32990.

Figure 53. This micrograph shows the fuzzy coat surrounding the nurse cell centriole. X43310.

Figure 54 to 56. Restructuring of nurse cell cytoplasm during normal differentiation results in the formation of membrane bound segregation bodies, several of which are illustrated here. Note the variety of organelles found within these bodies. X23950, X32300, X5130.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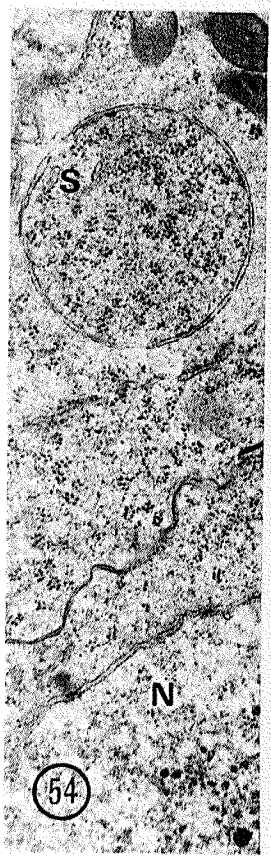
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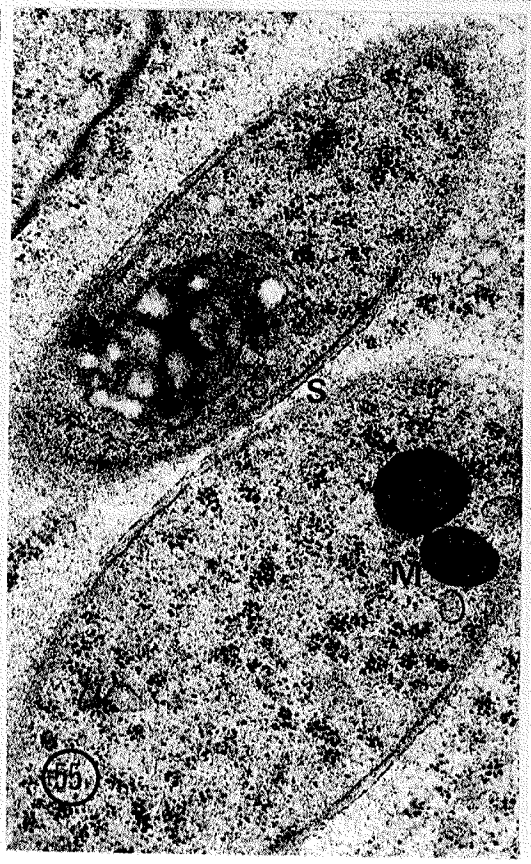
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Plate 11

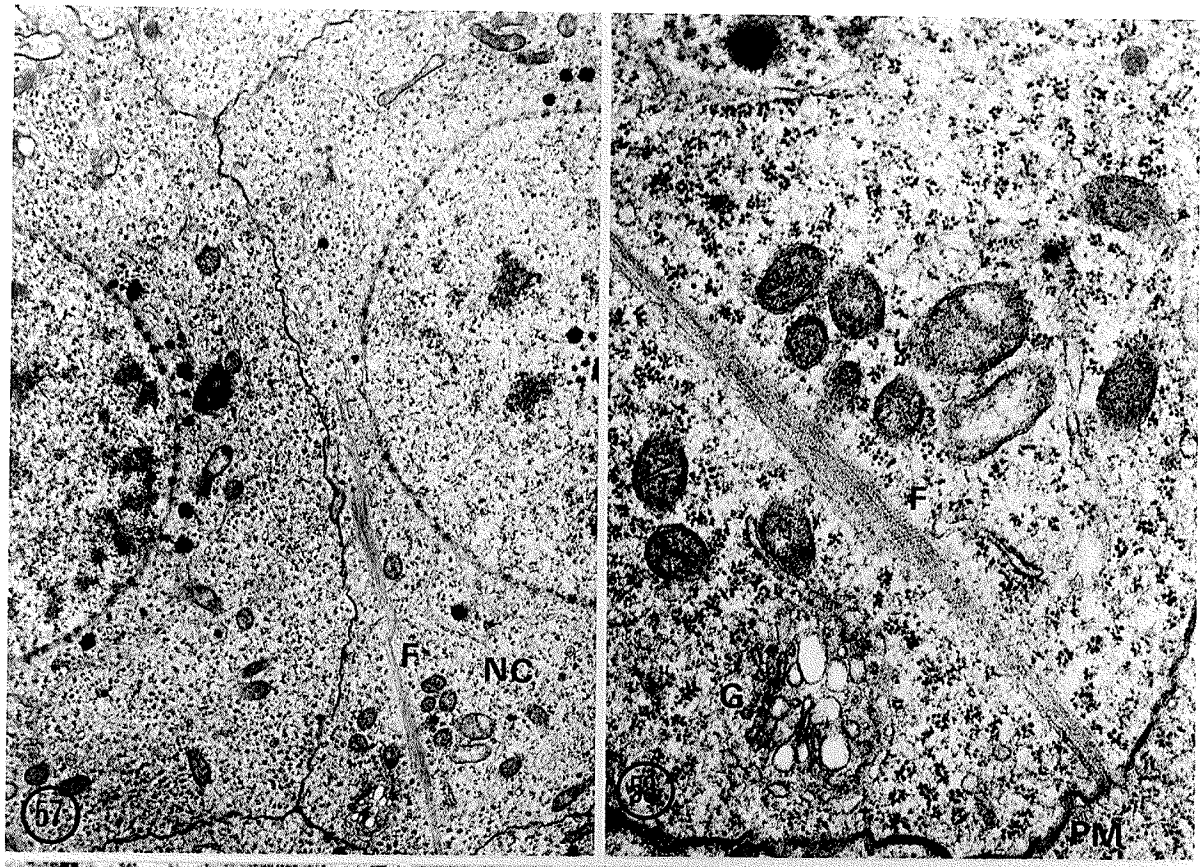
Nurse cell cytoplasm - fibrous bodies

Figure 57. This low power electron micrograph shows a fibrous element within the cytoplasm of a nurse cell. X10410.

Figure 58. This micrograph illustrates the plasma membrane association of a fibrous element. X25660.

Figure 59. Illustrated in this micrograph is the association of the fibrous element with a cytoplasmic membrane component. X17190.

Figure 60 and 61. These micrographs provide a low and higher magnification image of the fibrous elements free within the nurse cell cytoplasm. X32600, X66810.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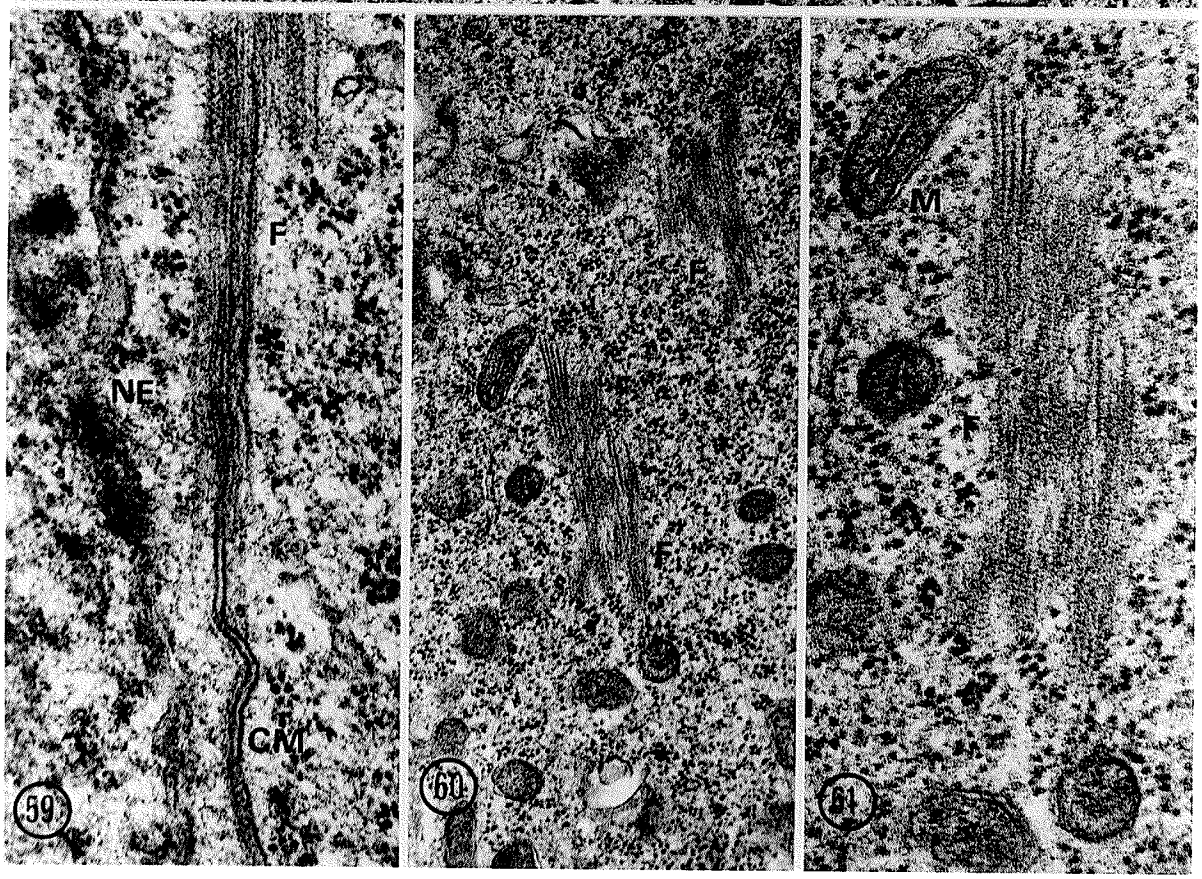
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Plate 12

Oocytes - early detection

Figure 62. This light micrograph shows the early developing oocyte region. Note nuclear features of early oocytes compared with more apical nurse cells. X1550.

Figure 63. H^3 - uridine incorporation at the beginning of the early differentiation phase is substantially less in oocytes than in the more apical nurse cells as shown in this dark-field autoradiograph. X550.

Figure 64 and 65. These electron micrographs illustrate the changes in nuclear features of the oocytes at the start of the early differentiation phase. Note loss of characteristic germ cell nucleolus, which is replaced by many small micronucleoli. X3920, X11750.

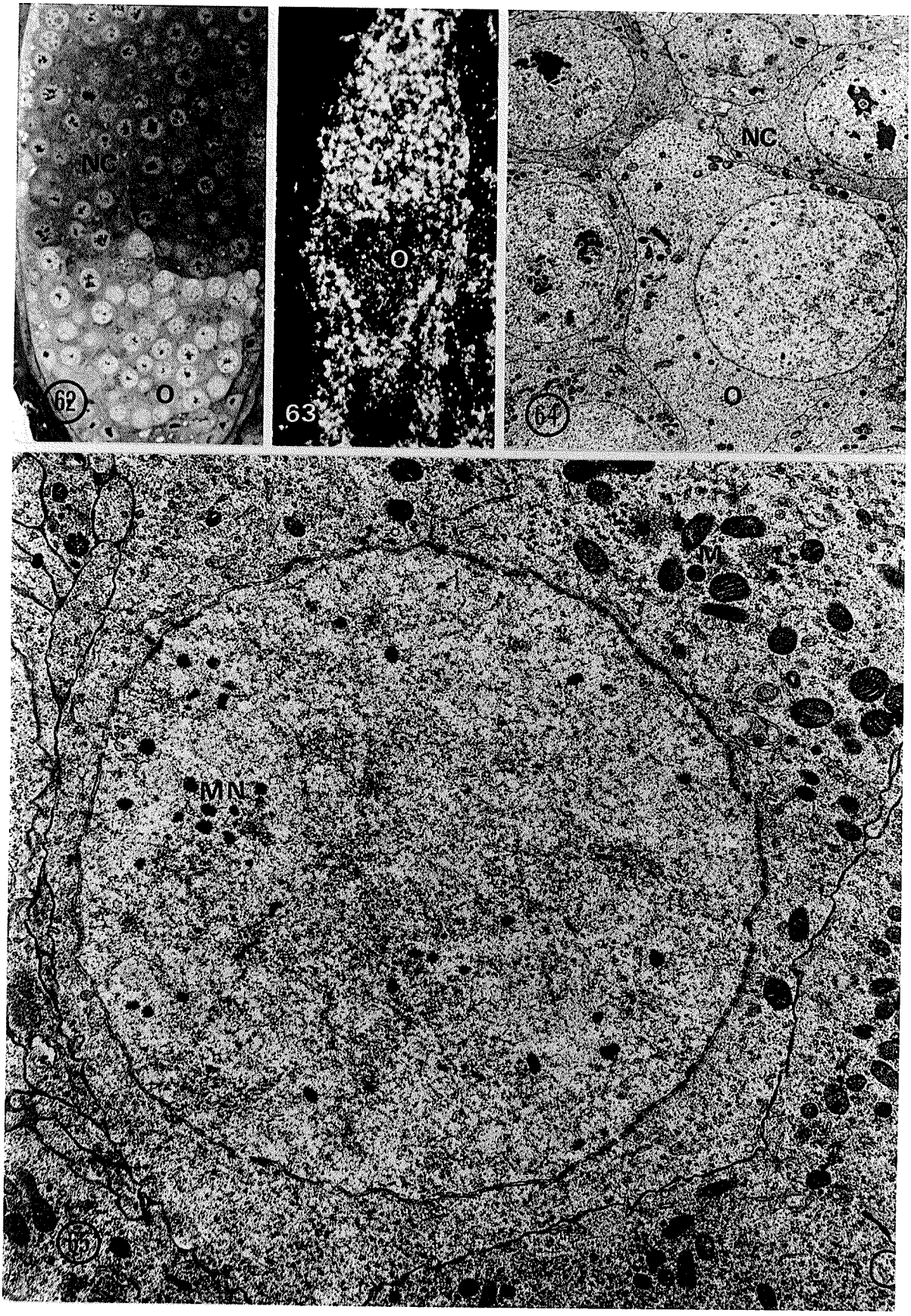
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Plate 13

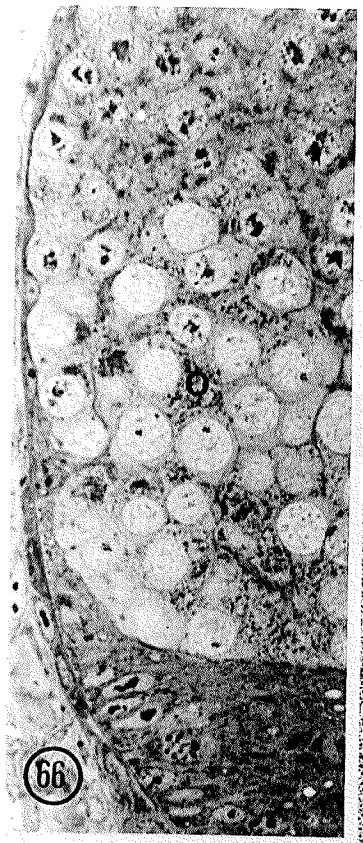
Oocytes - further nuclear differentiation

Figure 66. Shown in this light micrograph is the appearance of the oocyte nucleus as it enters the first meiotic prophase. Note condensing chromosomes in the nucleus at this time. X2630.

Figure 67. Further morphological nuclear differentiation is accompanied by further functional differentiation as indicated by this dark-field autoradiograph. At this stage, oocytes incorporate a negligible quantity of H^3 - uridine. X740.

Figure 68. EM examination of the oocyte nucleus at this stage reveals the condensing chromatin of the chromosomes and the appearance of synaptonemal complexes. Note the double synaptonemal complex in this oocyte nucleus. X19490.

Figure 69. This micrograph shows another synaptonemal complex which has the typical sub-structure of a central element surrounded by two lateral elements. X27390.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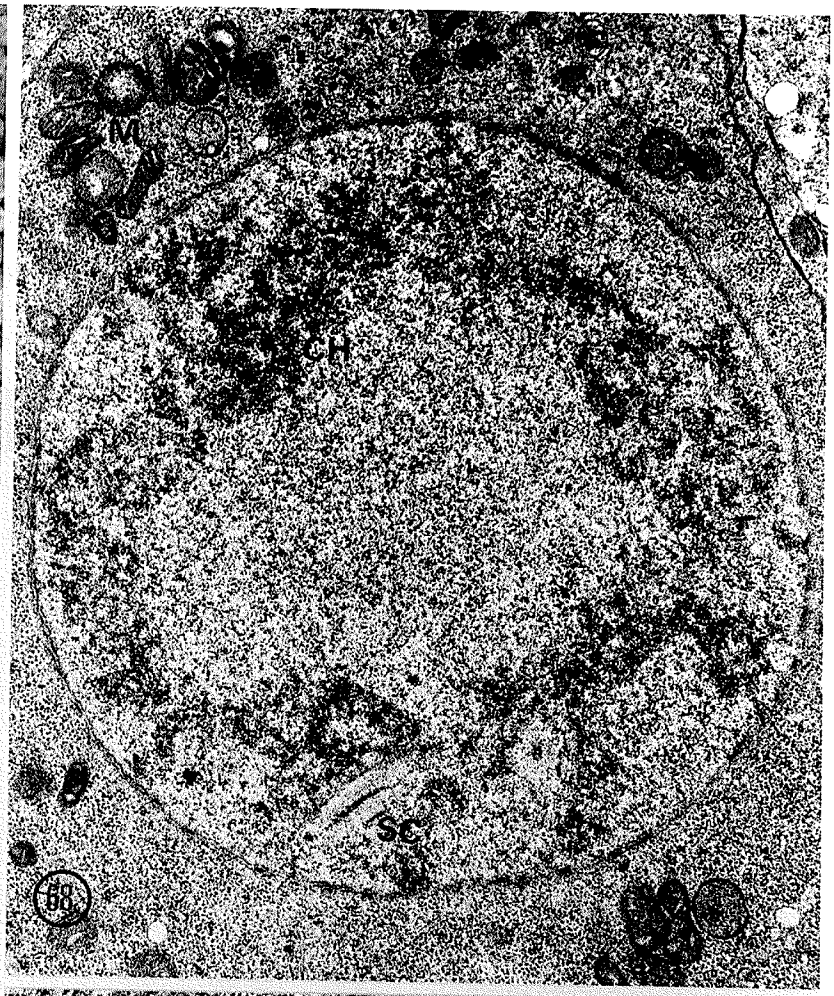
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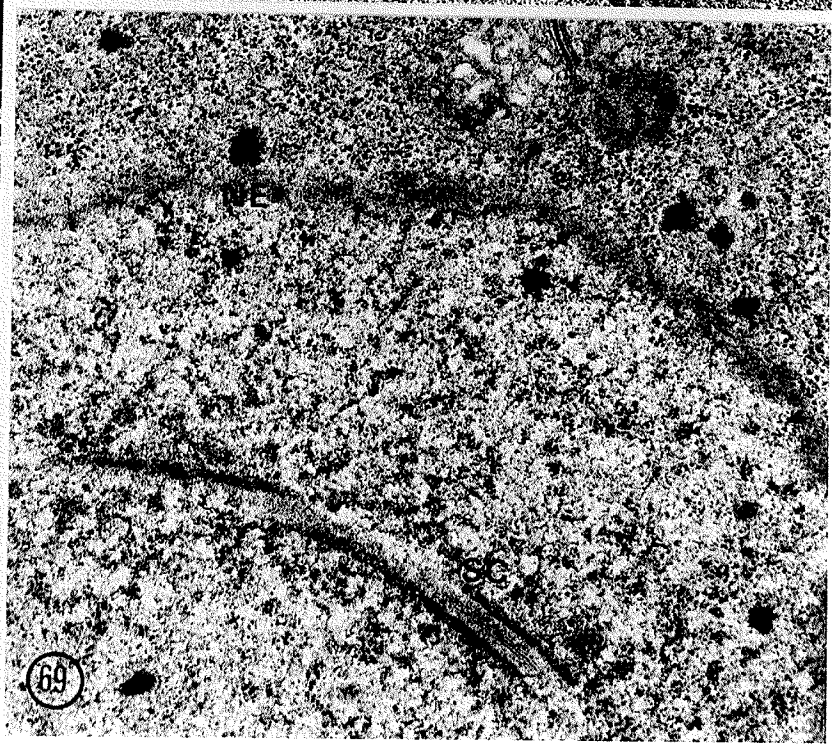
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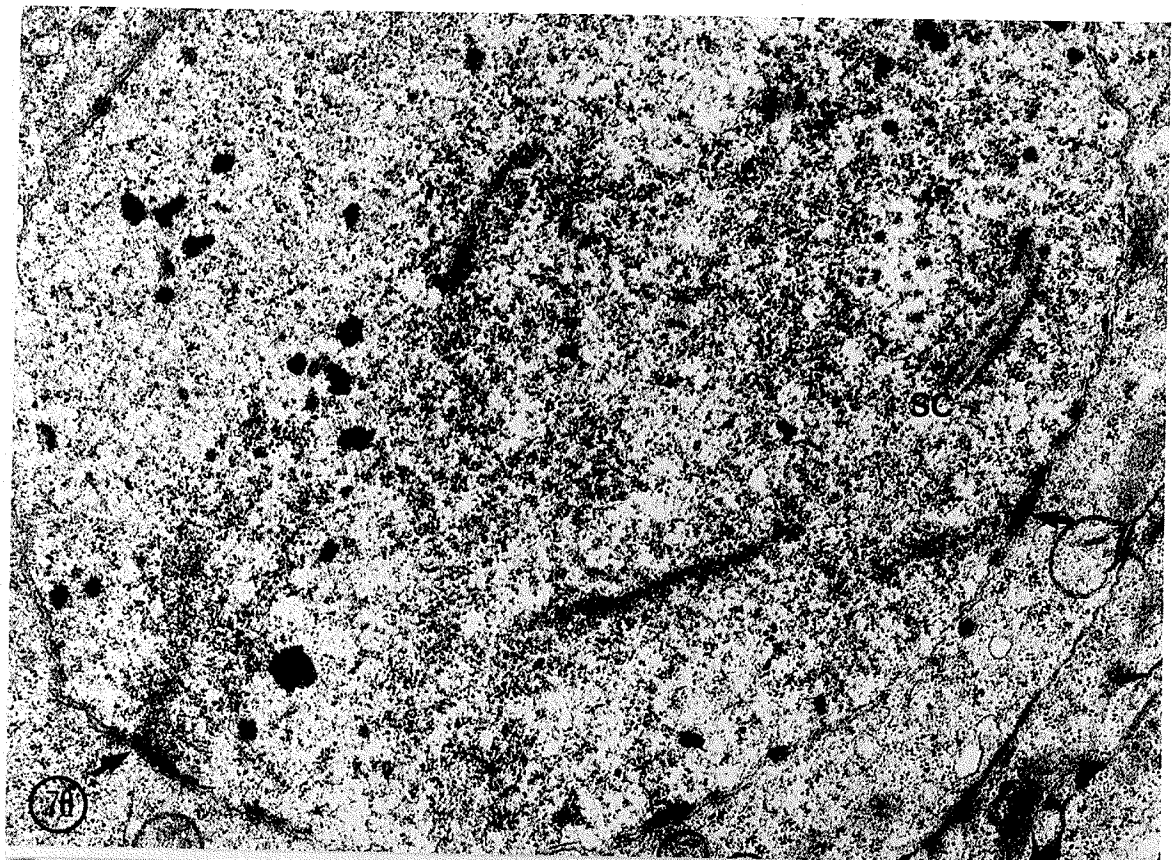
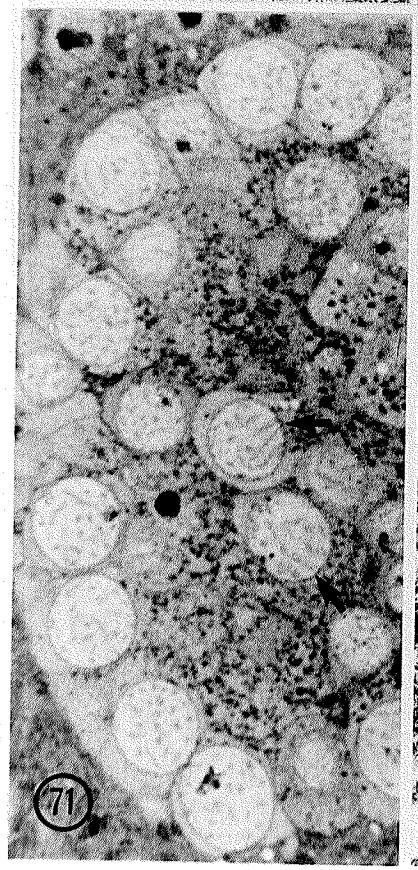
Plate 14

Oocyte - synaptonemal complexes

Figure 70 to 72. As suggested by light microscope examination, and confirmed with the electron microscope, synaptonemal complexes attach to the nuclear envelope. Several attachment sites are observed in these figures. X21770, X4140, X34030.


SC
70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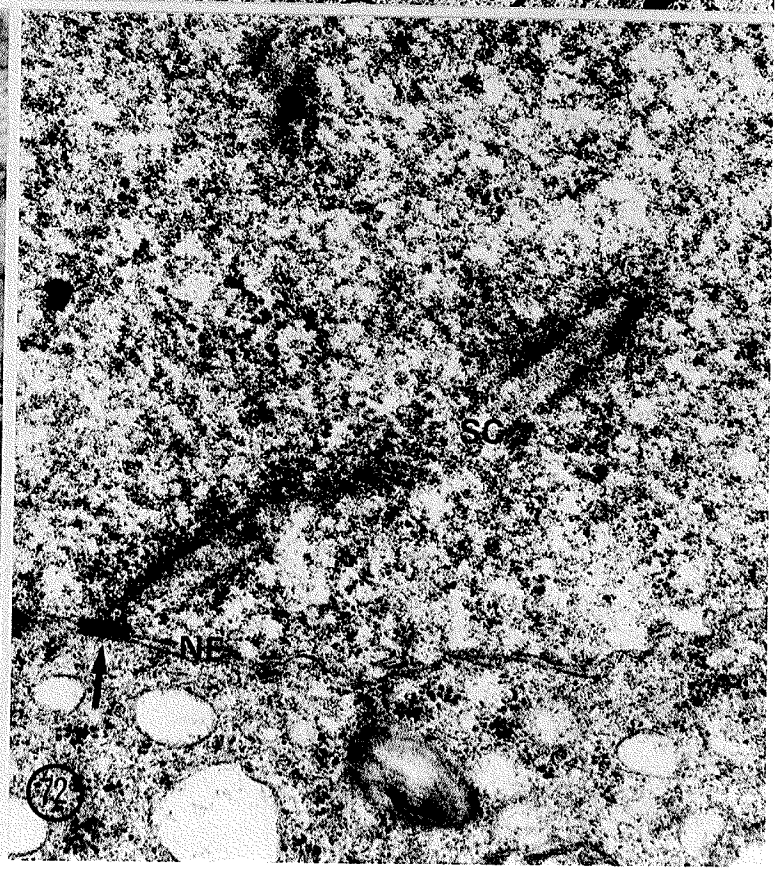
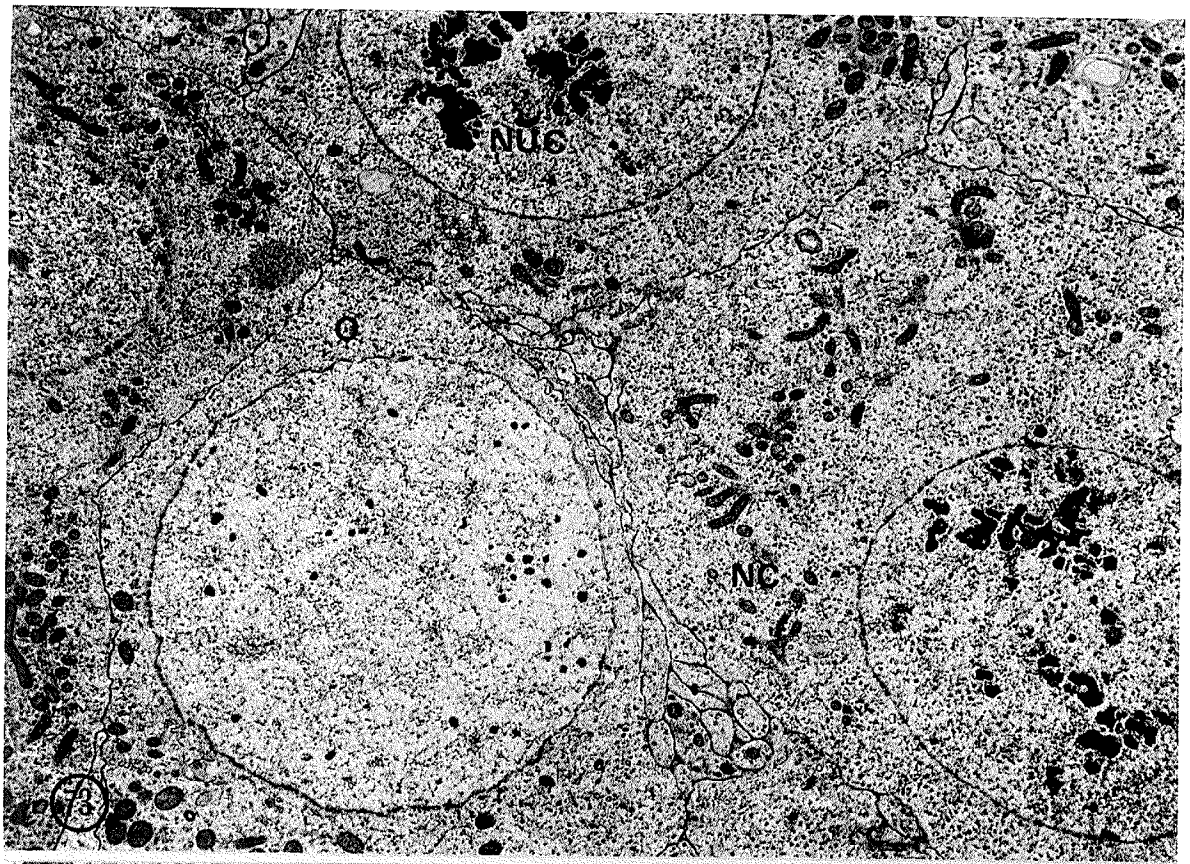

SC
NE
72

Plate 15

Oocyte - cytoplasmic features

Figure 73. This micrograph illustrates the difference between nurse cell cytoplasm and oocyte cytoplasm. X6790.

Figure 74 and 75. These micrographs reveal the characteristic features of oocyte cytoplasm such as anastomosing RER, clustering of mitochondria and other organelles and few free ribosomes. Nuage material, in nurse cells restricted to peri-nuclear position is found throughout oocyte cytoplasm. X15910, X15580.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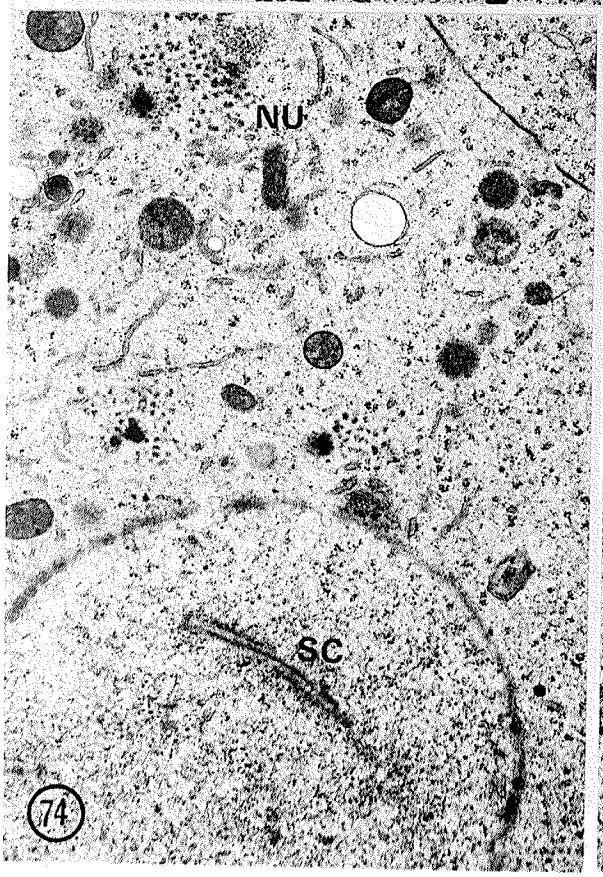
G
NC
73


NU
SC
74

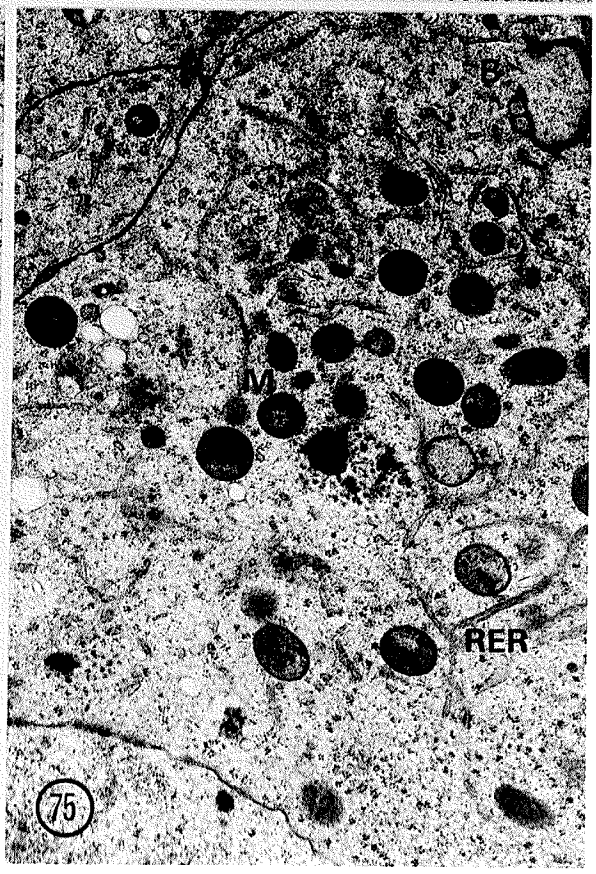

M
RER
75

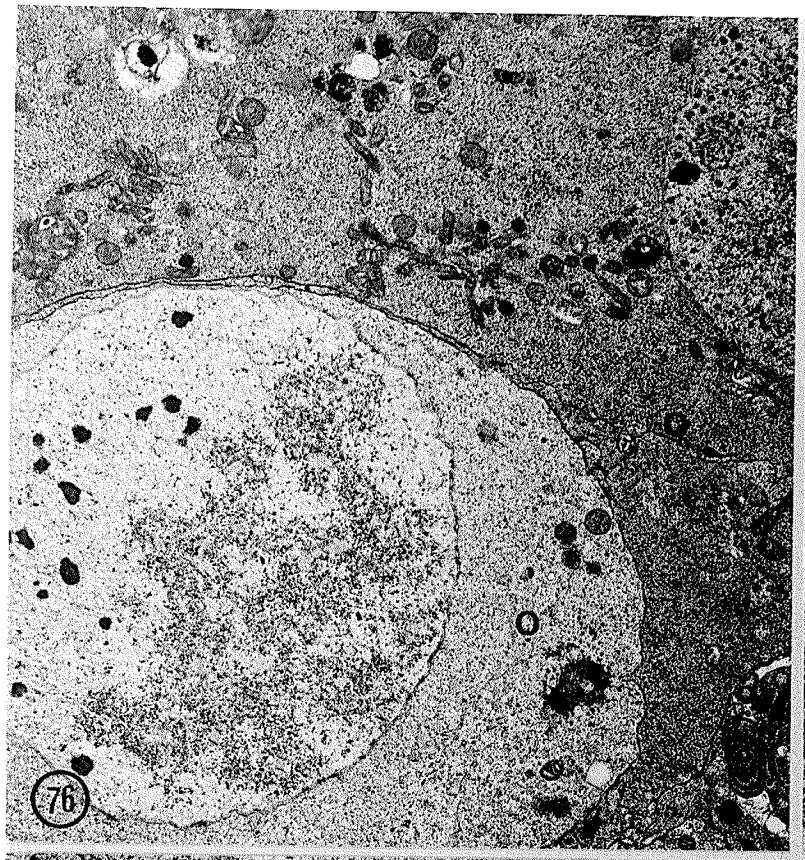
Plate 16

Oocyte cytoplasm

Figure 76. This micrograph illustrates the cytoplasmic density differences which exist between oocytes depending on their size and stage of development. X2160.

Figure 77. Oocyte centrioles do not have the fuzzy coat which is present on nurse cell centrioles. X40700.

Figure 78 and 79. Golgi complexes and Golgi vesicles are abundant in early and late differentiation stages, sometimes filling large areas of cytoplasm. X11600, X15820.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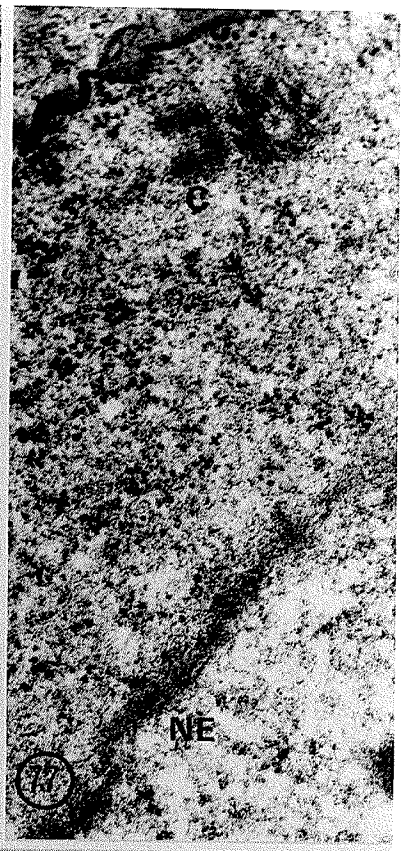
76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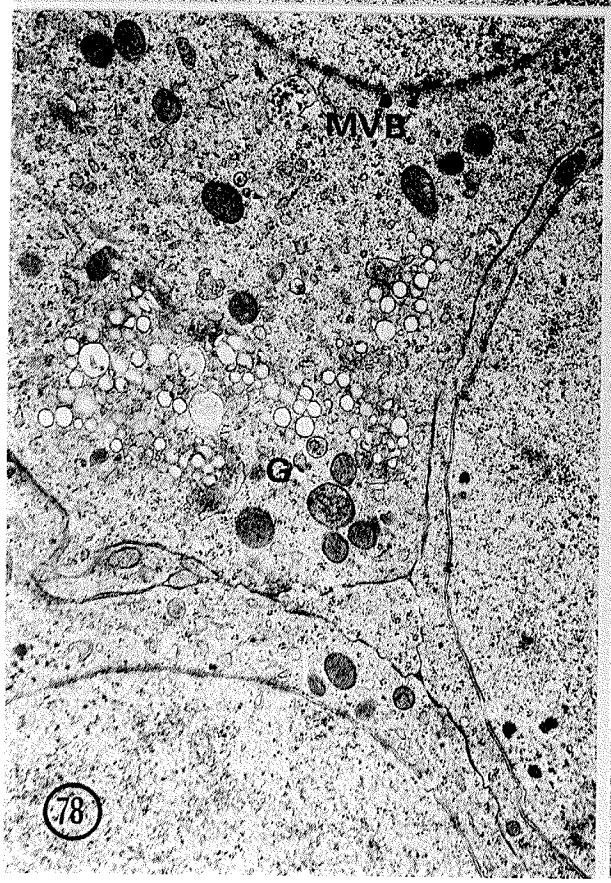
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G
78

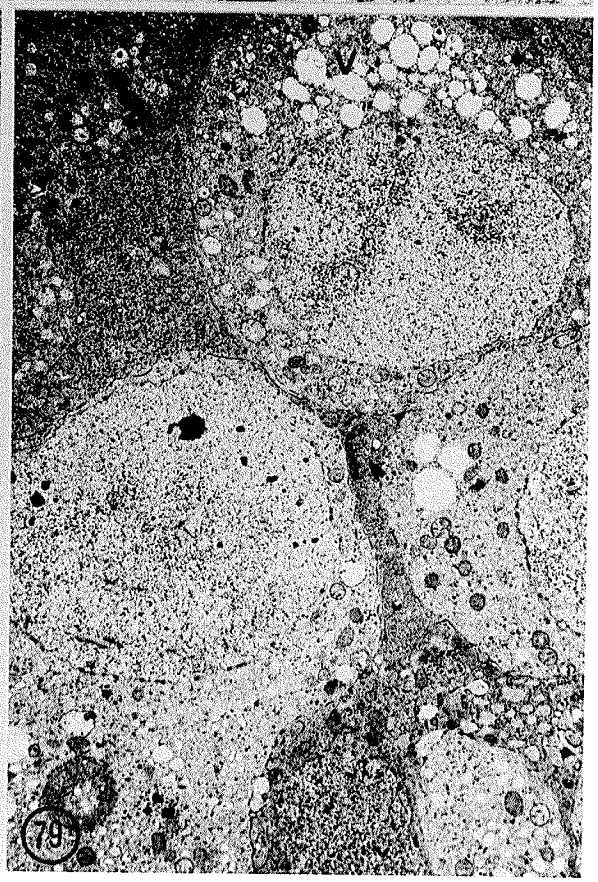

V
79

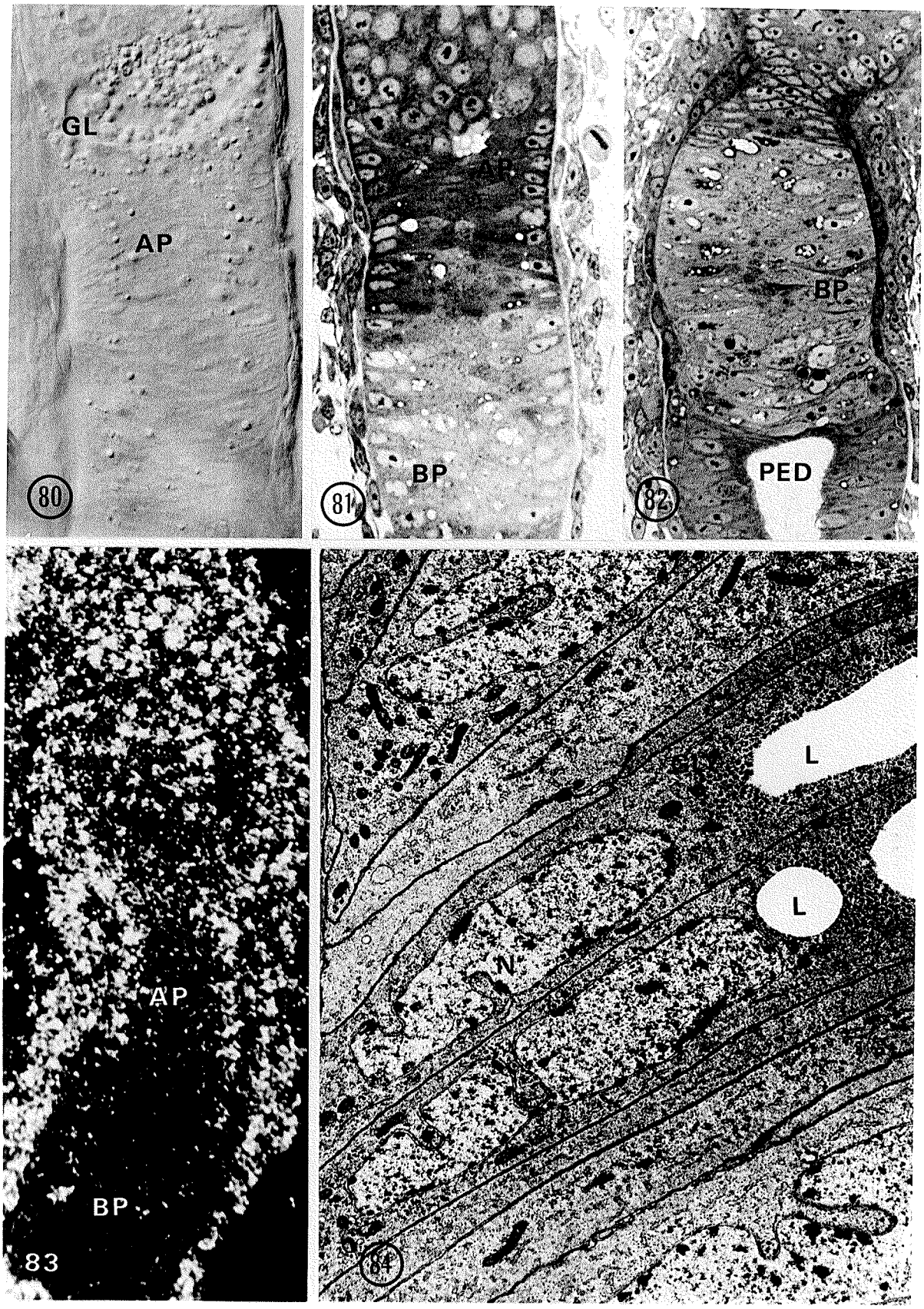
Plate 17

Prefollicular Cells - Proliferative phase

Figure 80, 81 and 82. Prefollicular region of the 5th instar ovariole can be divided into morphologically distinct apical (80, 81) and basal regions (81, 82). Apical area stains more intensely than the basal area. X1850, X2300, X1730.

Figure 83. This dark-field autoradiograph illustrates that although H^3 - uridine is incorporated into apical region nuclei, basal nuclei do not incorporate H^3 - uridine. X1850.

Figure 84. Ultrastructurally, prefollicular cells are characterized by an oval, often lobed nucleus and a prominent lipid droplet with associated glycogen. X13520.


GL
AP
80
AP
BP
81
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PED
82
AP
BP
83
L
L
N
84

Plate 18

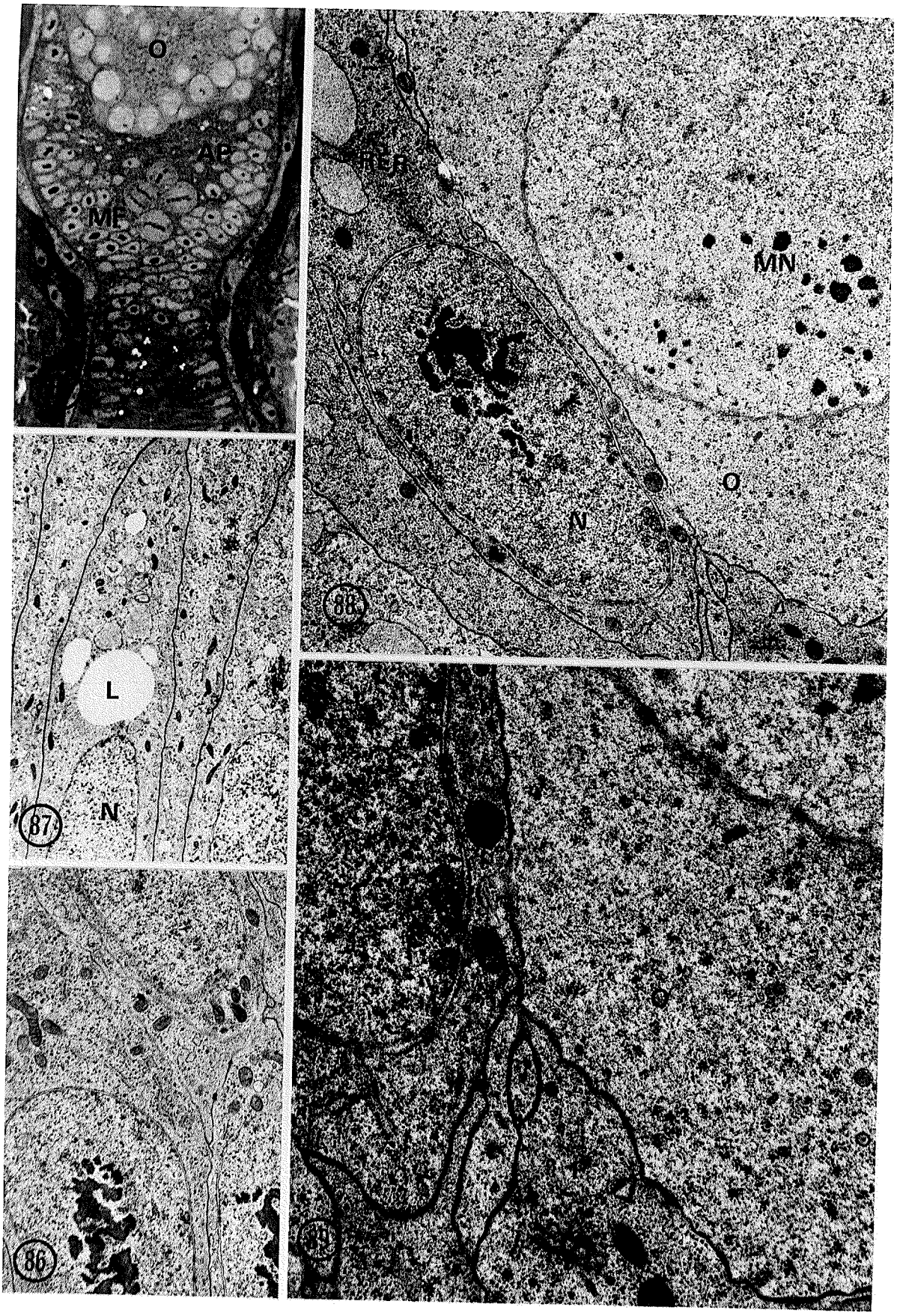
Prefollicular Cells

Early Differentiation Phase

Figure 85. Active mitosis in the apical prefollicular region results in growth of this area during the instar. X2180.

Figure 86, 88, 89. These micrographs illustrate the features of the differentiated apical prefollicular cells. Distended vesicles of RER have replaced the lipid droplet and glycogen as the most prominent cytoplasmic feature of the cell. As well, apical cells have lost their larval shape in preparation for their association with the oocytes(O). X8690, X12440, X25650.

Figure 87. Basal cells retain their larval cell features at this stage. X4610.


O
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MF
RER
MN
O
N
88
L
N
87
86
O

Plate 19

Prefollicular Cells

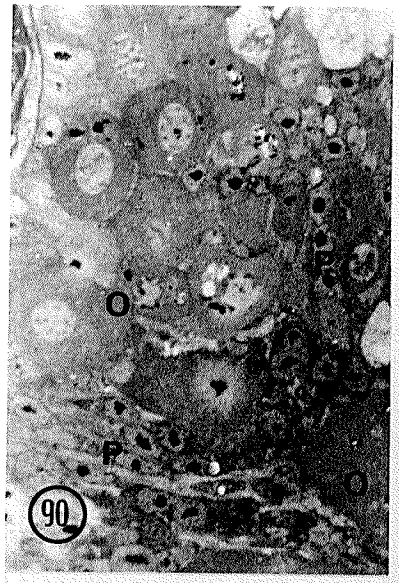
Late Differentiation Phase

Figure 90. This light micrograph illustrates the reciprocal associations between oocytes and prefollicular cells. X2230.

Figure 91. Inner sheath cells at the periphery of the tropharium are involved in primary follicle formation within this region. X12770.

Figure 92. Prefollicular cells also associate with the trophic cords near the oocyte. X6150.

Figure 93. The electron micrograph shows the complexity of the association of prefollicular cells with oocytes as the oocytes enlarge. X10200.



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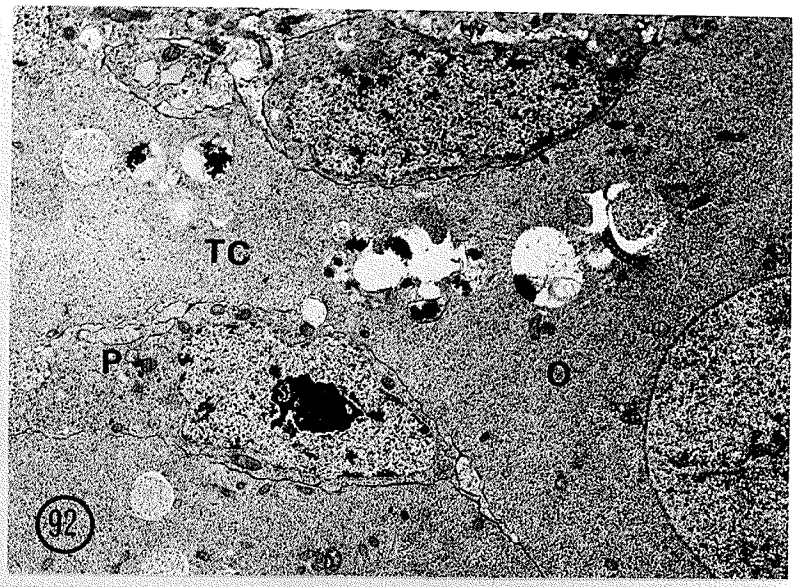

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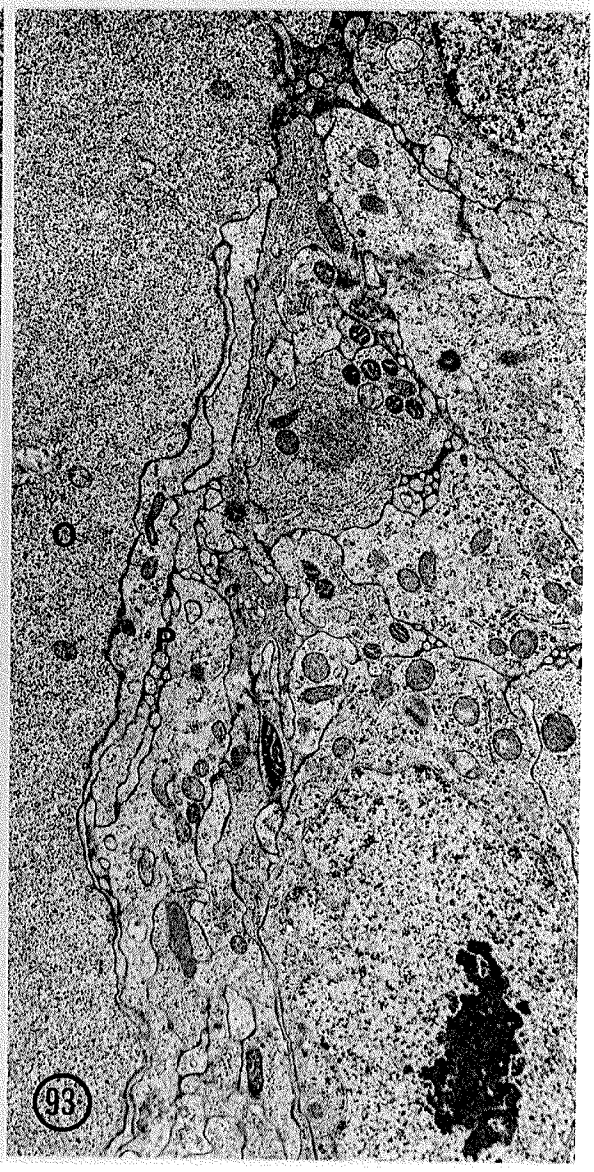
91



93

Plate 20

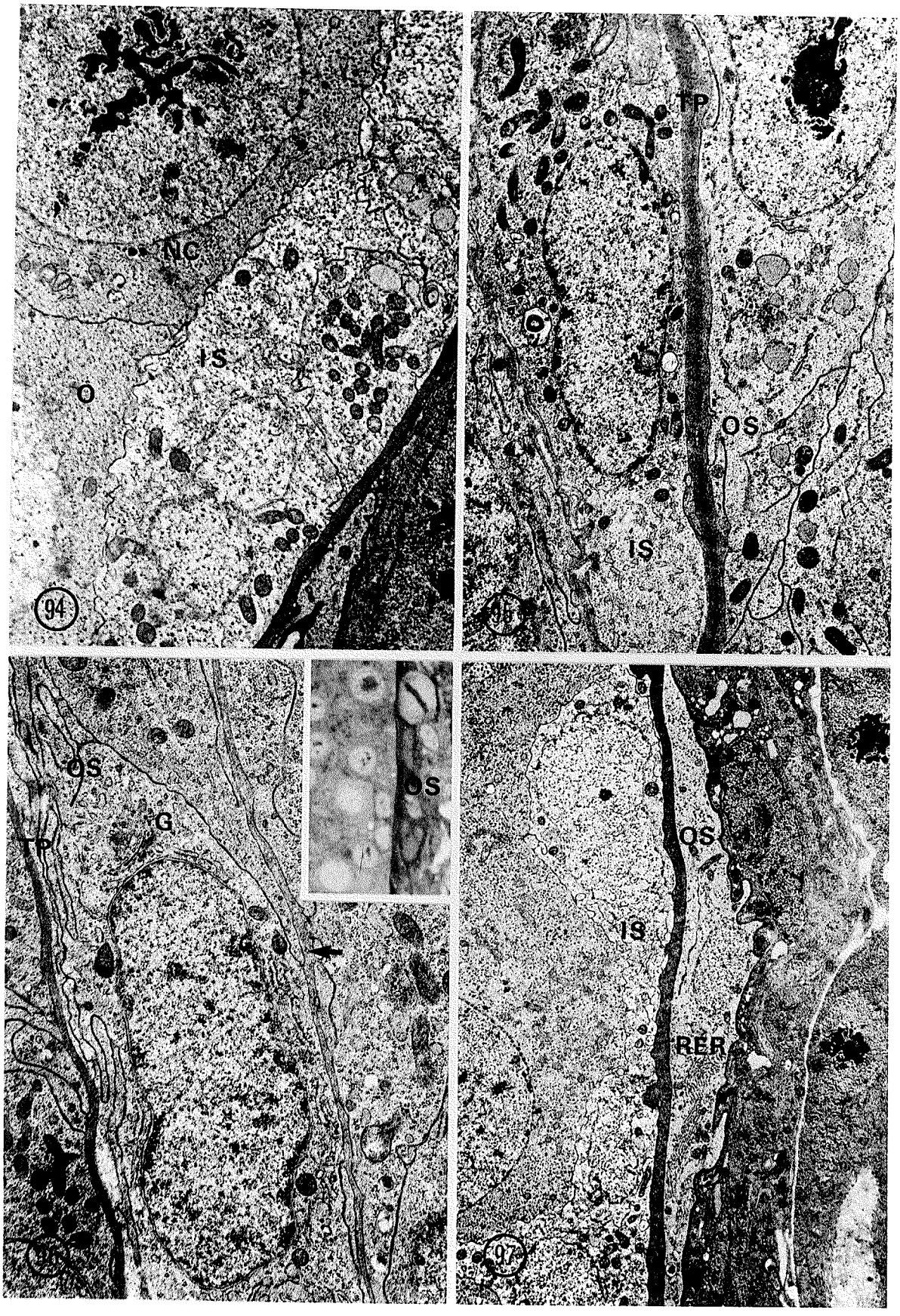
Ovarian Sheaths

Figure 94. Inner sheath cells in this electron micrograph can be contrasted with nurse cells and oocytes. X 9690.

Figure 95. Both inner and outer sheath cell features can be seen in this micrograph. They are separated by the non-cellular tunica propria. X8820.

Figure 96. This micrograph shows the nuclear and cytoplasmic features of the outer sheath cell. It is separated from the rest of the sheath by a thin lamina (arrow). The inset shows an outer sheath cell in metaphase. X11920, X1606.

Figure 97. This micrograph illustrates another outer sheath cell commonly found characterized by stacks of RER within its cytoplasm. X4920.


NC
IS
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TP
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G
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OS
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RER
97

Plate 21

Nurse Cell - Nurse Cell Bridges

Figure 98 and 99. These micrographs show two daughter cells which have finished nuclear division but remain interconnected through spindle mid-body. Note lateral membrane densities in the lower and higher magnifications (arrows). X12000, X23360.

Figure 100 and 101. Spindle microtubules disappear and the intercellular bridge has the morphology illustrated in these micrographs. Dense material within the bridge is the mid-body remnant. X8460, X44220.

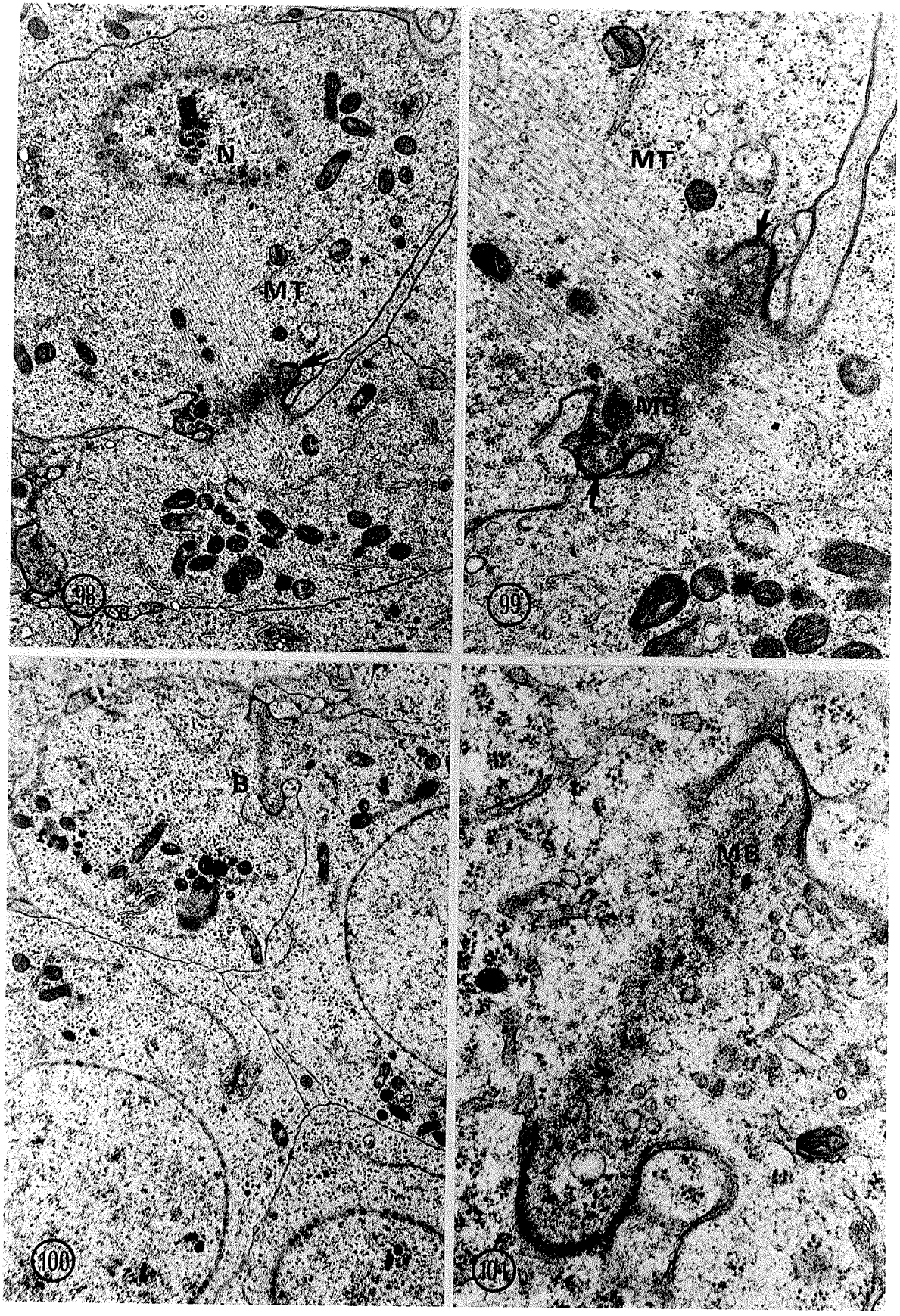

N
MT
98
MT
MB
99
B
100
MB
101

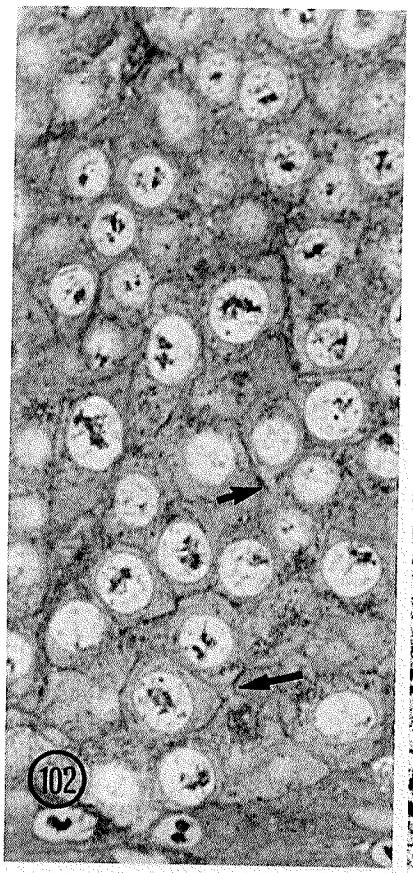
Plate 22

Nurse Cell - Nurse Cell Bridges

Figure 102. Intercellular bridges can be observed in light microscopy. X2630.

Figure 103 and 104. Various morphologies of intercellular bridges between nurse cells. Note membrane vesicles in the bridge region. X10570, X16920.

Figure 105 and 106. Two bridge system geometries are illustrated in these micrographs. Note in Figure 105 that a fusomal-like material fills the region of the bridges. X12260, X14120.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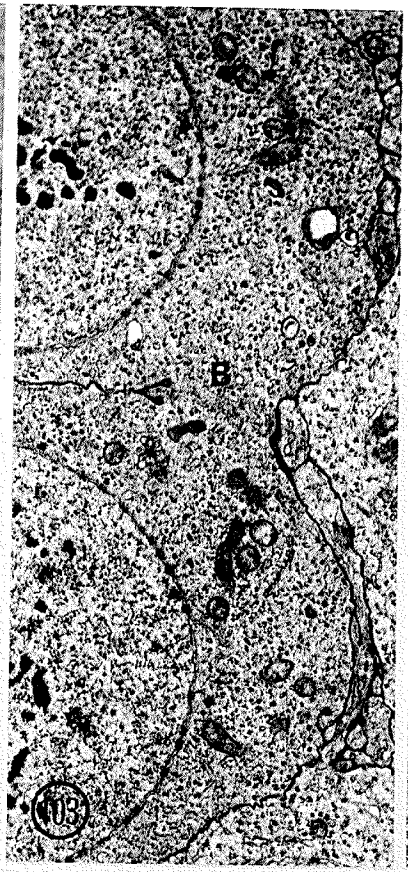
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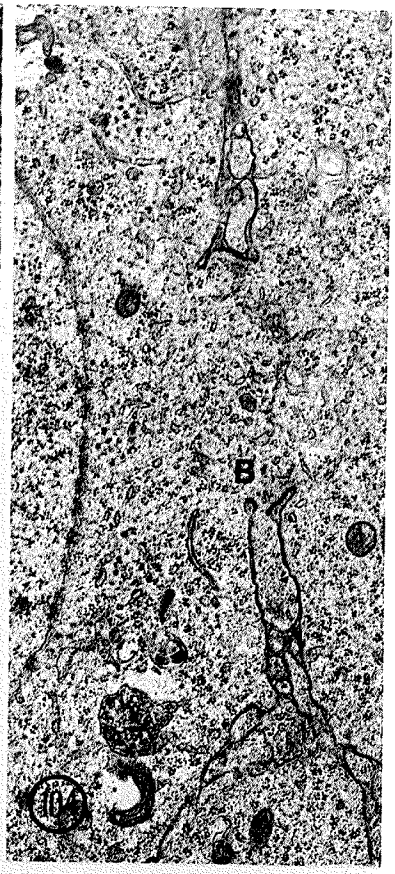
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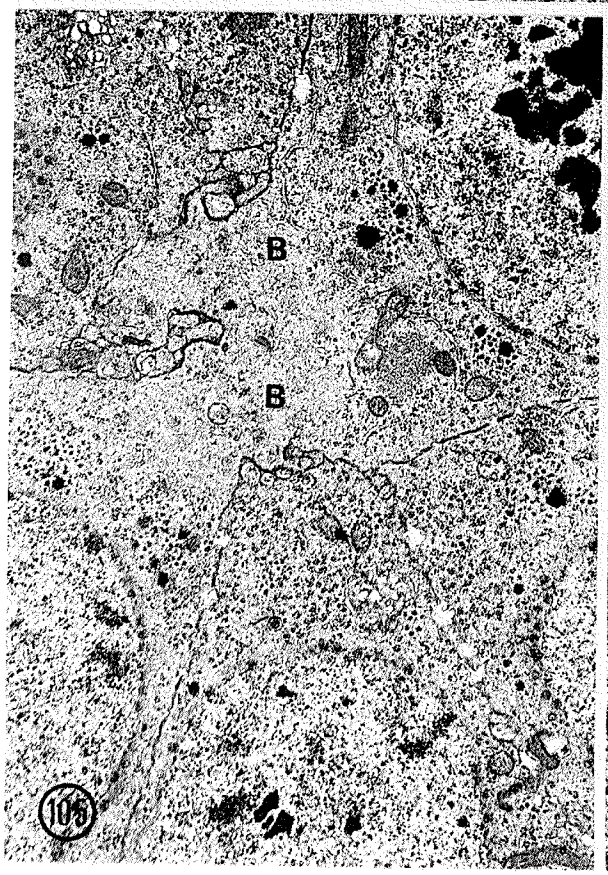
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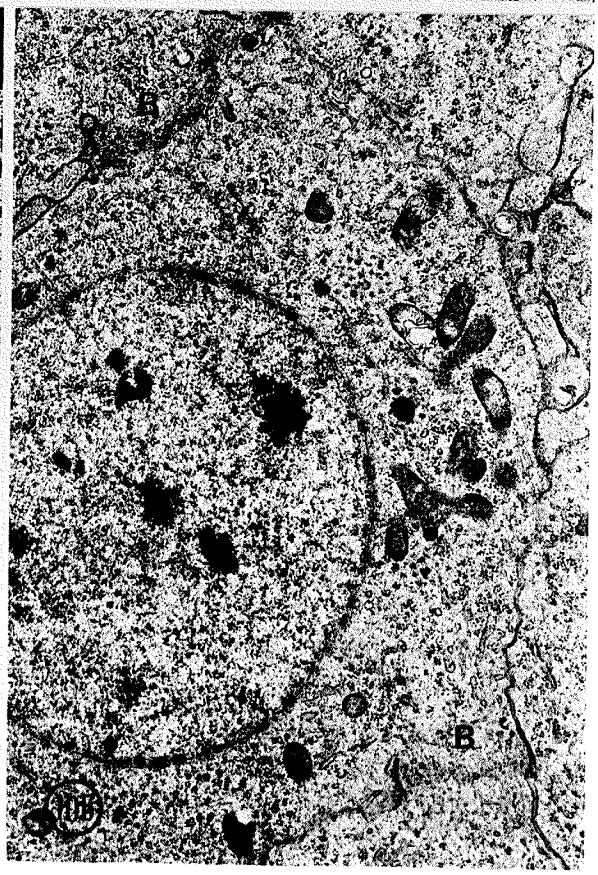
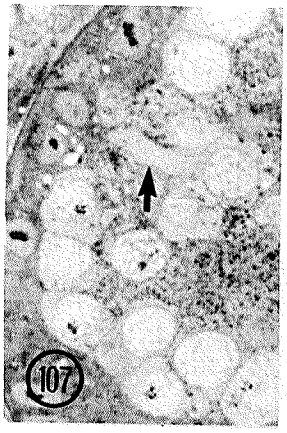

B
B
106

Plate 23

Oocyte - Oocyte Bridges

Figure 107 and 108. These light micrographs show oocyte cell processes which suggest oocytes also interconnected by bridge systems. X2770, X1990.

Figure 109 and 110. These electron micrographs show a system with six oocytes joined by a series of intercellular bridges and cell processes (⇦-evidence from adjacent sections indicates cytoplasmic confluency). Arrows outline the bridge system. X6800, X9080.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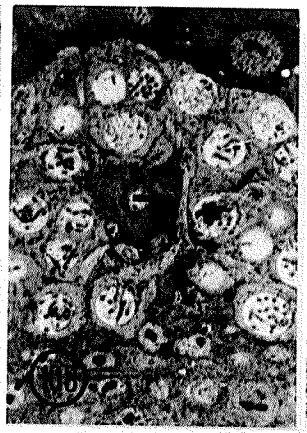
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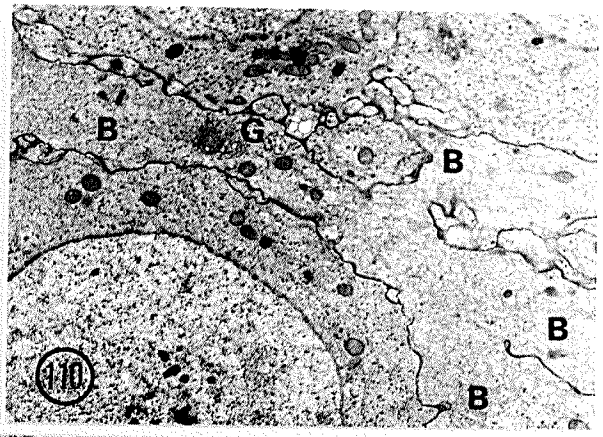
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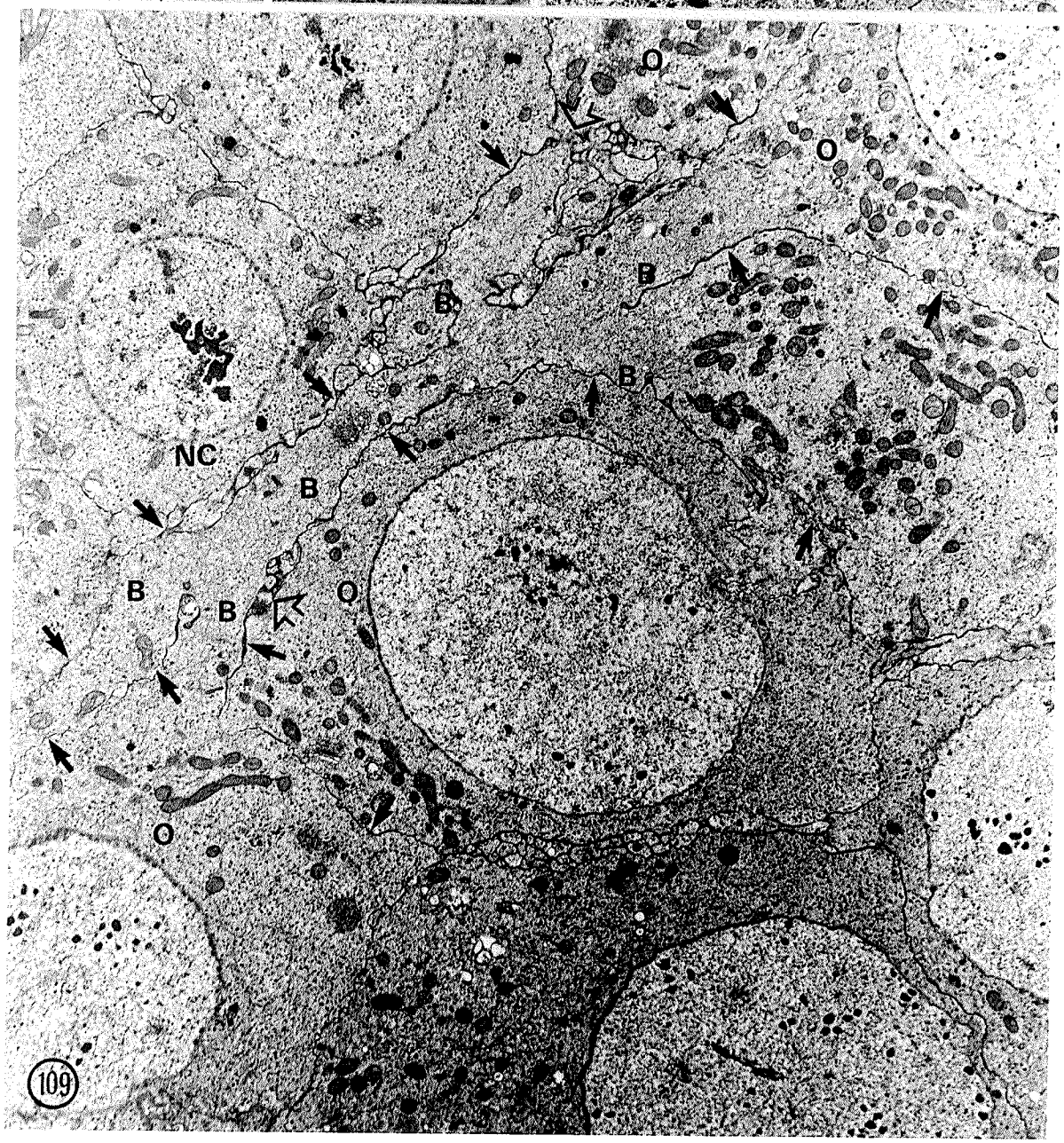
O
O
B
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B
NC
B
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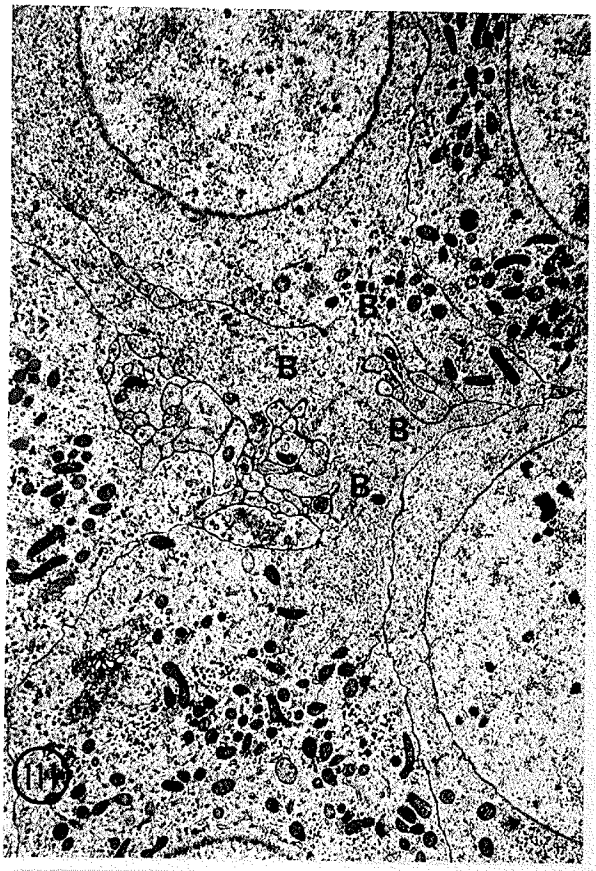
B
O
O
O
109

Plate 24

Oocyte - Oocyte Bridges

Figure 111 to 113. Two other oocyte-oocyte bridge systems, connecting two identifiable oocytes to a 3rd cell, are illustrated in these micrographs. Note Golgi complexes in the bridge system of Figure 113. X5730, X22750, X6830.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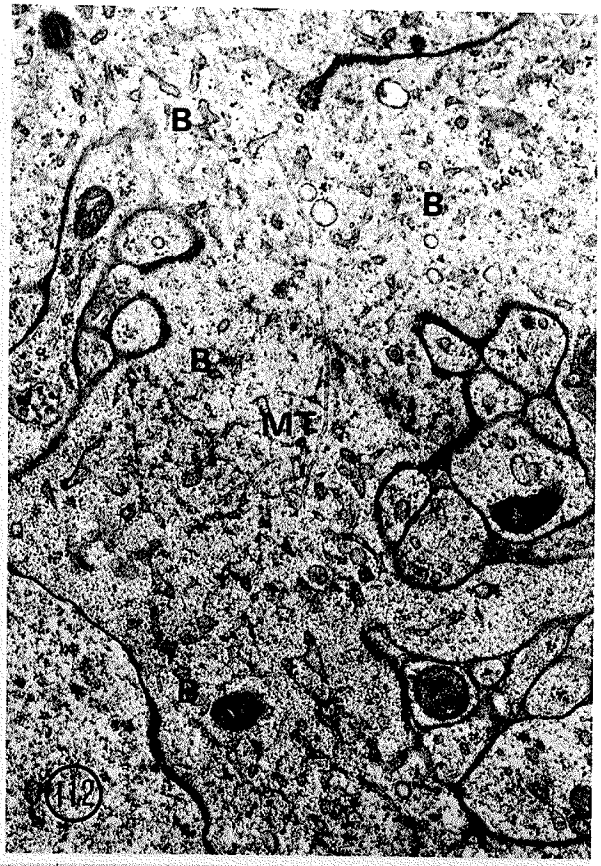
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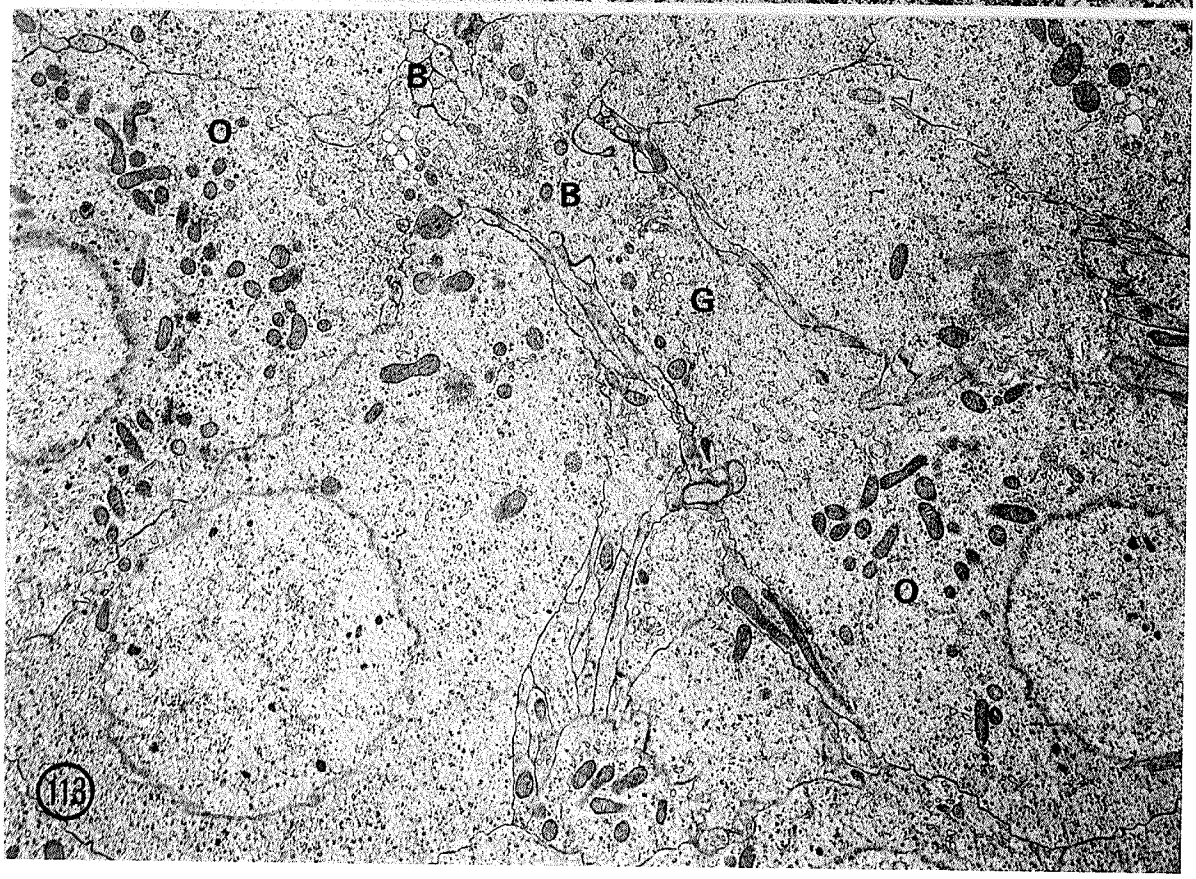
12


B
O
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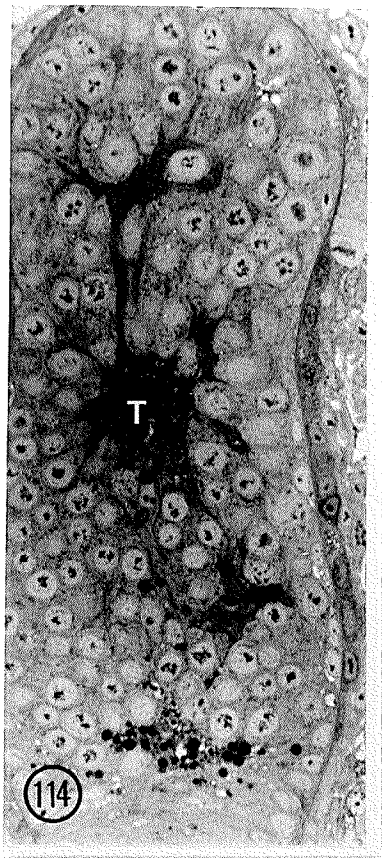
O
13

Plate 25

Trophic Core - Larval

Figure 114. Cell processes throughout the tropharium feed into the central trophic core as observed in this light micrograph. X1750.

Figure 115 to 117. These micrographs illustrate the tortuous arrangement of cell processes found in the trophic core. Germ and nurse cells surrounding the core have intercellular bridges connecting them to processes which lead into the core. Note bridge profiles present within the trophic core itself. X12530, X14180, X15580.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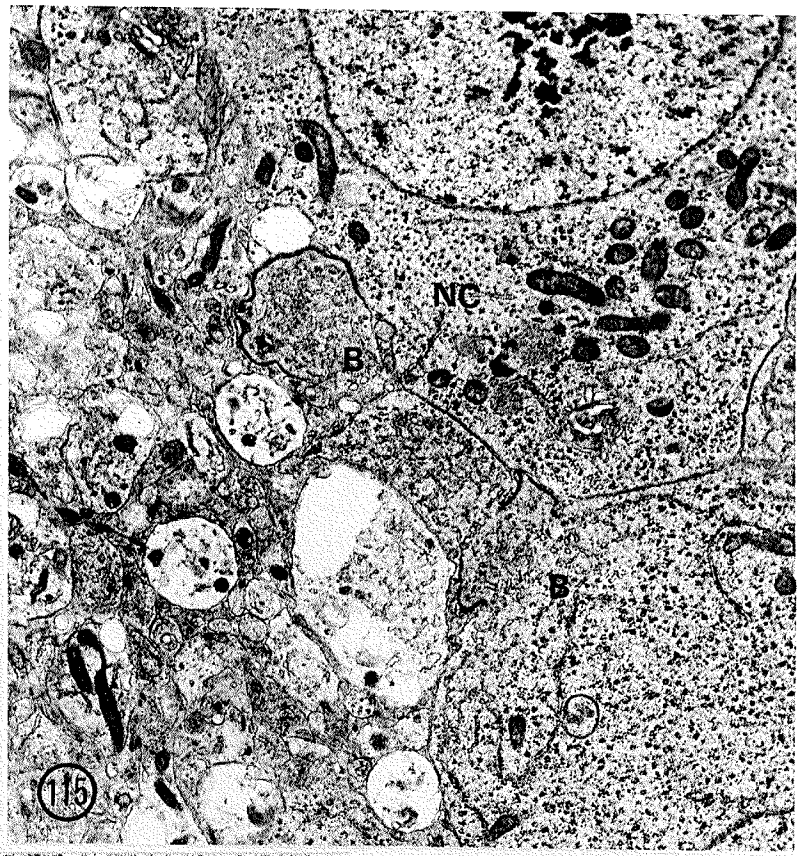
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B
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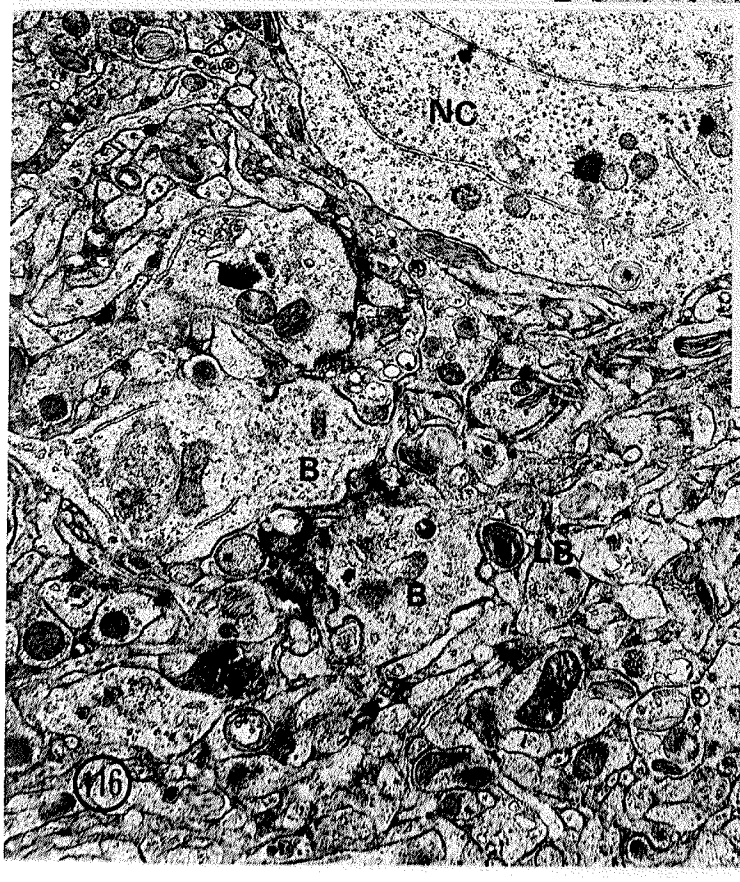
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LB
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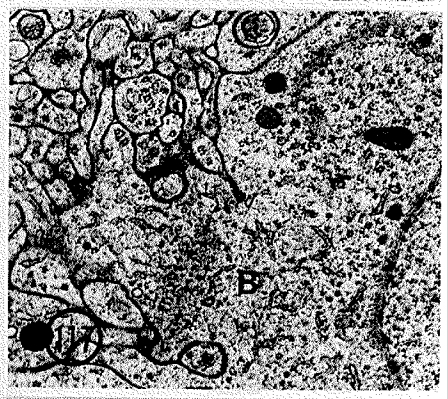

B
117

NC

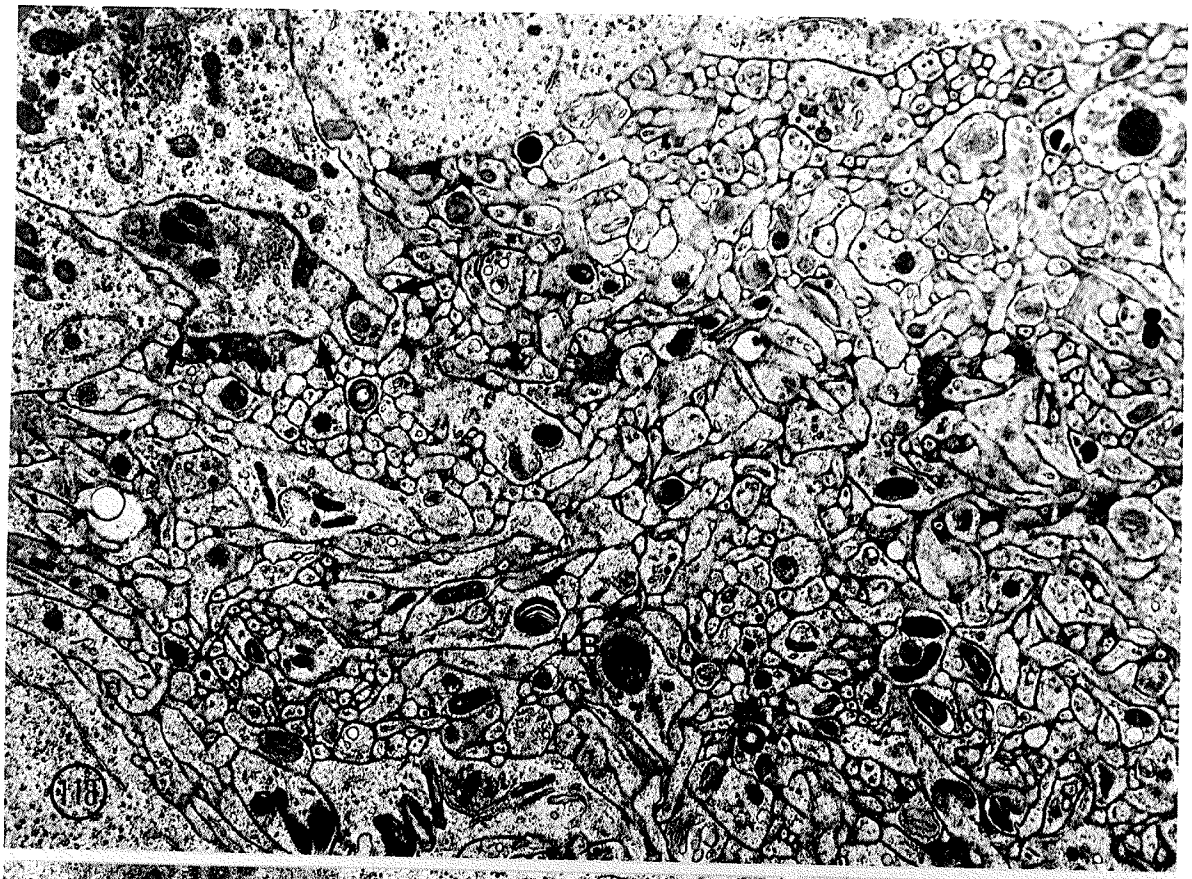
Plate 26

Trophic Core - Larval

Figure 118. Cytoplasmic extensions from nurse cells are seen to enter the core in this micrograph (arrows). X11240.

Figure 119. This micrograph shows the complex nature of the bridge systems at the periphery of the core. Note microtubules, ER and Golgi vesicles in the system. X14470.

Figure 120. This micrograph shows a centriole which was present within the core region. X33710.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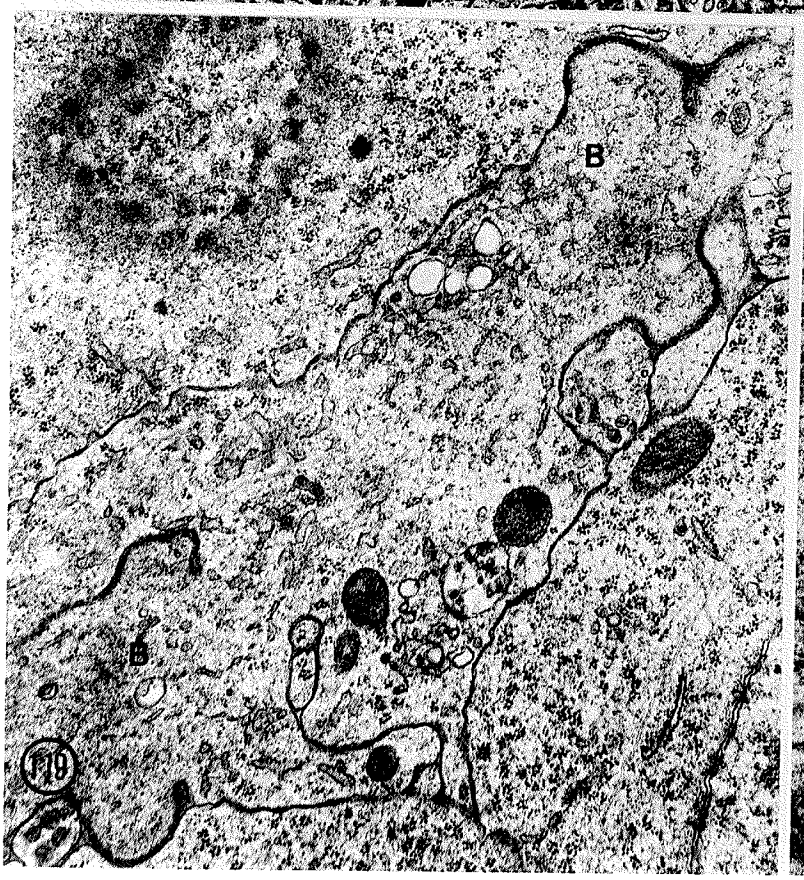

B
B
F19

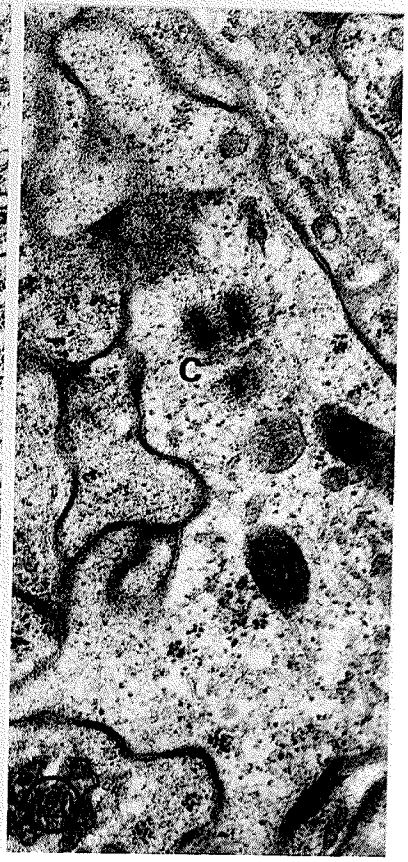

C

Plate 27

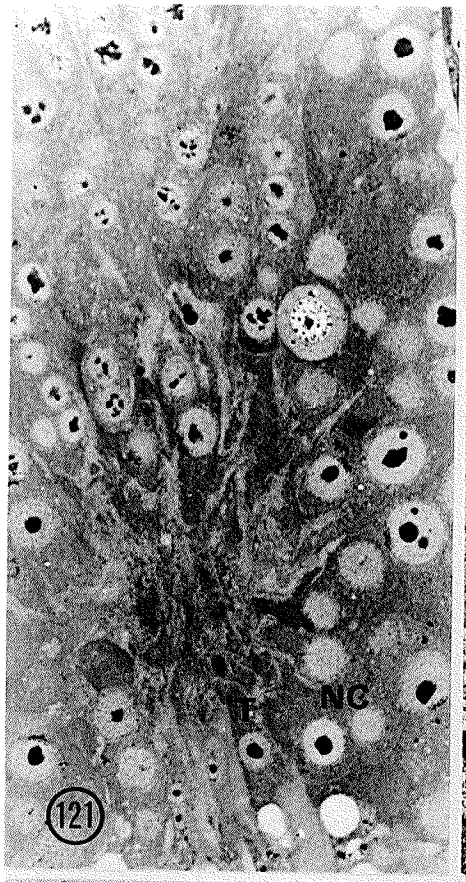
Trophic Core

Late Differentiation Phase

Figure 121. Restructuring events within the core are evident in thick sections. X1800.

Figure 122 to 123. Membrane fusions within the core result in a region which appears as shown in these micrographs. Amount of membrane in the core is reduced and large areas are free of membrane. X5210, X5120.

Figure 124. Bridge profiles are still observed in the peripheral region of the transforming core. X17260.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121


T
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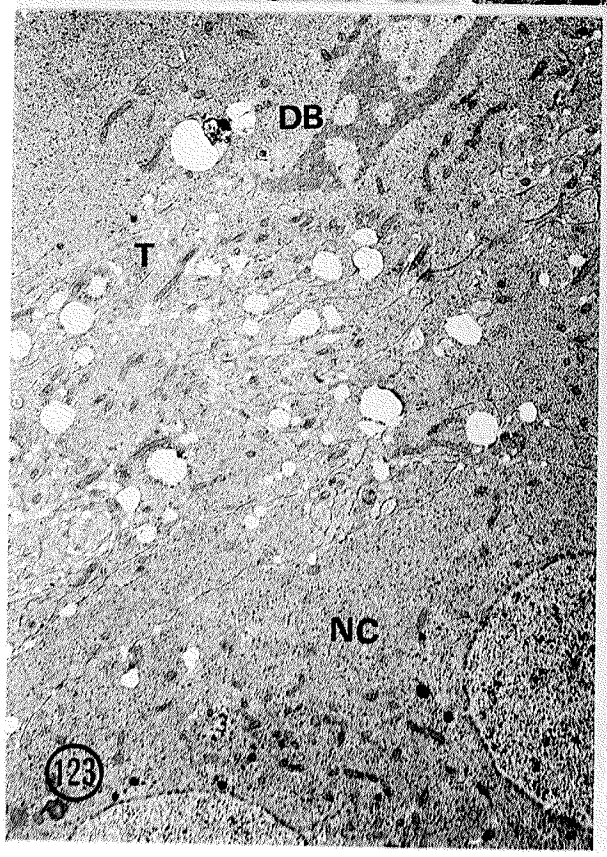
122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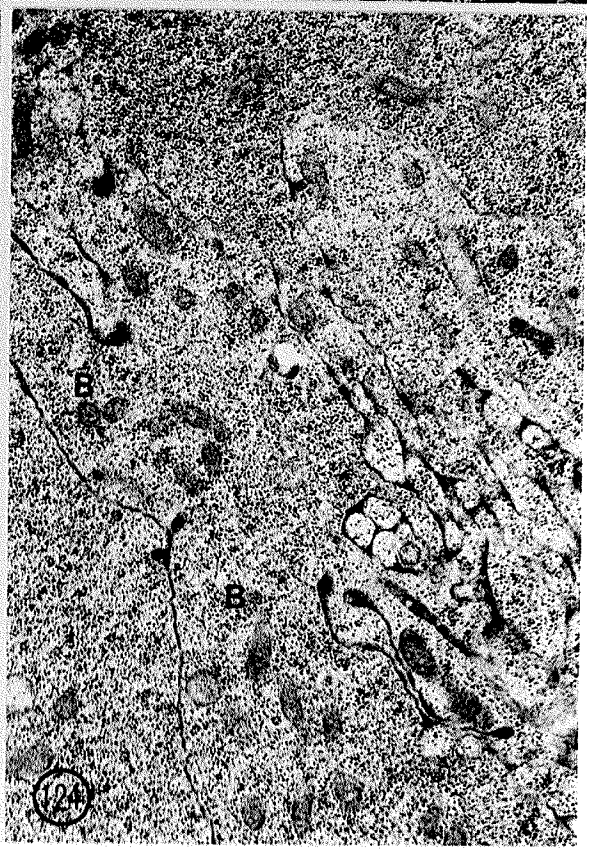
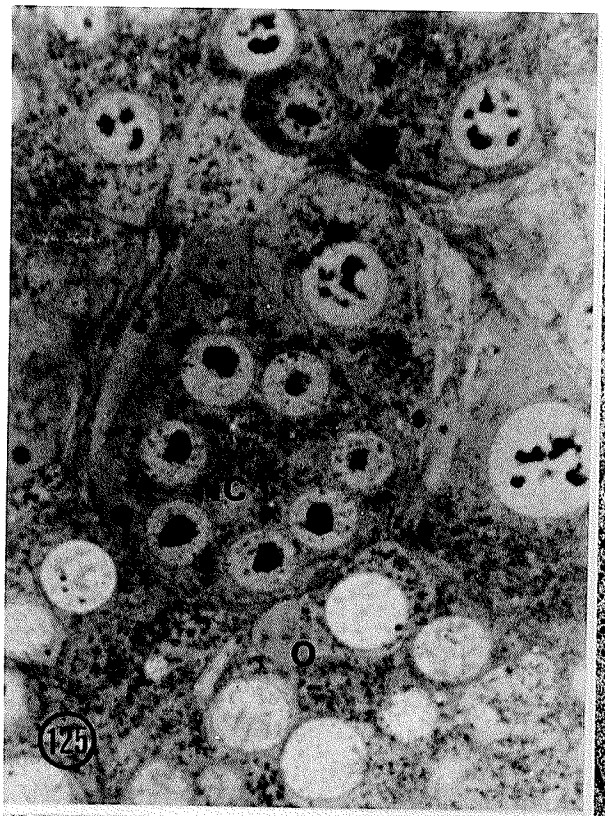

B
B
124

Plate 28

Nurse Cell - Nurse Cell Fusions

Figure 125 to 127. Nurse cell compartments within Zone III of the late differentiation phase ovary fuse resulting in groups of active nuclei existing in a common cytoplasm. X4060, X4636, X15310.

Figure 128. Nurse cell groups found in Zone III maintain contact with other groups or with the core through intercellular bridges. X11750.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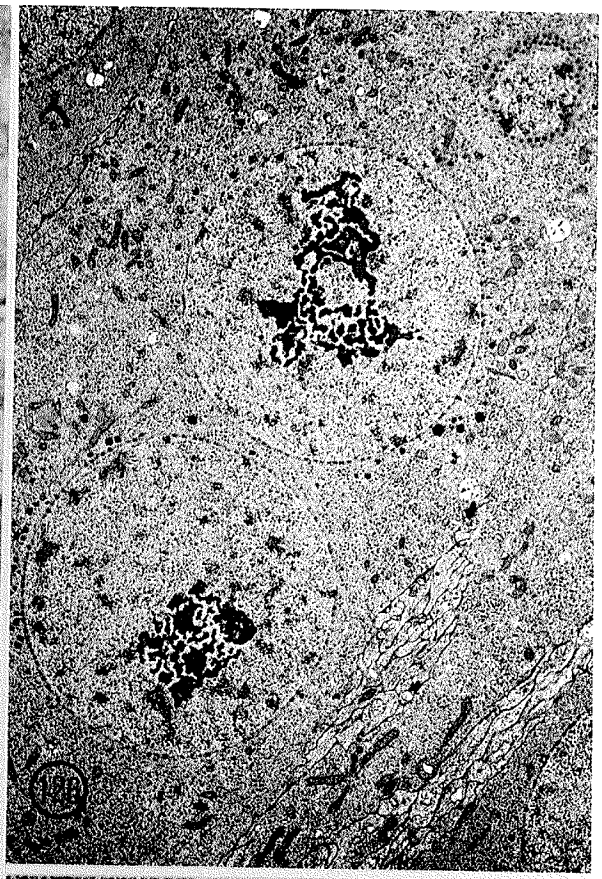
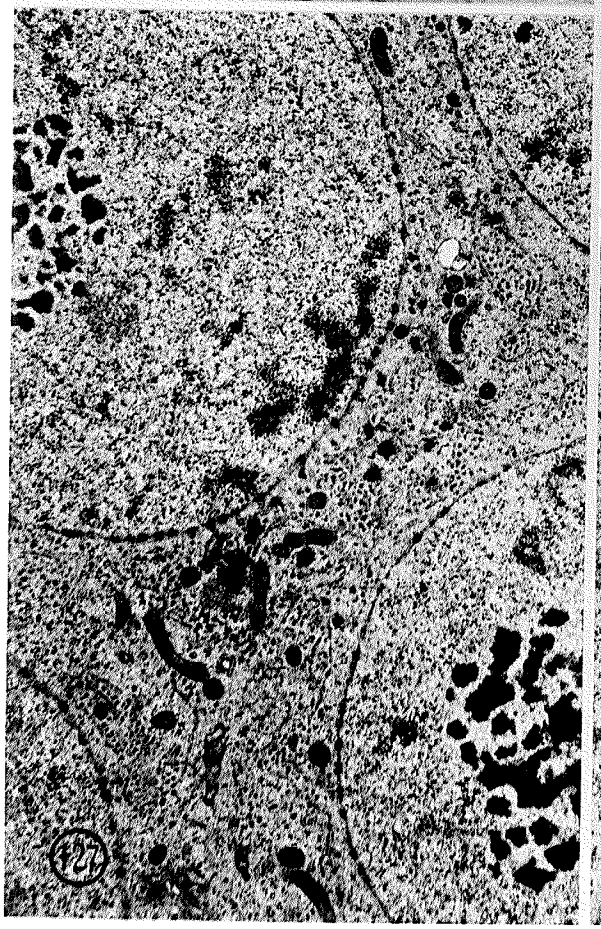

A
S
126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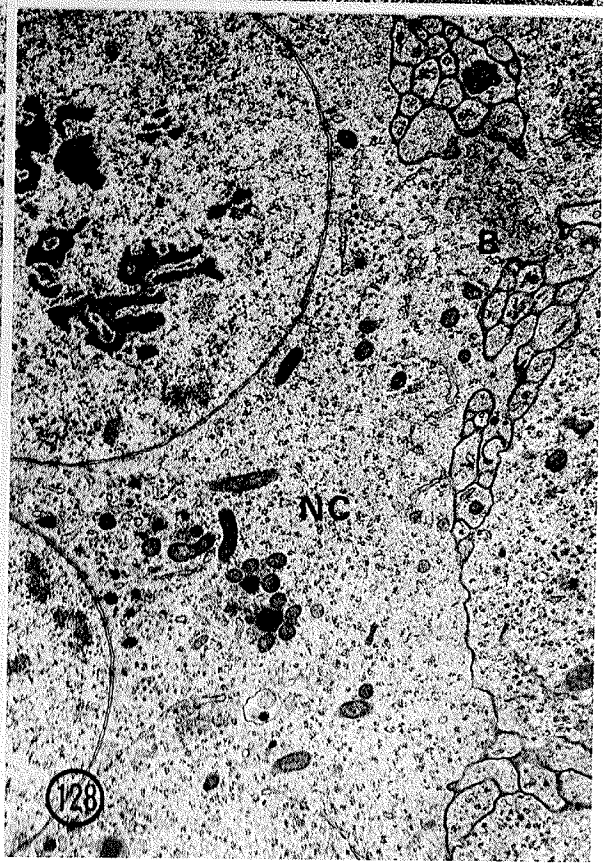

B
NC
128

Plate 29

Nurse Cell - Oocyte Interaction

The trophic cords

Figure 129 to 131. Early differentiated oocytes retain connections (arrows) to the trophic core (129, 130) which, during the late differentiation phase, form the trophic cords (131). X2010, X2080, X1180.

Figure 132. Bridge profile on the lateral edges of the trophic cords is observed in this micrograph. X7540.

Figure 133 and 134. These micrographs show an oocyte with trophic cord. Microtubule profiles can be seen in the cord (134). X7310.

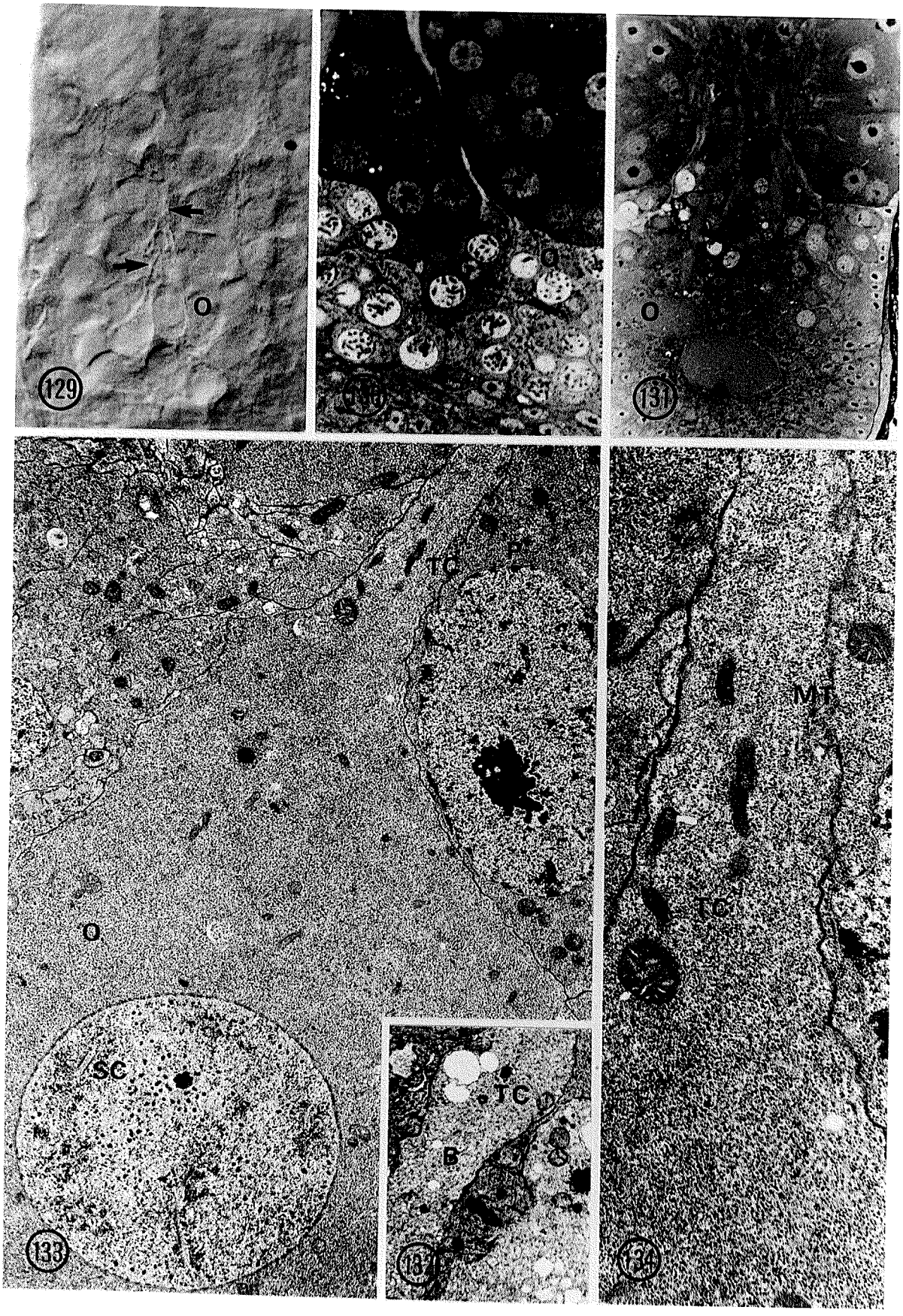
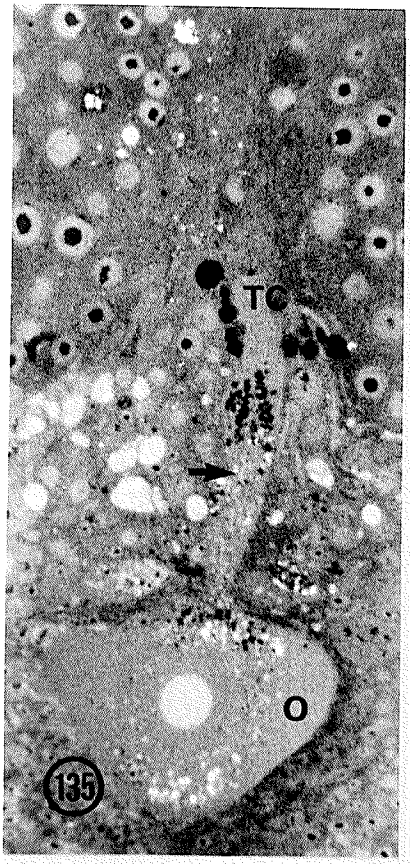

O
129
O
130
O
131
TC
O
SC
133
TC
B
132
MT
TC
134

Plate 30

Transport of material through cords

Figure 135 and 136. These micrographs show that material is being transported to the oocytes (arrows) in the final days of the 5th instar. X1100, X960.

Figure 137 to 139. These electron micrographs illustrate the various morphologies of the material being transported into the oocyte from the trophic core. X7640, X7600, X20300.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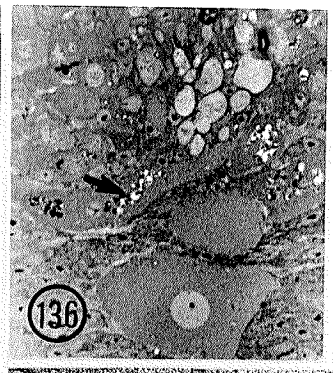
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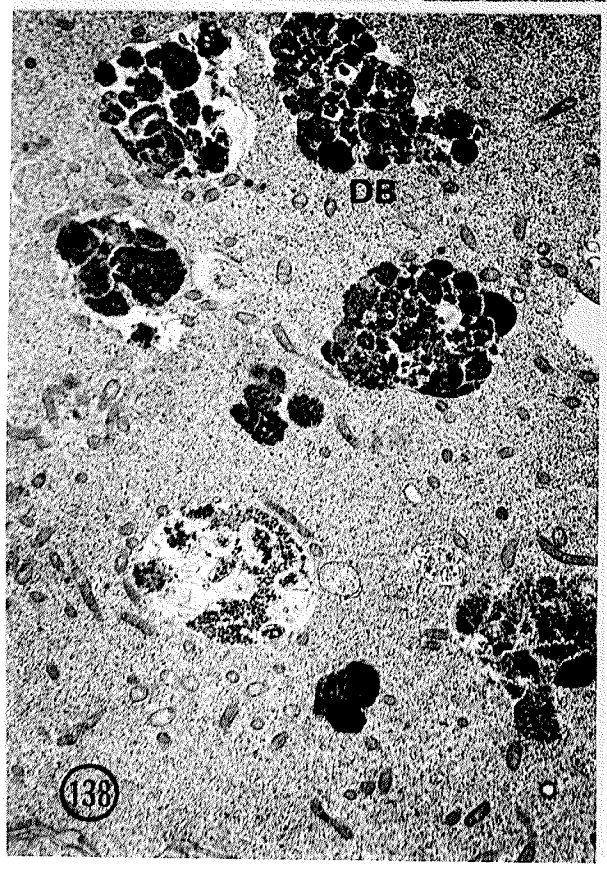
P
O
137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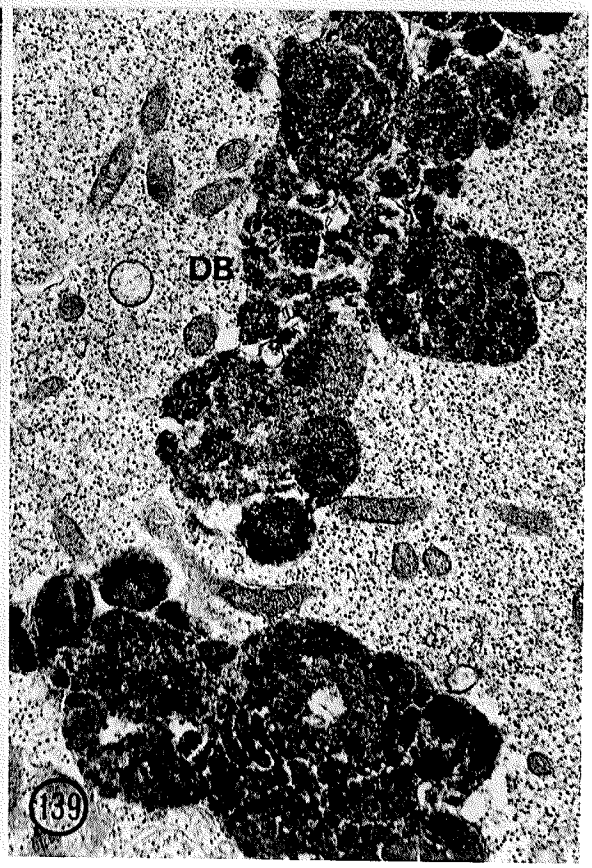

DB
139

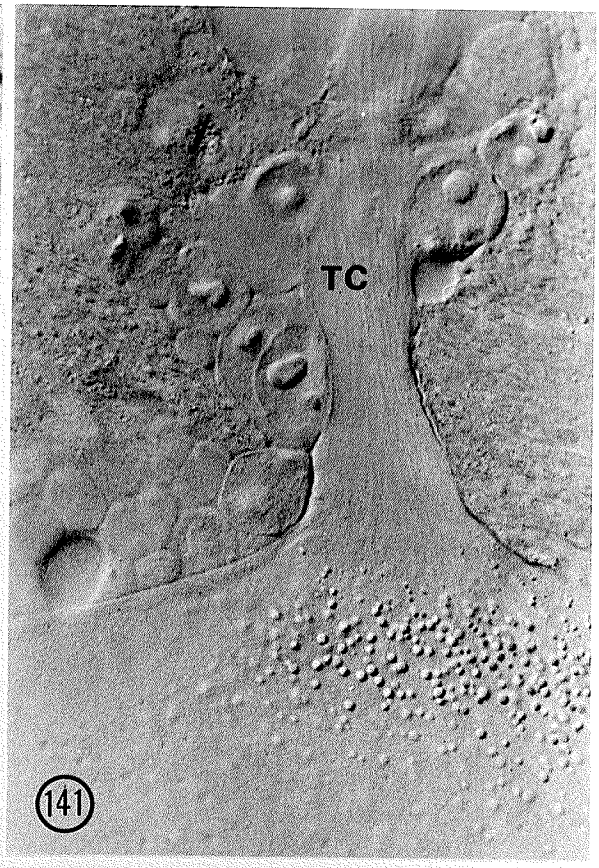
Plate 31

Trophic Cords

Figure 140 and 141. These Nomarski interference micrographs of a live adult ovariole demonstrate that oocytes both small (140) and large (141), retain their connections to the trophic core into the adult stage. X830, X2030.

Figure 142 and 143. The establishment of the vast array of microtubules present within the core and cords of the adult, is the final phase of larval-adult ovarian transformation. Weak birefringence of 5th instar core and cords just premoult and strong birefringence of the adult core and cords reflects this phenomenon. X560, X480.


O
140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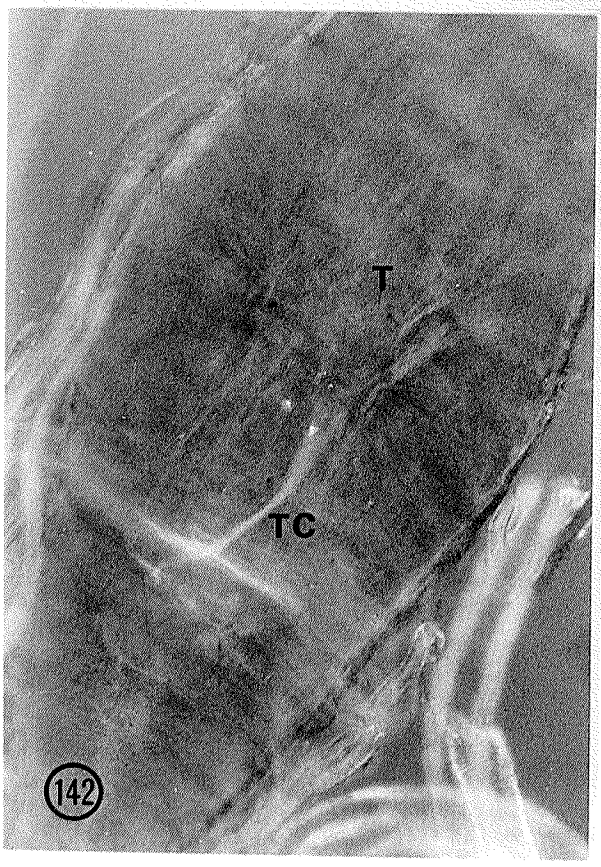
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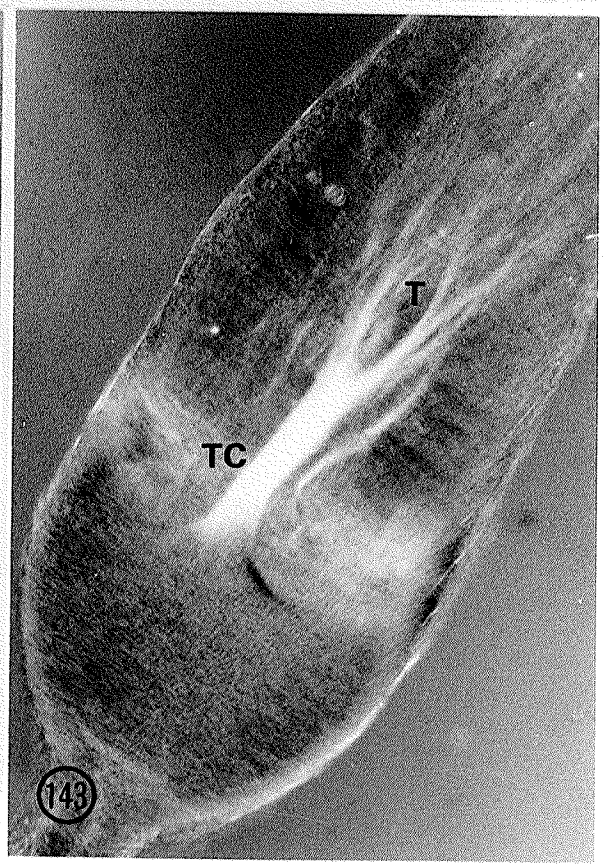

T
TC
143

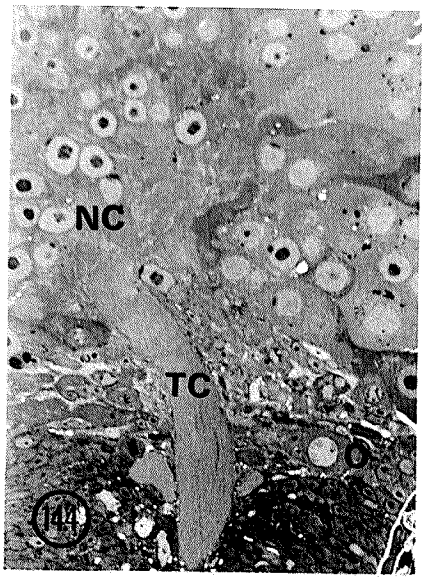
Plate 32

Transplants

Figure 144 and 145. Differentiated donor ovaries of 5th to 5th instar control transplant. X640, X610.

Figure 146. This Nomarski interference is of a recipient ovary of the 5th to allatectomized adult transplant. X690.

Figure 147, 148 and inset. These micrographs of both live and fixed material show the undifferentiated donor ovary of the 5th to allatectomized adult transplant. X1510, X2350, X2300.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O
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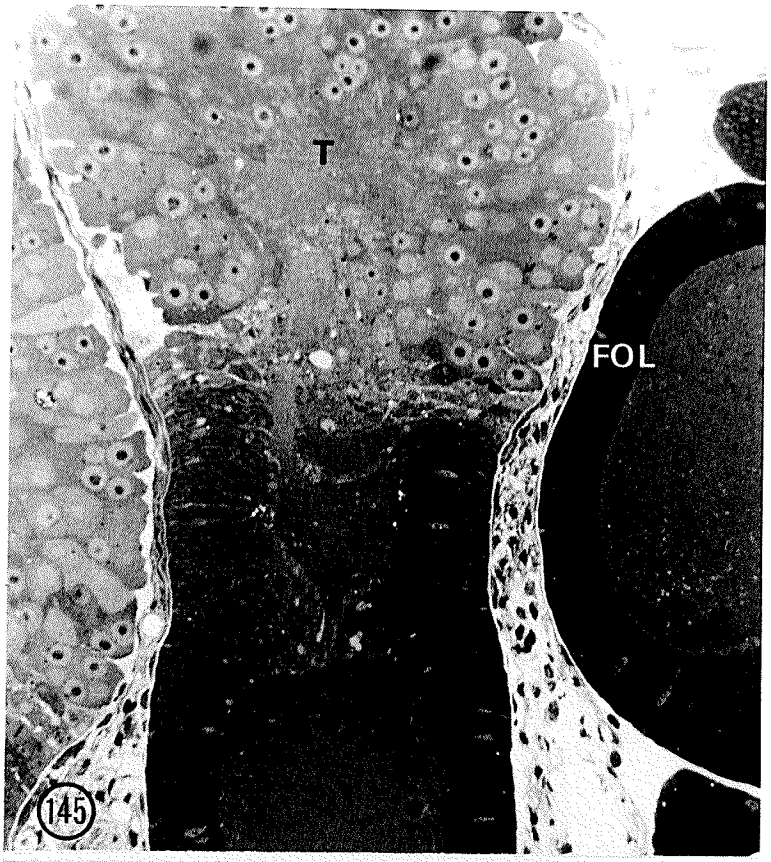
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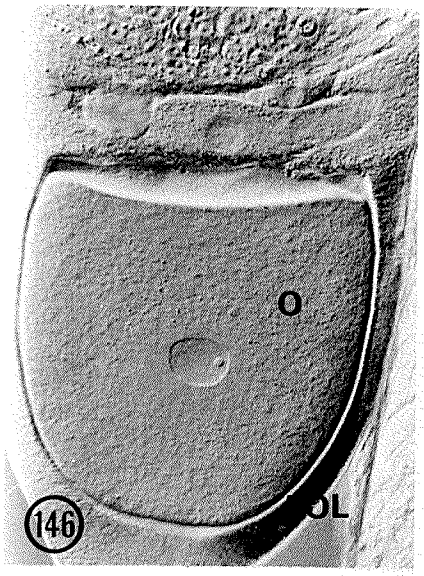

O
OL
146


GC
P
147

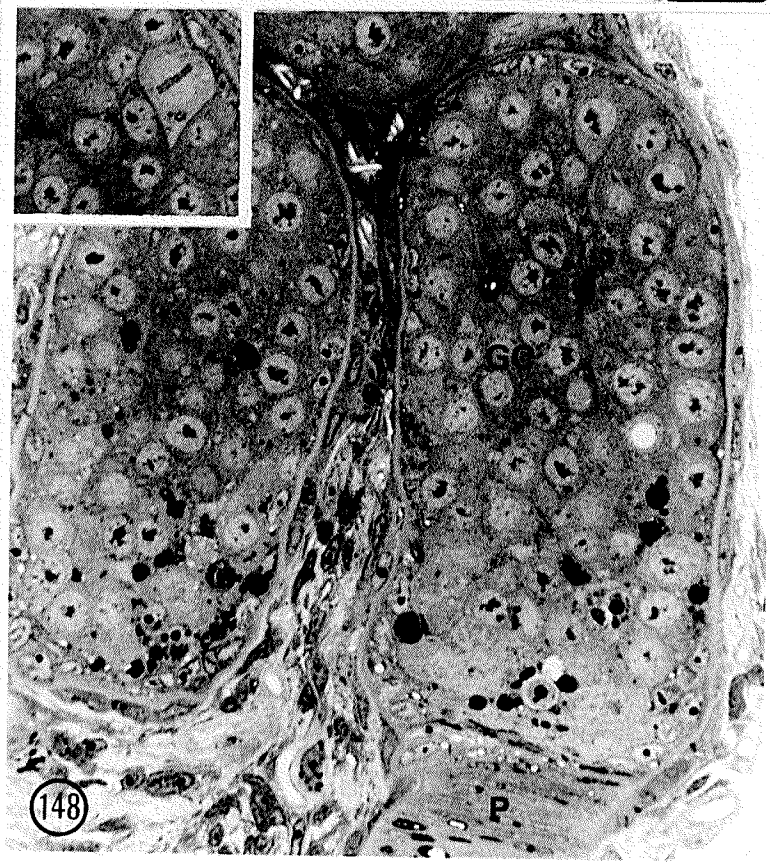
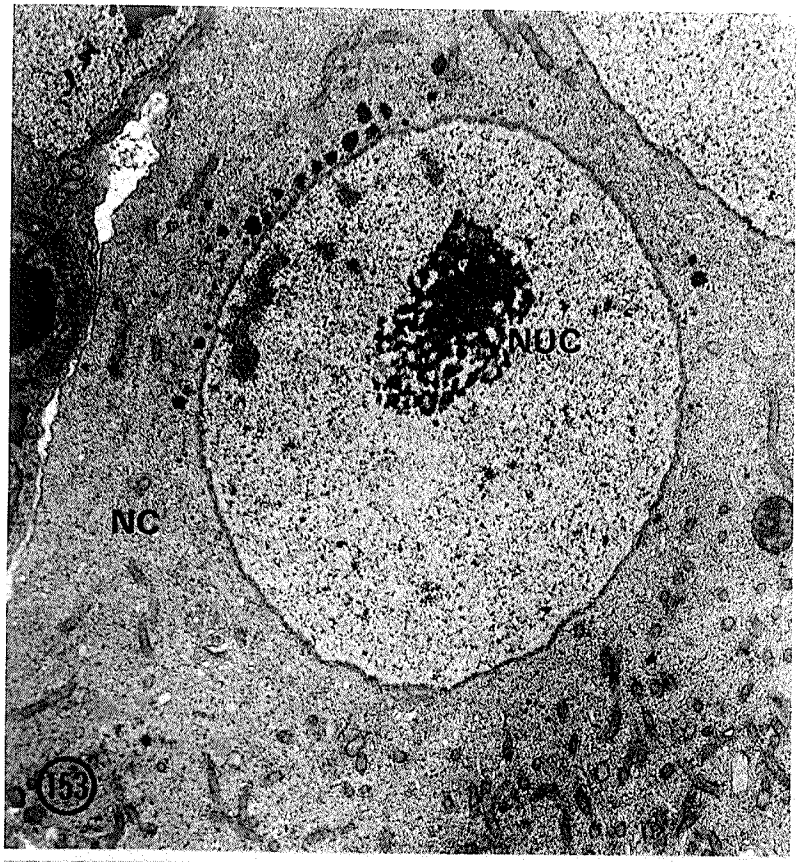

GC
P
148

Plate 34

Transplants

Figure 153 and 154. Nurse cells that differentiated in the donor ovary appear normal and functioning with condensed nucleolus and nucleo - cytoplasmic transport evident. X17630, X27080.

Figure 155 and 156. Oocytes that differentiated in the donor ovary appear normal having nuclei with synaptonemal complexes and cytoplasms which contain Golgi vesicles, mitochondria and few ribosomes. X30010, X23200.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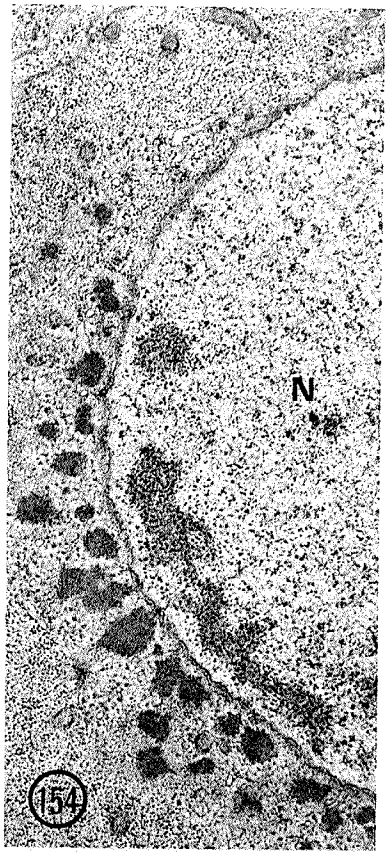
153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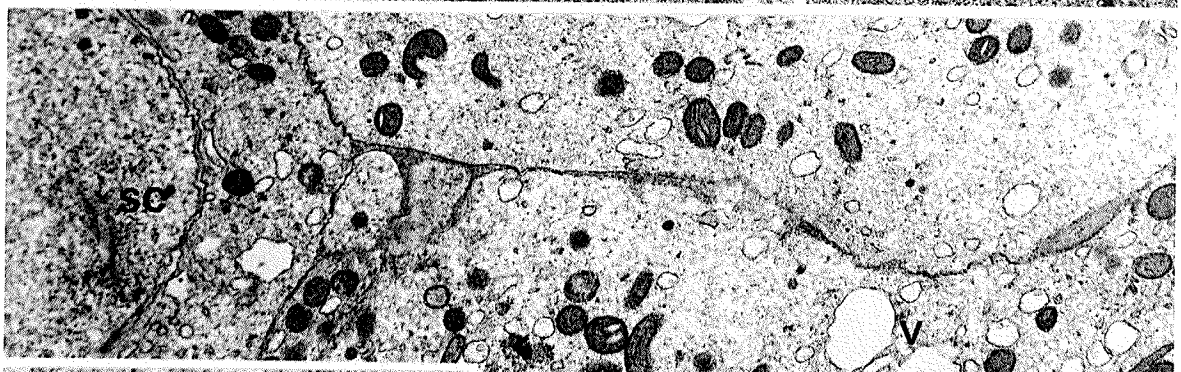
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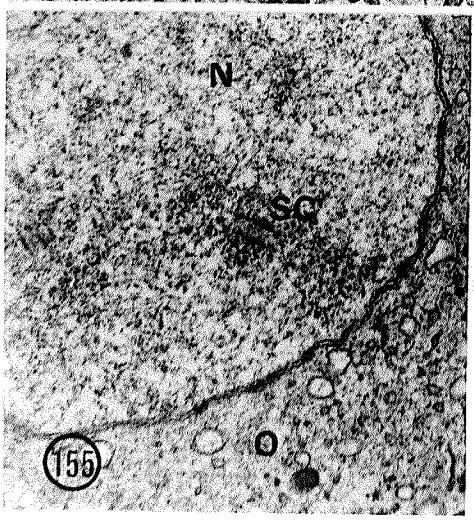
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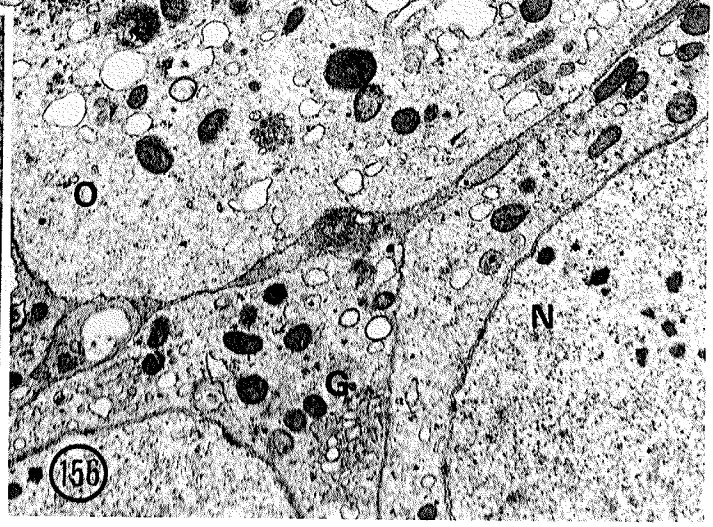
O
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156

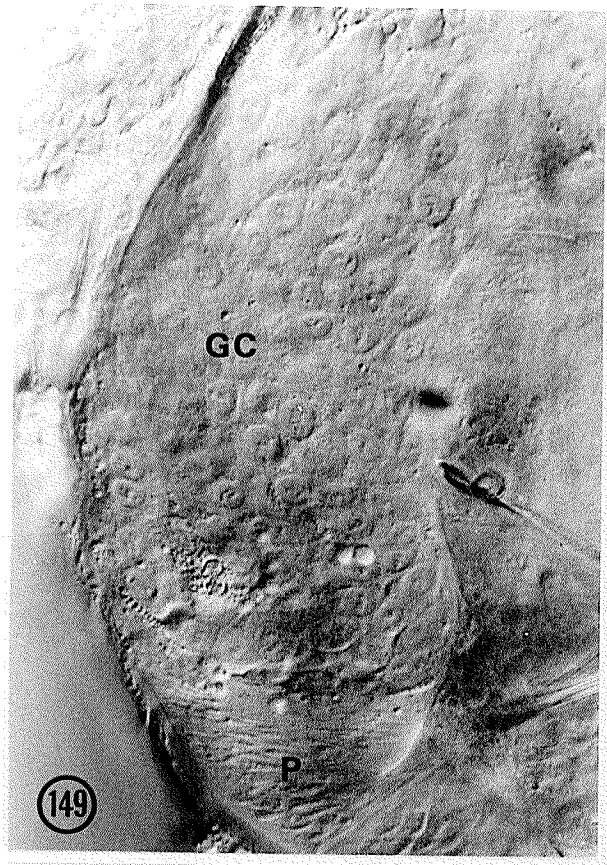
Plate 33

Transplants

Figure 149 and 150. These micrographs of live and fixed material show the undifferentiated donor ovary of the 5th to virgin adult transplant. X1850, X1860.

Figure 151. This thick section micrograph shows the partially differentiated donor ovary of the 5th to 4th instar transplant. X1700.

Figure 152. Active nurse cells can be found in the 5th to 4th donor ovary although the germarial lumen is still present. X7080.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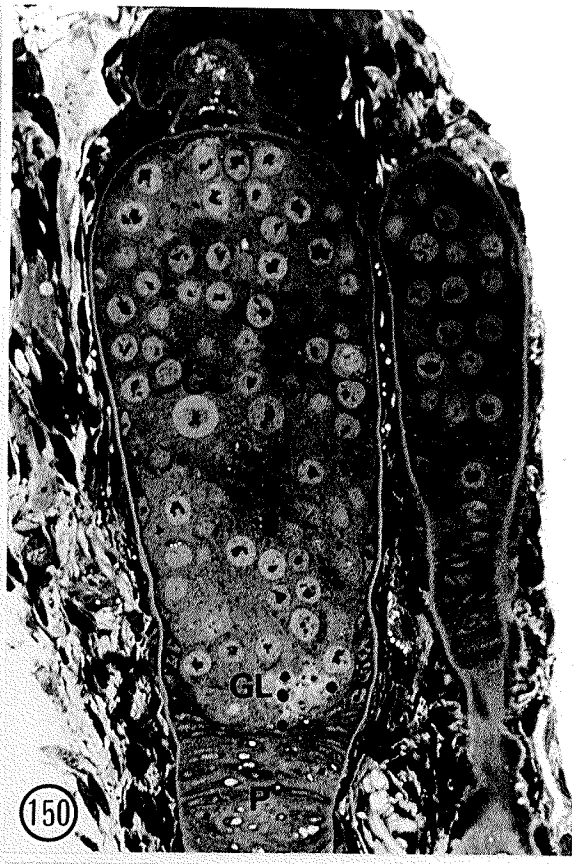
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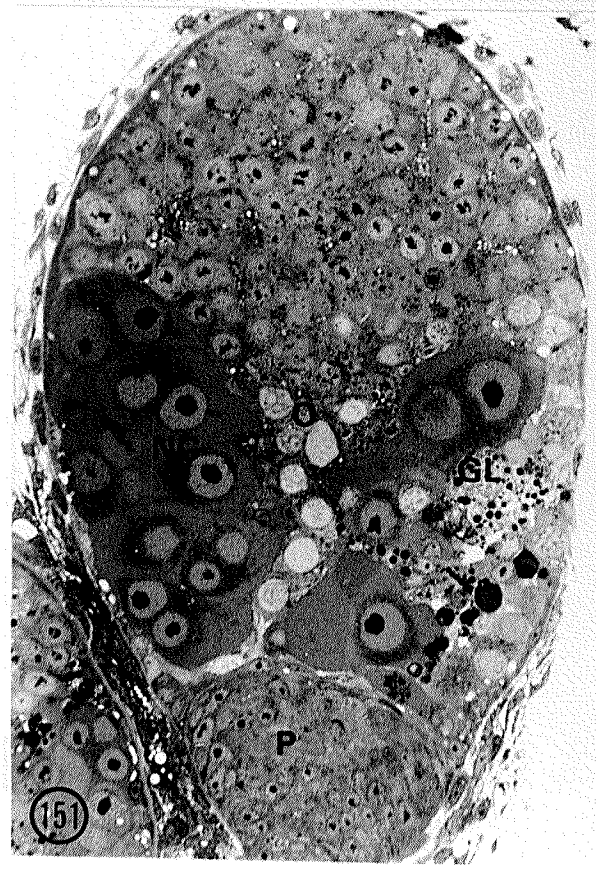
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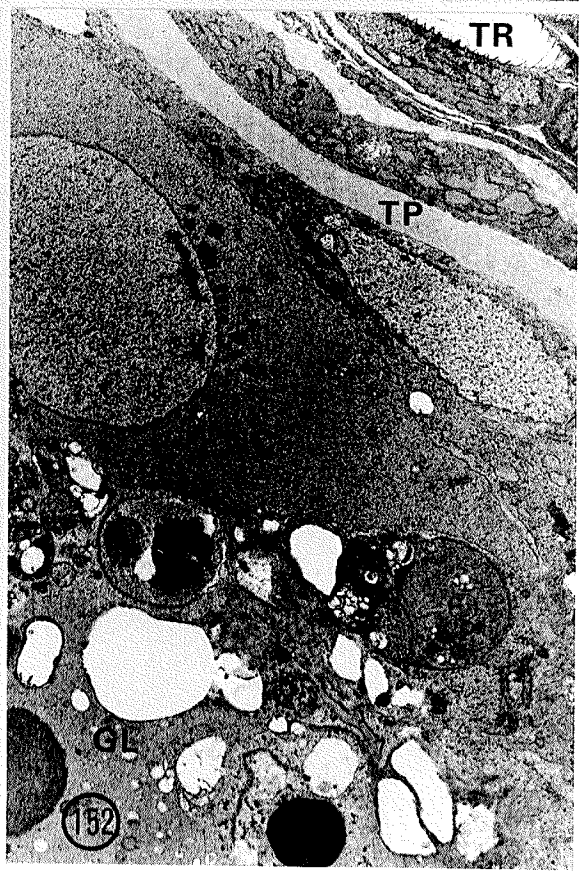
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