Degradation of Zymogen Granules by Lysosomes in Cultured Pancreatic Explants

JAMES H. RESAU, MS, LOUIS MARZELLA, MD, PhD, BENJAMIN F. TRUMP, MD, and RAYMOND T. JONES, PhD

From the Department of Pathology, School of Medicine and MIEMSS, University of Maryland, Baltimore, Maryland

Pancreatic explants from Syrian hamsters were maintained in culture for determination of the fate of the zymogen granules in the acinar cells. By 6 hours of culture the zymogen granule matrix in many cells becomes nonhomogeneous, and electron-lucent spaces appear. Autoradiography performed 48 hours after pulse labeling shows that several of the altered zymogen granules contain radioactively labeled proteins. Hence these altered granules are mature zymogen granules undergoing regression. Ultrastructural analyses indicate that the zymogen granules are degraded intracellularly in the lysosomes by autophagy, by direct fusion between the lysosomes and the zymogen granules, as well as by engulfment of intact zymogen granules by the lysosomes. Budding of cytoplasm into zymogen granules is also frequently observed. Acid phosphatase histochemistry and anti-amylase immunohistochemistry were used for demonstration of presence of hydrolases and of secretory material in the degenerating zymogen granules. Cycloheximide and low temperature inhibit the degradation of zymogen granules and enhance the short-term viability of the explant. Secretagogues stimulate secretion but have little or no effect on the degradation of zymogen granules or on explant viability. The lysosomes participate in the intracellular degradation of zymogen granules in acinar cells of pancreatic explants. The induction of these lysosomal catabolic processes correlates with the structural alteration of pancreatic acinar cells. The intracellular degradation of secretory membranes and products by the lysosomes may play a role in the adaptation of pancreatic acinar cells to injury. (Am J Pathol 1984, 115:139–150)

PHYSIOLOGIC and pathologic stimuli affect the composition and the size of cells in part by the modulation of lysosomal catabolic processes.[1] For example, in secretory cells a block in secretion promotes the intracellular degradation of secretory granules by a process termed crinophagy.[2,3] Crinophagy, as originally described by de Duve, refers to transfer of secretory products to the lysosomes for the purpose of degradation or of modification.[4] It has now become clear that newly synthesized secretory protein molecules may also be degraded in the endoplasmic reticulum and in the Golgi apparatus.[2] The intracellular degradation of secretory proteins thus has both a lysosomal (crinophagy) and a nonlysosomal component. Work from several laboratories has shown that the degradation of newly synthesized secretory proteins can be extensive and can occur in normal conditions.[3] It has been hypothesized that degradation serves to control the intracellular levels of secretory proteins at the post-translational level and to regulate phenotypic expression of cells.[3] Crinophagy has been observed in pancreatic acinar cells in vivo in response to cell injury. It has been proposed that the loss of zymogen granules by crinophagy is the first step in altered differentiation of acinar cells.[5] In the case of the pancreas, the possibility exists that crinophagy may cause the intralysosomal activation of zymogen enzymes. Pancreatic injury would result if these activated enzymes were subsequently secreted extracellularly by the lysosomes.

We have taken advantage of a recently developed method for the culture of pancreatic explants[6] to begin to characterize the mechanism of formation of crinophagic vacuoles and the relationship between crinophagy and structural and functional alterations of pancreatic explants.

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Address reprint requests to Benjamin F. Trump, Department of Pathology, University of Maryland MSTF 7-60, Baltimore, MD 21201.

Materials and Methods

Culture of Pancreatic Explants

Pancreatic explants from adult Syrian golden hamsters were grown in culture and analyzed at 6, 12, 24, and 48 hours. In experiments dealing with the effects of low temperature, the explants were maintained for periods of up to 14 days according to the method of Resau et al.[6] Briefly, explants were placed on Gelfoam sponge rafts (Upjohn) in 60-mm culture dishes with CMRL 1066 (GIBCO) serum-supplemented medium. The dishes were placed on trays in a controlled atmosphere chamber (BellCo) with a 45% O_2–5% CO_2–50% N_2 atmosphere. The chamber was placed on a rocker platform (BellCo) which oscillates 5–10 times each minute. The media were changed twice each week, and samples and/or media were taken from equivalent dishes at various time points. The chambers were kept in an incubator at appropriate temperatures (20–37 C). Each experiment utilized the pancreas from 6 hamsters.

Amylase Determination

Amylase activity in the culture medium was assayed by the method of Caraway[7] with some modifications. Diluted samples were added to a standardized starch solution in Tris buffer and incubated for 15 min at 37 C. The reaction was stopped, and the color developed with an H_2SO_4–I_2 mixture. The specimens were read at 575 nm with a Beckman spectrophotometer.

Preparation of Zymogen Granule Fraction

Pancreatic explants cultured for 24 hours were minced with scissors and diluted with cold 0.3 M sucrose 1:10 (wt/vl). Homogenization was performed in a glass-Teflon Potter-Elvehjem apparatus. The homogenate was centrifuged at 600*g* for 10 minutes. We centrifuged the supernatant at 1000*g* for 10 minutes to obtain a crude zymogen granule fraction.[8] This preparation was fixed in 4% formaldehyde–1% glutaraldehyde and prepared for electron-microscopic examination.

Light Microscopy

Explants were fixed in a phosphate-buffered 4% formaldehyde–1% glutaraldehyde mixture (4F-1G) and embedded in paraffin. We used hematoxylin and eosin (H&E) stains to assess histologic patterns. The viability of the explants was quantitated by morphometric analyses of H&E-stained sections with a Zeiss Videoplan analyzer.

Electron Microscopy

The explants were fixed in 4F-1G for routine ultrastructural analysis. They were postfixed in O_5O_4, *en bloc* stained, dehydrated, and embedded in plastic (Polybed 812). We stained sections 0.5 μ thick with toluidine blue to select viable areas of explants. Thin sections were placed on carbon coated grids, stained with uranyl acetate and lead citrate, and examined with a JEOL 100 B electron microscope at an accelerating voltage of 60 kv.

Immunohistochemical Demonstration of Amylase

The explants were fixed in 0.1 M phosphate-buffered glutaraldehyde (0.5%) at 4 C. The tissues were washed in phosphate buffer, dehydrated, and embedded in Polybed. Thin sections were mounted on nickel grids and etched with 10% H_2O_2 for 30 minutes.[9] After rinsing in H_2O and in a dilute ovalbumin solution, the sections were incubated for 60 minutes with anti-amylase (purchased from Sigma) diluted 1:50. The excess antibody was rinsed off with phosphate-buffered saline. The sections were incubated with protein A (Pharmacia) complexed to 200 Å gold chloride particles.[10] After rinsing, the sections were stained for 20 minutes with 0.5% uranyl acetate.

Acid Phosphatase Histochemistry

For the demonstration of acid phosphatase, a modified Gomori technique was used with lead as the capture ion. The explants were fixed for two hours in 1% formaldehyde–1% glutaraldehyde buffered with 0.1 M Na cacodylate and rinsed in buffer overnight. Sections were cut at approximately 5 μ with a cryostat and incubated in a Na β-glycerophosphate medium.[11] Control incubations were performed in a medium containing sodium fluoride.

Autoradiography

Pancreatic explants were prepared in a buffered salt solution (HBSS) containing 14 mM glucose and gassed with 95% O_2–5% CO_2. The explants were incubated for 3 minutes at 37 C in 2 ml HBSS containing 400 μCi of ^{3}H-tryptophan (3.8 Ci/mmol) to label zymogen granule contents. After three rinses in HBSS containing 3 mM tryptophan, the explants were transferred to complete culture medium for two final rinses. The culture media containing excess tryptophan were changed at 4, 8, and 24 hours. Explants were fixed after 2 and 48 hours of culture in 4F-1G. Sections of Polybed embedded explants (600 A) were picked up on 200-mesh copper grids coated with Formvar and carbon. The grids were coated with Ilford L-4 emulsion (diluted 1:10) by the touching

method. After 6 weeks of exposure the emulsions were developed for 2 minutes at 25 C with D-19 (Kodak) diluted 1:2. Following fixation with acid fixer (Kodak) and rinsing in distilled water, the grids were floated on a dilute NaOH solution[12]. The section were rinsed in distilled water and stained with uranyl acetate. Parallel light-miscroscopic autoradiography was performed on 5-μ-thick sections of paraffin-embedded material after 7 and 10 days of exposure.

Results

Light-Microscopic Study of Pancreatic Explants

The cultured pancreatic explants were examined by light microscopy at various intervals, up to 14 days, for monitoring of the histologic and cellular alterations and for determination of the amount of tissue necrosis. H&E-stained sections showed focal areas of eosinophilia in the acini by 4 hours of culture. By 8 hours the areas of necrosis were confluent and became clearly demarcated by 24 hours. At this point approximately one-third of the explant was viable and remained so for up to two weeks of culture. The acinar lumens began to dilate by 24 to 48 hours; some acinar cells appeared to flatten, and most showed vacuolization, particularly at the base of the acini. Plastic-embedded sections stained with toluidine blue were used for selection of the viable areas of explant for ultrastructural analysis and for evaluation of the intracellular distribution of the zymogen granules. The orientation of the zymogen granules toward the apex of the acinar cell was in general preserved by 48 hours of culture (Figure 1).

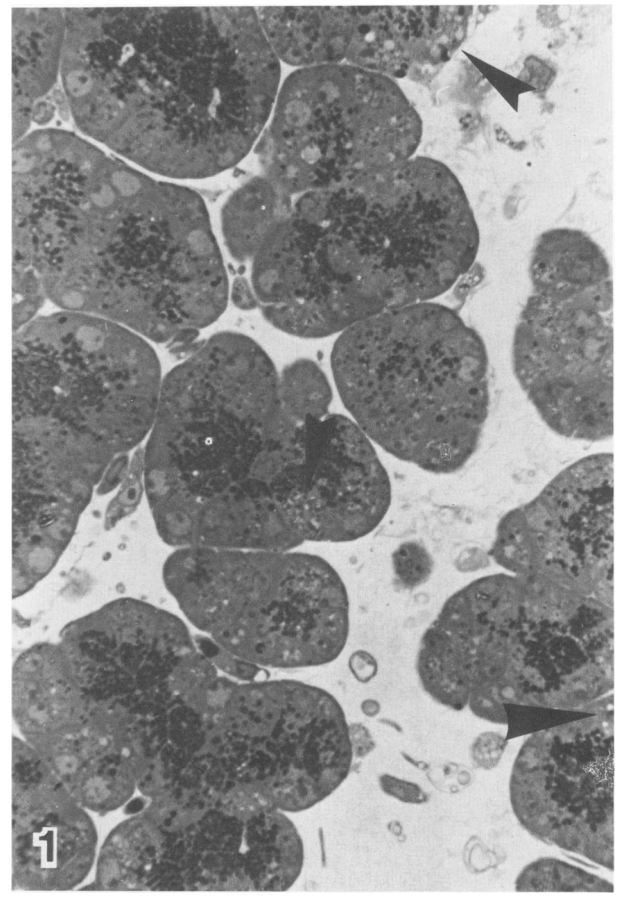


Figure 1 — Pancreatic explant after 24 hours of culture at 37 C. There are numerous small vacuoles (*arrowheads*) in the acinar cells in both apical and basolateral locations. (Toluidine-blue-stained, Epon-embedded tissue, ×480) (With a photographic reduction of 7%)

Ultrastructure of Crinophagic Vacuoles

The size and number of lysosomal profiles increases after 6 hours of culture. Large secondary lysosomes containing cytoplasmic components are seen predominantly in the basal portions of the acinar cells. By 12 to 24 hours the lysosomes increase in size, giving rise to the large vacuoles seen by light microscopy. Rough endoplasmic reticulum cisternae, zymogen granules, and zymogenlike material are by far the most frequently identifiable contents of the lysosomes (figure 2A). Regions of close apposition between the membranes of zymogen granules and secondary lysosomes were frequently seen (Figure 2B). Profiles such as those seen in Figures 2 and 3 suggest that the initial apposition between the two organelles is followed by the engulfment and sequestration of usually the smaller of the two organelles. Apposition and engulfment phenomena between two secondary lysosomes (Figures 2A and 7) or two zymogen granules (Figures 4D and E) were also frequently seen. Invaginations and engulfment of the surface membrane of zymogen granules also occurred. In these hybrid vacuoles morphologic signs of degradation of both the zymogenlike and trapped cytoplasmic material were apparent (Figures 3A and B; 4A–C). The sequestration of zymogen granules by parallel layers of cytomembranes was also seen (Figure 4F). These zymogen granules were sequestered either individually or together with other cytoplasmic organelles. To exclude the possibility that the hybrid organelles containing zymogenlike material and cytoplasmic organelles were due to section plane phenomena, we homogenized pancreas explants after 48 hours of culture. Zymogen granule fractions were prepared by differential centrifugation and examined by electron microscopy. Zymogen granules containing portions of cytoplasmic organelles could be seen in a zymogen subcellular fraction (Figure 4C). Profiles suggestive

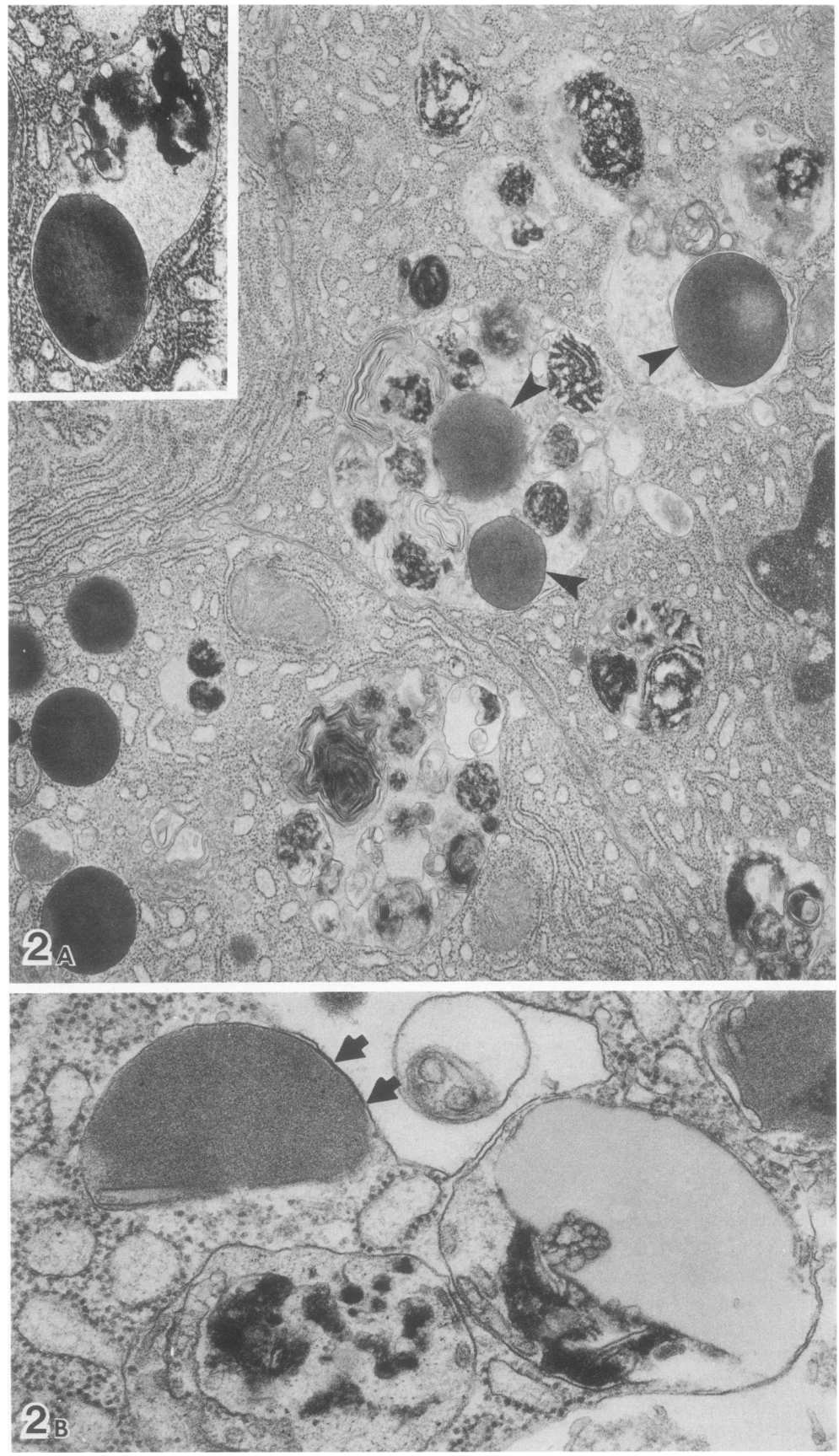


Figure 2—Sequestration of zymogen granules into secondary lysosomes. Engulfment of intact zymogen granules by secondary lysosomes is evident in **A** (*arrowheads*) and in the **Inset**. Zymogen granules and lysosomes can also fuse after becoming closely apposed and establish membrane continuity. In **B** (*arrows*) a zymogen granule and a lysosome appear to be in the process of fusing. (**A**, ×20,000; **Inset**, ×27,000; **B**, ×54,000)

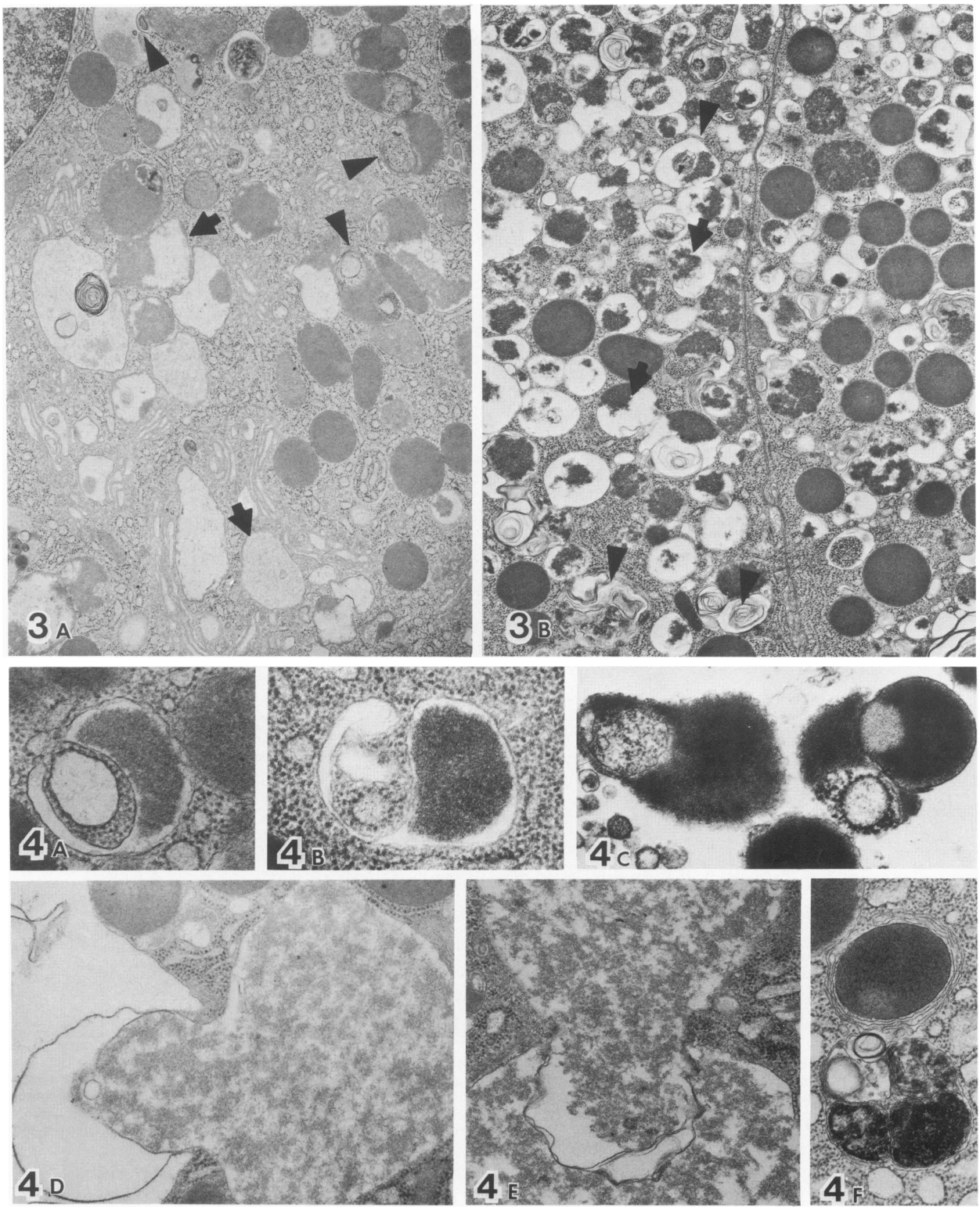


Figure 3 — Degradation of zymogen granules. Degenerative changes of zymogen granules become prominent by 24 hours of culture. The zymogen contents became nonhomogeneous and electron-lucent (*arrows*). Myelin figures and cytoplasmic contents in various degrees of ultrastructural preservations are seen in the zymogen granules (*arrowheads*). Fusion and engulfment phenomena involving zymogen granules and lysosomes as well as uptake of cytoplasm by the zymogen granules are occurring in the acinar cells in culture. (**A**, 48 hours of culture, × 13,500; **B**, 30 hours of culture, × 12,900) **Figure 4** — Mechanisms of degradation of zymogen granules. Invagination of zymogen granule membrane may lead to the uptake of cytoplasm by the zymogen granules (**A** and **B**). Zymogen granules containing cytoplasmic material are preserved after homogenization and fractionation of the explant (**C**). Invaginations of zymogen granule membrane can also lead to the engulfment of other cytoplasmic vesicles. **D** and **E** show the close apposition of membranes of two zymogen-containing vesicles. This is followed by the engulfment of one vesicle by the other. Sequestration of a zymogen granule by multiple membrane profiles is seen in **F**. Note the apparent continuity of this vacuole with a secondary lysosome. (**A**, × 40,500; **B**, × 40,500; **C**, × 40,500; **D**, × 27,000; **E**, × 27,000; **F**, × 21,600) (Both with a photographic reduction of 4%)

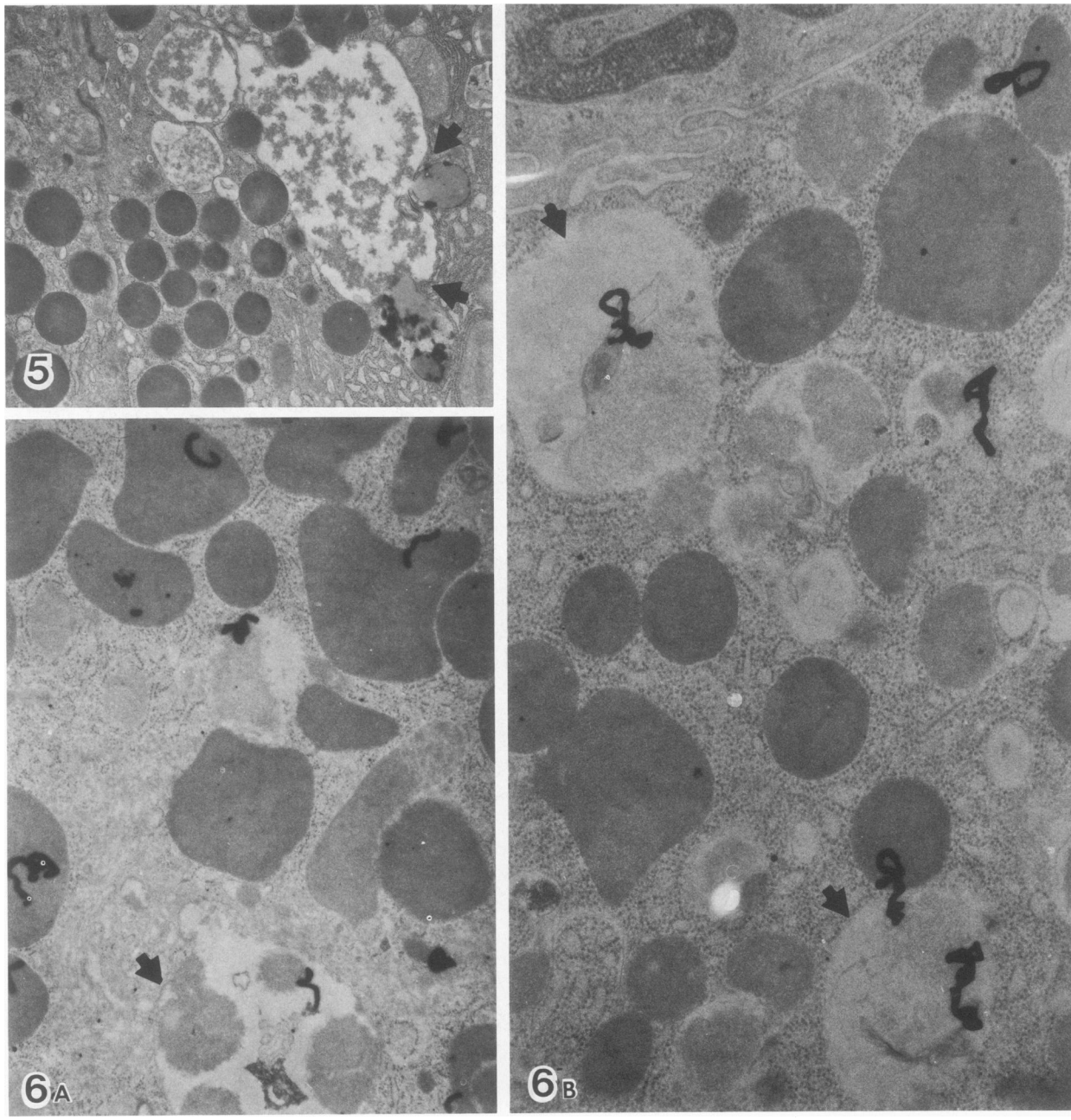


Figure 5—Mechanisms of degradation of zymogen granules. A large vacuole containing amorphous zymogenlike material is apparently fusing with two secondary lysosomes (*arrows*). (Six hours of culture, × 10,800) **Figure 6**—Demonstration of autoradiographic grains in degenerating zymogen granules. Pancreatic explants were pulse-labeled with ^{3}H-tryptophan at the beginning of culture. Forty-eight hours later autoradiography was performed. The presence of grains in the vacuoles with amorphous contents (*arrows*) indicates that zymogen granules are undergoing degeneration in the acinar cells. Condensing vacuoles have a similar ultrastructural appearance, but they would not contain labeled proteins. (**A**, × 13,500; **B**, × 27,000) (Both with a photographic reduction of 5%)

of fusion between secondary lysosomes and zymogen-containing vacuoles were also seen (Figure 5).

Degeneration of Mature Zymogen Granules

The ultrastructural appearance of the zymogen granules was progressively altered during explant culture. Areas of decreased electron density began to appear in the zymogen contents. These areas progressively enlarged and became more electron-lucent. Undoubtedly some of these profiles were condensing vacuoles. In order to exclude the possibility that the abundant electron-lucent vacuoles seen, for example, in Figure 3B represented only a block in the maturation of the condensing vacuoles, we performed auto-

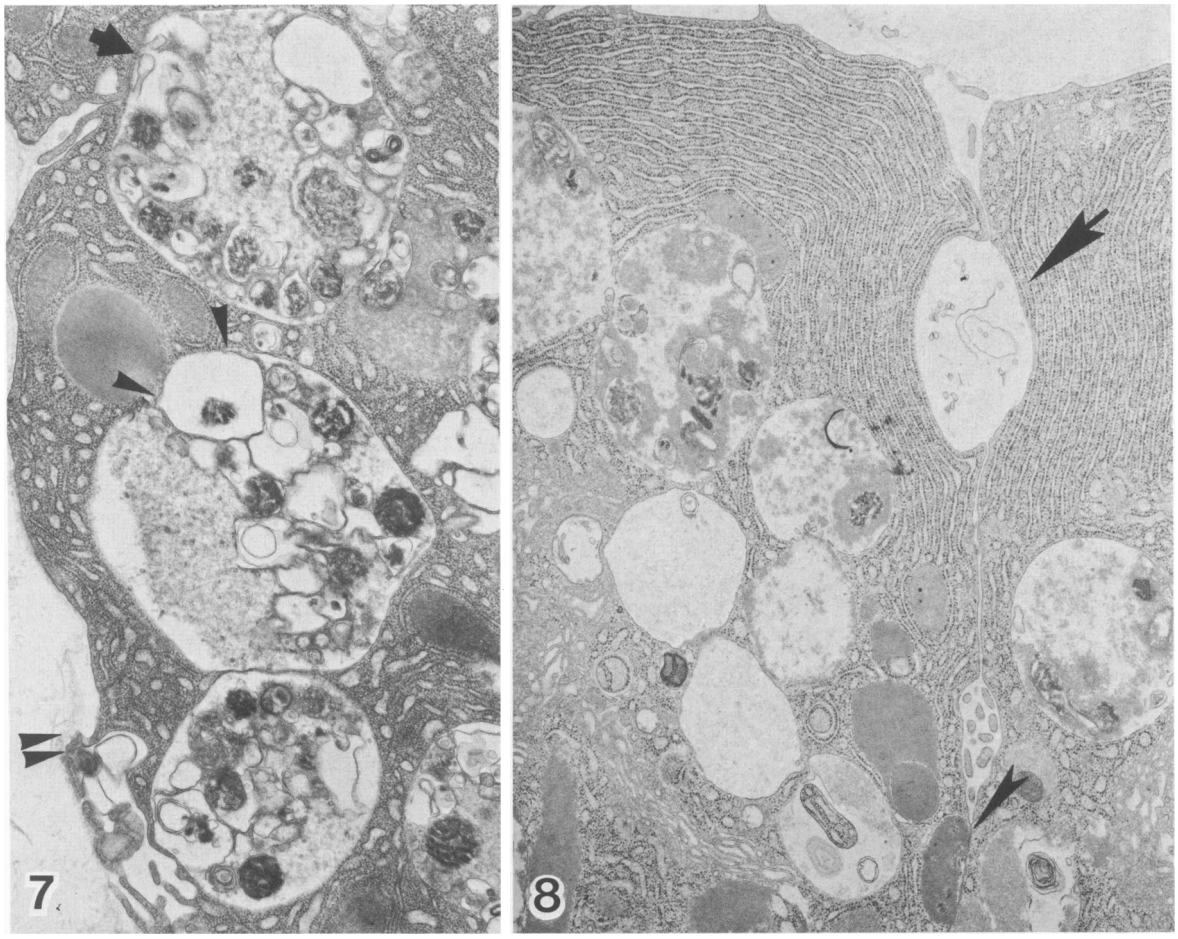


Figure 7 – Accumulation of large secondary lysosomes at the basolateral aspect of the acini. Notice that one of the lysosomes is compressing and deforming the intercellular margin (*arrow*). Myelinlike and electron-dense debris is present outside the cell (*double arrow*) near the basal lamina. Notice the apparent engulfment of a vesicle by a large secondary lysosome (*arrowheads*). (Forty-eight hours of culture, × 13,500)
Figure 8 – Apparent extrusion of lysosomal contents at the basolateral aspect of an acinar cell. Several vacuoles containing zymogenlike material and degraded cytoplasm are present. Some of these vacuoles are in close apposition to the intermembranous space (*arrowhead*). Fusion of one of the vacuoles with the plasma membrane may have occurred (*arrow*). (Forty-eight hours of culture, × 13,500)

radiography on the explants. Thirty minutes after the beginning of culture, the explants were pulse-labeled (3 minutes) with ^{3}H-tryptophan. After chasing with unlabeled tryptophan, the explants were maintained in culture for 48 hours. At this point autoradiography was performed. We reasoned that if the abundant electron-lucent vacuoles represented degenerating zymogen, at least some of them should contain radioactively labeled contents. As can be seen in Figures 6A and B, grains could be demonstrated in intact zymogen granules as well as in the zymogen granules showing ultrastructural signs of degeneration.

Exocytosis of Crinophagic Vacuoles

The morphologic alterations indicate that enhanced catabolism of acinar cell organelles, in particular endoplasmic reticulum and zymogen granules, is occurring in the explants. Several mechanisms for the uptake of zymogen material in the lysosomes were suggested by the morphologic observations. Of particular interest is the apparent capacity of lysosomes and zymogen granules to sequester other vacuoles or cytoplasmic contents by endocytic-like membrane invaginations. The possibility of exocytosis of degenerating zymogen material was suggested by the finding of large secondary lysosomes containing zymogen and membrane-like material often seen in close approximation to the basolateral aspect of the acinar cells (figures 7 and 8). A few images suggestive of fusion of these lysosomes with the lateral region of the acinar cell plasmalemma were also seen (Figures 7 and 8).

Demonstration of Lysosomal and Zymogen Markers in Crinophagic Vacuoles

To support the notion that the observed catabolic changes were mediated by a lysosomal process, we performed acid phosphatase histochemistry. As can be

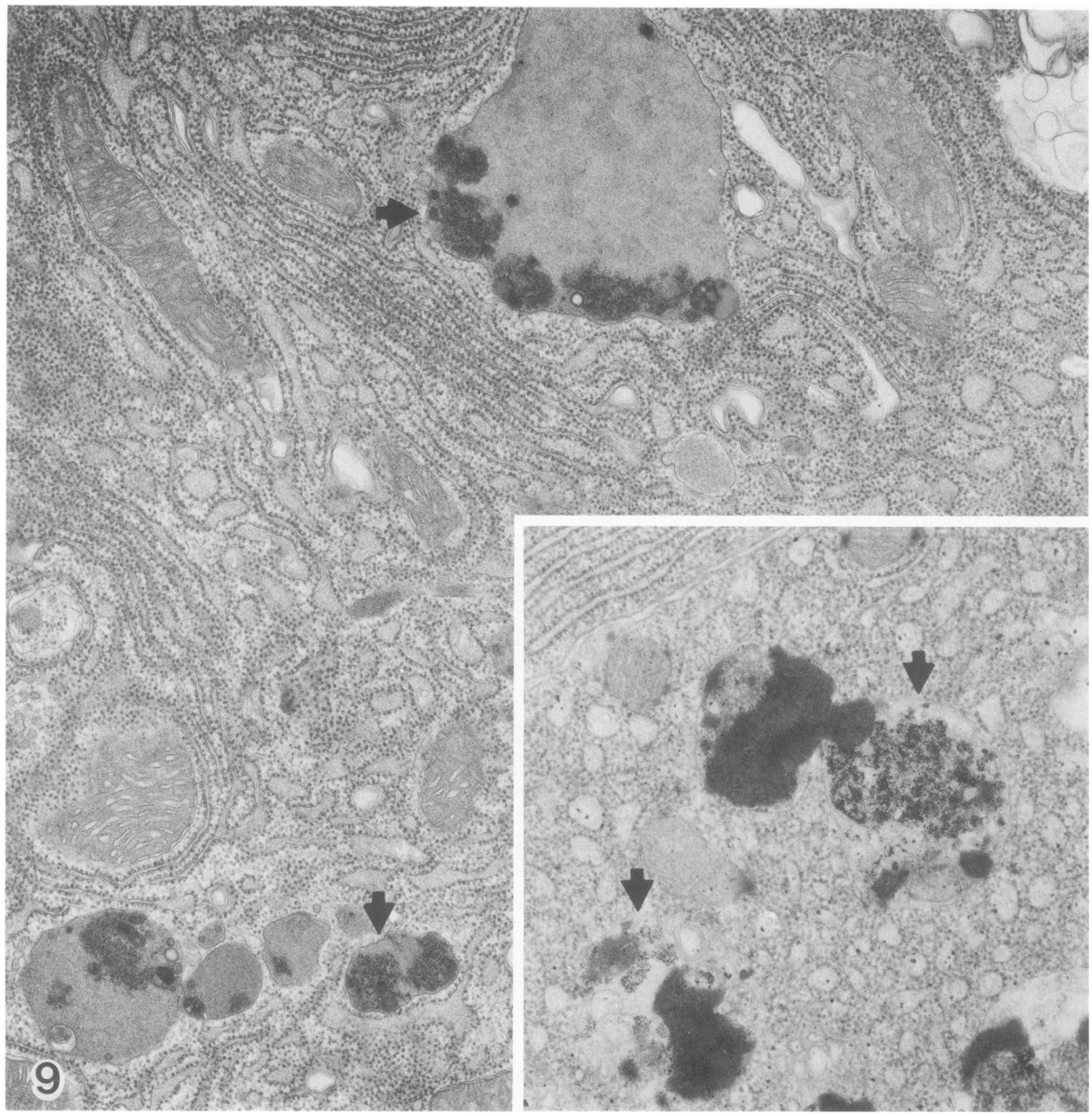


Figure 9—Acid phosphatase histochemistry. The transfer of lysosomal enzymes to degenerating zymogen granules and to vacuoles with mixed contents was demonstrated with a modified Gomori lead technique. Fine and coarse granular reaction product can be seen in the different types of vacuoles (*arrows*). (Twenty-four hours of culture, ×27,500; **inset**, ×40,500)

seen in Figure 9 and the inset, finely granular reaction product can be seen in the vacuoles containing zymogenlike material alone or together with other cytoplasmic organelles.

In order to demonstrate the presence of zymogen material in lysosomal vacuoles, we used a gold–protein A anti-amylase complex. The colloidal gold particles were seen over profiles of secondary lysosomes as well as zymogen granules and rough endoplasmic reticulum. As can be seen in Figure 10 and the inset (arrowheads), colloidal gold particles were present in the hybrid vacuoles containing cytoplasmic as well as zymogenlike material.

Effects of Secretagogues, Cycloheximide, and Low Temperature on Crinophagy and Explant Viability

In order to test whether the modulation of crinophagy in vitro would affect the viability of the explant, we used secretagogues, cycloheximide, and low temperature. The areas of viable explant were de-

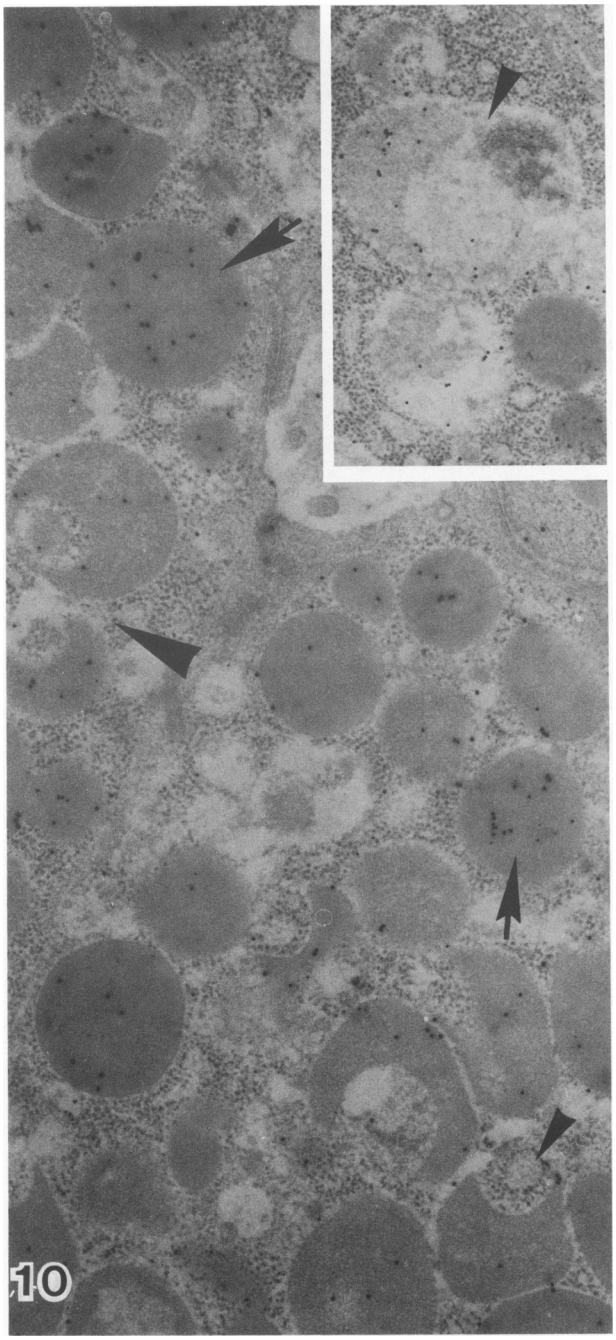


Figure 10—Immunocytochemical localization of amylase in degenerating zymogen granules. Colloidal gold protein A antibody technique was used for localization of amylase in the explants. Gold particles (200 Å) were preferentially localized over intact (*arrows*) as well as degenerating (*arrowheads*) zymogen granules. (Twenty-four hours of culture, ×27,000; **Inset**, ×27,000) (With a photographic reduction of 10%)

termined by morphometric analysis of H&E-stained sections with the use of standard morphologic criteria for acinar and ductal cell viability. The sections were analyzed by two observers. Observer and interobserver variability was approximately ± 10%.

Table 1—Effect of Secretagogues (Secretin, 1 U/ml; Carbachol, 10^{-4} M) and Cycloheximide (10^{-5} M) on the Viability of Explants Cultured at 37 C*

Time (hrs)	Secretagogues	Cycloheximide
6	0.96	0.84
12	1.00	0.80
24	0.72	1.60

* The area of viable explant was determined morphometrically. The numbers are percentages of the control value for each time point. These are means of two to six experiments.

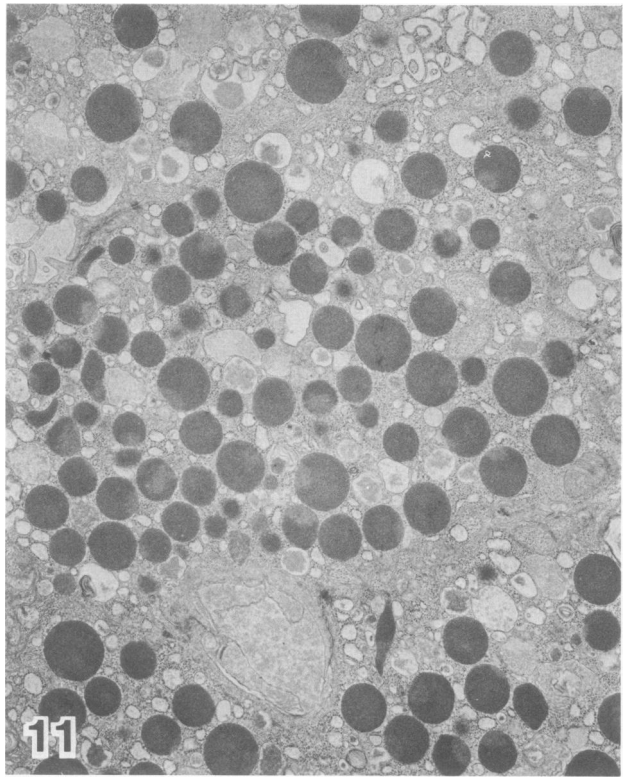
Secretagogues were selected with the expectation that the stimulation of secretion from the explants would have an inhibitory effect on crinophagy. Cycloheximide was selected because it inhibits the formation of autophagic vacuoles.[13] Culture at low temperatures (20 C) was performed because low temperature inhibits fusion of lysosomes with endocytic vacuoles.[14] In Table 1 is shown the viability of the explants cultured in the presence of secretin (1 U/ml) and 10^{-4} M carbachol or 10^{-5} M cycloheximide. The numbers are percentages of the control value normalized to 1 for each time point. At the dosage we have used, secretagogues did not enhance the viability of the explant for up to 24 hours. By this time point cycloheximide enhanced the viability of the explant by approximately 50%. The viability of the explant was enhanced two to threefold by culture at 20 C for 24 hours (Table 2). The protective effect of temperature was seen for up to 5 days of culture.

Parallel electron-microscopic analyses of explants cultured for 24 hours showed that the addition of secretagogues to the explants did not appreciably alter either the ultrastructure or the amount of crinophagy in the acinar cells. Cycloheximide (figure 11) inhibited the activation of both autophagy and crinophagy in the explants. Explants cultured at 20 C (Figure 12) also showed few vacuoles of the autophagic and crinophagic type. In addition, cycloheximide as well as culture at 20 C induced dilation and vesiculation of endoplasmic reticulum and Golgi apparatus.

Table 2—Effect of Temperature on the Percentage of Viability of Explants*

	Time (days)				
Temperature	1	2	5	7	14
37 C	30	35	35	35	30
20 C	85	50	50	35	0

* Numbers represent the percentage of viable tissue determined morphometrically.



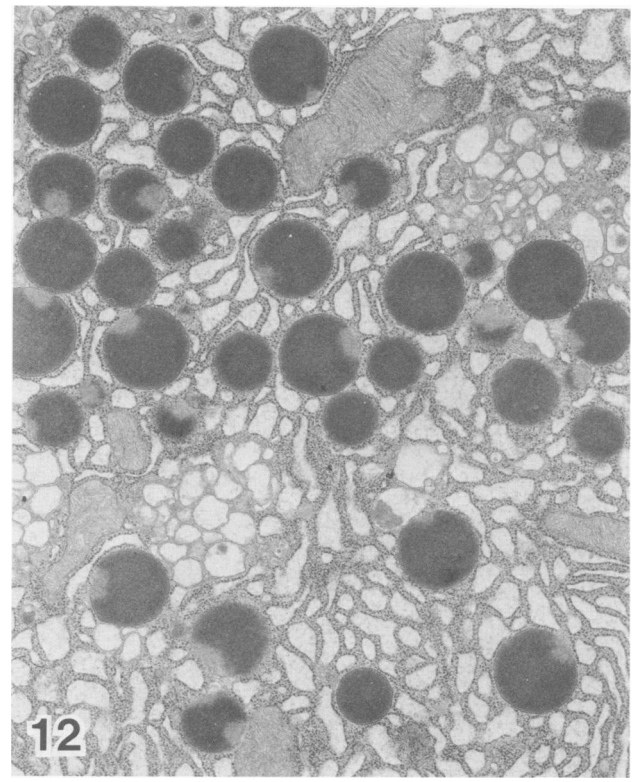


Figure 11 — Appearance of acinar cell after 24 hours of culture in the presence of cycloheximide (10^{-5} M). There is a decreased incidence of autophagy and degenerating zymogen granules. Compare with Figures 2 and 3. Signs of sublethal cell injury such as vesiculation and swelling of the endoplasmic reticulum and Golgi apparatus are seen. (× 10,800) **Figure 12** — Appearance of an acinar cell after 24 hours of culture at 20 C. This micrograph illustrates the inhibition of autophagy and crinophagy induced by low temperature. Notice the features of sublethal cell injury. (× 16,200) (With a photographic reduction of 4%)

The tissue culture media from control and secretagogue- and cycloheximide-treated explants were collected at 6, 12, or 24 hours, and the amount of amylase released by the explants into the media was determined. As can be seen in Table 3, by 12 hours of culture the secretagogues increased, whereas cycloheximide decreased, the release of amylase to the medium. By this time no difference in the viability of the explants was seen (Table 1). The same pattern of release of amylase was seen by 24 hours of culture. As far as can be judged from these observations, no positive correlation was seen during short-term culture (up to 24 hours) between explant viability and secretion of zymogen enzymes.

Discussion

In secretory cells, proteins and lipids targeted for secretion can be diverted from the secretory pathway to lysosomal catabolic pathways. This phenomenon is designated crinophagy.[2,15] Ultrastructural observations suggest that in secretory cells the secretory granules may fuse with lysosomes or may be taken up in autophagic vacuoles. In the cultured pancreatic explants a reliable marker for the transformation of zymogen granules into crinophagic vacuoles is the presence of degenerating cytoplasmic organelles derived from the fusion between zymogen granules and secondary lysosomes. Fusion of zymogen granules with primary lysosomes or secondary lysosomes lacking digestive remnants presumably also occurs and is responsible for the observed ultrastructural degeneration of the contents of mature zymogen granules in the cultured explants. The fusion phenomena we observed were often preceded by the formation of lysosomal membrane invaginations. These invaginations appeared to surround and engulf apparently intact zymogen granules. Similar conformational changes of lysosomal membranes have been observed in other systems and are thought to result in the uptake of cytosolic components into the lysosomes. The term "microautophagy" has been given to this process because it is hypothesized that it functions in the small-scale uptake and degradation

Table 3—Effect of Secretagogues (Secretin, 1 U/ml; Carbachol, 10^{-4} M), Cycloheximide (10^{-5} M), and Low Temperature on the Levels of Amylase in Explant Media

	Time		
	6 hour	12 hours	24 hours
Control	.73 (1.0)	2.02 (1.0)	4.78 (1.0)
Secretagogue	1.14 (1.6)	2.39 (1.2)	7.84 (1.6)
Cycloheximide	0.42 (0.6)	0.97 (0.5)	3.36 (0.7)
Low temperature	0.40 (0.5)	–	1.72 (0.4)

* The numbers in parentheses represent increase or decrease over control value for each time point. These are the means of three to five experiments.

of proteins in particular.[15] Our observations indicate that zymogen granules may be taken up by similar invaginations of lysosomal membranes. In fact, after the initial interaction between the zymogen granule and the lysosomal membrane, it appears that the relative size of the two vesicles determines how the engulfment proceeds. Profiles suggestive of engulfment of smaller lysosomes by larger granules were seen. Additionally, coalescence of large zymogen-containing vacuoles by the same engulfment phenomenon was also noted. The uptake of zymogen granules into autophagic vacuoles was seen less frequently. These observations identify new variations of the mechanisms of exchange of membrane and/or contents between vesicles of the exocytic type such as Golgi apparatus and lysosomes. Tracer studies indicate that the exchange pathways betwen Golgi apparatus and lysosomes are demonstrable in cells during physiologic conditions.[16,17] The finding that cycloheximide or low temperature inhibits crinophagy suggests that crinophagy may be regulated by the same regulatory mechanisms as other lysosomal catabolic processes. In explants treated with cycloheximide or low temperature, the formation of secondary lysosomes of the autophagic type was also suppressed. Because of this finding, we cannot exclude the possibility that morphologically unrecognized fusion between primary lysosomes and zymogen granules is occurring in the explants treated with cycloheximide or low temperature. It also remains to be determined whether or not the induction of crinophagy and autophagy can be dissociated in the pancreatic explants.

The explants treated with cycloheximide or low temperature manifested more prominent features of sublethal cell injury as well as an enhancement of viability in the short term. Since the suppression of crinophagy in the acinar cells is associated with enhanced cell viability, the question of whether crinophagy is a protective or deleterious process arises. Evidence of intraparenchymal activation of zymogen enzymes has been reported.[18] The crinophagic vacuoles could be a potential site for the intracellular activation of the zymogens. The induction of pancreatic injury would result if these crinophagic vacuoles were exocytosed.[19] Crinophagy may, on the other hand, function as an adaptive mechanism for the degradation of zymogen granules in situations where secretion is inhibited. For example, crinophagy may be useful during fasting. In fact, in rats fed a protein-free diet, numerous crinophagic as well as autophagic vacuoles are induced.[20]

The pancreatic explants we have studied undergo progressive structural alterations during long-term culture.[6] In particular, a reduction in the amount of zymogen granules and endoplasmic reticulum is observed in many acinar cells. The activation of crinophagy and autophagy we have described may play a role in these alterations. An example of structural and functional alteration of pancreatic acinar cells has been recently described by Rao et al.[21,22] These workers have characterized a population of cells which apparently arises from a pool of regenerating acinar cells and takes on structural and functional properties of hepatocytes in response to carcinogen exposure or the feeding of a methionine-deficient diet. It is interesting to note that *in vivo*, degenerating and regenerating acinar cells exhibit the morphologic features we have described, particularly autophagy and crinophagy, in parallel with loss of zymogen granules.[23,25] Crinophagy is a very prominent feature of the adaptation of pancreatic acinar cells to injury and may play a role in structural alterations of acinar cells. Crinophagic vacuoles are a potential site of zymogen enzyme activation and extrusion from the acinar cell.

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