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Development and Maintenance of the Oviductal Epithelium during the Estrous Cycle in the Bitch

H. G. VERHAGE, J. H. ABEL, JR., W. J. TIETZ, JR., AND M. D. BARRAU

Department of Physiology and Biophysics, Colorado State University, Fort Collins 80521

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The epithelium of the ampulla of the bitch oviduct was studied by light and electron microscopy during representative stages of the normal estrous cycle. Anestrus and early proestrus cells were characteristically low cuboidal and of uniform staining density. The disappearance of large patches of condensed chromatin from the nucleus and the enlargement of the nucleolus during midproestrus was the first evidence of differentiation in the parenchyma. Hypertrophy and ciliation of about 60% of the cells were characteristic of late proestrus. The apical cytoplasm of the ciliated cells contained a row of basal bodies, numerous mitochondria, and a complex network of fine filaments. Maximum secretory cell differentiation was not reached until midestrus at which time these cells were characterized by dilated cisternae of rough endoplasmic reticulum (RER), enlarged Golgi apparatus, and apical secretory granules. During metestrus there was an atrophy of the secretory cells, a loss of cilia, and an increase in the nucleocytoplasmic ratio. This marked the onset of a sequence of regressive events leading back to the anestrus state where low cuboidal basal cells are predominant. The results are correlated with ovarian steroid plasma levels. The differentiation sequence observed during proestrus and early estrus in the oviductal epithelium of the bitch is directly correlated with, and appears to be the result of, an estrogen surge which occurs at the onset of proestrus. The dedifferentiation sequence observed throughout metestrus is correlated with, and may be the result of, a progesterone surge which occurs during midestrus and reaches a peak early in metestrus. In addition, hormonal and morphological events are so timed that when the ova are released during early estrus, ciliation and secretion are at a maximum for ova/zygote transport and maintenance.

To date, no fine structural study of the bitch oviductal epithelium during the estrous cycle has been described. Excellent light and electron microscopic studies of the oviductal epithelium of the mouse, rat, rabbit, monkey, and human, however, have been reported. Frommel, as early as 1886, reported that the tubal epithelium of mammals in general consisted of mainly two types of cells, one ciliated, the other nonciliated and apparently secretory. Snyder (1924) and Novak and Everett (1928) reaffirmed Frommel's conclusion and also showed that the tubal epithelium undergoes cyclical changes in the human sex cycle. More recently, Fredricsson and Bjorkman (1962), Clyman (1966), and Brenner (1969a), utilizing electron microscopy, have demonstrated remarkable cyclic changes in both the secretory and ciliated tubal epithelial cells of primates. However, this does not appear to be universal for all mammalian systems. Brenner, in a recent review (1969b), discusses the controversy concerning the degree of differentiation observed in different species and even in the same species during the sexual cycle. He concludes that the reason

for the conflicting reports is due not only to species variation but also to length of the sexual cycle, section of the oviduct sampled, and the time of sampling relative to the overall cycle. For example, the estrous cycle in the rat and mouse is short and the cyclical changes observed are minimal. By contrast the bitch is somewhat unique in that the estrous cycle is extended (6–8 mo) and the ova remain in the oviduct for a period of at least a week (Evans and Cole, 1931). Therefore, the oviduct of the bitch was chosen as a model to more fully understand the cycling phenomenon of the oviductal epithelium during the estrous cycle and the relationship between hormone levels, cellular response and ova/zygote maintenance throughout the estrous cycle.

MATERIALS AND METHODS

In this study, 24 normal cycling Beagle bitches were used. Onset of protestrus was determined by the appearance of blood in the vulva. Bitches were placed with a male daily, with acceptance marking the beginning of estrus. Refusal of the male was used to mark the end of estrus and the beginning of metestrus. Whelping marked the onset of anestrus.

At the time of specimen collection, bitches were anesthetized with sodium pentabarbitol. A midventral incision was made to open the abdominal cavity. The dorsal aorta was freed of the fascia just posterior to the renal arteries. The external iliac arteries were clamped. To fix the oviduct *in situ* 3% glutaraldehyde in 0.1 M cacodylate buffer, 5% sucrose, pH 7.4 was perfused into the dorsal aorta immediately posterior to the renal arteries. Following perfusion, cold Karnovsky's fixative (1966) was injected into the oviductal bursa, the oviduct and ovary were surgically removed and immersed in the perfusate while the oviduct was dissected free from the ovary. The ampulla was dissected from each oviduct, fixed for 3 hr in cold Karnovsky's solution, rinsed, and postfixed for 1 hr in 1% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.4. The tissues were dehydrated in graded alcohols and propylene oxide and flat embedded in epon 812. Specific areas were selected, cut out, mounted, and orientated for cutting on metal chucks. One micron thick sections were cut on an LKB ultramicrotome, mounted on glass slides, stained with either 1% toluidine blue–0.1% sodium borate (Pearse, 1968) or PAS (Dibella and Hashimoto, 1966) and used for light photomicrography and cytomorphometric measurements. The height and width of 20 cells and 100 nuclei from each experimental group were measured with an eyepiece filar micrometer and the cellular and nuclear volumes determined by the formula $\pi r^2 h$. Random procedures were used on cells which were clearly sectioned through a longitudinal plane for each of the experimental groups. For electron microscopy silver to gold sections were cut on an LKB ultramicrotome, mounted on copper mesh grids, stained with uranyl acetate and lead citrate and micrographs taken with a Seimens Elmskop IA.

RESULTS AND OBSERVATIONS

During early proestrus the mucosal membrane is deeply infolded and the oviductal lumen is narrow and tortuous (Fig. 5). The epithelium at this time is made up of uniformly staining cuboidal cells with a nucleocytoplasmic ratio of 0.47 (Fig. 3) and a relatively low nuclear and cytoplasmic volume (Figs. 1 and 2). Occasionally a lighter staining ovoid ciliated cell is observed in the epithelium (Fig. 5); however they constitute less than 1% of the epithelium cell population (Fig. 4).

Studies of oviductal fine structure indicate that the epithelium is made up for the most part of undifferentiated basal cells (Horstmann and Stegner, 1966) at this time (Fig. 11). The lateral and basal surfaces of these cells are quite tortuous and infolded while the apical border is relatively smooth except for a few short microvilli which project into the lumen. In addition the apices of adjacent cells are attached to one another by well-developed junctional complexes. The cytoplasm is characterized by numerous free ribosomes, a few scattered mitochondria and a small indistinct Golgi apparatus. The nuclei are relatively small (Fig. 1), pleomorphic and deeply infolded, whereas the nucleoplasm is characterized by large patches of condensed chromatin and a relatively small nucleolus (Fig. 11).

Hypertrophy and the beginnings of cytodifferentiation are evident in the epithelial cells by midproestrus. Although this hyper-

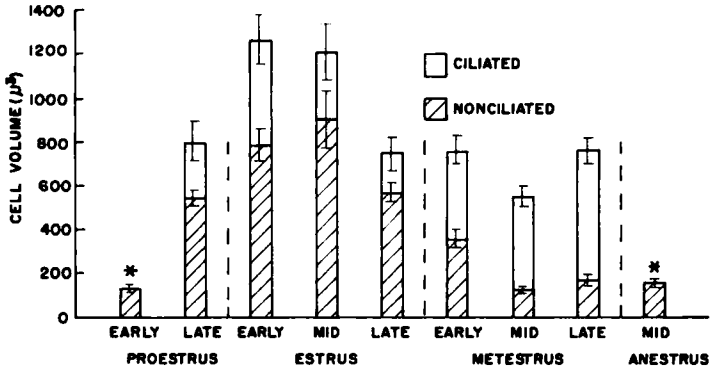


FIG. 1. Changes in the mean epithelial cell volume of the oviduct during different stages in the estrous cycle of the bitch. (*Ciliated cells were not present in sufficient numbers.)

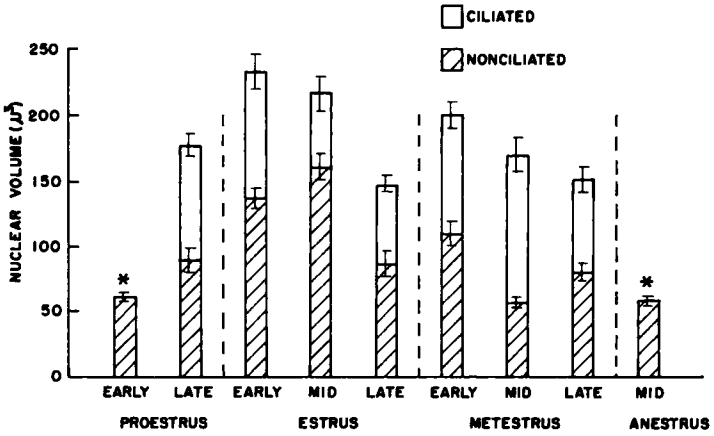


FIG. 2. Mean nuclear volume of the oviductal epithelium of the bitch. (*Ciliated cells were not present in sufficient numbers.)

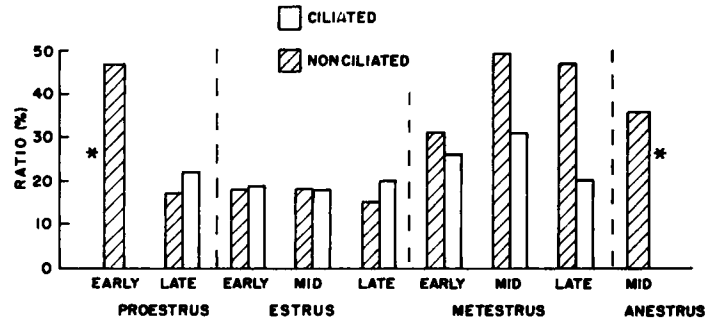


FIG. 3. Nucleocytoplasmic ratio of the epithelial cells of the oviduct of the bitch. (*Ciliated cells were not present in sufficient numbers.)

trophy is not readily apparent at the light microscopic level, it is at the ultrastructural level (Fig. 12). The nucleus is greatly enlarged, the amount of euchromatin relative to heterochromatin increases dramatically, and the nucleolus possesses increased amounts of both fibrous and granular material (Fig. 12). Changes, although not as

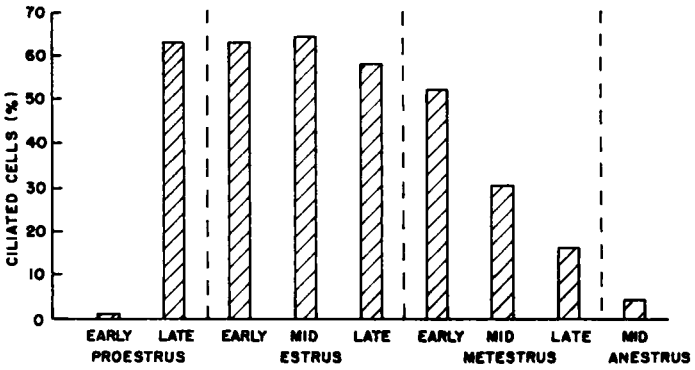
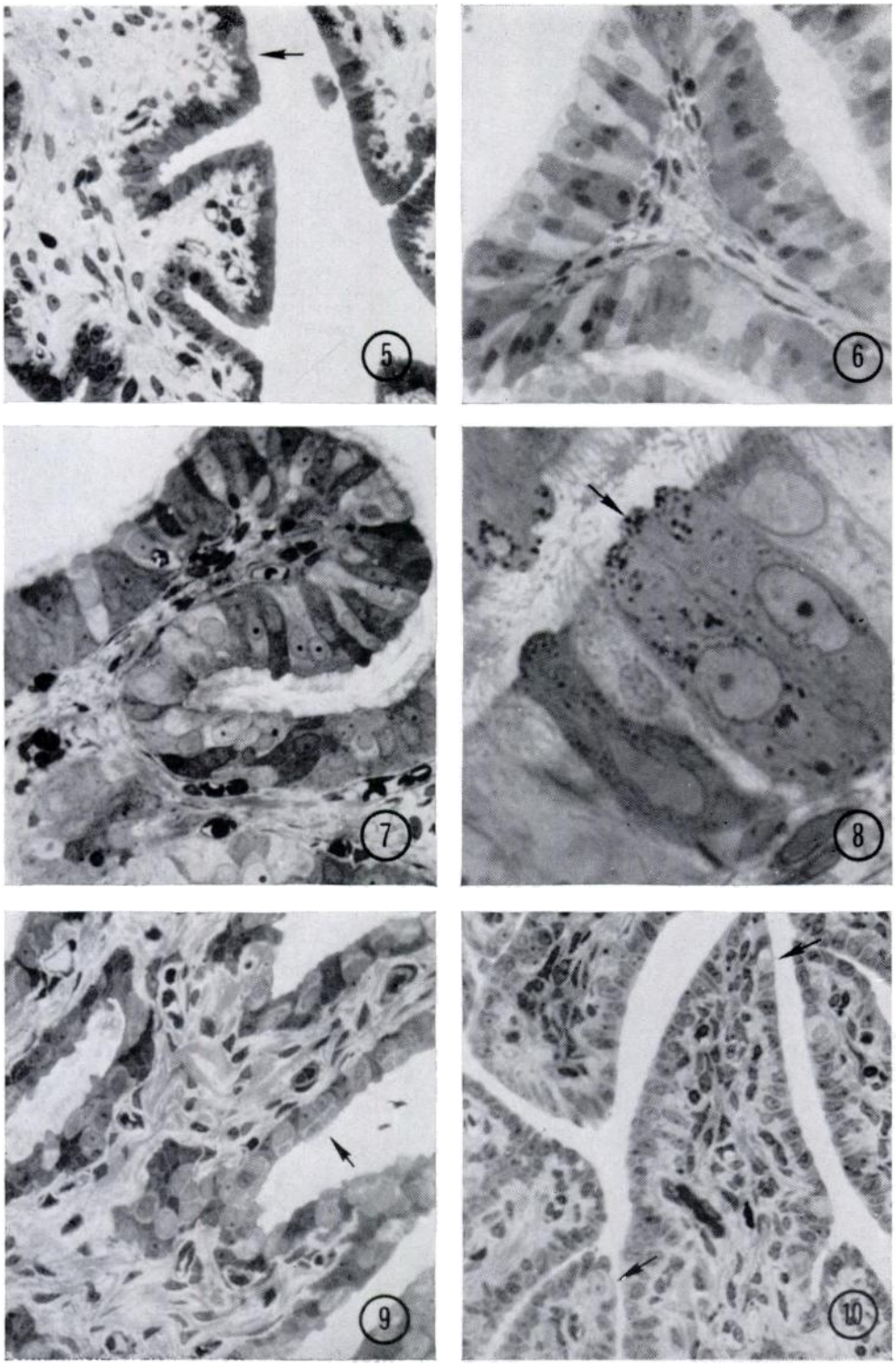


FIG. 4. Ciliated cells (%) present in the oviductal epithelium of the bitch during different stages in the estrous cycle.

dramatic, have also occurred within the cytoplasm. The lateral surfaces are less tortuous and the apical surface bulges into the lumen and possesses slightly elongated microvilli. The number of polyribosomal complexes and short profiles of RER within the cytoplasm also begins to increase. These observations suggest that cytodifferentiation begins predominantly with the nucleus and that the early nuclear events then trigger cytoplasmic differentiation.

By late proestrus the glands of the mucosal membrane are much larger and more complex and the amount of epithelium relative to stroma is much greater (Fig. 6). Hypertrophy and cytodifferentiation are very evident within the epithelial cells. Two distinct cell types are observed, a chromophobic ciliated cell which makes up 63% (Fig. 4) of the epithelial cell population and a more basophilic nonciliated cell. There has been a fourfold increase in nonciliated cell volume and a sixfold increase in ciliated cell volume (Fig. 1). In addition both cell types demonstrate a marked reduction in the nucleocytoplasmic ratio (Fig. 3). It is evident from this data that *de novo* synthesis of cilia has occurred in 63% of the epithelial cells. The details of the process of ciliogenesis in the dog oviduct will be given in the succeeding paper. These observations indicate that the ciliated cells differentiate and become mature more quickly than the nonciliated cells.

By early estrus the ciliated cells have undergone maximum hypertrophy (tenfold increase in cell volume, Fig. 1) and complete differentiation. These cells are now tall columnar, possess an apical nucleus as well as a large amount of basal cytoplasm (Fig. 7). The nucleocytoplasmic ratio has decreased to 0.19 (Fig. 3). The fine structural appearance of the ciliated cell does not appear to change throughout the first half of estrus (Figs. 13 and 14). The surfaces of the cell are now free of folds and no longer interdigitate extensively with adjoining cells. The apical border is lined by a row of basal bodies as well as numerous long, fingerlike microvilli and cilia which project into the lumen (Fig. 13). The cilia contain the typical $9 + 2$ microtubular arrangement and basal body structure as well as long, striated rootlets (Fig. 14). Associated with the ciliary apparatus in the apex of the cell are numerous mitochondria and a laterally oriented complex network of microfilaments which appear to be 50–80 Å in diam (Fig. 13). Just superior to the nucleus is a moderately large Golgi apparatus which consists of an increased number of flattened sacs and large and small vesicles. The greatly enlarged basal region of the cytoplasm now contains numerous poly-



ribosomal complexes as well as scattered mitochondria and short profiles of RER. The nucleus also reaches its maximum hypetrophy at this time (Fig. 2); however, the appearance of the nucleoplasm has not changed from that observed during late proestrus.

The secretory cells do not reach maximum hypertrophy (seven-fold increase in cell volume, Fig. 1) and cytodifferentiation until midestrus. These basophilic cells are also tall columnar; however, they are narrower and possess more basally placed nuclei (Fig. 7) than do the surrounding ciliated cells. Observations of the fine structure of the nonciliated cells during late proestrus and early estrus indicate that the amount of RER gradually increases and also becomes distended throughout the basal two-thirds of the cytoplasm. Concomitant with this increase in RER is also an increase in number and size of mitochondria and hypertrophy of the Golgi apparatus. This organelle development seems to reach its peak during midestrus at which time the RER are greatly enlarged (Fig. 15). The supranuclear Golgi apparatus forms a large cup-shaped mass consisting of numerous flattened cisternae, small vesicles and numerous large condensing vacuoles (Fig. 14). Several PAS-positive secretory granules are present at the apex of these cells (Fig. 8). The mitochondria seem to be distributed uniformly around the Golgi apparatus and among the greatly distended cisternae of RER (Figs. 14, 15). Thus, both epithelial cell types have reached full differentiation by the middle of estrus.

During the early stages of metestrus a gradual process of regression and dedifferentiation begins within the oviduct. The secretory cell volume is reduced to one-third while the ciliated cell volume is reduced to one-half of that found during estrus (Fig. 1). Even though atrophy has begun, the fine structure of these cells indicates that they are probably still active in transport and secretion (Fig. 16). The ciliated cells possess mature cilia, numerous apically distributed mitochondria and laterally oriented microfilaments just superior to the nucleus. The secretory cells possess somewhat distended cisternae, a moderately large Golgi apparatus and a few apical secretory granules. The number of mitochondria, the amount of RER and the size of the Golgi apparatus have decreased since estrus and they appear to be regressing together. In addition, it is at this time that the apex of the secretory cells begin to bulge beyond the surface of the surrounding ciliated cells. This data indicates that not only does the secretory

FIG. 5. Epithelium of the bitch oviduct during early proestrus. A few ovoid ciliated cells (arrow) are observed scattered among the undifferentiated basal cells. (Toluidine blue; 1 μm; $\times$270.)

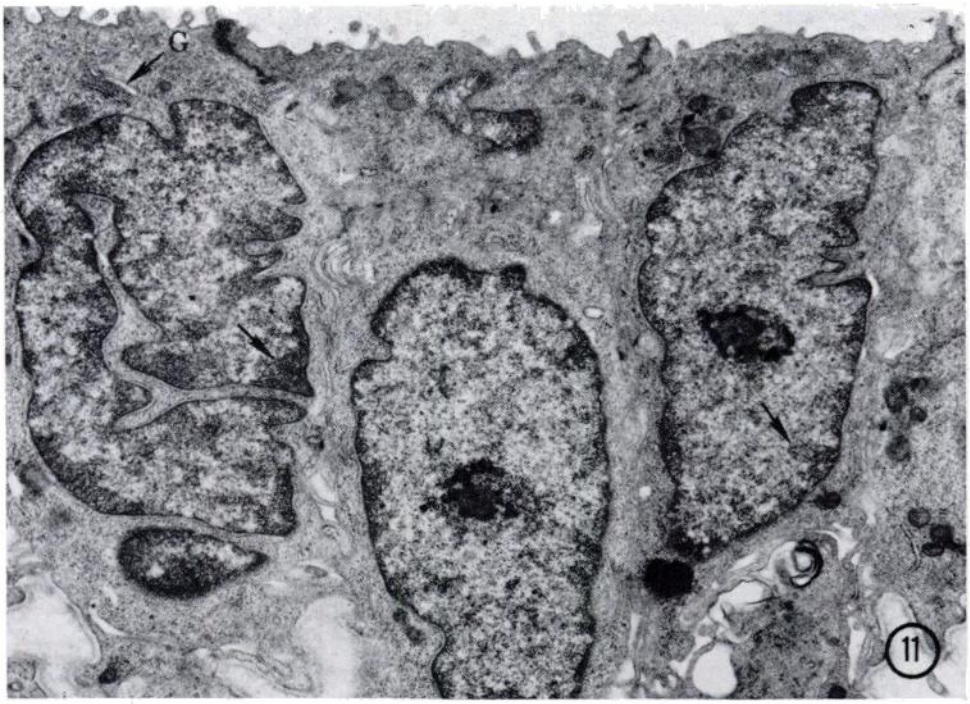
FIG. 6. Oviductal epithelium during late proestrus. Two distinct tall columnar cell types are apparent, ciliated and nonciliated. The ciliated cells are chromophobic and have a larger cell volume than the adjacent basophiles. (Toluidine blue; 1 μm; $\times$270.)

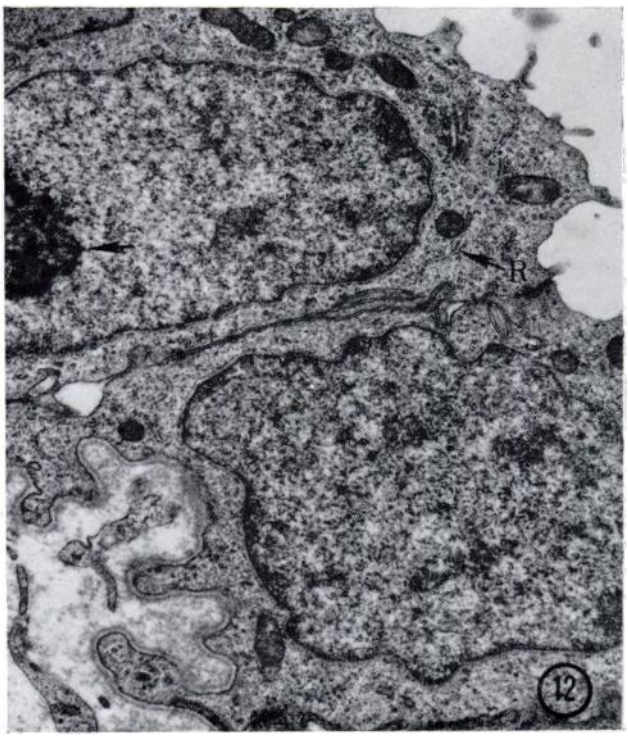
FIG. 7. Maximum hypertrophy has occurred in the epithelial cells by midestrus. The chromophobic ciliated cells have apically placed nuclei whereas the basophilic nonciliated cells have more basally placed nuclei. (Toluidine blue; 1 μm; $\times$270.)

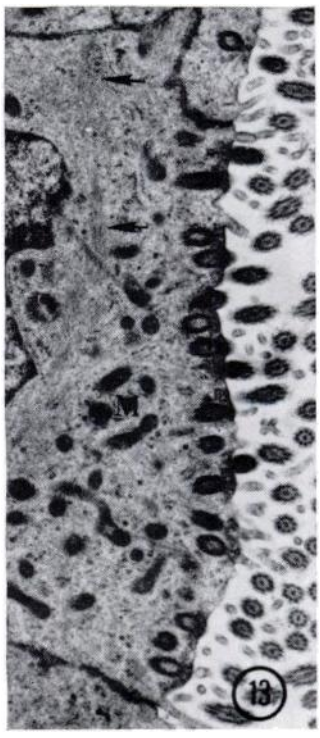
FIG. 8. Dark staining, PAS-positive, diastase resistant granules (arrow) are apparent at the apex of the nonciliated cells during estrus. (PAS; 1 μm; $\times$1050.)

FIG. 9. Atrophy has occurred in the epithelial cells by midmetestrus. The epithelium is cuboidal; the ciliated cells (arrow) possess basal nuclei and the basophilic nonciliated cells are thin and rodlike. There apparently has been a dramatic loss in the basal cytoplasm in all the epithelial cells. (Toluidine blue; 1 μm; $\times$270.)

FIG. 10. Epithelium from the oviduct of the bitch during anestrus. The cells are basically low cuboidal and similar to those of early proestrus (Fig. 5). A few ciliated cells are scattered in the epithelium. (Toluidine blue; 1 μm; $\times$270.)







cell reach maximum cytodifferentiation 2–3 days later than the ciliated cell, but it also dedifferentiates sooner than the ciliated cells.

Regressive changes are very apparent within the mucosal membrane and epithelium by midmetestrus (Fig. 9). The folds and glands of the mucosal membrane are less complex and contain more stroma. The highly basophilic cells now have a cell volume comparable to that found for the basal cells of early proestrus (Fig. 1) and make up 70% of the epithelial cell population. The remaining cells are chromophobic, ciliated and possess a cell volume two to three times that of the nonciliated cells. These cells possess a basally placed nucleus (Fig. 9), whereas during estrus the ciliated cells demonstrated an apical nucleus. The fine structure of the ciliated cells indicates that the ciliary apparatus is still intact and presumably functional. Numerous mitochondria are still apparent at the apex of the cells and the basal bodies and cilia appear intact (Fig. 18). Within the cytoplasm the polyribosomal complexes are not as numerous as those observed during estrus, the Golgi region is very small and the cytoplasm appears to be almost completely devoid of RER profiles (Fig. 18). These changes along with the dramatic reduction in basal cytoplasm indicate that the basal cytoplasm present during estrus was probably necessary for the production of microtubular and other proteins used during ciliogenesis and that once ciliogenesis has been completed the cell eliminates those cellular components needed in that process.

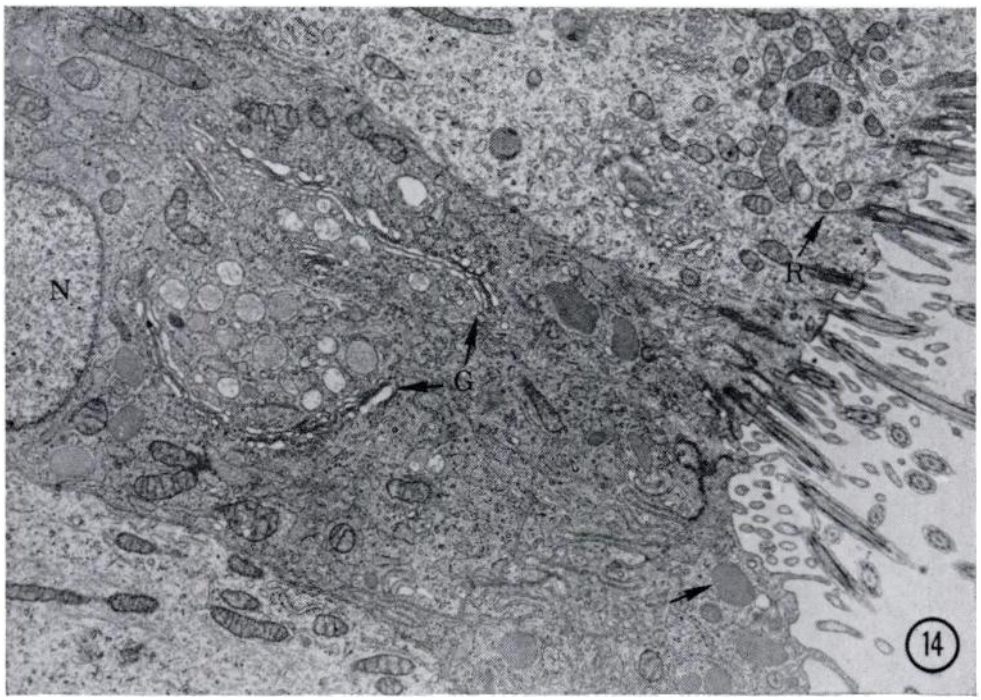
The fine structure of the nonciliated cells during midmetestrus indicates that they are remarkably similar to the basal cells observed during early proestrus. The cytoplasm displays numerous free ribosomes as well as a few scattered mitochondria and a very small Golgi apparatus. The nucleus is pleomorphic and contains a few deep folds while the nucleoplasm contains large areas of condensed chromatin (Fig. 18). The lateral surfaces, however, are much more tortuous than in the basal cells of early proestrus and form complex interdigitations with adjacent cells (Fig. 17). This suggests that membranes are more resistant to degradative processes than the cytoplasm. The epithelium during midmetestrus therefore consists of dedifferentiated secretory and ciliated cells which now are basophilic and appear very similar to the basal cells of early proestrus. In addition, approximately 30% of the epithelial cells are chromophobic, ovoid, have cilia extending from their apex and possess basal nuclei. This data indicates that regression is essentially complete in all the secretory as well as some ciliated cells, whereas regression is only partially complete in the remaining ciliated cells.

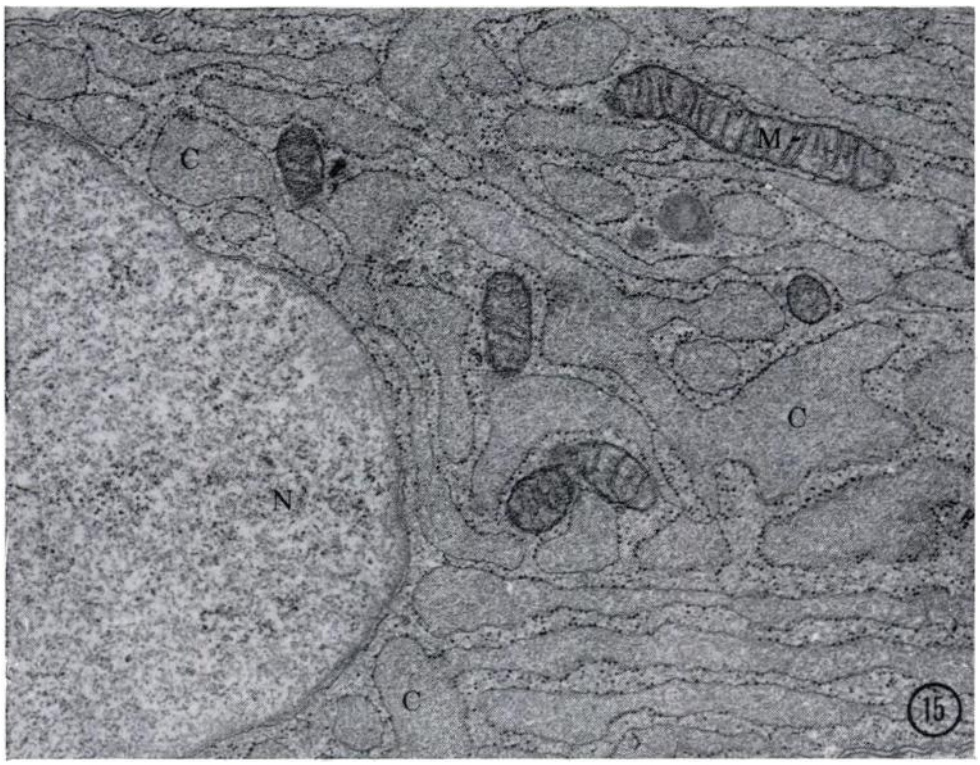
During the later stages of metestrus and all during anestrus the process of dedifferentiation continues, but at an ever declining rate. During anestrus the profile of the mucosal folds appears similar to those observed during early proestrus (Fig. 10). The number of ciliated cells decreases from 30% at midmetestrus to 16% by the end of metestrus and down to 4% by midanestrus (Fig. 4). The ciliated cells which remain

FIG. 11. Basal cells of early proestrus. They are characterized by little cytoplasm, numerous free ribosomes, a small Golgi apparatus (G), and a pleomorphic nucleus which displays large areas of condensed chromatin (arrows) (×10,000).

FIG. 12. Epithelium during midproestrus. A few short profiles of RER (R) and polyribosomal complexes are visible within the cytoplasm. The nuclei are ovoid and demonstrate mainly dispersed chromatin and an enlarged and prominent nucleolus (arrow) (×10,000).

FIG. 13. A fully differentiated ciliated cell during late proestrus. Numerous elongated microvilli and mature cilia extend into the lumen. Mitochondria (M) are abundant and a complex network of microfilaments (arrows) is present just below the row of basal bodies in the apical cytoplasm (×10,000).





throughout late metestrus and anestrus continue to have an appearance similar to those of midmetestrus (Fig. 19). The lateral surfaces of the nonciliated cells are less tortuous (Fig. 20) than those observed during midmetestrus and their apices no longer bulge beyond the surrounding ciliated cells (Fig. 19). This implies that membrane degradation does continue even after cytoplasmic degradation has been completed. The cytoplasm and nucleus appear similar to the nonciliated cells of proestrus.

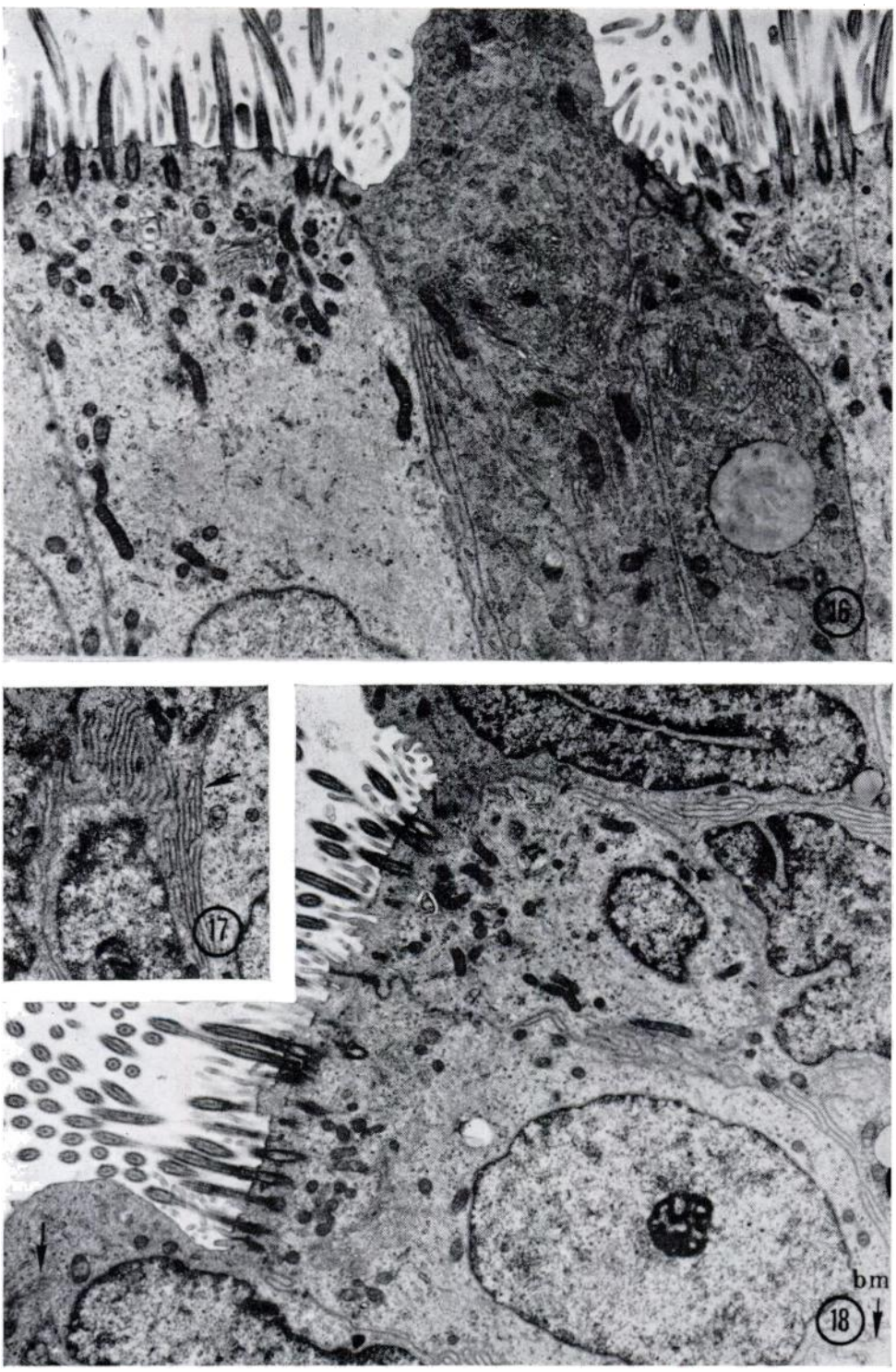
DISCUSSION

The results of this study show that the basal epithelial cells of the ampulla of the bitch oviduct undergo almost complete differentiation during the estrous cycle. The literature, however, indicates a variety of opinions and contradictions on the degree of cytodifferentiation and cycling within the mammalian oviductal epithelium. This conflict is probably due to species variation and the length of the sexual cycle. For instance, in the mouse, which has a 4-day estrous cycle, the oviductal epithelial cells undergo only minor fluctuations in cell height ('Espinase, 1935). An animal such as the rabbit which is in continuous estrus also displays only minor changes in the oviductal epithelium and those which do occur, occur primarily in the secretory cells (Borell *et al.*, 1956, 1957; Brower and Anderson, 1969). In primates, where the menstrual cycle approaches a month, the cyclical changes are more dramatic and involve the ciliated as well as the nonciliated cells. Snyder (1924) and Clyman (1966) reported that the cyclical changes observed in the human oviduct involved mainly the secretory cells and that there was no apparent change in the number of ciliated cells. However, both Brenner (1967a, 1969a) and Dabancens *et al.* (1972) have observed *de novo* synthesis of cilia in the primate oviduct. The duration of the estrous cycle of the bitch is at least six times longer than that for any other species studied to date. In addition such a complete differentiation as observed here in the bitch oviduct has not been reported previously for any normal cycling animal. The extended estrous cycle as well as species variation very likely accounts for the different degrees of cytodifferentiation and cycling observed in the mammalian oviduct. These results indicate that the bitch oviduct is an excellent model for the study of steroid induced cytodifferentiation as well as cycling behavior.

The sensitivity of the mammalian oviductal epithelium to ovarian steroids is well documented (Brenner, 1967b; McDaniel *et al.*, 1968); however, there has been no attempt to correlate plasma steroid levels with the observed morphological changes. Correlative studies on the ovarian steroids and LH levels, however, were carried out on the bitches used in this study (Spano, 1971; Spano *et al.*, 1971; Masken, 1972). Estrogen levels rise at the onset of proestrus, reach a peak early in estrus, then fall off sharply throughout the remainder of estrus. Plasma progesterone levels are low throughout proestrus, begin rising with the onset of estrus, reach a peak early in metestrus and decline gradually to a low point at the time of whelping. A plasma LH peak occurs within the onset of estrus, and ovulation occurs within a few hours after the LH peak. These data agree well with the results reported by Bell *et al.* (1971), Christie and Bell (1971), and Smith

FIG. 14. Epithelium at midestrus. The large secretory cell possesses a large well-developed Golgi apparatus (G) which is located just superior to the nucleus (N) and several secretory granules (arrow) within the apical cytoplasm. The ciliated cells possess a row of basal bodies from which well-developed cilia and striated rootlets extend (R) (×10,000).

FIG. 15. Basal region of a secretory cell at midestrus which demonstrates abundant cisternae of RER (C) as well as scattered mitochondria (M). (N, nucleus; ×21,000.)



and McDonald (1971). In the succeeding paper (Verhage *et al.*, 1973) evidence is presented that exogenous estrogen injected into prepubertal dogs resulted in functional ciliated and secretory cells. Differentiation of the ciliated cells was complete approximately 5–6 days after the first injection of estrogen whereas the secretory cells needed an additional 3–4 days. Therefore the lag period observed in the estrogen-treated prepubertal dog oviduct was approximately 6 days for ciliated cells and 10 days for the secretory cell. In the mature bitch, the elapsed time from early proestrus when plasma estrogen levels begin to rise, to early estrus when ciliated cell cytodifferentiation is complete is approximately 6 days. Maximum differentiation of the secretory cells occurred 3–4 days later. Considering the plasma estrogen levels, similar differentiation sequence in both the bitch and estrogen-treated prepubertal dog, and morphological changes observed in this study, one can only conclude that estrogen is responsible for the differentiation of the epithelial cells of the oviduct of the bitch.

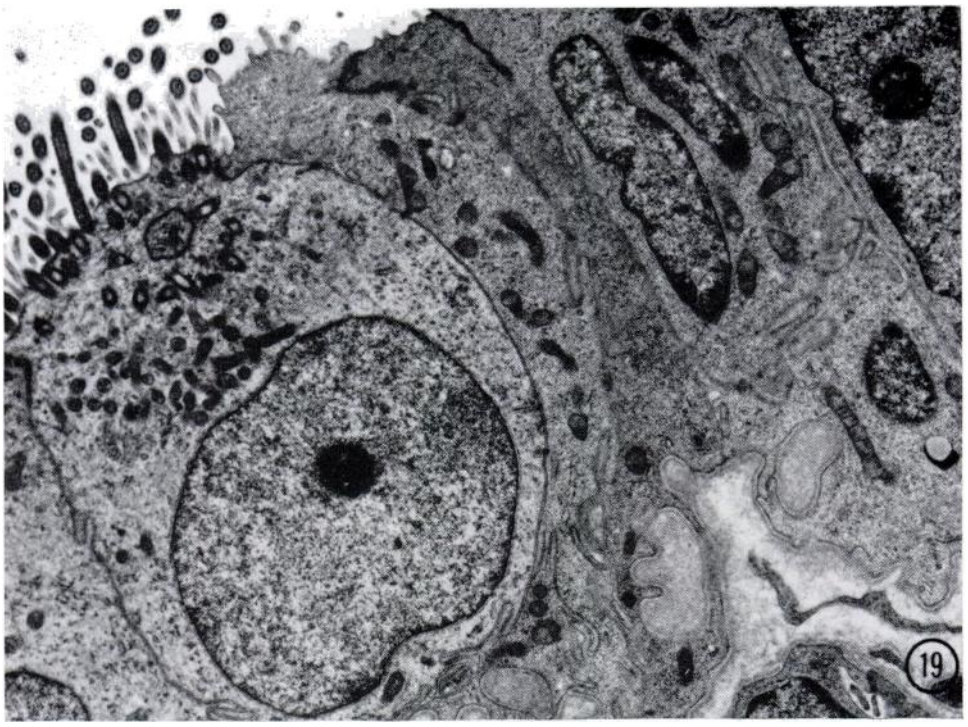
The mechanisms responsible for the dedifferentiation observed during metestrus are not as readily apparent. It could be the result of either the rapid disappearance of plasma estrogen during estrus, the rapid rise of plasma progesterone during estrus and early metestrus, or the relative amounts of the two ovarian steroids. The onset of dedifferentiation in the normal cycling bitch seems to correlate well with the surge in plasma progesterone levels during estrus and metestrus (Masken, 1972). In the succeeding paper (Verhage *et al.*, 1973) evidence is presented that progesterone may in fact be able to override estrogen's effect and result in dedifferentiation in not only the bitch but other mammals as well.

The cycling nature of the mammalian oviduct has resulted in controversies concerning epithelial cell types and the origins of these cell types. The epithelium of the mammalian oviduct has been reported to possess four distinct types of cells, namely, basal, ciliated, secretory, and peg or intercalary cells ('Espinase, 1935; Clyman, 1966. Dabancens *et al.*, 1972). The same four cell types were obseved in this study; however, it is questionable whether they are in fact distinct. During proestrus, the epithelial cells in the bitch oviduct appeared to consist entirely of basal cells which had a potential upon estrogen stimulation to develop into either ciliated or secretory cells. There was no evidence in this study for a transformation of ciliated cells into secretory cells as has been suggested by Martinek *et al.* (1967). In addition, secretory granules were not observed in ciliated cells or cilia projecting into the lumen from secretory cells as has been reported to occur in the rabbit oviduct (Nilsson, 1958; Merchant, 1969). Brower and Anderson (1969) occasionally observed a cilium extending from a secretory cell in the rabbit oviduct; however, its structure was not similar to that observed for the cilia extending from ciliated cells and thus they concluded that it was an anomaly and not evidence for transformation. Also, in this study, ciliogenesis occurred during late proestrus and by the end of proestrus 60% of the epithelial cells were ciliated. Furthermore, the number of ciliated cells re-

FIG. 16. By early metestrus the epithelium has begun to atrophy and the apex of the secretory cell bulges beyond the surface of the surrounding ciliated cells (×8500).

FIG. 17. A segment of an epithelial cell during midmetestrus showing the complex interdigitations (arrow) between adjacent cells (×8500).

FIG. 18. Epithelium during midmetestrus. The nonciliated cells possess a small Golgi apparatus (arrow), little RER and no secretory granules in the apex. The apices of the ciliated cells have remained intact; however, there has been a dramatic reduction in the basal cytoplasm as compared to estrus (Fig. 7) (bm, basement membrane; ×8500).



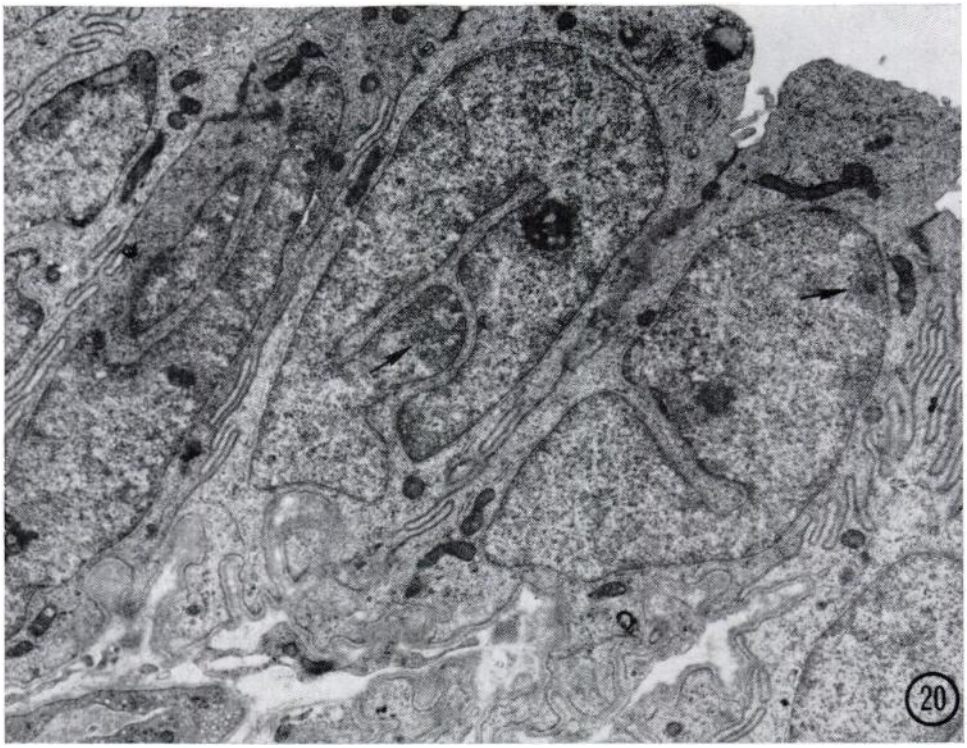


FIG. 19. Epithelium during late metestrus. The ciliated cell is ovoid, lacks basal cytoplasm but does possess an intact ciliary apparatus. The nonciliated cells are small and contain pleomorphic nuclei with large patches of condensed chromatin (×8500).

FIG. 20. Epithelial cells during anestrus. They are for all practical purposes similar to the cells of early proestrus described in Fig. 11 (arrows, condensed chromatin; ×8500).

mained constant during the period of time when the secretory cells reached maximum differentiation. During metestrus numerous ciliated cells were present at all stages whereas secretory cells were observed only during early metestrus. Thus these data indicate that in the oviduct of the bitch, the ciliated cells do not transform into secretory cells or vice versa. Although peg cells were observed during metestrus, they also appeared to be a stage in the dedifferentiation of the secretory and ciliated cells and therefore are not a specific cell type. No cellular extrusion or desquamation was observed during dedifferentiation as has been reported to occur in the bovine oviduct (McDaniel *et al.*, 1968). The results of this study indicate that during the estrous cycle of the bitch the epithelial basal cells of proestrus differentiate into either a ciliated or secretory cell and through the process of dedifferentiation return to the basal cell state by the end of anestrus.

The functional significance of the observed differentiation sequence is readily apparent. Hypertrophy and differentiation are maximal at a time when the ova are in the oviduct. Cilia are thought to function in the transport of both the ova and sperm (Pauerstein *et al.*, 1968; Gaddum-Rosse and Blandau, 1973). The oviductal secretions are thought to be necessary for both the maintenance and final maturation of both the ova and sperm (Reinius, 1970). Therefore the oviduct of the bitch seems to be an excellent model for investigation of the differentiation sequence in the epithelial cells. The reason for this is that the estrous cycle of the dog lasts from 6 to 8 mo. This is sufficient time to allow the epithelial cells not only to differentiate but also to dedifferentiate and return to a reserve or baseline state following steroid stimulation.

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