

# ESTROGEN-INDUCED CYTODIFFERENTIATION OF THE OVALBUMIN-SECRETING GLANDS OF THE CHICK OVIDUCT

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## ABSTRACT

The histological, ultrastructural, and biochemical changes occurring during hormone-induced cytodifferentiation of the ovalbumin-secreting glands in the chick oviduct have been studied. Marked perivascular edema is an initial response of the immature oviduct stroma to diethylstilbestrol administration and is accompanied by an interstitial migration of mononuclear cells. Mitotic activity in the immature mucosal epithelium increases within 24 hr, and glands begin to develop on days 2–4 as budlike invaginations into the sub-epithelial stroma. An immediate intracellular effect of the hormone is aggregation of previously dispersed ribosomes. Ribosomal zones in the nucleolus gain prominence, and there is a progressive development of rough endoplasmic reticulum in the epithelial cells. Extensive profiles of endoplasmic reticulum are present in the gland cells by day 6. Fine apical progranules appear in the epithelial cells on day 2, and ovalbumin can be measured immunochemically by day 3 at about the same time that new species of nuclear RNA have been identified. Ovalbumin granules form within condensing vacuoles in the Golgi zone and begin to be released into the lumina of the gland acini at about day 6 of the treatment.

The specialization of primitive reproductive tissue in response to hormonal stimulation during sexual maturation is one of the few instances in which dramatic cytodifferentiation in vertebrates is not restricted to the period of embryonic development. The mucosa of the magnum portion of the chick oviduct is a tissue admirably suited to the study of controlled hormone-induced differentiation. After exogenous estrogen stimulation, three distinct types of epithelial cells differentiate from previously indistinguishable immature epithelial cells of the chick oviduct mucosa (2). Furthermore, two of these cell types synthesize cell-specific proteins which may be used as markers for differentiation. The tubular gland cells produce ovalbumin, and the goblet cells synthesize avidin (14). The third type of epithelial cell which differentiates in response to estrogen is ciliated and evidently concerned with propulsion of material through the

oviduct. The purpose of the present investigation was to observe sequentially the histological, ultrastructural, and biochemical events which occur during estrogen-induced cytodifferentiation of the ovalbumin-secreting glands of the chick oviduct magnum.

## MATERIALS AND METHODS

5-day-old Rhode Island Red chicks were injected subcutaneously with 5 mg of diethylstilbestrol (DES) in sesame oil for 17 consecutive days. Chicks were sacrificed throughout the period of hormone treatment by subluxation of the cervical vertebrae. The oviducts were removed immediately after sacrifice, and a region corresponding to the location of the adult magnum was fixed in Zenker's-4% formaldehyde (1) or in phosphate-buffered 4% glutaraldehyde (4). For conventional light microscopy, blocks of oviduct were oriented in transverse or longitudinal planes and embedded in paraffin. Sections were

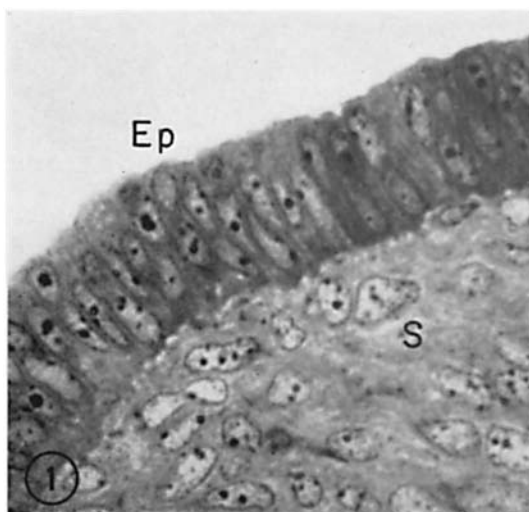


FIGURE 1 Light micrograph of immature chick oviduct (5 days old). Columnar epithelial cells (*Ep*) with prominent nucleoli are seated upon dense stroma (*S*). Stromal nuclei are more rounded and contain several nucleolar clumps. Araldite section stained with toluidine blue.  $\times 1000$ .

stained with hematoxylin and eosin or according to special methods detailed elsewhere (1). Some samples of glutaraldehyde-fixed oviduct were embedded in Araldite resin (Ciba 502) according to methods of Luft (16) after postfixation in 1% osmium tetroxide buffered with 0.1 M phosphate. Araldite sections of 1–2  $\mu$  were stained with 1% toluidine blue in 1% borax solution for light optical examination. Mitotic activity was determined by counting the number of mitoses in at least 500 cells in representative areas of two different oviducts. Araldite sections were mounted on copper mesh grids and stained with uranyl acetate (saturated aqueous) or lead citrate (31) for examination in an RCA EMU 3F electron microscope.

Quantitative determinations of ovalbumin were made on homogenates of oviduct magnum tissue removed at the same time as the tissue for histology. Ovalbumin was precipitated by specific antibody and quantified by protein estimates of the precipitate by the Folin-Lowry procedure (12). Agar gel diffusion studies on Ouchterlony plates demonstrated that the bulk of the precipitable protein was identical to ovalbumin (22).

## RESULTS

### *Immature Oviduct*

In the 5-day-old chick, the entire oviduct wall measures no more than 1 mm in thickness, and

there is a minimal external sleeve of fibrous tissue. The oviduct mucosa is formed by a thin layer of pseudostratified columnar epithelium which rests upon a compact stroma of polygonal cells and intermingled collagen (Figs. 1 and 2). The epithelial cell nuclei are elongate and display relatively prominent, centrally located nucleoli. Marginal chromatin is scant. The stromal cell nuclei are rounded and usually exhibit several clumps of nucleolar material. In the 5-day-old chick, occasional mitotic figures are observed in

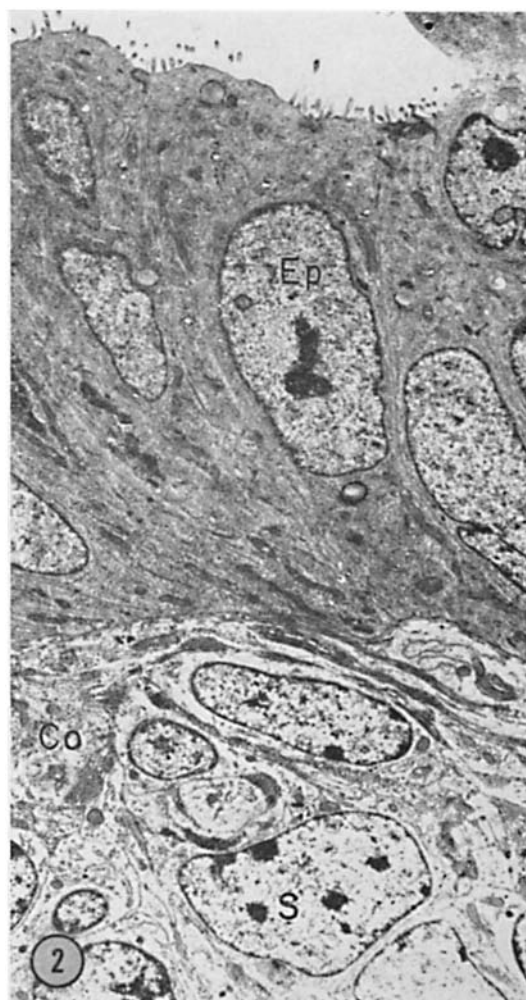


FIGURE 2 Immature oviduct in low magnification electron micrograph. Epithelial cell (*Ep*) microvilli are irregularly spaced. Stromal cells (*S*) are enmeshed in fascicles of collagen (*Co*).  $\times 6600$ .

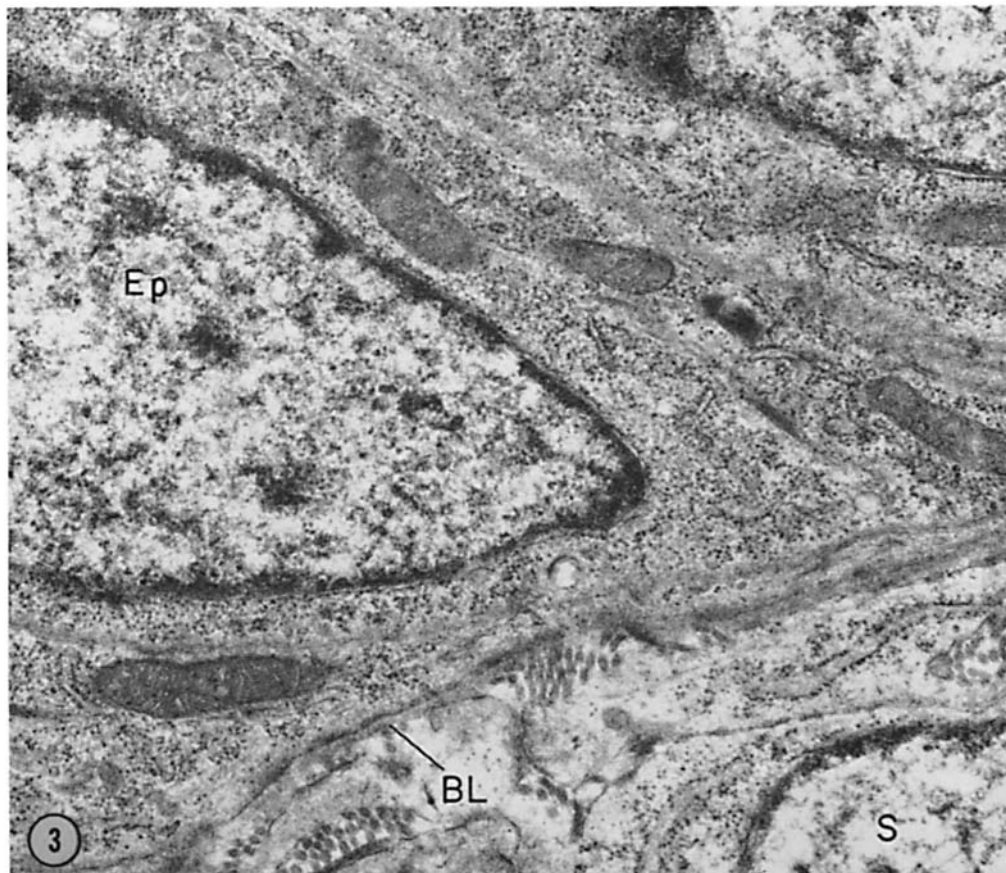


FIGURE 3 Detail of cytoplasm in immature epithelial cell (*Ep*) illustrating numerous dispersed ribosomes and paucity of endoplasmic reticulum. A fine basement lamina (*BL*) separates the epithelial layer from subjacent stroma (*S*).  $\times 31,000$ .

both stromal and epithelial cell layers of the oviduct. These findings are consistent with the slow, natural growth of the oviduct which ordinarily continues until sexual maturation at about 100 days of age (2).

Ultrastructurally, the surface epithelial cells of the immature oviduct mucosa are characterized by electron-opaque hyaloplasm with abundant dispersed ribosomes (Figs. 2 and 3). Rough-surfaced endoplasmic reticulum is sparse and occurs only in short profiles. The Golgi apparatus is not conspicuous, and there are few mitochondria. These immature epithelial cells are connected laterally by interlocking membrane plications, desmosomes, and junctional membrane complexes corresponding to the "terminal bars" of conventional histology. In advantageously oriented planes

of section, a dense plasmolemmal band, corresponding to the zonula occludens of the junctional complex (5), may be distinguished. Discrete arcades of tonofibrillae attach to desmosomes positioned more distally in the complexes. Short, irregularly spaced microvilli occur at the surface of immature oviduct epithelial cells (Fig. 2).

A very thin basement lamina separates the surface epithelium from stroma (Fig. 3). The stromal cells are compactly arranged, but discretely separated by irregular interstitial spacings packed with fascicles of collagen (Fig. 2). The nucleocytoplasmic ratio of the unstimulated stromal cells is high. The stromal cell cytoplasm is unremarkable with a few scattered mitochondria and short profiles of rough endoplasmic reticulum.

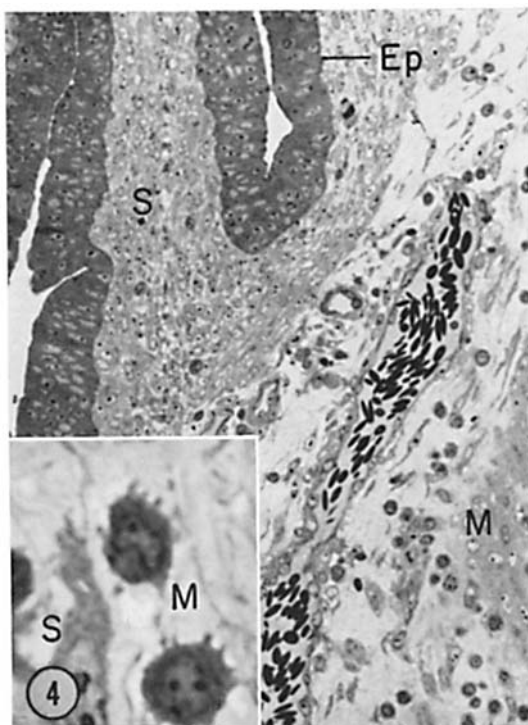


FIGURE 4 Perivascular edema and mononuclear cell infiltration following 24 hr of DES administration. Stroma (S) immediately subjacent to surface epithelium (Ep) remains compact. Deep stromal cells assume more elongate form, and nucleoli increase in prominence. Mononuclear cells (M) are identified by prickly cytoplasmic outline and relatively basophilic cytoplasm (insert). Araldite section stained with toluidine blue.  $\times 240$ ; insert,  $\times 1,340$ .

#### *Effect of DES Administration*

Changes are described only for the left oviduct. As in normal maturation of the adult hen, the right oviduct remains rudimentary after exogenous estrogen. The principal effect of DES injection is a rapid development of tubular glands from the parental layer of pseudostratified columnar cells. This development is preceded by a phase of stromal edema. Decreased density of stromal cells is evident within 24 hr after DES administration and proceeds upward from the deep-lying perivascular areas to involve the entire subepithelial region (Figs. 4–6). Initially, the interstitial fluid appears serous. However, in glutaraldehyde-fixed tissues, a well preserved eosinophilic material is evident at 48 hr. This material stains with the

ninhydrin-Schiff reaction and appears as a flocculent precipitate in electron micrographs. During the phase of interstitial edema, the stromal capillaries dilate impressively, and a clear separation of the endothelial cell nuclei is noted by light microscopy. This appearance correlates with an attenuation of the endothelial cell cytoplasm forming the capillary walls which is observed by electron microscopy. Desmosomal junctions between the endothelial cells generally appear to remain intact; however, the interstitial edema is soon accompanied by an interstitial migration of round mononuclear cells which originate from within vessels. These migratory cells must presumably pass through the junctional zones between endothelial cells (6, 19) and are most numerous by 48–72 hr. In Araldite sections stained with toluidine blue, mononuclear migratory cells are readily distinguishable from isolated stromal cells by a characteristic peripheral aggregation of their nuclear chromatin, a prickly outline of their

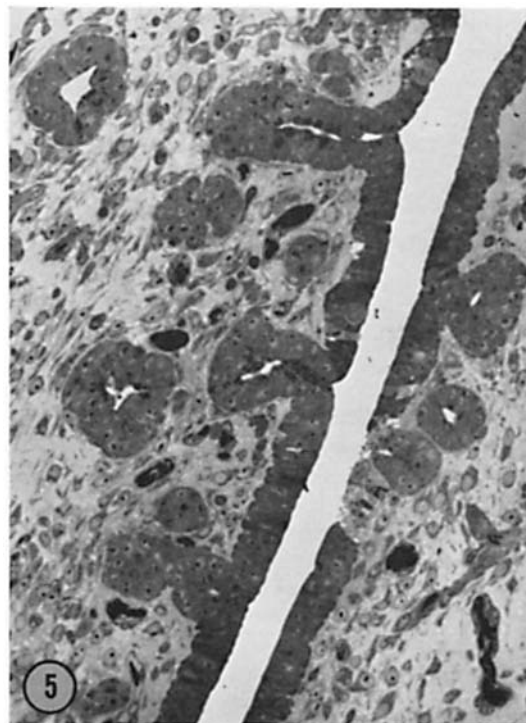


FIGURE 5 Early gland formation from surface epithelium following 4 days of DES administration. Stromal edema has advanced to involve subepithelial region.  $\times 250$ .

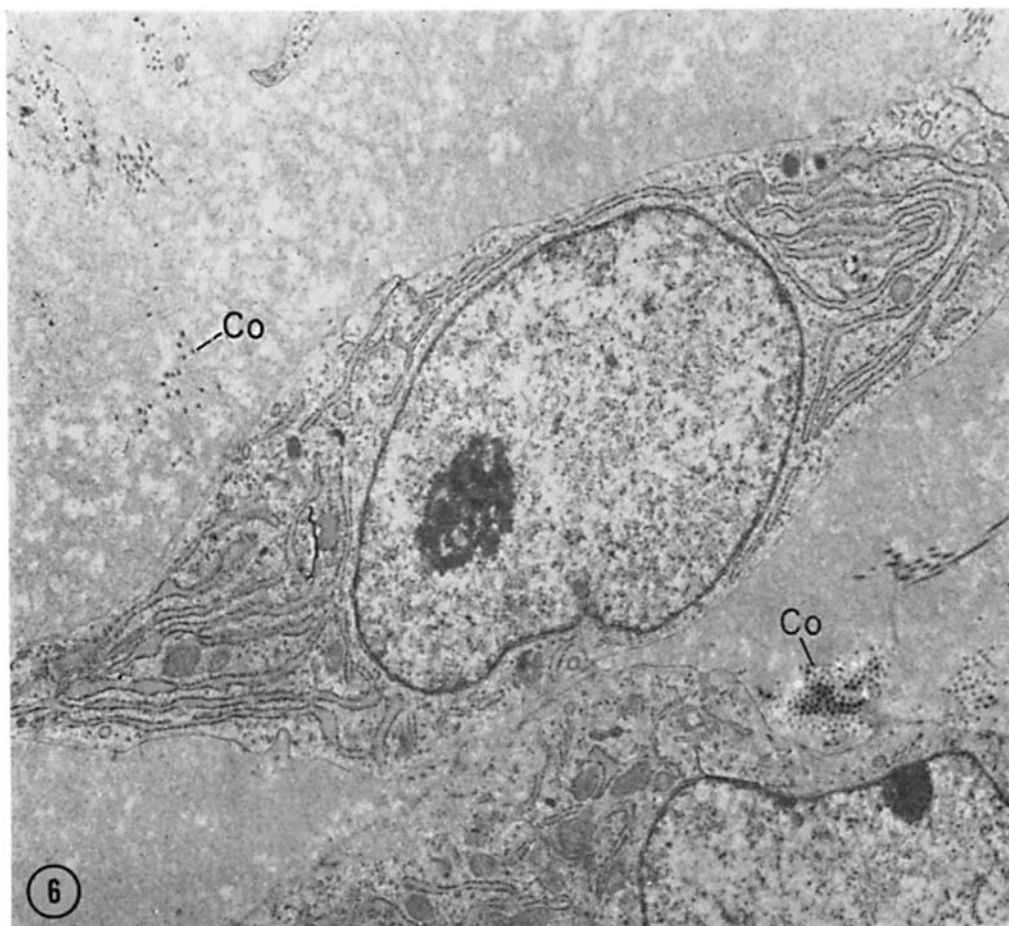


FIGURE 6 Electron micrograph of DES-activated stromal cell (48 hr). Nucleolus has gained prominence (compare to Fig. 2) and rough-surfaced endoplasmic reticulum is highly developed. Collagen bundles (*Co*) appear disrupted and separated by areas of exudate.  $\times 12,300$ .

cytoplasmic margins, and a strong cytoplasmic affinity for the basic dye (Fig. 4). Surface projections and irregular, membrane-bound cytoplasmic granules of the primary lysosome type (Fig. 7) suggest that many of the migratory cells are derived from circulating monocytes which possess similar structural features (29). There is, however, no overt evidence of phagocytosis, and many of the infiltrating mononuclear cells appear well endowed with stacked lamellae of rough surfaced endoplasmic reticulum (Fig. 8).

The stromal cell changes effected by DES administration are pronounced. The nuclei enlarge, but retain their general configuration and chromatin pattern. The increase in cytoplasmic

volume is proportionately greater, and is accompanied by a dramatic development of rough endoplasmic reticulum (Fig. 6). As interstitial edema progresses, the stromal cells lose their original polygonal outline and assume an elongate form. Study of conventional sections stained for connective tissue elements and of plastic sections stained with toluidine blue suggests that activated stromal cells gradually migrate to the oviduct perimeter where they condense to form bundles of smooth muscle. This view is also supported by lack of mitotic activity in the peripheral muscular bands during a stage of rapid thickening.

Mitotic activity in the immature surface epithelial layer begins to rise within 24 hr after the ad-

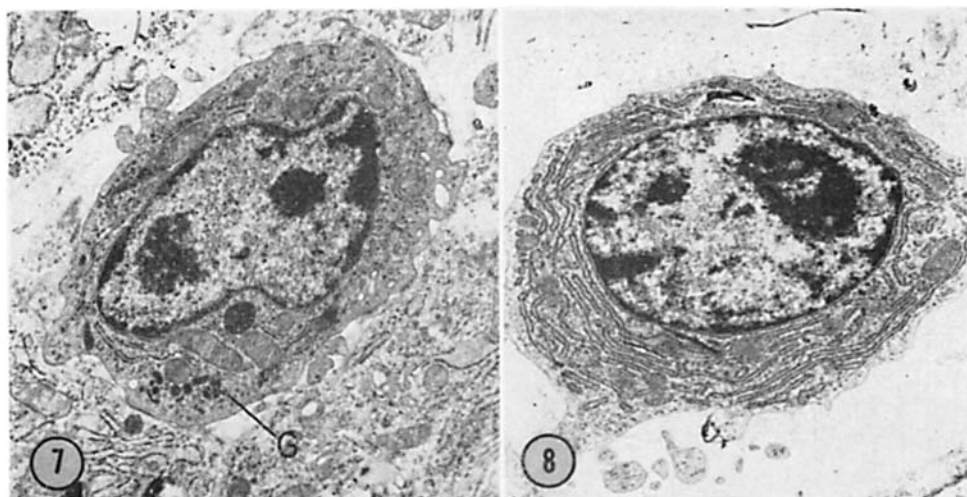


FIGURE 7 Monocytic cell displaying histiocytic features: ruffled cytoplasmic surface and irregular cytoplasmic granules (G) of the primary lysosome type. Chromatin densities are relatively coarse and peripheral in comparison to those of activated stromal cell (compare to Fig. 6).  $\times 11,500$ .

FIGURE 8 Monocytic cell displaying elaborate development of rough-surfaced endoplasmic reticulum.  $\times 11,500$ .

TABLE I  
*Mitotic Activity after Diethylstilbestrol (DES)*

Days of DES	Surface epithelium	Stroma	Tubular gland
0	2	1	—
1	12	2	—
2	14	4	—
4	18	2	19*
6	7	—	12
9	6	—	18
12	4	—	2
17	5	—	1

Numbers indicate actual mitoses per 500 cells counted in representative areas of two different oviducts. Stromal cells have largely been displaced by tubular glands by day 6.

\* At day 4 the early gland buds with high mitotic activity are only partially invaginated from the surface epithelial layer.

ministration of DES (Table I). Mitoses are most frequent in the surface layer of epithelium until day 4 at which time mitotic activity is highest in the newly developing tubular glands. However, mitoses continue to be frequent in cells of the surface epithelium which apparently differentiate into ciliated cells or goblet cells. By day 1 of DES, the epithelial nuclei enlarge over-all, but nucleolar

hypertrophy is most conspicuous. Ribosomal zones within the nucleolus (10, 28) appear to gain prominence (Fig. 9). An immediate and striking subcellular effect of the DES administration is an aggregation of ribosomes previously dispersed in the epithelial cell cytoplasm (Fig. 10). The aggregation of ribosomes into many small clusters may be indicative of their assembly into polyribosomes. This is followed by a progressive development of rough endoplasmic reticulum (Fig. 11). An increase of cytoplasmic pyroninophilia is noted by light microscopy on days 2-4, and corresponds to the period of ribosomal aggregation.

Granule-secreting glands originate as budlike invaginations of the original columnar epithelium into the edematous subepithelial stroma (Fig. 5). By day 9 of DES, the loose stroma has been replaced by tubular glands. A physical continuity of gland epithelium with the surface epithelium appears to persist in the form of desmosomes and junctional complexes (Figs. 12 and 13). Growth of the proliferating gland buds is rapidly accelerated by intraglandular mitoses which rise to a peak on day 9 (Table I). These mitoses often occur within cells containing secretory granules (18). The orientation of mitotic spindles during this period appears random, in both the parental sur-



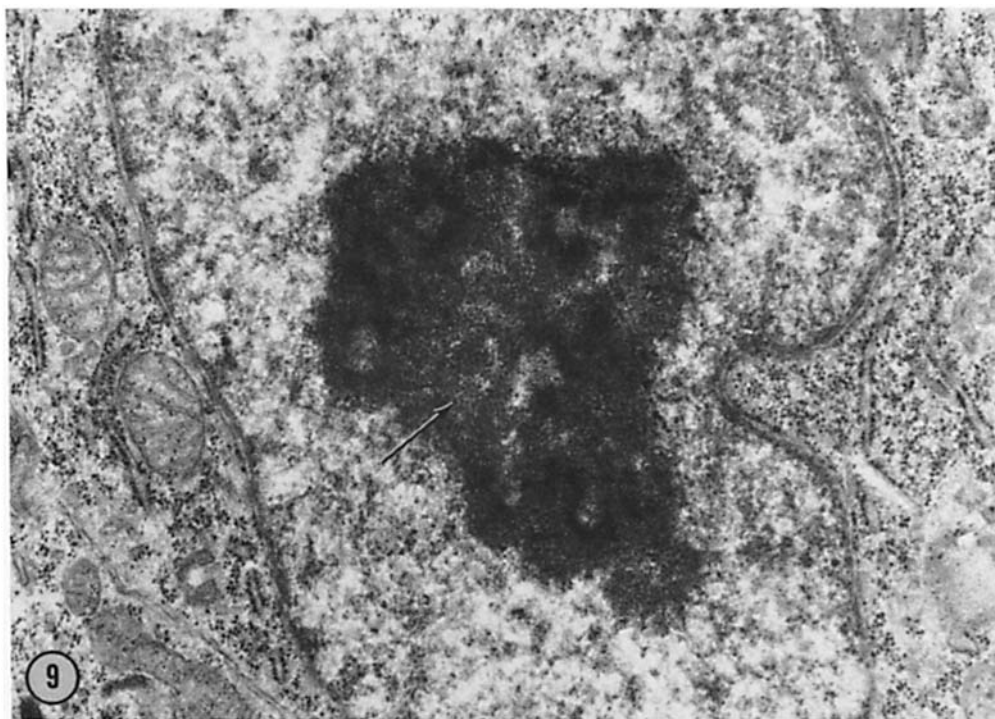


FIGURE 9 Detail of nucleolus in epithelial cell of developing gland (48 hr of DES). Note well developed particulate region (arrow). Individual particles resemble cytoplasmic ribosomes in size and density. Uranyl acetate and lead citrate stains.  $\times 36,900$ .

face layer and the underlying glands in the process of proliferation. Later in development, the origin of glands from the surface epithelium is no longer apparent by light microscopy, and orientation of mitotic spindle fibers parallel to the luminal border in dividing surface cells suggests that both progeny will remain in a superficial position (20). During the phase of rapid glandular proliferation, epithelial cell nuclei appear immature and resemble those in differentiating cells at the luminal surface. After day 6, however, disparate maturation of the deep glands is signaled by increased granule production, a diminution of nuclear size, loss of nucleolar prominence, and the appearance of dense chromatin aggregates at the nuclear periphery and around the nucleolus (Figs. 12, 14–16).

The uniformity of the DES-induced developmental stages produced within a relatively homogeneous group of newborn chicks permitted comparative observation of sequential stages in the process of secretory-granule formation. Immunofluorescent studies (14) have indicated that the tubular gland cells contain ovalbumin, and

the progress of granule development correlates well with quantitative measurement of ovalbumin (Table II). The earliest morphological evidence of granule formation appears 24–48 hr after DES administration. Fine apical progranules, presumably ovalbumin, appear in scattered groups of the original surface epithelial cells (Figs. 12 and 14). These granules measure no more than  $0.2 \mu$  in diameter and are barely detectable by light microscopy. Small amounts of immunoreactive ovalbumin are detected by day 3 (Table II). The appearance of well developed elongate profiles of endoplasmic reticulum (Fig. 11) does not occur until days 3–4 and is followed closely by hypertrophy of the previously inconspicuous Golgi zone (Figs. 17 and 18). By day 6, electron-opaque granules may be observed forming in vesicles of the Golgi zone (Figs. 18 and 19). Condensation vacuoles, which contain substance of lower electron opacity than the mature granules, arise from the concave surface of the Golgi lamellae (Fig. 19). Irregularities in their outline suggest continuous coalescence with small transport vesicles (11). At this time, the

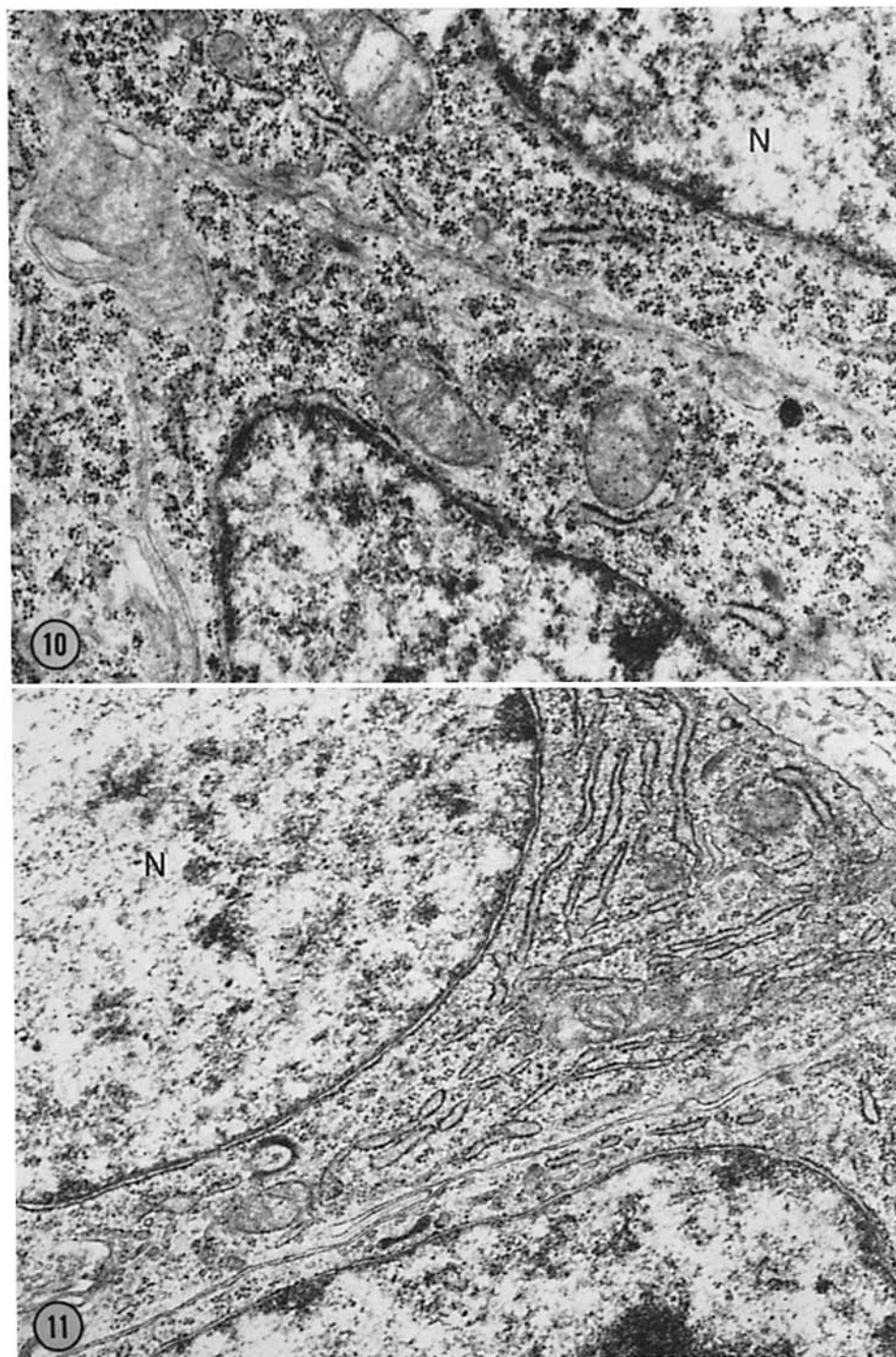


FIGURE 10 Aggregation of cytoplasmic ribosomes in epithelial cells which may be indicative of their assembly into polyribosomes after DES stimulation (48 hr). Compare to Fig. 3.  $\times 36,500$ .

FIGURE 11 Elaboration of rough surfaced endoplasmic reticulum in DES-stimulated epithelial cell of ovalbumin gland (6 days).



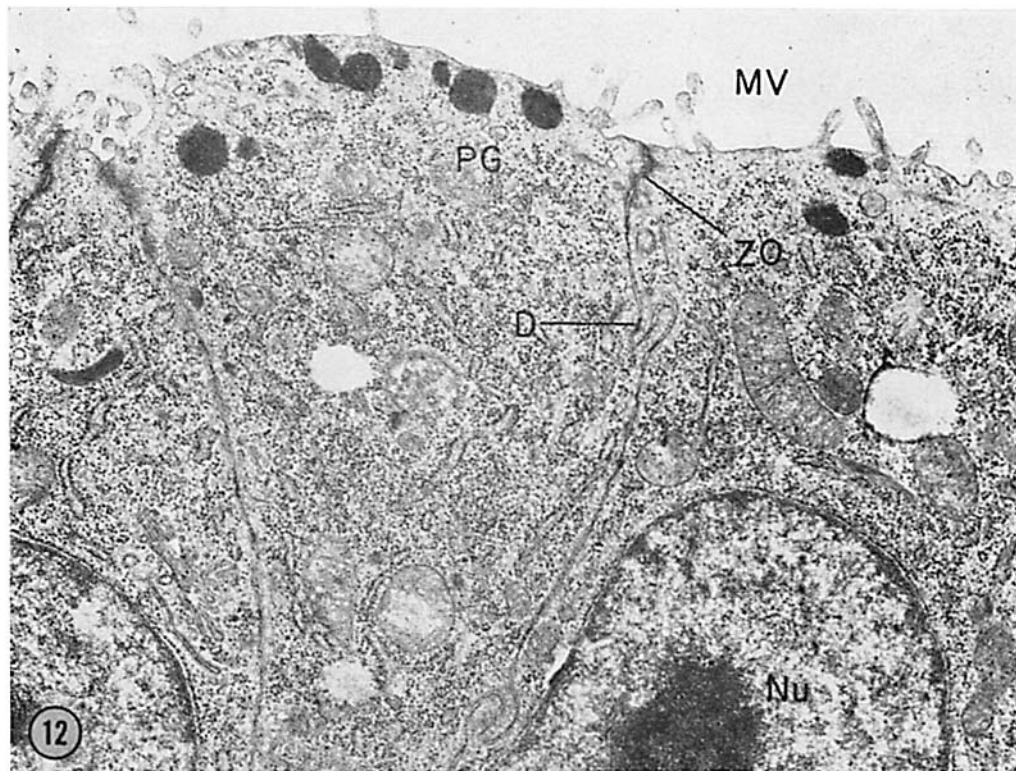


FIGURE 12 Formation of apical progranules (*PG*) in surface epithelial cells within 24 hr of DES administration. Elements of junctional complex between epithelial cells include dense zonula occludens (*ZO*), zonula adherens, desmosomes (*D*), and lateral plication of adjacent plasma membranes. Epithelial cell nucleolus (*Nu*) is relatively large. Note surface microvilli (*MV*).  $\times 23,800$ .

Golgi zone nearly always lies on the acinar aspect of the basally placed gland cell nucleus. During maturation of the oviduct, the average size of the condensation vacuoles increases (Fig. 20). Ovalbumin-granule formation continues throughout the period of estrogen stimulation, and the average granule diameter progressively increases (compare Figs. 13–15, 20–22). Intracytoplasmic granule accumulation continues through day 6, after which many granules appear to be released into the lumina of the acini (Figs. 13 and 21). Dilation of the rough endoplasmic reticulum and accumulation of flocculent material within the reticulum cisternae are features of maximally stimulated gland cells (Figs. 16 and 20).

Ovalbumin granules in the granular epithelial cells were best preserved in glutaraldehyde-fixed materials (4). After Zenker's-formalin fixation, many granules were extracted, leaving a foamy-appearing cytoplasm in paraffin sections. The

albumin granules stained strongly with phosphotungstic acid hematoxylin and weakly with the periodic acid-Schiff method both before and after diastase treatment. By electron microscopy, ovalbumin granules were uniformly electron opaque and deeply osmiophilic after the glutaraldehyde fixation (Figs. 13, 20, 21).

After the period of rapid glandular proliferation (Table III), glandular development ceases to be a major feature of the surface epithelial layer. The residual surface epithelial cells proceed to undergo a separate pathway of maturation. Cellular pleomorphism in the surface layer is evident by light and electron microscopy as early as day 6 (Fig. 22). Large cells which are superficially located in the surface layer of the mucosa display numerous mitochondria in a relatively electron-lucent cytoplasm. These cells begin to develop cilia on day 6 of DES. Other smaller cells located in a somewhat more basal position are char-

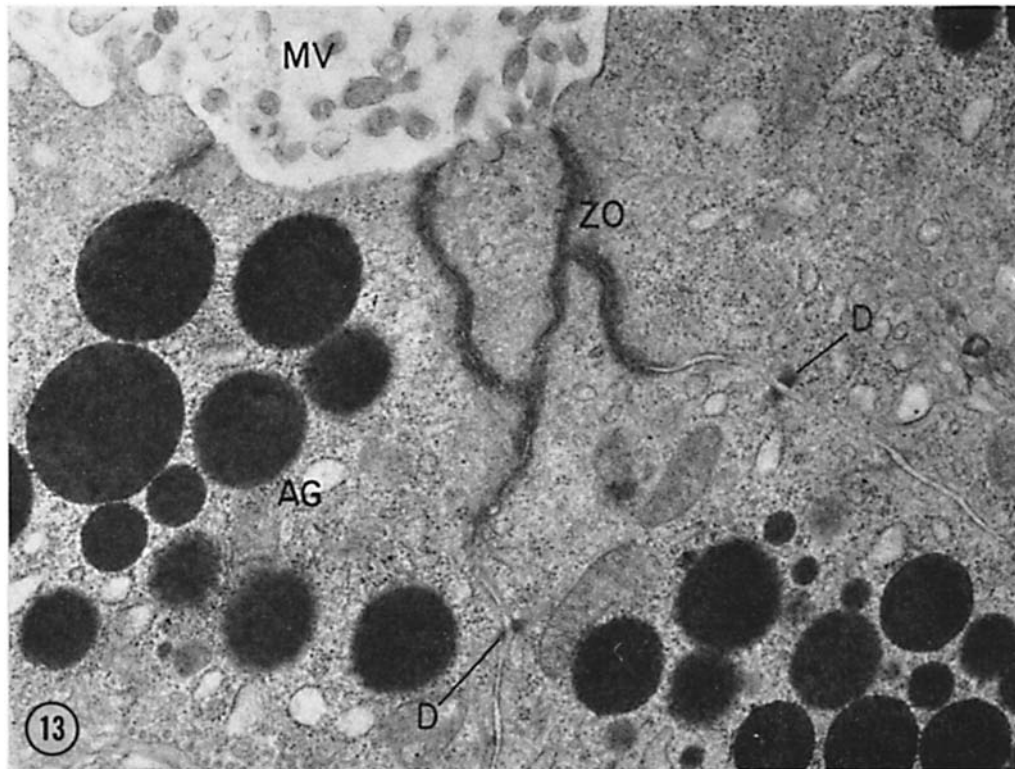


FIGURE 13 Ovalbumin granules (AG) concentrated in apical portion of young glandular epithelial cells after 6 days of DES stimulation. Zonula occludens (ZO) and desmosomes (D) of junctional zone persist. Numerous microvilli (MV) project into empty lumen of gland acinus.  $\times 25,550$ .

acterized by relatively electron-opaque cytoplasm and possess small, crenated nuclei. The latter cells develop into the intercalated goblet cells on about day 9 of DES. These goblet cells contain small granules which have a fibrillar appearance in the electron microscope (Fig. 23). The histological and ultrastructural changes during induction of avidin synthesis (14) in these goblet cells by progesterone are the subject of another communication. (O'Malley, B. W., P. M. Grimley, and P. O. Kohler. In preparation.)

#### DISCUSSION

The progressive morphological and biochemical specializations which occur during cytodifferentiation are probably the result of differential gene action. Although the primary stimuli which regulate gene expression during embryological differentiation are not well understood, gonadal hormones clearly provide at least the triggering

influence leading to overt cytodifferentiation of primitive cells in reproductive tissues during sexual maturation.

The regulation of protein synthesis by exogenous gonadal hormones in the immature chick oviduct has recently been studied in detail as an experimental model for investigating mechanisms of steroid action (15, 22-27). The administration of exogenous estrogen to the immature chick has been shown to result in impressive increases in oviduct size and weight (9, 21), which are proportional to the amount of hormone given. Previous studies (14, 22) have demonstrated that DES administration alone induces the synthesis of ovalbumin in the immature chick oviduct. Normally, ovalbumin is found only in the oviduct of mature chickens.

Estrogen stimulates marked increases in oviduct DNA, RNA, and general protein synthesis and induces the appearance of new species of messenger

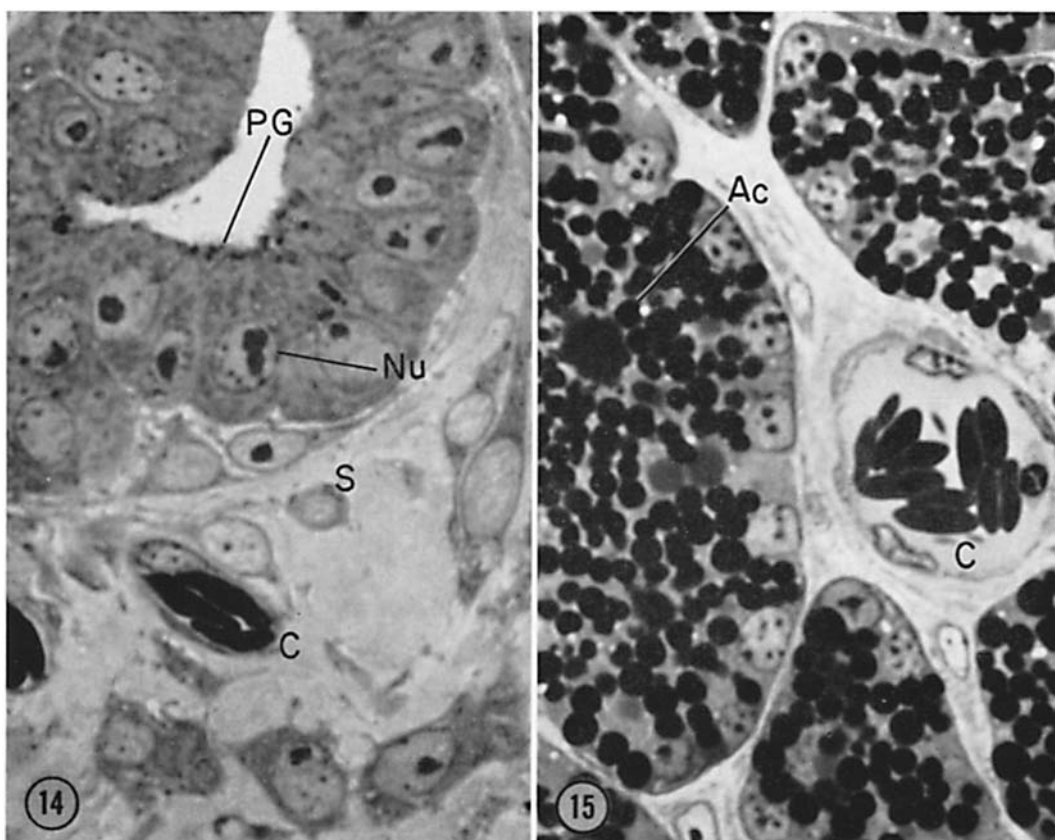


FIGURE 14 Light microscopic appearance of epithelial cells during early ovalbumin gland formation (4 days of DES). Epithelial cell nucleoli (*Nu*) are enlarged and prominent, and ovalbumin progranules (*PG*) accumulate along the luminal border of the cytoplasm. Note tendency of elongated stromal cells (*S*) to delimit advancing edge of epithelial cells. Fine capillary branch (*C*) is filled with erythrocytes. Araldite section stained with toluidine blue.  $\times 1,340$ .

FIGURE 15 Light microscopic appearance of more mature gland (12 days of DES). Large ovalbumin granules are distributed throughout cytoplasm; glandular acini (*Ac*) are filled with secretory material and the basally situated epithelial cell nuclei display relatively inconspicuous nucleoli. Interstitial capillary (*C*) is dilated and filled with nucleated erythrocytes. Araldite section stained with toluidine blue.  $\times 1,340$ .

RNA as measured by DNA-RNA hybridization techniques (27). The new species of mRNA are detectable by 2 days after DES and are present in larger quantities at 5 days. The induction of ovalbumin synthesis correlates temporally with the appearance of these new populations of nuclear RNA. The present investigation further demonstrates a good correlation between the initial appearance of ovalbumin, as determined by immunoprecipitation, and the ultrastructural

development of cytosecretory granules in the tubular gland cells.

Local tissue factors may play a crucial role in cytodifferentiation of the oviduct mucosa. The first effect of DES in this system is to produce a stromal edema and invasion of circulating monocytes. The increased permeability of vessels after estrogen has been studied extensively in the rat uterus (6). In the chick oviduct vessels, there is a cytoplasmic attenuation of the lining endothelial

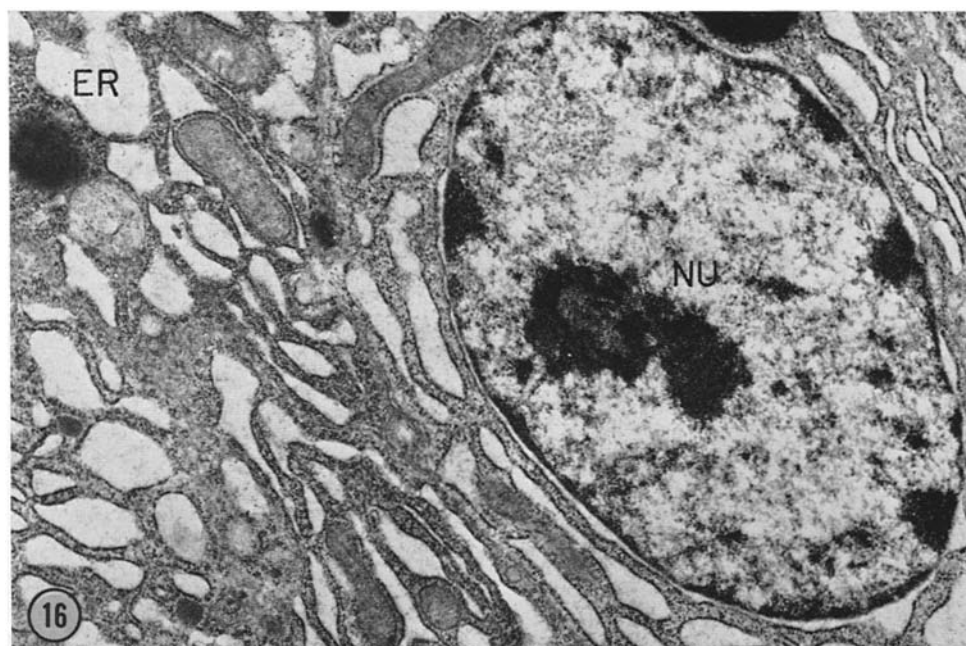


FIGURE 16 Nucleus of maximally stimulated epithelial cell (12 days of DES) exhibits a margination of dense chromatin to nuclear membrane and a relatively small nucleolar region (*Nu*) surrounded by heterochromatin. Cisternae of rough endoplasmic reticulum (*ER*) appear dilated. Compare to Figs. 9 and 11.  $\times 17,100$ .

TABLE II  
*Effect of Diethylstilbestrol (DES) on Ovalbumin  
Synthesis by the Oviduct*

Days of DES	mg ovalbumin per gram* oviduct
0	0
3	1.6
6	5.4
10	9.3
15	16.3

\* wet weight.

cells at 24 hr. From a purely physical standpoint, decreased stromal cell density might be expected to facilitate invagination and expansile growth of tubular glands which arise from the surface epithelial sheet. Less obvious and poorly understood are the more complex influences which mesenchymal cells evidently exert upon their epithelial neighbors (8, 32). Kallman and Grobstein (13) have shown that mesenchymal tissue can influence the differentiation of epithelial cells

of embryonic mouse pancreas, even in transfilter culture. Grobstein and Cohen (7) have introduced evidence that collagen itself may be instrumental in modeling glandular acini in organ cultures. Preliminary assays of the DES-stimulated oviduct suggest that collagenase activity may increase substantially during the period of glandular proliferation (Fullmer, H. personal communication.) It is thus conceivable that the migratory monocytes or activated stromal cells are involved in the elaboration of either lytic enzymes or trophic substances which modify the epithelial growth pattern.

The delayed appearance of the oviduct goblet cells until after the completion of tubular gland development suggests that goblet cell differentiation need not be a direct effect of DES, but could be influenced by the microenvironment of the cells, local tissue effectors, or removal of repressive influences. In the esophageal epithelium, it appears that the randomly determined position of identical daughter cells in either the basal or

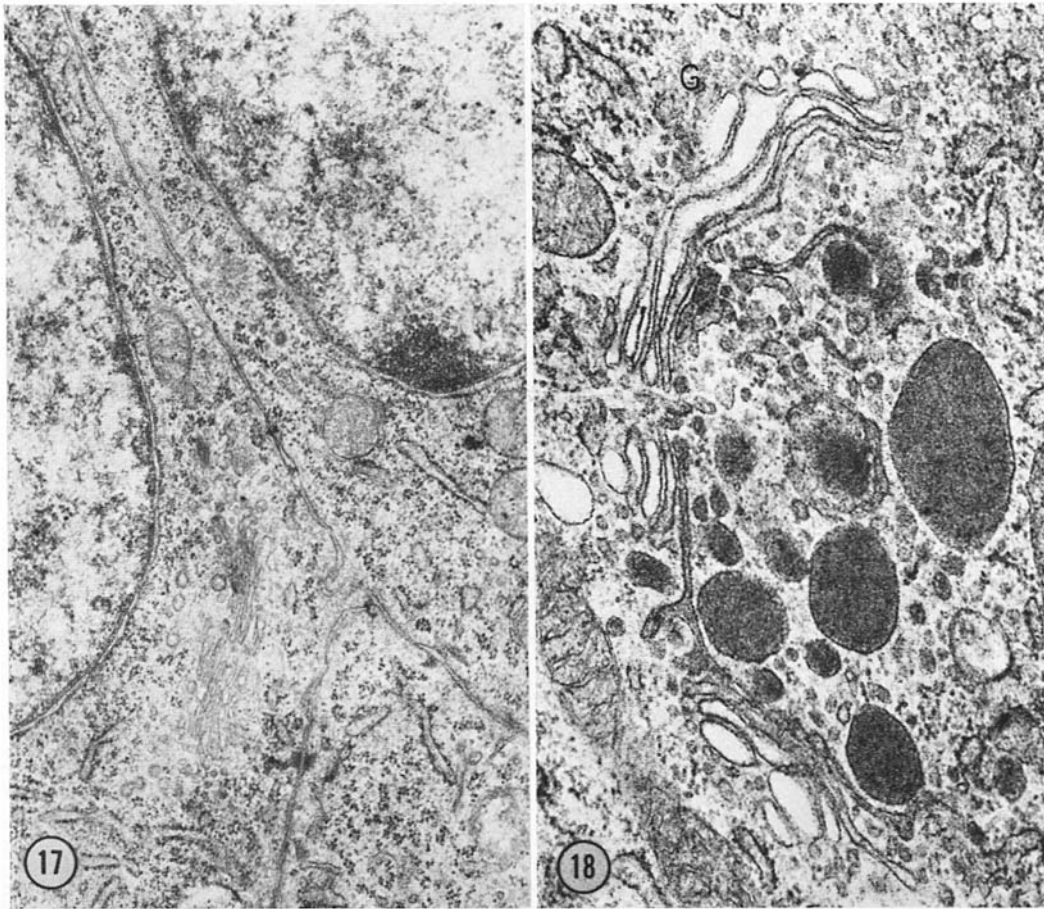


FIGURE 17 Early development of epithelial cell Golgi zone following DES administration (48 hr).  $\times 26,600$ .

FIGURE 18 Formation of granule densities within cisternae along concave face of Golgi zone (G) (6 days of DES).  $\times 33,000$ .

spinous layers determines whether a cell will proliferate or differentiate (17).

The finding of ovalbumin secretory granules in oviduct tubular gland cells after stimulation with DES alone differs from the previously reported findings of Brant and Nalbandov (2). These investigators examined the oviduct magnum at a single point in time after implantation of a stilbestrol pellet in the immature chick and found tubular gland formation, but no granule formation until progesterone or testosterone was also administered. Difficulty in fixing the tissue without dissolving the ovalbumin granules encountered by Brant and Nalvondov (2) and noted after Zenker's-

formalin in the present study may account for the difference in results, although the preparations, doses, and routes of administration were also different.

The immature chick oviduct affords a unique opportunity to trace the sequential events in synthesis of protein for export, under externally controlled conditions. The increasing proportion of oviduct weight which is represented as ovalbumin in the course of DES stimulation suggests that a relatively larger proportion of the proteins constituting the actual synthetic machinery must be produced early in differentiation. Morphological changes are consistent with this view. Initial

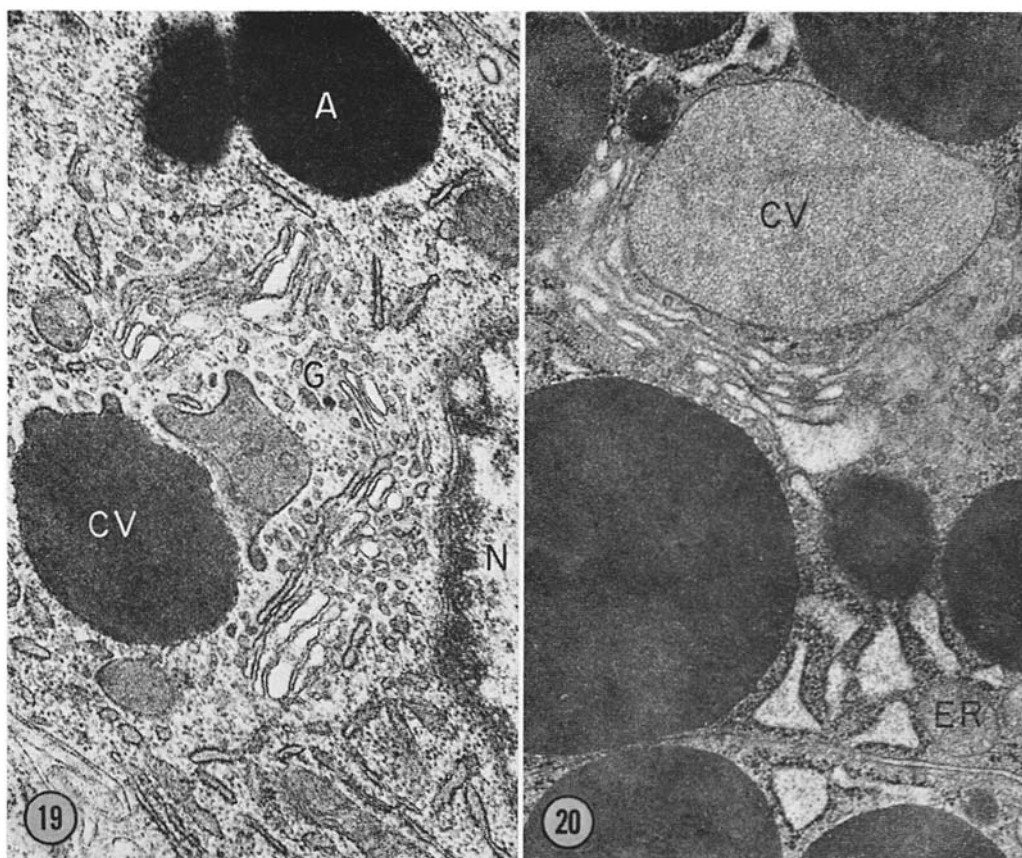


FIGURE 19 Irregular outline of condensing vacuoles (CV) in Golgi zone (G), suggesting continuous fusion with transport vesicles. Mature ovalbumin granule (A) above. (6 days of DES).  $\times 33,000$ .

FIGURE 20 Large condensing vacuole (CV) of type seen after maximal stimulation of glandular epithelium (13 days of DES). Note flocculent densities in dilated profiles of rough-surfaced endoplasmic reticulum (ER).  $\times 28,700$ .

events in the differentiation of the parental epithelial cells were nuclear enlargement, nucleolar hypertrophy, prominence of nucleolar ribosomes, and aggregation of cytoplasmic ribosomes. The progressive development of an elaborate network of rough endoplasmic reticulum, the enlargement of the Golgi apparatus, and the formation of condensing vacuoles observed in this differentiating tissue simply recapitulate the events of secretory maturation already well established in exocrine secretory cells studied under conditions of physiological stimulation (3, 11). These changes are similar to events noted in differentiating pancreatic rudiments (13) and morphogenesis induced in tadpoles by thyroid hormones (30).

The nuclear maturation, evident in differentiated glandular epithelial cells after 6 days, is of interest. In particular, the smaller nucleolar size must indicate a relatively decreased demand for new ribosome synthesis (28, 30). The peripheral clumping of dense "heterochromatin" evident at this stage may provide evidence of a decrease in the active volume of DNA. Since the oviduct has achieved less than one-third its maximal experimental weight by day 6 (Table II), continued intraglandular growth must occur. Although some electron micrographs suggest that immature basal cells of the surface strata may continue to differentiate into glandular epithelium as late as day 9, hyperplasia does not appear to be the major



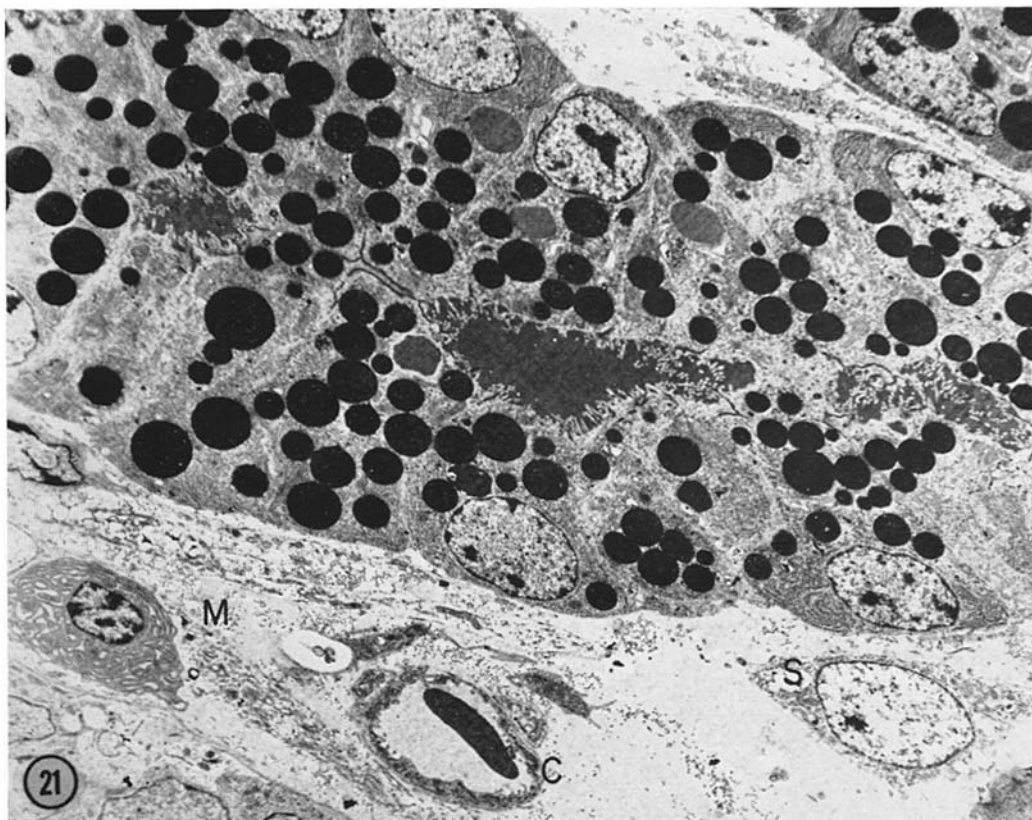


FIGURE 21 Low magnification view of maturing ovalbumin gland (9 days of DES). Secretion in gland lumen highlights numerous and regular microvilli at the apex of epithelial cells. Contrast of stromal (*S*) and wandering mononuclear cell (*M*) nuclei is evident. Capillary (*C*) wall is relatively thin.  $\times 3,500$ .

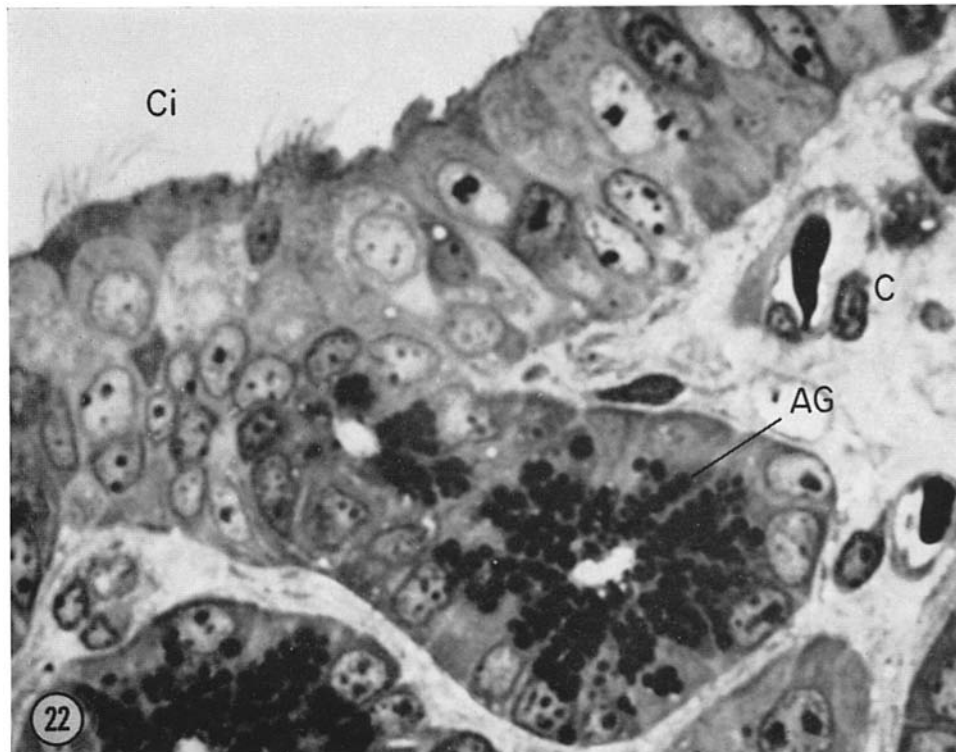


FIGURE 22 Pleomorphism of surface epithelial cells illustrated in light micrograph (6 days of DES). The first ciliated cells (*Ci*) have begun to appear. Ovalbumin granules (*AG*) are concentrated in apical regions of glandular epithelial cells, but exocrine secretion has not yet begun. Maturation of glandular nuclei and granules is intermediate between that illustrated in Fig. 14 and that shown in Fig. 15. Fine capillaries (*C*) are numerous in edematous stroma. Araldite section stained with toluidine blue.  $\times 1,340$ .



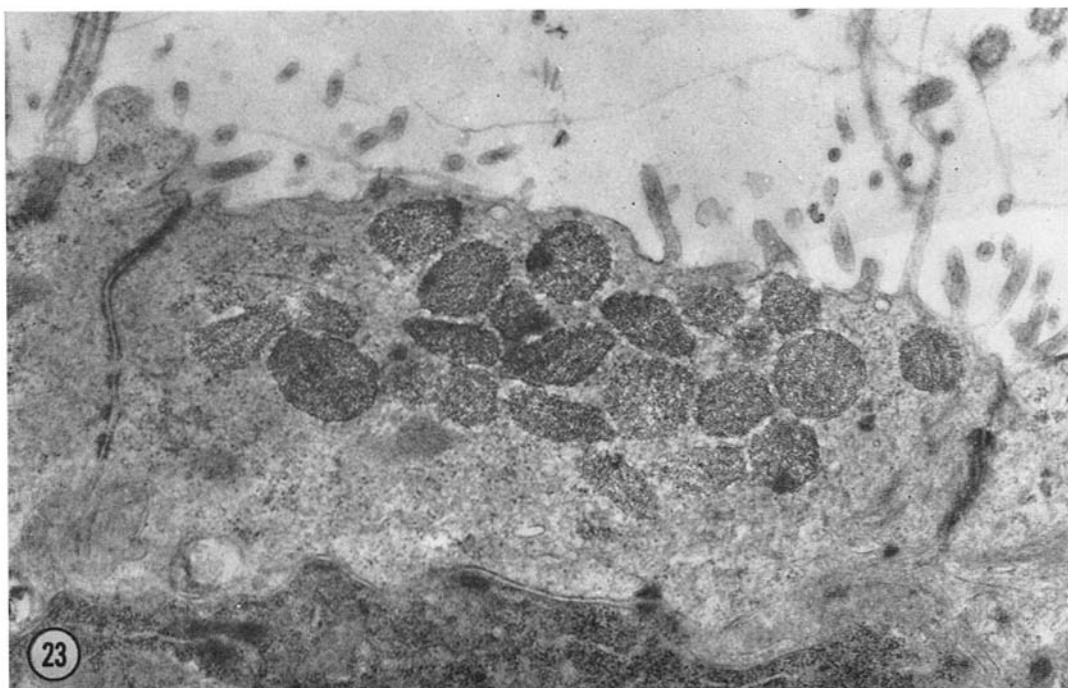


FIGURE 23 Appearance of goblet cell granules after prolonged estrogen stimulation (12 days). Fine striations characterize the granule matrix.  $\times 41,000$ .

pathway for further oviduct growth. The progressive enlargement of intracellular structures concerned with protein synthesis and indeed the progressive enlargement of the secretory granules themselves is strong evidence of cellular hypertrophy.

The stimulated oviduct may provide a useful system for assessing the differential alterations of

intracellular metabolism that must occur during the preferential augmentation of exocrine secretion.

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