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

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ABSTRACT

The postpartum involution of corpora lutea was examined by electron microscope cytochemistry of guinea pig ovaries previously fixed by vascular perfusion, a method which produces optimal preservation of steroid-secreting cells and yet maintains enzyme activity. The intracellular digestive apparatus was identified through the localization of two acid hydrolases, acid phosphatase (ACPase) and arylsulfatase. Other marker enzymes localized were thiamine pyrophosphatase (in Golgi cisternae) and alkaline phosphatase (along plasma membranes). Prolonged osmication was used to mark the outer Golgi cisterna. The results demonstrate that luteal cell regression is characterized by a striking increase in the number of lysosomes and the appearance of numerous, double-walled autophagic vacuoles. Both lysosomes and the space between the double walls of autophagic vacuoles exhibit ACPase and arylsulfatase activity. In contrast to earlier periods, just before and during regression, Golgi complex-endoplasmic reticulum-lysosomes (GERL) is markedly hypertrophied, displaying intense acid hydrolase activity. On the basis of various criteria, GERL is proposed to function in the formation of lysosomes and autophagic vacuoles. Lysosomes seem to develop from GERL as focal protuberances of varying size and shape, which detach from the parent structure. Double-walled autophagic vacuoles, often large and complex in structure, initially are produced as GERL cisternae envelop small areas of cytoplasm. Lytic enzymes, perhaps furnished by the engulfing membranes and trapped lysosomes, presumably bring about digestion of the contents of these vacuoles, producing first aggregate-type inclusions, then, as the contents are further degraded, myelin figure-filled residual bodies. ACPase activity occasionally appears within smooth endoplasmic reticulum tubules and cisternae in advanced regression, possibly suggesting that lytic enzymes utilize this membrane system as an access route to GERL. These data indicate that cellular autophagy is a prominent mechanism underlying luteal cell involution during normal postpartum

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degeneration of guinea pig corpora lutea. Furthermore they suggest that in regressing luteal cells GERL is responsible for packaging acid hydrolases into lytic bodies.

KEY WORDS luteal cell involution · autophagy · electron microscope cytochemistry · acid hydrolases · GERL · lysosomes · Golgi complex

The degenerating corpus luteum of the guinea pig is an ideal system in which to study cell death as it occurs under physiological conditions, since this tissue involutes in response to hormones produced by the uterus. Furthermore, the decline of the corpus luteum in the guinea pig is gradual (39), permitting the mechanisms underlying cellular regression to be examined in detail.

Even though lysosomes and autophagic vacuoles have been noted in degenerating luteal cells of various animals, little attention has focused on the importance of autophagy in luteolysis. Moreover, studies dealing with this topic have often come to differing conclusions. For example, van Lennep and Madden (43) in a study on human corpora lutea reported that autophagy was probably only a minor component in the overall process of involution; in contrast, it was proposed in two studies on sheep that autophagy played a substantial role in the destruction of corpora lutea (18, 30). Our recent observations on guinea pigs (39) indicate that, as luteal cells age during normal postpartum luteolysis, they accumulate large numbers of lysosome-like dense bodies and apparent autophagic vacuoles, suggesting that autophagy might indeed be an important aspect of luteolysis. The lytic nature of these bodies, however, was not confirmed in that report. Whether lysosomes proliferate in luteal cells during involution also remains ambiguous, since aging luteal cells have been reported to contain lysosomes in both increased (3, 5, 27, 41) and unchanged (18) numbers.

The genesis of autophagic vacuoles is of particular interest since their origin has not been clearly established for most tissues, including the corpus luteum. Several organelles have been implicated as the source of autophagic vacuoles in nonovarian tissues. They include the Golgi complex (17, 28), smooth endoplasmic reticulum (smooth ER) (1, 11, 13, 35), and a specialized region of smooth ER, termed Golgi complex-endoplasmic reticulum-lysosomes (GERL) (see Review in reference 32). Quatacker (42) has proposed that the membranes enclosing autophagic vacuoles in regressing human corpora lutea arise from plasma membranes. On the other hand, in the earlier report from our laboratory (39), it was suggested that most autophagic vacuoles in involuting luteal cells of guinea pigs are derived from GERL. It should also be noted that even though lysosomes may be involved in luteal cell degeneration, their origin has not been demonstrated previously in these cells.

It thus seems important to evaluate the role of lytic bodies in luteal cell destruction and to determine how they arise. Such information could be gained through the localization of appropriate marker enzymes, e.g., acid phosphatase (ACPase) or arylsulfatase, in luteal cells by electron microscope cytochemistry. This approach should identify which structures participate in luteolysis and may also reveal their source. However, as noted in the companion paper (40), few cytochemical studies on luteal cells exist (18, 29, 30), primarily because it is difficult to prepare these cells for electron microscopy in a way that preserves both fine structure and enzyme activity. This problem has been resolved in the present study by using tissue fixed by vascular perfusion, a technique capable of optimally preserving steroid-secreting cells (4), yet maintaining enzyme activity (9, 19).

In this report, the degenerative processes that underlie luteal cell senescence and death are studied by electron microscope cytochemistry. Lysosomes and other structures belonging to the intracellular digestive apparatus were identified by cytochemical incubation for ACPase and arylsulfatase. The localization of both enzymes was necessary, since proof that a structure is involved in intracellular digestion rests on the demonstration that it contains more than one acid hydrolase (7). Both the Golgi complex and plasma membrane have been proposed as sources of autophagic vacuoles; thus, procedures were carried out for the localization of thiamine pyrophosphatase (TPPase) (a marker for inner Golgi cisternae) and alkaline phosphatase (ALPase) (a plasma membrane marker), and the tissue was subjected to prolonged treatment with aqueous OsO_4 (marks the outer Golgi cisterna).

Among other observations, the findings of the present study demonstrate that, during involution,

acid hydrolase activity is prominent in GERL, in lysosomes, and between the double walls of autophagic vacuoles. Furthermore, both lysosomes and autophagic vacuoles appear to arise from GERL in regressing luteal cells of guinea pigs.

MATERIALS AND METHODS

Corpora lutea were obtained from a total of 36 Hartley guinea pigs: 10 at 60–68 days of gestation (near term), 3 on the day of parturition, and in the postpartum period, 7 at 5 days, 1 at 8 days, 6 at 10 days, 6 at 16 days, and 3 at 20 days. The fixatives, method of fixation, and incubation conditions (including controls) used for the various cytochemical procedures were identical to those described in the companion report (40).

RESULTS

The fine structure of luteal cell regression after pregnancy in guinea pigs has been described in detail in an earlier publication from this laboratory (39). The following sections, which describe cytochemical reactions of the Golgi complex, GERL, autophagic vacuoles, and lysosomes, include pertinent morphological details as background for the cytochemistry. It should be noted that luteal cells at a similar stage of regression, regardless of the actual age of the corpus luteum, show identical cytochemical staining properties. Control incubations proved to be negative for the various cytochemical reactions described below.

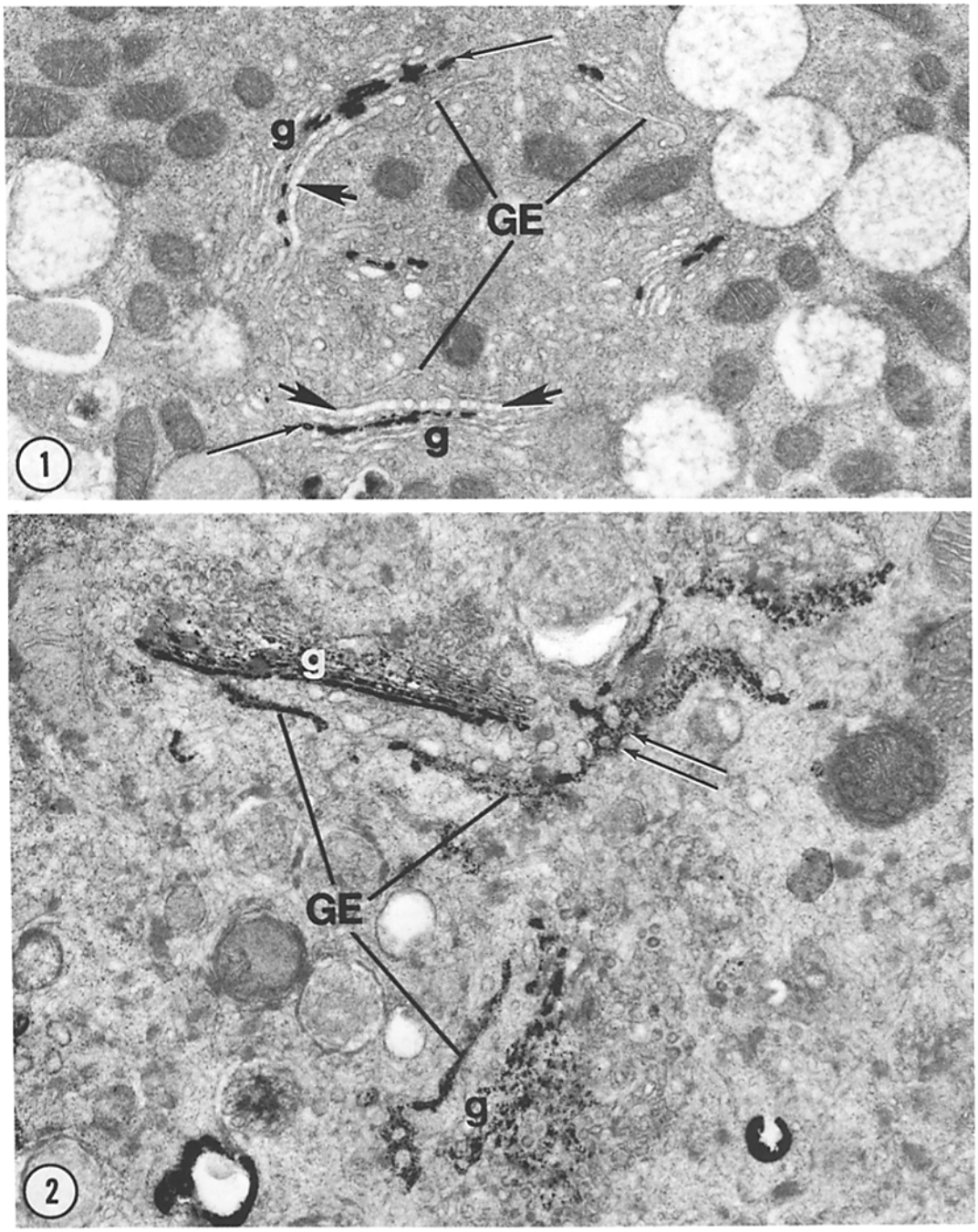
Golgi Complex

The Golgi complex in regressing luteal cells remains well developed. The Golgi stacks and their constituent cisternae display an ultrastructure similar to that described for luteal cells at 25–33 days of gestation (40). After cytochemical incubation for ACPase activity, reaction product is observed in Golgi cisternae (Figs. 4*a*, *c–e* and 5), all of which show activity to a degree that is uniform for a given experiment. The reaction varies from scant to moderately intense, and is usually less pronounced than that in GERL (see below). Inner Golgi cisternae are reactive for TPPase (Fig. 1), but occasionally other cisternae may exhibit activity. The Golgi complex also shows ALPase activity; usually the reaction is most intense in the inner cisterna (Fig. 2). Although Golgi cisternae in aging luteal cells tend not to stain after prolonged osmication, the outer cisterna sometimes does. Occasionally, a slightly widened cisterna (or tubule) or unknown origin is interposed between the inner element of a Golgi stack and GERL (Fig. 3*b*). This cisterna is devoid of both ACPase (Fig. 4*d*) and TPPase (Fig. 1) activity. It is clearly distinguishable from GERL since it is expanded in width and has "thin" limiting membranes, whereas the cisternae of GERL, as described below, are usually narrow and have thickened membranes.

GERL

Even though luteal cells are filled with smooth ER, certain smooth-surfaced cisternal elements near the Golgi complex exhibit morphological and cytochemical features that suggest that they are analogous to the GERL system described in neurons (36) and elsewhere. Since this GERL-like system is hypertrophied at this stage, it will be more fully characterized here than elsewhere (39, 40). These GERL-like cisternae are situated next to, or some distance from, the inner (trans) face of a Golgi stack (Fig. 3). The intervening cytoplasm often contains small tubules or vesicles (Fig. 3*a*) that do not occur between Golgi cisternae. In aging luteal cells as in other cell types (14, 20, 21, 22), these GERL-like cisternae show specializations that serve to distinguish them from other smooth membrane-bounded organelles; e.g., they may contain moderately dense material (Fig. 3*d*) and/or a central dense line (Fig. 4*f*); they often display a "thickened" (denser) limiting membrane (Fig. 3*a*), which sometimes appears to be coated (Fig. 3*c*); and, they often are narrower in width (about 25 nm) than are tubules (50–60 nm) or cisternae (40–45 nm) of smooth ER. Although their origin remains uncertain, the possible derivation of these GERL-like cisternae from ER is suggested by their occasional continuities with ER (Figs. 3*b*, *d*, and 4*a*). The structures, with which GERL is continuous, are judged to be ER by their characteristic diameter (45–60 nm), pale contents, thin limiting membranes, position outside the Golgi stack, and by their similarity to other smooth ER in the vicinity (and by exclusion, since alternative possibilities are not readily obvious). Finally, these cisternae display cytochemical properties (see below) similar to those of GERL systems described elsewhere (36). Thus, smooth-surfaced structures conforming to these criteria will be considered to constitute GERL in luteal cells.

In striking contrast to that in 25–33 days of pregnancy (40), GERL in regressing luteal cells



FIGURES 1 and 2 Golgi complex, cisterna of unknown origin, and GERL of aging luteal cells, showing typical reaction for TPPase and ALPase. All cytochemical material shown in this report has been stained en bloc only.

FIGURE 1 The inner cisternae of the Golgi complex (*g*) typically show (long arrows) TPPase activity. In contrast, both the cisterna of unknown origin (short arrows) and GERL (*GE*) are negative for this enzyme. Near term. × 20,000.

FIGURE 2 After incubation for ALPase activity (pH 9, with β-glycerophosphate as substrate), reaction product characteristically fills the inner one or two cisternae of the Golgi stack (*g*); the remaining saccules exhibit scattered deposits. GERL (*GE*), which seems to follow a twisting course through the cytoplasm and sometimes bears fenestrations (arrows), is also strongly ALPase-reactive. Near term. × 25,000.

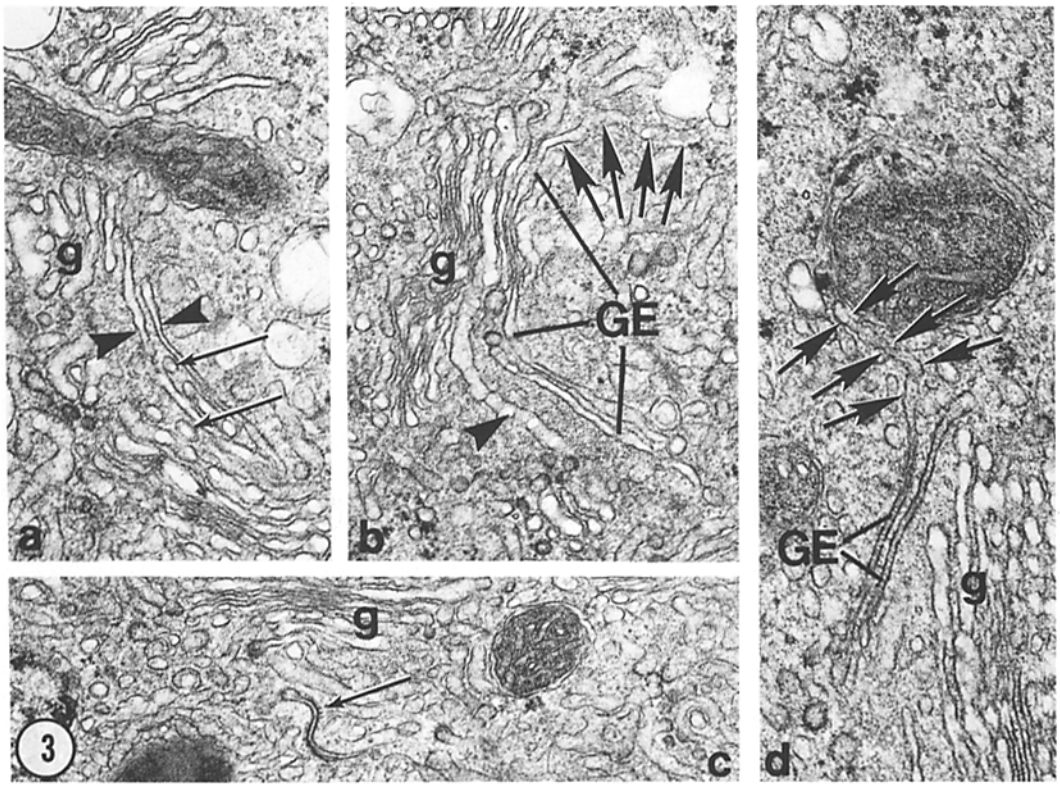
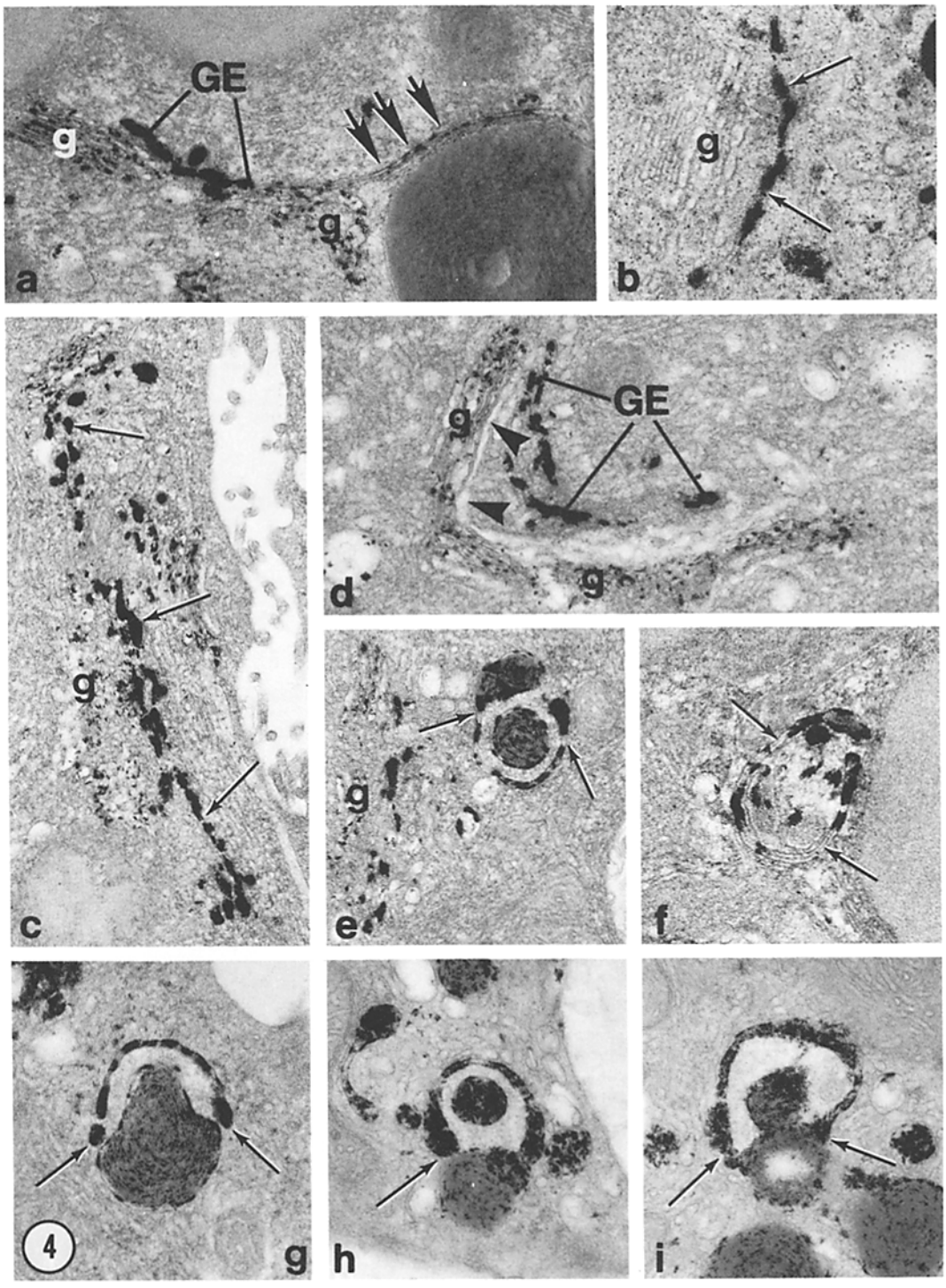


FIGURE 3 GERL from regressing luteal cells, illustrating the unique, fine-structural features that distinguish it from other smooth membranes in luteal cells. (*a*) GERL (between arrowheads) frequently has limiting membranes that are "thicker" (denser) than those of many other cytoplasmic organelles, including smooth ER. Small vesicles (arrows) occur between GERL cisternae, and between GERL and Golgi cisternae (*g*), but not between Golgi saccules. × 38,000. (*b*) Images in which GERL (*GE*) appears to be in continuity with a tubule of smooth ER (arrows) suggests the possibility that GERL derives from ER. Note that GERL is separated from the Golgi stack (*g*) by a widened cisterna (arrowhead), whose origin is uncertain. × 33,000. (*c*) GERL membranes (arrow) are sometimes coated, whereas Golgi cisternae (*g*) and smooth ER membranes have not been observed to bear a similar coating. Note the density of the membranes that limit GERL. × 29,000. (*d*) One of the two GERL cisternae (*GE*) shown here appears to be continuous with a tubule of smooth ER (between arrows), again suggesting the possibility that GERL takes origin from ER. Note that these GERL cisternae contain a moderately dense material, whereas Golgi cisternae (*g*) are electron-transparent, and smooth ER tubules and cisternae have a pale appearance. × 50,000. Note the narrowed width of GERL in figures *a*, *c*, and *d*. All, near term.

consistently displays intense ACPase activity (Figs. 4*a*, *c*, *d*, and 5*d* and *e*). This strong ACPase reaction emphasizes the hypertrophied condition of GERL in these cells. Dense accumulations of reaction product fill small (coated or smooth) vesicles (Fig. 5*d*) and larger vacuoles (Fig. 5*e*), which presumably represent nascent lysosomes, that project from GERL. Circular and U-shaped profiles of GERL cisternae, containing reaction product between their double walls, are noted close to (Fig. 4*e*) as well as some distance from (Fig. 4*f* and *g*) Golgi stacks. A central dense line is observed in some GERL cisternae even in cytochemical preparations (Fig. 4*f*). U-shaped GERL profiles are noted near (Fig. 4) and in continuity with (Fig. 4*h* and *i*) dense bodies. In addition to ACPase, GERL shows intense arylsulfatase activity (Fig. 4*b*). GERL is also strongly reactive for ALPase activity (Fig. 2). As at 25–33 days of gestation (40) and as in other cell types, neither incubation for TPPase activity (Fig. 1) nor prolonged immersion in aqueous OsO_4 results in staining of GERL in regressing luteal cells.

Lysosomes and Autophagic Vacuoles

When compared with luteal cells during the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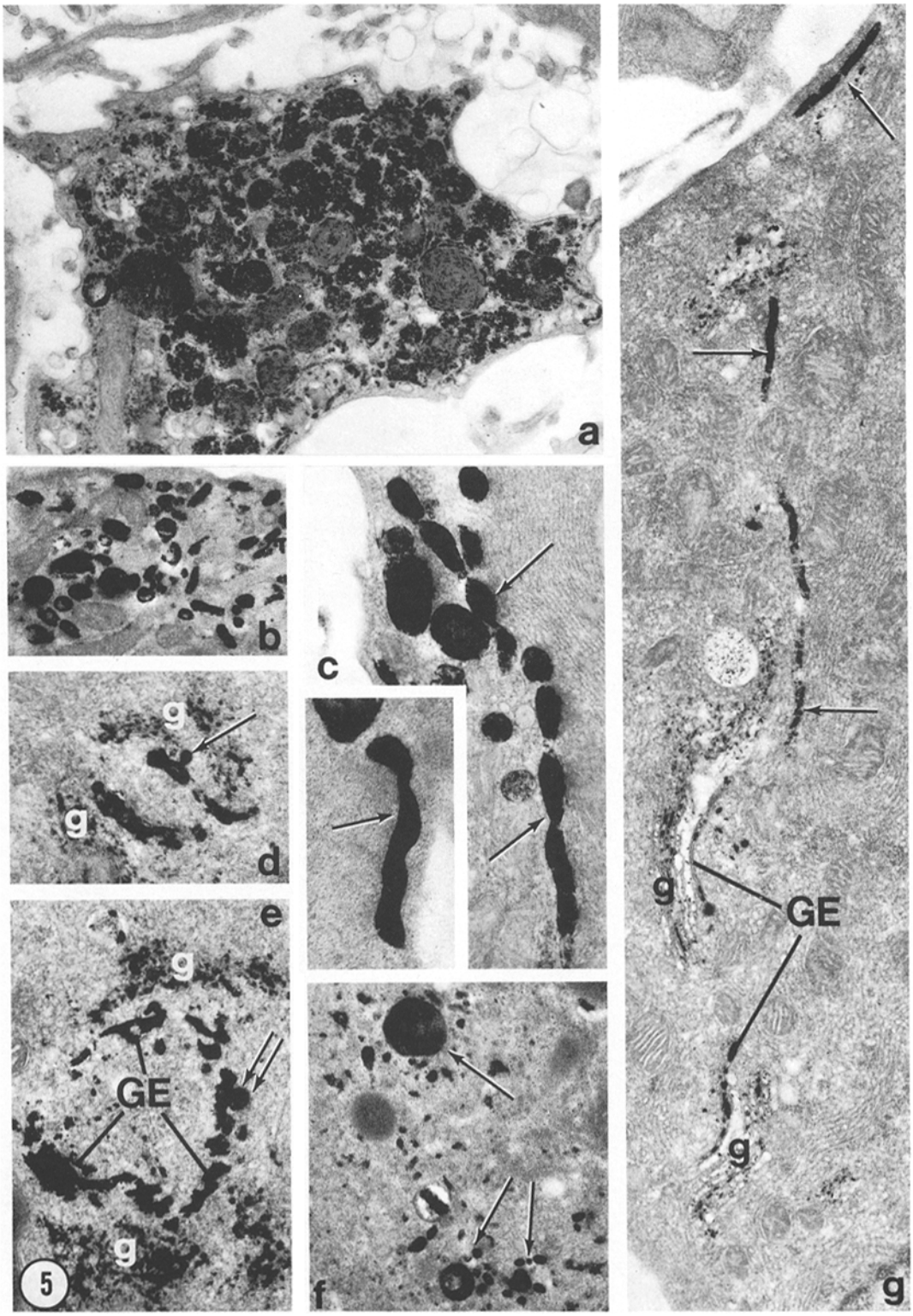
period of maximal progesterone secretion (40), aging luteal cells contain markedly increased numbers of lysosomes (Fig. 5*a*). These bodies are ACPase (Fig. 5*a*) and arylsulfatase (Fig. 5*b*) reactive. Throughout involution, they are often observed trapped in autophagic vacuoles (Fig. 6*h*). As regression proceeds, highly pleomorphic lysosomes appear, including the unusual beaded (Fig. 5*c*) and elongated (Fig. 5*g*) types. Elongated lysosomes sometimes occur in proximity to GERL (Fig. 5*g*). Frequently, several small, acid hydrolase-positive vesicles are noted surrounding a larger reactive body in the Golgi-GERL region (Fig. 5*f*).

In contrast to earlier stages (40), autophagic vacuoles are prominent in luteal cells during involution (Fig. 6*a*). These vacuoles are unusually pleomorphic in the guinea pig (39), and constitute several more or less distinct groups, each of which has been identified by a descriptive phrase to simplify reference to it: intricate (Fig. 6*c*), double-membrane (Fig. 6*f*), and simple (Fig. 6*i*). For further details concerning these bodies, see Paavola (39). The present study reveals that the first two types are stongly reactive for ACPase and arylsulfatase (Fig. 6*a*, *b*, *d*, and *g*), whereas the simple form is typically nonreactive (Fig. 6*j*). It is striking that the predominant type of autophagic vacuoles in regressing luteal cells is enclosed by double membranes. After cytochemical incubation for acid hydrolases, reaction product fills the space between the double walls of these vacuoles (Fig. 6). In instances where the reaction product is less dense, the space between the double walls, as well as the central dense line of intricate (Fig. 6*d* and *e*) and double-membrane (Fig. 6*h*) type autophagic vacuoles, can be discerned, confirming that these acid hydrolase reactive inclusions are identical to those described previously in conventional preparations (39). The material filling the space between the double walls is sometimes not apparent in cytochemically reacted tissue, probably because the tissue was stained en bloc only. Images seen in the present study suggest that, as the contents of double-walled autophagic vacuoles are digested, they are transformed into aggregate-type inclusions (Fig. 7), which through further digestion eventually become residual bodies. The possible interrelationships between these various autophagic vacuoles, GERL and lysosomes are summarized in Fig. 8.

ACPase reaction product is sparse in multivesicular bodies and does not occur free in the cytoplasm of involuting luteal cells before their engulf-

FIGURE 4 GERL from involuting luteal cells, reacted for acid hydrolase activity. After cytochemical incubation for lytic activity, deposits of lead phosphate fill GERL cisternae, clearly revealing its morphology and extent. Golgi stacks, which exhibit varying degrees of activity, are indicated in each figure at (*g*). (*a*) The possibility of connections between GERL and ER, such as those in Fig. 3*b* and *d*, are also suggested in cytochemical preparations. The GERL (*GE*) cisterna shown here appears to be in continuity with an ER-like structure (arrows). Reacted for ACPase. × 31,000. (*b*) After incubation for arylsulfatase activity, reaction product is accumulated in GERL (arrows), demonstrating that this organelle possesses more than one acid hydrolase. × 47,000. (*c*) In contrast to that in younger cells, GERL as shown here is hypertrophied in aging luteal cells. Reacted for ACPase. × 23,000. (*d*) Occasionally, a nonreactive cisterna (arrowheads) of unknown origin separates GERL from a Golgi stack, in instances when both GERL (*GE*) and Golgi cisternae (*g*) show lytic activity. Reacted for ACPase. × 29,000. (*e*) This U-shaped GERL cisternae (between arrows) is situated near a Golgi stack. From an examination of serial sections, it was determined that similar U-shaped profiles are derived from cup-shaped bodies. Reacted for ACPase. × 25,000. (*f*) In lightly reacted specimens, the central dense line (arrows) of GERL cisternae can be discerned. The moderately dense material that fills the cisternal space is not always readily apparent in cytochemical preparations, probably because the tissue was stained en bloc only. Reacted for ACPase. × 37,000. (*g*) U-shaped GERL profiles (arrows) may be located some distance from a Golgi stack, sometimes occurring in close proximity to dense bodies. Reacted for ACPase. × 32,000. (*h*) In some instances, one edge (arrow) of a U-shaped GERL profile appears to be unattached to a dense body. Observations on serial sections indicated that such images represent a cup-shaped structure attached by one lip to a (usually spherical) dense body. Reacted for ACPase. × 19,000. (*i*) Both sides of a U-shaped profile (GERL cisterna) can be attached to a dense body. Here again, a study of serial sections indicated that the U-portion of such structures represents a completely closed (most likely) or a partially closed (less likely) cup. Reacted for ACPase. × 19,000. (*a*) 5 days postpartum; (*b* and *f*) 10 days postpartum; (*c–e* and *g–i*). Near term.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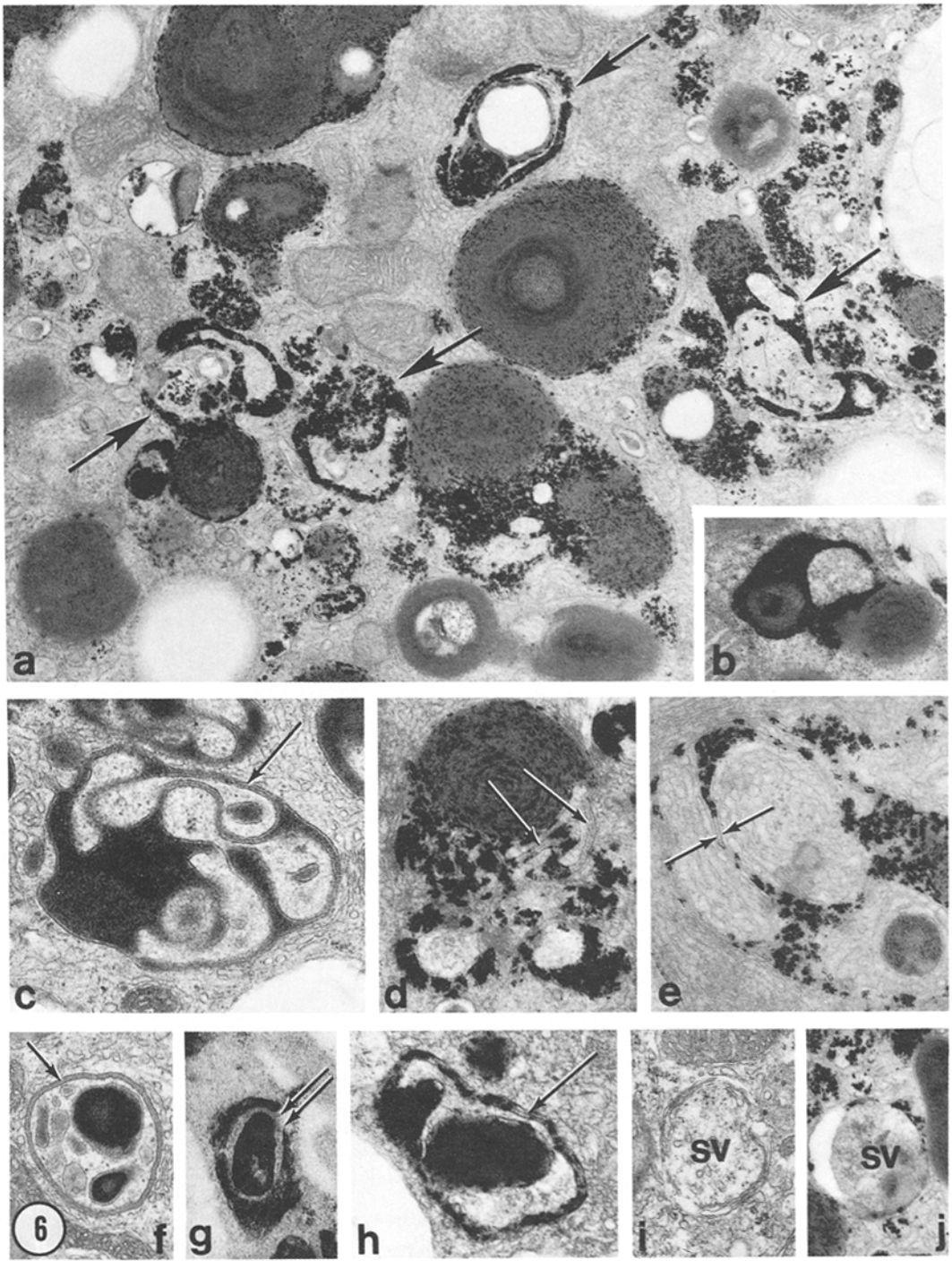
ment by macrophages. Neither lysosomes nor autophagic vacuoles show TPPase or ALPase activity, and only rarely stain after prolonged treatment with OsO_4.

The complex relationships between GERL, autophagic vacuoles and lysosomes require further comment. It might be claimed that the double-membrane type of autophagic vacuole (Fig. 6*f*) is merely the result of an appropriate plane of section through an intricate-type autophagic vacuole (Fig. 6*c*) or through a cup-shaped GERL cisterna (Fig. 4*e*). In a similar vein, it might be suggested that the compartments within intricate-type vacuoles are merely surface depressions that contain tongues of cytoplasm. 90 consecutive serial thin sections were used to examine these possibilities. Even though some data concerning this point were presented previously (39), more extensive data are offered here to clarify further these relationships. 62 double-membrane type vacuoles (Fig. 6*f*) were followed in the serial sections, and were examined: all were roughly sphere-shaped, completely closed, discrete entities, unattached to other inclusions. Their interiors were not subdivided into compartments. 87% of these double-walled inclusions contained dense bodies, other autophagic vacuoles, or GERL cisternae, that were not attached to their walls. 40 intricate-type vacuoles (Fig. 6*c*) were studied: they also were independent structures, whose multiple compartments were completely walled off from the surrounding cytoplasm, and contained various inclusions. 45 GERL-type cisternae, exhibiting central densities and dense contents, were examined: 73% were cup-shaped; 20% were almost completely closed spheres with a small opening communicating with the cytoplasm, suggesting the possibility of eventual closure; and, 7% were slightly curved discs. A number of cup-shaped GERL-like structures were observed in partial continuity with dense bodies, being attached by one edge to a dense body (such a body could be represented by Fig. 4*h*, where one edge of a cisternal-like structure appears unattached). In addition, 150 lysosome-like dense bodies of varying shape, size, and electron-opacity were studied in these serial sections; all were separate entities and the most common shape was a sphere. Also noted were elongated and beaded lysosomes that extended for considerable distances through the cytoplasm. Some of these lysosomes were observed attached to or surrounding lipid droplets. These results clearly demonstrate that these various structures can exist as independent entities, entirely separate from each other.

Some Comments on the Cytochemical Behavior of the ER and Plasma Membrane

In regressing luteal cells, as at earlier stages, the smooth ER is nonreactive for both TPPase and ALPase, shows glucose-6-phosphatase activity (Paavola, unpublished observations), and can stain after extended treatment with OsO_4. However, in marked contrast to the time of maximal progesterone secretion (40), the smooth ER of an occasional terminally regressing luteal cell displays intense ACPase activity (Fig. 7). Such a finding, to the best of my knowledge, has not been previously reported in luteal cells. Luteal cells, other

FIGURE 5 Regressing luteal cells, showing proliferation of lysosomes, pleomorphic lysosomes, and the relationship of developing lysosomes to GERL. GERL is indicated by *GE* and Golgi stacks by *g* in these figures. (*a* and *b*) The dramatic increase in quantity of the common spherically shaped lysosome in aging luteal cells is illustrated in this figure from an ACPase preparation; large numbers of reactive bodies have accumulated in an armlike extension of luteal cell cytoplasm. These bodies display ACPase (*a*) and arylsulfatase (*b*) activity. × 17,000; 19,000. (*c*) Highly pleomorphic lysosomes appear during involution, including beaded types that show both ACPase (arrows) and arylsulfatase (*inset*, arrow) activity. × 22,000, (*inset*) 37,000. (*d* and *e*) During luteolysis, GERL cisternae frequently display small vesicles (*d*, arrow) or larger vacuoles (*e*, arrows) attached to their membranes. These protuberances show intense lytic activity, thus appearing to represent developing lysosomes. Both, reacted for ACPase. × 37,000; 29,000. (*f*) Clusters of small acid hydrolase-positive vesicles commonly surround, and through fusion may possibly give rise to, larger reactive bodies (arrows) in aging luteal cells. Reacted for arylsulfatase. × 21,000. (*g*) Occasional elongated lysosomes (arrows) are noted in close association with GERL, leading to the speculation that such bodies may possibly originate from GERL cisternae. Reacted for ACPase. (*a* and *g*) Near term; (*b–f*) 5 days postpartum.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than those that are in a state of advanced regression, do not show ACPase activity within smooth ER tubules or cisternae.

After incubation for ALPase at pH 9 with Na-β-glycerophosphate as substrate, the plasma membrane shows deposits of reaction product along its external surface. The cytochemical reactions of the plasma membrane for ALPase and TPPase under diverse conditions (varying pH, substrates, inhibitors) are similar to those described for luteal cells at 25–33 days of gestation (40).

DISCUSSION

In the present study, the cytochemical events underlying the normal involution of luteal cells in guinea pigs after pregnancy are described in material fixed by vascular perfusion. The decline and death of luteal cells in this animal are brought about by hormones produced by the uterus; thus these findings provide further insight into how cells regress in response to physiological signals. This report also offers evidence on the origin of autophagic vacuoles and lysosomes in degenerating luteal cells, and on the possible pathways that acid hydrolases follow en route to these lytic bodies.

The fine structural events leading to the postpartum destruction of corpora lutea in guinea pigs were detailed in a previous publication from this laboratory (39), in which it was suggested that the most striking changes occurring in luteal cells during involution were the accumulation of apparent autophagic vacuoles and the dramatic increase in the number of lysosome-like dense bodies. The cytochemical findings reported here substantiate that claim by establishing that these bodies contain ACPase and arylsulfatase. The localization of both enzymes was necessary, since a structure cannot be regarded as a true digestive body unless it is shown to contain more than one acid hydrolase (7). Because these inclusions satisfy that condition, they thus belong to the intracellular digestive apparatus and probably represent the morphological bases for autolysis.

The extent to which lytic bodies are involved in regression of corpora lutea has remained elusive. For example, although autophagic vacuoles of widely differing morphology have been described in ultrastructural studies on luteal cells of various animals (see Reference 39), their actual participation and relative importance in luteolysis are still uncertain. Similarly, the role of lysosomes in luteolysis is disputed. Two recent cytochemical studies on regressing corpora lutea of sheep at the end of the estrous cycle came to differing conclusions: Gemmell et al. (18) maintained that lysosomes were no more abundant during involution than at other times, whereas McClellan et al. (30) reported that they increased markedly during regression. The marked proliferation of autophagic vacuoles and lysosomes in degenerating luteal cells of the present study provides compelling evidence that in guinea pigs they are involved in luteolysis, and substantially strengthens the claim that autophagy is a prominent mechanism in

FIGURE 6 Autophagic vacuoles in regressing luteal cells. (*a*) The marked accumulation of autophagic vacuoles that takes place in luteal cells during involution is clearly illustrated in this larger field from an ACPase preparation. Note that in these cells the predominate type of autophagic vacuole is enclosed by double walls (arrows). × 18,000. (*b–e*) The interior of the most elaborate of the autophagic vacuoles in luteal cells, the intricate type, is divided into compartments. The double walls of this vacuole typically enclose a moderately dense material and a central dense line (*c*, arrows). Incubation for arylsulfatase (*b*) or ACPase (*d*) reveals that lytic enzymes are located between the double walls and in the dense portions of this vacuole. The central density (*d*, arrows) and the space separating the double walls (*e*, arrows; reacted for ACPase) are also evident in lightly-reacted cytochemical specimens. × 28,000; 24,000; 23,000; 25,000. (*f–h*) Regressing luteal cells contain another type of autophagic vacuole, whose interior is not subdivided into compartments, the double-membrane type. The double walls of these bodies also display a moderately dense content and a central density (*f*, arrow). Arylsulfatase (*g*, arrows), as well as ACPase (*h*, arrow) occur in the space between the walls. When reaction product is less intense, the central dense line of these bodies is apparent (*h*, arrow). × 19,000; 51,000; 37,000. (*i* and *j*) On the other hand, simple autophagic vacuoles (*i, sv*) characteristically lack reaction product after incubation for acid hydrolases (*j, sv;* reacted for ACPase). They thus seem to be of less importance in autolysis. × 20,000; 20,000. (*a–c, e, h* and *j*) Near term; (*b* and *g*) 5 days postpartum; (*f* and *i*) 16 days postpartum.

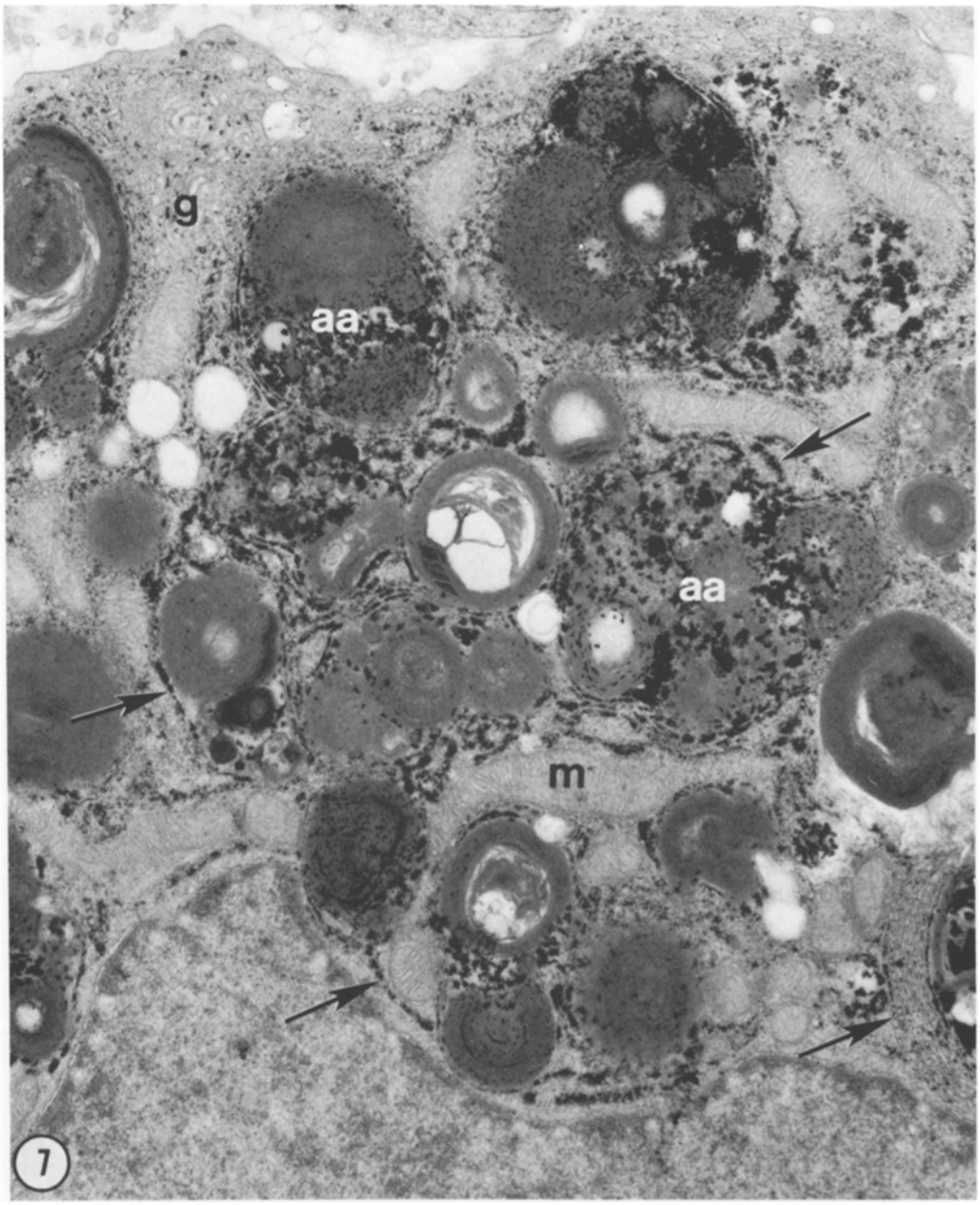

FIGURE 7 Smooth ER of terminally regressing luteal cell, showing ACPase activity. During advanced involution, cytochemically demonstrable lytic activity appears for the first time within tubules and cisternae of the smooth ER (arrows). Note that the reaction product is confined to the cavities of smooth ER, and does not occur free in the cytoplasm. This aged luteal cell also contains several large, ACPase-positive aggregate-type inclusions (*aa*). Profiles of nonreactive smooth ER (not included in this field) identified this cell as a luteal cell. (*g*) Golgi complex; (*m*) mitochondrion. Near term. × 17,000.

the involution of corpora lutea. This finding contrasts with that of van Lennep and Madden (43) in humans, and agrees with that of McClellan et al. (30) in sheep.

Another conceivable mechanism for luteolysis, which is not supported by the present results, is the release of lysosomal enzymes into the cytoplasm. It has been reported that, as the corpus

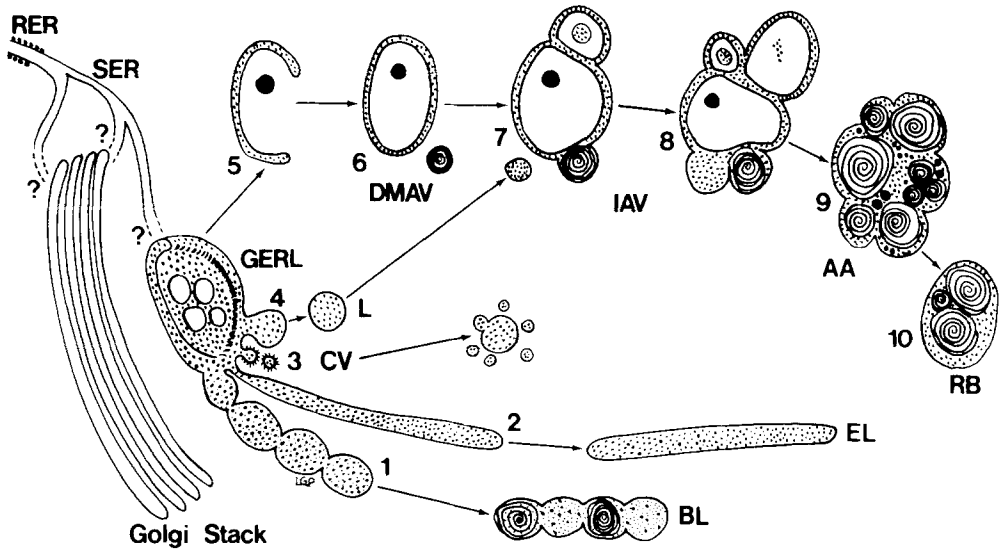

FIGURE 8 This diagram summarizes the ways in which GERL appears to participate in the formation of lytic bodies during postpartum luteolysis in guinea pigs. Although the pathway that lytic enzymes follow to reach GERL remains uncertain (*?*, *RER*-rough ER; *SER*-smooth ER), GERL is responsible for packaging acid hydrolases into lytic bodies in these cells. Lysosomes are thought to arise from GERL by the growth and detachment of beaded (*1, BL*), elongated (*2, EL*), vesicular (coated or smooth) (*3, CV*), and rounded vacuolar (*4, L*) structures. The small vesicles may possibly fuse, forming larger lysosomes. As cup-shaped GERL cisternae (*5*) wall off areas of cytoplasm by becoming completely closed spheres, they produce double-membrane type autophagic vacuoles (*6, DMAV*). These double-membrane type autophagic vacuoles may then enlarge, possibly developing into autophagic vacuoles of the intricate variety (*7, 8, IAV*). The inner limiting membrane of double-walled autophagic vacuoles apparently breaks down and digestion ensues, converting the vacuole into an aggregate inclusion (a lysosome) (*9, AA*). Further digestion may take place, ultimately producing a residual body (*10, RB*), filled with myelin figures.

luteum ages, lysosomes become more fragile (8); thus, they are more susceptible to rupture. However, in the present work, ACPase activity was never observed free in the cytoplasm of luteal cells, arguing against a generalized intracellular release of lytic enzymes. Rather, advanced involution was found to be dominated by highly pleomorphic, intensely acid hydrolase-reactive, membrane-bounded inclusions.

The pathway that acid hydrolases follow en route to their final destination in lysosomes or other lytic bodies is not yet resolved. According to one view (1, 12), lytic enzymes are handled in a manner similar to that described for various secretory proteins (23–25); i.e., after synthesis on bound ribosomes, they enter rough ER cisternae, eventually reaching the Golgi zone, where packaging occurs. Alternatively, Novikoff (32) and coworkers suggest that acid hydrolases may move directly from ER to GERL, where they are processed, bypassing the Golgi complex entirely. The current finding of ACPase activity in smooth ER tubules of an occasional moribund luteal cell indicates that lytic enzymes can gain access to this compartment, perhaps via the many continuities linking rough and smooth ER in these cells (39). But how these enzymes then reach GERL is unclear. ACPase activity often occurs in both GERL and Golgi cisternae, and, even though kinetic data are lacking, this evidence seems to suggest that lytic enzymes might move from ER through the Golgi complex to GERL. But an ACPase-negative cisterna is sometimes interposed between GERL and the Golgi complex when both are ACPase-positive (Fig. 4*d*), a finding which makes it difficult to envision an uninterrupted flow of enzymes from Golgi cisternae to GERL. Lytic activity could also occur in both structures if lytic enzymes moved (*a*) from ER to GERL, then to Golgi cisternae, or (*b*) from ER to both GERL and Golgi cisternae. Even though the pathway to GERL is complicated, it is clear from the marked hypertrophy and intense lytic activity of this organelle in luteal cells during luteolysis that it has

the primary responsibility for the final packaging of acid hydrolases. The role of the Golgi complex in this process, however, requires further clarification.

Two further questions that merit comment are the origin of lysosomes and the source of autophagic vacuoles in senescent luteal cells. ACPase-reactive vesicles and vacuoles, presumably representing nascent lysosomes, bulge from GERL (Fig. 5), strongly suggesting that such bodies are derived from these cisternae. They seem to develop as focal, knoblike swellings that eventually detach from the parent GERL cisterna. As shown in Fig. 5*g*, elongated lysosomes occur in intimate association with GERL, a relationship which makes it tempting to suggest that they also develop from GERL. Similar mechanisms for lysosome formation have been proposed for several nonovarian cell types (6, 14, 15, 22, 31, 34, 36). Only those smooth membranes adjacent to a Golgi stack appeared to give rise to lysosomes in the current study; a different situation occurs in neurons and other cells, where ER located some distance from the Golgi complex has been reported to form lysosomes (22, 26). The clustering of small acid hydrolase-positive vesicles around dense bodies (Fig. 5*f*), together with images indicating the possibility of fusion between these bodies, suggests that larger lytic bodies might arise by fusion of smaller units.

Several organelles have been implicated as the source of the membranes enclosing autophagic vacuoles in nonovarian tissues, including the inner (17) and outer (28) Golgi cisternae, Golgi-derived vesicles (16), smooth ER (1, 11, 13, 31), and a specialized region of smooth ER, GERL (6, 22, 32, 36). Quatacker (42) favored the view that the plasma membrane formed autophagic vacuoles in degenerating luteal cells of humans. On the other hand, a recent report from our laboratory (39) suggested that GERL gave rise to the predominate type of autophagic vacuole in guinea pig luteal cells as follows: GERL cisternae engulf small areas of cytoplasm, becoming in the process completely closed, double-walled autophagic vacuoles (double-membrane type), which then may enlarge or become structurally more complex, developing into intricate-type autophagic vacuoles. The results presented here provide support for that suggestion, since GERL cisternae and the double walls of autophagic vacuoles display several features that point to a common origin. For example, both show intense lytic enzyme activity between their double walls; both frequently have thickened limiting membranes; and both show dense material or central dense lines in the space separating their double walls (apparent even in some cytochemical preparations). Smooth ER and Golgi cisternae, other possible sources of double-walled autophagic vacuoles, are somewhat less attractive candidates. Neither display dense contents or central densities in their cisternae. Smooth ER has a thin limiting membrane, and Golgi cisternae are commonly TPPase-positive, whereas autophagic vacuoles are negative for this enzyme. Although smooth ER occasionally shows ACPase activity in the final stages of luteal cell regression, this is long after most double-walled autophagic vacuoles have made their appearance. It therefore appears unlikely that autophagic vacuoles arise primarily from ER or Golgi cisternae in these cells. It thus is concluded that GERL supplies the membranes of most autophagic vacuoles in regressing luteal cells of guinea pigs. The role of GERL in the formation of such bodies in luteal cells has not previously been documented in the literature. One somewhat similar suggestion (30) has appeared, citing an earlier abstract (37) of the current report. GERL also appears to give rise to double-walled autophagic vacuoles in neurons of mammals (2, 32, 33) and in degenerating neurons of frog larvae (6).

A separate question is how lytic enzymes, derived from GERL, become associated with autophagic vacuoles. The overwhelming majority of autophagic vacuoles in the present study displayed acid hydrolases between their double walls, as did their precursor, GERL. This finding suggests that lytic enzymes are acquired early in development and carried along with the developing vacuole. The hydrolases contained between the double walls may possibly gain access to the interior of the vacuole through a disintegration or alteration of the inner membrane, a process also suggested by others (1, 17, 32). This would convert the vacuole into a lysosome. Actual disappearance of this inner membrane was not observed, but its dissolution may occur very rapidly and would be difficult to recognize in electron micrographs. Additional hydrolases may be derived by other means, e.g., from lysosomes trapped in these vacuoles. In contrast to the present situation, autophagic vacuoles (termed cytosegresomes or isolation bodies) in other tissues often do not exhibit lytic enzymes between their double walls when first formed, gaining them later through fusion with preexisting lysosomes or Golgi vesicles (1, 10, 28).

Finally, in my earlier report (39), it was not clear how aggregate-type inclusions related to the other lytic bodies present in degenerating luteal cells. In the present work, however, stages possibly representing the transformation of double-walled autophagic vacuoles to aggregate-type inclusions have been observed. Thus, one can now postulate that a stepwise progression leads from GERL to residual body, as described in the legend to Fig. 8.

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