THE CORPUS LUTEUM OF THE GUINEA PIG

II. Cytochemical Studies on the Golgi Complex, GERL, and Lysosomes in Luteal Cells during Maximal Progesterone Secretion

LAURIE G. PAAVOLA

From the Department of Anatomy, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

ABSTRACT

This study characterizes the cytochemical properties of the Golgi complex, the structure which corresponds to Golgi complex-endoplasmic reticulum-lysosomes (GERL), and the granule population in luteal cells of guinea pigs at the time of maximum progesterone secretion, in material fixed by vascular perfusion, a method particularly suited for preserving both fine structure and enzyme activity. The distribution of several marker enzymes was determined by electron microscope cytochemistry. Acid phosphatase (ACPase) and arylsulfatase were used to identify structures containing lysosomal proteins. To resolve specific problems, additional cytochemical markers were employed: localization of thiamine pyrophosphatase (TPPase) (in the Golgi complex) and alkaline phosphatase (ALPase) (a plasma membrane marker), and prolonged osmication (a generally accepted method of marking the outer cisterna of the Golgi complex). The results demonstrate that at the time of peak steroid secretion the Golgi complex in luteal cells, in marked contrast to that of most other cell types, typically displays intense ACPase activity in all of its cisternae. Similarly, all Golgi cisternae stain after prolonged osmication and may show TPPase activity. On the other hand, GERL in luteal cells of this age, unlike that in most cells, commonly shows low levels of, or lacks, ACPase activity. However, GERL resembles that of other cell types in being TPPase-negative and in being unstained by treatment with aqueous OsO_4. GERL and some Golgi cisternae are reactive for ALPase. The granule population in luteal cells of this stage consists of lysosomes, multivesicular bodies, electron-transparent vacuoles, and microperoxisome-like bodies. These results form a base line with which luteolytic changes described in the companion study (Paavola, L.G. 1978. The corpus luteum of the guinea pig. III. Cytochemical studies on the Golgi complex and GERL during normal postpartum regression of luteal cells, emphasizing the origin of lysosomes and autophagic vacuoles. *J. Cell. Biol.* **79**:59–73.) can be compared.

KEY WORDS Luteal cells · Golgi complex · GERL · lysosomes · electron microscope cytochemistry · acid hydrolases · phosphatases

The corpus luteum of the guinea pig ovary provides an excellent system for the study of cellular senescence and death as it occurs under physiological conditions. Luteal cells function in the synthe-

sis and secretion of progesterone for a limited period of time, then undergo regression in response to hormones produced by the uterus. The extensive cell death accompanying the slow involution of the corpus luteum in this animal permits the mechanisms underlying cellular regression to be followed in depth. Recent observations from this laboratory (47) indicate that normal postpartum degeneration of luteal cells in guinea pigs is characterized at the fine structural level by the accumulation of apparent autophagic vacuoles and lysosome-like dense bodies. The prominence of such bodies at the time of involution suggests that autophagy may have a central role in luteal cell degeneration (47). The lytic nature of these bodies, however, was not confirmed in that study. Moreover, the source of similar bodies has not been firmly established in luteal or most nonluteal tissues. It should be possible to determine whether these bodies are part of the intracellular digestive apparatus, and to investigate their origins, by following changes in the distribution of appropriate marker enzymes, such as acid phosphatase (ACPase) or arylsulfatase, in luteal cells by electron microscope cytochemistry.

Cytochemical studies on luteal cells, however, have been hampered in the past by the difficulty of preparing these cells for electron microscopy in a manner that maintains both enzyme activity and fine structure. The smooth endoplasmic reticulum (smooth ER), a prominent organelle in these cells, is particularly difficult to preserve. Fixation by immersion is generally unfavorable for most cytochemical procedures involving luteal tissue, since to preserve these cells adequately by this method, prolonged exposure to the fixative is necessary which leads to the inactivation of many enzymes. In the present study, this problem has been approached using material fixed by perfusion, a technique capable of providing optimal preservation of steroid-secreting cells (12), yet maintaining enzyme activity (14, 22).

This and the companion study characterize the cytochemical behavior of various organelles in luteal cells of guinea pig ovaries at the time of maximal steroid release during pregnancy, and throughout involution. In the present study, the distribution of several marker enzymes was determined by electron microscope cytochemistry in luteal cells during the period of peak progesterone secretion, 20–33 days of gestation (10, 29), to establish a normal base line that could serve as an aid in evaluating regressive changes occurring during luteolysis. ACPase and arylsulfatase, the main probes in this study, were used to identify structures containing lysosomal proteins. Other marker enzymes used to resolve specific problems were thiamine pyrophosphatase (TPPase) (for the Golgi complex) and alkaline phosphatase (ALPase) (a plasma membrane marker). In addition, prolonged osmication was carried out (generally marks the outer Golgi cisterna).

One of the unexpected findings of this study was the presence of ACPase activity throughout all Golgi cisternae, and its paucity in the structure which corresponds to Golgi complex-endoplasmic reticulum-lysosomes (GERL). This finding is in contrast to most other published studies, in which GERL is strongly positive for this enzyme, while Golgi cisternae are commonly negative.

MATERIALS AND METHODS

The 25–33-day-pregnant Hartley guinea pigs used in this study were obtained from Perfection Breeders (Douglassville, Pa.). The stage of gestation was verified as previously described (47). A total of 25 animals was used in this work.

All corpora lutea examined in this study were fixed in situ by vascular perfusion with: (*a*) full- (1% paraformaldehyde-1% glutaraldehyde-0.01% 2,4,6-trinitrocresol) or half-strength formaldehyde-glutaraldehyde-cresol (32) in 0.1 M cacodylate buffer at pH 7.4, a fixative previously determined to provide optimal preservation of luteal cell morphology (47), (*b*) 1.5% glutaraldehyde-0.067 M cacodylate, pH 7.4, or (*c*) 4% paraformaldehyde in 0.1 M cacodylate buffer. The basic perfusion technique, developed in this laboratory, was described elsewhere (47). In this study, the ovary was perfused first with Krebs-Ringer-bicarbonate buffer (containing 2 mg/ml glucose, pH 7.4) for 2–3 min to wash out the blood, then perfused for 2.5–30 min with fixative *a* or *b*, or for 10 min with *c*. After perfusion-fixation, the ovary was excised, and the corpora lutea were removed, freed of thecal tissue and sliced. The tissue fixed with *a* or *b* was placed in buffer (0.1 M cacodylate containing 5% sucrose, pH 7.4); that fixed with *c* was placed in fresh fixative for 30 min. All tissue was washed thoroughly with several changes of cold buffer. In general, tissue used for cytochemistry was held in buffer no longer than 24 h.

Morphology

For morphology, slices of fixed corpora lutea were diced into smaller pieces, which were processed as previously described (47). Pale gold-to-silver thin sections were prepared with a diamond knife. The thin sections were stained for 0.5–2 min in lead citrate (52), and examined in a Philips 300 electron microscope.

Cytochemistry

For cytochemical procedures, 25–40 μm nonfrozen sections of perfusion-fixed luteal slices were prepared with a Smith and Farquhar TC-2 tissue sectioner (DuPont Instruments-Sorvall, DuPont Co., Wilmington, Del.), and collected in 0.1 M cacodylate buffer, pH 7.4, containing 5% sucrose. The incubations were carried out at 37°C or room temperature with constant agitation, and the incubation media contained 5% sucrose or sucrose sufficient to give a final osmolarity of 300–350 mosM. Incubation media for ALPase and TPPase were renewed every 30–60 min. The progress of the cytochemical reactions was monitored with the light microscope by treating incubated nonfrozen sections with 2% ammonium sulfide for 10 min. All substrates were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Acid Phosphatase

Nonfrozen sections were incubated at pH 5 in the Barka and Anderson (3) modification of the Gomori (25) medium, which contained 11.5 mM Na-β-glycerophosphate as the substrate, 2.4 mM $Pb(NO_3)_2$, and 40 mM Tris-maleate buffer. Before incubation in the complete medium, the sections were washed for 30 min in cold 40 mM Tris-maleate buffer containing 5% sucrose, at pH 5. Incubation times varied from 30–90 min. Control sections were incubated in a complete medium containing 10 mM NaF or in a medium lacking substrate, or were heated to 60°C for 15 min before incubation in a complete medium.

Arylsulfatase

Nonfrozen sections were incubated for 90 min at pH 5.4 in the Goldfischer (23) medium, as modified by Decker (13). It contained 4.8 mM *p*-nitrocatechol sulfate, 55.8 mM $Pb(NO_3)_2$, and 80 mM acetate buffer. Before incubation in the complete medium, the sections were washed for 30 min in cold 100 mM acetate buffer, pH 5.4. After incubation, the sections were treated with 2% buffered ammonium sulfide to enhance the reaction product. For controls, tissue was incubated without substrate, or heated then incubated in a complete medium. In the present study, arylsulfatase activity is best preserved by a 2.5–3-min perfusion with fixative *a* or *b*. The activity of this enzyme is completely suppressed by fixation with 4% paraformaldehyde, under the conditions used here.

Thiamine Pyrophosphatase

Nonfrozen sections were incubated at pH 7.2 or 9 for 1.5–2 h in the Novikoff and Goldfischer (43) medium, which was modified to contain 2.2 mM TPP chloride, 2 mM $Pb(NO_3)_2$, and 3 mM $MnCl_2$, in Tris-maleate buffer (at pH 9, $MgCl_2$ was substituted for $MnCl_2$). L-*p*-Bromotetramisole (0.2 mM) or 5 mM L-cysteine was included in the complete medium to suppress the hydrolysis of TPP chloride by ALPase (see below). The lower levels of lead nitrate and $MnCl_2$ tended to minimize spurious precipitates in the medium. The medium was filtered through Whatman no. 50 filter paper immediately before it was used. Fixation by perfusion for 2.5–3 min with fixative *a* best preserves the cytochemical activity of this enzyme in luteal cells. Controls included deletion of substrate of $MnCl_2$ ($MgCl_2$) from the medium, or incubation of heated sections in a complete medium.

Alkaline Phosphatase

It was necessary to study ALPase activity, since sections incubated in a TPPase medium lacking L-*p*-bromotetramisole of L-cysteine showed dense deposits of reaction product along plasma membranes and activity throughout GERL and the Golgi complex. To determine whether these precipitates represented TPPase activity or the tail of ALPase activity (7), nonfrozen sections were incubated at pH 7.2 or 9 for 90 min in the Hugon and Borgers (31) medium, which contained 11.6 mM Na-β-glycerophosphate as the substrate, 3.9 mM $Pb(NO_3)_2$, and 40 mM Tris-maleate buffer. Control sections were incubated in a complete medium containing 0.2 mM L-*p*-bromotetramisole (8) or 5 mM L-cysteine (49), known inhibitors of ALPase.

After cytochemical incubation, the sections were washed three times, 5 min each, with cold 0.05 M veronal-acetate buffer containing 7% sucrose, and then prepared for electron microscopy as described above. Pale gold-to-silver thin and 0.25 μm thick sections, stained en bloc only or poststained 30 s with lead citrate (52), were viewed in the electron microscope at 60–100 kV.

Osmium Reduction

Tissue previously fixed by perfusion was minced into small pieces and placed in 2% aqueous OsO_4 for 40 h at 40°C (21). The OsO_4 solution was renewed once at 24 h. After this treatment, the tissue was prepared for electron microscopy.

RESULTS

The fine structure of luteal cells in the guinea pig at the time of maximum progesterone secretion has been described in detail in a previous publication from this laboratory (47), and the present study will be confined to the cytochemistry of the Golgi complex, GERL, and lysosomes. Where pertinent, aspects of their morphology will be mentioned as background for the cytochemical findings. The control incubations proved to be negative for all of the cytochemical procedures used.

Golgi Complex

During the period of peak steroid secretion, the Golgi complex in luteal cells is extensive, occurring as stacks of four to six closely spaced, flattened, parallel cisternae, whose content is typically electron-transparent. All Golgi cisternae display perforations, but the number and size vary according to the position of the cisterna within the stack. The outer cisterna has a few widely spaced, small fenestrations, measuring ~30–35 nm in diameter, scattered irregularly over its surface (Fig. 1*a*). The middle saccules exhibit many 50–60 nm fenestrations, which are separated from each other by ~60–100 nm and are arranged in rather uniform rows (Fig. 1*c*). The innermost element displays many large fenestrations, that are about the same size (85 nm) or slightly larger than the space separating them (Fig. 1*b*). A smooth-surfaced tubule or vesicle often protrudes through the fenestrations of this element (Fig. 1*b*).

At this stage, the most striking localization of ACPase activity is to the Golgi complex, where dense precipitates of lead phosphate accumulate in several cisternae (Figs. 2 and 3), completely filling the cisternal cavity (Fig. 3). The several Golgi stacks seen in each luteal cell invariably

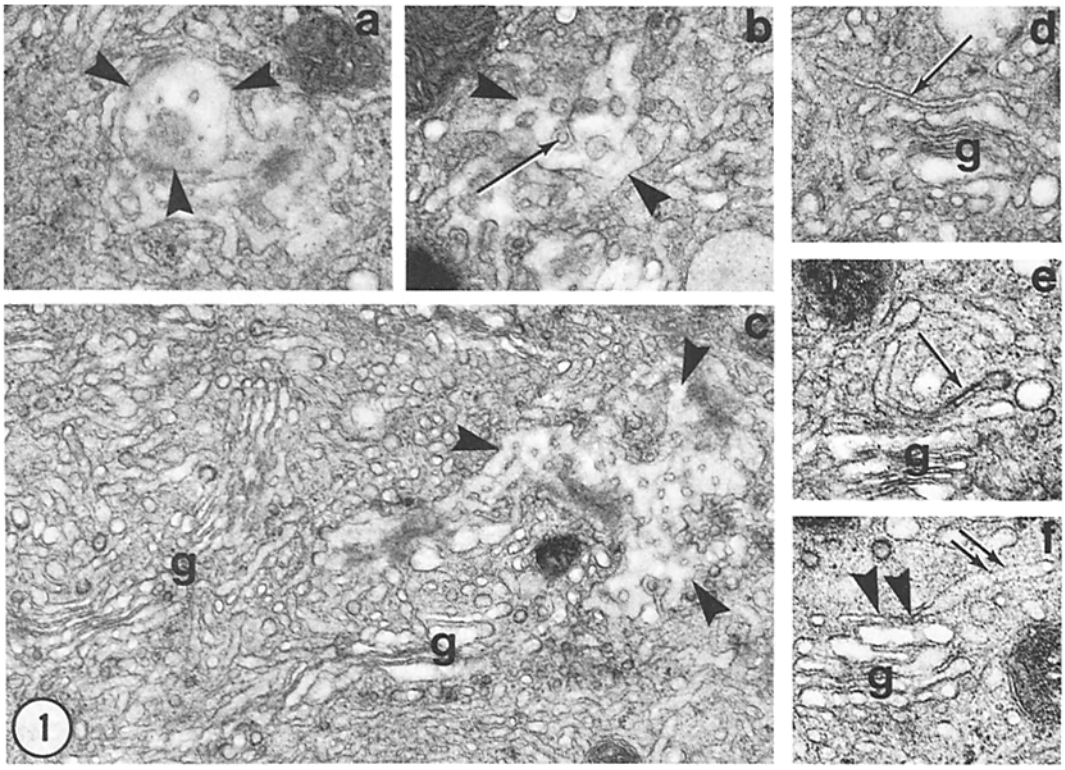


FIGURE 1 Golgi complexes of luteal cells during the period of maximum progesterone secretion. (*a*) The outer cisterna (between arrowheads) of a Golgi stack, showing characteristic appearance when seen en face, bears only a few small, widely spaced fenestrations. × 29,000. (*b*) In contrast, the innermost element (between arrowheads) of a Golgi stack typically displays many large, closely spaced perforations. A smooth-surfaced tubule often protrudes through a fenestration, as indicated at the arrow. × 25,000. (*c*) Middle cisternae, one of which occurs en face in this field (between arrowheads), also have many fenestrations, which are arranged in regular rows and are somewhat smaller than those of the inner element. This middle cisterna, seen en face, connects with one of two nearby Golgi stacks (*g*) seen in a more typical orientation. × 25,000. Golgi stacks are indicated at (*g*) in (*d–f*). (*d*) GERL cisternae (arrow) commonly are narrowed in width and display limiting membranes that are thicker than those of most other organelles. × 32,000. (*e*) GERL membranes are sometimes coated (arrow). × 34,000. (*f*) GERL cisternae (arrowheads) occasionally show possible continuities with ER-like tubules (double arrows). × 34,000. All, 26 days of gestation.

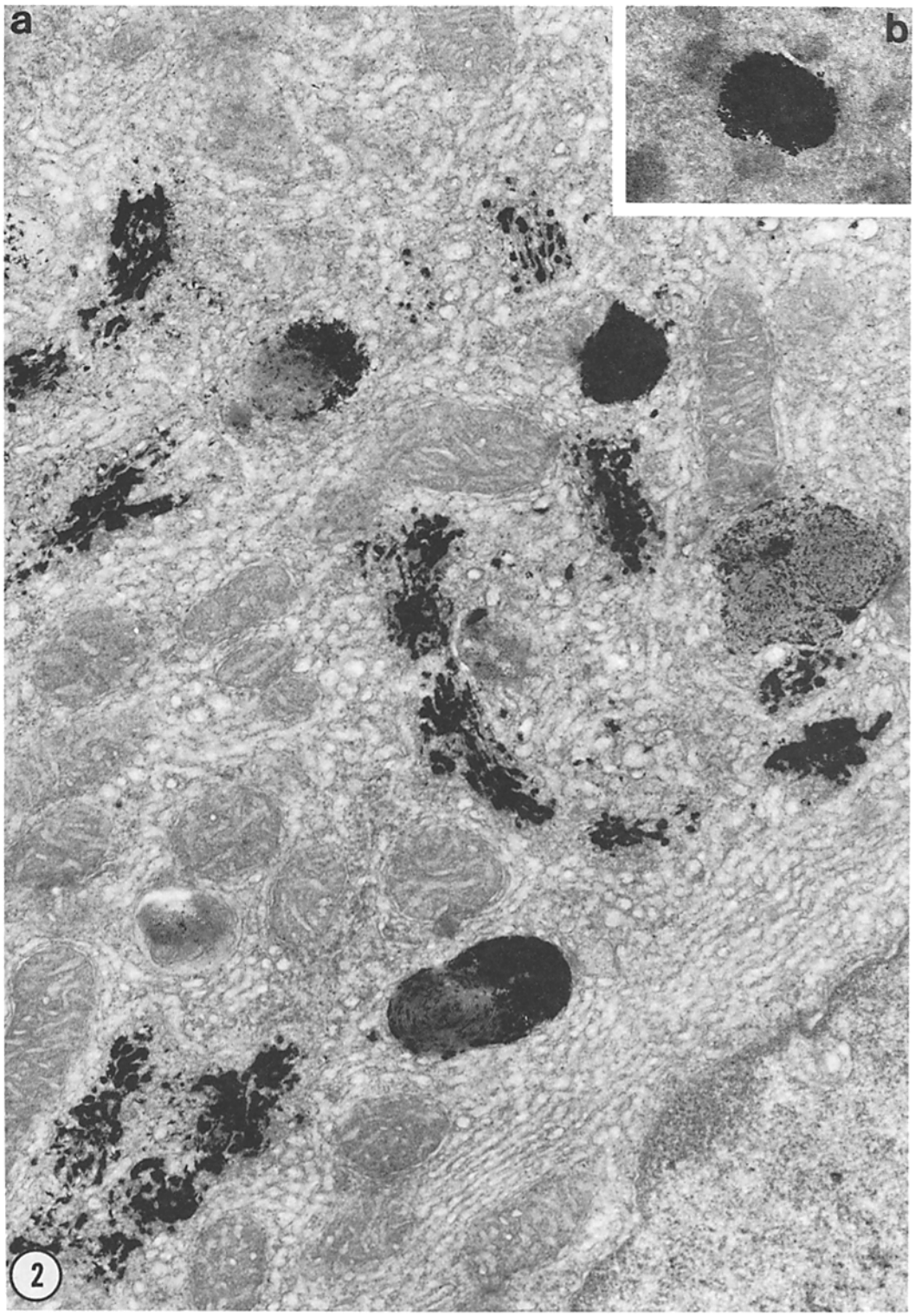


FIGURE 2 Luteal cells, reacted for acid hydrolase. (*a*) This cell shows the typical appearance after ACPase localization. Lysosomes contain sparse to dense deposits of reaction product. More striking, however, is the intense ACPase reaction displayed by the several Golgi stacks in the field. All cytochemical material shown in this report is stained en bloc only. × 28,000. (*b*) Arylsulfatase preparation showing an intensely reacted lysosome. × 37,000.

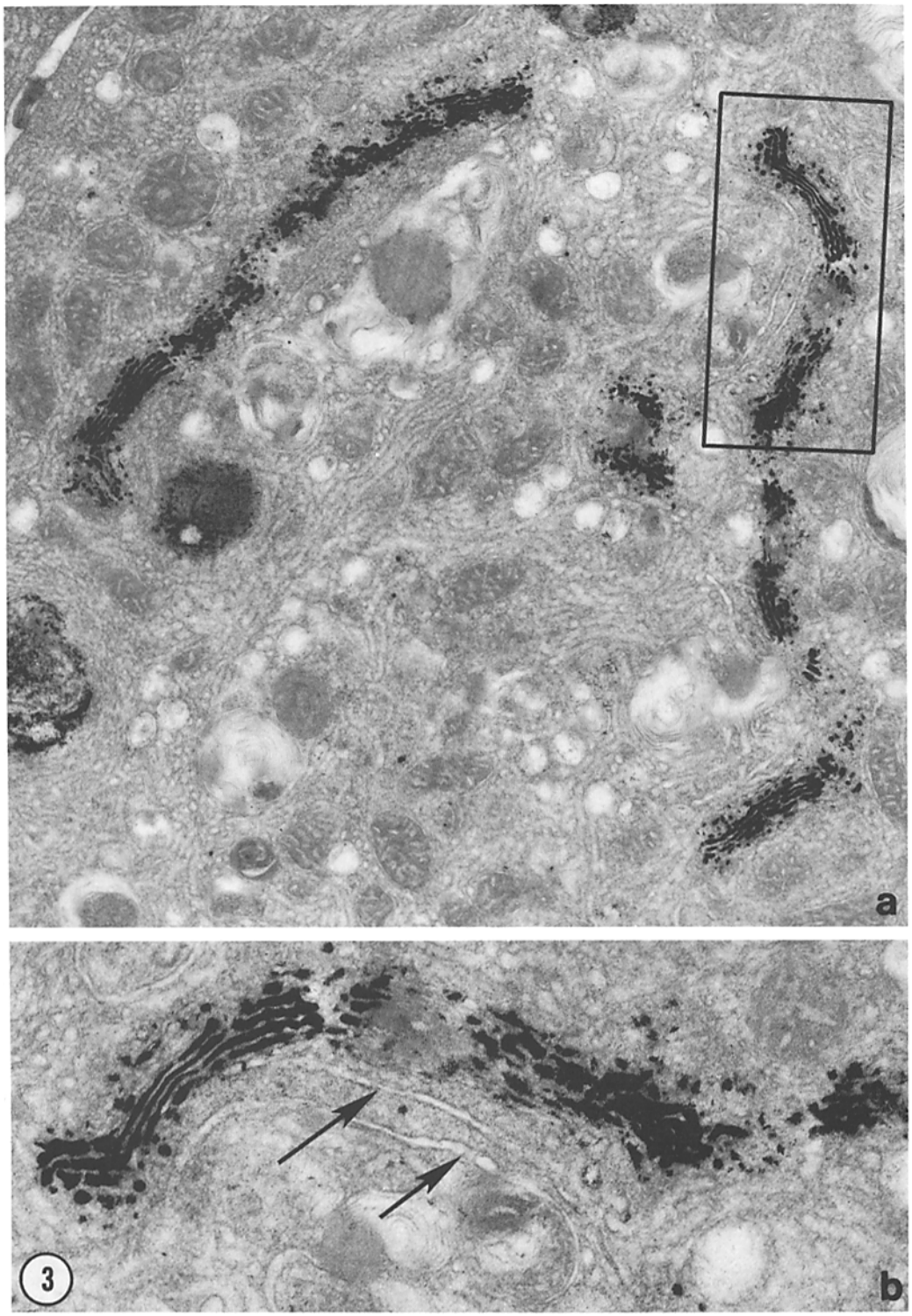


FIGURE 3 Golgi complex of a luteal cell at the time of maximum progesterone secretion, reacted for ACPase. (*a*) As is characteristic of this period, all cisternae of this extensive Golgi complex show prominent ACPase activity. The area enclosed by the rectangle is shown at higher magnification in the figure below. × 22,000. (*b*) Two cisternae (arrows) which correspond to GERL parallel this Golgi stack; both of these GERL cisternae lack ACPase activity. On the other hand, the closely spaced Golgi saccules are strongly reactive. × 49,000. 25 days of gestation.

show reaction product. The Golgi complex also shows TPPase activity. As in other cell types, the inner element is reactive for this enzyme but, in addition, several of the remaining saccules also often show activity (Fig. 5). Similar results were obtained in tissues fixed with fixative *a* or *b*. In en face views, the morphology of the inner element (Fig. 5, *inset*) resembles that described by Novikoff et al. (45) in neurons of rat dorsal root ganglia. The Golgi complex in some TPPase-incubated tissue lacks reaction product, a situation that may be the result of fixation, since this enzyme is readily inactivated by aldehydes (24). One or more Golgi cisternae and some Golgi vesicles contain reaction product after cytochemical incubation for ALPase; the inner cisternae show the most intense reaction (Fig. 6). Prolonged treatment with aqueous OsO_4 heavily stains all cisternae of the Golgi complex (Fig. 7). This staining pattern appears to be independent of the location of the cell in the tissue block.

GERL

Although luteal cells contain abundant smooth ER, certain smooth-surfaced elements located adjacent to the Golgi complex display features that suggest that they correspond to the GERL system described in rat neurons (30, 40, 45) and other tissues (15, 27, 28). A more comprehensive description of these Golgi-associated smooth membranes is given in the companion paper (48), which covers a time when they are highly developed and therefore more conspicuous. In luteal cells of 25–33-day-pregnant guinea pigs, the smooth-surfaced cisternae that seem to bear a special relationship to the Golgi complex: (*a*) occur in proximity to the inner face of a Golgi stack, and commonly follow the shape of the inner element, often being separated from it by a variable space that contains tubules or vesicles (see Fig. 9 in reference 47); (*b*) have limiting membranes which are typically thicker (denser) than those of most other organelles, including smooth ER (Fig. 1*d*), and are occasionally coated (Fig. 1*e*); and (*c*) sometimes appear to be continuous with ER-like structures (Fig. 1*f*). In most tissues, GERL is defined by its distinctive cytochemical behavior (ACPase-positive). Even though many of the structures described as GERL in this study do not meet that criterion, the fact that some of the Golgi-associated, GERL-like cisternae show traces of ACPase activity (see below) lends support to the suggestion that similar nonreactive cisternae are part of the GERL system in these luteal cells. For other details regarding GERL in luteal cells at this stage, see Paavola (47).

In contrast to most cell types, the structure corresponding to GERL in luteal cells of this age is commonly unreactive after incubation for ACPase activity. Two such nonreactive GERL cisternae are seen paralleling the inner face of the Golgi complex in Fig. 3. Occasional GERL cisternae display sparse, scattered deposits of reaction product (Fig. 4*a–d*), but only rarely does GERL exhibit intense ACPase activity (Fig. 4*e*). Note that, in each instance, the cisternae described as belonging to the GERL system, whether nonreactive or reactive, are narrow in width and have membranes that appear to be thicker (denser) than those of most other cytoplasmic organelles. GERL in luteal cells is reactive for ALPase activity (Fig. 6) but, as in other cell types, typically lacks TPPase activity (Fig. 5) and does not stain after prolonged osmication (Fig. 7).

Lysosomes

The identity of the lysosomal population was established by cytochemical incubation for the acid hydrolases, ACPase and arylsulfatase. Lysosomes with more or less homogeneous contents, or displaying myelin figures, occur in modest numbers and vary in morphology, but are usually irregularly spheroid. Most of these bodies are reactive for ACPase and arylsulfatase (Fig. 2) but the amount of reaction product they contain varies. Reaction product in multivesicular bodies (MVBs) is limited to slight stippling. Lysosomes and MVBs are generally nonreactive for TPPase (Fig. 5) and ALPase (Fig. 6), and only occasionally stain following extended osmication. As noted in other tissues (1), a diffuse precipitate often occurred over lipid droplets and the background cytoplasm of luteal cells treated with $(NH_4)_2S$ after incubation for arylsulfatase activity. Such treatment, however, was helpful because it intensified the reaction product, making it more conspicuous.

Use of Alkaline Phosphatase Localization to Clarify the TPPase Results

Nonspecific ALPases can hydrolyze various substrates at neutral pH, including TPP chloride (7); thus, these enzymes could give rise to reaction product that could potentially be interpretated as resulting from TPPase activity. To eliminate this possible source of confusion, the distribution of

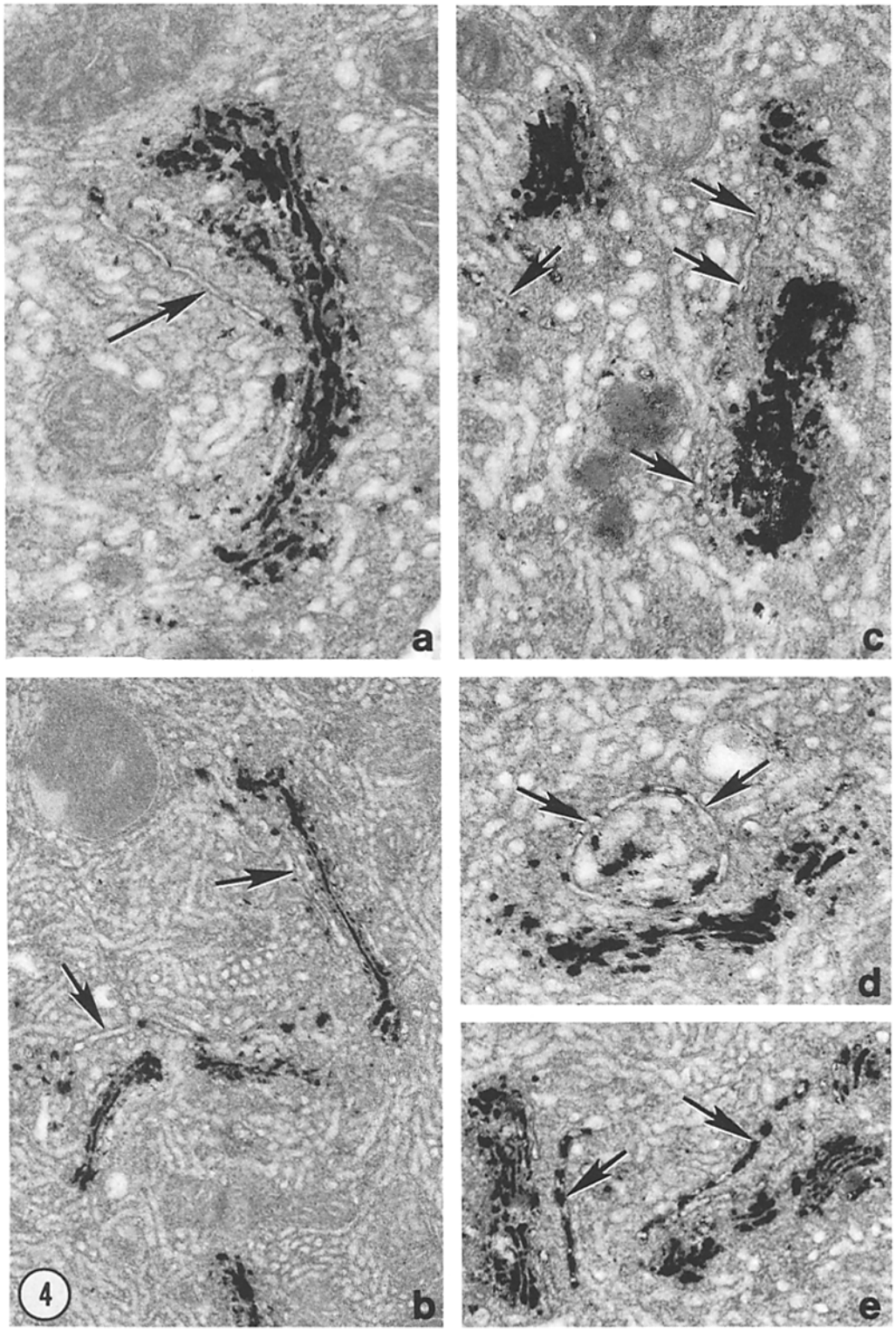


FIGURE 4 GERL cisternae in luteal cells of this age occasionally show low levels of ACPase activity or, rarely, more intense activity. This is illustrated in these figures, each from a different luteal cell. Fig. 4*a–d* show scant reaction product in GERL (arrows). Golgi cisternae, on the other hand, are strongly reacted. Fig. 4*e* is a rare example of heavy activity in GERL (arrows). (*a*, *b*, *d*, and *e*) 25 days of gestation; (*c*) 26 days of gestation. (*a*) × 39,000, (*b*) × 38,000, (*c*) × 28,000, (*d*) × 40,000, and (*e*) × 33,000.

ALPase in luteal cells has been examined after incubation in a classical ALPase medium (at pH 9 and 7.2, with β-glycerophosphate as substrate), and in a TPPase medium (at pH 7.2 and 9, with TPP chloride as substrate). Parallel cytochemical incubations were carried out, in which L-cysteine or L-*p*-bromotetramisole (known inhibitors of ALPase) was included in the medium. The results of these studies follow.

Cytochemical incubation in an ALPase medium at pH 9 or 7.2 results in dense precipitates along plasma membranes and in subplasmalemmal vacuoles, as well as in GERL and some Golgi cisternae (Fig. 6). Inclusion of an ALPase inhibitor in the medium suppresses this activity at both neutral and alkaline pH, indicating that nonspecific ALPases were responsible for the deposition of reaction product in all of these sites.

After incubation at pH 7.2 with TPP chloride as substrate (with no ALPase inhibitors in the medium), reaction product occurs in Golgi cisternae, GERL, some cytoplasmic granules, and along plasma membranes. In the presence of an ALPase inhibitor, the reaction of the Golgi complex is more or less unchanged, that of the plasma membrane is somewhat reduced, and that in GERL is abolished (Fig. 5). This indicates that the Golgi reaction results from TPPase action, whereas that in GERL appears to be related to ALPase activity. Persistance of a plasma membrane reaction under these conditions suggests that this organelle bears TPPase or another phosphatase capable of cleaving TPP chloride. With TPP chloride as substrate at pH 9, reaction product is observed scattered along plasma membranes and, rarely, in some Golgi cisternae. This activity appears to be only partially susceptible to inhibition by L-cysteine or L-*p*-bromotetramisole, suggesting that both TPPase and ALPase may be responsible for their deposition.

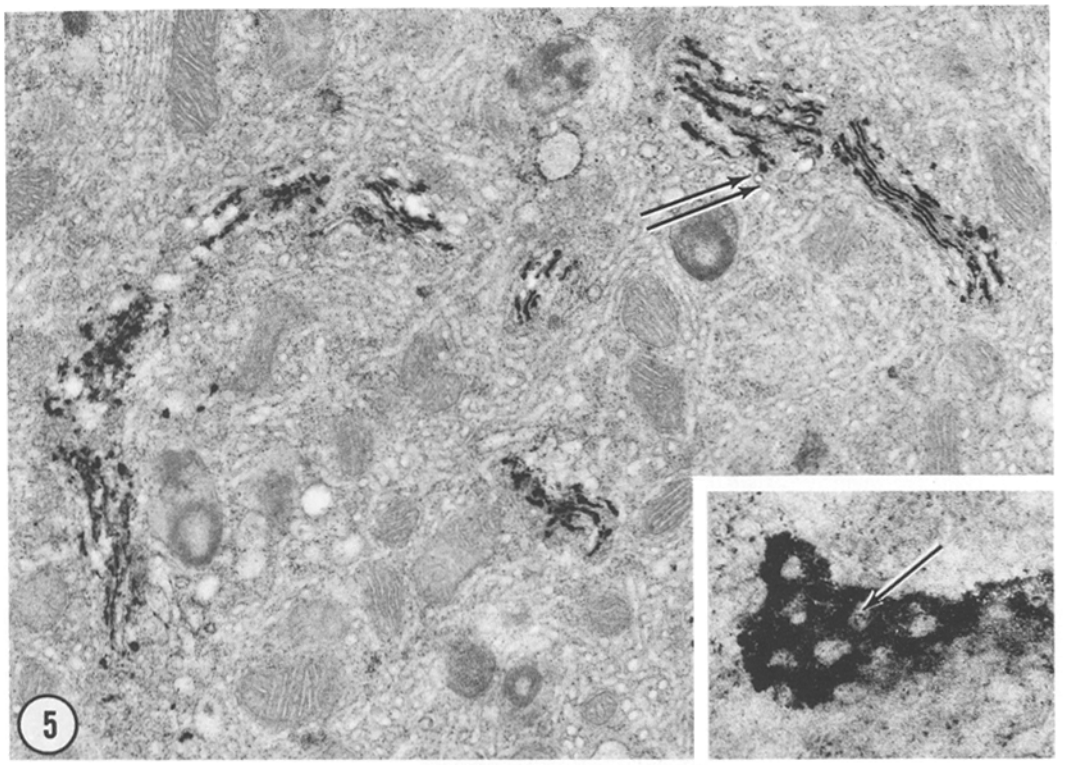
DISCUSSION

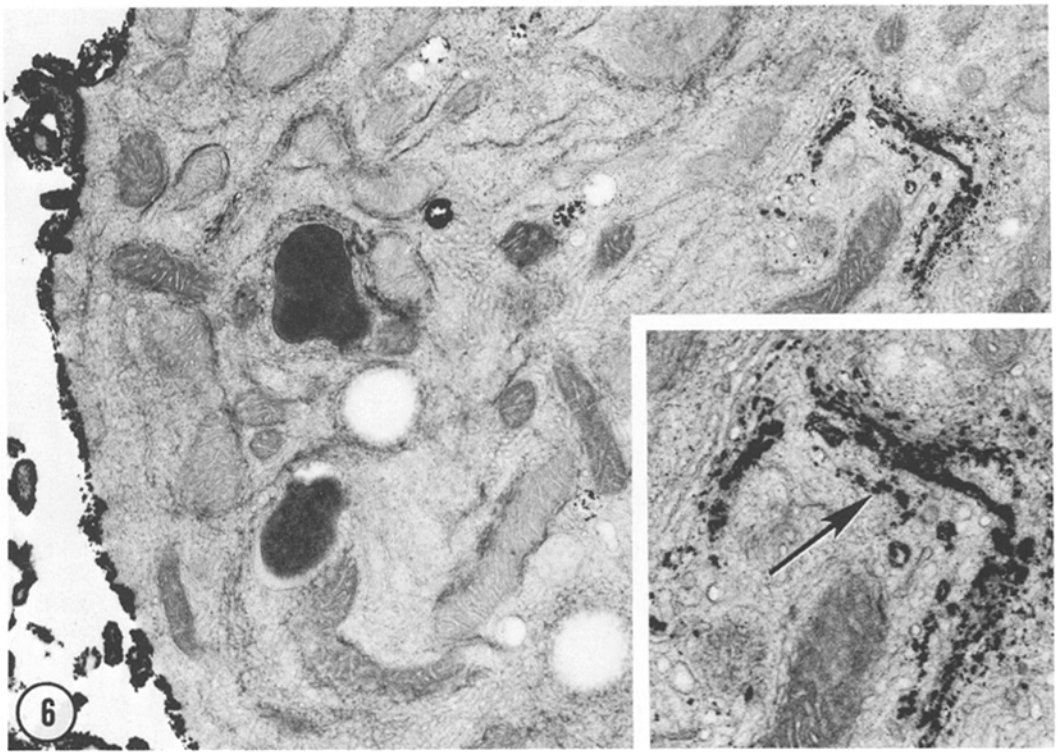
The present study is the first to describe in detail the cytochemistry of the Golgi complex, the structure which corresponds to GERL, and lysosomes at the time of peak progesterone secretion in luteal cells fixed by vascular perfusion. It defines GERL in luteal cells of this age and establishes a normal base line with which changes occurring during luteolysis can be compared.

The results of the present study indicate that, during the period of maximum progesterone release, the Golgi complex in luteal cells of pregnant guinea pigs shows cytochemical and staining properties different from those of most published studies. For example, ACPase activity occurs throughout all cisternae of the Golgi complex in these luteal cells. This finding contrasts with observations on the corpus luteum of the rat during late pregnancy (37) and of sheep during the estrous cycle (6, 38), where ACPase activity appeared to be confined to the inner cisternae of the Golgi complex or to GERL. Considerable evidence also indicates that ACPase activity is absent from the Golgi complex or is limited to the inner one or two cisternae in most nonovarian tissues as well (5, 9, 17–20, 26, 30, 36, 42, 54, 55). In only one study dealing with small neurons of mice (9) has a pattern of ACPase activity similar to that observed in the present work been described for the Golgi complex. Another unusual finding in the current work was the occurrence of TPPase activity in all cisternae of the Golgi complex in many luteal cells. In most other tissues that have been studied, TPPase activity in the Golgi complex is generally observed in the inner cisternae (9, 11, 13, 16, 17, 19, 24, 26, 45, 50). Equally unusual is the finding that prolonged treatment with OsO_4 stains all cisternae of the Golgi stack in these luteal cells. In many other tissues, this treatment preferentially stains the outer cisterna (13, 21, 36, 45, 51). Staining of all Golgi cisternae after prolonged osmication has been reported in only one other instance, epididymal cells of the hypophysectomized rat (19).

The role of the Golgi complex in luteal cells remains an enigma. One combined light microscope and biochemical study has suggested that maximal development of this organelle coincides with peak steroidogenic activity of the corpus luteum (39). In other cell types, this organelle participates in the packaging of secretory proteins (33–35), addition of carbohydrate residues to proteins (4, 53), and sulfation of glycosaminoglycans (56). In leukocytes, it is active in the formation of lysosomes (1, 2). Because the Golgi complex is markedly ACPase positive in luteal cells of the present study, one might speculate that it is involved in the manufacture of lysosomes. But, in cells other than leukocytes, the Golgi complex may not have a direct role in lysosome formation (41). Furthermore, unless a second acid hydrolase can be demonstrated in the Golgi complex, the functional significance of the observed ACPase activity remains obscure.

GERL, a series of unique smooth-surfaced ele-





ments located at the inner face of the Golgi complex, was first observed by Novikoff (40) in neurons of rat spinal ganglia, and subsequently described in various neurons (13, 30, 45) and other cells (42, 44, 50). In those studies and others, GERL displayed morphological and cytochemical characteristics that distinguished it from other smooth-surfaced structures in the cell. For example, GERL is limited by membranes that are often thickened (15, 28) or coated (30), and GERL cisternae may show dense content or central densities (27). In addition, GERL in some cases seemed to show possible continuities with the endoplasmic reticulum (44). In all of those studies, GERL possessed prominent ACPase activity after cytochemical incubation. Luteal cells of the current report contain certain smooth-surfaced elements, closely associated with the Golgi complex, which exhibit some of those features; e.g., they have thickened limiting membranes that are sometimes coated, and occasionally appear to be in continuity with ER-like structures. On the basis of these criteria and others (48), these Golgi-associated smooth elements in luteal cells appear to correspond to GERL in other cells. In contrast to previous observations on other cell types, GERL in luteal cells of this age commonly lacks ACPase activity (Fig. 3), or, if present (Fig. 4), the level of activity appears to be lower than that in other cells, including older luteal cells (48). These findings suggest that GERL may lack, or show low levels of, ACPase activity under certain conditions (concentration perhaps below that detectable by cytochemical methods).

It might be questioned whether an ACPase-negative cisterna should be called "GERL," since in the past this structure has been defined largely on the basis of its role in lysosome formation. Also, it is not clear whether all cells contain GERL systems. The ACPase-negative cisternae of the current report, however, are morphologically identical to cisternae that can be clearly identified as GERL; i.e., both cisternae occupy the same position in relation to the Golgi complex, both are narrow in width, and both have thickened limiting membranes. It seems possible that the nonreactive GERL cisternae described here represent a different physiological state of GERL, and that their lack of activity reflects a period of relative quiescence in the packaging of acid hydrolases into lytic bodies. The overall low ACPase activity of GERL in luteal cells of this age may be related to a modest level of lysosome production, a possibility given some support by the relative paucity of lysosomes and autophagic vacuoles in these cells. The more intense ACPase activity in GERL of occasional luteal cells may represent a sporadic production of lysosomes, or incipient cell death, since some luteal cells die during pregnancy (47). It thus appears that, at the time of peak steroid secretion, the GERL system in luteal cells is not particularly well developed.

Other possible explanations for the lack of activity in GERL of these luteal cells should be considered. It might be claimed that GERL lacks ACPase activity because the enzyme has been inactivated by the aldehydes used to fix the tissue. However, in cells that contain nonreactive GERL cisternae, the Golgi complex shows pronounced activity, making that possibility unlikely, unless the ACPases in these two organelles differ in their sensitivity to the fixative. Furthermore, during regression of these cells, GERL is strongly reactive for ACPase (48); therefore, unless this enzyme has different properties in luteal cells of different ages, it seems likely that the low activity observed in the present study represents existing levels. It could also be suggested that GERL cisternae are so closely applied to a Golgi stack that they are mistaken for Golgi saccules. It is, however, possible to distinguish GERL from Golgi cisternae by their TPPase content and affin-

FIGURE 5 Golgi complex, reacted for TPPase, pH 7.2 (with L-*p*-bromotetramisole included in the medium). Reaction product can be found in all Golgi cisternae; GERL (arrows), however, is nonreactive for this enzyme. × 19,000. (*inset*) Reaction product outlines the fenestrations (arrow) in this en face view of the inner element. × 70,000.

FIGURE 6 Luteal cell, reacted for ALPase at pH 9, β-glycerophosphate as substrate. Reaction product typically accumulates along the external surface of the plasma membrane, in some subplasmalemmal vacuoles, as well as in GERL and some Golgi cisternae. 30 days of gestation. × 18,000. (*inset*) At higher magnification, it can be seen that GERL (arrow) is ALPase reactive, as are the inner Golgi cisternae, but the outer Golgi cisternae are nonreactive. × 29,000.

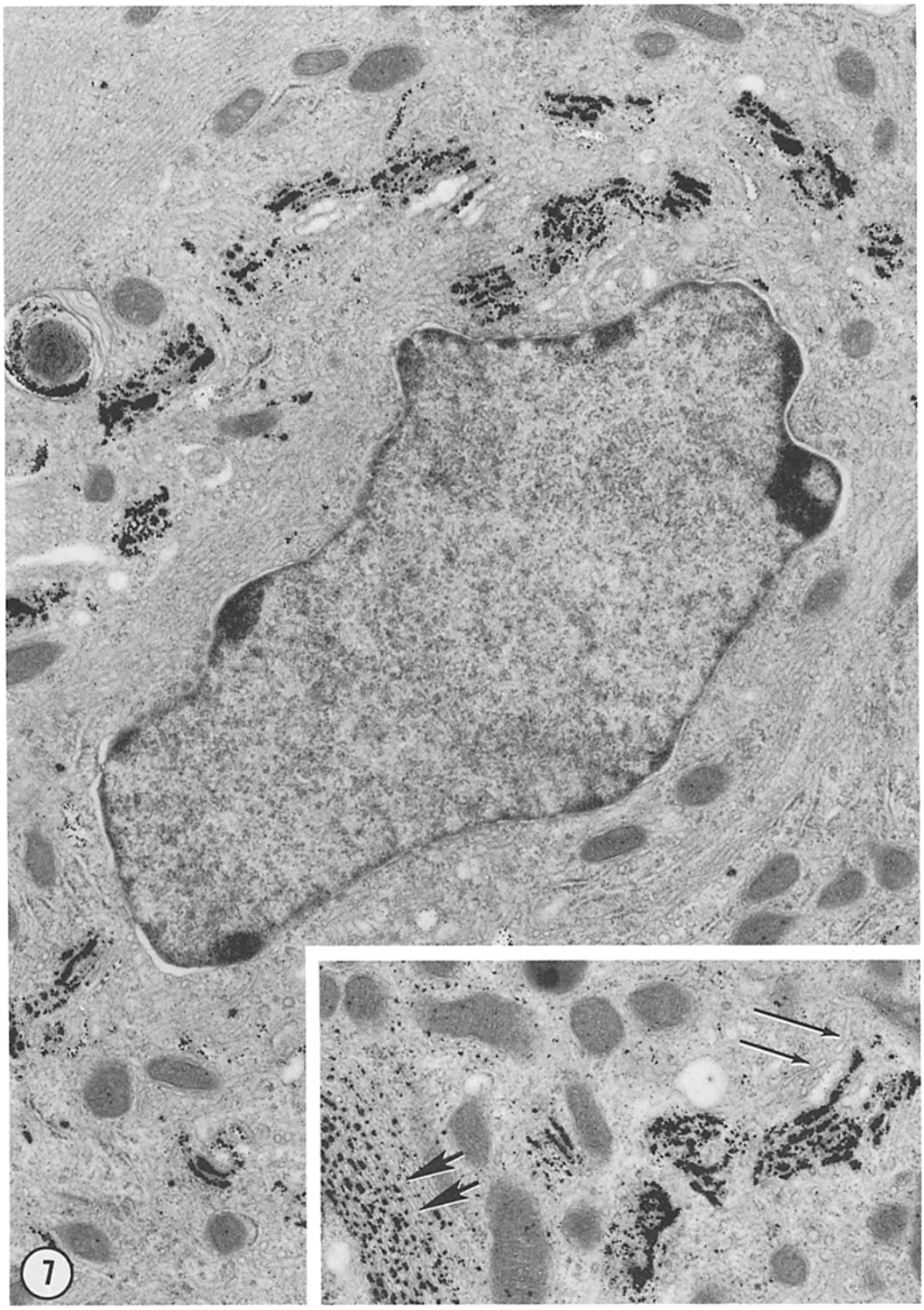


FIGURE 7 Luteal cell, after prolonged treatment with OsO_4. All cisternae of the several Golgi stacks in this cell show the characteristic staining with osmium. 26 days of gestation. × 16,000. (*inset*) In addition to Golgi cisternae, smooth ER (short arrows) may also be stained by this treatment, but GERL cisternae (long arrows) remain unstained. × 21,000.

ity for OsO_4, since certain Golgi cisternae stain after these procedures (noted above), whereas GERL does not (9, 13, 16, 26, 45, 50). After use of these methods, the same number of cisternae stain (Figs. 5 and 7) as do after ACPase incubation (Fig. 3), indicating that GERL cisternae are not overlooked by virtue of a close relationship to a Golgi stack.

Luteal cells of many species display a heterogeneous population of cytoplasmic granules. The identity of these various granules is best confirmed by cytochemistry since they are often difficult to classify in conventional electron micrographs. The current results indicate that guinea pig luteal cells at 25–33 days of gestation contain occasional lysosomes (reactive for acid hydrolases) and a number of multivesicular bodies and electron-transparent vacuoles. In addition, catalase-positive microperoxisomes are present in these cells (Paavola, unpublished observations). Luteal cells of some species have inclusions thought to contain relaxin. Such a class of granules could not be identified in this study. Guinea pig serum shows little relaxin before day 40 of pregnancy; subsequently, serum levels increase markedly (46). It is possible that relaxin-bearing inclusions are present in insufficient numbers at the age examined here to be readily detected in electron micrographs.

In summary, luteal cells at the peak of functional activity have a well-developed, strongly ACPase-positive Golgi complex, a modest GERL system that lacks or shows low levels of ACPase activity, and relatively few lysosomes. These results provide a normal basis of comparison for identifying changes in these cells related to involution, which is the subject of the companion paper (48).

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