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Title: ULTRASTRUCTURAL AND CYTOCHEMICAL STUDIES OF
THE OVIDUCT

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The ultrastructure of the oviduct and the hormonal induced alterations in the cellular organelles of prepubertal and ovariectomized rabbits have been studied with electron-microscopic histochemical methods.

The pseudostratified epithelium of the oviduct in prepubertal rabbits contained both ciliated and secretory cells. The secretory cells showed apocrine projections filled with electron-dense secretory granules. The oviductal cilium consisted of the characteristic 9+2 fibrils in the central core. The cytoplasm of the ciliated cells contained only a few granular endoplasmic reticulum as compared to the secretory cells. Multivesicular and dense bodies resembling lysosomes were present in the apical cytoplasm.

Cells of the oviductal epithelium underwent characteristic changes in response to oophorectomy and ovarian steroids. The
alterations seen six weeks after oophorectomy in the fine structure of the ciliated and secretory cells of the fimbria have been described. The most striking feature observed following oophorectomy was the disappearance of cilia and the occurrence of rather bizarre forms of basal bodies and mitochondria in the apical cytoplasm. Polysomal formations were less frequent indicating a reduced level of protein synthesis. The secretory cells contained poorly developed endoplasmic reticulum, relatively inactive Golgi apparatus and occasionally a few electron-dense secretory granules.

The most obvious change after hormonal stimulation was the regeneration of cilia and basal bodies in the ciliated cells from all the regions of the Fallopian tube and the frequent extrusion of mucin-like secretory granules into the tubal lumen. Extreme dilation of the cisternae of the granular endoplasmic reticulum, the presence of numerous polyribosomes and many secretory granules were observed in the cytoplasm of the secretory cells. The contribution of the granular endoplasmic reticulum and Golgi apparatus in the formation of secretory granules was discussed.

Evidence in support of the view that estrogens stimulate the growth of the cilia has been presented in this study. Stages in the formation of cilia and basal bodies were also observed. An interesting feature hitherto not reported was the presence of cross-striated rootlets in some ciliated cells in response to estrogen
This study has reported for the first time the estrogen and progesterone induced changes in the subcellular localization of the hydrolytic enzymes, adenosine triphosphatase and acid phosphatase in the Fallopian tube of rabbits. ATPase activity was found to be localized on the cell membranes, outer membranes of cilia and microvilli, and peripheral fibrils of cilia and basal bodies. The presence of this enzyme on the inner membrane of mitochondria, the membrane of the cristae and in the matrix of mitochondria was also demonstrated.

The activity of ATPase in the oviduct epithelium was significantly reduced following oophorectomy. Estrogen and progesterone induced significant increases in the activity of ATPase on the cell membranes, cilia and microvilli. There appeared to be a synergistic reaction when the two hormones were given simultaneously. Deposits of the reaction product were also observed in the Golgi apparatus, in the luminal membrane of the capillary endothelium, and in the micropinocytic vesicles of the smooth muscle cell and capillary endothelium. It is suggested that one of the mechanisms by which ovarian steroid hormones exert their effect on the oviductal cell is the utilization of energy from high energy phosphate bonds on the cell membranes for transport of materials between individual cells.

Preliminary studies on the fine structure localization of acid phosphatase in the oviductal epithelium have revealed the activity of
this enzyme in the Golgi saccules, secretory granules, secretory material, outer cell membrane and microvilli. Acid phosphatase activity was also present within the large membrane-bound bodies, most of which were similar to the dense bodies described in other cells.

Reinterpretation of fine structure changes in the rabbit Fallopian tube has been presented in accord with current advances in oviduct physiology and biochemistry. The possible roles of adenosine triphosphatase and acid phosphatase and the pitfalls in electron microscopic studies of cytochemistry were also discussed.
Ultrastructural and Cytochemical Studies of the Oviduct

by

Ramesh Kadbet Nayak

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ULTRASTRUCTURAL AND CYTOCHEMICAL STUDIES OF THE OVIDUCT

INTRODUCTION

The mammalian oviduct is a dynamic structure with many functions in reproduction. It provides not only a suitable environment for the spermatozoa and the ovum, but also for the fertilization process and cleavage during the early stages of development.

However, perhaps because of its intermediate position between the ovary and uterus, the oviduct has received comparatively little attention and has been regarded by some investigators simply as a transportation organ. Only recently, ultrastructure descriptions of oviducts in a few species have been reported. The responsiveness of the oviduct to spontaneous and experimentally induced alterations in the hormonal environment has afforded the investigators invaluable opportunities for inquiring into basic problems of structural and functional relationship.

Since the advent of the electron microscope in 1932, biologists have been able to study the ultrastructure of tissues and have attempted to relate structure and function at subcellular level. The relatively recent improvement in ultramicrotomy, fixatives, embedding techniques and electron stains have enabled the investigator to observe much greater cellular detail with the electron microscope.
Over the past 30 years, the localization of enzymes in cells has been intensively studied with the light microscope using a variety of staining methods. Related methods for use with the electron microscope, however, are still in a very early stage of development. Nevertheless, ultrastructural localization of enzymes can be depicted with much greater precision than by light microscopy. Recent studies by various workers combining both electron microscopy and enzyme histochemistry have produced a correlation between cell chemistry and fine structure and have produced a functional meaning to ultrastructural information. This new approach offers many opportunities in the field of reproductive physiology and biochemistry.

In developing enzyme staining techniques for electron microscopy, a compromise must be sought between the conflicting requirements of adequate preservation of enzyme activity and of the fine structure of cells. The fine structure should survive incubation in the chemically and osmotically active staining media. Also, the enzymatically active sites must be precisely delineated, not obscured, by the deposition of an electron opaque stain. Barrnett and Palade (1953) and Barrnett (1959) have discussed in some detail the application of histochemical techniques to electron microscopy. They state that each specific application of staining methods to electron microscopy undoubtedly requires individual consideration. Osmium tetroxide which is the most widely used fixative for electron microscopy,
seriously reduces the activity of many enzymes (Novikoff, Burnett and Glickman, 1956). Even if sufficient activity remains after brief osmium tetroxide fixation, the results have to be interpreted with caution in view of the possibility that the enzyme has been totally inhibited in some sites.

Sabatini, Bensch and Barnett (1963) and Sabatini, Miller and Barnett (1964) introduced aldehyde fixation for preservation of cellular ultrastructure and enzyme activity. Since the publication of these papers, many investigators have localized enzymes at the electron microscope level and obtained impressive results.

The effect of hormonal environment upon the morphology and enzyme systems is of considerable interest to a physiologist. An approach which promises to avoid the handicaps of interpretation of tissue homogenate data is the application of enzyme morphology to hormone stimulated tissue. Since phosphatase enzymes are known to be involved in a wide variety of chemical reactions occurring in tissue functions such as metabolism and transport, it is believed that histochemical studies of these enzymes would provide meaningful information regarding the functional capabilities of various tissues. The objective of this investigation is to elucidate the effect of steroid hormones on ultrastructural changes and some phosphatase activity of the Fallopian tube.
REVIEW OF LITERATURE

The ultrastructure and histochemistry of the female reproductive system is a subject of fascinating interest. Indeed, morphologic responses to the changing hormonal environments in reproduction have held the attention of biologists for many decades. Early studies on the structure of the mammalian oviduct were confined to the use of the light microscope (Moreaux, 1913; Novak and Everett, 1928; Corner, 1932; Hadek, 1955). Since the advent of the electron microscope, the ultrastructure of the oviduct has been reported but the information was rather scarce until recently. Fine structure in mammalian oviducts has been observed in rats (Borell et al., 1959), cattle (Bjorkman and Fredricsson, 1961), humans (Bjorkman and Fredricsson, 1962; Clyman, 1966) and sheep (Abdalla, 1969). Excellent reviews are now available on the ultrastructure of the oviduct in the rat, mouse, rabbit, guinea pig, ruminants, and primates including man (Brenner, 1969; Nilsson and Reinius, 1969).

Electron microscope studies of the rabbit oviduct were first published by Borell and his associates (1956). They found that in mature rabbits there were only a few secretory granules in the secretory cells lining the ampulla. The secretory cells were ordinarily found to protrude only slightly beyond the free surface of the ciliated cells. During estrus, the free surface of the secretory cells bulged far
into the tubal lumen. In pregnant animals, the bulges of the secretory cells were ruptured with numerous granules released into the lumen. They also noticed that ovariectomy induced atrophy in secretory cells but caused no significant change in oviductal ciliated cells. This seems to contradict with the earlier report of Flerko (1954) who found a complete loss of cilia from the rabbit oviduct after ovariectomy and a complete restoration of cilia following estrogen treatment.

Nilsson (1958) found that during estrus the rabbit oviduct epithelium contained numerous bulging secretory cells filled with secretory granules. The cytoplasm of the ciliated cells, on the other hand, contained only a few granular endoplasmic reticulum. Among the three types of secretory granules observed by Nilsson and Rutberg (1960), a few were of the estrous type, e.g. dense and homogeneous; the other types were less dense but contained patches of distinct electron-dense material.

Hashimoto and his associates (1959a) reported the changes in the epithelial cells of the isthmus of the oviduct in mature rabbits before and after ovulation. They noticed that after ovulation the endoplasmic reticulum dilated remarkably and the secretory granules increased in size and decreased in density in the secretory cells. In a later paper, they (Hashimoto et al., 1959b) described the changes of the epithelium of the fimbriae. Fine structure of the
ciliated cells in the fimbriae was found to be the same as that of the isthmus region. However, the fimbriae also contained non-ciliated epithelial cells without any secretory granules.

Odor (1969) recently reported, without electron-micrographs, her preliminary observations of estrogen effect on ciliogenesis in the infundibulum of the rabbit oviduct. She found that following ovariectomy, the height of epithelium in the fimbriae markedly decreased and secretory activity was absent. The number of ciliated cells in the fimbriae decreased to about half of that found in the same region of intact rabbits. Disappearance of cilia and basal bodies also occurred in the ciliated cells following ovariectomy. The percentage of normal ciliated cells remained low after three to five days of estrogen treatment. With eight or ten daily hormonal injections the oviduct contained cilia with lengths comparable to those found in normal animals.

Reports on the cyclical variations in the mammalian oviductal epithelium are conflicting. Hashimoto et al. (1964) and Clyman (1966) found no cyclic changes in the ciliated cells of the human oviduct while Pernkopf and Pichler (1953) and Schultka (1963) reported cyclic variations. One study of the rat oviduct showed no cyclic change (Deane, 1952). Flerko (1954) reported cyclic loss and regeneration of cilia. Recently Brenner (1969) found that in the Rhesus monkey the oviductal cilia shed and regenerate during the menstrual cycle.
Cellular hypertrophy and ciliogenesis occur in the early follicular phase. During the luteal phase the ciliated cells of the fimbriae atrophy and the cilia become shredded.

Brower and Anderson (1969) reported electron microscopic findings of the cytological events associated with the secretory process in the rabbit oviduct. Ciliated cells were found to be predominant in the infundibulum. These cells made up about half of the folded epithelium of the ampulla and isthmus. They described two types of secretory granules in the secretory cells of the precoitus oviduct. One was a large, oval electron-lucent type and the other was small, circular and uniformly electron dense. They did not find secretion in the precoitus oviduct but at 24 hours postcoitum, numerous secretory granules were found in the process of extruding their contents from the goblet-shaped secretory cells.

The identification and localization of enzymes at the subcellular level is one of the most recent and rapidly developing fields of cytochemistry. For detailed reviews one should refer to the papers by Goldfischer et al. (1964) and Marchesi and Barret (1964). Among the pertinent texts and reviews which include enzyme histochemistry at the electron microscopic level are those by Burstone (1962), Barka and Anderson (1963) and Goldfischer et al. (1964).

Histochemical observations with the light microscope on human Fallopian tube have been reported by Fawcett and Wislocki (1951) and
Fredricsson (1959). More recently Abdalla (1963) found that acid phosphatase activity in the oviduct of the ewe was localized mostly in the apical parts of the non-ciliated cells and their cytoplasmic projections. He concluded that this enzyme might be concerned with the formation of secretion. However, tests conducted by Bjorkman and Fredricsson (1961) for the demonstration of lipid, non-specific esterase and acid phosphatase in the oviduct of the cow did not give such information.

The first application of histochemical methods to study enzymes at electron microscope level was reported by Sheldon et al. (1955) who employed the Gomori metal-salt technique for the demonstration of acid phosphatase in mouse intestinal epithelium. Since the publication of this paper many investigators have reported enzyme distribution in cellular organelles. Tice and Barnett (1963) found that in rat testes the acid phosphatase was associated with the dense bodies of Sertoli cells and with the Golgi apparatus of all spermatogenic cells. Acid phosphatase has also been shown in the lysosomes of rat and human prostate glands. Hopkins and Baker (1968) reported the fine structural localization of acid phosphatase in the prolactin cells of the eel pituitary. They found acid phosphatase activity within the Golgi cisternae and developing secretory granules and within the large membrane-bound bodies similar to the dense bodies in other cell types. More recently Henzl et al. (1968) found that in
ovariectomized rabbit, estrogen treatment intensified the acid phosphatase activity within the Golgi complex and lysosomes in epithelial and endothelial cells of uterus. They suggested that under certain conditions acid phosphatase bound in lysosomes may play a role in degrading acid mucopolysaccharides in the capillaries.

Essner et al. (1958) and Spater et al. (1958) were the first to demonstrate the plasma membrane ATP-ase activity at the electron microscope level. Spater and his associates (1958) found high ATP-ase activity in the deeply infolded membranes at the base of the renal tubule cells. They postulated that this enzyme may play significant roles in active transport of molecules across the cell membrane and also in cell activities such as pinocytosis. Henzl et al. (1968) reported the influence of estrogen on the subcellular localization of ATP-ase in the endometrium of rabbit. They noticed only sporadic ATP-ase activity in the cell membrane of some cells and in the microvilli of the low columnar epithelium of ovarietomized rabbits. After five days of estrogen treatment there was a significant increase in ATP-ase activity in cilia and microvilli, and around some secretory granules and along the entire length of the cell membranes as well as on the basement membrane of the columnar layer. The enzyme activity in endometrial small blood vessels was more pronounced in the basement membrane of endothelium.
and on cell membranes of perithelial cells. Positive reaction also occurred on cell membranes of some stromal cells close to the epithelium. Prolonged estrogen treatment, however, led to a slight decrease in ATP-ase activity.

It seems clear that there is a paucity of information on the specific role of ovarian steroids in the fine structure and enzymatic activity of the cells of the mammalian oviduct. This study is designed to observe the effect of exogenous estrogen and progesterone, given singly or in combination, on various cellular organelles and on the activity of adenosine triphosphatase (ATP-ase) in prepubertal rabbit oviduct. The information should explain some of the hormonal effects on reproduction in general and the mechanism of hormone action at the subcellular level in particular.
MATERIALS AND METHODS

Immature, female rabbits of the New Zealand White strain obtained from the Oregon State University Small Animal Laboratory were used in this study. These animals were in apparent good health and were under close supervision throughout this investigation. Rabbits were maintained on a diet of Purina laboratory chow and water ad libitum.

Two series of experiments were performed.

Experiment 1

Animals were assigned randomly to four groups of four rabbits each. The following hormone treatments were given to animals in Group 1 for seven days.

1. Control, 0.1 ml propylene glycol.
2. Estradiol benzoate, $^1$ 5 µg in 0.1 ml propylene glycol.
3. Progesterone, $^2$ 2 mg in 0.1 ml propylene glycol.
4. Estradiol benzoate, 5 µg and progesterone, 2 mg in 0.1 ml propylene glycol each.

Crystalline hormones were used. They were dissolved in propylene glycol $^3$ and administered intramuscularly. The animals

$^1$ Nutritional Biochemical Corporation, Cleveland, Ohio.
$^2$ Sigma Chemical Company, St. Louis, Missouri.
$^3$ Robinson Laboratory Inc., San Francisco, California.
were sacrificed 24 hours after the final injection and the oviducts 
were removed immediately for morphological and cytochemical stud-
ies.

Animals in Group 2 and Group 3 were treated similarly as for 
Group 1, except that they were sacrificed 14 days and 28 days, re-
spectively after the final injection. The age of all these rabbits at 
the time of autopsy was eight weeks and their body weight ranged 
from 2.1 to 2.6 kg.

**Experiment 2**

The animals in Group 4 were ovariectomized at eight weeks; 
treatments similar to those given Group 1 were initiated on the 40th 
day after surgery. The rabbits were sacrificed 24 hours after the last 
injection. The weight of these animals varied between 2.8 to 3.3 kg.

Samples of tissue from the oviduct were collected from four 
regions; the fimbriae, ampulla, isthmus, and uterotubal junction 
and processed for electron microscopic and cytochemical studies. 
After the removal of the oviduct, subsequent work was carried out 
at 0-4°C to avoid inactivation of enzymes.

**Fixation**

After excision, the tissue blocks approximately 1 mm$^3$ each 
in size, were transferred to vials containing 3% glutaraldehyde buf-
fered to pH 7.4 with 0.1 M solution of Na cacodylate and fixed
for one hour at 0-4°C (Sabatini, Bensch and Barrnett, 1963). Following fixation, unbound glutaraldehyde was removed from the tissue in order to avoid interference with either the enzymatic reactions or the postfixation with osmium tetroxide. This was accomplished by rinsing the tissue four times for 15 minutes each in cold 0.1 M Na cacodylate buffer, pH 7.4 containing 0.22 M sucrose. The tissues were then left overnight in the last change of buffer for further treatment.

**Histochemical Technique**

**Adenosine Triphosphatase**

The activity of the enzyme, adenosine triphosphatase, was demonstrated at subcellular level by employing the standard method of Wachstein and Meisel (1957).

Stock solutions were prepared on the same day of the experiment and the final incubation mixture was prepared immediately before use in the following order, with thorough mixing after the addition of each component.

- Tris maleate buffer 0.2 M, pH 7.2 20 ml
- Phosphate ester substrate 125 mg/100 ml 20 ml
- Lead nitrate 2% 3 ml
- Magnesium sulfate 0.1 M 5 ml
Distilled water 2 ml

Tissue blocks, fixed with glutaraldehyde and washed overnight with cacodylate buffer, were incubated in the medium at room temperature for 50 minutes.

**Acid Phosphatase**

The activity of the enzyme, acid phosphatase, was demonstrated at subcellular level by employing Barka and Anderson's (1962) modification of the original Gomori lead phosphate method (Gomori, 1952).

The final incubation medium was prepared immediately before use in the following order:

- Tris maleate buffer 0.2 M, pH 5 10 ml
- Distilled water 10 ml
- Substrate solution (1.25% Sodium beta-glycerophosphate, pH 5) 10 ml

Twenty ml of 0.2% lead nitrate were added drop by drop to the mixture with continuous stirring. The freshly prepared solution was clear and did not produce any nuclear staining artifacts. Tissue blocks fixed and washed as described, were incubated at room temperature for 20 minutes.

Thin sections of tissue blocks were also used for incubation. This was accomplished by using the unfrozen sections cut at 45 microns thickness with a tissue chopper (Smith and Farquhar, 1965).
Glutaraldehyde fixed tissue strips 5 to 8 mm long were stabilized with 7% agar. Tissue strips were placed on polyethylene filter paper cushion. Cooled agar was placed on the mounted tissue strip until it was covered on all sides. When the agar was solidified, the two sides were trimmed that lie perpendicular to the cutting blade. The sections were cut with a Schick injectable razor blade cleaned with 70% alcohol and were carefully collected in 0.1 M cacodylate buffer (pH 7.4) containing 0.22 M sucrose and washed overnight in buffer at 4°C, to leach out traces of fixative. Frozen sections were not utilized for incubation, since it has been shown that considerable disruption of fine structure occurs, attributable to freezing and thawing during sectioning (Smith and Farquhar, 1966).

For control experiments, tissue sections and blocks were incubated by omitting the substrate from the incubation medium and in media in which ATP was replaced with equimolar concentrations of sodium beta-glycerophosphate. Also other control preparations were obtained by inactivating enzymes through pretreatment of specimens for one hour in buffered 1% osmium tetroxide prior to incubation in the substrate containing media.

Post-fixation

After incubation in the respective media all sections and blocks of tissue were washed twice in cold cacodylate buffer over
a period of 30 minutes. They were then refixed in 1% osmium tetroxide buffered to pH 7.4 containing 0.22 M sucrose and embedded in plastic as described below.

Dehydration and Embedding

The osmium fixed tissues were dehydrated in graded dilutions of ethanol: ten minutes each in 50%, 70% and 95% of ethanol and three changes of ten minutes each in 100% ethanol. The tissue was then rinsed twice in propylene oxide for 20 minutes each. Following dehydration, the tissues were infiltrated with 1:1 mixture of propylene oxide and epon mixture with catalyst for four hours. The epon embedding mixture was made up by using Epon 812, dodecenyl succinic anhydride, methyl nadic anhydride, and 2,4,6-tri (dimethyl aminomethyl) phenol (Ladd Research Industries Inc.). The resin components were made into stock mixtures A and B as described by Luft (1961).

1. Mixture A: Epon 812 62 ml
   DDSA 100 ml
2. Mixture B: Epon 812 100 ml
   NMA 89 ml
3. DMP-30 1.5%

Tissues were embedded in mixtures of six parts of A and four parts of B. Immediately before use, the accelerator DMP-30 was
added at 1.5%, V/V and stirred thoroughly.

Finally, the tissue blocks were transferred from the infiltration mixture to plastic beam capsules filled with complete epon mixture. The plastic was then polymerized at 60°C.

**Staining, Sectioning and Viewing the Tissue**

The polymerized tissue blocks were sectioned on a Porter Blum Serval MT-2 ultramicrotome with a glass or diamond knife. Sections about 500 to 700 Å thick were cut and mounted on uncoated and formvar coated 300 or 500 mesh copper Athene grids. Sections were later stained with saturated uranyl acetate for 20 minutes and then with Reynold's lead citrate (Reynolds, 1963).

Stained and unstained sections were examined with an electron microscope (RCA 2 D, 3 F or 3 G) operated at 50 KV, with a 50 micron objective aperture, or with a Philips EM 300 electron microscope operated at 60 KV, and a 30 micron objective aperture. The negatives obtained were further enlarged photographically and printed on Kodak polycontrast or Kodabromide paper for detail interpretation.
RESULTS

Immature Rabbit

Ultrastructure

The epithelium of the ampullary region of the Fallopian tube in the immature rabbit is pseudostratified in all of the sections examined. There is considerable variation in the length and width of the cells and the position of nuclei within the cells. Two types of cells are observed—ciliated and secretory cells. Fine structure of the ciliated and secretory cells of the ampulla region are illustrated in Figures 1-17. A typical basement membrane is seen separating the epithelium from the lamina propria (Figures 3 and 15).

Ciliated Cell. The lateral cell surface of each ciliated cell is irregular and extensive foldings of the cell membrane are often encountered (Figures 8 and 9). The membranes of the adjacent cells near the luminal surface, show both tight junctions and desmosomes (Figures 1 and 10). The nuclei are usually situated in the apical cytoplasm and are very irregular in outline with deep indentations. The nuclei are composed of granular dense chromatin located within the less dense ground substance. Sometimes patches of chromatin are seen in the nucleoplasm. Chromatin patches are often distributed
in contact with the inner nuclear membrane. Depending on the plane of sectioning, the nucleus normally shows a distinct electron-dense nucleolus (Figures 1 and 9).

The nuclear envelope consists of the inner and outer membranes and the perinuclear space (Figures 8, 9 and 10). The mitochondria are numerous, especially in the supranuclear region. Generally, they are spherical, ovoid or elongated in shape and are abundant in the apical and basal cytoplasm. Their fine structure resembles the general basic pattern as described by Palade (1952). The inner membrane of mitochondrion is thrown into the cristae which contain the intracristal space. The matrix is often finely granular in appearance (Figures 7 and 12).

The Golgi apparatus is usually located at the supranuclear or juxtanuclear position and consists of parallel membrane lamellae, dilated membrane bound vesicles and small vesicles (Figures 8, 10 and 11). In the Golgi region there are sometimes electron-dense circular bodies which may be lysosomal in nature. Endoplasmic reticulum is poorly developed or almost inconspicuous with very narrow cisternae. Free ribosomes occur singly or as clusters or rosettes. Microfibrils and microtubules are abundant in the cytoplasm (Figures 1 and 13).

The apical surface of the ciliated cell is provided with a few short cilia and microvilli but many basal bodies are present in the
apical cytoplasm (Figures 1 and 2). The basal bodies have an electron-dense knob-like structure but striated rootlets have not been regularly observed in the ampulla epithelium (Figure 4). Only in one case a rootlet-like structure is seen extending downward into the cytoplasm from the proximal end of a basal body (Figure 14). A cross sectional view of an individual cilium shows a central core of typical 9+2 pattern of organization with nine peripheral double filaments, two central single filaments and a surrounding ciliary membrane (Figure 5). The profiles of the two central filaments are circular, those of the nine peripheral filaments are shaped like figures of eight. The peripheral filaments have short dense protrusions extending from their one side, the arms. This complex is referred to as the axoneme. The ciliary membrane is an extension of the cell membrane or plasma membrane covering the rest of the cell. The basal body of the cilium situated beneath the cell membrane is a hollow cylindrical structure. In transverse section, a basal body has a circular outline and the wall of the cylinder is seen to be composed of nine sets of triplet fibrils with each triplet set at an angle to the axis of the basal body. The orientation of the triplets in cross section thus appears in a pinwheel fashion similar to the centriole of the cell (Figures 20 and 94). The microvillus differs from the cilium in that the microvillus lacks a central core of fibrils. Instead, each microvillus consists of a central core of electron-dense material
clothed by the surface membrane. Dense bodies and multivesicular bodies, probably lysosomal in nature, are sometimes seen in the apical cytoplasm or in close association with the Golgi apparatus (Figures 1, 10, 42 and 43).

**Secretory Cell.** The apical portion of the secretory cell often has balloon-like bulges projecting into the lumen and this has been referred to as the apocrine projection or secretory cell protrusion (Figures 1, 2 and 8). These projections can be ruptured or dissociated from the cell and sometimes lie free in the tubal lumen. The balloon-like bulges of the secretory cell contain a few homogeneous electron-dense secretory granules which range in size from 0.3 to 0.6 μ. The second type of secretory granules is homogeneous and less dense and contain patches of electron-dense material (Figures 6 and 9).

The nuclei of the secretory cells, in most cases are situated basally, but their position varies. As in the case of the ciliated cells, the nuclei are very irregular in shape. Portions of the nucleus are sometimes in close apposition to the granular endoplasmic reticulum (Figure 6). The cytoplasm of the secretory cells also contains spherical, oval and elongated mitochondria which are more frequently observed in the apical region (Figure 1). The Golgi apparatus is usually situated in the supranuclear part of the cell (Figures 1 and 8).

Granular endoplasmic reticulum which encloses irregular spaces
containing a homogeneous substance is well developed and is more extensively developed in the secretory cells than in the ciliated cells. In these cells with prominent endoplasmic reticulum continuity may be seen between the lumina of the cisternae of the endoplasmic reticulum and the perinuclear space (Figure 8). This structural relationship provides a potential intercommunication between these two important organelles. Close association between the granular endoplasmic reticulum, secretory granules and the Golgi apparatus can be seen in Figures 8, 9 and 11. The surface is provided with numerous finger-like microvilli, which are sometimes branched (Figure 1).

Morphologically, two different types of cells are seen in the basal portion of the ampulla epithelium (Figure 3). One type resembles the secretory cell and contains well developed endoplasmic reticulum, mitochondria and few circular dense secretory granules. The nuclei of these cells are irregular in shape and each has a distinct dense nucleolus. The other type of cell resembles the ciliated cell and contains abundant free ribosomes and mitochondria and poorly developed endoplasmic reticulum.

Stromal Ultrastructure. The stroma is composed of many connective tissue cells, capillaries, and connective tissue space with sparse distribution of collagen fibrils. The connective tissue cell contains a nucleus and other organelles such as granular endoplasmic reticulum, ribosomes and mitochondria. Connective tissue cell is
the source of collagen and its prominent granular endoplasmic reticulum is in keeping with this protein secretory function (Figures 3 and 15).

The outlines of the connective tissue cells are irregular in comparison with the epithelial cells. The connective tissue cells do not associate with each other and therefore do not form desmosomes. The nucleus of the connective tissue cell is irregular in shape and the nuclear membrane is a double membrane structure. The chromatin is irregularly distributed throughout the nucleoplasm and the nucleolus is indistinct. The cytoplasm of the connective tissue cells consists of long processes which extend quite a distance from the nucleus. Lipid droplets are also present in the connective tissue cell and its elements.

The stromal capillary consists of the endothelial cells which is surrounded by a closely applied basement membrane. There is space between the capillary and the connective tissue cells. Each endothelial cell has a thickened area which contains the nucleus along with granular endoplasmic reticulum, ribosomes, and occasional mitochondria. Few flask-shaped invaginations or micropinocytic vesicles are present on the inner and outer surface of the endothelial cell. These vesicles are also known as caveolae. Capillary pores or fenestrations have not been observed in the capillary endothelium.

Smooth Muscle Cell. The smooth muscle cell (Figures 16 and
17) has a distinct plasma membrane and is invested on its outer surface by a thin but distinct, dense and finely fibrillar basement membrane. The smooth muscle cell contains a prominent nucleus, few ribosomes, mitochondria and granular endoplasmic reticulum. Mitochondria, usually situated near the nucleus, are small spherical or oval in shape and do not show features of high metabolic activity. The cytoplasm is filled with myofilaments arranged parallel to the long axis of the cell. Dense bodies are observed between the fine filaments. The intercellular space between the smooth muscle cells contains collagen fibrils. Continuity between the smooth muscle cells often occurs by means of an intercellular or protoplasmic bridge.

**Cytochemistry**

Results on the subcellular localization of ATPase are illustrated in Figures 8 to 16. The final reaction product resulting from enzymatic hydrolysis of ATP occurs as a fine granular electron-dense precipitate along the outer and lateral cell membranes, outer membrane of microvilli and cilia. Evidence of ATPase activity is absent on the basal cell membranes, basement membrane and stromal cell components. Frequently, the reaction product occurs on the endothelium and red blood cell membrane of the stromal capillary. Fine granular deposits of the reaction product are also distributed on the peripheral fibrils of cilia and basal bodies (Figure 14).
Occasional deposits of the reaction product are also seen in the Golgi apparatus (Figure 11) and mitochondria (Figure 12). In the mitochondria, the reaction product is present in the cristae and matrix. It occurs as clumps of electron-dense precipitate sometimes extending from the inner membrane into the matrix. The tight junction, desmosome and the nuclear envelope are devoid of enzymatic activity.

**Immature Rabbit Following Estrogen Treatment**

**Ultrastructure**

Figures 18 to 22 demonstrate the fine structure of the epithelial cell of the oviduct in response to estrogen treatment. The cytoplasm of ciliated cells is richly provided with polyribosomes and expanded profiles of the granular endoplasmic reticulum. The apical cytoplasm also contains dense bodies resembling lysosomes and multivesicular bodies. Mitochondria often appear swollen and fragmented with vacuolated matrices. Stages in the formation of cilia are observed in some ciliated cells. The cytoplasm of these cells contains numerous centrioles and dense bodies known as "ciliary precursor bodies." The association between the dense body and a centriole can be seen in Figure 22. The dense body near a centriole seems to have a tubular structure. Some dense bodies are seen close to the Golgi apparatus.
indicating that these "ciliary precursor bodies" might have originated from the Golgi complex.

In the estrogen treated rabbit, morphological changes apparently related to the secretory process are observed in the secretory cells of the oviductal epithelium. The apical regions of the cells contain well developed dilated cisternae of the granular endoplasmic reticulum, numerous ribosomes and secretory granules in the process of passing into the tubal lumen. The cytoplasm contains mitochondria and lysosomes. Most of the secretory granules are of the less dense type but dense homogeneous type are also observed in the apical cytoplasm. The secretory granules can be seen to open singly or to coalesce both at the cell surface and deeper within the cell (Figures 18 and 21).

The basal cytoplasm of the epithelial cell contains numerous ribosomes, mitochondria and a well developed granular endoplasmic reticulum containing homogeneously distributed fibrillar material. The most striking feature is the widening of the intercellular spaces of the lateral cell membranes (Figure 19).

The connective tissue cell of the subepithelial zone contains a prominent nucleus, numerous ribosomes, Golgi apparatus, mitochondria, lipid droplets and well developed granular endoplasmic
reticulum. The interstitial space is filled with collagen fibrils.

The cytoplasm of the smooth muscle cell is particularly rich in polyribosomes and possess a well developed granular endoplasmic reticulum which forms dilated cisternae containing fibrillar material. A prominent Golgi apparatus and numerous mitochondria are also observed in the cytoplasm. Evidence of increased pinocytic activity occurs on the plasma membrane of the smooth muscle cell (Figures 23 and 24). The interstitial space between the smooth muscle cells contain abundant collagen fibrils.

Cytochemistry

As compared to the control group, ATPase activity is markedly pronounced on the cell membranes of the epithelial cell (Figure 21). ATPase activity also appears to be much more pronounced on the outer membranes of cilia and microvilli, peripheral fibrils of cilia and basal bodies and in the Golgi saccules.

ATPase activity is also demonstrated by the presence of sparse electron-dense deposits on the plasma membrane and basement membrane of smooth muscle cell (Figure 24). Intense deposits of the reaction product have also been observed on the luminal membrane of capillaries and on the surface of erythrocytes.
Immmature Rabbit Following Progesterone Treatment

Ultrastructure

Figures 25 to 35 demonstrate the effect of exogenous progesterone on the ultrastructure of the epithelial cells and smooth muscle cells of the Fallopian tube. The most striking feature of the effect of progesterone on the epithelial cells is the extreme dilation of the cisternae of the granular endoplasmic reticulum. Another striking feature frequently observed is the continuity between the perinuclear space and the cavities of the endoplasmic reticulum.

The apical cytoplasm of the ciliated cell contains numerous mitochondria, microtubules and polyribosomes. Well developed Golgi apparatus and expanded profiles of the endoplasmic reticulum can often be seen in the apical cytoplasm (Figures 26, 28 and 29). The most significant observation is the active ciliogenesis in response to progesterone treatment. Numerous "ciliary precursor bodies" and basal bodies are present in the apical cytoplasm (Figures 28 and 29). Close association appears between the microtubules and the basal bodies. A microtubule with cross-striated appearance is seen in close association to a dense "ciliary precursor body" (Figure 29). Dense bodies resembling lysosomes are also present in the cytoplasm of the ciliated cells. These observations clearly
indicate marked ultrastructural changes in the ciliated cells in response to progesterone.

Pronounced morphological changes are also observed in the secretory cells. The most striking observation is the occurrence of prominent and markedly dilated granular endoplasmic reticulum, which form large cisternae containing fine fibrillar material. The cytoplasm is richly provided with free ribosomes, mitochondria and secretory granules of varied density (Figures 25, 26, 27 and 28). There is a prominent Golgi apparatus consisting of lamellae, clusters of small vesicles and large vacuoles with contents of varying density (Figure 30). The intimate morphological relationship of the Golgi elements, endoplasmic reticulum and secretory granules is often observed. Vesicles formed by dilation and budding of the tips of the Golgi saccules occur frequently in close proximity to endoplasmic reticulum. Formation of secretory granules is observed in the Golgi region (Figure 30).

Secretory granules of varied size and density are found in the cytoplasm. Most of the granules are moderately electron-dense containing small dense spots (Figures 27, 28, and 29). Secretory granules often possess a distinct plasma membrane devoid of a regular internal structure. The apical portions of the secretory cells are often filled with goblet-shaped granules when the elapsed time after the last day of injection of the hormone is prolonged.
Increased coalescence of the secretory granules occurs throughout
the cytoplasm at this time. The electron micrographs illustrate
clearly apocrine, microapocrine and merocrine types of secretion.
An interesting cytological feature is the occurrence of a cilium in
a secretory cell (Figure 25).

The basal cytoplasm contains markedly dilated cisternae of
the granular endoplasmic reticulum and numerous ribosomes. For-
mation of secretory granules also occurs in the basal cytoplasm in
close association with the granular endoplasmic reticulum. Secre-
tory granules in this region are of the dense homogeneous type.
Some mitochondria in this region appear ballooned and exhibit dis-
rupion of their matrices. Widening of the lateral cell membrane
is frequently encountered. Portions of the connective tissue cells
contain large lipid droplets, well developed granular endoplasmic
reticulum with dilated cisternae and numerous polyribosomes which
occur singly or in clusters. The interstitium contains abundant
collagen fibers (Figures 31 and 32).

Significant ultrastructural changes are also observed in the
smooth muscle cells. Numerous elongated and branched mitochondria
and clusters of ribosomes occur in close association with each other.
The cytoplasm of these cells also contains microtubules and dense
bodies resembling lysosomes. The Golgi apparatus seen near the
nucleus contains many vesicles and a few lamellae. The cytoplasm
of the smooth muscle cells contain well formed myofilaments and small dense bodies of unknown nature (Figures 33, 34 and 35). Micropinocytic vesicles are numerous along the plasma membrane. The interstitial space is filled with collagen fibrils. Figure 34 illustrates the intimate morphological relation of a lipid droplet, mitochondria and ribosomes.

**Cytochemistry**

The final reaction product resulting from the enzymatic hydrolysis of ATP is distributed as a fine granular or globular deposits of lead phosphate on the cell membrane, outer membrane of microvilli and cilia (Figures 27 and 28). ATPase activity occurs sporadically on the basal cell membrane (Figure 32). Evidence of ATPase activity is completely absent in the stroma. As compared to the control group, ATPase activity appears to be more pronounced on the cell membrane, cilia and microvilli. However, the enzymatic activity on the lateral cell membrane is relatively less intense when compared to the estrogen treated group. Fine granular precipitate of the final reaction product has also been observed on the axoneme complex of cilia and basal bodies, and mitochondria.

The stroma is completely devoid of enzymatic activity even though an occasional deposit has been noticed on the outer membrane of the connective tissue cells. Reaction product occurs sporadically on
the plasma membrane and basement membrane of smooth muscle cells (Figures 33, 34 and 35).

**Immature Rabbit Following Estrogen and Progesterone Treatment**

**Ultrastructure**

Figures 36 to 41 illustrate the combined effect of estrogen and progesterone on the fine structure of epithelial and smooth muscle cells. The apical cytoplasm of the ciliated cell contains many microtubules, clusters of ribosomes and vesicular profiles of the endoplasmic reticulum. The mitochondria are spherical, oval or elongated in shape and have a dense matrix (Figure 36). "Ciliary precursor bodies" and basal bodies are observed in the apical cytoplasm (Figures 36 and 38). Multivesicular bodies and dense bodies resembling lysosomes have also been observed in the apical cytoplasm.

The secretory cells also have marked changes in the fine structure as compared to the control group. The apical cytoplasm of these cells is richly provided with the expanded profiles of the endoplasmic reticulum containing fibrillar material of varying density. Disrupted portions of the plasma membrane can be seen in the tubal lumen (Figure 37). Secretory cells also contain a well developed Golgi apparatus. The intimate morphological relation between the Golgi saccules, granular endoplasmic reticulum and secretory
granules is illustrated in Figure 39.

The basal cytoplasm also contains dilated cisternae of the endoplasmic reticulum, dense mitochondria and numerous ribosomes. Dilation of the intercellular space between the cell membrane has been observed. The nucleus in this region has deep indentations (Figure 40). Portions of stroma contain connective tissue cells which have prominent nuclei, well developed granular endoplasmic reticulum with dilated cisternae and numerous ribosomes. Collagen fibers are abundant in the interstitium (Figure 40).

The cytoplasm of smooth muscle cells contain numerous mitochondria, polyribosomes and granular endoplasmic reticulum located near the nucleus (Figure 41). Evidence of increased micro-pinocytic activity occurs on the plasma membrane of the smooth muscle cell. The interstitial space between smooth muscle cells is filled with increased amount of collagen fibrils. Close association between mitochondria and lipid droplets has been observed in the smooth muscle cell.

**Cytochemistry**

The final reaction product representing ATPase activity occurs on the outer and lateral cell membranes and outer membranes of microvilli and cilia. It has also been observed on the peripheral fibrils of cilia and basal bodies. Intense deposits of reaction product
occur at these sites as compared with the controls (Figure 36). As in the previous groups, evidence of ATPase activity on the basement membrane and stromal cell elements is absent. Distribution of the reaction product in the smooth muscle cell is the same as described for the previous groups. However, ATPase activity appears to be more pronounced than in the controls.

**Oviductal Regions Other Than Ampulla**

**Isthmus**

**Ultrastructure.** Figures 44 and 45 demonstrate the fine structural alterations in the epithelium in response to exogenous estrogen. The epithelium consists of both secretory and ciliated cells as observed in the ampulla and fimbriae region (Figures 42 and 43). The secretory cell is engorged with goblet-like less dense secretory granules which contain small patches of electron-dense material. Coalescence of the secretory granules with the loss of its membrane occurs throughout the cytoplasm. At the luminal surface these secretory granules come into contact with the outer cell membrane which is eventually disrupted and releases secretory product into the tubal lumen. The morphological features observed in the secretory cells of the isthmic region are similar to those of the ampulla.

The ciliated cell shows numerous microvilli in their free
surface. Basal bodies and "ciliary precursor bodies" are present in the apical cytoplasm. The most significant observation is the occurrence of a cross-striated rootlet-like structure which extends obliquely into the cytoplasm from the proximal end of a "ciliary precursor body" (Figure 45). The apical cytoplasm of the ciliated cell also contains mitochondria and clusters of ribosomes.

Ultrastructure of the epithelium in response to exogenous progesterone is illustrated in Figures 46 and 47. The ciliated cell contains basal bodies and "ciliary precursor bodies" and the tubal lumen appears to be filled with homogeneously distributed secretory material (Figure 46). Figure 47 clearly demonstrates the extrusion of several secretory granules into the tubal lumen.

Fine structural alterations in the secretory cell in response to combined treatment of exogenous estrogen and progesterone can be seen in Figure 48. The cytoplasm is completely filled with mucin-type secretory granules and extrusion of several of these into the tubal lumen are observed in the apical pole. The nuclei in these cells are basally situated and are irregular in shape.

Uterotubal Junction

Ultrastructure. As in the case of ampulla and isthmus region, the epithelial cell of the tubal junction contains both ciliated and secretory cells. The apical region of the ciliated cell contains a
prominent nucleus which is irregular in shape (Figure 49). No difference in the morphological features is observed as compared to the ciliated cells from other regions of the Fallopian tube. Figures 50 to 52 illustrate the alterations in morphological features of the epithelial cell in response to exogenous estrogen. The most striking feature observed is the dilation of the endoplasmic reticulum and active growth of many cilia.

Results of the progesterone stimulated epithelium of the tubal junction are illustrated in Figures 53 to 55. Marked alterations in the morphology of the secretory cells have been observed. The secretory cells are filled with goblet-like granules and many of these can be seen in the process of extrusion of their contents into the lumen. An interesting observation is the occurrence of remnants of disrupted plasma membrane and intact secretory cells in the tubal lumen. Electron-dense bodies resembling lysosomes are observed in the apical cytoplasm of the ciliated cells. The cytoplasm of the ciliated cells is richly provided with polyribosomes, mitochondria and microtubules. The apical cytoplasm also contains dense "ciliary precursor bodies" (Figure 54).

Cytochemistry. As compared to the controls estrogen stimulated epithelium shows intense deposits of the final reaction product along the lateral cell membrane, outer membrane of the microvilli and cilia (Figures 49, 50, 51 and 52). Fine granular deposits of the
reaction product are also distributed on the basement membrane and stroma (Figure 51). As compared to the estrogen stimulated group, ATPase activity appears to be less intense in the progesterone stimulated epithelium.

Figure 56 illustrates the distribution of the ATPase reaction product in the capillary stimulated with estrogen and progesterone. Fine discrete deposits of the reaction product occur on the endothelium and outer surface of red blood cells. Sparse deposits of the reaction product are distributed on the outer membrane of connective tissue cells. An occasional deposit is seen in the connective tissue space.

Ovariectomized Rabbit

Ultrastructure

Results of the effect of ovariectomy on the ultrastructure of the oviductal epithelium from the fimbriae are illustrated in Figures 57 to 60. After ovariectomy, both the ciliated cells and secretory cells show several alterations in their fine structure. The epithelium becomes atrophied and most of the cilia disappear. Very few microvilli are observed in the apical surface. Changes in the basal bodies are also evident. The apical cytoplasm contains relatively inactive Golgi apparatus and fewer ribosomes scattered throughout the cytoplasm.
without forming any clusters. Mitochondria become very irregular in shape and usually do not possess distinct cristae. The matrix of the mitochondria is dense and sometimes contains vesicles and vacuoles (Figures 57 and 58). Endoplasmic reticulum is very poorly developed and polysomal formations are infrequent. The monosomal state of the ribosomes indicates a low level of protein synthesis. Electron-dense lysome-like bodies are frequently encountered in the apical cytoplasm. Atrophy of cells is evident from the increased nucleocytoplasmic ratio. Patches of chromatin forming discrete clumps which are seen in normal epithelial cells seldom appear in the nuclei. Occasionally, continuity between the cavities of the endoplasmic reticulum and the perinuclear cisterna has been observed. Stages in the formation of cilia and basal bodies are no longer present in the apical cytoplasm. In some ciliated cells all the cilia have disappeared except for one or two basal bodies.

The secretory cells also atrophy after ovariectomy and the secretory granules disappear from the cytoplasm. Very few ribosomes occur in a polysomal state. In comparison with the well developed granular endoplasmic reticulum of the normal rabbit oviduct, the granular endoplasmic reticulum appears to be almost obliterated in some cells following ovariectomy. The Golgi apparatus is relatively inactive and mitochondria become swollen and appear to have structural deficiencies. Few short microvilli appear at the
apical surface of the secretory cells.

Ultrastructure of the smooth muscle cell from the ampulla region following ovariectomy is illustrated in Figure 61. The cytoplasm of the smooth muscle cell contains few oval and elongated mitochondria and ribosomes. Nucleoli are few and small. The endoplasmic reticulum is poorly developed and decreased pinocytic activity occurs on the plasma membrane of the smooth muscle cell.

Cytochemistry

In the oviduct epithelium of the ovariectomized rabbit, considerably less ATPase activity occurs on the cell membrane of some cells and on the outer membrane of microvilli. Mitochondria, Golgi apparatus and other intracellular organelles lack ATPase activity. This is indicated by the absence of the reaction product in these organelles (Figures 57, 58, 59 and 60). Occasional deposits of the reaction product occur on the plasma membrane of smooth muscle cells.

Ovariectomized Rabbit Following Estrogen Treatment

Ultrastructure

The influence of estradiol benzoate on the ultrastructure of ovariectomized rabbit oviduct epithelium from the fimbriae region is illustrated in Figures 62 to 65. As compared with the ovariectomized
animal, the apical surface of oviductal ciliated and secretory cells contains numerous microvilli. The apical cytoplasm of the secretory cells from the fimbriae consists of many electron-dense circular secretory granules. The less dense type of secretory granules are few in number. The cytoplasm contains many mitochondria and free ribosomes occurring singly or in clusters. Polysomal formations, however, are frequently encountered. The most striking feature observed is the extreme dilation of the cisternae of the granular endoplasmic reticulum, the lumina of which contain homogeneously distributed flocculent precipitate of moderate electron density (Figure 64). Mitochondria appear to have a similar structure to those found in intact animals. Figure 65 demonstrates the potential intercommunication between the perinuclear space and the cisternae of the endoplasmic reticulum.

Growth and early formation of cilia are observed in the ciliated cell (Figures 62 and 63). Microfibrils and microtubules are found in the apical cytoplasm.

Smooth muscle cells of the fimbriae region display many interesting features. The myofilaments are not well developed and not compact as compared to smooth muscle cells from other regions of the Fallopian tube (Figure 66). Very few micropinocytic vesicles and dense bodies occur. The mitochondria are sometimes very elongated and contain longitudinal and transverse cristae. Polysomal
formations and granular endoplasmic reticulum containing dense material are frequently encountered.

Fine structural alterations in the ampulla epithelium of ovariectomized rabbits receiving exogenous estrogen are illustrated in Figures 67 to 73. The ciliated cells have increased number of cilia and microvilli on their free surfaces (Figure 70). Various stages of ciliogenesis are often observed. In the cytoplasm, the following alterations are frequently found: mitochondria are more numerous in the apical cytoplasm and polysomal formations are frequent. The granular endoplasmic reticulum and the Golgi apparatus are well developed. Numerous basal bodies, "ciliary precursor bodies" and centrioles are frequently observed (Figures 67, 69 and 72). Multivesicular and dense lysosome-like bodies are also present in the cytoplasm. An interesting morphological feature that has been previously mentioned is the occurrence of cross-striated rootlets extending from the proximal end of the basal body into the cytoplasm (Figures 70 and 71).

The apparent intensification of morphological signs of secretion after administration of estradiol benzoate in the intact rabbit (Figure 18) is also observed in ovariectomized rabbits treated with estrogen (Figures 67 and 71). The formation of secretory blebs and the occurrence of secretory material in the tubal lumen are frequently observed under these experimental conditions. Sometimes the apical
portion of the ciliated cells appears to protrude and separate into the lumen. The apical portion of the secretory cells is often filled with goblet-like secretory granules and the typical extrusion of these into the lumen are observed (Figure 71). Most of the secretory granules are of the less dense type and contain patches of electron-dense material. This probably indicates the transformation of the electron-dense type into the less dense mucin type secretory granules. Coalescence of the secretory granules occurs both at the cell surface and throughout the cytoplasm.

The intercellular spaces between the epithelial cells often become widened, sometimes to considerable dimensions in the basal region (Figures 68 and 73). The cytoplasm of the secretory cell contains numerous ribosomes and well developed vesicular profiles of the granular endoplasmic reticulum and Golgi apparatus.

Figures 75 to 80 illustrate the ultrastructural features observed in the isthmic region of the oviduct from an ovariectomized rabbit treated with estradiol benzoate. Many cilia and basal bodies are present in the apical surface of the ciliated cells. Dense fibro-granular aggregates can be seen in close association with the basal bodies (Figure 76). Few of the dense bodies have a tubular structure. An interesting observation is the occurrence of electron-lucent secretory granules in the apical cytoplasm of the ciliated cell (Figure 79). The secretory cells of the isthmic region seem to be engorged with
less dense type of granules and typical extrusion of these can be seen in Figure 75. Extrusion of cell contents and nuclei has also been observed in this region (Figure 80). The intimate morphological relation of the mitochondria and lipid droplets in the smooth muscle cell can be seen in Figure 78.

**Cytochemistry**

After seven days of estrogen treatment, ATPase activity in the epithelial cells of the fimbriae is highly pronounced on the cell membrane, and the outer membranes of microvilli and cilia. Deposits of the reaction product have also been observed in the Golgi lamellae. The intensity of the reaction product appears to be much more pronounced on the cell membrane when compared to the ovariectomized untreated group. Fine granular deposits of the reaction product are distributed on the plasma membrane and basement membrane of the smooth muscle cell (Figure 66) with sparse deposits in the intercellular space.

ATPase activity is distinctly more pronounced on the cell membrane and the outer membranes of microvilli and cilia of ampulla epithelium of ovariectomized animals injected with exogenous estrogen. This is demonstrated by the presence of heavy deposits of lead phosphate reaction product at these sites (Figures 67, 69, 70 and 71). Fine deposits of the reaction product are distributed on the peripheral
fibrils of the basal body and cilia (Figures 68 and 72). Enzymatic activity occurs sporadically in the saccules of Golgi apparatus and on the epithelial basement membrane. Dense deposits of the reaction product also coat the luminal membrane of capillary endothelium (Figure 73). In the mitochondria, the reaction product resulting from the hydrolysis of ATP is found in the matrix, or in relatively larger clumps extending over the cristae and inner membrane and into the matrix space. Heavy deposits of the reaction product are distributed on the plasma membrane and basement membrane of smooth muscle cells (Figure 74).

Figures 75, 76 and 78 illustrate the distribution of ATPase activity in the isthmic region of an ovariectomized animal treated with estrogen. ATPase activity is demonstrated on mitochondria, basal bodies, peripheral fibrils of cilia and the membrane of secretory granules by the presence of an electron-dense precipitate in these organelles. Intense ATPase activity has also been observed on the cell membrane and on capillary endothelium. Fine granular deposits coat the cell membrane of erythrocytes. Most of the micropinocytic vesicles of the endothelium also show ATPase activity.
Ultrastructure

Figures 81, 82 and 83 demonstrate the ultrastructural alterations observed in epithelial cells of the fimbriae region of an ovariectomized animal treated with progesterone. As compared to ovariectomized untreated group, the ciliated cells from progesterone treated animals have many cilia and microvilli at the apical surface.

The free surface of the secretory cells bulges markedly into the tubal lumen. The secretory blebs contain a few electron-dense granules at the apical surface and some dilated cisternae of the granular endoplasmic reticulum. Pinched off portions of the secretory blebs and remnants of plasma membrane are also observed in the tubal lumen.

The fine structure of the epithelial and smooth muscle cells from the ampulla of an ovariectomized rabbit treated with progesterone are illustrated in Figures 84 to 90. As in the fimbriae region, the ciliated cells have many basal bodies at the apical surface and formation of cilia can be seen. The apical cytoplasm contains numerous microtubules and polyribosomes. Most of the mitochondria appear to be swollen and contain vacuolated and disrupted matrices.

The secretory cells contain numerous secretory granules of
varying size and density and a well formed granular endoplasmic reticulum. Most of the secretory granules are of the less dense type and contain patches of dense material. Dilation of the cisternae of the endoplasmic reticulum and the close relationship between endoplasmic reticulum and secretory granules are observed. The mitochondria appear to be vacuolated with poorly defined internal structure.

Coalescence of secretory granules is observed throughout the cytoplasm. Several unusual features of the release of the secretory granules have been observed. In one case, the secretory product is apparently released into the lumen after the dissolution of the plasma membrane (Figure 85) while in others the secretory material is extruded into the lumen without loss of any part of the cytoplasmic components. Sometimes a secretory granule bounded with a distinct membrane approaches and attaches to the surface membrane of the cell, and finally extrudes through small opening in the plasma and secretory membranes.

The cytoplasm of the smooth muscle cells contains numerous polyribosomes, mitochondria and a well developed granular endoplasmic reticulum. A well formed Golgi apparatus can be seen in the perinuclear region (Figure 90). The number of micropinocytic vesicles appear to be greatly increased along the plasma membrane.
Cytochemistry

In ovariectomized rabbits receiving progesterone treatment, the distribution of the ATPase activity in the fimbriae epithelium is the same as observed in the estrogen treated group (Figures 81 and 82), but the ATPase activity in the progesterone treated group seems to be more intense when compared to ovariectomized controls. This is demonstrated by the presence of reaction product on the plasma membrane and outer membranes of cilia and microvilli. Sparse deposits of the reaction product also occur as a fine granular precipitate on the basal cell membrane, basement membrane and stromal cell elements (Figure 83).

Distribution of the reaction product in the ampulla epithelium is the same as described for the fimbriae region. ATPase activity is seen on the cell membrane, microvilli and cilia (Figures 84 and 85). The axial filament complex of cilia and basal bodies contain fine deposits of the reaction product.

The most striking cytochemical observation in the ampulla region is the occurrence of ATPase activity in the micropinocytic vesicles along the plasma membrane of smooth muscle cell (Figures 87 and 88).
Figures 91 to 100 illustrate the fine structural features in epithelial cells from the fimbriae region after estrogen and progesterone treatment. The apical cytoplasm of the ciliated cells is provided with numerous mitochondria, polyribosomes and microtubules. A greatly increased number of basal bodies and stages in the formation of cilia are observed at the apical surface. The cytoplasm also contains a well developed Golgi apparatus, granular endoplasmic reticulum, electron-dense lysosome-like bodies and many centrioles.

The most striking feature of the treated ciliated cell morphology is the extensive dilation of the endoplasmic reticulum and the active growth of cilia and microvilli (Figures 92 and 95). The nuclei of the epithelial cells are very irregular in shape and dense patches of chromatin and distinct nucleoli are often seen.

The cytoplasm of the secretory cells contains numerous mitochondria, expanded profiles of the endoplasmic reticulum and few electron-dense secretory granules. The secretory blebs extend far beyond the free surface of the ciliated cell into the tubal lumen (Figure 93). Pinched off portions of secretory cells containing secretory
granules, ribosomes and endoplasmic reticulum can sometimes be seen in the tubal lumen.

Figures 101 to 104 illustrate the fine structural features in epithelial cells from the ampulla region of a rabbit treated with exogenous estrogen and progesterone. The apical cytoplasm of the ciliated cells from ampulla contains numerous mitochondria, polyribosomes and microtubules. Early formation of cilia and basal bodies are observed at the apical surface. The secretory cells contain numerous granules of the less dense nonhomogeneous type. Dissolution and bulging out of the plasma membrane has been frequently observed (Figure 103). The cisternae of the endoplasmic reticulum seem to contain a homogeneously distributed fibrillar material. Coalescence of the secretory granules and the typical extrusion of their contents also occur.

Cytochemistry

The ATPase activity in fimbriae and ampulla epithelial cells from ovariectomized animals receiving both hormones is distinctly more pronounced on the cell membrane, on the luminal surface of the capillaries and also on the plasma membrane of the smooth muscle cell. This is demonstrated by the presence of electron-dense lead phosphate deposits which are frequently found to fill the entire intercellular space of the lateral cell membrane. The intensity of the
reaction product appears to be significantly greater when compared to ovariectomized controls (Figures 95 to 103). ATPase activity also occurs on the stromal cell membrane, luminal membrane of capillary, outer surface of the red blood cell of the stromal capillary and in the Golgi saccules of the secretory cell. This is demonstrated by the presence of electron-dense reaction product at these sites.

Ovariectomized Rabbit Uterotubal Junction Following Estrogen and Progesterone Administration

Figures 106, 107 and 108 illustrate the ultrastructural and cytochemical features of the epithelium from uterotubal junction of an ovariectomized rabbit treated with estrogen. Growth of cilia and microvilli is observed at the apical surface. The apical cytoplasm of the ciliated cell has many mitochondria some of which appear to be swollen and possess vacuolated matrices. The nucleus is very irregular in shape with extensive indentation and is often fragmented. The cytoplasm of the secretory cells is filled with goblet-like secretory granules. Release of secretory material into the lumen can be seen in Figure 107. Pinched off portions of secretory cells containing many secretory granules are often found in the lumen. Extreme dilation of the cisternae of the endoplasmic reticulum can be seen in the basal cytoplasm. The lumens of the cisternae of the endoplasmic reticulum contain a flocculent precipitate of homogeneous
density. Large intercellular vacuoles and goblet-like secretory granules are also found in the basal cytoplasm. Widening of the intercellular spaces between the plasma membranes is especially prominent in this region (Figure 108).

Figures 109 and 110 illustrate the ultrastructural features of the epithelium from the uterotubal junction of an ovariectomized rabbit stimulated with progesterone. Extensive infoldings and interdigitations of the membranes of adjacent cells are commonly encountered. A single basal body at the cell surface between two secretory granules has been observed (Figure 109). The well developed granular endoplasmic reticulum has dilated cisternae containing fibrillar material. Nuclei and other intracellular organelles have been found in the tubal lumen. The illustrations demonstrate typical apocrine and merocrine types of secretion. The ciliated cells contain numerous basal bodies and ribosomes in clusters. Dense "ciliary precursor bodies" are seen in the apical cytoplasm (Figure 110). Among the fine structural changes mitochondrial alterations are the most frequent. They appear to be swollen and have vacuolated matrices.

Figures 111 to 114 reveal many interesting fine structural changes in the ciliated and secretory cells of the uterotubal junction of an ovariectomized rabbit stimulated with estrogen and progesterone. The apical surfaces of ciliated cells contain numerous basal bodies
and cilia. Clusters of ribosomes, dense lysome-like bodies, numerous mitochondria and a well developed Golgi apparatus are also observed in the apical cytoplasm. Most mitochondria appear to have a normal structure even though some of them contain dense bodies, myelin figures and vacuoles. Fine structure of a multivesicular body can be seen in Figure 112. An interesting observation is the presence of a cilium in a secretory cell (Figure 114). The basal body of this cilium is visible but its axial filament complex is not well developed. The secretory cell contains numerous secretory granules which seem to be fused with each other. Active release of the secretory material into the lumen has also been observed (Figures 111 and 112).

The subcellular localization of ATPase activity in the epithelium of uterotubal junction of ovariectomized rabbits treated with estrogen or estrogen and progesterone together is illustrated in Figures 106, 107, 108, 111, 112, 113 and 114. Intense deposits of lead phosphate reaction product, representing ATPase activity can be seen on the cell membranes of the epithelial cells from the tubal junction. Fine granular deposits of the reaction product resulting from hydrolysis of ATP are also distributed on microvilli, cilia and secretory material in the process of extrusion. Deposits of lead phosphate are sparsely distributed on the basement membrane of the epithelial cells. The capillary luminal membrane contains heavy
deposits of the final reaction product. Fine granular deposits of the reaction product are also distributed in the Golgi elements (Figure 113).

The distribution and intensity of the reaction product resulting from the hydrolysis of ATP in the uterotubal junction epithelium appears to be the same as that found in the fimbriae and ampullae epithelial cells from ovariectomized animals receiving estrogen singly or estrogen and progesterone together. There also appears to be a synergistic effect of estrogen and progesterone on ATPase activity of the cell membrane of uterotubal junction epithelial cells. This is comparable and similar to the observations of the ATPase activity of the cell membrane from the ampulla epithelium of ovariectomized rabbits stimulated with estrogen and progesterone.
DISCUSSION

Few dense lysosome-like bodies and multivesicular bodies have been observed in the apical cytoplasm of the ciliated cells from both the fimbriae and ampulla regions of the oviduct. These organelles have not been described in previous electron microscopic studies of the oviduct (Borell et al., 1956; Nilsson, 1958; Hashimoto et al., 1959a; Brower and Anderson, 1969; Odor, 1969).

Fine structure of the dense bodies resembles that of lysosome which has been shown to contain various hydrolases such as acid phosphatase. Early studies by Clyman (1966) indicated the occurrence of lysosomes in the cytoplasm of the human oviductal epithelium.

The presence of microfibrils and microtubules was frequently encountered in the apical cytoplasm of oviductal ciliated cells. Since various proteins often aggregate to form fibrillar patterns, one may speculate that these microfibrils of elongated protein molecules may serve as a diffuse protoplasmic skeleton and give both resilience and rigidity to the cell. The true nature and significance of the microtubules are not clear. It has been suggested by Toner and Carr (1968) that these organelles could be concerned either with the maintenance of the characteristic shape of a cell or with the establishment of diffusion channels through the cytoplasm.

In the oviductal epithelial cells, the nuclei were frequently
found to be irregular in shape, with deep infoldings of the nuclear membranes. These infoldings probably associate with a regulatory mechanism facilitating nucleocytoplasmic exchange and the deep indentations may lead to separation of portions of the nuclei or the phenomena known as amitosis.

The fine structures of the electron-dense and electron-lucent granules were found to be similar to those described for the precoitus oviduct (Nilsson, 1958; Brower and Anderson, 1969). Hashimoto et al. (1959b) did not observe secretory granules in what they called "non-ciliated cells." The present study demonstrates the occurrence of both ciliated and secretory cells in the fimbriae region of the oviduct. This is in agreement with the findings reported in Rhesus monkeys by Brenner (1969).

Only a very few reports have been found in the literature concerning changes in oviductal epithelium following ovariectomy or ovarian steroid administration. The present study has revealed considerable alterations in the fine structure and the results are in general agreement with the findings of Brenner (1969) in Rhesus monkeys and of Odor (1969) in mature rabbits. Borell and his associates (1956), however, did not find any changes in the ciliated cells of the rabbit oviduct following oophorectomy.

Regeneration of the ciliated epithelium has been noticed in ovariectomized rabbits receiving estrogen treatment. The results
support the view that estrogens stimulate the growth of cilia in the ciliated cells (Flerko, 1954; Brenner, 1969; Odor, 1969). The findings of this study indicate a similar mechanism of ciliogenesis might occur in the rabbit oviduct as that described in the vertebrate rod cell (Tokuyasu and Yamada, 1959), in protozoa (Roth and Shigenaka, 1964), and in the oviducts of fetal mice (Dirksen and Crocker, 1965) and Rhesus monkeys (Brenner, 1969). In this type of ciliogenesis, a centriole migrates to the cell surface and after attachment a ciliary bud is formed. Later, the fibrils of the cilium are developed and elongated within the ciliary bud.

Ovarian steroids also stimulate the development of microvilli. The lumen surface contained only a few short microvilli in ovariectomized animals but had numerous long microvilli in animals receiving hormone treatment. Microvilli increase the surface area of the epithelial cells and appear to be associated with the metabolic potential of the oviductal epithelium. They are also probably involved in some phases of discharge of the cellular contents into the tubal lumen (Nilsson, 1959) or in the absorption of luminal constituents (Burgos and Wislocki, 1958).

One of the interesting observations is the presence of cross-striated rootlets in the ciliated cells of oviducts from rabbits receiving estrogen treatment. In his review on the biology of mammalian oviduct, Brenner (1969) pointed out that cross-striated rootlets have
not been observed in mammalian oviducts except those of man and Rhesus monkey. More data is needed to clarify the role of steroids on the formation of this organelle.

Another striking observation in the ciliated cells is the growth of cilia in response to ovarian steroids. Results of the present study regarding the enhancing effect of progesterone on ciliogenesis do not, however, agree with the findings of Andrews (1951) in human and Brenner (1969) in the Rhesus monkey. According to Andrews (1951) the human oviductal epithelium is low and mostly deciliated at the time of delivery. Estrogen causes an intense proliferation of the ciliated epithelium but progesterone exerts an inhibitory effect. Brenner (1969) has not presented direct evidence of the role of progesterone on cilia in Rhesus monkey oviduct but he is of the opinion that the underdevelopment of cilia during the luteal phase is probably due to the inhibitory effect of progesterone.

Another interesting feature of the hormonal influence in the rabbit oviduct is the considerable widening of the intercellular space between the adjacent cells under estrogenic influence. This is probably a manifestation of the general accumulation of fluid in the tissue in response to the influence of estrogen. Dilation of the cisternae of the endoplasmic reticulum in the secretory cell also occurs. This change is similar to the observations in the uterine epithelium of the rat after administration of estrogen (Mueller et al., 1958) and in
liver cells (Porter et al., 1960).

In this study, electron microscopy of the secretory cells has revealed many fine structural alterations in response to hormonal influence. The most striking observation after estrogen or estrogen and progesterone administration is the presence of numerous vesicular profiles of endoplasmic reticulum and secretory granules. Secretory granules were mostly of the mucin-type and the release of many of these into the lumen has been frequently observed. The results of this study agree with the study of Borell et al. (1956) who first reported that changes in the appearances of the secretory cells during the different phase of the reproductive cycle are controlled by the ovarian hormones.

It is also observed that the endoplasmic reticulum becomes markedly dilated and filled with a fine amorphous material in animals receiving progesterone treatment. Similar findings have been reported by Björkman and Fredricsson (1962) and Clyman (1966) on the human Fallopian tube.

Dilation of endoplasmic reticulum also occurs in the smooth muscle cells of the oviduct after estrogen treatment, with the formation of large cisternae containing a fine precipitate. The increase in the number of ribosomes in smooth muscle cells correlates well with biochemical data. Greenman and Kenny (1964) have isolated large numbers of ribosomes from estrogen stimulated rat
myometrium. Electron microscopic studies have revealed clustering of ribosomes associated with protein synthesis after three hours of estrogen stimulation, while most ribosomes lay singly before the hormone treatment (Friederici and DeCloux, 1968). One may speculate that the myoglobin of smooth muscle cells is synthesized on clustered ribosome complexes. Occasionally, close association between lipid droplets and mitochondria has been observed in the smooth muscle cell. It is suggested that an active process of fat metabolism takes place probably under the action of fatty acid oxidases in mitochondria (Palade and Schidlowsky, 1953).

According to many investigators smooth muscle cell has a secretory function in addition to its contractile property (Haust and More, 1966; Jennings et al., 1966; Ross and Klebanoff, 1967; Friederici and DeCloux, 1968). Jennings et al. (1966) have reported the formation of collagen by proliferating smooth muscle cells in the connective tissue developing in aortic grafts in dogs. In the estrogen stimulated uterus, Moses and Catchpole (1955) observed a significant increase in extracellular mucoproteins. An increase in interstitial fine filaments and collagen has also been observed in the estrogen stimulated uterus (Morgan, 1963). Friederici (1967) is of the opinion that these are produced by the fibroblasts, capillary endothelial cells or pericytes. The enlarging smooth muscle cells undoubtedly require additional basement membrane, which contains
glycoproteins and collagen (Kono and Colowick, 1961). Micrographs of basement membrane, however, show evidence of a fine membrane structure with no periodic repeating pattern as observed in collagen.

In the present study close association between the fibrils and the basement membrane of the smooth muscle cell has been observed. Fibrils have also been found in close proximity to smooth muscle cells merging with the basement membrane in the rat myometrium (Friederici and DeCloux, 1968). It has therefore been suggested that smooth muscle fibers, in addition to fibroblasts, may participate in the secretion of collagen and noncollagenous extracellular proteins (Ross and Klebanoff, 1967). Some observations of the present study support the view of Friederici and DeCloux (1968) that this synthesis is enhanced by the influence of estrogen. In the case of rat myometrium the synthesis has been found to take place 18 hours after its administration (Friederici and DeCloux, 1968).

Stromal ultrastructure has been described for the human Fallopian tube by Clyman (1966). However, Clyman has not described the changes in the stromal ultrastructure in response to hormonal environments. In the present study, more collagen and variable ground substance in the oviductal stroma have been found when stimulated with estrogen. The connective tissue cells contained numerous ribosomes and vesicular profiles of endoplasmic reticulum. It seems that the connective tissue cells, resembling
fibroblasts, secrete the collagen found in the interstitium.

Earlier investigators maintained that ciliated cells are absent in the epithelium lining the isthmic region of the rat and mouse Fallopian tube (Espinasse, 1935). The present study has clearly demonstrated the presence of ciliated and secretory cells throughout the tubal epithelium including the uterotubal junction. Borell et al. (1959) found the Fallopian tube of the rat likewise lined by ciliated and secretory cells throughout.

Although the Fallopian tube has been considered as a transportation organ for ovum, spermatozoa and early cleavage stages, the important function of the tube seems to be the formation of secretory granules. Endoplasmic reticulum and Golgi apparatus seem to be the most important organelles involved in the formation of secretory granules.

The granular endoplasmic reticulum has been described as formed by three main components, the ribosomes coating its outer surface, the membrane and the contents of cisternae. The particles have been isolated and have been shown to be extremely rich in RNA (Palade and Siekevitz, 1956). There is good evidence that proteins synthesized at the ribosomes become segregated within the cisternae (Porter, 1961). The observations on the mechanism of secretion have been described in the guinea pig pancreas by Siekevitz and Palade (1958a, b). It has been shown that in the guinea pig pancreas,
proteins newly synthesized at the ribosomes find their way into the cisternae of the granular endoplasmic reticulum, presumably across the limiting membranes, and that thereafter they appear as condensed intracisternal granules. The subsequent transport of the intracisternal material toward the Golgi apparatus through the channels of granular endoplasmic reticulum appears to be supported by findings indicating a continuity between both cell organelles. These studies have indicated that the Golgi apparatus plays a role in the packaging of secretory products of the cell, for exportation or as storage for later use. Many examples of the secretory mechanism have been described in the review article by Kurosumi (1961) and DeRobertis et al. (1965).

A similar sequence of events could be postulated in relation to protein synthesis in the Fallopian tube where continuities between the channels of the granular endoplasmic reticulum and Golgi elements have been occasionally seen. Close association has often been observed between the granular endoplasmic reticulum, Golgi apparatus and enveloping secretory granules in the secretory cell of the rabbit oviduct. This is in accord with the recent observations by Brower and Anderson (1969).

Increase in secretory activity in various regions of the Fallopian tube has been found to respond to estrogen and progesterone in a similar manner. The increase in secretory activity is
evidenced by the presence of numerous mucin-type of secretory granules. Extrusion of several granules into the tubal lumen and marked dilation of the cisternae of the endoplasmic reticulum have also been observed in response to hormonal stimulation. Similar results have been reported on the cyclical changes in the secretory cell of human oviduct (Björkman and Fredricsson, 1961; Clyman, 1966) and rabbit oviduct (Borell et al., 1956; Brower and Anderson, 1969).

Electron micrographs of the secretory cell in response to hormonal stimulus presented in this study clearly demonstrate the different means of extrusion of secretory material into the lumen. For example, an extrusion may consist of the membrane-bounded contents of a single granule, or of a number of granules fused together. Secretory blebs have been found separated and lie free in the lumen. Occasionally, extrusion of cell contents and nuclei has been observed. According to Brower and Anderson (1969), the protrusions are not lost at all but these are only special configurations, perhaps resulting from the pressure of surrounding cells, or from trauma. The present investigation demonstrates the separation of the secretory bleb and the presence of nuclei and other cytoplasmic debris in the tubal lumen. This finding supports the view that extrusion of cell contents and nuclei does occur in the oviduct, and this might be a normal part of the cell cycle as pointed out by Restall (1966).
According to Weiss (1953), typical protein secretory cells have a well developed ergastoplasm. Bjorkman and Fredricsson (1961) reported that typical carbohydrate secreting cells such as in the fructose-secreting part of the rabbit prostate gland have little endoplasmic reticulum but numerous ribosomes in the cytoplasm. In this study both of these components have been found in the secretory cells of the rabbit oviduct. This supports the view of Brower and Anderson (1969) for the formation of two types of secretion or of a secretion with two chemically different components in rabbit oviducts.

The presence of a large amount of granular endoplasmic reticulum and ribosomes in the cytoplasm of the secretory cells may suggest that the membrane system takes part in the production of secretory granules (Nilsson, 1958; Brower and Anderson, 1969). Nilsson (1958) suggested that the secretory granules are synthesized in the membrane-bounded spaces called secretion spaces which can be found anywhere in the cytoplasm. Nilsson (1958) did not find any relationship between the secretion spaces and structures of the Golgi zone or the granular endoplasmic reticulum. In the present study close association between the Golgi apparatus, endoplasmic reticulum and secretory granules has been noticed.

In the present study, vesicles budding off from the endoplasmic reticulum have been observed occasionally. The proteins transported
in the Golgi apparatus may be coupled in varying proportions to polysaccharides to form muco- or glycoproteins which eventually pass into the secretory granules. Favard (1969) stated "The Golgi apparatus is an organelle which has its own individuality. It synthesizes polysaccharides which can be exported directly or coupled in the Golgi saccules with proteins coming from the cavities of the endoplasmic reticulum." Radioautographic studies, for example, have revealed that it is indeed in the Golgi apparatus that polysaccharide synthesis and possible subsequent coupling with proteins take place (Neutra and Leblond, 1966a,b).

According to some investigators the secretory cells and ciliated cells of the rabbit oviduct have the ability to transform from one type to the other (Moreaux, 1913; Westman, 1930). Only in one case, in this study, has a cilium been found in a secretory cell, and conversely, only a few secretory granules were noticed in the ciliated cells. These observations appear to be cytological events occurring in response to the influence of steroid hormones. However, there seems to be no transformation between the two distinct cell types.

Specific activators and inhibitors (other than Mg$^{++}$) have not been used in this study but several assumptions may be made regarding the specificity of the enzyme reported here. First of all, it may be assumed that the hydrolysis of ATP is not due to a non-specific alkaline phosphatase. Ahmed and King (1960) have reported
that 0.001 M arsenate strongly inhibits placental alkaline phosphatase and this inhibition could not be reversed by Mg++. Since the tissues in the present experiment were washed in 0.1 M dimethyl arsenic acid (Sodium cacodylate) for a period of at least 24 hours prior to incubation, it may be assumed that alkaline phosphatase is inhibited. Also when the oviductal tissues were incubated in the Gomori medium for the demonstration of alkaline phosphatase, no reaction product was seen.

The lack of the reaction product when tissues were incubated in the absence of ATP, with a lead phosphate precipitate (cloudy control), and in equimolar concentrations of sodium beta-glycero-phosphate, indicated that the final reaction product observed in the experimental tissues is not due to a nonspecific binding of lead with protein but represents the genuine activity of the enzyme ATP-ase (Figure 105).

It has been suggested that the commercially available adenosine nucleoside phosphate substrates contain inorganic phosphate contaminants and that a nonspecific hydrolysis of the substrate might occur before or during the cytochemical reaction (Tice and Barnett, 1963). Therefore, in the present study, tissues were incubated in a medium containing precipitated lead phosphate. This cloudy control experiment demonstrated a sparsely and diffusely localized electron-opaque precipitate, scattered randomly in the epithelium.
and stroma, showing no particular association with cell membranes, cilia, microvilli or other intracellular organelles.

The reaction product observed in the present experiments is, therefore, formed by the enzymatic hydrolysis of ATP substrate. This hydrolysis could be due to one or more related glutaraldehyde resistant ATPase-like enzymes.

In the present study cytochemical methods were applied at the electron microscope level to delineate the hormone-induced changes in the subcellular localization of hydrolytic enzymes ATPase, and acid phosphatase, because of their known contribution in cellular metabolism. Their steroid dependence has been established through biochemical and light microscopic investigations (Connel et al., 1967; Gross, 1964; Hayashi and Fishman, 1961; Watanabe and Fishman, 1964; Murdoch and White, 1968).

In relation to the previous studies on different tissues, the results obtained in the present investigation reveal the localization of ATPase-like activity on the plasma membranes, cilia, and microvilli.

In general, ATPase is known to play an important role in many of the biological processes involving energy utilization from high energy phosphate bonds including oxidative phosphorylation. It has been shown that this activity is necessary to bring about early estrogenic effects (Aaronson et al., 1965). It has been suggested that
ATPase is involved in the transport of Na\(^+\) and K\(^+\) (Post et al., 1960; Farquhar and Palade, 1966) and other substances across the cell membranes (Hoff and Graff, 1966). Recent studies by many investigators (Marchesi and Barrnnett, 1964; Torrack and Barrnnett, 1964) have clearly indicated that ATPase play an important role in mechanisms controlling capillary function.

The evidence presented in the present study suggests that one of the mechanisms by which estrogens exert their effect on the oviductal cell is the utilization of energy from high energy phosphate bonds on the cell membranes in transport of materials between individual cells. With the first subcellular demonstration of ATPase-like enzyme distribution in oviductal cells, the mechanism by which steroid hormones exert their action on the cellular level has been elucidated. In the present study, the infoldings of the lateral cell membrane have been found to contain high enzymatic activity. In the epithelial cells, considered to be the site of active transport (Leaf and Hayes, 1961), deep infoldings are present between adjacent cells. According to Novikoff et al. (1962) these membranes hydrolyze ATP very rapidly.

In the present study ATPase activity in the mitochondria and Golgi apparatus has also been demonstrated. Biochemical data on membranes isolated from disrupted mitochondria suggest that the ATPase of mitochondria is localized in the membranes rather than
in the matrix (Siekevitz et al., 1958). The precise intramitochondrial localization of ATPase at the electron microscope level is controversial. In the present study, ATPase activity occurred on the inner membrane, membrane of the cristae and in the matrix. Recently, Ogawa and Mayahara (1969) reported the precise intramitochondrial localization of ATPase activity with special reference to the inner membrane particles in normal rodent cardiac mitochondria. The outer membrane and spaces were devoid of enzymatic activity. However, Lazarus and Vethamany (1966) have described ATPase activity in the matrix of mitochondria but other investigators have found that ATPase activity was limited in the inner or cristal membranes (Schulze and Wollenberger, 1962; Shiose and Sears, 1966).

Cheetham et al. (1970) have recently demonstrated the presence of the nucleosidephosphatase activity in the Golgi apparatus fraction isolated from rat liver. Novikoff et al. (1962) and Goldfischer et al. (1964) have discussed in some detail the existence of nucleoside phosphatases in the Golgi apparatus. The enzyme activities are revealed in the saccules and vesicles but the precision of the technique is unable to determine whether the corresponding enzymes are situated in the membrane or inside the saccules or vesicles. An enzymatic heterogeneity was evident among the individual elements of the Golgi apparatus.
Tice and Barnett (1963) found both di- and triphosphatase activities in the Golgi apparatus in rat testes. In the present study, a distinctly more pronounced reaction product was seen in the Golgi apparatus after stimulation with estrogen. As Goldfischer et al. (1964) have reported, the roles of phosphatases in the Golgi remain unknown. However, one may speculate that some active process is involved in the utilization of energy from high energy phosphate bonds.

Nelson (1958) found ATPase activity in the rat epididymal sperm to be localized to the nine longitudinal fibers of the outer axial bundle of spermatozoa. Nagano (1965), however, reported that in the rat sperm tail the ATPase activity was present both in the tail filament complex and on the surface membrane of the mitochondrial helix of the middle piece. Lansing and Lamy (1961) found ATPase activity in the peripheral filaments of the rotifer cilia and no activity in the region near the base of the cilium.

The present study reports for the first time the localization of ATPase activity on the ciliary membrane, the outer peripheral fibrils and the axoneme complex of the basal body in the rabbit oviduct. These results are consistent with the biochemical properties of the components of cilia and flagella (Gibbons and Rowe, 1965, 1967). The biochemical analysis, however, showed that the properties of the ATPase from the membrane and dynein fractions (proteins
forming the arms of the peripheral fibers) were different. These studies have revealed that the dynein ATPase was activated by both Ca\textsuperscript{++} and Mg\textsuperscript{++}, while the membrane ATPase was activated by Mg\textsuperscript{++} but not Ca\textsuperscript{++}. In the present study, Mg\textsuperscript{++} activated ATPase has been studied and this has been demonstrated by the presence of the reaction product on the ciliary membrane.

The enzyme ATPase localized in cilia is probably involved in energy forming reactions related to contractile cellular mechanisms. Authorities on both cilia and flagella agree that the movements performed by these organelles are the result of active processes along the length of the structure and not passive movements resulting from movement on the base within the cell. There is an increasing body of evidence (Satir, 1967) that the ciliary fibrils do not change in length during movement, but merely change their relative positions by sliding along one another. According to Sleigh (1969) bending of cilia and flagella depends on the sliding along one another of peripheral doublets of constant length, and that the force required to perform the movement is derived from the formation by the arms of links between each doublet. The energy for the formation of links between adjacent doublets is made available by ATPase activity of the dynein which forms the arms. The complex structure of oviductal cilia and the distribution of ATPase lend support to the view expressed by these investigators (Satir, 1967; Sleigh, 1969).
Many tissues known to be actively engaged in fluid transport show a histochemical localization of nucleoside phosphatase activities restricted to, or predominantly associated with, cell membranes in electron microscope preparations. These tissues include the mammalian kidney tubules (Goldfischer et al., 1964; Wachstein and Besen, 1964), gall bladder (Kaye et al., 1965), colon (Otero-Vilardebo et al., 1964), urinary bladder (Bartoszewicz and Barrnett, 1964), ciliary epithelium (Kaye and Pappas, 1965), corneal epithelium and endothelium (Kay and Tice, 1964), frog epidermis (Farquhar and Palade, 1966), placental epithelium (Connell, 1967), salt glands (Abel, 1969), and gustatory epithelium (Iwayame, 1969). Since biochemical studies indicate that cation-activated adenosine nucleoside phosphatases are associated with plasma membranes (Hoffman, 1962), it is suggested here, as in the aforementioned investigations, that ATPase activity, distributed on the plasma membranes of the oviduct epithelium, regulates the transport of electrolyte and water across the membrane and also the passage of macromolecules.

In the bovine oviduct epithelium, tests for the demonstration of acid phosphatase did not give information that could be correlated with the ultrastructural organization (Björkman and Fredricsson, 1961). Abdalla (1968), however, using light microscopic histochemical techniques found acid phosphatase activity in the apical parts of the non-ciliated cells and their cytoplasmic projections. In this
investigation, the dense bodies found in the oviductal epithelium are identified as lysosomes on the basis of their cytochemically demonstrable acid phosphatase activity and by such fine structural features as their delimitation by a unit membrane and their electron-dense matrix. Acid phosphatase activity was also found in the Golgi sacculles, microvilli, secretory granules and secretory product (Figures 115 to 118). This is in accord with the recent observations in other cell types (Smith and Farquhar, 1966; Hopkins and Baker, 1968). On the basis of these findings, it has been suggested that acid phosphatase is associated with the formation of the secretory product.
SUMMARY

The ultrastructure and electron microscopic localization of ATPase activity in the oviduct epithelium of rabbit have been investigated. The epithelial cells of the fimbriae, ampulla, isthmus and uterotubal junction contained both the ciliated and secretory cells. Numerous mitochondria, ribosomes and a well developed Golgi apparatus were seen in the apical cytoplasm of the ciliated cells of the oviduct from prepubertal rabbits. Multivesicular bodies and dense bodies resembling lysosomes were also observed. The secretory cells contained a well developed granular endoplasmic reticulum and protrusions filled with some electron-dense secretory granules.

The alterations seen after oophorectomy and hormone replacement therapy in the fine structure of the epithelium, stroma and smooth muscle cells of the oviduct have also been investigated by the application of an electron-microscopic histochemical staining method. Marked change was found in the fine structure of oviduct ciliated cells after ovariectomy. Most of the cilia disappeared and abnormal mitochondria and basal bodies were observed. The secretory cells became atrophied and contained poorly developed endoplasmic reticulum and occasionally a few dense secretory granules. The cilia and basal bodies of the ciliated cells were found to be similar in structure to
the cilia and basal bodies in other cells (Fawcett and Porter, 1954). Endoplasmic reticulum became dilated under the influence of steroid hormones.

One of the most interesting morphological features noticed in the present study is the occurrence of cross-striated rootlets in oviduct ciliated cells in response to estrogen treatment. It is suggested that estrogen not only stimulated the growth of cilia but also the formation of ciliary rootlets.

Ultrastructure of the smooth muscle cells of rabbit Fallopian tubes has also been described. The fine structure of smooth muscle cells was found to be similar to that described for human Fallopian tube (Clyman, 1966). A marked increase in the number of mitochondria has been observed in smooth muscle cell under hormonal influence. Polyribosomes were frequently encountered. The endoplasmic reticulum was prominent. The fine structure of the smooth muscle cells and hormonally induced alterations in these cells have been discussed in relation to their function.

The present study has demonstrated the presence of ciliated and secretory cells throughout the tubal epithelium including the fimbriae and uterotubal junction. It has been suggested that the transport of ovum has been brought about by the peristaltic contractions of smooth muscle of the tubal wall and ciliary movement. Evidence for this view has been demonstrated in the complex
structure of cilia and smooth muscle cells of the rabbit oviduct.

This study has reported for the first time the subcellular localization of adenosine triphosphatase and acid phosphatase in the oviduct epithelium of intact and ovarietcomized rabbits and their response to estrogen and progesterone. By combining electron microscopy and enzyme histochemistry the results obtained in this study provide evidences to relate ultrastructure and function of cellular organelles. The final reaction product resulting from the hydrolysis of ATP was deposited on the lateral and outer cell membranes. ATPase activity was also demonstrated by the presence of the reaction product on the outer membrane of cilia and microvilli, and the peripheral fibrils of cilia and basal bodies.

The fine structural localization of ATPase in the mitochondria and Golgi apparatus has also been demonstrated. It is concluded from the present experiments that the reaction product was formed by the enzymatic hydrolysis of ATP, which could be due to one or more related glutaraldehyde resistant ATPase-like enzymes. Limitations in the phosphatase cytochemistry with electron microscopy has been discussed.

The distribution of ATPase at different sites of the oviduct and its biological role have been discussed with regard to recent biochemical and cytochemical findings. It is concluded that one of the mechanisms by which ovarian steroid hormones exert their effect
on the oviductal cell is the utilization of energy from high-energy phosphate bonds on the cell membranes in transport of materials between individual cells and across the membranes of the capillary and smooth muscle cells. The enzyme localized in cilia is probably involved in energy forming reactions related to cellular contractile mechanisms.

Acid phosphatase activity was found on the outer cell membrane, Golgi saccules, and occasionally on the secretory granules and secretory material. Further ultrastructural, cytochemical and biochemical studies on the oviducts of other species would not only lend support to our findings on the sensitivity of the secretory and ciliated cells to estrogen and progesterone but would also yield valuable information on the reproductive phenomena.

The present investigation has demonstrated that electron microscopic and cytochemical studies not only supplement light microscopic and biochemical findings but also contribute substantially to a better understanding of oviductal morphology and physiology at subcellular levels.
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APPENDIX
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BB</td>
<td>Basal body</td>
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<tr>
<td>BM</td>
<td>Basement membrane</td>
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<td>CB</td>
<td>Ciliary precursor body</td>
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<td>Ciliated cell</td>
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<td>Cross-striated rootlet</td>
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<td>ICB</td>
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<td>TJ</td>
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Figures 1-17. Tissue specimens were taken from ampulla region of the oviduct of eight-week old rabbit. Following glutaraldehyde fixation in cacodylate buffer, the tissue specimens were incubated in Wachstein-Meisel medium with or without ATP as the substrate.

Sections were then fixed in osmium tetroxide in cacodylate buffer and processed for electron microscopy in the usual way. Thin sections were doubly stained with uranyl acetate and lead citrate.
Figure 1.  Apical region of the epithelial cell. The micrograph illustrates two types of cells in the ampulla epithelium: one ciliated (CC) and two non-ciliated secretory cells (SC). The apical cytoplasm of the ciliated cells has numerous mitochondria (M) and ribosomes (R). Note the numerous microvilli (MV), few cilia (CI) and basal bodies (BB) at the apical surface. Dense lysosome-like bodies (LY) and the large nucleus (N) are present in the apical cytoplasm. The secretory cell protrusion contains a few moderately electron-dense circular secretory granules (SG). The apical cytoplasm of the secretory cell also contains many mitochondria and a well developed granular endoplasmic reticulum (GER). The nucleus of the secretory cell is large and irregular in shape. (11, 600 X)
Figure 2. Apical portion of ciliated epithelial cell and a portion of oviduct lumen. Few cilia and basal bodies are seen in the apical cytoplasm of the cell. Note the presence of a circular secretory granule at the base of two cilia (single arrow) in the vicinity of two basal bodies. A prominent Golgi apparatus (G) is present in juxtanuclear position. The tubal lumen contains portions of a secretory cell (double arrow) with secretory granules and other intracellular organelles. (7, 260 X)
Figure 3. Basal portion of the epithelial cell. Morphologically, two types of cells are clearly seen in this region. The basal portion of the secretory cell (SC) has extensive endoplasmic reticulum, few mitochondria and electron-dense circular secretory granules. The ciliated cell (CC) has numerous mitochondria and free ribosomes scattered throughout the basal cytoplasm. A thin basement membrane (BM) separates the epithelium from the lamina propria which contains a connective tissue cell (CT) and collagen fibers (CO) in the intercellular spaces. (8, 800 X)
Figure 4. Apical region of ciliated cell from ampulla showing the beginning of the formation of cilia with its knobbed kinetosomes (K). (76, 590 X)

Figure 5. Transverse section through cilia and microvilli at the apex of the ampullary region. Note the typical 9+2 central core of cilia and the differences between cilia (CI) and microvilli (MV). The cilia have a complex central cores which are absent in the microvilli. (46, 800 X)
Figure 6. Portion of a secretory cell showing secretory granules which contain homogeneously distributed moderately electron-dense material and patches of more electron-dense material. Note the close association between the granular endoplasmic reticulum, the secretory granules and the nuclear membrane. The secretory granule has a distinct plasma membrane (PM) which coalesces with the membrane of an adjacent secretory granule. The nucleus contains an electron-dense granular material, presumably RNA. (79, 920 X)

Figure 7. Portion of the apical region of a ciliated cell. This micrograph shows the fine structure of the mitochondria. Note the inner and outer membrane, cristae and evenly distributed finely granular material. The cytoplasm contains clusters of ribosomes (R) and an electron-dense material (arrow) which presumably play some role in the formation of cilia. (106, 400 X)
Figure 8. Apical region of an epithelial cell from the ampulla incubated with ATP as the substrate. The final reaction product is localized in a beaded pattern as a discrete granular electron-dense lead phosphate precipitate along the lateral and external cell membrane (CM), outer membrane of cilia and microvilli. A few scattered deposits of the reaction product are present in the Golgi apparatus. The secretory granules, endoplasmic reticulum, nuclear membrane and nucleoplasm appear to be devoid of ATPase activity. Note the intercommunication between the perinuclear cisternae and the lumena of the endoplasmic reticulum (arrow). (12,800 X)
Figure 9. Portion of secretory and ciliated cells. The secretory cell contains electron-lucent (LG) and electron-dense (DG) granules. The nucleus of the secretory cell is situated basally, and is irregular in shape. Few mitochondria occur in the cytoplasm but the endoplasmic reticulum and Golgi apparatus are well developed. The ciliated cell has numerous mitochondria especially in the supranuclear zone. Many dense basal bodies occur in the apical cytoplasm. The endoplasmic reticulum is inconspicuous with narrow cisternae. The nucleus of the ciliated cell is irregular in shape; the nucleolus (NU) is distinct and electron-dense. The distribution of the final reaction product is the same as described for Figure 8. (8,800 X)
Figure 10. Supranuclear region of the ciliated cell. The Golgi (G) apparatus consists of a stack of parallel saccules with dilated tips and group of vesicles of varied diameters. Sparse deposits of electron-dense lead phosphate reaction product occur on the lateral cell membrane. Note the desmosome (DE) connecting the two cells. A dense lysosome-like body and a multivesicular body (arrow) are seen near the Golgi complex. Many clusters of ribosomes are present in the cytoplasm. (12, 320 X)

Figure 11. Portion of secretory cell showing the close association between the granular endoplasmic reticulum and the Golgi complex. The cisternae of the endoplasmic reticulum contain granular material of homogeneous density. Sparse ATPase activity is demonstrated by the presence of electron-dense lead phosphate precipitate in the Golgi complex and lateral cell membrane. (18, 150 X)
Figure 12. Portion of the epithelial cell showing the intramitochondrial localization of ATPase. Note the reaction product also occurs in the endoplasmic reticulum. (28, 160 X)

Figure 13. Portion of ciliated cell showing the presence of aggregates of fine fibrils (F) in the cytoplasm. Note the reaction product localized on the cell membrane. (46, 575 X)
Figure 14. Apical region of ciliated cell showing ATPase activity on the cilia and basal body. ATPase activity is demonstrated by the presence of an electron-dense lead phosphate reaction product. Note the presence of a rootlet-like structure (arrow) extending downward into the cytoplasm from the proximal end of a basal body. (28, 160 X)

Figure 15. Basal portion of the epithelial and subepithelial zone. The stroma contains connective tissue cells with a prominent nucleus and lipid droplets. Collagen fibrils are present in the intercellular space. Sparse distribution of the reaction product occurs as a granular precipitate on the lumenal membrane of capillary endothelium (EN). (8, 800 X)
Figure 16. Portion of smooth muscle cell and capillary. Adjacent smooth muscle cells are separated by a connective tissue space of variable width, in which collagen fibers (CO) can be seen at a number of points reinforcing the basement membrane which surround individual cells. The cytoplasm contains myofilaments (MYF). The nucleus is indented. There are few mitochondria, a well developed granular endoplasmic reticulum, free ribosomes, and lipid droplets (L). ATPase activity is demonstrated by the presence of an electron-dense lead phosphate reaction product on the capillary lumenal membrane and the outer surface of erythrocyte (ER). Fine granular precipitate of the reaction product resulting from hydrolysis of ATP also occurs on the basement membrane, plasma membrane and in the intercellular space. (7, 260 X)

Figure 17. This micrograph clearly demonstrates the fine structure of the smooth muscle cell. The cytoplasm of the smooth muscle cell contains a prominent nucleus, myofilaments and dense bodies (DB) of unknown nature. Note also the presence of few mitochondria and ribosomes in the cytoplasm. Few micropinocytic vesicles (PV) occur on the plasma membrane. (11, 600 X)
Figure 18. Portion of apical region of epithelial cell. Note the release of secretory material into the tubal lumen (LU) and the numerous cilia. (11,600 X)

Figure 19. Basal portion of the epithelial cell. The basal cytoplasm of the epithelial cell contains numerous mitochondria, ribosomes and a lipid droplet (L). Note the artifact in the large mitochondrion (arrow) and the widening of the intercellular spaces of the lateral cell membrane. A portion of the cytoplasm of the connective tissue cell contains well developed endoplasmic reticulum, numerous ribosomes and swollen mitochondria (M). The intercellular space is filled with collagen fibers. (11,600 X)
Figures 18-24. Tissue specimens were taken from ampulla region of the oviduct of eight-week old rabbits which received exogenous estrogen, 5 µg daily for seven days.

Following glutaraldehyde fixation, the tissue sections were incubated in Wachstein-Meisel medium with or without ATP as the substrate. Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were doubly stained with uranyl acetate and lead citrate.
Figure 20. Apical portion of ciliated and secretory cell. Sparse distribution of ATPase activity is demonstrated by the presence of electron-dense precipitate of lead phosphate on the microvilli, cilia and basal body. Note the transverse section of the basal body shows nine peripheral triplet fibrils and no central fibrils. (37, 400 X). The inset depicts heavy deposits of the reaction product on the outer cell membrane and microvilli. (28, 160 X)

Figure 21. Portion of secretory cell. ATPase activity in the Golgi saccules, lateral cell membrane and a mitochondrion (M) is demonstrated by the presence of an electron-dense lead phosphate precipitate. (34, 475 X). The inset shows dense deposits of the reaction product in the lateral cell membrane situated at the apical region. (10, 400 X).
Figure 22. Portion of ciliated cell. This micrograph illustrates the association between the centriole and a dense body (fibrogranular aggregate). Few dense bodies are seen in the vicinity of Golgi apparatus (G). (22, 800 X)
Figure 23. Longitudinal section of smooth muscle cell. Evidence of ATPase activity is absent in this preparation. Note the dilated cisternae of the granular endoplasmic reticulum. (11, 600 X)

Figure 24. Longitudinal section of smooth muscle cell. Sparse deposits of the final reaction product are distributed as a precipitate on the plasma membrane and basement membrane. The cytoplasm of the smooth muscle cell contains numerous mitochondria, polyribosomes and granular endoplasmic reticulum. A lysosome (LY) is seen in the cytoplasm. (16, 400 X)
Figures 25-35. Tissue specimens were taken from ampula region of the oviduct of eight-week old rabbits which received 2 mg exogenous progestrone daily for seven days.

Following glutaraldehyde fixation the tissue sections were incubated in Wachstein-Meisel medium with or without ATP as the substrate. Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were doubly stained with uranyl acetate and lead citrate.
Figure 25. Portion of secretory cell. Distribution of ATPase activity appears as a granular precipitate of reaction product on the cell membrane, and microvilli. The cytoplasm of the secretory cell contains dilated cisternae of the granular endoplasmic reticulum, electron-dense secretory granules and mitochondria. Note the close association between the cisternae of the endoplasmic reticulum, Golgi apparatus and a secretory granule. The lumens of the endoplasmic reticulum contain homogeneously distributed fibrillar material. An interesting cytological feature is the formation of cilia (CI) in the secretory cell. (11, 600 X)

Figure 26. Apical portion of secretory cell showing sparse distribution of the final reaction product on the cell membrane, microvilli and cilia. Note many dense secretory granules and the release of secretory material into the tubal lumen (LU). The lumen also contains RNA particles (arrow). The most noticeable feature is the marked dilation of the cisternae of the endoplasmic reticulum in both the ciliated and secretory cells. (11, 600 X)
Figure 27. Apical portion of secretory cell. This micrograph illustrates the presence of heavy deposits of electron-dense lead phosphate reaction product on the outer cell membrane and microvilli. Most of the secretory granules contain small patches of dense material (DM). (10, 260 X)

Figure 28. Apical portion of secretory and ciliated cell. Distribution of the electron-dense reaction product resulting from hydrolysis of ATP occurs as a fine granular precipitate on the outer cell membrane, outer membrane of cilia and microvilli. The lumen contains portions of secretory cells, indicating apocrine type of secretion. Note the extensive indentation of the nucleus. (10, 260 X)
Figure 29. Lumen of oviduct and lumenal faces of a secretory and ciliated cells. This micrograph illustrates clearly the presence of dense precursor bodies which presumably play some role in the formation of cilia. Note that microtubules (MT) are associated with the basal body and the dense granules. The tubal lumen contains secretory cell fragments, indicating apocrine type of secretion. (11,600 X)

Figure 30. Portion of secretory cell. Note the extensive development of the dilated cisternae of the endoplasmic reticulum, numerous ribosomes and a prominent Golgi apparatus. Close association between the Golgi elements and the granular endoplasmic reticulum is apparent. Formation of secretory granule can be seen in the vicinity of the Golgi apparatus. A dense body, probably a fixation artefact, is present in one mitochondrion. (11,600 X)
Figure 31. Basal portion of the epithelial cell. Note the basal cytoplasm contains extremely dilated cisternae of the endoplasmic reticulum and numerous ribosomes. The lumens of the endoplasmic reticulum contain amorphous granular material. Dense homogeneous secretory granules are present in the cytoplasm. A distinct basement membrane (BM) separates the epithelium from the lamina propria. A portion of the cytoplasm of the connective tissue cell shows dilation of the cisternae of the granular endoplasmic reticulum. (10, 260 X)

Inset: Note the numerous polyribosomes and widening of the intercellular space of the cell membrane. (22, 800 X)

Figure 32. Basal portion of epithelial cell and subepithelial zone. Sparse distribution of the reaction product occurs on the cell membrane and basement membrane. The basal cytoplasm contains dilated cisternae of the endoplasmic reticulum, few dense secretory granules and mitochondria. The stroma is composed of a connective tissue cell, a large lipid droplet, and an intercellular space containing collagen fibers. (16, 400 X)
Figure 33. Portion of longitudinally sectioned smooth muscle. Sparse distribution of the reaction product occurs on the basement membrane and plasma membrane of smooth muscle cell. The cytoplasm of the smooth muscle cell contains numerous mitochondria and ribosomes. An intercellular bridge (ICB) with protoplasmic continuity can be seen. Note the fixation artefact lying against the nuclear membrane. (11, 600 X)

Figure 34. Portion of longitudinally sectioned smooth muscle cell. This micrograph illustrates the close association between a lipid droplet and two mitochondria. Note the numerous micropinocytic vesicles on the plasma membrane. ATPase activity is demonstrated by the presence of dense deposits of the reaction product on the plasma membrane and basement membrane. (11, 600 X)
Figure 35. Longitudinally sectioned smooth muscle cell. Distribution of the reaction product is the same as described for Figures 33 and 34. Note the numerous rosettes of ribosomes and mitochondria. The cytoplasm of the smooth muscle cell also contains myofilaments, granular endoplasmic reticulum and microtubules (MT). The plasma membrane can be seen to dip into shallow pockets, known as micropinocytic vesicles. Dense bodies are seen interspersed between myofilaments. Note the well developed Golgi apparatus (G) and the lipid droplet (L) at the juxtanuclear position. (17, 750 X)
Figures 36-41. Tissue specimens were taken from ampulla region of the oviduct of eight-week old rabbits which received exogenous estrogen and progesterone for seven days.

Following glutaraldehyde fixation the tissue sections were incubated in Wachstein-Meisel medium with or without ATP as the substrate. Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were stained with uranyl acetate and lead citrate.
Figure 36. Portion of apical region of ciliated and secretory cell. Heavy deposits of the final reaction product are distributed along the cell membrane, cilia, microvilli and basal body. A tight junction (TJ) is present and the mitochondria apparently lack enzymatic activity. (24, 560 X)

Figure 37. Apical region of the secretory cell. ATPase activity occurs on the cell membrane and microvilli. The disrupted plasma membrane also shows electron-dense deposits of the final reaction product. Note the presence of dilated cisternae of the endoplasmic reticulum. (25, 560 X)
Figure 38. Apical region of ciliated and secretory cell. The apical cytoplasm of the ciliated cell contains numerous basal bodies and a dense lysosome. The apical region of the secretory cell is filled with secretory granules of different size and density. The tubule lumen is filled with cross sections of cilia and microvilli. (11,600 X)

Figure 39. This micrograph illustrates the formation of a secretory granule in the Golgi region (arrow). Note the intimate association between the Golgi saccules, endoplasmic reticulum and the secretory granule. (11,600 X)
Figure 40. Basal region of the epithelial cell and subepithelial zone. Evidence of enzymatic activity is absent. The basal cytoplasm contains dilated cisternae of the endoplasmic reticulum, together with mitochondria and numerous ribosomes. The lumens of the endoplasmic reticulum contain fibrillar material of homogeneous density. The connective tissue cell of the stroma contains a prominent nucleus and dilated cisterne of the endoplasmic reticulum. The intercellular space is filled with collagen fibers. (11,600 X)

Figure 41. Longitudinal section of smooth muscle cell. The final reaction product occurs as dense deposits along the plasma membrane (PM), basement membrane (BM) and intercellular space (IS). Note that the cytoplasm is well provided with mitochondria, granular endoplasmic reticulum and polyribosomes. (11,600 X)
Figures 42-56. Tissue specimens were taken from regions other than ampulla of the oviduct of eight-week old rabbits. Following glutaraldehyde fixation the tissue specimens were incubated with or without ATP as the substrate.

Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were doubly stained with uranyl acetate and lead citrate.
Figure 42. Portion of ciliated and secretory cell from fimbria. Note sparse distribution of final reaction product on the cell membrane, microvilli and cilia, and the dense lysosome-like bodies (LY), multivesicular bodies (MVB) and a myelinated body (MB). The apical cytoplasm of the ciliated cell contains many basal bodies, mitochondria and ribosomes. The Golgi apparatus is situated in the juxtanuclear position. (18, 150 X)

Figure 43. Secretory cell from fimbria. Note the balloon-like bulge. This micrograph clearly demonstrates the fine structure of the multivesicular bodies (MVB) situated in the perinuclear region. (18, 150 X)
Figure 44. Portion of epithelial cell from isthmus showing morphological alterations in response to estrogen. Note the secretory cell is engorged with goblet-like secretory granules containing aggregations of dense material. (5,068 X)

Figure 45. Apical region of secretory and ciliated cell from isthmus showing morphological alterations in response to estrogen. Coalescence of secretory granules with loss of membrane occurs throughout the cytoplasm. At the free surface these secretory granules pass through the outer cell membrane and into the tubal lumen. The apical cytoplasm of the ciliated cell contains basal bodies, cilia and dense bodies (ciliary precursor bodies). Note the presence of a cross-striated rootlet-like structure (arrow) which extends obliquely into the cytoplasm from the proximal end of a dense body. (10,260 X)
Figure 46. Portion of apical region of ciliated cell from isthmus showing morphological alterations induced by progesterone treatment. Note especially the growth of cilia and presence of dense ciliary precursor bodies (CB). Note a dense body enclosed by a distinct membrane (arrow). The tubal lumen is filled with homogeneously distributed secretory material and secretory granules. (11,600 X)

Figure 47. Apical portion of ciliated and secretory cell from isthmus showing fine structural changes induced by progesterone treatment. Note extrusion of secretory material into tubal lumen. (11,600 X)
Figure 48. Portion of secretory cell from isthmus showing fine structural changes induced by estrogen and progesterone treatment. Note the apical cytoplasm of the secretory cells filled with mucin type secretory granules and the basally situated indented nuclei. (5, 600 X)
Figure 49. This micrograph illustrates the fine structure and localization of ATPase activity in the ciliated cell from uterotubal junction of an intact control rabbit. ATPase activity is shown by the sparse granular deposits of reaction product on the cell membrane. (10, 260 X)

Figure 50. Uterotubal junction of an intact animal treated with exogenous estrogen. Note the heavy deposits of final reaction product along the cell membrane, outer membrane of cilia and microvilli. The nuclei are very irregular in shape and sometimes contain an electron-dense nucleoli. (5, 068 X)
Figure 51. Basal region of an epithelial cell from uterotubal junction of an intact animal treated with exogenous estrogen. Note the final reaction product on the basement membrane and stroma. The cytoplasm of the connective tissue cell contains dilated cisternae in the endoplasmic reticulum. (10, 260 X)

Figure 52. Cell at uterotubal junction of an intact animal treated with exogenous estrogen. Note ATPase activity along the infoldings of the lateral cell membrane, and the striking feature of dilation of the cisternae of the endoplasmic reticulum in the secretory cell. (21, 600 X)
Figure 53. Secretory cell from uterotubal junction of an intact animal treated with exogenous progesterone. Granular deposits of the reaction product are distributed along the cell membrane, microvilli and secretory material. Note the release of several secretory granules into the lumen. (11,600 X)

Figure 54. Portion of ciliated cell and secretory cell from uterotubal junction in response to progesterone treatment. Note the reaction product, the dilated cisternae of the granular endoplasmic reticulum and the dense lysome-like bodies in the Golgi region. The lysosomal sac has apparently ruptured (arrow). Smaller dense bodies (ciliary precursor bodies) are present in the apical cytoplasm. (11,600 X)
Figure 55. Apical portion of a ciliated and a secretory cell from uterotubal junction of an animal treated with exogenous progesterone. Note ATPase activity associated with secretory material, cell membrane and microvilli. Note also the growth of cilia and microvilli and a well developed Golgi apparatus. (16, 400 X)

Figure 56. Portion of stromal capillary and connective tissue cell from uterotubal junction treated with exogenous estrogen and progesterone. Note ATPase activity on the erythrocyte (ER), capillary endothelium and stromal cell membrane. The well-developed dilated cisternae of the endoplasmic reticulum are filled with a fine granular material. The intercellular space is completely filled with collagen fibers (CO). (15, 600 X)
Figures 57-61. Tissue specimens were taken from fimbria and ampulla region of the oviduct six weeks after ovariectomy. Following glutaraldehyde fixation the tissue specimens were incubated in Wachstein-Mesisel medium with ATP as the substrate.

Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were stained with uranyl acetate and lead citrate.
Figure 57. Apical region of a ciliated cell from fimbria. Note all the cilia have disappeared and abnormal basal bodies are present near the surface of the cell. The apical cytoplasm contains mitochondria with poorly defined cristae, dense lysosome-like bodies, and free ribosomes. The large nucleus has a distinct nuclear membrane. Aggregates of chromatin and distinct nucleoli are absent. (17,750 X)

Figure 58. Portion of a secretory and a ciliated cell from fimbria. The fine structure of the granular endoplasmic reticulum of the secretory cell appear to be disrupted with the cisternae no longer discernible (arrow). ATPase activity is observed only sporadically on the cell membranes of some cells. This is illustrated by the presence of sparse deposits of the reaction product on the lateral cell membrane. (24,580 X)
Figure 59. Apical portion of secretory cell from fimbria. Note particularly the atrophy of the secretory cell and the disappearance of secretory granules from the cytoplasm. An aggregate of the organelles (arrow) can be seen in the lumen. Microvilli are present but appear to be shorter than those found in intact animals. (17,750 X)

Figure 60. Basal portion of the epithelial cell from fimbria. Note the intracellular space contains electron-dense reaction product resulting from the hydrolysis of ATP. Large nuclei occupy most of the cytoplasm and have distinct nuclear membranes. Only few ribosomes can be seen. The bizarre form of mitochondria with granule-like cristae is clearly demonstrated. (17,600 X)
Figure 61. Longitudinal section of smooth muscle cell from ampulla. Occasional deposit of the reaction product can be seen on the plasma membrane and basement membrane (arrow). Capillary endothelium and basement membrane apparently lack ATPase activity. Note the cytoplasm of the smooth muscle cell contains few mitochondria and ribosomes. Polysomal associations are infrequent. Very few micropinocytic vesicles occur on the plasma membrane of the smooth muscle cell. (10, 260 X)
Figures 62-74. Fimbriae, ampulla and isthmus of ovariectomized rabbit after seven days treatment with exogenous estrogen, 5 μg/day. Following glutaraldehyde fixation the tissue specimens were incubated with ATP as the substrate.

Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were stained with uranyl acetate and lead citrate.
Figure 62. Apical region of ciliated and secretory cell from fimbria. Note the dense reaction product on the outer cell membrane, microvilli and cilia. The most striking observation following estrogen treatment is the growth of cilia, numerous microvilli and the appearance of electron-dense secretory granules. (15, 600 X)

Figure 63. Apical region of ciliated cell from fimbria. This micrograph illustrates the development of cilia in an estrogen treated animal. Note the mitochondria restored to their normal appearance in ovariectomized animal following estrogen treatment. (35, 760 X)
Figure 64. Basal portion of the epithelial cell from fimbria. The ATPase activity is highly pronounced along the entire length of the lateral cell membrane but absent in desmosome (arrow). Note the extensive dilation of cisternae of the granular endoplasmic reticulum which contain material of homogeneous density. (17, 600 X)

Figure 65. Portion of secretory cell from fimbria showing the continuity between the perinuclear cisternae and the lumens of the endoplasmic reticulum. A secretory granule can be seen in the vicinity of granular endoplasmic reticulum. At this magnification, the typical structure of mitochondria is also evident. (35, 760 X)
Figure 66. Portion of smooth muscle cell from fimbria. The final reaction product occurs as a fine granular precipitate on the plasma membrane, basement membrane and in the intercellular space. Myofilaments are not as compact as in the smooth muscle from the ampulla. Aggregates of ribosomes are also seen in the cytoplasm. A few longitudinally oriented cristae have been observed in mitochondria. (25,560 X)

Figure 67. Portion of ciliated and secretory cell from ampulla. ATPase activity is demonstrated on the cell membrane, microvilli and cilia. The apical cytoplasm of the ciliated cell contains numerous basal bodies and small dense granules. The cytoplasm of the secretory cell contains electron-dense (DG) and electron-lucent secretory granules (LG). (11,600 X)
Figure 68. Apical region of epithelial cell from ampulla. Note ATPase activity on the basal body and mitochondria (arrow) and the widening of the intercellular space of the lateral cell membrane. (10, 260 X)

Figure 69. Portion of secretory and ciliated cell from ampulla. Heavy deposits of final reaction product are seen along the lateral cell membrane. The cytoplasm of the ciliated cell contains several lysosome-like bodies and numerous mitochondria. (8, 400 X)
Figure 70. Apical region of ciliated cell from ampulla. Note the bases of the cilia and particularly the formation of cross-striated rootlets (CR). These latter structures seem to be associated with estrogen treatment. This micrograph also demonstrates the distribution ATPase activity. (22, 800 X)

Figure 71. Apical region of secretory and ciliated cell from ampulla. The fine granular deposits of the final reaction product resulting from the hydrolysis of ATP also occur on the secretory material. Note also the cross-striated rootlet and the extrusion of secretory material into the lumen. (16, 400 X)
Figure 72. Portion of ciliated cell from ampulla showing group of centrioles and dense ciliary precursor bodies (arrow). These centrioles appear to migrate toward the surface of the cell and form the basal bodies. (24,584 X). The inset shows a fine precipitate of lead phosphate on the basal bodies. (36,180 X)

Figure 73. Basal region of epithelial cell from ampulla. Deposits of the final reaction product occur on the basement membrane and basal cell membrane of the epithelium and luminal membrane of the capillary. Widening of the intercellular space between the cell membrane can be clearly seen in this illustration. (11,600 X)
Figure 74. Cross section of smooth muscle cell from ampulla. Note intense deposits of the final reaction product on the plasma membrane, basement membrane of the smooth muscle cell and in the intercellular space. (24,580 X)

Figure 75. Apical portion of secretory cell from isthmus showing the release of several secretory granules into the lumen. The plasma membrane of the secretory granule appears to contain deposits of final reaction product (arrow). (16,400 X) Inset: Note ATPase activity in the basal bodies and cilia. (31,600 X)
Figure 76. Portion of ciliated and secretory cells from isthmus. The apical surface of ciliated cell contains besides cilia, also numerous microvilli. Note also the close association of the small dense bodies and the basal bodies. Fine granular deposits of reaction product occur on the peripheral fibers of basal body and in the mitochondria. (11,600 X) Inset: To show the intramitochondrial localization of ATPase. (31,600 X)

Figure 77. Epithelial cell of isthmus. Note the ATPase activity in the mitochondria and widening of the intercellular space. (11,600 X)
Figure 78. Portion of longitudinal section of smooth muscle cell. Note intimate relation of mitochondrion and lipid droplet, and micropinocytic vesicles (arrow) along the plasma membrane. (11,600 X) Inset: Note the final reaction product on the surface of an erythrocyte, and on the endothelium of a blood vessel. (11,600 X)
Figure 79. Portion of a secretory and a ciliated cell from isthmus. Note the rare case of the occurrence of secretory granules in the cytoplasm of ciliated cell. (22,800 X)

Figure 80. This micrograph shows the nuclei and secretory granules found to lie free in the tubal lumen. (11,600 X)
Figures 81-90. Fimbria and ampulla of ovariectomized rabbit after seven days treatment with exogenous progesterone, 2 mg/day. Following glutaraldehyde fixation the tissue specimens were incubated with or without ATP as the substrate.

Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were stained with uranyl acetate and lead citrate.
Figure 81. Longitudinal and cross sections of cilia and microvilli from fimbria. Note the final reaction product on the outer membrane of cilia and microvilli. Inset: illustrates the growth of cilia. (47,520 X)

Figure 82. Portion of epithelial cell from fimbria. Note the final reaction product along the lateral cell membrane. (25,560 X) Inset: Shows the ATPase activity on the cell membrane and microvilli of a secretory bleb. (10,250 X)
Figure 83. Basal portion of epithelial cell from fimbria. ATPase activity can be seen on the basal cell membrane and basement membrane. Note the basal cytoplasm contains groups of numerous mitochondria and a well-developed granular endoplasmic reticulum. (25, 560 X)

Figure 84. Portion of secretory cell from ampulla. Note the reaction product on the cell membrane and microvilli. The cytoplasm of the secretory cells contain numerous secretory granules of different size and density. (10, 960 X)
Figure 85. Portion of secretory cell from ampulla. Note the unique feature of the presence of the nine granules, each containing numerous small dense granules in a less-dense background (arrow). Five of the granules appear to release their contents into the tubal lumen. A pinched off portion of a secretory cell is seen in the lumen. (10,960 X)

Figure 86. Portion of secretory and ciliated cells from ampulla. Note the numerous basal bodies, polyribosomes, granular endoplasmic reticulum and microtubules in the cytoplasm of the ciliated cell. Electron-dense secretory granules are situated just below the outer cell membrane (arrow) and are in the process of releasing their contents into the lumen (10,260 X)
Figure 87. Cross section of smooth muscle cell from ampulla. This micrograph illustrates the localization of ATPase activity in the numerous micropinocytic vesicles, basement membrane and plasma membrane. (17,750 X)

Figure 88. Cross section of smooth muscle cell from ampulla. Note the numerous mitochondria, polyribosomes, and the intercellular bridge between the smooth muscle cells. (10,260 X)
Figure 89. Portion of smooth muscle cell and capillary from ampulla. Capillary is completely filled by an erythrocyte. The endothelium contains numerous micropinocytic vesicles, well developed granular endoplasmic reticulum and polysomes. The interstitium between the capillary and the smooth muscle cell is filled with collagen fibers. (16,400 X)

Figure 90. Longitudinal section of smooth muscle cell. The cytoplasm of the smooth muscle cell contains numerous mitochondria and polyribosomes. Note also the Golgi apparatus (G) and granular endoplasmic reticulum. Numerous micropinocytic vesicles occur on the plasma membrane. (16,400 X)
Figures 91-104. Fimbria and ampulla of ovariectomized rabbit after seven days of treatment with exogenous estrogen (5 μg/day) and progesterone (2 mg/day). Following glutaraldehyde fixation the tissue specimens were incubated with ATP as the substrate.

Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were stained with uranyl acetate and lead citrate.
Figure 91. Apical region of a ciliated and a secretory cell from fimbria. Deposits of reaction product can be seen on the cell membrane, microvilli and cilia. The cytoplasm of the ciliated cell contains numerous mitochondria and polyribosomes. The secretory bleb contains dense secretory granules, polyribosomes and vesicular profiles of the endoplasmic reticulum. (11,600 X)

Figure 92. Portion of ciliated cell from fimbria. Note the marked dilation of the cisternae of the granular endoplasmic reticulum and the active formation of cilia at the apical surface. Note the cytoplasmic protrusion of the ciliated cell also contains dilated cisternae and ribosomes. (16,400 X)
Figure 93. Portion of the apical region of a secretory and a ciliated cell from fimbria. Note the free surface of the secretory cell bulges far beyond the surface of the ciliated cell. Besides the secretory granules the bleb also contains well developed cisternae. (16, 400 X)

Figure 94. Apical region of ciliated cell from fimbria. Note the reaction product on microvilli and cilia, and numerous basal bodies in the apical cytoplasm. (22, 800 X)
Figure 95. Apical portion of epithelial cells from fimbria. Note the cytoplasmic projections (arrow). The reaction product is clearly demonstrated along the cell membrane of a part of the secretory cell found in the tubal lumen. (11,600 X)

Figure 96. Apical portion of ciliated cell from fimbria. A group of centrioles and a lysosome-like body can be seen in the cytoplasm. It is of interest to note that the inner membrane of the dilated endoplasmic reticulum contains deposits of the final reaction product. (22,500 X)
Figure 97. Basal portion of the epithelial cell from fimbria. Note the reaction product on the cell membrane, and that the lateral cell membrane is dilated at many points. (11, 600 X)

Figure 98. Basal portion of the epithelial cell from fimbria. Note the localization of the final reaction product on the cell membrane, basement membrane, stromal cell membrane and connective tissue space. (22, 800 X)
Figure 99. Portion of stromal capillary. Note the reaction product on the surface of the erythrocyte and capillary endothelium. ATPase activity also occurs in the micropinocytic vesicles. (10, 200 X)

Figure 100. Portion of capillary and connective tissue cell from fimbria. Note the lead phosphate deposits on the stromal cell membrane, connective tissue space and an erythrocyte. (11, 600 X)
Figure 101. Apical region of secretory cell from ampulla. Note heavy deposits of reaction product on the cell membrane, microvilli and cell membranes situated in the cytoplasm. (17,750 X)

Figure 102. Portion of secretory cell from ampulla. Note particularly the intercommunication (arrows) between the perinuclear cisterna, lumens of the endoplasmic reticulum and the cell membrane. Deposits of the reaction product can also be seen on the cell membrane. (16,400 X)
Figure 103. Apical portion of secretory cell from ampulla showing a cytoplasmic projection. (22, 800 X)

Figure 104. Portion of secretory cell from ampulla. Note the reaction product in the Golgi cisternae and the cell membrane. Note also secretory granules of a low density and with small dark spots. (17, 750 X)
Figure 105. Cloudy control specimen (incubation in the medium with a fine lead phosphate deposit) from ampulla. The lead phosphate deposits in these cloudy controls, when compared with enzymatically deposited lead phosphate, are randomly localized as large aggregates which have no preferential association to the cell membranes, cilia or microvilli. (11,600 X)
Figures 106-114. Uterotubal junction of ovariectomized rabbit after seven days of treatment with estrogen (Figures 106-108), progesterone (Figures 109-110) and estrogen and progesterone (Figures 111-114). Following glutaraldehyde fixation the tissue specimens were incubated with or without ATP as the substrate.

Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were stained with uranyl acetate and lead citrate.
Figure 106. Apical portion of a secretory and a ciliated cell. Note the deposits of the reaction product on the cell membrane, microvilli and cilia. Goblet-like secretory cell contains several granules of low density with small patches of dense material. (11, 600 X)

Figure 107. Apical portion of a secretory cell. Note the dense deposits of the reaction product on the cell membrane and the discharge of secretory material from one of the secretory granules. (16, 400 X)
Figure 108. Basal portion of epithelial cell showing the distribution of the reaction product on the basement membrane, capillary lumenal membrane and connective tissue space. Note also the extreme dilation of the intercellular spaces of the cell and the cisternae of the endoplasmic reticulum. (8,400 X)
Figure 109. Portion of a secretory cell showing the release of the contents of several secretory granules. Note the extensive infoldings of the lateral cell membrane and the presence of a basal body (BB) between two secretory granules. (16,400 X)

Figure 110. Portion of a secretory and a ciliated cell. Several granules of a low density and with small dense spots are noticed in the apical parts of the cells. This micrograph demonstrates clearly the pinching off process of a secretory bleb and the release of secretory material into the lumen. Note the tubal lumen contains a portion of nucleus (arrow) and other intracellular organelles. (11,600 X)
Figure 111. Apical region of secretory and ciliated cells. Note the reaction product occurs along the cell membrane, microvilli and cilia. The cytoplasm of the secretory cell contains numerous secretory granules of low density which are usually found coalescing with each other. Note the cytoplasm of the ciliated cell contains dense lysosome-like bodies, Golgi apparatus, numerous mitochondria and polysomes. Note particularly the 'fragmented' nucleus (N). (11,600 X)
Figure 112. Apical portion of epithelial cells. Note the multivesicular body (MVB) and the lysosome-like body (LY) in the ciliated cell and the extrusion of secretory material. (22, 500 X). The inset shows the extensive development of granular endoplasmic reticulum and ribosomes in smooth muscle cell. (21, 600 X)
Figure 113. Portion of a secretory and a ciliated cell. Note the intense ATPase activity along the cell membrane, microvilli and secretory material. Fine granular deposits of the reaction product can also be seen in Golgi elements. (22, 800 X)

Figure 114. Portion of apical region of epithelial cells. Note also the dense deposits of the reaction product and their distribution showing the unique growth of a cilium near a secretory granule. (31, 600 X)
Figures 115-118. Tissue specimens were taken from fimbria and ampulla region of the oviduct of intact or ovariectomized animal. Following glutaraldehyde fixation the tissue specimens were incubated in Barka-Anderson medium with sodium beta-glycerophosphate as the substrate for demonstration of acid phosphatase activity.

Sections were then fixed in osmium tetroxide and processed for electron microscopy in the usual way. Thin sections were stained with uranyl acetate and lead citrate.
Figure 115. Apical portion of a secretory cell from fimbria of an intact animal. Acid phosphatase activity is demonstrated by the presence of an electron-dense granular precipitate of the reaction product on the cell membrane and microvilli. (18, 150 X) The inset shows the localization of acid phosphatase activity in a dense body situated in the perinuclear region. (22, 800 X)

Figure 116. Apical region of a secretory cell from ampulla showing the fine granular deposits of the reaction product on the cell membrane, microvilli and secretory material. Note the great extent of extrusion of a secretory granule. (22, 800 X) The inset illustrates the localization of acid phosphatase activity in a dense body near the nucleus from a ciliated cell. (22, 800 X)
Figure 117. Apical portion of a secretory cell from ampulla of an ovariectomized rabbit treated with exogenous estrogen. Note the acid phosphatase activity on the cell membrane, microvilli and secretory material. (35,760 X)

Figure 118. Portion of a secretory cell from ampulla of an ovariectomized rabbit treated with exogenous estrogen. Note the acid phosphatase activity in the Golgi saccules (arrow) and secretory granules. (17,600 X)