OBSERVATIONS ON THE FINE STRUCTURE OF LUTEIN CELLS

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ABSTRACT

Corpora lutea from the period of delayed implantation and from early postimplantation stages of the armadillo, mink, and rat were fixed in buffered osmium tetroxide–sucrose or potassium permanganate. After rapid dehydration, the portions of the corpora lutea were embedded in either methacrylate or epoxy resin. Examination of the lutein cells by electron microscopy revealed the presence, in the better preserved material, of an extensive development of tubular agranular endoplasmic reticulum. Although the membranes of the endoplasmic reticulum are the most striking feature of the lutein cells of both stages of the three animals examined, very numerous large mitochondria with cristae that exhibit a variety of forms tending toward villiform, and protrusions and foldings of the lutein cell margins on the pericapillary space are also characteristic of these cells. Certain minor differences in the lutein cells of the species examined are also noted. No indications of conversion of mitochondria into lipid, of accumulation of lipid in the Golgi area, or of the protrusion of lutein cells into spaces between the endothelial cells, as suggested by other authors, were noted in these preparations. Some of the difficulties inherent in the visualization of the secretory activity of cells producing steroid hormones are briefly discussed.

Early studies on lutein cells have been primarily concerned with the villiform nature of the cristae of the mitochondria (2, 23) and have not dealt extensively with the endoplasmic reticulum. However, Lever (23), in his general description of the parenchymal cells of the corpora lutea of the rat, commented on the numerous circular profiles of apparently vesicular elements within the cytoplasm. He suggested that these elements were Golgi-form sacs. Seemingly vesicular elements within the cytoplasm were also a common feature of micrographs of the adrenal cortex (24). However, later reports on steroid-producing structures noted the presence of tubular rather than vesicular elements. Muta (27) reported tubules of the endoplasmic reticulum in the interstitial tissue of the mouse ovary. Ross *et al.* (29) noted the presence of tubular agranular endoplasmic reticulum in the fetal zone of the human fetal adrenal. With the appearance of Christensen and Fawcett's (5, 6) particularly clear demonstration of an extensive network of agranular tubules of the endoplasmic reticulum in the interstitial cells of the opossum testis, it became apparent that a tubular form of endoplasmic reticulum might be a common feature of well preserved parenchymal cells of steroid-producing structures. Consequently, particular attention was paid to the preservation and interpretation of the membranes of the endoplasmic reticulum in the study of the corpus luteum reported here.

Porter and Yamada (28), in their study of the pigmented epithelium of the frog retina, pointed out the relative scarcity of information on the agranular form of the endoplasmic reticulum. Ito (19) emphasized the difficulty of preservation of agranular reticulum in gastric parietal cells of several animals. Yamada and Ishikawa (36) reported the presence of agranular reticulum in the

lutein cells of the mouse corpus luteum. These recent reports make the determination of the extent to which an agranular endoplasmic reticulum is a common feature of lutein cells particularly pertinent.

MATERIALS AND METHODS

Corpora lutea from armadillos, mink, and rats were used in this study. The highly functional corpora from the early postimplantation stages and the less studied but probably functional corpora from delayed implantation were selected. The armadillos and rats were killed by abrupt vertebral fracture and the mink by asphyxiation. The corpora lutea were removed as rapidly as feasible and placed in appropriate fixatives. The mink and rat uteri were flushed for blastocysts, and the armadillo uteri were everted, then dipped in saline solution. If neither blastocysts nor implantation sites were observed, the corpora lutea were discarded. In the rat, there are two sets of corpora present during delay of implantation; the corpora selected for study were those of the second pregnancy. These corpora were taken on the 7th to 13th day of the second pregnancy.

The portions of the corpora lutea placed in cold Caulfield's (3) osmium-sucrose mixture or Dalton's (7) chrome-osmium mixture were fixed for 1 hour. Portions of corpora lutea placed in modified Luft's (25) mixture (1.2 per cent $KMnO_4$ in 0.9 per cent NaCl) were fixed for 2 hours. Following fixation, the tissues were rinsed in cold dilute ethanol and dehydrated rapidly in increasing concentrations of ethanol. Following dehydration, the tissues were either embedded in a methacrylate mixture (3 parts butyl to 1 part methyl) or placed in propylene oxide, then embedded in Ciba 502 epoxy resin. Sections were cut with glass knives in a Porter-Blum microtome. Methacrylate-embedded sections were mounted on celloidin-coated grids and covered with evaporated carbon. Sections from epoxy-embedded material were generally placed on celloidin-coated grids without carbon. Occasionally uncoated grids were used for sections of epoxy-embedded material. Various staining methods were employed. The potassium permanganate method of Lawn (22) and Dalton and Zeigel's (8) modification of Watson's (33) lead hydroxide method proved particularly useful. Micrographs were taken of corpora from 14 armadillos (10 delay, 4 implanted), 12 rats (9 delay, 3 implanted), and 5 mink (2 delay, 3 implanted). The electron microscope used in this study was an RCA EMU-3F.[1]

[1] The purchase of this instrument was facilitated by grant RG-7206 from the National Institutes of Health, United States Public Health Service.

OBSERVATIONS

In routine paraffin preparations, the lutein cells from the corpora of the three species used in this study are characteristically large, mildly acidophilic, irregular in outline, and superficially uniform in appearance. The lutein cells of the armadillo are particularly large (36 μ maximum diameter) and show little variation in size between the delay and early implantation periods. The lutein cells of the rat are relatively small and regular in outline. By day 7 post coitum, lutein cells in the corpora of the second pregnancy of rats in lactational delay approach the size of lutein cells in corpora of rats in early implantation (15 μ in diameter). In contrast, the corpora lutea of the mink are only partially developed during the delay period. The lutein cells of even the more highly luteinized corpora are much smaller (19 μ) than the lutein cells from mink in which implantation has occurred (33 μ).

Elaboration of the Cell Margin

All lutein cells examined had elaborations of the cell membrane and adjacent cytoplasm. These elaborations vary from blunt microvillous protrusions to compound folds involving a relatively large amount of cytoplasm (Fig. 1). Irregular folds over the greater part of the cell surface are most characteristic. The elaborations thus constitute a ruffled border rather than a series of microvillous projections. Basally the folds occasionally include mitochondria, but generally only ground cytoplasm and endoplasmic reticulum are included in the folded region. In mink and armadillo lutein cells, the folds generally extend over much of the surface, while the rat lutein cells have relatively smooth surfaces between adjacent parenchymal cells. The elaborations of the cell margins face on the space surrounding the sinusoidal capillaries and are particularly highly developed in regions where there is an extensive interval between the capillaries and the parenchymal cells. The endothelial cells constituting the capillaries are separated from the pericapillary space by a diffuse basement membrane. A similar membrane is sometimes discernible on the surface of the lutein cells. Discontinuities in the endothelial cells have been observed only in micrographs of rat corpora lutea embedded in methacrylate. The areas of the greatest folding of the cell margins did not ap-

pear to be closely associated with the endothelial cells, and in no instance were portions of the lutein cells either insinuated into the capillary lumen or imbricated with the endothelial cells. The pericapillary space is somewhat limited in rat corpora lutea and is extreme in mink corpora manganate. It should be noted that in our hands permanganate fixation produced swelling (relative to the picture after the osmium-containing fixatives) of both the mitochondria and the cytoplasm. The depth of the ectoplasmic areas is greater in permanganate-fixed tissue, and the

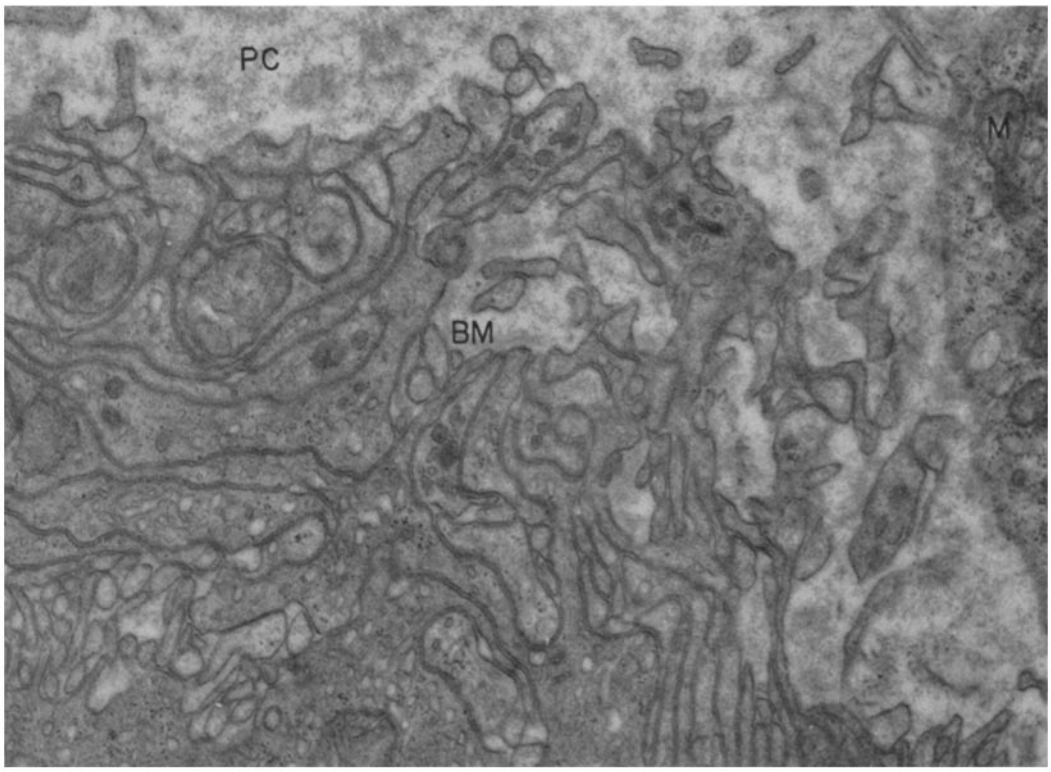


FIGURE 1

The folding of the margin of the cell is clearly depicted in this tangential section of the periphery of a lutein cell from an armadillo in an early postimplantation stage. An endothelial cell with prominant mitochondria (*M*) is at the top right of the picture. A portion of the pericapillary space (*PC*) separates the lutein cell from the endothelial cell. It is into this space that the folds of the margin protrude. The intercellular substance composes a diffuse basement membrane (*BM*) around these protrusions and the endothelial cell. Caulfield's-fixed, epoxy-embedded. × 29,000.

in late pregnancy, at which time there may be a separation of several microns between the endothelial cells and the lutein cells.

In occasional lutein cells there is a peripheral zone in which the cytoplasm is devoid of mitochondria. Only membranes of the agranular endoplasmic reticulum and surrounding ground cytoplasm can be discerned in these regions. Such ectoplasmic areas are easily detected in micrographs of tissue fixed in potassium permanganate. It should be noted that in our hands permanganate fixation produced swelling (relative to the picture after the osmium-containing fixatives) of both the mitochondria and the cytoplasm. The depth of the ectoplasmic areas is greater in permanganate-fixed tissue, and the foldings of the cell margin are more restricted and tend to be stubbier and more microvillous in nature.

Endoplasmic Reticulum

The most striking feature of micrographs of the lutein cells of the corpora lutea is the presence throughout the cytoplasm of numerous profiles of agranular, vesicular or tubular, elements of

the endoplasmic reticulum (Fig. 2). The profiles from cells fixed in Caulfield's solution and embedded in epoxy resin are of short tubular elements. These elements are most numerous in the regions rich in mitochondria, but also extend into the area of abundant Golgi membranes and, to a lesser extent, into the ectoplasmic zone when such a zone is present. The branched tubular nature of these elements can be discerned in sections where the diameter of the tubules is small (Fig. 3), and in relatively thick sections (Fig. 4). The profiles are generally short, but can frequently be identified through a series of consecutive sections, indicating that the tubules are probably long and tortuous; the latter feature makes it difficult to follow them in any single micrograph. In thicker sections the profiles are more elongate, and branching is more frequent. The latter feature suggests that the tubules are continuous, but the continuity of more than a few of these elements is difficult to establish with certainty. The tubules vary in mean diameter from a minimum of $\sim$ 35 mμ to $\sim$ 65 mμ, with little variation in any given cell. In permanganate-fixed tissue, the diameter of the tubules of the reticulum is reduced, and portions of the tubules appear collapsed. The profiles obtained in such sections are only rarely vesicular, being more commonly branched and irregular. Occasionally continuous membrane pairs are sufficiently extensive to suggest a cisternal rather than a tubular organization (Fig. 5).

The abundance of the tubules of the endoplasmic reticulum is such that they are in proximity to all the elements within the cell. They are in particularly intimate association with the mitochondria. Ribosomes are usually present in small numbers in the cytoplasmic matrix. In some instances ribosomes are also present in association with some of the membranes of the endoplasmic reticulum. When reticulum with and without ribosomes is present in the same cell, the diameter of the tubules is smaller in those areas where ribosomes are associated with the membranes.

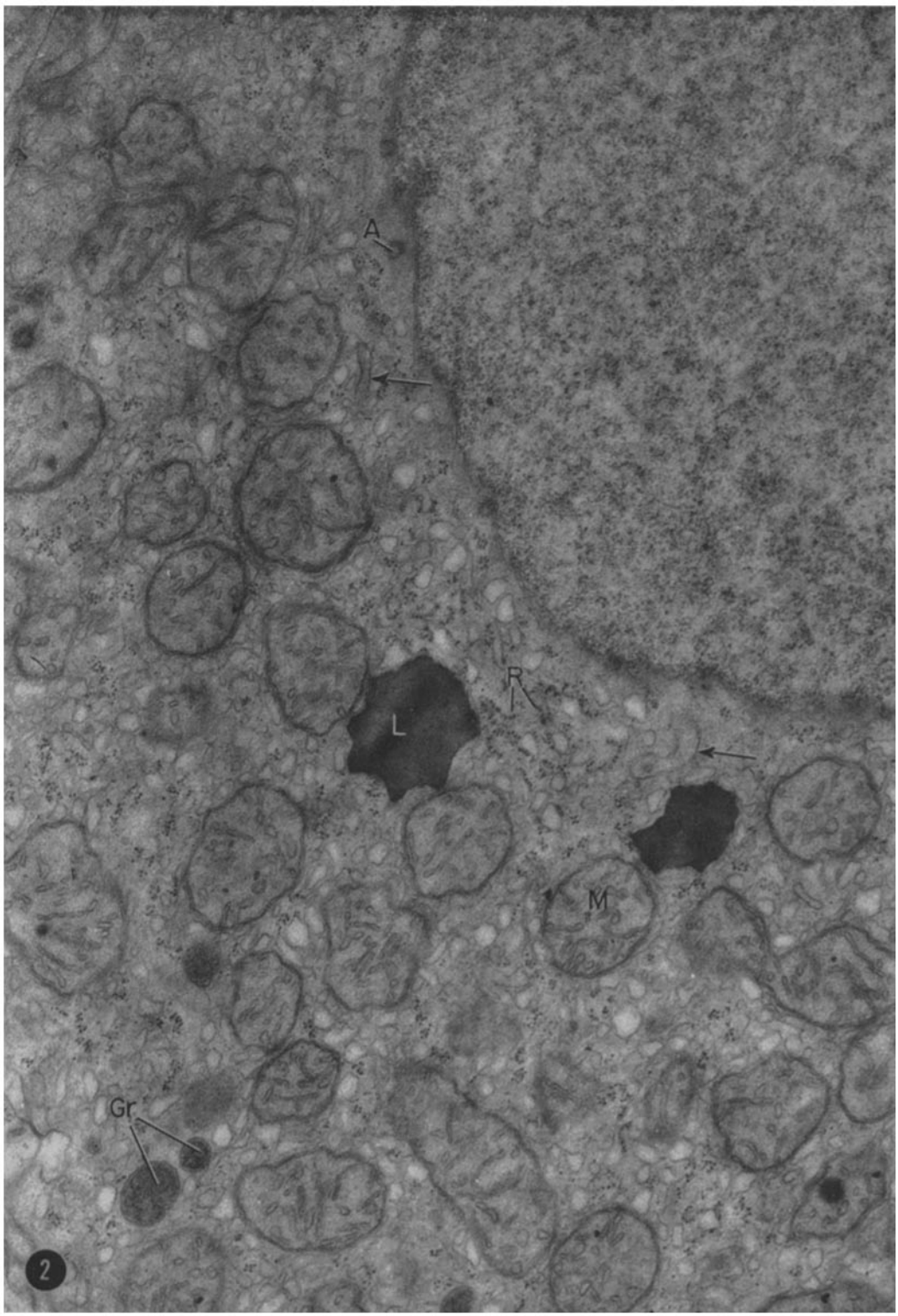
There is considerable variation in form of agranular reticulum. In lutein cells showing signs of swelling of the mitochondria, interruptions of the cell membrane, or other evidence of poor preservation, the form of the endoplasmic reticulum is invariably vesicular. In some instances adjacent cells have different forms of endoplasmic reticulum. Cells with a more dense cytoplasm tend to have tubular endoplasmic reticulum of irregular diameter and tortuous form. Cells with a less dense cytoplasm have more vesicular profiles of the endoplasmic reticulum, and frequently ribosomes are more abundant in these cells. Any swelling which occurs in fixation tends to exaggerate the variations. The tubular nature of the endoplasmic reticulum is most readily preserved in the lutein cells from the corpora lutea of mink in delayed implantation (Fig. 6). The tubules are most difficult to preserve and probably most tortuous in the lutein cells of the rat corpus luteum. Variations in morphological type of endoplasmic reticulum between the lutein cells of the same corpus are rare in mink, less rare in the armadillo and rat.

Mitochondria

Mitochondria are extremely numerous in lutein cells. They are apparently much more numerous in these cells than they are in the interstitial cells of the testis, and appear to be almost as abundant as in the cells of the adrenal cortex. In preparations viewed with the phase microscope the mitochondria appear largely granular, with relatively few elongate or rod-shaped forms. In electron micrographs the majority of mitochondria are roughly oval in outline, although the actua

FIGURE 2

The abundant agranular endoplasmic reticulum appears largely vesicular, but its tubular nature can be seen at the arrows. The nuclear annuli (*A*) are readily apparent where the nuclear envelope is obliquely sectioned. Although some of the ribosomes (*R*) are associated with membranous structures in this lutein cell from rat in delayed implantation, most of the ribosomes are free in the cytoplasm. Typical large lipid droplets (*L*) and granules (*GR*) are also seen. *M*, mitochondria; note the presence of both short and elongate profiles of the cristae. Caulfield's-fixed, epoxy-embedded, lead hydroxide-stained. × 29,000.


A
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surface may be irregular rather than describing a smooth arc. Differences in outline of individual mitochondria occur within a single lutein cell, and the diameter of these relatively large mitochondria varies from 500 mμ to 800 mμ. Occasionally stacks of disc-shaped mitochondria are present (Fig. 7). Some of these mitochondria of lutein cells is more dense than is the ground cytoplasm of these cells. Intramitochondrial granules are frequently present. These granules are considerably less dense than the frank lipid droplets within the lutein cells. No particular association between the lipid droplets in the cytoplasm and the mitochondria is apparent.

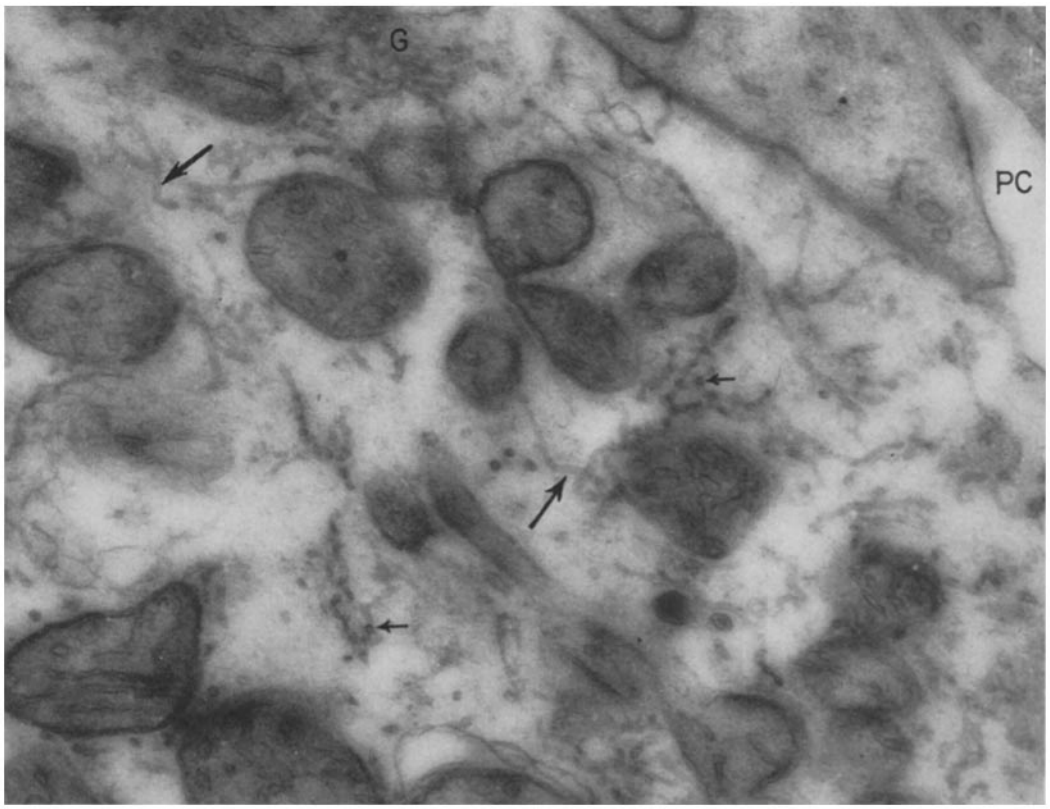


FIGURE 3

The tubules of the agranular endoplasmic reticulum are relatively slender in this micrograph of a lutein cell from an armadillo in the period of delayed implantation. The large arrows point to long profiles of the tubules, the small arrows to cross-sections of these tubules. A small portion of the pericapillary space (*PC*) is in the upper right of the picture. Face views of fenestrated cisternae of the Golgi complex (*G*). Caulfield's-fixed, epoxy-embedded. × 36,000.

are curved, having one convex and one concave surface, and approach the form described by Christensen and Chapman (4) as "cup-shaped."

The cristae mitochondriales are tubular and villiform, and frequently describe a tortuous path within the mitochondria. Occasional mitochondria have lamelliform cristae, and in a few lutein cells mitochondria with concentric lamelliform cristae are found. The matrix of the mitochondria Neither are there any indications of intermediate forms between mitochondria and lipid droplets. Frequently the mitochondria are in close association with the tubular elements of the endoplasmic reticulum. In permanganate-fixed preparations short sections of the endoplasmic reticulum of rather small diameter may parallel the surface of individual mitochondria.

Consistent differences between the mitochondria

of the three species are apparent. The mitochondria of rat lutein cells are the most uniformly oval in outline and have the greatest number of intramitochondrial granules. Mitochondria from armadillo and mink lutein cells show greater variation in outline, type of cristae, and density

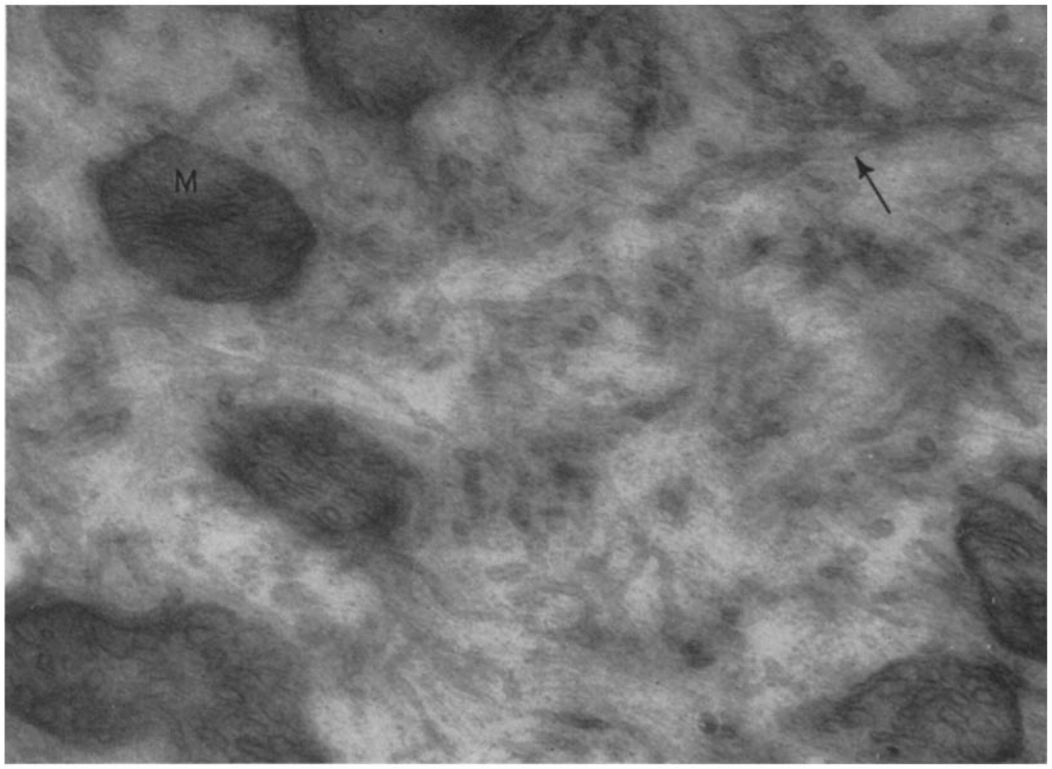


FIGURE 4

A relatively thick section of a portion of a lutein cell of an armadillo in the period of delayed implantation. The tubular meshwork formed by the agranular endoplasmic reticulum fills the cytoplasm. Note the elongate profiles at the arrow. *M*, mitochondria. Caulfield's-fixed, epoxy-embedded. × 61,000.

of the matrix. Lamelliform cristae are most commonly encountered in the armadillo lutein cells.

Golgi Complex

The Golgi complex of lutein cells is characterized by the presence of stacked membranes and a relative paucity of vesicles. Up to three or four membrane pairs are usually present in one unit. The stacked membranes of the Golgi are readily distinguished from the vesicular and tubular components of the endoplasmic reticulum (Fig. 6). Lutein cells from the armadillo have an unusually extensive, distinct Golgi region. Within this region, which may be as large as 12 μ in diameter, are numerous Golgi membranes and membranes of the endoplasmic reticulum, but mitochondria are largely absent (Fig. 8). The paucity of mitochondria has been observed with the light microscope (15). In the rat and mink lutein cell, the Golgi complex is relatively small and does not constitute a separate region. Recently Davis and Enders (9) used the position of the Golgi complex as an indication of polarity of parenchymal cells in the parathyroid. There is no common polarity of cells within the corpus luteum by this criterion.

Cytoplasmic Inclusions and Nucleus

Frank lipid droplets are a common feature of the cytoplasm of lutein cells. Except for occa-

sional cells, these droplets are not numerous. The rat has more lipid droplets than either of the other two animals. In the armadillo, large laminar myelin bodies are occasionally observed. These bodies are frequently sufficiently large to be well within the range of the light microscope. With the light microscope, myelin bodies are seen to contain phospholipid as determined by

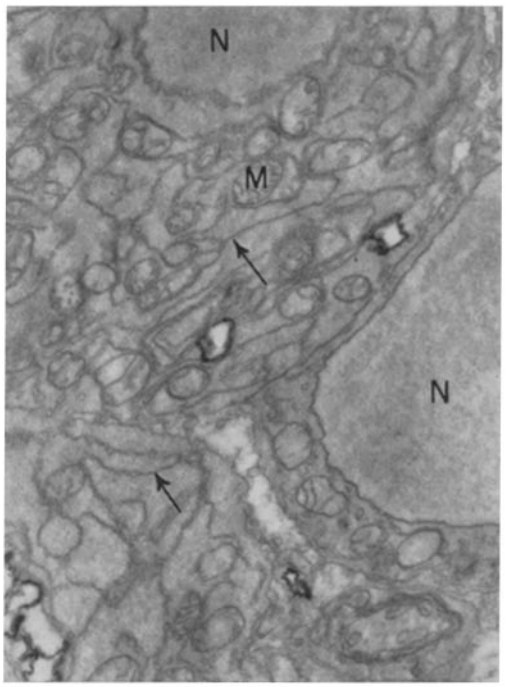


FIGURE 5

The profiles of the endoplasmic reticulum (arrows) can be followed for relatively long distances in this low magnification micrograph, indicating that the endoplasmic reticulum may be more cisternal than tubular in these cells. *N*, nucleus; *M*, mitochondria. Armadillo lutein cells, potassium permanganate–fixed, epoxy-embedded. × 11,000.

the Elftman method (14). In lutein cells from all three animals, inclusions with a granular matrix and a distinct limiting membrane are present. These granules are not so dense as the lipid droplets and have a more limited size range. The granules range in size from ~ 200 mμ to ~ 375 mμ and, although they are almost as common as lipid droplets, they are never numerous nor are they concentrated in any particular portion of the cell. A conspicuous feature of micrographs of Caulfield's-fixed material is the presence of numerous nuclear annuli similar to those described by Watson (34). The outer diameter of these annuli ranges consistently between 80 mμ and 90 mμ. However, in micrographs of permanganate-fixed materials, annuli are absent but nuclear pores are found.

DISCUSSION

Since agranular endoplasmic reticulum in the tubular form is present in all the better preserved corpora, and the vesicular form is present in sections with such indications of poor fixation as swollen mitochondria and interrupted membranes, it is probable that the tubular form of agranular endoplasmic reticulum is a normal component of the lutein cells of the animals examined. Ito (19) has observed that the agranular reticulum is more difficult to preserve than the reticulum with associated ribosomes. He was, nevertheless, able to demonstrate tubular reticulum in parietal cells, using buffered osmium tetroxide fixative and methacrylate embedding. A similar method was used by Porter and Yamada (28) in demonstrating the reticulum in cells of the pigment layer of the retina of the frog. Christensen and Fawcett (6) used methacrylate-embedded material to demonstrate the reticulum in opossum testicular interstitial cells, but used a chromate-dichromate–buffered osmium tetroxide fixative. In the study reported here, both the use of permanganate as a fixative and the use of epoxy resin as an embedding medium tended to improve retention of tubular organization. Perhaps the widespread use of rapid dehydration which has occurred in recent years is as important (or more important) to the preservation of these easily damaged membranous structures as the fixative and embedding medium.

The observation of tubular agranular reticulum in corpora lutea from the armadillo, mink, and rat, species in three widely different orders, and the similar observation of reticulum by Yamada and Ishikawa (36) in the corpora lutea of the mouse suggest that this form of reticulum is common to lutein cells and has some special functional significance. A folded irregular cell margin and numerous mitochondria with tubular villiform cristae are also characteristic of lutein cells. The corpora lutea from animals with implanted blastocysts were presumably active, since in all instances animals in the first half of pregnancy only were used. Experimental produc-

tion of implantation in the rat by administration of exogenous estrogen (21, 31, 35) during delayed implantation indicates that the corpora lutea are functional during this period. Assay of armadillo blood by the Hooker-Forbes method (32) indicates that in this species also the corpus luteum is functional during the delay period.

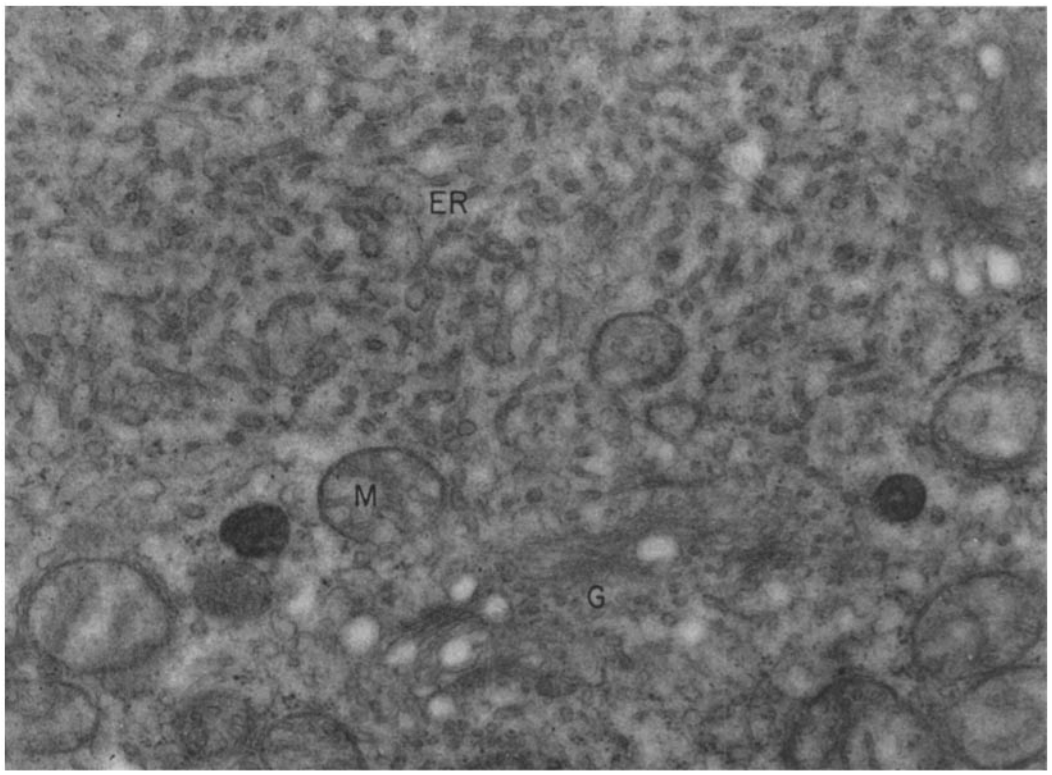


FIGURE 6

The tubules of the agranular endoplasmic reticulum are apparently quite tortuous, as indicated by the relatively short profiles seen in this micrograph of a mink lutein cell from the period of delayed implantation. The Golgi membranes (*G*) are readily distinguished from the agranular endoplasmic reticulum (*ER*). *M*, Mitochondria. Dalton's-fixed, methacrylate-embedded. × 37,000.

Mink corpora, however, are only partially luteinized prior to implantation (16), and whether they are functional or not during the delay period remains to be established. It appears, therefore, that with one possible exception (mink in delay) the corpora lutea examined in this study were in a functional state.

Structural criteria of functional activity have always been difficult to discern with regard to the corpus luteum (13). In order to determine whether any or all of the common features observed in the lutein cells studied are necessarily associated with functional activity in the cell, or whether their establishment is a part of the process of luteinization and is not necessarily associated with functional activity, it would be necessary to separate these two features. This separation could be accomplished by examining corpora lutea in hypophysectomized rats in the presence or absence of lactogenic hormone, since the rat corpus luteum is dependent on the presence of LTH for functional activity but not for luteinization (1).

Porter and Yamada (28) have recently emphasized that agranular endoplasmic reticulum is generally present in cells synthesizing lipid. Christensen and Fawcett (6) reviewed the information concerning participation of the microsomal fraction in the synthesis of steroids. Although

the precise pathway has been only partially established, the implication of the agranular reticulum in the synthesis of testosterone is particularly strong. Biochemical and cytological evidence concerning the synthesis of adrenal steroids (30) and progesterone (18) is somewhat more equivocal. The preponderance of agranular

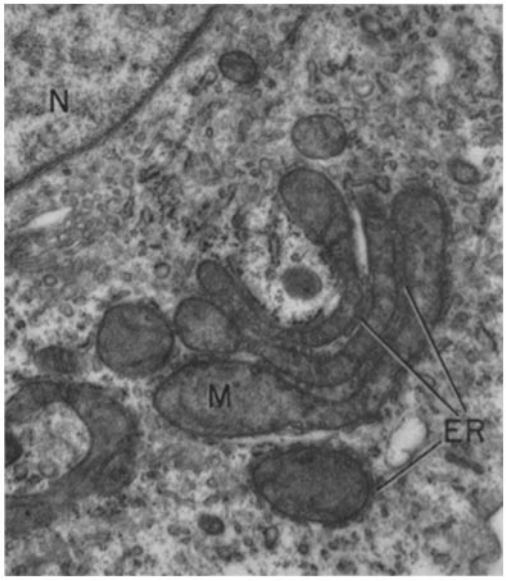


FIGURE 7

A stack of "cup-shaped" mitochondria (*M*) are seen in this lutein cell from a postimplantation mink. Note the close association of some of the membranes of the endoplasmic reticulum (ER) with the mitochondria. *N*, nucleus. Caulfield's-fixed, methacrylate-embedded, lead hydroxide–stained. × 23,000.

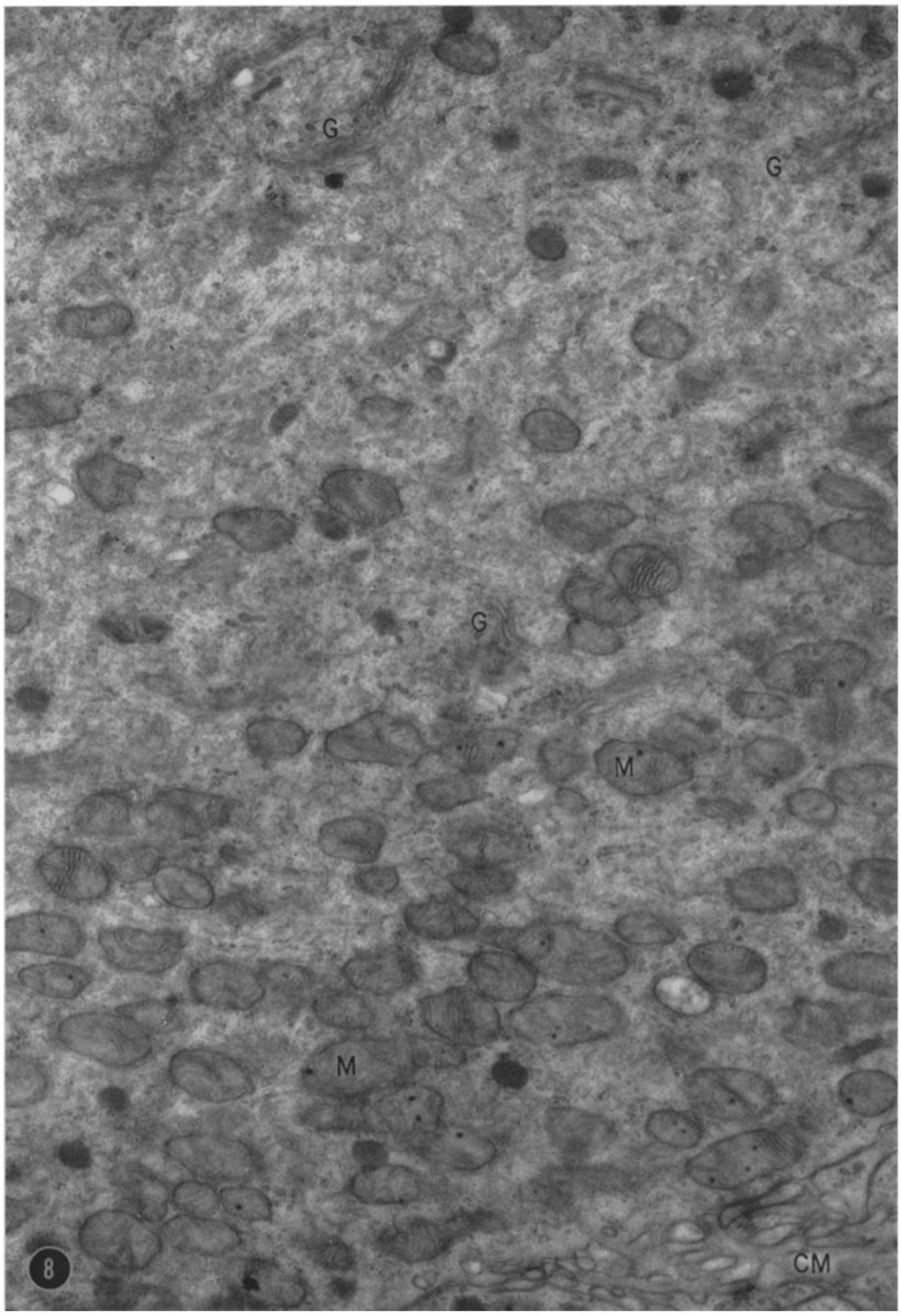
endoplasmic reticulum within the lutein cells of the corpora lutea and the relatively large percentage of parenchymal cells in an individual corpus should make this tissue particularly useful for a study of the synthetic activities of the agranular reticulum.

Everett (17) showed that the storage of cholesterol in lutein cells of the rat is apparently related to the LH and LTH ratios. Studies by Deane (11) on the ovary of the cyclic rat and by Dawson and Velardo (10) on the ovary of the pseudopregnant rat demonstrated the difficulty in relating the amount of stored lipid to secretion of progesterone. Karnovsky and Deane (20) discussed the difficulties inherent in histochemical identification of lipids after formalin fixation. In her summary article on intracellular lipids, Deane (12) draws attention to the following: (*a*) droplets visualized in steroid cells probably represent "stores of potential precursor materials that may be converted into steroid hormones when the proper stimulus occurs"; (*b*) to assess cell activity, primary emphasis must be placed on cell droplet size; and (*c*) "the corpus luteum frequently fails to display visible droplets when it is apparently secreting most rapidly."

The resolution of lipid droplets of less than 200 mμ by the electron microscope has as yet added little to our understanding of the relation of these droplets to secretory activity. As previously mentioned, lipid droplets are abundant in only a few cells in armadillo and mink corpora lutea. In these cells and in lutein cells of rat corpora lutea in which lipid is more abundant, the droplets are frequently large (0.1 to 0.4 μ) and probably represent stores of potential precursor material. However, little of the chemical nature of these droplets can be determined by electron microscopy, with the possible exception of the extent of unsaturation (26). Furthermore, most small lipid droplets are found scattered throughout the cytoplasm without particular association with any of the organelles or areas. No indications of conversion of mitochondria to lipid, as suggested by Lever (23), have been observed. Nor was there any evidence, in the armadillo, mink, or rat, for apocrine secretion by protrusion of the lutein cells into the capillary lumina, as suggested by Yamada and Ishikawa (36) in their report on the mouse corpus luteum. The observed separation of the lutein cells from the capillaries makes such a secretory mechanism highly im-

FIGURE 8

Numerous Golgi membranes (*G*) are present throughout the Golgi region in this portion of a lutein cell from an armadillo in early postimplantation. Mitochondria (*M*) are relatively scarce in this region, however, and are more common toward the periphery of the cell (bottom of picture). The folding of the cell margin (*CM*) is clearly seen in the lower right corner. Caulfield's-fixed, epoxy-embedded. × 17,500.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probable. It appears that more information is necessary before a meaningful secretory pattern for lutein cells can be postulated.

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