THE ULTRASTRUCTURE OF THE RAT OVARIAN GERMINAL EPITHELIUM AND ITS PERMEABILITY TO ELECTRON MICROSCOPICALLY DEMONSTRABLE TRACER MOLECULES

by

RANALD ROSS DONALDSON

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Department of ANATOMY

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date April 6, 1976

ABSTRACT

The detailed fine structure of normal rat ovarian germinal epithelium was studied by means of transmission and scanning electron microscopy. The germinal epithelium possesses features suggestive of an absorptive and/or secretory capacity, a marked protein synthetic ability, and possible steroid metabolism. These and other cellular features were in turn indicative of a possible germinal epithelial involvement in transcellular movement of particulate substances.

In order to investigate the permeability of the germinal epithelium to molecules from the peritoneal cavity, two electron microscopic tracer molecules, horseradish peroxidase (HRP) and ferritin, were injected intraperitoneally. The results indicate that there is a differential movement of these two molecules across the germinal epithelium, presumably related to the difference in their molecular dimensions. The predominant route of movement of HRP is extracellular, apparently by diffusion through the intercellular clefts. Ferritin movement, on the other hand, is intracellular, via a vesicular transport mechanism associated with pinocytotic activity at the apical surface of the germinal epithelial cells.

It is concluded that the germinal epithelium is a metabolically active tissue which plays both a passive and an active role in the movement of molecules from the peritoneal cavity.

TABLE OF CONTENTS

Table of Contents (Cont.)

LIST OF FIGURES

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INTRODUCTION

A. Previous Studies of the Germinal Epithelium

The functional significance of the germinal epithelium of the mammalian ovary was first investigated in terms of its possible oogenic potential (Waldeyer, 1870, quoted by Franchi et al., 1962). As clearly evident by the name accorded it by early workers, the germinal epithelium was considered to be a source of ovarian germ cells (Franchi et al., 1962). Subsequent studies have demonstrated that primordial germ cells which give rise to definitive oocytes arise either from stem cells which give rise to endodermal cells, or from the endoderm of the embryonic secondary yolk sac, near the site of the allantoic evagination (Witschi, 1948). These germ cells then actively migrate in an amoeboid fashion, perhaps assisted by histiolytic action, to the region of the gonadal blastema by way of the dorsal mesentery of the developing gut (Witschi, 1948; Pinkerton et al., 1961). The gonadal blastema is initially a thickened region of the coelomic mesothelium and underlying mesenchyme on the ventromedial aspect of the urogenital ridges. This mesothelium later becomes the germinal epithelium. It is now well established that all definitive germ cells are progeny of the primordial germ cells which first populate the ovary. The germinal epithelium does not contribute to the germ cell population at any stage of ovarian development (Witschi, 1963; Franchi, 1970).

Currently, a number of investigators believe that the

germinal epithelium is active mainly during prenatal, and perhaps early postnatal development. At these times it is believed to be a source of precursors of follicular (later, granulosa) cells of the developing ovarian cortex (Franchi, 1970). This is disputed, however, by others who suggest that the follicular cells are derived from mesenchymal and not mesothelial (germinal epithelial) cells (Franchi, 1970). Apart from the developmental function of the germinal epithelium, its functional activity in the postnatal animal (exclusive of earlier studies of potential germinal activity) has not been seriously considered.

Most recent studies of the germinal epithelium have been concerned with its descriptive ultrastructure, both during intra-uterine and postnatal development (Wischnitzer, 1965; Gondos, 1969; Weakley, 1969; Papadaki and Beilby, 1971; Jeppesen, 1975; Merchant, 1975; Pelliniemi, 1975). Papadaki and Beilby (1971) and Weakley (1969) have suggested that germinal epithelial cells, on the basis of their ultrastructural features, may transfer material through the cytoplasm. The direction of movement, however, could not be ascertained from this morphological data alone. These workers observed pinocytotic vesicles, vacuoles, microvilli and an abundance of ribosomes, rough endoplasmic reticulum, and mitochondria within the germinal epithelial cells. Since such features are often prominent in cells able to carry on transport and synthetic processes, they considered their assumptions warranted.

Of those studies of the germinal epithelium conducted to

date, only Chiquoine's work (1961) involved an experimental component in an ultrastructural study (light microscopic studies will be mentioned later). To determine whether or not the germinal epithelium had an oogenic function, mice were injected intraperitoneally with colloidal gold, an electron microscopically demonstrable tracer substance. If the germinal epithelial cells were labelled with gold and gave rise to germ cells (which have distinctly recognizable histological, histochemical, and ultrastructural features), then Chiquoine would have expected to find particulate gold tracer within the cells so derived. Because no oocytes were labelled, Chiquoine concluded that "vital staining of the germinal epithelium provides no evidence for an oogenic function on the part of the germinal epithelium in the postnatal animal." This resolved the conflicting results of earlier light microscopic tracer studies (Latta and Pederson, 1944; Jones, 1949), which were limited in part by virtue of inherent technical restrictions. Chiquoine's description of tracer localization was brief, stating that aggregates of gold particles were randomly scattered within the germinal epithelial cells. No mention was made of how the gold tracer might have entered the cells, nor whether its course was followed over a period of time using a series of animals.

Zuckerman (1951) has also made mention of the uptake of particulate matter by the germinal epithelium. He said that it "is highly phagocytic, and in the normal animal picks up debris from red blood cells or any particulate matter of suitably small size that is introduced into the peritoneal

cavity." Unfortunately, this paper presented no substantiating evidence for that statement, nor did it mention the experimental animal to which that statement referred.

Chiquoine's study, although presumably not intended to illustrate tracer movement across the germinal epithelium, did show that germinal epithelial cells can take up colloidal gold. This uptake could represent the first step of a cell-mediated transport. However, since then there have been no reports in the literature concerned with tracer movement across the germinal epithelium. Such movement could conceivably be either by passive diffusion or by active transport. It could occur across the germinal epithelium in a unidirectional or bidirectional fashion, either intracellularly or extracellularly. Any such transport properties could be of potential physiological and developmental significance to the ovary. This applies especially to the oocytes in that certain substances, some perhaps of a deleterious nature in terms of oocyte development, might be able to pass or be transported from the peritoneal cavity into the substance of the ovary.

Transport could also be operative over slightly shorter distances in the case of substances actually manufactured within the germinal epithelial cells and transferred to subjacent cells of the tunica albuginea ovarii and cortex. Weakley (1969) suggests the possibility of reciprocal induction by mutual transfer of substances, such as amino acids and proteins, between the germinal epithelial cells and underlying follicle cells in early development.

Accordingly, in the present study, exogenous electron microscopic tracers were chosen in an attempt to examine and characterize the germinal epithelium in terms of its active and/or passive transport properties.

B. Electron Microscopic Tracers

The use of electron microscopically demonstrable tracers to elucidate pathways of normal molecular uptake, transfer, and release from cells has become of increasing value in recent years. Currently used tracers represent a considerable range of molecular size and weight, and as such constitute a graded series of molecular probes with which cells and tissues may be investigated. In both qualitative and quantitative determination of molecular movement characteristics, selection of a tracer depends also on a number of other important factors. Firstly, the dosage required should not be inimical to the continued normal physiological functioning of the animal. Also, molecules of the tracer should be of a uniform size, so as to allow accurate assessment and quantification of results. In addition, the tracer should not be metabolized by the tissues nor should it be rapidly cleared from the body.

Electron microscopic tracers may be classified into two main groups. The first consists of molecules which are detectable by virtue of their natural electron opacity. Once having exposed tissues to this type of tracer, routine processing and examination will reveal the presence (or absence) of these molecules. Tracers of this type may be of either biological or non-biological origin. They are exemplified by lanthanum

(Revel and Karnovsky, 1967), carbon (Leak, 1971), latex spheres (Leak, 1971), ferritin (see p. 8), dextrans (Simionescu and Palade, 1971), glycogen (Simionescu and Palade, 1971), and colloidal suspensions of gold (Chiquoine, 1961) and mercuric sulphide (Odor, 1956).

The second group of tracers is enzymatic in nature. The site of their localization within a tissue or cell is visible only subsequent to the exposure of the tissue to a substrate appropriate to the particular enzyme used as a tracer. What is then seen is the electron microscopically visible end product of the reaction, which should be localized as a precipitate within the immediate vicinity of the enzyme. Tracer molecules of the second type must be by definition of biological origin, and are mainly peroxidatic enzymes: horseradish peroxidase (HRP) (see p. 8), myeloperoxidase (Graham and Karnovsky, 1966a), lactoperoxidase (Graham and Kellermeyer, 1968), microperoxidase (Feder, 1970), catalase (Goodenough and Revel, 1971), myoglobin (Anderson, W.A., 1972b), and cytochrome c (Karnovsky and Rice, 1969). Experimentally the sites of localization of these enzymes within a tissue or cell are typically visualized via a reaction involving hydrogen peroxide and 3,3'-diaminobenzidine (DAB) (Graham and Karnovsky, 1966b; Hirai, 1975). As an example the reaction sequence of HRP with H_2O_2 and DAB is shown below (modified after White et al., 1973). To show that DAB is initially a hydrogen donor in the following reaction, it is represented as $DABH_2$.

$$HRP\text{-}H_2O + H_2O_2 \longrightarrow \underset{\text{(complex I)}}{HRP\text{-}H_2O_2} + H_2O$$

$$HRP\text{-}H_2O_2 + DABH_2 \longrightarrow \text{complex II} + DABH\cdot$$

$$\text{complex II} + DABH\cdot \longrightarrow HRP\text{-}H_2O + DAB$$

The reaction sequence involves the formation of consecutive complexes which have not yet been precisely defined. The overall result of the reaction is the regeneration of HRP, which will continue to catalyze the same reaction, and the formation of oxidized DAB, which forms an insoluble precipitate at the sites of reaction (Seligman et al., 1968). The precipitate is a brown pigment at the light microscopic level and an electron opaque substance at the electron microscopic level.

The aforementioned criteria for choosing tracers, as well as the successful experience of other workers with certain of these tracers, were taken into consideration when choosing tracer molecules with which to investigate the germinal epithelium. It was decided to use two tracer molecules, ferritin and HRP. By virtue of their respective molecular dimensions and weight they would be expected to have different transfer characteristics, and thus bound a sizeable range of possible tracers which could be used.

Ferritin, the larger of the two tracers, is a heme protein containing 20-24% iron which was first isolated from horse spleen (Ainsworth and Karnovsky, 1971). The molecule consists of a spherical shell of protein surrounding a core of ferric hydroxide micelles. The total molecular diameter is 110 Å, whereas the core diameter is approximately 55 Å (Ainsworth and Karnovsky, 1971). The core is the electron dense part of the molecule. In order to avoid misinterpretation of results it

must therefore be kept in mind that the entire molecule is not seen in electron micrographs. The molecular weight of ferritin is about 462,000 (Ainsworth and Karnovsky, 1971).

The second tracer, HRP, has also been extensively used in tracer studies. The reason for the efficacy of this tracer is aptly described by Graham and Karnovsky (1966b): "the method is sensitive because enzymatic activity has an amplifying effect; thus a few molecules of protein at a site can generate a much larger amount of reaction product upon incubation." HRP has a molecular diameter of about 40 Å and a molecular weight of about 40,000 (Klapper and Hackett, 1965). Commercially available HRP contains a number of components which are separable by starch electrophoresis. The amino acid composition, absorption spectrum, size, and enzymatic activity of these components are all quite similar (Klapper and Hackett, 1965). Catalytic activity of the five purified peroxidase fractions was identical in the two assays carried out by Klapper and Hackett (1965). They concluded that the total enzymatic activity of unfractionated HRP would not be affected by different relative amounts of the fractions.

HRP and ferritin have been used as tracers in a number of different experimental situations. One or other or both of them have been used to study the permeability of capillaries in cardiac and skeletal muscle (Karnovsky and Cotran, 1966; Karnovsky, 1967; Bruns and Palade, 1968), lung (Schneeberger-Keeley and Karnovsky, 1968; Clementi, 1970), intestine (Clementi and Palade, 1969), cerebrum (Reese and Karnovsky, 1967),

thymus (Gervin and Holtzman, 1972), ovary (Anderson, W., 1972a; Payer, 1975), and renal glomerulus (Farquhar and Palade, 1961; Farquhar et al., 1961; Webber and Blackbourn, 1970). They have also been used to study permeability and absorption in proximal renal tubule (Graham and Karnovsky, 1966b; Maunsbach, 1966), mesothelium (Karnovsky and Cotran, 1966; Cotran and Karnovsky, 1968; Kluge, 1969), pericardium (Kluge and Hovig, 1968; Kluge, 1969), ovarian follicle (Anderson, W., 1972a; Payer, 1975), oocyte (Anderson, E., 1967, 1972), parietal layer of Bowman's capsule (Webber and Blackbourn, 1971), post-ovulatory zona pellucida (Hastings et al., 1972), urinary bladder epithelium (Wade and Discala, 1971), and cultured and normal tumor cells (Ryser et al., 1962).

Ferritin can also be linked, using bifunctional conjugating agents, to antibody, in order to localize sites of antigen-antibody reactions. Protein compounds labelled with ferritin have included antifibrinogen (Wylie, 1964), enzymes (Benjaminson et al., 1966), and antiviral globulin (Morgan et al., 1961).

C. Scope of the Present Study

A description of the normal ultrastructure of pre- and postpubertal rat germinal epithelium will be presented, as the epithelium of this species has not yet been adequately described in the literature in terms of its fine structure.

The present study is also concerned with the movement of two tracers, HRP and ferritin, from the peritoneal cavity into and across the germinal epithelium. By observing the localization

of these tracers with the electron microscope, it was anticipated that the active and/or passive transport capacities of the germinal epithelium might be elucidated.

MATERIALS AND METHODS

Female albino rats of the Wistar strain were used in this study. They ranged in age from 29 days to 17 months, thus representing both pre- and postpubertal animals. Food (Purina Rat Chow) and water were provided ad libitum. Adult animals were selected without regard to the exact stage of the ovarian cycle.

HRP (type II, Sigma Chemical Company, St. Louis, Missouri) and ferritin (horse spleen, 2X crystalline, cadmium free, Nutritional Biochemical Corporation, Cleveland, Ohio, or ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio) were chosen as the electron microscopic tracers. HRP was used in dosages varying from 8-100 mg/100 g body weight and in concentrations ranging from 10-40 mg/ml isotonic saline. Ferritin dosages of 20-200 mg/100 g body weight, at concentrations of 10-100 mg/ml isotonic saline, were used. Animals received an intraperitoneal injection of one or other of the tracers in the right lower quadrant of the abdomen. After specific periods of time these animals were anesthetized and sacrificed. In addition, some animals were anesthetized and their ovaries directly immersed in ferritin at a concentration of 100 mg/ml isotonic saline. In either case the anesthetic consisted of a sequential combination of intraperitoneally injected sodium pentobarbital (concentration: 3.3%; dosage: 0.2 ml/100 g) followed by subcutaneously injected sodium phenobarbital (concentration: 2.0%; dosage: 0.2 ml/100 g).

Animals injected with HRP were sacrificed at 45 min, 65 min,

2 hr, 4 hr, and 5 hr. Post-injection sacrifice times for animals receiving ferritin injections were 30 min, 45 min, 1 hr, 2 hr, 4 hr, 4½ hr, and 24 hr. Post-immersion sacrifice times for anesthetized animals with their ovaries immersed in ferritin solution were 15 min, 30 min, 1 hr, 1½ hr, 2 hr, and 3½ hr.

To expose the ovaries, a midline incision was made through the ventral abdominal wall into the peritoneal cavity from a position just rostral to the vaginal opening as far as the subcostal line. Fixative was immediately introduced into the peritoneal cavity at this point in those animals which had received prior injections of tracer. Lateral incisions were then made for approximately 1 cm on either side of the caudal end of the first incision. The abdominal wall flaps so created were then retracted and most of the small intestine displaced to the outside of the abdominal cavity. This manoeuvre exposed the ovaries. Each was situated near the end of each horn of the uterus, separated from it by the highly coiled oviduct. A tendinous band, travelling within the broad ligament from the dorsal aspect of each ovary to a point on the dorsal body wall adjacent to the ipsilateral diaphragmatic crus, maintained each ovary in position. This band was cut and the uterine horn, oviduct, and ovary raised off the dorsal body wall so that the ovary, contained within its bursa, could be removed.

The relationship of the oviducal mesenteries to the mammalian ovary is highly variable (Beck, 1972). There may be complete anatomic independence of these structures, with much of the

surface of the ovary freely exposed, such as is seen in the human and deer (Beck, 1972). On the other hand the ovary may be completely surrounded by a closed bursa derived from the oviducal mesenteries, such as that seen in the golden hamster (Clewe, 1966). The situation that prevails in the rat resembles most closely that seen in the golden hamster. The important difference, however, from the point of view of intraperitoneal tracer introduction, is that the periovarian bursa of the rat has on its ventromedial side a small opening by which the peritoneal cavity and the periovarian space may communicate. Due to this direct communication it was not necessary to surgically remove or retract the bursa in order to expose the ovarian epithelial surface more fully. Even though this procedure might have expedited tracer movement, it would have added unnecessary surgical complications to the procedure. These complications could be avoided by increasing the time of exposure to tracer, which would overcome the restriction on bulk fluid movement which a single small bursal opening would be expected to pose.

Immediately after severing its attachments the encapsulated ovary was removed and placed in fixative. The bursa was then carefully dissected away to promote a more rapid fixation of the germinal epithelium. After preliminary experiments, intravascular perfusion fixation was deemed to be of no added advantage in preservation of germinal epithelial cell ultrastructure. Like all epithelia, the germinal epithelium is not vascularized. Thus it would not be subject to the action of

the fixative by diffusion from blood vessels any sooner than it would be with immersion fixation.

In those anesthetized animals in which the ovaries were immersed in ferritin solution, the basic surgical procedure was similar to that just described. However, the period of time between exposing the ovaries and sacrificing the animal was extended to correspond to sacrifice times of animals receiving intraperitoneal tracer injections. After dividing the dorsal tendinous ligaments, adipose tissue surrounding each ovary and its adnexa was teased away. Each uterine horn, together with its attached oviduct and ovary, was raised so that the ovary could be placed in a small plastic container filled with ferritin solution (the container was intra-abdominally situated). The periovarian bursa was removed in some animals prior to this step. The small intestine was repositioned within the abdominal cavity and the cut edges of the incision approximated. After the desired period of immersion the ferritin solution was displaced by fixative. After brief in situ fixation the ovaries were removed and placed in fresh fixative for a further period of time.

After removal of the ovaries, all experiments were terminated by cutting the inferior vena cava and allowing the animals to exsanguinate.

The fixative used in this study consisted of a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer adjusted to pH 7.3 (a 1:1 dilution of the fixative described by Graham and Karnosvsky, 1966a). Ovaries were

fixed for 3-20 hr at room temperature (20° C). After fixation, the procedures used for processing tissues for light and transmission electron microscopy diverged from those used for preparing specimens for scanning electron microscopy.

For light and electron microscopic studies, fixed ovaries were embedded in 7% agar, sectioned on a Sorvall TC-2 tissue chopper at 200 μm, rinsed several times in cold 0.1 M cacodylate buffer, and stored in buffer at 4° C for 1-48 hr.

Sections from ovaries which had been exposed to HRP were then transferred from buffer to an incubation solution which contained the substrate for HRP. The solution consisted of 0.05% 3,3'-diaminobenzidine tetrahydrochloride in 0.05 M tris buffer containing 0.01% hydrogen peroxide at pH 7.6 (Graham and Karnovsky, 1966b). The sections were exposed for 40 min at room temperature and were then again rinsed in 0.1 M cacodylate buffer.

All sections were then post-fixed in buffered 1% osmium tetroxide for 1 hr, rinsed in distilled water, and stained *en bloc* in a saturated aqueous solution of uranyl acetate for 1 hr. Tissues were then dehydrated through an ascending ethanol series and propylene oxide, infiltrated *in vacuo*, and embedded in a 1:1 mixture of epon-araldite polymerized at 60° C.

Thick (0.5 μm) and thin (silver-grey) sections were cut on an LKB Ultrotome III, using either glass or diamond knives. Thick sections were stained with 1% aqueous toluidine blue and were used for preliminary light microscopic identification and orientation of areas of interest. Thin sections were mounted

on uncoated copper grids and examined in a Philips 200 or 300 electron microscope. All thin sections were first examined without additional membrane staining to avoid misinterpretation of results due to possible lead citrate staining artefacts. In the case of sections not stained with lead citrate, the operating voltage of the microscope was reduced from 60 kV to 40 kV to enhance contrast.

For scanning electron microscopic studies the following procedure was used. After initial fixation, ovaries were rinsed in several changes of cold 0.1 M cacodylate buffer. After being stored in buffer at 4° C for 1-48 hr, ovaries were post-fixed for 1 hr in buffered 1% osmium tetroxide. They were then rinsed in distilled water and dehydrated through an ascending ethanol series. The ethanol was then substituted by iso-amyl acetate by processing the tissues through an ethanol-iso-amyl acetate series of increasing iso-amyl acetate concentration. Iso-amyl acetate is a polar solvent miscible with carbon dioxide, which is used as the transitional fluid in the critical point drying procedure. Carbon dioxide prevents ice crystal formation during the procedure. Ovaries were critical point dried, mounted on aluminum stubs, and coated with gold in a vacuum evaporator. Specimens were examined in a Cambridge Stereoscan Model S4 microscope.

Control animals, representing the same age groups as the experimental animals, were also prepared for transmission and scanning electron microscopic examination by the methods just described. Control animals received either one of two treatments.

Some animals received no intraperitoneal injection corresponding to a tracer injection. They were anesthetized and their ovaries removed and processed for examination. This would be expected to show the normal structure of the germinal epithelium and reveal any endogenous ferritin or ferritin-like molecules. As well, when reacted in the incubation medium, any endogenous peroxidase activity would be shown. By comparison with the experimental results, any changes due to the tracer injection by virtue of either its volume or its composition would also be revealed.

The second group of animals received an intraperitoneal injection of isotonic saline of a volume similar to that of tracer administered to an animal of the same weight. After a period corresponding to the sacrifice time of an animal receiving a tracer injection, the ovaries of the control animal were processed. In this way any changes due to the saline could be detected. Any other changes seen in the germinal epithelium could then be attributed to the tracer itself or to the saline and tracer in combination with one another. As in the first control group, some of the ovarian tissue was also reacted for endogenous peroxidase activity.

RESULTS

A. Normal Germinal Epithelium

The following results apply to all animals, both pre- and postpubertal, receiving either no intraperitoneal injection (other than anesthetic) or an injection of saline alone.

1. General Epithelial Morphology

As seen with the light and transmission electron microscopes, ovarian germinal epithelium in section ranges from a simple squamous to a simple cuboidal type, with all possible intermediate variations (fig. 1). This finding was corroborated and extended by scanning electron microscopic studies which showed overall surface morphology of the germinal epithelial cells as well as the regional distribution of cell shape. This correlated with the degree of maturation of underlying follicles (see Discussion). Cuboidal cells are found in the "valleys" or crypts between follicles. The cuboidal cells give way in a graded fashion to squamous cells overlying the follicles (fig. 2). Subsequent examination of sectioned material showed that no cytological differences other than shape distinguish these two cell types. Typical examples of cuboidal and squamous cells are shown in figures 3 and 4.

In all specimens examined, the germinal epithelial cells seem to represent a continuous cellular covering for the ovary. Occasionally, in scanning electron micrographs, sites of apparent individual cell necrosis are seen (fig. 2). In contrast, more

extensive areas of denudation of epithelial cells have been observed in studies of the human ovary (Papadaki and Beilby, 1971).

The epithelial cells lie on a basement membrane of variable thickness (figs. 1,3,4). They are separated from the region of the developing follicles by the tunica albuginea ovarii in both pre- and postpubertal animals. No continuity is observed between the germinal epithelial cells and the follicular cells of the cortex. The basement membrane merges imperceptibly with the contents of the intercellular clefts and with the interstitial matrix of the tunica albuginea ovarii. Collagen fibres are randomly scattered throughout the basement membrane but seem to be more abundant in its deeper regions (figs. 1,3,4).

2. Cell Membrane Features

   a. Lateral cell membrane: intercellular clefts and junctions.

The most striking feature of the intercellular clefts of the germinal epithelium is the extreme variability of their course (figs. 1,3,4). This variability is a function of the interdigitation of cytoplasmic processes of irregular size and shape from adjacent cells. There is no common pattern for all intercellular clefts. A relatively short and direct cleft is the exception, and usually the clefts are tortuous, convoluted, or even labyrinthine in their complexity (fig. 5). Many of the cells may overlap one another for considerable distances.

The most obvious way in which germinal epithelial cells are adherent is via the interdigitating processes just mentioned.

This is a purely mechanical interlocking. Such union does not require the maintenance of a constant intercellular distance for its function, as do some more specialized types of junctions. Nonethelesss, membranes often parallel one another quite closely along the path of a cleft, regardless of its complexity. Thus a very tortuous cleft may display a fairly regular intercellular distance. However, in some clefts, especially along the basal half of their course, membranes are often greatly separated from each other. These regions sometimes look merely like large gaps. Usually, however, the appearance is suggestive of large polymorphic vacuoles either fusing with or budding off one or both of the membranes bounding the cleft (figs. 1,3). The density of the contents of these "vacuoles" is identical to that of the intercellular substance of the more closely approximated regions of the clefts. Therefore, these areas probably represent sections through large spaces which exist due to irregularities in the shape and pattern of interlocking cytoplasmic processes.

Pinocytotic invaginations and vesicles appear quite often along the course of the membranes bounding the intercellular clefts. These will be discussed further in section 4, under Organellar Structure.

Small punctate regions or foci of very close membrane approximation, and in some cases fusion, are frequently seen along some of the clefts (fig. 6). Their appearance in section suggests that they may represent macular forms of gap junctions or occluding junctions, or sections of zonular junctions of the same types. Often there is an increase in the cytoplasmic

density subjacent to this focal type of junction. There are often multiple randomly scattered foci along a cleft, their numbers usually being greater near the peritoneal end. Less numerous are extended versions of the focal junctions just described, which similarly may be multiple along a single cleft (fig. 7). Again there is a difficulty in ascertaining whether adjacent membranes are just extremely close or actually fused. Occasionally, long regions of apparent membrane fusion are seen (fig. 8). These are similar to those observed in mesothelium by Cotran and Karnovsky (1968). However, even these have sometimes been resolved to actually be composed of two distinct cell membranes separated by a small but definite intercellular space (fig. 9).

b. Apical cell membrane.

Cell apical surface modifications appear mainly as microvilli and pinocytotic vesicles and invaginations. The numbers and distribution of microvilli are quite random from cell to cell and are best appreciated with scanning electron micrographs of the cell free surface (fig. 10). In section the microvilli are seen to contain ground cytoplasm and an internal skeleton of microfilaments parallel to the long axis. Pinocytotic invaginations are seen to open on to the free surface in both transmission and scanning electron micrographs (figs. 3,4,11). The size of the invaginations is variable, as is their distribution. Irregular cytoplasmic evaginations of the free surface, as described in other species (Gondos, 1969; Papadaki and Beilby, 1971), are sometimes seen. Cilia are seen only infrequently,

even in scanning electron microscopic studies.

c. Basal cell membrane.

The contour of the cell basal surface varies from cell to cell, being relatively straight in some and highly irregular in others. Pinocytotic vesicles open on to this surface, although they are fewer in number than at the free surface. There are no junctional specializations along the basal membrane.

3. Nuclear Structure

Germinal epithelial cell nuclear shape reflects that of the cell (figs. 3,4). Nuclei have overall shapes in section ranging from flat and elongated to almost circular. The relative proportion of the cell occupied by the nucleus varies with the plane of section. The nuclear contour is usually irregular (figs. 1,4). Cytoplasmic projections of varying dimensions extend into the nucleus (fig. 1), and sometimes cause the nucleus to assume a pseudolobulated form. Mitochondria and vesicles are sometimes seen in these extensions. The nucleus is delineated by the usual bilamellate nuclear envelope, along which nuclear pores are found. Some of these pores are closed by a thin diaphragm. Ribosomes are adherent to the outer lamella of the envelope (fig. 7). Most of the chromatin is circumferentially distributed against the inner aspect of the nuclear envelope, with some located as coarse clumps throughout the nucleus (figs. 1,3,4). All nuclei examined were in the interphase state. Nucleoli are rarely cut in section (fig. 4).

4. Organellar Structure

Rough endoplasmic reticulum is found in variable amounts throughout the germinal epithelial cell cytoplasm. It is usually closely associated with mitochondria, with which it may form large aggregates (figs. 1,3,4,7). The contents of the cisternae appear to be of about the same density as the cytoplasm. The degree of dilation of the cisternae varies both within a cell and from cell to cell. Smooth endoplasmic reticulum is very seldom seen.

Both the mitochondria related to the rough endoplasmic reticulum and those distributed randomly throughout the cytoplasm have a similar appearance. The matrix is denser than the surrounding cytoplasm and the contents of most vesicles, and in section mitochondrial shape varies from circular to ovoid to elongate (figs. 3,4). Cristae appear as flattened lamellae whose orientation within the mitochondria is not specific.

The Golgi complex consists of a variable number of lamellae of different shape and size. Usually only one Golgi complex is observed in each cell. It is situated in a lateral or superior paranuclear position in most instances. It is not observed between the nucleus and the basal cell surface. Vesicles presumably derived from the Golgi complex are seen about its entire perimeter and are not preferentially related to any particular aspect of the complex (fig. 4 inset). The degree of filling and electron density of the contents of these vesicles is variable.

Ribosomes are very numerous. As well as being closely

associated with the endoplasmic reticulum and nuclear envelope, they are observed in large numbers free in the cytoplasm and occasionally in polyribosomal groupings.

Cytoplasmic vesicles are seen throughout the cell and at all cell surfaces. They are of highly diverse size and shape. Many of the vesicles are near the apical and lateral cell surfaces and may be derived from pinocytotic activity. They could also be pinocytotic invaginations sectioned in a plane other than that in which their opening to a surface can be seen. The amount and distribution of electron dense material within a vesicle ranges considerably, as previously noted for Golgi vesicles. Some vesicles contain what appear to be smaller vesicles inside them, thus forming multivesicular bodies (fig. 4 inset). Somewhat denser structures similar to these have been identified as autophagic vacuoles in human germinal epithelium (Papadaki and Beilby, 1971).

Cytoplasmic inclusions which may represent lipid stores are seen in some cells (figs. 3,4). Their size and distribution within a germinal epithelial cell is not constant and in section usually no more than two or three are ever noted within a cell. The subjacent stromal and granulosa cells usually contain more lipid inclusions than the epithelial cells.

Microfilaments are seen in the cytoplasm of some germinal epithelial cells, usually as bundles with no particular orientation.

B. Tracer Experiments

In all animals exposed to tracer, either HRP or ferritin, no departure from the normal ultrastructure of the germinal epithelium or subjacent ovarian tissue is seen. The only difference noted is the presence of tracers within these tissues. Although both HRP and ferritin appear in the ovary within 45 min of exposure to tracer, they differ markedly in their depth of penetration. HRP is found not only in the germinal epithelium but also deep within the tunica albuginea ovarii within this period of time. Ferritin on the other hand appears only in germinal epithelial cells at the end of 45 min, and requires several hours before it appears in the cells and interstitium of the tunica albuginea ovarii.

Endogenous peroxidase activity is limited to the cytoplasm of erythrocytes within ovarian blood vessels. No electron dense particles resembling ferritin are seen.

1. HRP Distribution

HRP itself can not actually be seen very well in an electron micrograph. Rather it is the electron dense enzymatic reaction product which is visualized as the tracer. Adhering to convention, however, the reaction product will be referred to as HRP. The visibility of the reaction product is further enhanced after its reaction with osmium tetroxide during post-fixation (Graham and Karnovsky, 1966b).

HRP is observed on the germinal epithelial cell apical surface, in the intercellular clefts, in pinocytotic invagina-

tions, at all epithelial (and some fibroblast) cell surfaces, in vesicles and multivesicular bodies of epithelial cells and fibroblasts, in the basement membrane, and in the interstitium of the tunica albuginea ovarii (fig. 12). The pattern of tracer uptake was similar in all experiments.

HRP is observed to be most abundant in the extracellular regions of the ovary. There is a marked extracellular gradient of HRP density from the epithelial free surface through the intercellular clefts, basement membrane, and interstitium (fig. 13). The extent of the gradient varies with the time of exposure to HRP. Collagen fibres are especially well visualized in the basement membrane and interstitium due to the negative staining effect of HRP.

Pinocytotic invaginations containing variable amounts of HRP are seen at all surfaces of the germinal epithelial cell, particularly the apical and lateral surfaces. Such invaginations are also seen, but much less frequently, at the epithelial cell basal surface and at the surface of fibroblasts. Occasionally HRP was not seen at the apical surface, even though it appeared elsewhere throughout the epithelium and subjacent tissues (fig. 14). This local removal of HRP was probably attributable to excessive washing of tissue in buffer prior to post-fixation, and has been noted in other studies where HRP has been used as a tracer (Cotran and Karnovsky, 1968).

Vesicles containing HRP are found throughout germinal epithelial cells. They constitute a variable but reasonably

small proportion of the total number of cell vesicles. Even after exposure to HRP for 5 hrs, the proportion of tracer-containing vesicles was still quite small. Some vesicles are completely filled with the tracer, which appears either to be amorphous or to have a coarse granular appearance. In other vesicles which are not completely filled with HRP, the tracer appears as small clumps similar to those often seen at the cell apical surface, or as an inner circumferential coating. Vesicles containing HRP are sometimes seen in fibroblasts (fig. 12), but not in capillary endothelial cells.

Peripherally, vesicles are more numerous near the apical and lateral cell surfaces than the basal surface. Apical surface vesicles are of a fairly uniform size and contour, as are those at the basal surface. Vesicles near the lateral cell surface may either be similar to those seen at the other cell surfaces, or they may be quite variable in size and shape. The distribution of vesicles at the apical and lateral surfaces parallels that of the pinocytotic invaginations described earlier. After excessive buffer washing the proportion of apical surface vesicles containing HRP is greatly reduced (fig. 14). This would not be expected of true vesicles and suggests that many so-called "vesicles" devoid of tracer subsequent to excessive washing are in fact pinocytotic invaginations continuous with the cell apical surface in another plane. There are, however, other tracer-containing vesicles within the cells whose contents are not removed by prolonged washing (fig. 12). Of these vesicles some are quite close to the lateral cell surfaces

bounding the intercellular clefts. Certain of these could be true vesicles derived from the lateral cell surface, whereas others could be invaginations similar to those seen at the apical surface. In either case they could have been filled with tracer by diffusion from the intercellular clefts. The possibility exists that they could also be vesicles from the apical surface emptying their contents into the intercellular clefts.

As earlier observed, the irregular interlocking of cytoplasmic processes from adjacent epithelial cells often gives rise to the appearance of large invaginations into the cell from the lateral cell surfaces. Sectioning through such an invagination in a plane other than that in which it is continuous with the membrane of the intercellular cleft may cause the artefactual appearance of tracer-filled vesicles of variable contour. Some of these "vesicles"may be quite removed from the lateral cell surface, depending on the length of the invagination. As seen in fig. 15, these invaginations can be very long. In this particular case, sectioning in another plane could easily result in the creation of an apparent vesicle deep within the cell. On occasion the appearance is also created of large tracer-filled vesicles to which are connected smaller vesicles. These smaller vesicles seem to be either forming from, or emptying their contents into, the large vesicle (fig. 15 inset). However, such large vesicles are always situated near intercellular clefts, never deep within a cell. They are also seen in control material.

There still remain a number of tracer-filled vesicles within the cell which are so situated that it would be difficult to envisage their connection with any of the cell surfaces. They are on average smaller and more regular in outline than would be expected of "vesicles" derived from section of lateral surface invaginations. One would have to invoke the existence of very long and numerous surface membrane invaginations of small and regular diameter penetrating deeply into the cell which, when cut in cross-section, would appear as vesicles. If this were the case, then oblique and longitudinal sections of such channels should be seen in some micrographs, and they are not. To completely eliminate this possibility serial sections were examined. This demonstrated the independence of these vesicles from any cell surface. There are thus some tracer-filled vesicles which would seem to constitute definite evidence of the vesicular uptake of HRP. Few vesicles are seen to open onto the basement membrane, suggesting that if tracer transport does occur, it is either not in the direction of the basal surface or occurs to only a minor extent in that direction. It is not possible to tell whether or not such vesicles actively transport tracer from the cell apical surface or to or from the cell lateral surfaces.

Within the intercellular clefts HRP may be reasonably homogeneous (figs. 16,17), or it may present a granular appearance (fig. 18). The presence of the tracer within the clefts emphasizes their tortuosity. The continuity of HRP from the cell free surface to the basement membrane varies within different

clefts. It seems to be a function of the presence of junctions of the types mentioned earlier (compare figs. 16,17,18). Unfortunately, the presence of HRP within a cleft often seems to obscure the lateral membrane surfaces. Thus junctional specializations may be difficult to discern and interpret along such clefts. In some clefts where definite focal junctions or extended versions thereof can be seen, HRP seems to be found on either side of the point of close cell approximation. It cannot be distinguished whether HRP is present within the junctional area itself or whether it has diffused around the junction to appear on both sides of it (fig. 17). When two or more focal junctions are seen along a cleft, the region between adjacent junctions is sometimes devoid of tracer (fig. 18). This suggests that some junctions are definitely not permeable to HRP. It also suggests that these particular junctions are not simple maculae occludentes, otherwise HRP could have diffused around them to fill the intervening space. These junctions may perhaps represent cross sections through an annular junction between adjacent lateral cell surfaces. Such a local ring of occlusion would prevent HRP from being localized in its interior and would lead to the appearance of a region such as shown in fig. 18. Again, freeze-fracture studies would probably be the most suitable method for positively establishing the nature of the junctions of the germinal epithelium.

2. Ferritin Distribution

Ferritin localization in ovaries of animals injected with

ferritin and in animals whose ovaries were directly immersed in ferritin is essentially similar. It is found intra- and extracellularly, with the notable exception that it is not found in the intercellular clefts of the germinal epithelium.

Subsequent to ferritin exposure, a variable number of germinal epithelial cells are seen to contain ferritin within membrane-bounded vesicles and multivesicular bodies (fig. 19). Ferritin molecules are not usually observed in epithelial intercellular clefts (see Discussion). Some fibroblasts of the tunica albuginea (fig. 20) and some capillary endothelial cells (fig. 21) also contain ferritin within vesicles. A possible sequence of the pinocytotic events underlying ferritin uptake is shown in fig. 22. It is realized that the vesicle containing ferritin in fig. 22 could actually be an invagination sectioned in a plane in which it is not connected to the surface. Nevertheless, as ferritin is found deep to the germinal epithelium and is probably not transported through the intercellular clefts (see Discussion), it is assumed that the sequence shown in fig. 22 actually mirrors the initial events in vesicular uptake of ferritin. Occasionally, epithelial cells and fibroblasts appear to contain free ferritin molecules in the cytoplasm. Ferritin is sometimes seen in granulosa cell vesicles, but not in the zona pellucida or in oocytes. Blood plasma within capillaries and venules of the tunica albuginea (fig. 21) and follicular fluid (fig. 23) are frequently seen to contain free ferritin molecules. Ferritin molecules are always seen to be discrete particles. They do not form the type of amorphous clumps

characteristic of HRP.

Both the number and distribution of ferritin-containing vesicles within a cell and the number and distribution of ferritin molecules within a single vesicle exhibit wide variation. In spite of their variable numbers, ferritin-containing vesicles rarely represent more than a relatively small proportion of all cell vesicles. The number of ferritin molecules within a vesicle ranges from as few as one to as many as several thousand, and both extremes may occur in a single cell. Vesicles with the highest ferritin concentration are found in the fibroblasts of the tunica albuginea ovarii (fig. 20). Vesicles of comparable size were not as frequently observed in control material. There is not always a direct correlation between the size of a vesicle and the number of ferritin molecules within it. Often a vesicle of large dimensions contains fewer molecules than a much smaller vesicle. On average, ferritin-containing vesicles seem to be larger than other cell vesicles. This could indicate that they arise from fusion of smaller vesicles, as large vesicles are not seen to open to the cell apical surface directly. In contrast to the HRP experiments, ferritin is not found in vesicles or invaginations associated with the membranes bounding the intercellular clefts. This would seem to support the argument that vesicles from the apical surface probably do not empty into the clefts.

Even though large vesicles were not often seen to empty into the basement membrane, the appearance of ferritin in the deeper regions of the ovary and its virtual absence in the intercellular clefts would seem to imply that vesicular transport

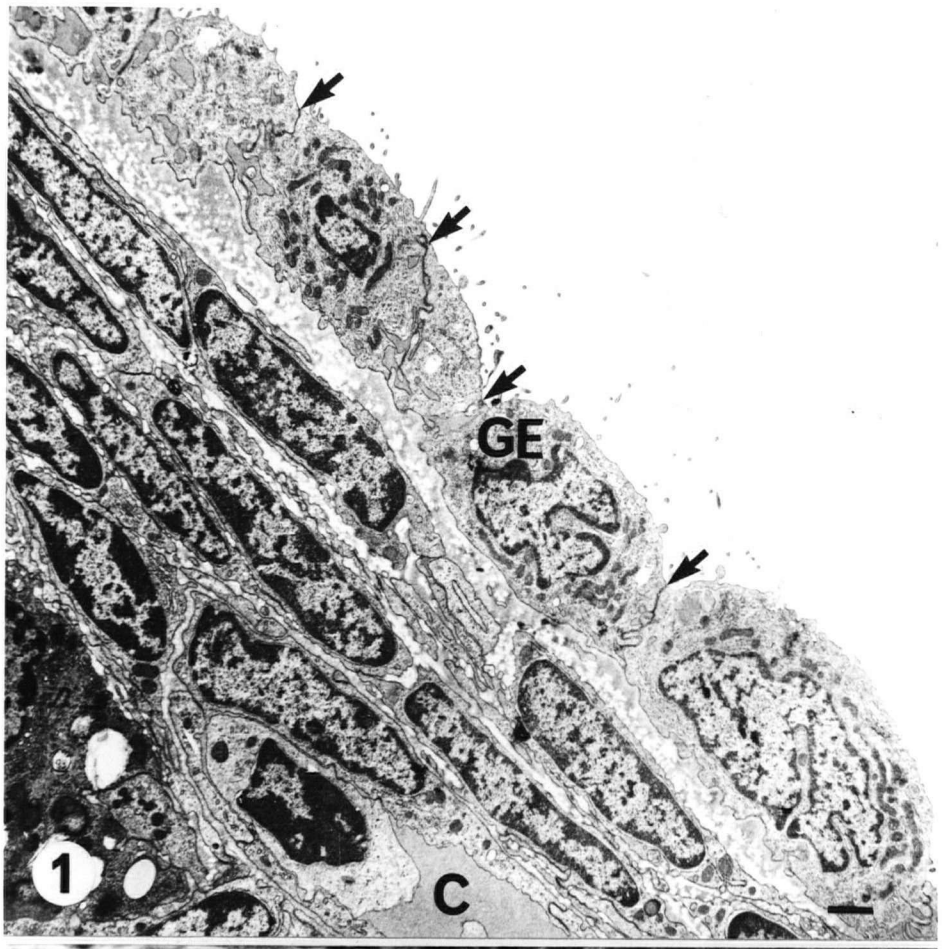
is the method by which ferritin leaves the germinal epithelial cells.

The distribution of free ferritin molecules in the basement membrane, interstitium, blood plasma, and follicular fluid seems to be random. No preferred routes of tracer movement are evident. Ferritin is extracellularly much less abundant than HRP and, because of its sparseness, does not assume an obvious gradient of distribution like that of HRP. Even though much more ferritin is contained intracellularly within vesicles, no cellular gradient of tracer distribution is evident. This is due to vesicle variation in number, size, and ferritin content both within a cell and from cell to cell. However, the depth to which ferritin penetrates the ovary increases as time of exposure to ferritin increases. This is indicated by its presence within fibroblast vesicles of successively deeper regions of the tunica albuginea (fig. 20) and within the follicular fluid of developing follicles of the outer cortex (fig. 23). Ferritin penetration thus seems to be a time-dependent process.

C. Figures

Figure 1: Overview of germinal epithelium and part of underlying tunica albuginea ovarii. One germinal epithelial cell is indicated (GE). Note the irregular nuclear contour in these cells, the complex intercellular clefts (arrows), and the underlying basement membrane of variable thickness. The presence of a capillary (C) very near to the surface is also seen. Scale bar in this and all succeeding micrographs indicates 1 μm. Uranyl acetate and lead citrate staining. X 5,800.

Figure 2: Scanning electron micrograph of the ovarian epithelial surface showing the regional distribution of cell shape. Sites of apparent individual cell necrosis are indicated (arrows). It is not known whether such sites occur normally in the germinal epithelium or whether they are artefactual. X 420.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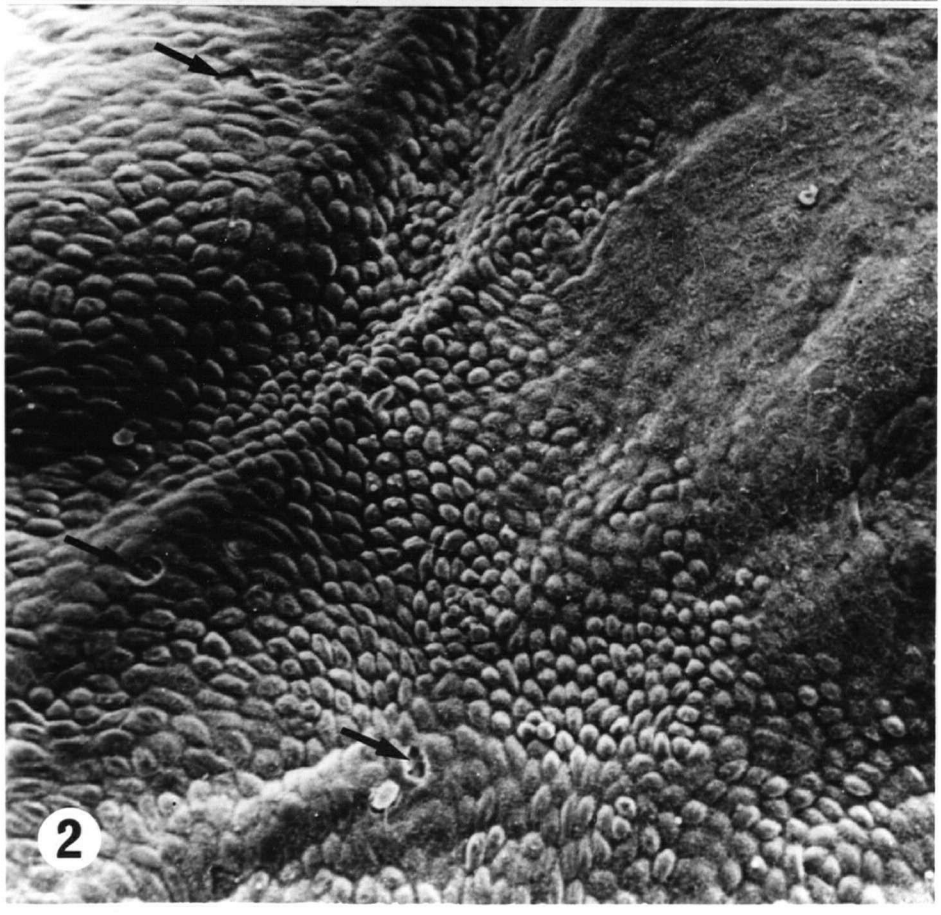
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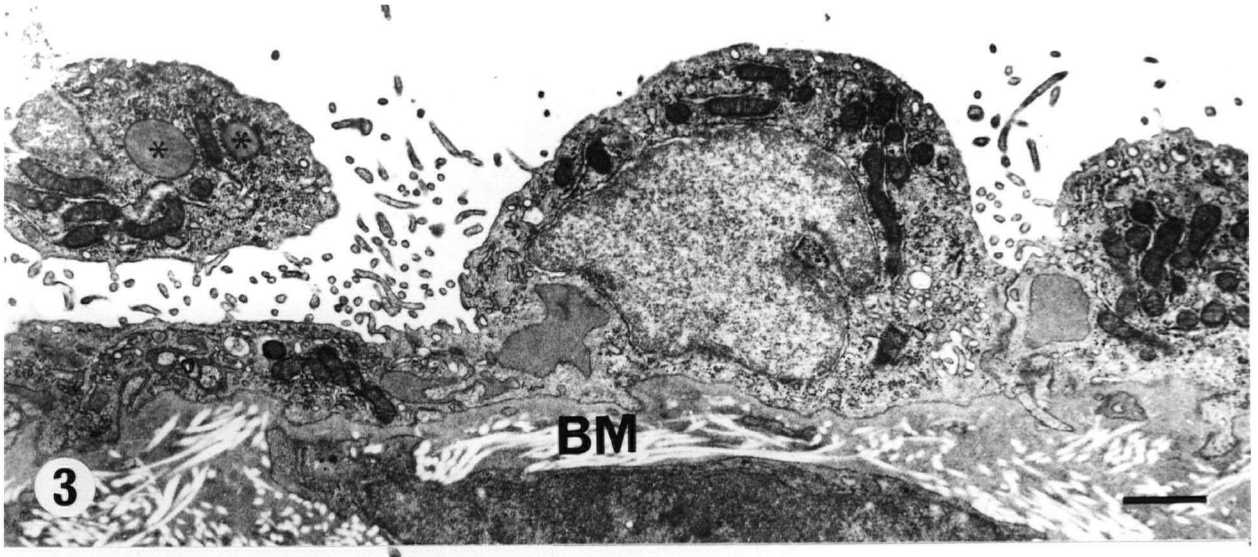
Figure 3: Typical cuboidal germinal epithelial cells. Cell on left, which appears to be detached from rest of epithelium due to plane of sectioning, contains lipid inclusions (asterisks). Rough endoplasmic reticulum, ribosomes, and mitochondria are abundant. Microvilli are also numerous. Note random arrangement of collagen fibers in basement membrane (BM) and highly variable course and appearance of intercellular clefts. Uranyl acetate and lead citrate staining. X 10,600.

Figure 4: Typical squamous germinal epithelial cell containing same organelles as cuboidal cell in fig. 3. A nucleolus (N) is also present. Uranyl acetate and lead citrate staining. X 10,600.
Inset shows Golgi complex (G), mitochondria, and numerous intracellular vesicles of variable content and electron density. A multivesicular body (arrow) is also seen. Uranyl acetate and lead citrate staining. X 19,000.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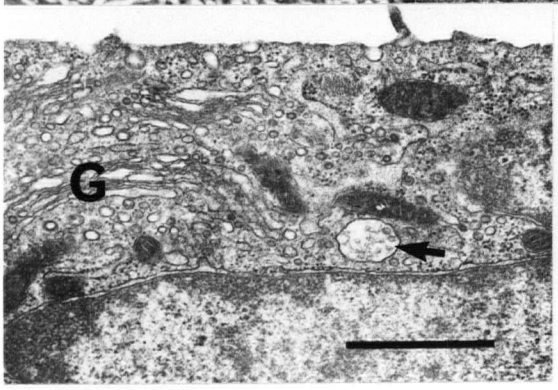
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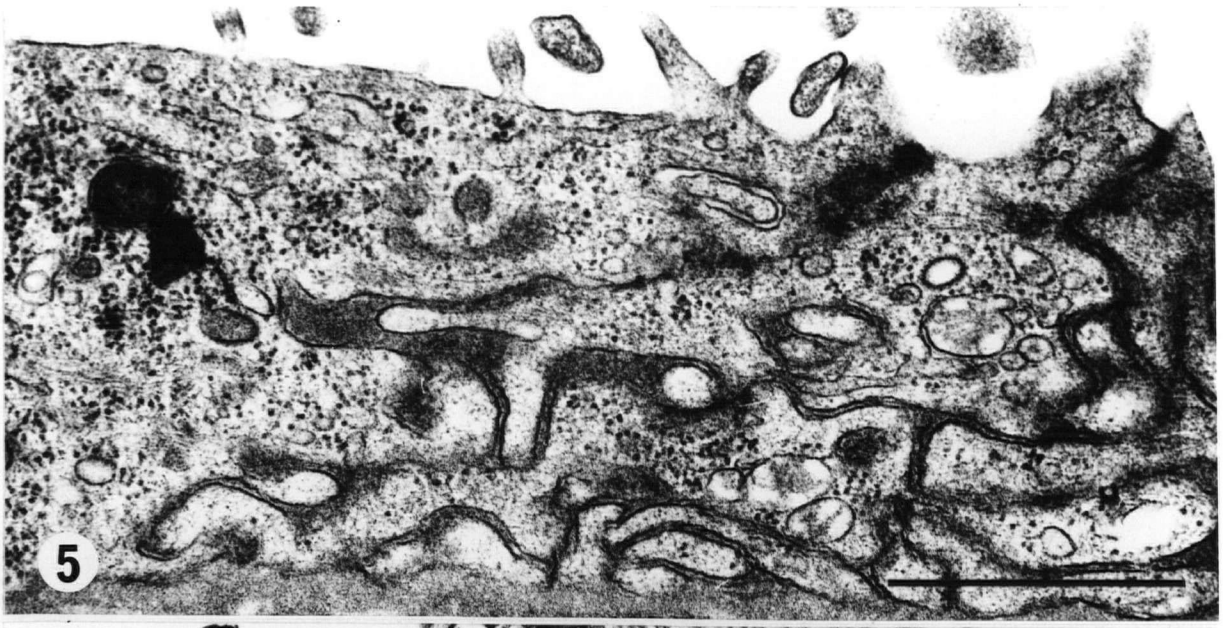
Figure 5: Germinal epithelium. Intercellular cleft, showing complexity of interdigitation of adjacent cell surfaces. Uranyl acetate and lead citrate staining. X 37,800.

Figure 6: Germinal epithelium. Small punctate junctions, which may be gap junctions or macular tight junctions, are indicated (arrows). Uranyl acetate and lead citrate staining. X 56,400.

Figure 7: Germinal epithelium. Extended regions of cell approximation are shown (arrows). These may be gap junctions. Uranyl acetate and lead citrate staining. X 32,400.

Figure 8: Germinal epithelium. Long region of apparent membrane fusion, creating appearance of a pentalaminar junction. Uranyl acetate and lead citrate staining. X 65,200.

Figure 9: Germinal epithelium. Detailed structure of a junction which appeared to be pentalaminar at a lower magnification. A definite space can be seen to separate the two external leaflets of apposing cell membranes. Uranyl acetate and lead citrate staining. X 235,000.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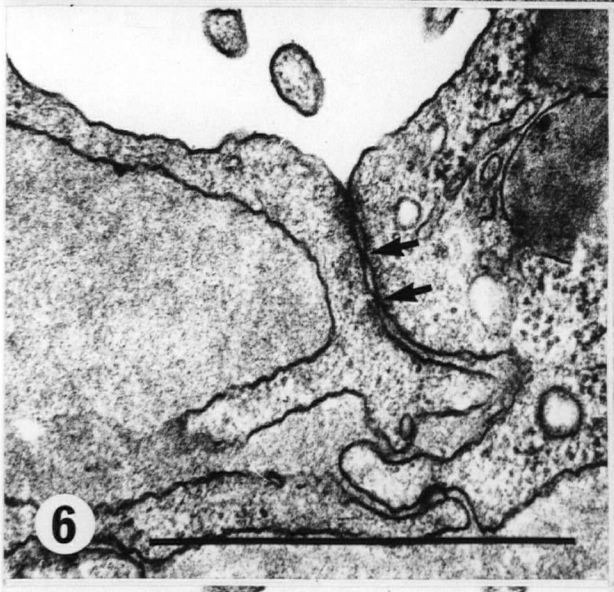
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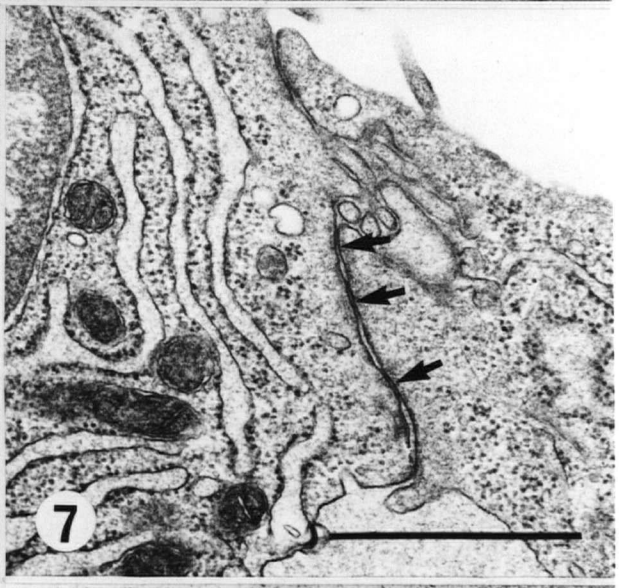
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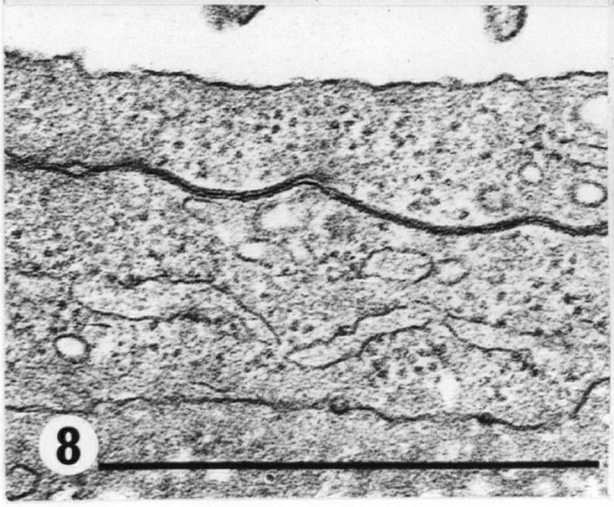
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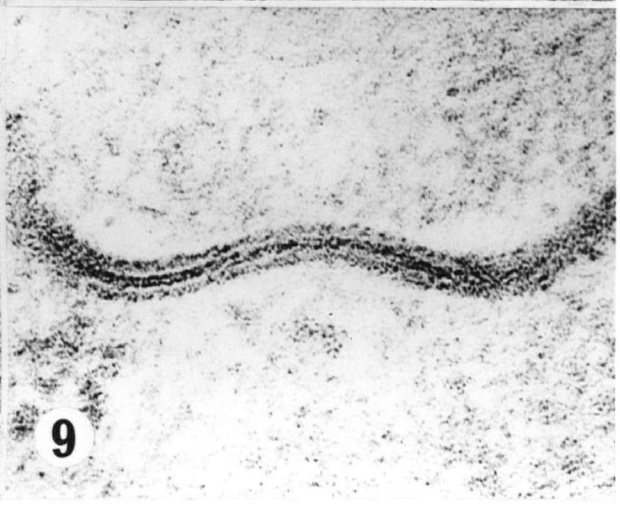
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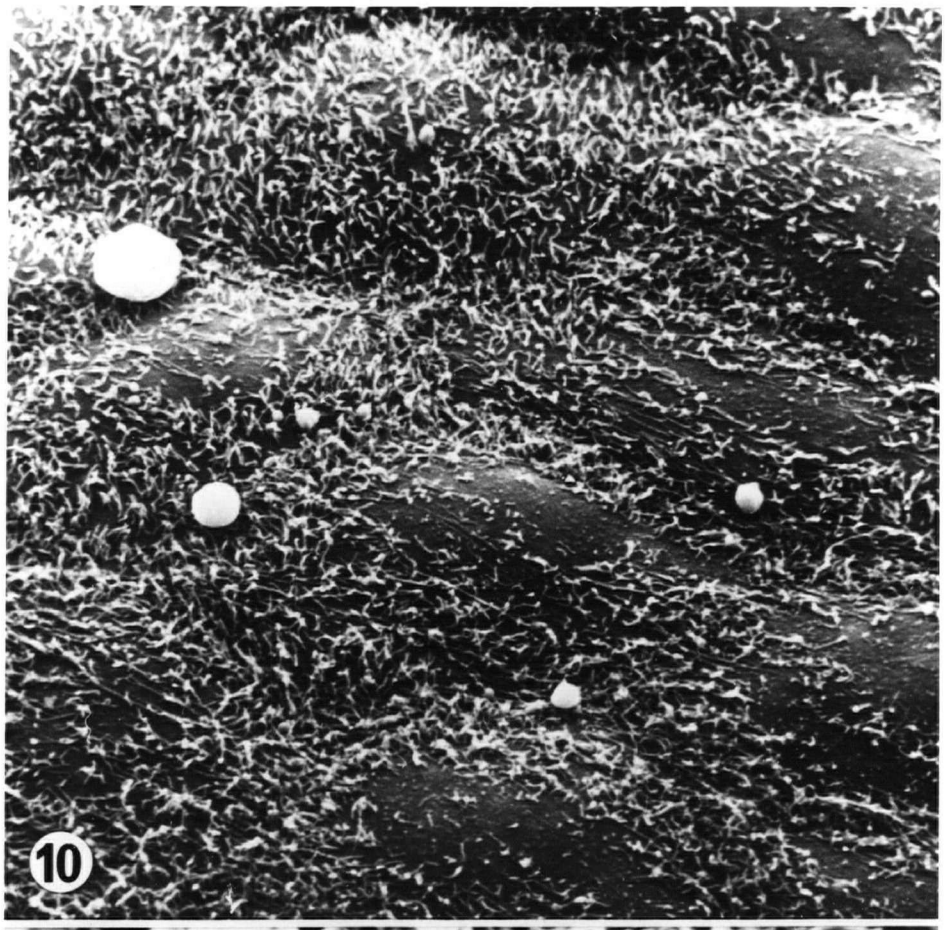
Figure 10: Scanning electron micrograph of the germinal epithelium, showing the numbers and distribution of microvilli. X 3,000.

Figure 11: Scanning electron micrograph showing pinocytotic invaginations of a germinal epithelial cell apical surface. Microvilli can also be seen. X 15,000.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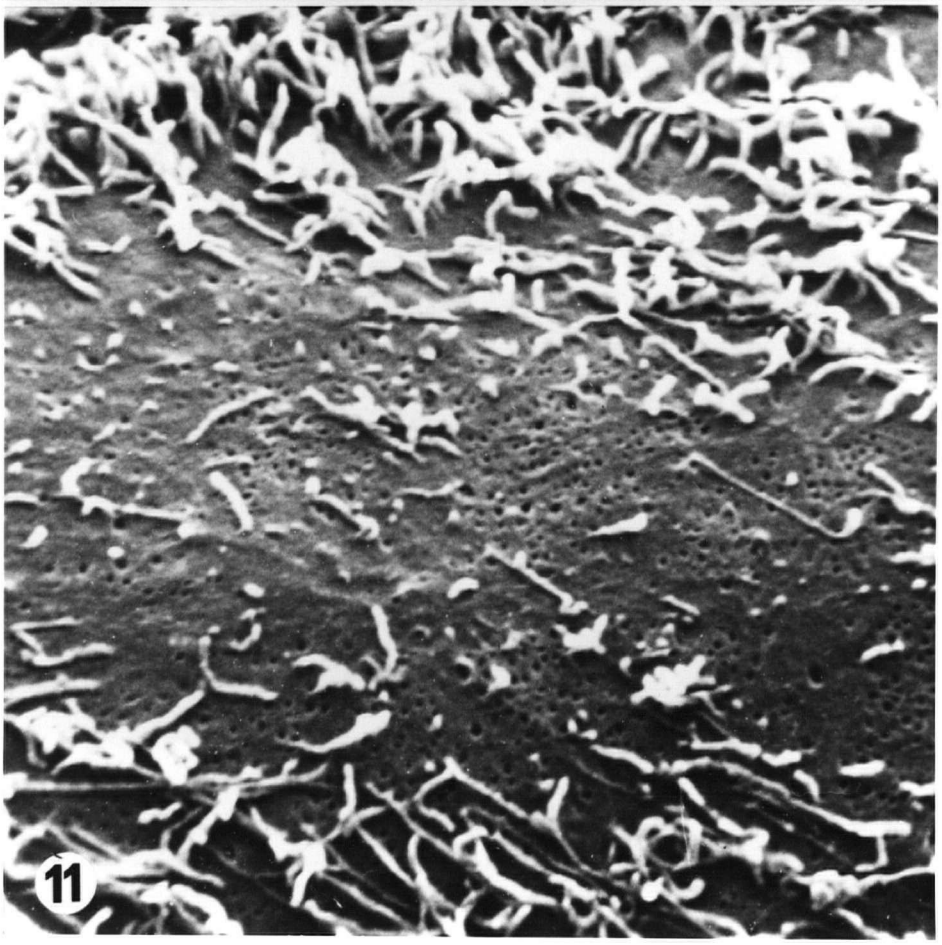
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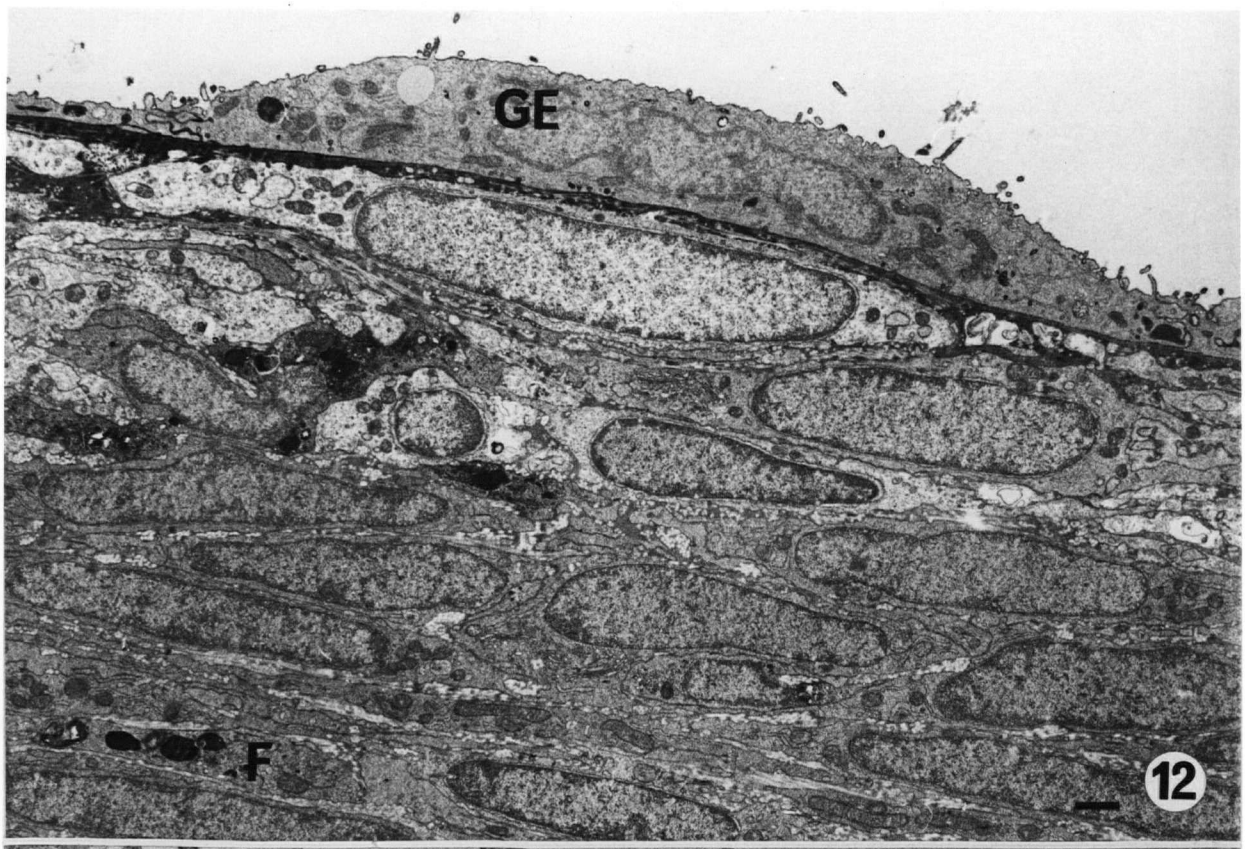
Figure 12: Overall distribution of HRP. Note its presence on apical surface, in intercellular clefts, in pinocytotic invaginations and vesicles of germinal epithelial cells (GE) and fibroblasts (F), in basement membrane, and in interstitium of tunica albuginea ovarii. Uranyl acetate and lead citrate staining. X 5,800.

Figure 13: Extracellular gradient of HRP. Density of HRP decreases from germinal epithelial cell (GE) apical surface through intercellular clefts, basement membrane, and interstitium. Uranyl acetate and lead citrate staining. X 16,600. Inset shows intercellular clefts (arrows) of a control specimen, similarly stained with uranyl acetate and lead citrate, for purposes of comparison re. electron density of clefts. X 14,300.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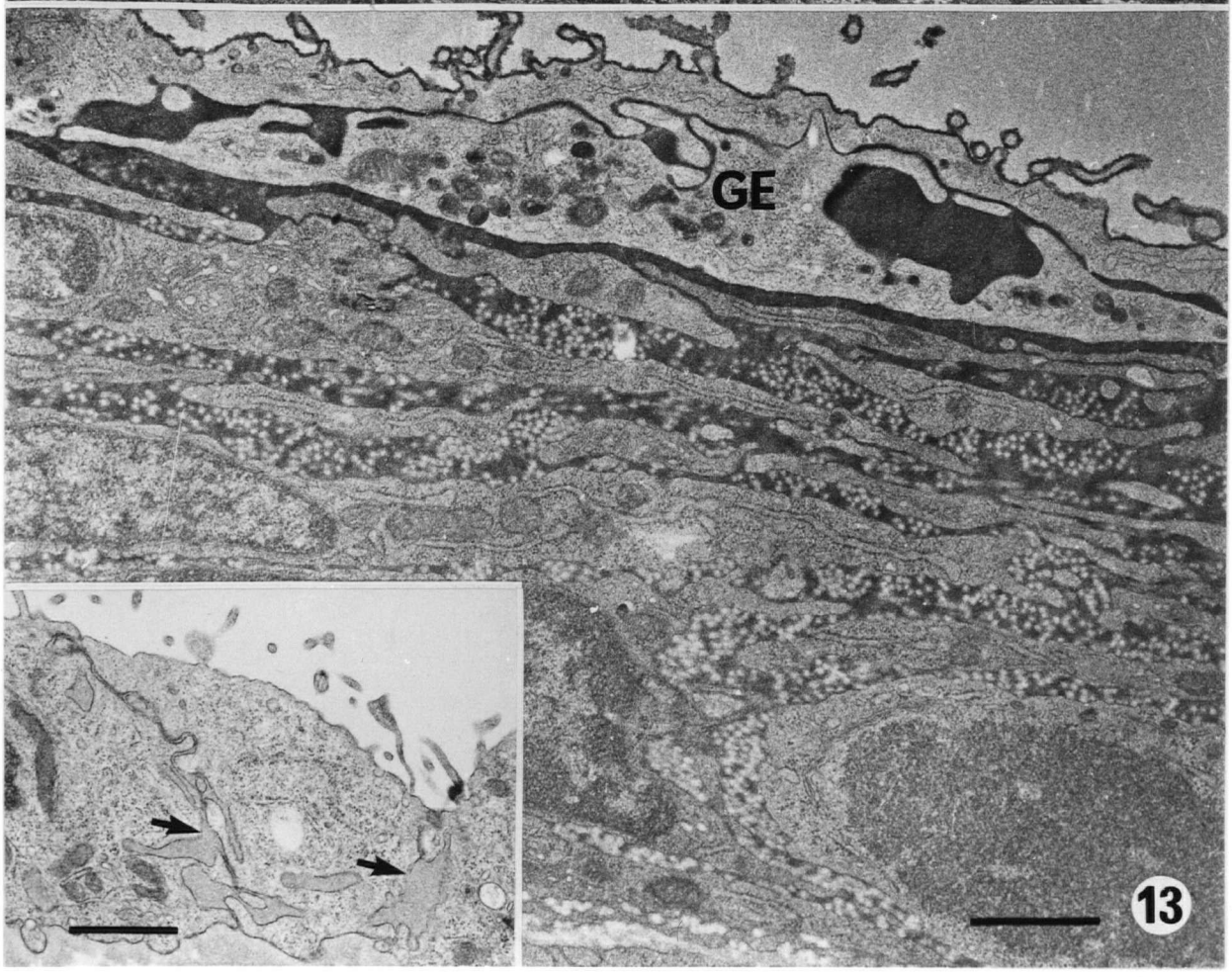
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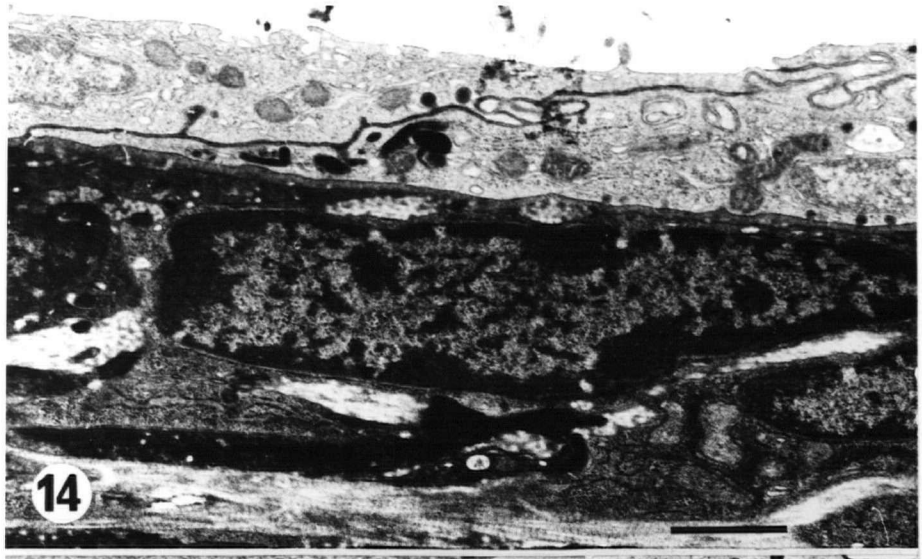
Figure 14: Micrograph illustrates removal of HRP from germinal epithelial cell apical surface after excessive buffer washing. Note that HRP is still found in deeper regions of germinal epithelium and subjacent tissues. Uranyl acetate and lead citrate staining. X 14,500.

Figure 15: Germinal epithelium. Note extended invagination of intercellular cleft (asterisk). Sectioning in another plane could result in creation of an apparent HRP-containing vesicle deep within cell. Uranyl acetate and lead citrate staining. X 24,300. Inset shows large "vesicle" (arrow) near intercellular cleft to which are connected smaller vesicles. Uranyl acetate and lead citrate staining. X 19,400.

Figure 16: Intercellular cleft showing continuity of HRP from peritoneal surface above to basement membrane below. Tracer-containing vesicular profiles (arrows) may actually be pinocytotic invaginations similar to those seen at the apical and basal surfaces of the germinal epithelial cells. Uranyl acetate and lead citrate staining. X 73,200.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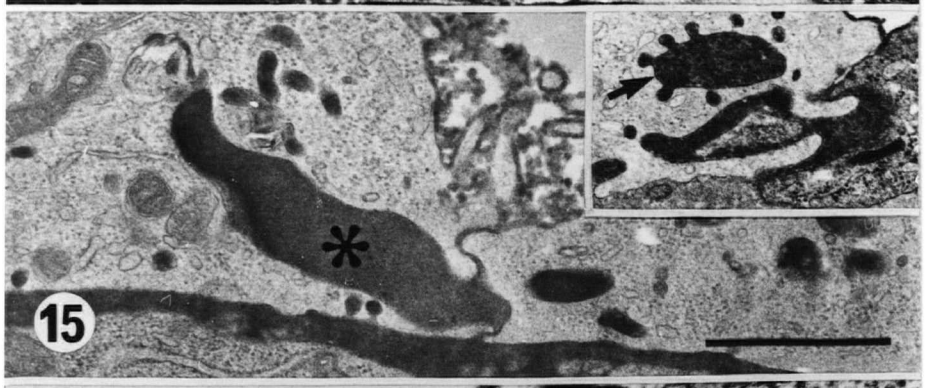
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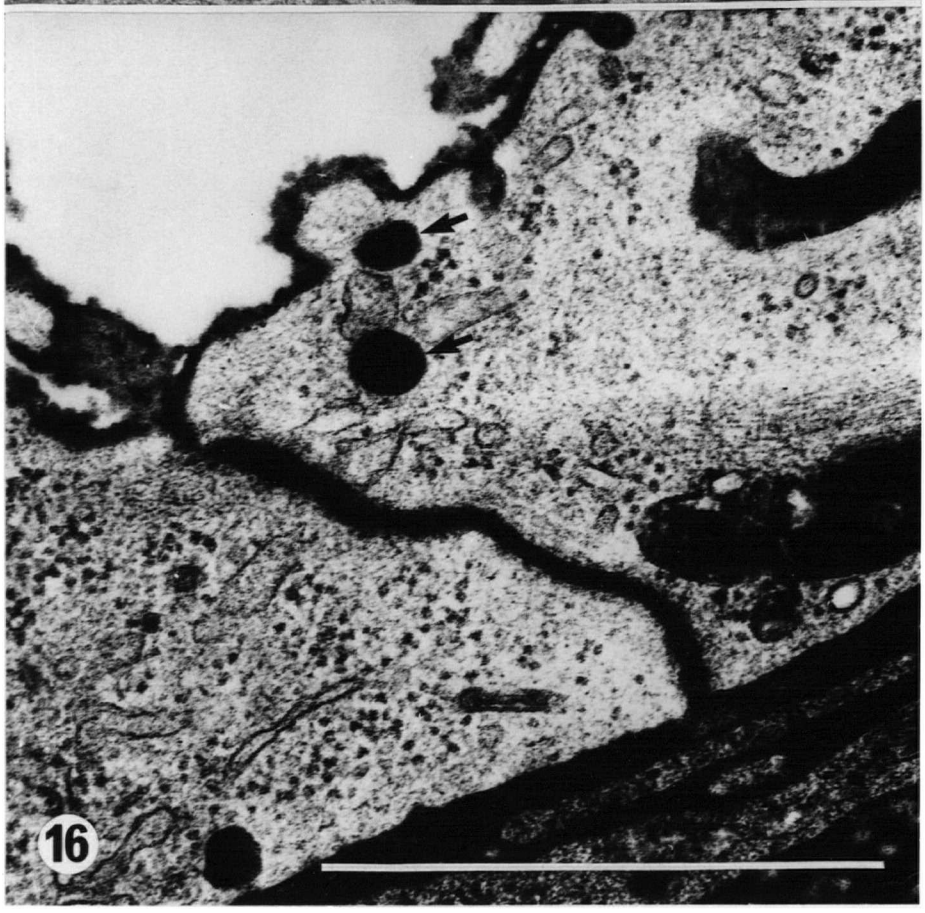
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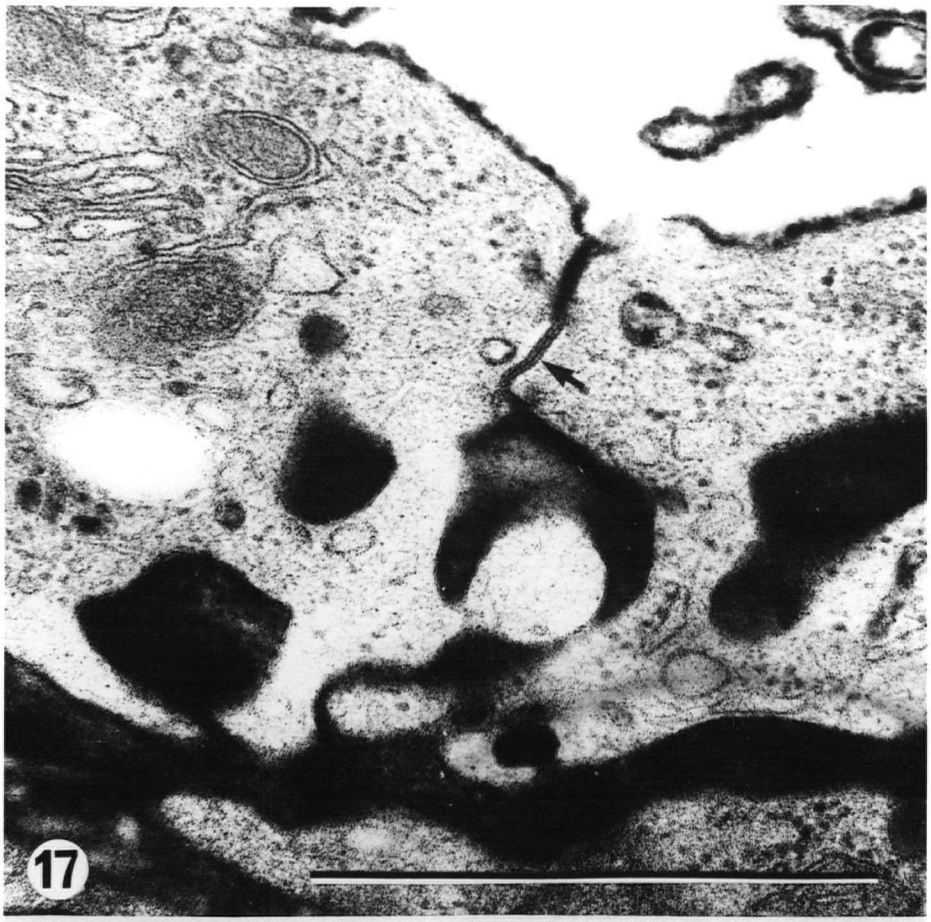
Figure 17: Germinal epithelium. Intercellular cleft showing possible region of membrane fusion (arrow) near apical end. Note the deposition of HRP in adjacent areas. Uranyl acetate and lead citrate staining. X 73,200.

Figure 18: Intercellular cleft showing region of exclusion of HRP (arrow), suggesting that some junctions are not permeable to this tracer. Vesicle (V) in lower right corner is a variation of that seen in fig. 15 inset. Uranyl acetate and lead citrate staining. X 73,200.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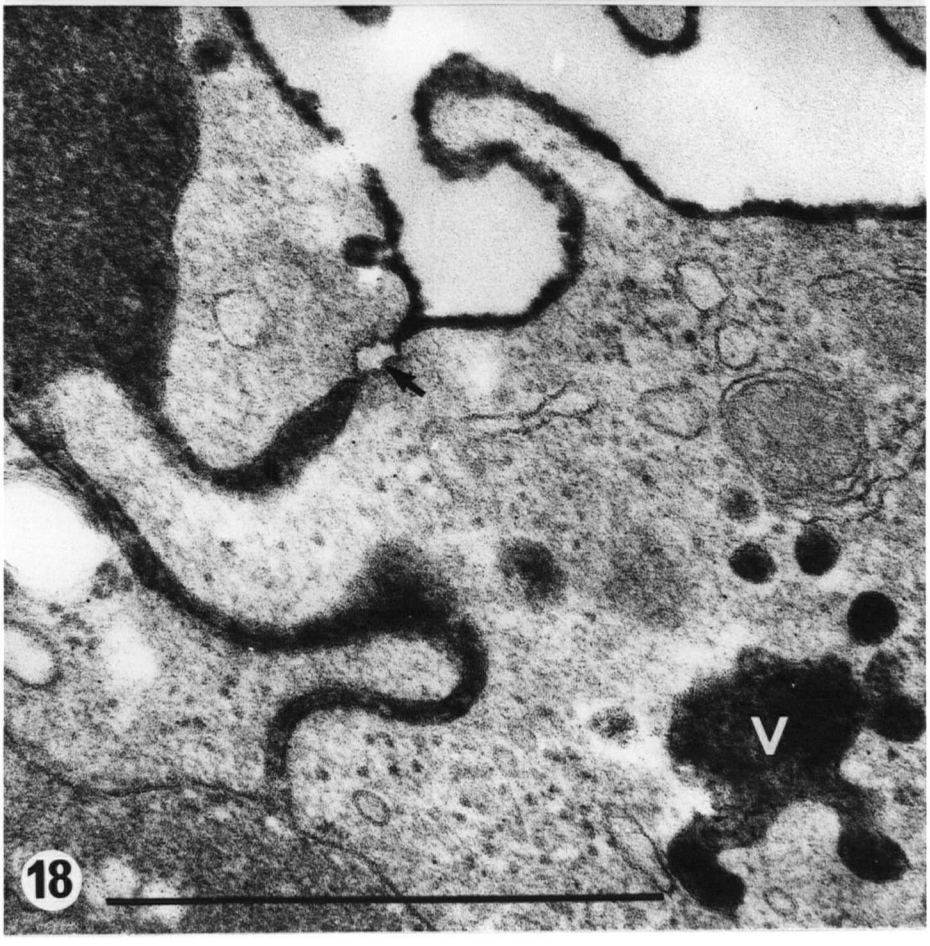
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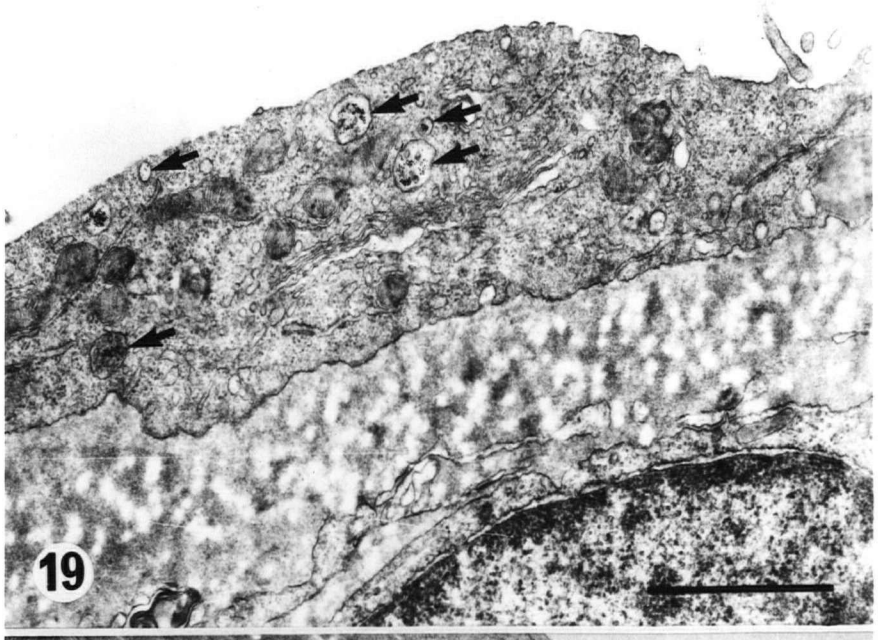
Figure 19: Germinal epithelial cell (above) showing vesicles and multivesicular bodies containing ferritin (arrows). Free ferritin particles are also seen in the underlying basement membrane. Uranyl acetate staining. X 24,300.

Figure 20: Overview of germinal epithelium (GE) and subjacent tissues. The presence of large ferritin-containing vesicles (arrows) in fibroblasts (F) of the tunica albuginea ovarii is well demonstrated. Uranyl acetate staining. X 19,400.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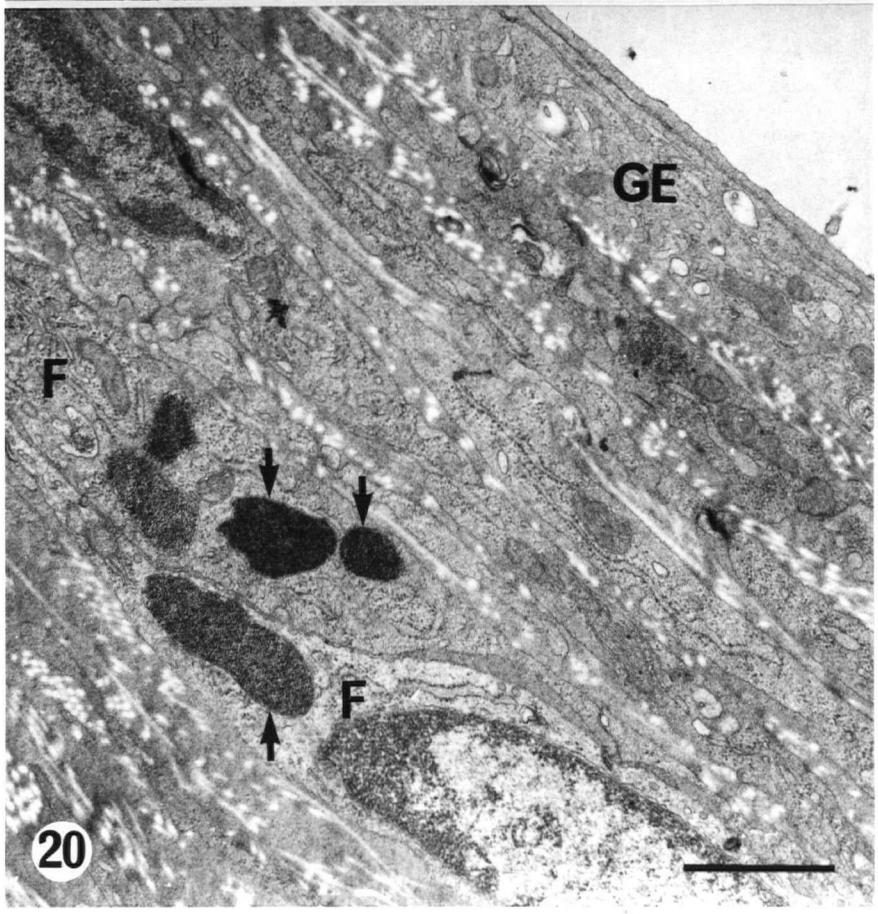
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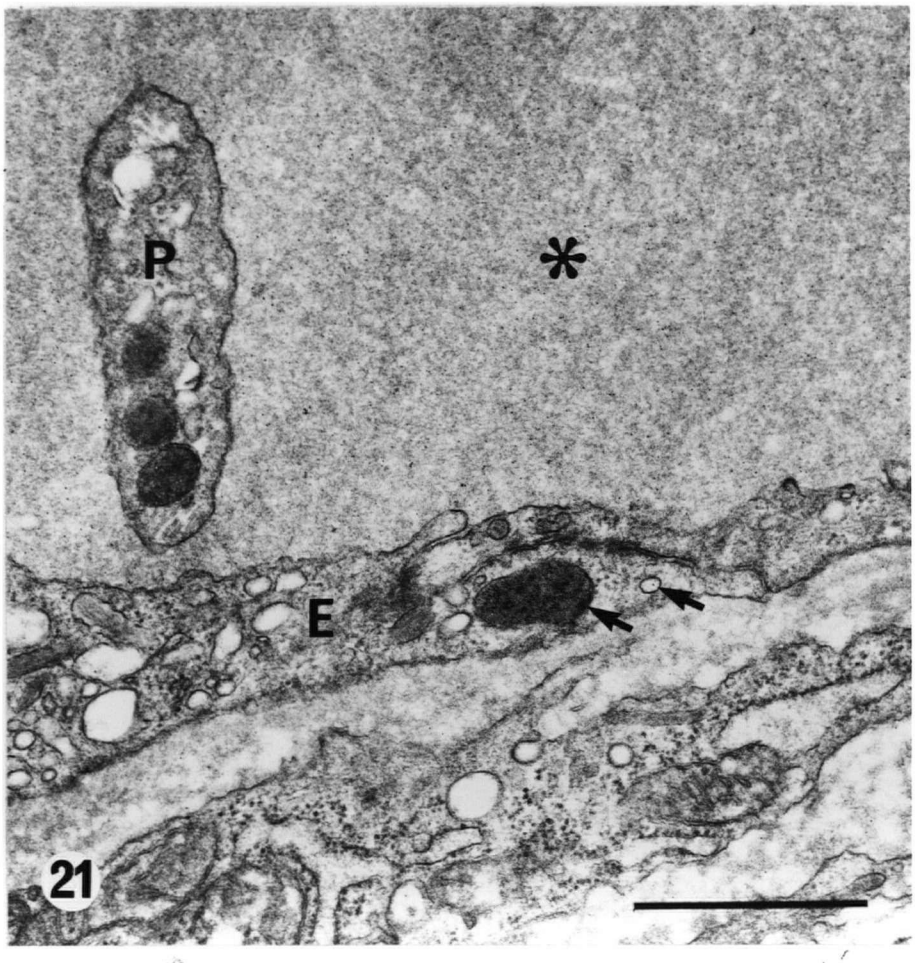
Figure 21: Ferritin is present in capillary endothelial cell (E), vesicles (arrows), and in luminal plasma (asterisk). Note platelet (P) within lumen of capillary. Uranyl acetate staining. X 30,500.

Figure 22: Apical surface of germinal epithelial cell. A possible sequence of the pinocytotic events underlying ferritin uptake is shown (arrows). Uranyl acetate and lead citrate staining. X 88,400.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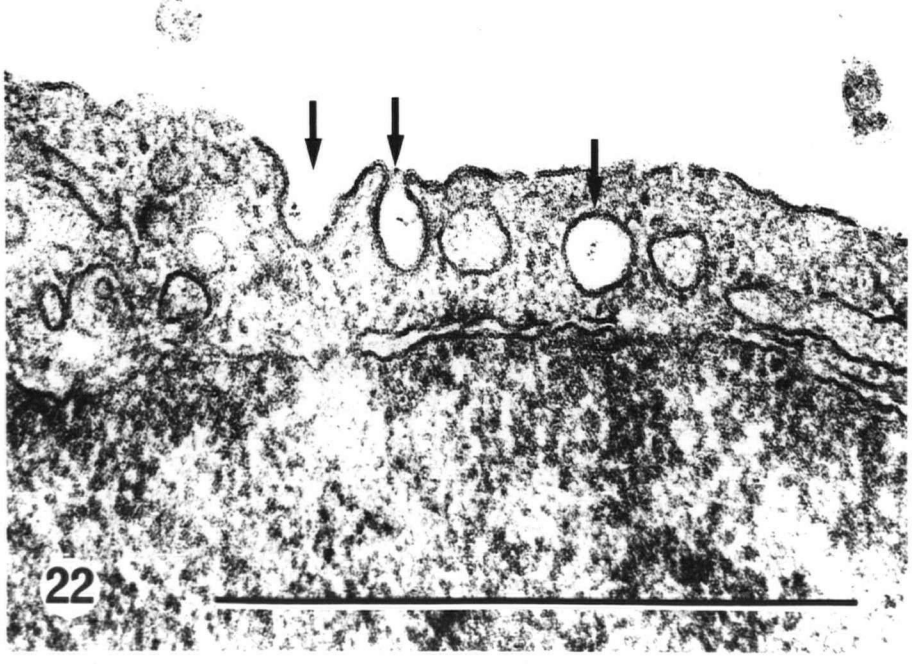
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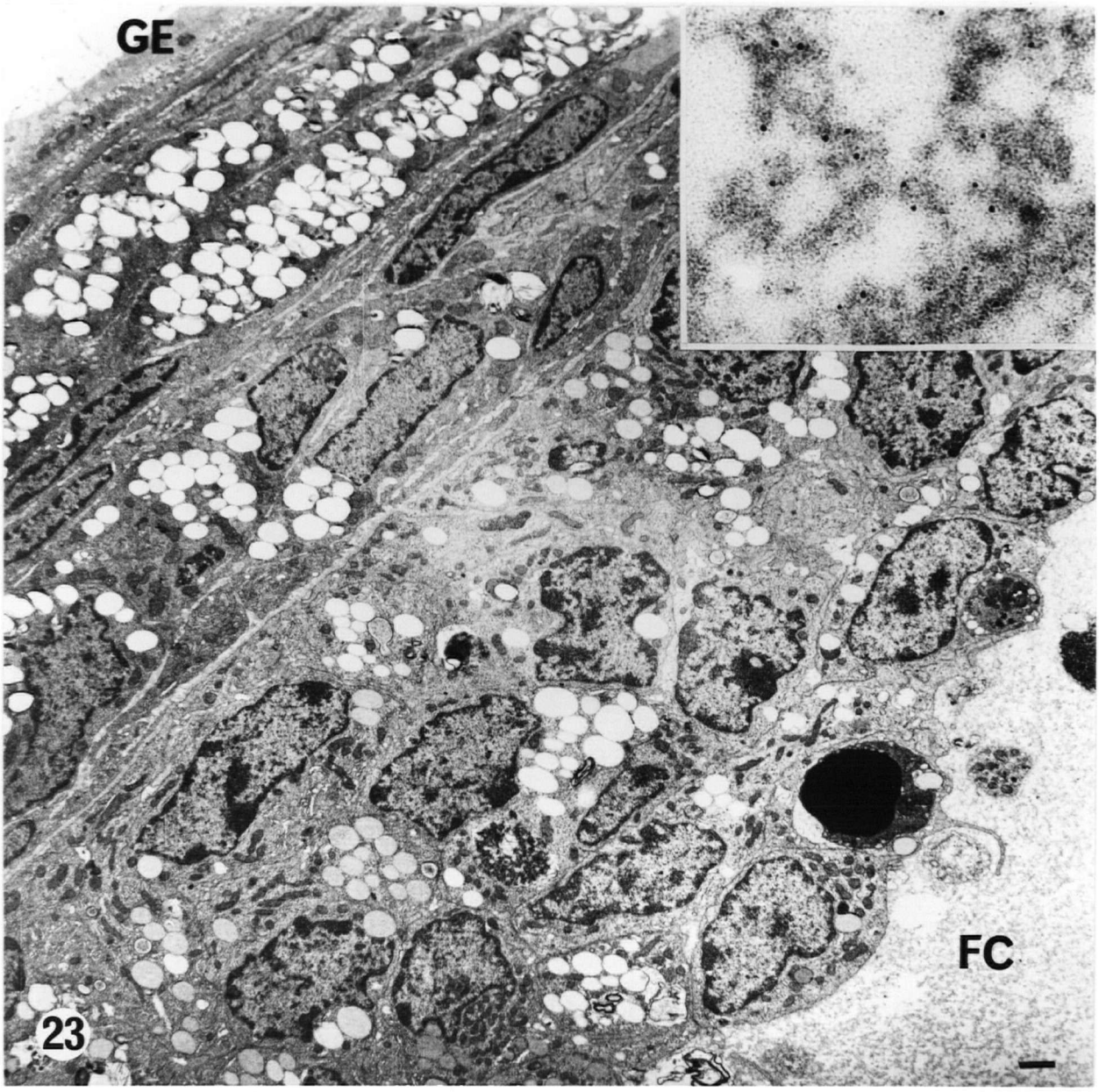
Figure 23: Overview showing germinal epithelium (GE), tunica albuginea ovarii, and granulosa cells surrounding follicular cavity (FC). Uranyl acetate and lead citrate staining. X 4,700. Inset shows ferritin in follicular fluid at high magnification. Uranyl acetate and lead citrate staining. X 117,000.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DISCUSSION

The fine structure of rat ovarian germinal epithelium as described in this study compares quite well with that of species previously reported (Wischnitzer, 1965 - mouse; Gondos, 1969 - rabbit; Weakley, 1969 - hamster; Papadaki and Beilby, 1971 - human). No major ultrastructural differences were encountered in comparing the germinal epithelium of pre- and postpubertal animals. This statement applies both to control and to tracer-exposed animals.

HRP and ferritin travel across the epithelium by two predominantly different routes. HRP movement is mainly extracellular in both pre- and postpubertal animals, whereas ferritin transport is intracellular in both groups.

The following discussion examines several major features of normal germinal epithelial ultrastructure, subsequent to which are described the methods of transfer of HRP and ferritin.

A. Germinal Epithelial Cell Shape

Variation in germinal epithelial cell shape from squamous to cuboidal may be explained on the basis of follicular growth. Presumably, the squamous cells represent "stretched" cuboidal cells. The cuboidal cells may deform according to the stresses imposed upon them by cell growth and multiplication occurring in underlying developing follicles. Such follicular, and hence ovarian, volume increase would imply a concurrent increase in ovarian surface area. This is especially marked in the rat, as it is polyovular, with a number of follicles developing to

maturity simultaneously. The increase in surface area, if the ovary is to remain covered by the germinal epithelium, requires a change in epithelial cell shape. The nature of this change is an increase in the area covered by a cell, i.e., a cell must "stretch" from a cuboidal to a squamous shape. It is possible that the convoluted infoldings of the intercellular clefts between cuboidal cells may contribute to the required increase in cell surface area. This is difficult to assess, as the clefts between squamous cells are still quite convoluted. They do not appear to have become more direct in their course, as might be expected.

Some authors suggest that perhaps an increase in epithelial cell number is also involved in epithelial accommodation to ovarian expansion (Wischnitzer, 1965; Weakley, 1969). However, no mitotic stages other than interphase were observed in the germinal epithelium of this study, even at the apex of Graafian follicles, where such division might be most expected. It thus seems unlikely that epithelial cell multiplication could be a major response to an increase in ovarian surface area. This is not to imply that the epithelial cells have lost their capability to replicate by mitotic division, merely that this would not seem to be a normal response to ovarian volume increase. That epithelial cells are still able to undergo mitosis is indicated by the presence of centrioles in association with the cilia seen in the present study. Subsequent to ovulation the epithelial cells must presumably undergo mitosis in order to re-establish the integrity of the epithelium over the corpus luteum. Otherwise,

regions denuded of epithelial cells should have been of quite common occurrence. To the contrary, no such regions were observed in the specimens examined.

As well as a passive alteration in epithelial cell shape, recent studies have implicated the possible involvement of an active component (Jeppesen, 1975). Such a hypothesis is supported by the observation in the present study of microfilaments in some germinal epithelial cells, which suggests that the cells may have an intrinsic ability to contract. Post-ovulatory re-establishment of cuboidal cell shape could thus be explained on the basis of a contractile event. As a corollary to this, cell "stretching" could be a function of the relaxation of an internal system of contractile elements. Microfilaments are abundant in the germinal epithelial cells of the fetal guinea pig ovary, where it has been proposed that they contract the superficial layer of the stratified epithelium (Jeppesen, 1975). The result of this contraction is that the more basally situated cells are forced into the gonadal anlage where they become associated with the sex cords as prefollicular cells. Jeppesen noted that microfilaments are observed only when changes in cell shape are occurring during development. This could explain why microfilaments are not often seen in adult germinal epithelial cells, as the epithelium has essentially completed its development.

B. Intercellular Junctions

Junctional specializations of types similar to those

observed in rat germinal epithelium have been noted in previous studies of both germinal epithelium and mesothelium. Focal regions of apparent membrane fusion observed in this study (see Results) appear similar to junctions which have been identified in the germinal epithelium of other species as tight junctions or junctional complexes (Papadaki and Beilby, 1971), "typical junctional complexes (terminal bars)" (Gondos, 1969), or terminal bars (Wischnitzer, 1965). These terms are usually reserved to describe junctions which are, or have as one of their components, circumcellular zones of molecular occlusion. Weakley (1969) was explicit in avoiding the term "tight junction" when describing the junctions of hamster germinal epithelium. As well as fused focal junctions, she also noted focal regions where membranes did not seem to be fused but were separated by a small gap of about 20 Å, similar to the situation observed in many of the focal junctions of the present study. It is difficult to assess these junctions in structural, and hence functional, terms with regard to the degree of membrane separation without resorting to more specialized techniques. For instance, freeze fracture studies of the focal junctions which seem to be separated by a small intercellular space could indicate whether or not these junctions are gap junctions. The appearance of membrane fusion could in some cases be artefactual, due to obliquity of sectioning through a junction, compounded by the tortuosity of the intercellular clefts. Such an interpretation could indeed explain some of the focal fusion of adjacent germinal epithelial cell membranes seen in this study. However,

examination of serial sections also suggests that some of these focal points of fusion are in fact quite real and not artefactual. Such fusion is not extensive and it is doubtful that it is continuous about the entire periphery of a cell as an occluding junction. As well, if some germinal epithelial cells are joined by zonular tight junctions, then one would expect to observe such junctions in all intercellular clefts if the epithelium is to constitute an effective barrier to diffusion. In fact, intercellular clefts devoid of any junctional specialization are sometimes noted.

In light of the results of the present study with regard to tracer movement, it is extremely unlikely that the areas of epithelial cell contact are tight junctions in the sense that they prevent intercellular molecular diffusion. The interpretation given to the focal fusion of membranes seen in this study is thus the same as that given to junctions of similar appearance in mesothelium (Cotran and Karnovsky, 1968) and capillary endothelium (Karnovsky, 1967). Namely, these small points of membrane fusion are most likely maculae occludentes, or "spots of occlusion." Their function would thus seem to primarily involve cell adherence rather than regulation of molecular movement through the intercellular spaces of the germinal epithelium.

Teleologically, one might reason that the germinal epithelium does not constitute an impervious barrier which protects the ovary and its contained oocytes because the same junctions on which the effectiveness of such a barrier depends could also

make ovulation a more difficult process. That is, such junctions could constitute a mechanical hindrance to the release of oocytes. Even if such a barrier did allow ovulation, every time an oocyte was released potentially harmful substances could enter the ovary. These substances could diffuse from the peritoneal cavity through the epithelial break at the apex of the ruptured Graafian follicle from which the oocyte was expelled. The substances would then be in the cavity of the corpus luteum, and unless all the constituent cells of that structure were joined by occluding junctions, which they do not seem to be, molecular movement through the ovary could continue unhindered. This would defeat the whole purpose of an impermeable epithelium. The situation would be exacerbated in the rat, which is polyovular, and would thus present numerous openings into the ovary from the peritoneal cavity through several ruptured Graafian follicles.

C. Microvilli

The presence of microvilli has been noted in previous studies of the germinal epithelium (Wischnitzer, 1965; Gondos, 1969; Weakley, 1969; Papadaki and Beilby, 1971), as well as in the present study. Studies of microvilli of the peritoneal mesothelium, which is of the same embryological origin as the germinal epithelium, have also been conducted (Cotran and Karnovsky, 1968; Andrews and Porter, 1973). Most authors believe that microvilli serve to enhance the absorptive or secretory surface-to-volume ratio of a cell. If this is the case, it

would indicate that the germinal epithelium, by virtue of its numerous microvilli, may be involved in the translocation of material either to or from the peritoneal cavity.

Another interesting proposal for the function of peritoneal microvilli has been put forth by Andrews and Porter (1973). They believe that the regions between adjacent microvilli entrap the slippery mucins secreted by the peritoneal mesothelial cells. The exudate may be held either by purely physical means or by binding of the aqueous component of the exudate by negatively charged acid mucopolysaccharides which coat the cell free surface. "The result of such entanglement and binding would be a slippery liquid cushion layer which could function in protecting the underlying thin mesothelium from surface abrasion arising from normal movement of internal organs over one another." Such a mechanism could assist in the maintenance of the integrity of the germinal epithelium. Even though the epithelium may be protected in part from gross damage by the periovarian bursa, movement of the ovary within the bursa would still necessitate the presence of microvilli. This theory also allows the postulation of one possible function for the proteins presumably synthesized and secreted by the germinal epithelial cells. These proteins could be a component of the serous exudate which lubricates the surfaces of the intraperitoneal viscera.

Although no specific attempts were made to preserve or stain such a mucinous surface coating in the present study, there did from time to time appear to be amorphous remnants

of such a coating adherent to the peritoneal surface of the germinal epithelium.

D. Protein Synthesis

The germinal epithelial cells are rich in rough endoplasmic reticulum, mitochondria, and free ribosomes. Golgi complexes are also present. These features are often associated with synthetic metabolic activity, some of which could be concerned with lipid metabolism. In addition, the abundance of ribosomes and rough endoplasmic reticulum indicates a high degree of peptide synthesis. Usually, when ribosomes occur singly within the cytoplasm rather than as polysomes or rough endoplasmic reticulum, the manufactured peptides are retained for endogenous use (Lentz, 1971). Rough endoplasmic reticulum in a cell is commonly concerned with the manufacture of protein for export (Lentz, 1971). These observations on both free ribosomes and those associated with the endoplasmic reticulum are together suggestive of a high intrinsic metabolism of the germinal epithelial cells coupled with the synthesis of protein for export. The abundance of mitochondria also attests to the high energy requirements of these cells, presumably for synthetic or transport functions. In cells whose prime function is protein export the Golgi complex is prominent as the site of packaging and concentration of proteins synthesized in the rough endoplasmic reticulum. Proteins are subsequently transported and released in the form of membrane bound vesicles. As previously noted (see Results), the morphology of the Golgi complex varies

considerably from one germinal epithelial cell to another. Numerous small vesicles of variable electron density and content are often seen in the vicinity of the Golgi complex. Because such vesicles are also seen to be irregularly scattered throughout much of the rest of the cytoplasm, it is difficult to assess whether or not they are also a product of the activity of the Golgi complex.

Weakley (1969) observed the indentation of the basal surface of the germinal epithelial cells of the hamster by follicular cell processes containing many ribosomes. There seemed to be no intervening basement membrane and the cells were apparently in direct contact. She suggested that this could indicate either amino acid transfer from epithelial to follicular cells, or protein transfer in the opposite direction. No such cytoplasmic processes were observed in the present study. Considering the amount of peptide synthetic machinery in the germinal epithelial cells, it seems unlikely that these cells would transfer free amino acids rather than completed peptides. Also in view of this epithelial preponderance of synthetic organelles and their presumed production of a large number of peptides, it seems unlikely that follicular cells would be transporting peptides to the germinal epithelial cells. It would seem to be much more reasonable to suspect protein transfer to occur from the germinal epithelial cells to either the underlying follicular cells or to the overyling peritoneal cavity. The polarity of intracellular organelles is not consistent enough to indicate any major route of secretory activity.

Autoradiographic studies may prove useful in further investigation of protein synthesis, transport, and function.

E. Lipids

Lipid inclusions similar to those seen in this study have also been observed in the germinal epithelial cells of the hamster (Weakley, 1969) and human (Papadaki and Beilby, 1971). It is possible that such inclusions are associated with steroidal activity since their morphology is similar to that observed in subjacent cortical cells. Germinal epithelial cells are believed to give rise to follicular cells during development. A high degree of steroidal activity is manifest in these follicular cells. Similar activity in the germinal epithelium, although not as pronounced, could represent the retention of an epithelial capability for steroid metabolism (Weakley, 1969; Papadaki and Beilby, 1971).

Certain enzymes have been histochemically localized within germinal epithelial cells. The concentration of 17β-hydroxysteroid dehydrogenase is quite marked. Also present are the 16α and 16β forms, the levels of which are much lower (Baillie et al., 1966). These enzymes are catalytic for specific reactions in the metabolism of 16- and 17-hydroxysteroids, which are probably of an estrogenic nature (Baillie et al., 1966). It is difficult to reconcile the presence of these enzymes within the germinal epithelial cells, as the cells have not been found to demonstrate Δ^5-3β-hydroxysteroid dehdrogenase activity (Baillie et al., 1966). This is an essential enzyme in the

initial stages of metabolism of the 16- and 17-hydroxysteroids. It would seem to be highly unlikely that the enzymes of later stages of the metabolic pathways would be present in the absence of the enzyme required to synthesize their precursors.

It may be that the histochemical techniques used to investigate Δ^5-3β-hydroxysteroid dehydrogenase activity are not sensitive enough to reveal its presence in the germinal epithelial cells. The possibility also exists of an alternate metabolic pathway which does not require this enzyme. And finally, the substrates for 17β-, 16α-, and 16β-hydroxysteroid dehydrogenase catalysis could be synthesized in other cells and transferred to the germinal epithelial cells for final processing.

Weakley (1969) noted that in the germinal epithelium of the hamster there is an increase in the number of lipid inclusions with development. Such inclusions are present in many embryonic tissues but usually disappear during differentiation. Because of the increase in lipid inclusions in the epithelial cells, she suggested that "they represent a specific product of the differentiated cell rather than nutrient material to be used by the developing tissue." The presence of lipid inclusions, their increasing numbers during development, and the localization of specific enzymes within the germinal epithelial cells all point to a definite epithelial involvement in steroid metabolism. However, this involvement is presumably of a much lesser degree than is seen in follicular and luteal cells. This is attested to by the lower cytoplasmic lipid

content of epithelial cells and by either the absence or very small number of ultrastructural features usually associated with cells whose prime function is steroidogenic. These latter features include tubular mitochondrial cristae and smooth endoplasmic reticulum. It is possible that some smooth endoplasmic reticulum may have been mistaken for small intracellular vesicles.

F. HRP Movement and Localization

The results of the present study indicate that the germinal epithelium of the rat is readily permeable to HRP. The movement of HRP seems to be predominantly through the intercellular clefts. Vesicular transport of HRP across the germinal epithelium could not be proven, even though there does seem to be a small but definite population of tracer-filled cytoplasmic vesicles which do not appear to be connected to any of the cell surfaces. However, in terms of the numbers of these vesicles, their size, and their HRP content, it would seem that the role of vesicular transport in moving HRP and other molecules of a similar size across the germinal epithelium is minor in comparison to intercellular diffusion. These results are similar to those found by Cotran and Karnovsky (1968) in their study of rat mesothelium, which is of similar embryological origin to the germinal epithelium.

The movement of HRP through the intercellular clefts is probably in the nature of a passive diffusion process. Support is lent to this conclusion by the work of Cotran and Karnovsky

(1968) on mesothelium, which is ultrastructurally, and thus presumably physiologically, quite similar to the germinal epithelium. In mesothelium exposed to HRP either during or after fixation there was still intercellular localization of HRP. Any active HRP-transporting mechanism in the intercellular clefts would obviously have been rendered non-functional, thereby leaving simple diffusion as the only means by which HRP could be translocated. Small vesicles filled with HRP in this same study were always directly connected to the apical surface or intercellular clefts or were in close apposition to them, again suggestive of filling by diffusion (see Results). There were no tracer-containing vesicles deep within the mesothelial cells. In addition, studies of follicular permeability to intravascularly-injected HRP have shown that HRP may appear in the intercellular clefts of rat germinal epithelium after leaving the circulation (Anderson, W., 1972a). Thus there is movement of HRP not only from the basement membrane toward the peritoneal surface but also in the opposite direction, as revealed in the present study. To explain these movements on the basis of the operation of active transport mechanisms, one would have to postulate the existence of either an active transport mechanism moving the same type of molecule bidirectionally. Alternatively, two different mechanisms, each transporting the same type of molecule, but in different directions, could be postulated. In either case this is not likely, as it would be of no obvious benefit to a cell or tissue. The results do suggest that HRP movement in the

intercellular clefts is not restricted as to direction, and would thus seem to support a diffusion theory for HRP movement through the germinal epithelium.

The possibility that the intercellular clefts may have been filled with tracer by vesicles from the apical surface emptying their contents into the clefts has already been noted (see Results). This factor was also taken into account by Cotran and Karnovsky (1968). They showed that exposure of mesothelium to HRP either during or subsequent to initial fixation (and preceding post-fixation), which would halt vesicular transport, did not measurably reduce the amount of HRP in the intercellular clefts. These results demonstrate that if there is a vesicular contribution to cleft filling it is inconsequential. Presumably the same may hold true for the germinal epithelium.

G. Ferritin Movement and Localization

The results suggest that the movement of ferritin through the germinal epithelium is an intracellular process mediated by vesicular transport. A similar cytoplasmic vesicular transport of ferritin is the sole means of ferritin movement through capillary and endothelial cells subsequent to intravascular ferritin injections (Anderson, W., 1972a; Payer, 1975). There is no evidence whatsoever of intercellular passage of ferritin through the germinal epithelium.

It has been noted that free ferritin particles are occasionally seen within epithelial cells and fibroblasts and apparently in intercellular clefts. Their appearance in

these locations is regarded as artefactual for a number of reasons. Firstly, these situations were observed infrequently in this study. If the cytoplasm or the intercellular clefts were common sites of ferritin movement, one would expect to observe free intracytoplasmic and intercellular ferritin particles on a constant basis. Such is not the case. In addition, free ferritin particles, when present within a cell, are not limited in occurrence to the cytoplasmic matrix. They may appear indiscriminately both within the matrix and within most cell organelles and in or on the membranes of those organelles. This suggests that ferritin particles may have been "smeared" across a section and are superficially situated. The source of these particles cannot be definitely established. They could be derived from sectioned vesicles which contain large numbers of ferritin molecules. Some of these molecules may have been displaced during the sectioning process to subsequently appear randomly over a section. Excessive ferritin at the peritoneal surface may also have been displaced by sectioning. Finally, the density of intravesicular ferritin is always substantially greater than the density of free ferritin, indicating the overwhelming predominance of vesicular transport.

The penetration of ferritin through the epithelium and subjacent regions is much slower than the movement of HRP. This is likely a function of their differing molecular size and weight and consequent differences in their modes of movement. Such temporal differences have been noted in other studies employing these two tracers (Payer, 1975).

The presence of ferritin in the follicular fluid of some developing follicles indicates that the follicular basement membrane does not constitute a barrier to ferritin movement. This contradicts the results of a previous study (Anderson, W., 1972a). Using the same strain of rat as was used in this study, Anderson found that intravascularly-injected ferritin seemed to accumulate at the level of the follicular basement membrane and did not pass through it. No explanation can be put forth at present to rationalize the observed differences in follicular permeability. The differences do not seem to be related to the time of exposure to ferritin, as the times in the present study and in Anderson's study were similar.

Anderson (W., 1972a), in his study of follicular permeability, used a variety of intravascular tracers. He shows the presence of Thorotrast (molecular diameter 70 Å) in intracellular clefts and large phagocytic vacuoles of the germinal epithelium. These vacuoles are in close proximity to the clefts and some appear as though they could be sections through irregularities in these clefts (see Results). It is suggested by Anderson that Thorotrast does not pass through the entire length of the clefts into the peritoneal cavity, due to the presence of junctions in the clefts. Nevertheless, focal and extended junctions were often observed at the basal end of intercellular clefts in material examined in the present study. Presumably these types of junctions would also have been present in the experimental animals used by Anderson, as some of his animals were of the same strain as used herein. If Thorotrast passed

either through or around these basally-situated junctions, then it would be likely that it would also pass through junctions at the peritoneal ends of the clefts, as there are no zonal regions of occlusion there. Additionally, as previously mentioned, some clefts appear to have no junctional specializations at all. It would thus be expected that if Thorotrast entered the basal end of such a cleft, it would eventually diffuse to the peritoneal end and into the peritoneal cavity. The upper limit of molecular size in terms of passive intercellular diffusion would therefore seem to be between 70 Å (Thorotrast) and 110 Å (ferritin).

The overall distribution of ferritin in the current study parallels in many respects that seen in previous studies of the ovary in which ferritin was introduced via inatravascular injection (Anderson, W., 1972a; Payer, 1975).

The observation that there are more large vesicles in fibroblasts subsequent to ferritin exposure than are seen in control material suggests that ferritin may have an inducing effect on vesicular formation.

CONCLUSION

On the basis of its ultrastructural features, the germinal epithelium appears to be a highly active tissue. It possesses structures suggestive of an absorptive and/or secretory capacity (microvilli), a marked protein synthetic activity (ribosomes and rough endoplasmic reticulum), and possible steroid metabolism (lipid inclusions). That the epithelium may be actively or passively involved in the movement of tracers has also been demonstrated.

Exposure of the peritoneal surface of the ovary to HRP and ferritin, and subsequent electron microscopic localization of these tracers, indicate that there are two distinct routes of molecular movement from the peritoneal cavity across the germinal epithelium, according to the size of the molecule translocated. Molecules of the order of 40 Å diameter, such as HRP, appear to freely diffuse through the intercellular clefts of the epithelium. Larger molecules, comparable in size to ferritin (110 Å), are excluded from the clefts and seem to be restricted to intracellular passage across the germinal epithelium. "Such a cytoplasmic route could provide a means of screening substances destined for the underlying tissues, or of altering them chemically before they are allowed to proceed further (Weakley, 1969)." Because only the ferric hydroxide core of ferritin is seen with the electron microscope, it is not possible to observe whether changes in the apoprotein portion of the ferritin molecule occur during its passage through the epithelium. Such a study would probably be amenable to investigation by immunohistochemical techniques.

It can be concluded that there is ready access of substances the size of ferritin or smaller into the substance of the ovary through the germinal epithelium. From this it follows that substances which permeate the epithelium and subjacent tissues may, if not altered during their transepithelial passage, influence the normal development of the oocyte. The effects exerted on the oocyte by these substances may or may not be detrimental. At the level of the epithelium, smaller molecules which move extracellularly are likely not subject to any biochemical screening procedure which could either prevent their passage or alter their molecular structure. Hence their effects, if any, on the oocyte would be direct. Larger molecules, which must pass through the epithelial cells, could be subject to intracytoplasmic modification of their structure, or their passage could be prevented entirely. The magnitude of the effect of these molecules on the oocyte would be a function of the degree of intracellular alteration of their molecular structure.

If an exogenous substance enters the peritoneal cavity, the possible effects of such a substance on the oocyte must be examined at several stages. Firstly, in terms of adverse effects, foreign molecules could interfere with normal oocyte development and metabolism. This could lead to a reduction in oocytic viability and a subsequent failure of the oocyte to reach maturity. Secondly, such molecules could alter the oocytes, but in a less radical manner. Oocytic development, ovulation, and fertilization could occur, but the zygote so

formed might not be viable, leading to an increased rate of spontaneous abortion. Finally, the effects of exogenous molecules could be so subtle as to allow development of the fertilized oocyte to term. However, the resultant progeny could manifest congenital abnormalities of varying degrees of severity due to alterations in the oocyte induced by the exogenous molecules.

In terms of the possible clinical significance of the findings of this study, there are a number of situations in which exogenous molecules may enter the peritoneal cavity and thus come into contact with the germinal epithelium. Most of these situations are associated with the entry of bacteria into the peritoneal cavity. The bacteria are the actual source of the exogenous molecules, which they release as either exotoxins or endotoxins. Positive identification of some exotoxins as enzymes has been made and their composition and size found to be similar to enzymes in general (Davis et al., 1973). The normal range of enzyme molecular weight is from 12,000 to more than 1 million (Lehninger, 1970). It would then appear that of the known bacterial exotoxins, a number are of a molecular weight less than that of ferritin (462,000). They may thus be able to pass through the germinal epithelium. Another source of exogenous molecules could be the digestive tract subsequent to perforation of an organ. This would allow not only bacteria to enter the peritoneal cavity, but also various products of digestion and excretion. The routes by which toxin-producing bacteria and non-bacterially derived

exogenous substances may enter the peritoneal cavity are enumerated below (Ellis and Calne, 1976):

1. from the exterior via an infection at laparotomy or a penetrating wound.
2. from intra-abdominal viscera:
   a) gangrene of a viscus, e.g. acute appendicitis, acute cholecystitis, diverticulitis or infarction of the intestine.
   b) perforation of a viscus, e.g. perforated duodenal ulcer, perforated appendicitis, rupture of intestine from trauma.
   c) post-operative leakage of an intestinal suture line.
3. via the blood stream as part of a septicemia (pneumococcal, streptococcal, or staphylococcal).
4. via the female genital tract as in acute salpingitis or a puerperal infection.

It is thus seen that the germinal epithelium is a metabolically and synthetically active tissue. Whether or not it is capable of modifying some of the molecules to which it is permeable remains to be demonstrated. Such processes could be of considerable significance in the maintenance of a normal environment for oocytic development, especially as there are definite situations in which exogenous molecules may enter the peritoneal cavity.

LITERATURE CITED

Ainsworth, S.K. and M.J. Karnovsky. 1971. An ultrastructural staining method for enhancing the size and electron opacity of ferritin in thin sections. J. Histochem. Cytochem. 20: 225-229.

Anderson, E. 1967. Observations on the uptake of HRPO by the developing oocytes of the rabbit. J. Cell Biol. 35: 160 A.

Anderson, E. 1972. The localization of acid phosphatase and the uptake of HRP in the oocyte and follicle cells of mammals. In: Oogenesis. J.D. Biggers and A.W. Schuetz, eds. University Park Press, Baltimore, pp. 87-117.

Anderson, W.A. 1972a. Permeability of ovarian blood vessels and follicles of juvenile rats. Microvasc. Res. 4: 348-373.

Anderson, W.A. 1972b. The use of exogenous myoglobin as an ultrastructural tracer. Reabsorption and translocation of protein by the renal tubule. J. Histochem. Cytochem. 20: 672-684.

Andrews, P.M. and K.R. Porter. 1973. The ultrastructural morphology and possible functional significance of mesothelial microvilli. Anat. Rec. 177: 409-426.

Baillie, A.H., M.M. Ferguson, and D.M. Hart. 1966. Developments in Steroid Histochemistry. Academic Press, London.

Beck, L.R. 1972. Comparative observations on the morphology of the mammalian periovarial sac. J. Morphol. 136: 247-254.

Benjaminson, M.A., S.L. Bazil, F.J. D'Oria, and S.L. Carito. 1966. Ferritin-labelled enzyme: a tool for electron microscopy. Nature (London). 210: 1275-1276.

Bruns, R.R. and G.E. Palade. 1968. Studies on blood capillaries. II. Transport of ferritin molecules across the wall of muscle capillaries. J. Cell Biol. 37: 277-299.

Chiquoine, A.D. 1961. An electron microscope study of vitally stained ovaries and ovaries from argyric mice. Anat. Rec. 139: 29-35.

Clementi, F. 1970. Effects of horseradish peroxidase on mice lung capillaries' permeability. J. Histochem. Cytochem. 18: 887-892.

Clementi, F. and G.E. Palade. 1969. Intestinal capillaries. II. Structural effects of EDTA and histamine. J. Cell Biol. 42: 706-714.

Clewe, T.H. 1966. Absence of a foramen in the ovarian bursa of the golden hamster. Anat. Rec. 151: 446.

Cotran, R.S. and M.J. Karnovsky. 1968. Ultrastructural studies on the permeability of the mesothelium to horseradish peroxidase. J. Cell Biol. 37: 123-138.

Davis, B.D., R. Dulbecco, H.N. Eisen, H.S. Ginsberg, and W.B. Wood. 1973. Microbiology. 2nd ed. Harper & Row, New York.

Ellis, H., and R.Y. Calne. 1976. Lecture Notes on General Surgery. 4th ed., revised reprint. Blackwell Scientific Publications, London.

Farquhar, M.G. and G.E. Palade. 1961. Glomerular permeability. II. Ferritin transfer across the glomerular capillary wall in nephrotic rats. J. Exp. Med. 114:699-716.

Farquhar, M.G., S.L. Wissig, and G.E. Palade. 1961. Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall. J. Exp. Med. 113: 47-66.

Feder, N. 1970. A heme-peptide as an ultrastructural tracer. J. Histochem. Cytochem. 18: 911-913.

Franchi, L.L. 1970. The ovary. In: Scientific Foundations of Obstetrics and Gynecology. E.E. Philipp, J. Barnes, and M. Newton, eds. William Heinemann Medical Books Ltd., London, pp. 107-131.

Franchi, L.L., A.M. Mandl, and S. Zuckerman. 1962. The development of the ovary and the process of oogenesis. In: The Ovary, Vol. I. S. Zuckerman, A.M. Mandl, and P. Eckstein, eds. Academic Press, New York, pp. 1-88.

Gervin, S.W. and E. Holtzman. 1972. The fate of exogenous peroxidase in the thymus of newborn and young adult mice. J. Histochem. Cytochem. 20: 445- 462.

Gondos, B. 1969. Ultrastructure of the germinal epithelium during oogenesis in the rabbit. J. Exp. Zool. 172: 465-480.

Goodenough, D.A. and J.P. Revel. 1971. The permeability of isolated and in situ mouse hepatic gap junctions studied with enzymatic tracers. J. Cell Biol. 50: 81-91.

Graham, R.C., and M.J. Karnovsky. 1966a. Glomerular permeability. Ultrastructural cytochemical studies using peroxidases as protein tracers. J. Exp. Med. 124: 1123-1134.

Graham, R.C. and M.J. Karnovsky. 1966b. The early stages of absorption of injected horseradish peroxidase into the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. 14: 291-302.

Graham, R.C. and R.W. Kellermeyer. 1968. Bovine lactoperoxidase as a cytochemical protein tracer for electron microscopy. J. Histochem. Cytochem. 16: 275-278.

Hastings, R.A., A.C. Enders, and S. Schlafke. 1972. Permeability of the zona pellucida to protein tracers. Biol. Reproduc. 7: 288-296.

Hirai, K-I. 1975. Diaminobenzidine in cytochemistry. Acta Histochem. Cytochem. 8: 31-40.

Jeppesen, T. 1975. Surface epithelium of the fetal guinea pig ovary: a light and electron microscopic study. Anat. Rec. 183: 499-516.

Jones, R.M. 1949. The use of vital staining in the study of the origin of germ cells in the female rat, Mus norwegicus. J. Morphol. 84: 293-333.

Karnovsky, M.J. 1967. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J. Cell Biol. 35: 213-236.

Karnovsky, M.J. and R.S. Cotran. 1966. The intercellular passage of exogenous peroxidase across endothelium and mesothelium. Anat. Rec. 154: 365.

Karnovsky, M.J. and D.F. Rice. 1969. Exogenous cytochrome c as an ultrastructural tracer. J. Histochem. Cytochem. 17: 751-753.

Klapper, M.H. and D.P. Hackett. 1965. Investigations on the multiple components of commercial horseradish peroxidase. Biochem. Biophys. Acta. 96: 272-282.

Kluge, T. 1969. The permeability of mesothelium to horseradish peroxidase. A light and electron microscopic study, with special reference to the morphology of pericardial Mast cells. Acta Pathol. Microbiol. Scand. 75: 257-269.

Kluge, T. and T. Hovig. 1968. Pericardial absorption of exogenous peroxidase in rats. An electron microscopical study. Acta Pathol. Microbiol. Scand. 73: 521-536

Latta, J.S. and E.S. Pederson. 1944. The origin of ova and follicle cells from germinal epithelium of the ovary of the albino rat as demonstrated by selective staining with India ink. Anat. Rec. 90: 23-35.

Leak, L.V. 1971. Studies on the permeability of lymphatic capillaries. J. Cell Biol. 50: 300-323.

Lehninger, A.L. 1970. Biochemistry. Worth Publishers, Inc., New York.

Lentz, T.L. 1971. Cell Fine Structure. An Atlas of Drawings of Whole-Cell Structure. W.B. Saunders Company, Philadelphia.

Maunsbach, A.B. 1966. Absorption of ferritin by rat kidney proximal tubule cells. Electron microscopic observations of the initial uptake phase in cells of microperfused single proximal tubules. J. Ultrastruct. Res. 16: 1-12.

Merchant, H. 1975. Rat gonadal and ovarian organogenesis with and without germ cells. An ultrastructural study. Dev. Biol. 44: 1-21.

Morgan, C., R.A. Rifkind, K.C. Hsu, M. Holden, B.C. Seegal, and H.M. Rose. 1961. Electron microscopic localization of intracellular viral antigen by the use of ferritin-conjugated antibody. Virology. 14: 292-296.

Odor, D.L. 1956. Uptake and transfer of particulate matter from the peritoneal cavity of the rat. J. Biophys. Biochem. Cytol. 2: (suppl.) 105-108.

Papadaki, L. and J.O.W. Beilby. 1971. The fine structure of the surface epithelium of the human ovary. J. Cell Sci. 8: 445-465.

Payer, A.F. 1975. Permeability of ovarian follicles and capillaries in mice. Am. J. Anat. 142: 295-318.

Pelliniemi, L.J. 1975. Ultrastructure of the early ovary and testis in pig embryos. Am. J. Anat. 144: 89-112.

Pinkerton, J.H.M., D.G. McKay, E.C. Adams, and A.T. Hertig. 1961. Development of the human ovary - A study using histochemical techniques. Obstet. Gynecol. 18: 152-181.

Reese, T.S. and M.J. Karnovsky. 1967. Fine structural localization of a blood-brain barrier to exogenous peroxidase. J. Cell Biol. 34: 207-217.

Revel, J.P. and M.J. Karnovsky. 1967. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J. Cell Biol. 33: C7-C12.

Ryser, H., J.B. Caulfield, and J.C. Aub. 1962. Studies on protein uptake by isolated tumor cells. I. Electron microscopic evidence of ferritin uptake by Ehrlich ascites tumor cells. J. Cell Biol. 14: 255-268.

Schneeberger-Keeley, E.E. and M.J. Karnovsky. 1968. The ultrastructural basis of alveolar-capillary membrane permeability to peroxidase used as a tracer. J. Cell Biol. 37: 781-793.

Seligman, A.M., M.J. Karnovsky, H.L. Wasserkrug, and J.S. Hanker. 1968. Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmiophilic reagent, diaminobenzidine (DAB) J. Cell Biol. 38: 1-14.

Simionescu, N. and G.E. Palade. 1971. Dextrans and glycogens as particulate tracers for studying capillary permeability. J. Cell Biol. 50: 616-624.

Wade, J.B. and V.A. Discala. 1971. The effect of osmotic flow on the distribution of horseradish peroxidase within the intercellular spaces of toad bladder epithelium. J. Cell Biol. 51: 553-558.

Weakley, B.S. 1969. Differentiation of the surface epithelium of the hamster ovary. An electron microscopic study J. Anat. 105: 129-147.

Webber, W.A. and J. Blackbourn. 1970. The permeability of the immature glomerulus to large molecules. Lab. Invest. 23: 1-7.

Webber, W.A. and J. Blackbourn. 1971. The permeability of the parietal layer of Bowman's capsule. Lab. Invest. 25: 367-373.

White, A., P. Handler, and E.L. Smith. 1973. Principles of Biochemistry. McGraw-Hill Book Company, Toronto.

Wischnitzer, S. 1965. The ultrastructure of the germinal epithelium of the mouse ovary. J. Morphol. 117: 387-400.

Witschi, E. 1948. Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. Contrib. Embryol. Carnegie Inst. Wash. 32:67-80.

Witschi, E. 1963. Embryology of the ovary. In: The Ovary. H.G. Grady and D.E. Smith, eds. The Williams and Wilkins Company, Baltimore, pp. 1-10.

Wylie, J.C. 1964. Identification of fibrin with ferritin-conjugated antifibrinogen. Exp. Mol.Pathol. 3: 468-474.

Zuckerman, S. 1951. The number of oocytes in the mature ovary. Recent Prog. Horm. Res. 6: 63-109.