OBSERVATIONS ON THE FINE STRUCTURE OF LUTEIN CELLS

II. The Effects of Hypophysectomy and Mammotrophic Hormone in the Rat

ALLEN C. ENDERS, Ph.D., and W. R. LYONS, M.D.

From the Department of Anatomy, Washington University, School of Medicine, St. Louis, and the Department of Anatomy, University of California School of Medicine, San Francisco

ABSTRACT

Corpus luteum formation was induced in 26-day-old rats which were subsequently hypophysectomized and injected with mammotrophic hormone (MH, LTH). Sections of corpora lutea from these animals were examined with the electron microscope and compared with similarly prepared (Caulfield's fixed, Araldite embedded) corpora from normal pregnancy and from controls, the latter consisting of corpora prior to hypophysectomy and corpora from uninjected rats 7 to 14 days after hypophysectomy. Lutein cells from corpora lutea of injected animals and of normal pregnancy are characterized by abundant, tortuous, tubular agranular endoplasmic reticulum and by mitochondria, many of which are disc-shaped with dense matrices and both villiform and lamelliform cristae. The endoplasmic reticulum is most abundant in lutein cells from pregnant animals, in which cells it is in the form of thin, highly tortuous tubules. The form of the lipid droplets seen in cells of stimulated animals varies greatly. Marginal foldings of the lutein cells on the perivascular space were found in all instances. Lutein cells from hypophysectomized animals have a less highly developed agranular endoplasmic reticulum. The mitochondria have irregular outlines and a relatively lucid matrix. The lipid droplets in these cells show less tendency to be extracted, but are not so large or abundant as in the cells of onset controls. Granules believed to contain lipid pigments are common in the lutein cells of these control animals. It is suggested that lutein cells from corpora lutea which are actively secreting progesterone may be readily distinguished from lutein cells from non-active corpora by means of the multiple characteristics enumerated. It is further suggested that mammotrophic hormone has a general effect on the metabolism of lutein cells rather than solely affecting a specific organelle, the abundance or composition of which may be the limiting factor in the production of progesterone.

In a previous paper active lutein cells from three species were studied. It was found that these lutein cells were characterized by the presence of marginal folding on the perivascular space, extensive agranular endoplasmic reticulum, and abundant mitochondria with numerous villiform cristae. It is the intention of the present study to examine the structure of inactive as well as active corpora lutea.

Since the early studies on a corpus luteum–stimulating factor (2, 13, 15) and the dependence of the corpus luteum of the rat on this luteotrophic factor (1, 14), the function of mammotrophic

hormone (MH) (prolactin, LTH)[1] in stimulating the corpora lutea to secrete progesterone has been repeatedly documented. Evans, Simpson, Lyons, and Turpeinen (14) used the deciduoma reaction as a means of detecting progesterone secretion in hypophysectomized rats in which the corpora lutea had been stimulated by injections of MH. Recently, Rennels (24) also used the deciduoma test in hypophysectomized animals in correlating the cytology of pituitary transplants with the luteotrophic activity of such transplants. Direct observation of the corpora lutea has also been used to detect luteotrophic activity. Browning, Larke, and White (5, 6) have used the vascularity of the corpora lutea of intra-ocular transplants as a means of assaying luteotrophic activity. The well known variation in lutein cell dimension between MH-stimulated and unstimulated animals (16, 17) has been used recently by Wolthuis (29) as the basis of a sensitive quantitative test for luteotrophic activity. He found an almost linear inverse relationship between the number of lutein cell nuclei per unit area and the logarithm of the dose of MH.

In this study, corpora lutea from hypophysectomized animals in the presence (physiologically active) or absence (morphologically distinct, but physiologically inactive) of MH have been examined using the induction of deciduomata as an indication of the presence of progesterone secretion.

MATERIALS AND METHODS

Long-Evans rats were injected subcutaneously with 5 IU of PMS[2] on day 26, to induce corpus luteum formation. On day 30, these animals were hypophysectomized and subcutaneously injected daily with MH for 7 or 14 consecutive days. Three days prior to necropsy, the left ovary was inspected for the presence of newly formed corpora lutea, and a thread was inserted into and out of the lumen of a short length of the proximal left uterine horn. Some animals were killed at the time of hypophysectomy to determine the condition of the corpora at the initiation of the experiment (onset controls). Other animals were killed 7 and 14 days after hypophysectomy and used as non-stimulated controls (terminal controls). The types and groupings of the animals are listed in Table I.

At necropsy, the animals were examined for the presence of corpora, the absence of pituitary fragments, and the presence or absence of a gross decidual reaction. Corpora and portions of the uterus were fixed in Grollman's and Lascano's fluids for examination with the light microscope. Individual corpora

TABLE I

The Effects of Ovine Mammotrophin (MH) on Corpora lutea and Uterus in Rats Injected with 5 IU of Equine Gonadotrophin

All rats except the onset controls were hypophysectomized.

No. of rats	Regimen	Average body wt. change	Corpora lutea microscopic	Deciduoma gross	Right uterus microscopic
6	Onset control		New		
3	7-day terminal control	−2.3	−	−	−
3	7-day terminal control	+1.67	−	0	−
3	14-day terminal control	−1.3	−	0	2 − 1 0
2	75 μg MH, 7 days	−1.0	(±)	(±)*	+
1	75 μg MH, 7 days	0.0	−	−	(±)
1	2 mg MH, 7 days	+4.0	+	(−)*	+
5	2 mg MH, 7 days	+4.6	+	+	+
3	2 mg MH, 14 days	+4.0	+	+	+

Explanation of symbols: 0, not examined; −, no evidence of stimulation; +, positive evidence of stimulation.

* Microscopically barely +.

[1] The term "mammotrophic hormone" (MH) is used here because the mammotrophic effect of this substance is more widespread throughout the class Mammalia than is the luteotrophic effect of this substance which we are studying in this paper. LTH, luteotrophic hormone.

[2] PMS, pregnant mare's serum gonadotrophin.

and halved corpora were fixed in Caulfield's (8) fluid for 1 hour, dehydrated rapidly in cold ethyl alcohol, and embedded in Araldite 502 epoxy resin. Sections of this material were stained with the lead-staining method of Dalton and Zeigel (10) or of Reynolds (25), and examined with an RCA-EMU 3F electron microscope.

Corpora from 3 of the animals receiving MH for 7 days were fixed in Grollman's fixative for 24 hours, and then examined with the electron microscope to determine the presence of lipids which might have been extracted during normal fixation. Corpora lutea fixed in 1.2 per cent potassium permanganate were also examined for lipid droplets. In addition to the corpora from experimental animals, corpora from 3 animals of the Long-Evans strain in normal pregnancy (day 11) and 3 in lactation (day 7) were also fixed in Caulfield's fluid for examination.

DESCRIPTION

Lutein Cells from Stimulated Animals

Characteristic of the lutein cells of stimulated corpora lutea is their relatively large size and uniform character compared to the lutein cells in corpora of onset and terminal controls. The lutein cells from animals given 2 mg MH per day for 14 days were appreciably larger than the lutein cells from animals given 2 mg MH for 7 days. Lutein cells from either of these groups were not so large as the cells from normal pregnancy (corpora lutea from day 11 of normal pregnancy were used for comparison in this series).

A number of features are common to the lutein cells of corpora both from pregnant and from lactating animals, and also from hypophysectomized animals injected with MH. In general, the cell margins of adjacent lutein cells are closely apposed, relatively smooth, and lack desmosomes or other junctional complexes. Irregular foldings and protrusions of the margins of the cells on the perivascular space provide an increased cell surface in these regions. The extent of the folding, however, is quite variable.

The mitochondria of stimulated forms are large and numerous, with a matrix which is of greater density than the background cytoplasm. The external form of the mitochondria varies from round to concavoconvex discs, which vary from lenticular to dumb-bell–shaped in cross-sectional outline. Disc forms with an attenuated central region are particularly characteristic of the lutein cells of active corpora lutea, but constitute less than half of the chondriome even in these cells. The cristae of the mitochondria of active lutein cells are numerous; many of them are villiform, but lamelliform cristae are also present. Stacks of closely apposed lamelliform cristae are fairly common. No particular orientation of the cristae with regard to the long axis of the mitochondria can be determined, except that in regions of attenuation the cristae are usually situated parallel to one another and to the surface of the mitochondrion.

The Golgi regions of rat lutein cells tend to surround the nucleus rather than occupy one pole of the cell. The cisternae enclosed by the Golgi membranes are usually mildly dilated in stimulated cells. No dense material is visualized within the cisternal elements. The Golgi vesicles are distributed in the surrounding cytoplasm, and the total extent of this element is frequently difficult to determine, in part because of the nature of the endoplasmic reticulum.

Probably the most striking feature of lutein cells is the abundance of agranular endoplasmic reticulum (Fig. 1). For the most part, this organelle is in the form of highly tortuous tubular elements with numerous dilations. In some instances, the element appears more vesicular in individual sections. Apparently, the tubules are both highly tortuous and irregular in shape. Consequently, the internal dimensions of individual portions of the endoplasmic reticulum vary considerably, but between definite limits. The abundance of agranular endoplasmic reticulum is such that the intratubular space frequently appears to be nearly as large as that occupied by the ground cytoplasm. Ribosomes, most of which are not associated with the endoplasmic reticulum, are surprisingly abundant throughout the cytoplasm. One or more regions in which the ribosomes are associated with the endoplasmic reticulum are frequently seen in cross-sections of entire cells. The elements of the endoplasmic reticulum are often in parallel cisternal array in these regions.

The lipid droplets found in active lutein cells show considerable variation. In all of our preparations, the lipid droplets show indications of having been partially extracted.

Differences among Active Lutein Cells

Aside from the size characteristics already mentioned, the differences between pregnant, lactating, and hypophysectomized MH-injected animals were most apparent in the nature of the

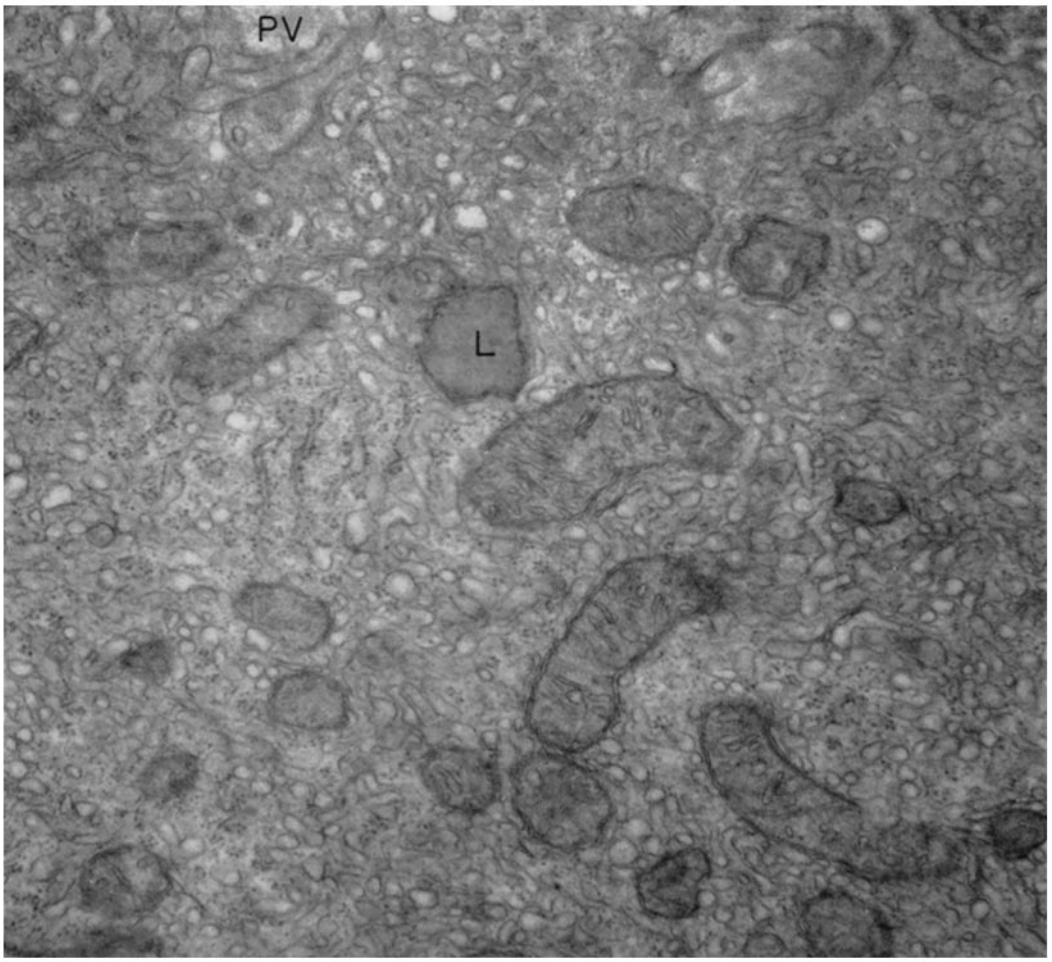


FIGURE 1 Peripheral region of a lutein cell of a rat on day 11 of normal pregnancy. Note the granular endoplasmic reticulum in the middle of the picture, and the tubular agranular endoplasmic reticulum ramifying throughout the rest of the cell. *PV*, perivascular space; *L*, lipid. The tissue in this and all subsequent micrographs was fixed in buffered osmium tetroxide plus sucrose and embedded in Araldite 502. × 28,500.

lipid inclusions. Hypophysectomized animals given MH for 7 days have numerous lipid droplets in the lutein cells (Fig. 2). In many of the cells, the lipid is highly extracted, giving a lacy appearance to the cytoplasm seen in cross-section (Fig. 3). Although little more than a limiting membrane remains to indicate the previous presence of lipid in the Caulfield's fixed material, a thin but dense strip in permanganate-fixed material and the presence of numerous large, unextracted lipid droplets in material fixed in Grollman's for 24 hours make it apparent that these areas are the remains of extracted lipid droplets. In other cells of the MH-injected animals, only the central region of the lipid droplets is extracted. Although some areas of lacy extracted lipid droplets are present in the lutein cells of animals receiving MH for 14 days, most cells have smaller, less extracted lipid droplets. In the lutein cells of pregnant animals the lipid droplets are not numerous and show relatively less tendency to be extracted.

The endoplasmic reticulum shows the greatest regularity in diameter (uniformly small), and occupies relatively the most space in the lutein cells

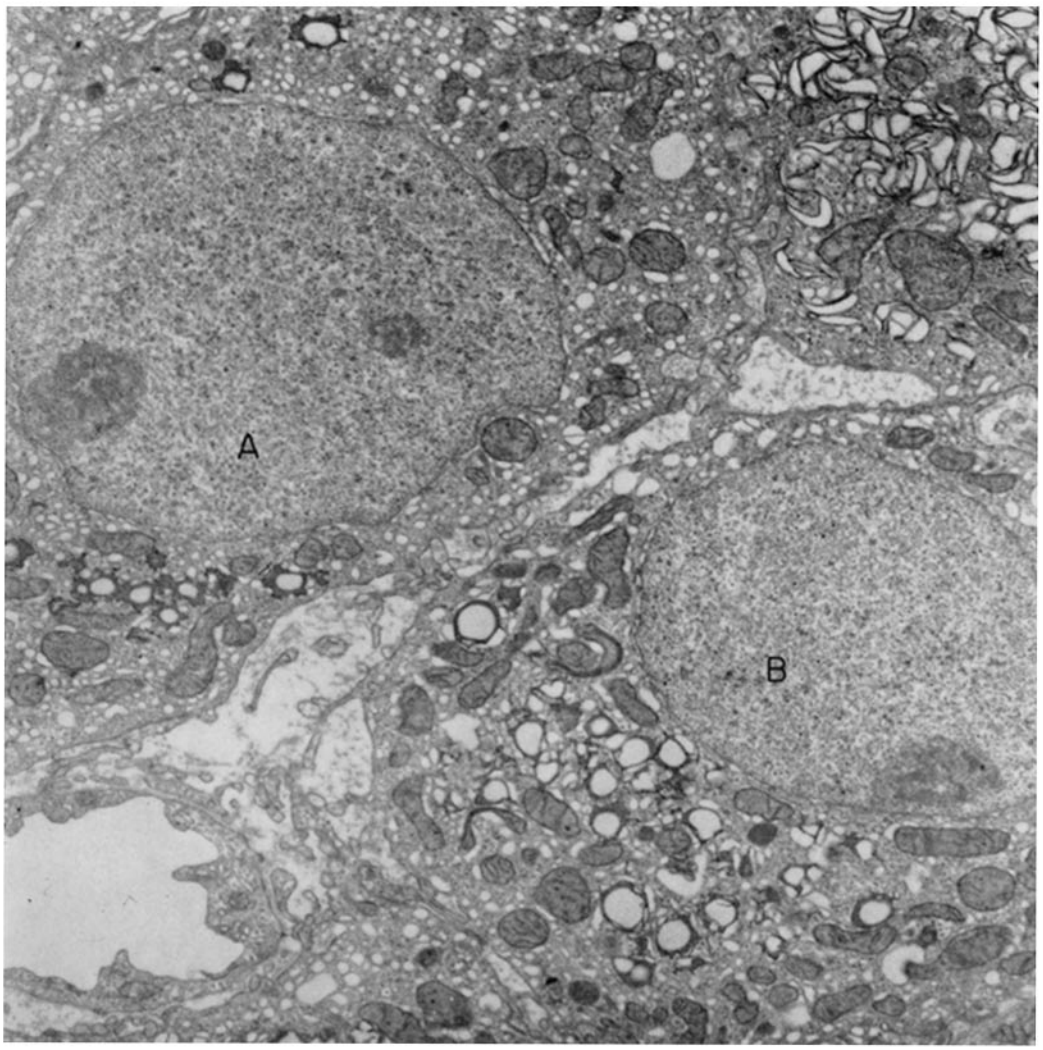


FIGURE 2 Lutein cells from a hypophysectomized animal that received 2 mg MH for 7 days. Note the variation in lipid droplets—partially leached in cell *A*, more leaching in cell *B*, and highly leached "lacy" areas in cell *C*. × 9000.

of the pregnant animals (Fig. 1). In consequence, the mitochondria appear less numerous per unit of area in these lutein cells. The Golgi zones of the small lutein cells of the animals given MH for 7 days are relatively compact, and form a central area near one end of the nucleus, where a pair of centrioles is commonly situated. The largest number of mitochondria with attenuated central regions is found in the animals receiving MH for 14 days and in pregnant animals (Fig. 4). Occasional cells from the animals injected with MH have regions in which the dense packing of membranes of the endoplasmic reticulum excludes other cytoplasmic elements (Fig. 5).

Onset Control and Terminal Control Lutein Cells

The lutein cells of the onset controls are small and somewhat variable in size and shape. The cell margins are not so highly folded on the perivascular space as are the margins of other lutein

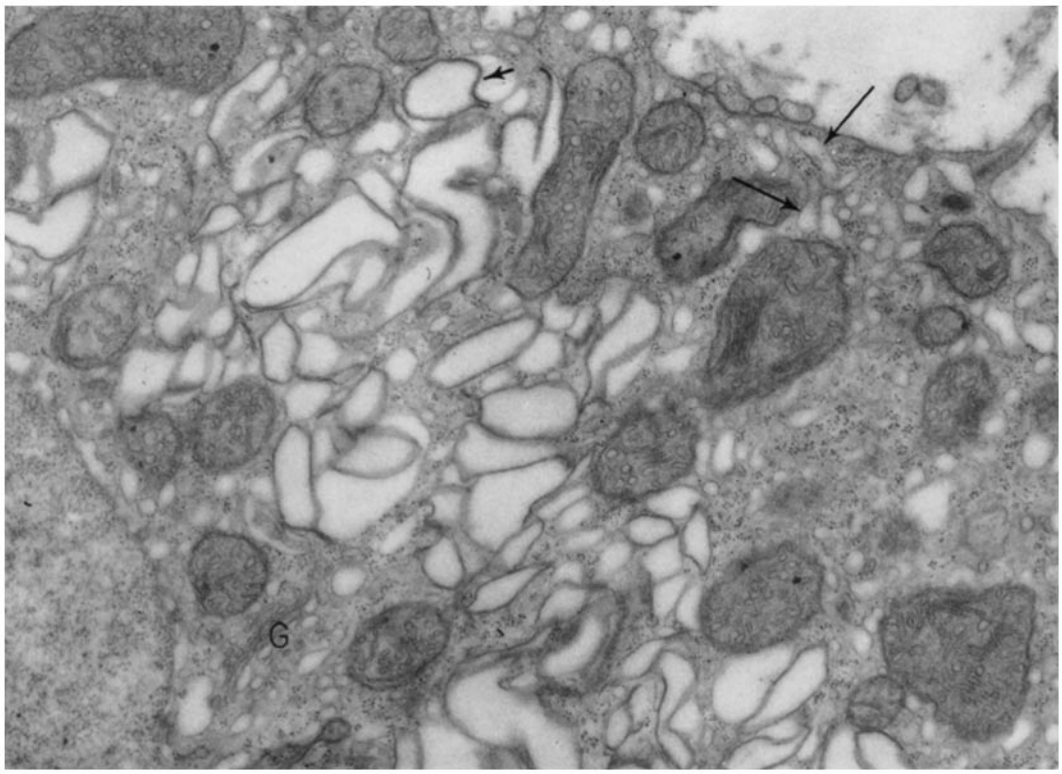


FIGURE 3 Higher magnification of a cell similar to cell *C* in Fig. 2. The "lacy" regions are extracted lipid droplets. Note residual lipid at small arrow. Note the presence of both villiform and lamelliform cristae in the mitochondria, and that the mitochondrial matrix is dense. Although numerous ribosomes are present in the cytoplasm, the endoplasmic reticulum is largely agranular. The tubular nature of the reticulum can be seen in the upper right (large arrows). *G*, Golgi. × 22,500.

cells. The mitochondria of these cells are numerous, tend to be of irregular rounded shape, and small. The matrix of the mitochondria is of low density, in striking contrast to the matrix of mitochondria in active corpora. The cristae are irregular, many of them being villiform (Fig. 6).

The Golgi membranes are condensed, with only mild dilation of the cisternae. The endoplasmic reticulum is not so conspicuous a feature of these non-stimulated cells, but, nevertheless, agranular endoplasmic reticulum is present in a vesiculotubular form throughout the cytoplasm. Ribosomes are numerous, appearing in clusters in the cytoplasm, and occasionally associated with the membranes of the endoplasmic reticulum. Numerous lipid droplets of moderately large size with irregular outlines and great density are present in these cells. Although some of these droplets show appreciable evidence of extraction, others do not and many of the droplets are very dense.

Lutein cells from terminal control animals are similar to lutein cells of onset control animals in that the cells and their mitochondria are small (Fig. 7). However, the perivascular spaces are more highly developed in terminal controls and the folding of the cell margins on these spaces is extensive (Fig. 8). The endoplasmic reticulum is also more extensive in the terminal controls than in the onset controls. The lipid present in these lutein cells is less abundant and the droplets are smaller and show less tendency to be extracted, as well as a lower density. Characteristic of the terminal controls is the presence of numerous granules, many of which have areas of secondary density and have the morphological characteristics of lysosomes or initial stages in formation and

lipofuscin (Fig. 9). These granules probably contain lipid pigments, but no histochemical tests were run to determine whether they were lipochromes, lipofuscin, "luteolipin," ceroid, or "brown degeneration pigment," all of which have been reported in steroid-secreting cells (18, 23).

The differences in structure of the different lutein cells are summarized in diagrammatic fashion in Fig. 10.

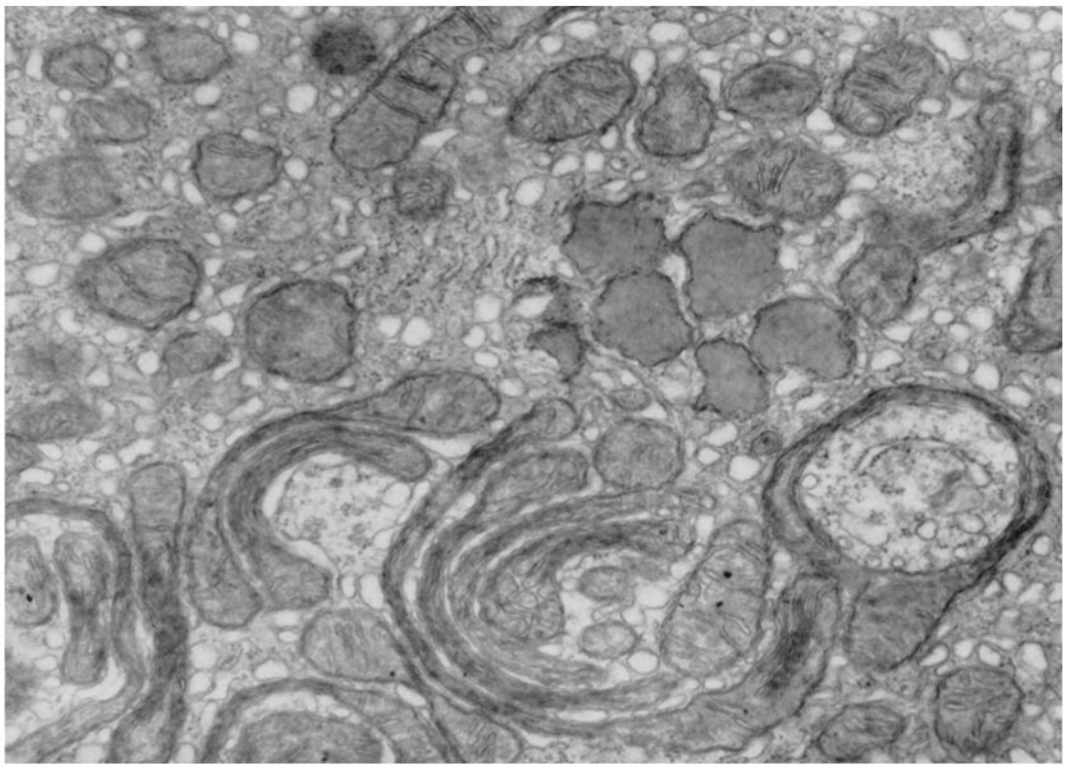


FIGURE 4 Region of a lutein cell of a rat on day 11 of normal pregnancy, showing mitochondria of the cupped disc type. Note that the elongate cristae lie parallel to the surface in the attenuated central regions of the mitochondria at the bottom of the picture. × 26,000.

DISCUSSION

The preceding description of lutein cells is based on the principal type of lutein cell that is found in the corpora lutea of each of the different sets of animals and located primarily in the compact outer zone of the corpora. Appreciable variation in cell structure occurs throughout the corpora lutea. For example, the cells in the central, less compact region of corpora from pregnant animals are "denser" than the lutein cells from the periphery of the corpora lutea. Despite evident differences among cells in a single corpus luteum, it was not possible in this study to distinguish between thecal as opposed to granulosal lutein cells.

By using the same preparative procedures for all corpora lutea, uniform treatment was assured. However, cells from the onset controls and terminal controls did not appear to be as well fixed on the whole as did those from the MH-injected and pregnant animals. In order to obtain an accurate image of the morphology of the organelles, the better fixed cells were especially carefully examined. The selection of well preserved cells from the control animals may tend to minimize the differences observed, since these cells may be those which were least affected by lack of luteotrophic factor.

The difference in cell size between the control

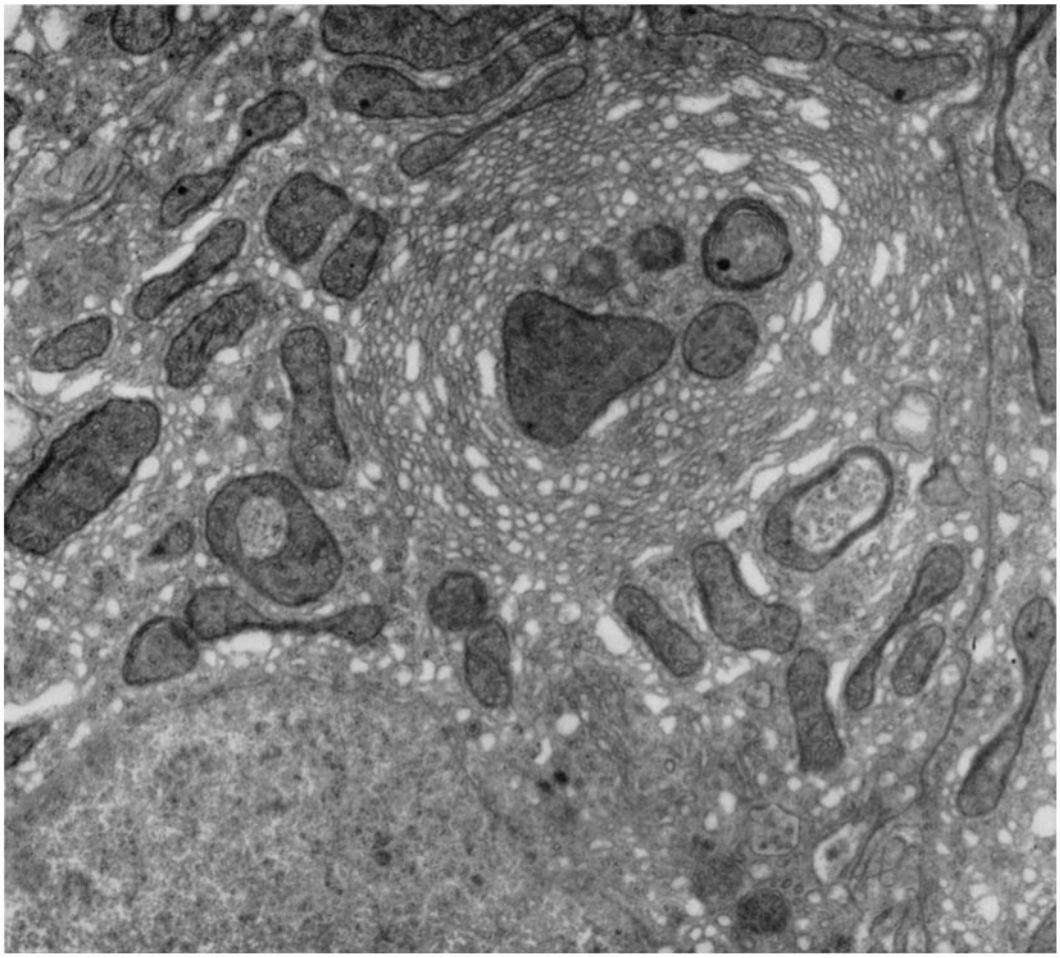


FIGURE 5 Lutein cell from a hypophysectomized rat that received 2 mg MH daily for 14 days. Note the annular arrangement of the agranular membranes of the endoplasmic reticulum and the presence of disc- and concave disc-shaped mitochondria. × 17,000.

animals and the animals injected with MH is marked. Examination of organelles in these cells indicates that the changes in cell size are accompanied by changes in structure of the organelles and lipids. No single organelle, however, shows changes of such character and consistency from cell to cell that these changes alone can be used as a definite indication of the presence or absence of progesterone secretion by the corpus luteum in which they are situated. If the several characteristics of active lutein cells enumerated above are considered together, then lutein cells from inactive corpora lutea can be distinguished readily from lutein cells from active corpora lutea. The multiple changes produced by MH may be taken as an indication of a general effect of MH on the metabolism of lutein cells, rather than an effect on a specific organelle, the abundance or composition of which is rate-limiting in the production of progesterone.

Appreciable agranular endoplasmic reticulum is present in the lutein cells from onset control and terminal control animals. However, this form of reticulum is more abundant and more typically tubular in the corpora lutea of animals with natural or exogenous luteotrophic hormone. The participation of the microsomal fraction in synthesis of mevalonic acid from acetoacetyl CoA

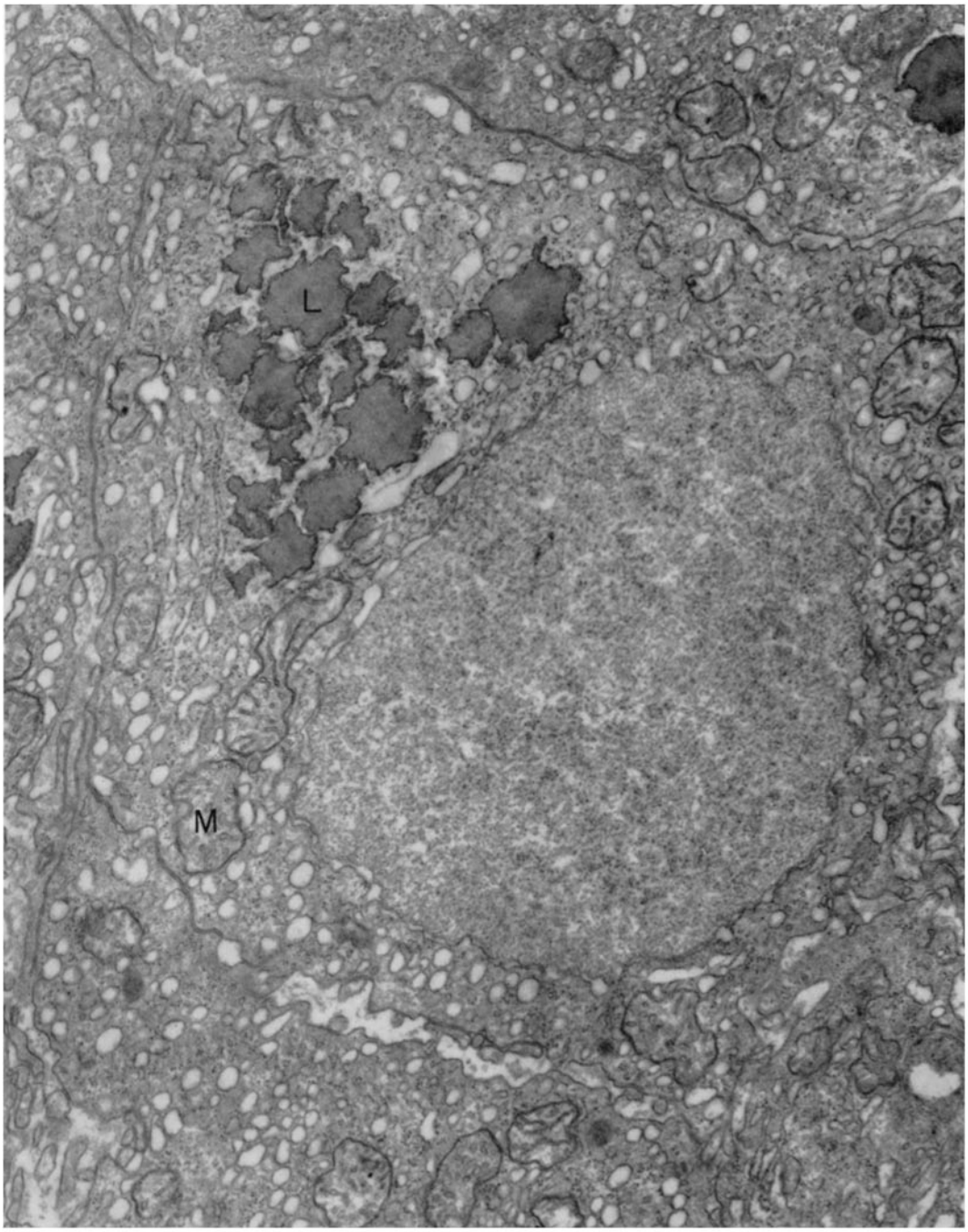
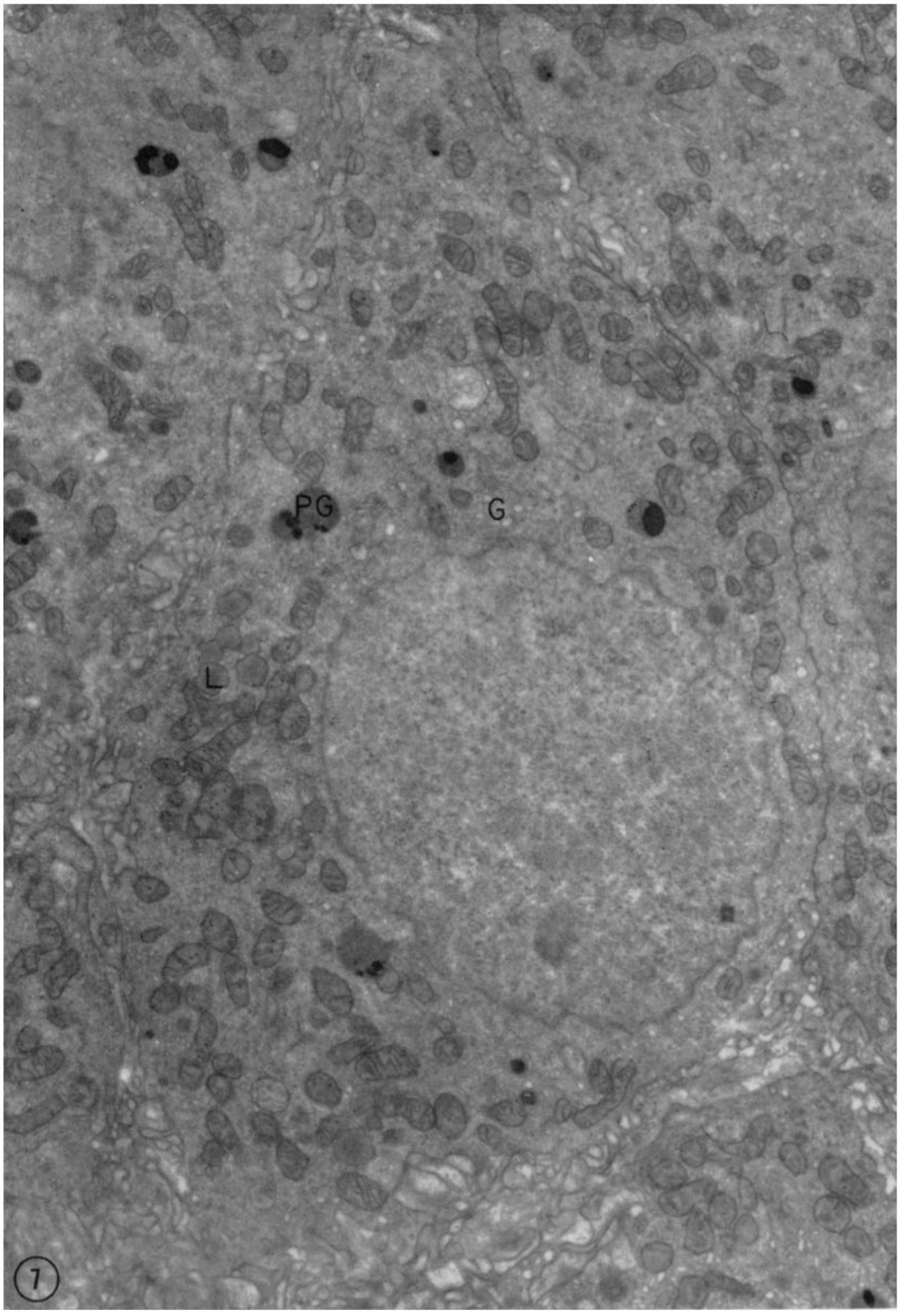


FIGURE 6 Lutein cells from an onset control, showing typical mitochondria (*M*) with irregular outlines and relatively lucid matrix. Note also the large lipid droplets (*L*). × 18,500.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in liver cells (7), the evidence for enzymes in this fraction which are involved in androgen biosynthesis (22), and the general abundance of agranular endoplasmic reticulum in all of the cells believed to secrete steroids (9, 12, 26) have led to speculation that some of the enzymes necessary for steroid synthesis are largely restricted to this agranular endoplasmic reticulum in lutein cells compared to intestinal epithelium, and the small quantities of lipid involved in the normal function of the corpus luteum would indicate that most of the endoplasmic reticulum is not subserving the function of lipid transport in lutein cells. Whatever function the agranular endoplasmic reticulum may

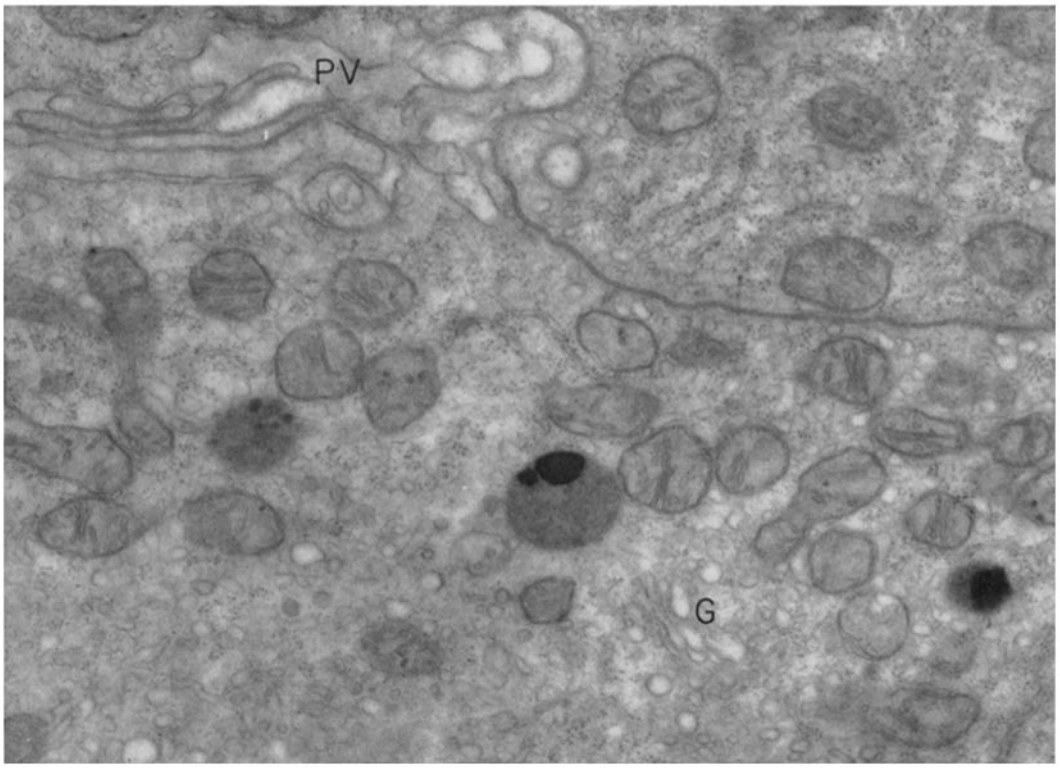

FIGURE 8 Lutein cells from a rat hypophysectomized 7 days prior to necropsy. Note the compound structure of the granule in the center of the picture. *G*, Golgi; *PV*, perivascular space. × 29,000.

element. Recently, evidence of participation of the endoplasmic reticulum in the transport and resynthesis of triglycerides (27) and synthesis of phospholipids (19), has also been adduced. Furthermore, morphological evidence has been obtained that the agranular endoplasmic reticulum may be associated with intracellular fat transport in the intestinal epithelium (21). (In other cells, however, caveolae have been implicated in the transport of lipids (28)). The relatively large amount of be concerned with in lutein cells, the abundance of this element, specially in normal pregnancy, indicates that it must have a particular physiological significance for these cells.

The variation in lipid droplets both from cell to cell and from group to group within the stimulated and non-stimulated major groupings is striking. Much of the lipid is highly extracted. It is generally established that the lipid droplets of steroid cells contain a mixture of different lipids

FIGURE 7 Lutein cells from a rat hypophysectomized 7 days prior to necropsy. Note the presence of numerous lipid pigment granules (*PG*), and the relatively normal appearance of the mitochondria. *G*, Golgi, *L*, lipid. × 12,500.

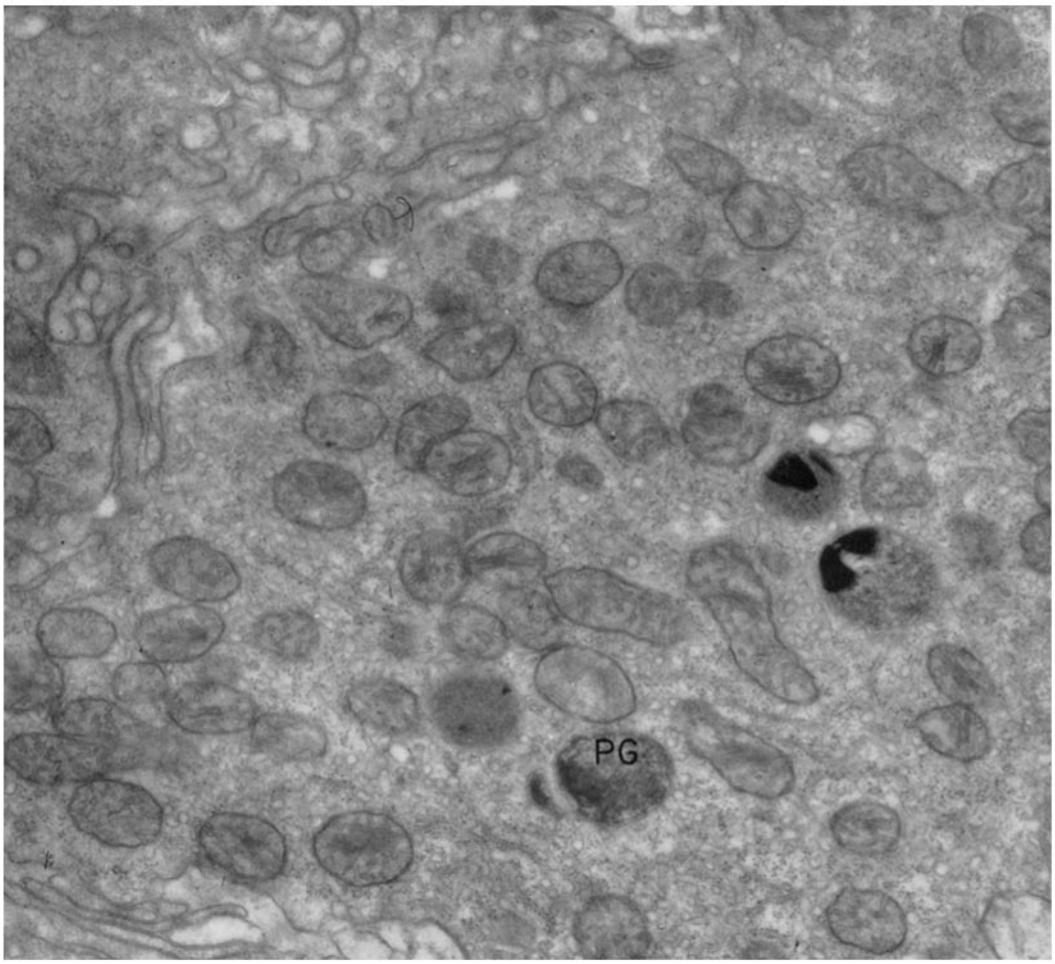

FIGURE 9 The tubular nature of the endoplasmic reticulum is somewhat more apparent in this picture than in the previous figure. Note that here also the mitochondria are apparently relatively short and normal in appearance compared to those of the MH-stimulated animals. *PG*, lipid pigment granule. × 23,000.

(11). The work of Bahr (3) indicates that cholesterol is not rapidly colored by osmium. On the other hand, osmium tetroxide unites in a 1:1 molar ratio with fatty acids exhibiting a single unsaturation and in higher ratios to those with more sites of unsaturation (20). It is possible, therefore, that the images seen in the micrographs obtained from this material give some indication of the composition of the droplets as well as the droplet size. But study of the differential extraction which occurs in preparation of tissues for electron microscopy and of differences in density of image is not, at present, in a stage that makes reasonable a detailed analysis of these droplets from observations in micrographs.

Bjorkman (4) described the partial luteinization of the granulosa cells of preovular and early postovular follicles in the rat. The onset controls in this study also exhibit a partial luteinization in the absence of appreciable quantities of MH. These observations add further support to the contention that the changes occurring at ovulation result in lutein cells with the potential for the production of progesterone already established. If these cells are well maintained by endogenous or

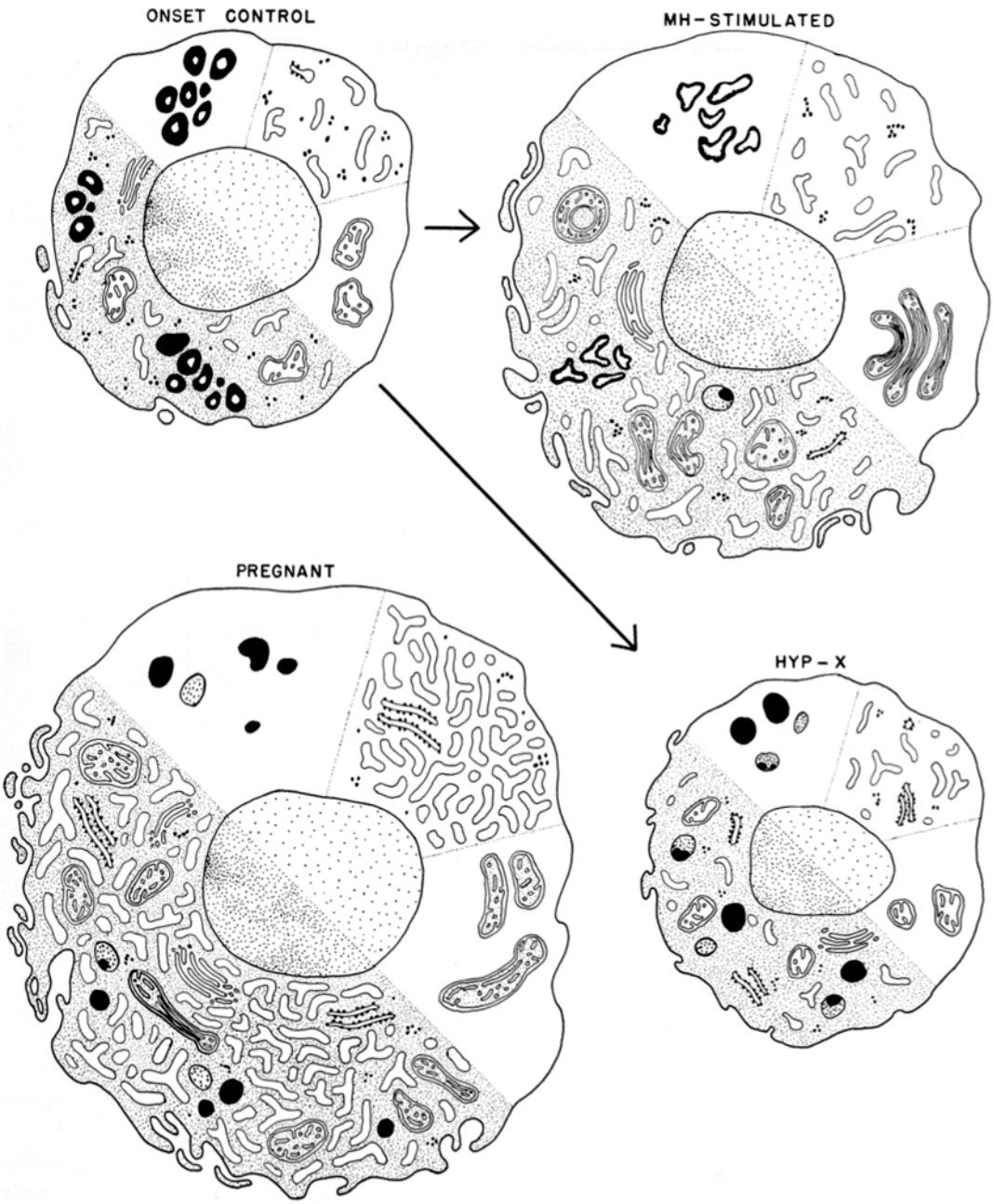

FIGURE 10 This figure illustrates diagrammatically the relative cell size and the differences in the organelles and lipid inclusions of lutein cells in different functional states. The wedges on the right of each cell represent, in clockwise direction, the lipid, endoplasmic reticulum, and mitochondria, respectively. The lower left half of each cell represents a section of the cell with all the normal constituents.

The cell at the upper left is a lutein cell of a corpus luteum formed as the result of injection of PMS (onset control). At the upper right is a cell after stimulation with MH following hypophysectomy. This cell represents a composite of the results obtained with daily injections of 2 mg MH for 7 days and 14 days. The cell at the lower left is a lutein cell from normal pregnancy. The cell at the lower right is a lutein cell after 2 weeks of hypophysectomy, without hormone replacement.

Note in the normal pregnancy and MH-stimulated cells the abundance of agranular endoplasmic reticulum and the large mitochondria with a dense matrix and tendency toward a disc shape. Note also the abundance of large lipid droplets in the non-stimulated cells. For a more complete description of the differences, see text.

exogenous luteotrophic factors, they will produce progesterone.

We wish to express our thanks to Mr. Nazir Ahmad for his technical assistance. The study was supported by Grants G-21829 and GB-1166 from the National Science Foundation, and grant AM-02248 from the National Institutes of Health.

Received for publication, August 19, 1963.

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