# Molecular Pathology of In-Vivo Inhibition of Protein Synthesis

## *Electron Microscopy of Rat Pancreatic Acinar Cells in Puromycin-Induced Necrosis*

Daniel S. Longnecker, M.D., Hisashi Shinozuka, M.D., Ph.D., and Emmanuel Farber, M.D., Ph.D.

PANCREATIC ACINAR CELLS, which serve the specific function of synthesis, storage, and secretion of large amounts of protein in the form of digestive enzymes, seem to be particularly susceptible to damage by compounds affecting protein synthesis and secretion.[1,2] The effects of several such agents on the pancreas have been studied ultrastructurally.[3–8] Single amino acid deficiency and protein deprivation have also been shown to induce ultrastructural changes in the pancreatic acinar cells.[9,10]

The administration of multiple-dose courses of puromycin, an agent which inhibits protein synthesis in many organs including the pancreas, results in necrosis of exocrine cells of the pancreas in rats.[11] In a light microscopic study, early structural changes were noted after 4 hr. of treatment, and frank acinar cell necrosis was evident by 24 hr. In addition, puromycin induces necrosis of acinar cells of the salivary gland, chief cells of the stomach, Paneth cells of the intestine, and intestinal crypt cells.[11–13]

Induction of necrosis by puromycin is of interest because of its selective toxicity for relatively few cell types, several of which serve the specific function of producing digestive enzymes. Ultrastructural studies of the pancreatic lesion after administration of puromycin were undertaken as one step toward understanding the mechanism of its induction, and in the hope that an analysis of structural alterations may serve as a guide to further study of biochemical alterations.

### Materials and Methods

Male Wistar rats (Carworth, Inc.) were used in all experiments. They ranged in weight from 84 to 190 gm. and were fed Wayne Lab Blox (Allied Mills, Inc.). They were deprived of food overnight before the experiments began.

From the Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, Pa.

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Address for reprint requests: Dr. Longnecker, Department of Pathology, College of Medicine, University of Iowa, Iowa City, Iowa 52240.

Puromycin dihydrochloride (Nutritional Biochemicals Corp.) was injected either subcutaneously or intraperitoneally in aqueous solution, in a concentration of 10 mg./ml., which had been neutralized with NaOH. Multiple doses of 40 mg./kg. body weight each were given at 1-hr. intervals. Experiments were timed from the injection of the first dose. The injection schedules and duration of experiments are summarized in Table 1. Five control animals received injections of 0.85% saline given according to a schedule matching that of the corresponding experimental group.

Table 1. Puromycin Injection Schedule

| Rat | No. of hourly puromycin doses * | Duration † (hr.) | Route ‡ | Weight (gm.) |
|---|---|---|---|---|
| 1 | 4 | 4 | SC | 180 |
| 2 | 4 | 4 | SC | 190 |
| 3 | 6 | 6 | SC | 126 |
| 4 | 4 | 6 | IP | 84 |
| 5 | 8 | 10 | SC | 150 |
| 6 | 8 | 10 | SC | 140 |
| 7 | 8 | 10 | IP | 140 |
| 8 | 8 | 10 | IP | 140 |
| 9 | 6 | 24 | SC | 124 |
| 10 | 6 | 24 | SC | 124 |

* Each puromycin injection was 40 mg./kg. body weight.
† Hours from first injection of puromycin to tissue sampling.
‡ SC, subcutaneous injection; IP, intraperitoneal injection.

Tissue was removed from the tail of the pancreas while the animal was under ether anesthesia. For the light microscopic studies, tissue was fixed in Stieve's solution, and sections were stained with hematoxylin and eosin. For electron microscopic examination, small pieces of tissue were fixed in 1% osmium tetroxide in 0.1 M Sörensen's phosphate buffer at pH of 7.4.[14] The tissues were dehydrated at room temperature in a graded series of ethanol solutions and were embedded in Epon 812.[15] Ultrathin sections were cut on a Porter-Blum MT2 ultramicrotome with glass knives and were stained with lead hydroxide by Method A of Karnovsky.[16] Thin sections (1 $\mu$) of plastic-embedded tissue were stained with toluidine blue [17] and served in orientation during the study of ultrathin sections. Sections were examined in a Philips 100B electron microscope with a 60-kv acceleration voltage.

## Results

The ultrastructure of normal pancreatic acinar cells has been described by several investigators.[18–22] The principal ultrastructural features of pancreatic acinar cells in our control rats were in accord with the previous descriptions, and we will refer to the findings in control rats only in contrast to the experimental groups.

Nearly all acinar cells were altered to some extent in the puromycin-treated rats; however, the severity of the lesion varied greatly from cell to cell. In general, animals killed 4 and 6 hr. after puromycin treatment showed mild alterations of cell organelles, and only a small proportion of cells showed severe alterations. In the animals killed after 10 and 24 hr.

the alterations were more advanced, and many cells showed frank necrosis. There was no regression of the lesion in rats killed 24 hr. after initiation of treatment, even though they had received the last injection of puromycin 19 hr. before sampling. In accord with previous observations by light microscopy, there was more diffuse involvement of the pancreas following subcutaneous injection of puromycin than following intraperitoneal injection. By the latter route there were more severe lesions in subserosal acinar cells than in cells lying deep in the pancreas.

#### Endoplasmic Reticulum

In control rats, rough endoplasmic reticulum was arranged in an orderly fashion, exhibiting parallel arrays of elongated cisternae around the nucleus (Fig. 1). The lumens of the cisternae were generally narrow and were devoid of any visible material. The earliest change observed in the experimental animal was the segmental dilatation of cisternae of rough endoplasmic reticulum. This was followed by segmentation of cisternae with the formation of rough-surfaced vesicles (Fig. 2–4). This vesiculation of rough endoplasmic reticulum was not uniform in rats killed at 4 hr., and many cells showed well-preserved parallel cisternae. By 6, 10, and 24 hr., the vesiculation progressed to a generalized form and involved the majority of acinar cells. Concurrent with the formation of cisternal vesiculation, there was progressive dilatation of cisternae of variable degree (Fig. 4 and 5). The most severe dilatation was found in rats of the 24-hr. group in which dilated cisternae were separated only by extremely attenuated cytoplasmic septums (Fig. 6). Perinuclear spaces were also markedly dilated.

The second change in the endoplasmic reticulum was the appearance of electron-dense spherical granules within the cisternal spaces (Fig. 2–4). Even at 4 hr. a small number of granules was seen in basal, supranuclear, and apical parts of acinar cells. Later these granules appeared in dilated cisternae either singly or in groups of 2–6. The granules, ranging from 100 to 200 mμ in diameter, were amorphous and stained more intensely with lead hydroxide than the zymogen granules. Such granules were not seen in sections from control rats.

#### Lipid

Focal accumulations of an electron-dense amorphous material having the appearance of lipid were seen in small numbers of the basilar portion of acinar cells of control rats (Fig. 1). There was a distinct increase in size and number of such deposits in the animals treated with puromycin (Fig. 2). An increase in the amount of lipid was evident at 4 hr. and progressed

slightly through the 10-hr. groups. At 24 hr., cytoplasmic lipid droplets appeared to be fewer than were observed at 10 hr., but were increased over the control group. Small lipid deposits tended to conform to the profile of the surrounding endoplasmic reticulum, but large deposits were globular and displaced the granular reticulum.

#### Cytoplasmic Granules

Lesions consisting of groups of small, irregular granules were observed in the cytoplasm of acinar cells as early as 4 hr. after initiation of puromycin treatment (Fig. 3). The individual granules varied from approximately 30 to 90 mμ and were not membrane bound either singly or in groups (Fig. 3 and 4). They were amorphous and electron dense, but less dense than the intracisternal granules described above. Occasionally, fragments of rough endoplasmic reticulum were seen among these granules (Fig. 4). A variable number of such lesions was present in the cells, but their number and size increased with time. Although these lesions were seen anywhere in the basilar two-thirds of acinar cells, there seemed to be a high incidence in the vicinity of Golgi complexes.

#### Mitochondria

The changes of mitochondria were quite variable from cell to cell. There were no consistent mitochondrial changes which could be correlated with the progression of time or with other types of cell damage. A few mitochondria from all experimental groups showed swelling and irregularity of the outer membrane associated with smudging of the matrix and loss of cristae (Fig. 5 and 7). However, even in the experimental animals of the 10- and 24-hr. groups, many acinar cells showed well-preserved mitochondria (Fig. 4). Mitochondria were frequently trapped in autophagic vacuoles or were seen in the necrotic areas. In both situations, they showed marked distortion of shape, loss of cristae, and an accumulation of electron-dense material in the matrix (Fig. 15).

#### Autophagic Vacuoles

In control rats, acinar cells contained a few small, dense bodies (lysosomes) which were single-membrane-limited bodies of different shapes containing dense material. In addition, acinar cells contained small autophagic vacuoles (cytosegresomes) which were single- or double-membrane-limited bodies containing clearly recognizable cytoplasmic organelles. In experimental animals, however, the distinction between dense bodies and autophagic vacuoles was extremely difficult because of degradation of cytoplasmic organelles in these vacuoles. Thus we will refer to all such membrane-bound bodies as autophagic vacuoles.

By 4–6 hr. after puromycin injection, there was a distinct increase in number and size of autophagic vacuoles (Fig. 3 and 7). They tended to be spherical and ranged in greatest diameter from 0.5 to 1.5 $\mu$. Vacuoles contained dense amorphous material or membranous debris (Fig. 3 and 7). A few vacuoles contained well-preserved endoplasmic reticulum, mitochondria, and zymogen granules. By 10 hr., there was a further increase in the number and size of these autophagic vacuoles (Fig. 9–11). They frequently measured up to 3 $\mu$ in greatest diameter. They were surrounded by a single or double membrane, but frequently the limiting membrane was not visible about the complete circumference (Fig. 9). The contents of these vacuoles varied considerably—some vacuoles contained intensely osmiophilic membrane and whorled osmiophilic bodies, some contained mitochondria or zymogen granules, and others contained markedly distorted endoplasmic reticulum with intracisternal granules.

By 24 hr., some of these vacuoles had become so large that it was difficult to ascertain whether they were part of autophagic vacuoles in intact cells or, rather, represented necrotic cells (Fig. 13). They contained dense concentric membranous structures suggestive of altered endoplasmic reticulum, intracisternal granules, various dense osmiophilic bodies, and distorted mitochondria.

#### Golgi Complex

The Golgi complexes in control rats showed typical lamellar stacks of cisternae and variably distended vacuoles and vesicles (Fig. 1). There were no consistent alterations of these structures in experimental rats except for dilatation of cisternae (Fig. 7). Abundant small vesicles were present adjacent to the dilated cisternae and were seen as communicating buds of both granular endoplasmic reticulum and large Golgi vesicles. The number of such small Golgi-associated vesicles appeared to be increased in puromycin-treated rats.

#### Zymogen Granules

Normal-appearing zymogen granules persisted in acinar cells of puromycin-treated rats at 4 and 6 hr. In rats killed at 10 hr. there was an overall decrease in number of zymogen granules, and some of those present were smaller in size than most zymogen granules in normal cells. There was further decrease in number of zymogen granules in treated rats killed at 24 hr. Even though the rats had been starved nearly 40 hr. by the end of this experiment, there was abundant zymogen in the control. Occasional examples of discharge of zymogen granules into the acinar lumens were noted in both the puromycin-treated and control rats.

#### Nucleus and Nucleolus

In control rats, acinar cells contained 1 or 2 nuclei which showed a round contour with relatively homogeneous nucleoplasm (Fig. 1). Chromatin could be seen around the nuclear membrane and around the nucleolus. Nucleoli were relatively small and compact. In rats killed at 4 and 6 hr. there was some accentuation of the chromatin pattern around the nuclear membrane and around the nucleoli. This accentuation of the chromatin pattern was more pronounced in rats killed 10 and 24 hr. after initiation of puromycin treatment. In severe cases, chromatin was completely clumped along a portion of the nuclear membranes to form crescentic or ovoid masses (Fig. 8). Shrunken nuclei with condensed chromatin were frequently found in the autophagic vacuoles. A breakdown of the nuclear membrane was observed in necrotic cells (Fig. 15).

No characteristic and consistent changes were observed in the nucleolus. In the nucleus which showed clumped chromatin, the nucleolus also showed clumping of its components to give small electron-dense masses (Fig. 8).

#### Acinar Cell Membrane

No alteration was noted in the basilar cell membrane or in the relationship of acinar cells to adjacent cells. Microvilli of normal appearance persisted on the luminal surface in all experimental groups.

#### Centroacinar Cells

Centroacinar cells are located normally within the acini of pancreas and participate in the formation of the acinar lumen.[19] They are characterized by a cytoplasm containing very little endoplasmic reticulum, few and small mitochondria, and a predominance of relatively low electron-dense matrix. Cytoplasmic organelles of centroacinar cells showed little alteration after puromycin treatment. A striking change was the frequent appearance of membrane-bound inclusions of varying size in the cytoplasm (Fig. 12 and 14). These were limited by a single or, occasionally, a double membrane and contained cytoplasmic organelles of acinar cells with various degrees of degradation. Acinar cell origin of the contents was readily apparent with the presence of recognizable zymogen granules and parallel arrays of densely packed rough endoplasmic reticulum with frequent intracisternal granules. In general, the contents of these inclusions had the same appearance as those of autophagic vacuoles in acinar cells.

## Discussion

It is evident from this study that the administration of puromycin induces a series of alterations in several organelles of pancreatic acinar cells.

The granular endoplasmic reticulum, the organelle concerned with synthesis and transport of exocrine enzymes, showed 2 distinct changes—vesiculation and dilatation of the cisternae, and the appearance of intracisternal granules. The development of cytoplasmic granular lesions and an increase in the number and size of autophagic vacuoles were constant changes in the cytoplasm as a response to puromycin treatment. Noted less frequently were alteration of mitochondria, hyperplasia of Golgi vesicles, lipid accumulation, and alteration of nuclei.

Vesiculation and dilatation of cisternae of endoplasmic reticulum has been reported to occur in a variety of cells as a response to injuries of various kinds.[23] Its specificity in regard to functional alteration is not clear. It has been suggested that the vesiculation of endoplasmic reticulum may reflect a shift of the normal osmotic relationship between various cell compartments,[23] a mechanism that seems to be appropriate in the case of puromycin since the antibiotic is known to induce discharge of peptides into microsomal vesicles.[24]

The appearance of a large number of intracisternal granules in pancreatic acinar cells after puromycin treatment is of considerable interest. The morphologic appearance of these granules is similar to those found in the pancreas of normal guinea pigs by Palade[21] and in the pancreas of dogs by Ichikawa.[22] In agreement with other investigators,[7] such granules were never observed in control rats. Furthermore, in rats, intracisternal granules similar to those seen in the present study have been described in certain pathologic conditions. Ekholm, Edlund, and Zelander[7] described these granules in the pancreas of rats after treatment with ethionine, and Hruban *et al.*[5] after treatment with β-3-furylalanine. On the basis of a biochemical analysis of the intracisternal granules of guinea pigs, Siekevitz and Palade[25] postulated that these granules are composed of enzyme protein. Although we have not determined the nature of these granules in puromycin-treated rats, we would speculate that they represent enzyme protein and/or puromycin-altered polypeptides. The formation of a large number of intracisternal granules in rat pancreas suggests that there is a compromise of transport of enzyme protein from cisternal spaces to Golgi vacuoles, as has been speculated by Hruban *et al.*[5] A mild dilatation of Golgi vacuoles and hyperplasia of Golgi vesicles after puromycin treatment may also reflect disturbed transport and storage of exocrine enzyme protein. Consistent with this suggestion is the observation of a similar type of change in the pancreas following β-3-furylalanine administration and protein deprivation in rats,[5,10] situations in which exocrine protein synthesis and transport are considered to be altered.

The unique lesion induced by puromycin was the development of groups of small dense granules in the cytoplasm (Fig. 3 and 4). We are

not aware of any previous description of such a lesion. The nature of these granules is not clear, nor is the significance of this lesion in relation to puromycin treatment. On the basis of morphology 2 possible mechanisms for the development of this lesion are suggested: (1) The lesion may represent an accumulation of abnormal secretory products in the cytoplasm due to a puromycin-induced aberration in the transport and/or secretory process. The observation that these lesions occur in close association with the rough endoplasmic reticulum, and frequently in the vicinity of Golgi complexes, favors this view. However, we have observed that this lesion also occurs in the basal portion of acinar cells without any apparent association with the Golgi complexes. Conceivably, these granules may originate from the intracisternal granules discussed above, although the staining characteristics of these granules differ from those of intracisternal granules. (2) Alternatively, these granules may represent breakdown products of pre-existing organelles, particularly membranes such as endoplasmic reticulum. Indeed, small fragments of endoplasmic reticulum were seen frequently in the granular lesions (Fig. 4).

An increase in the number and size of autophagic vacuoles in the acinar cells was a progressive change during puromycin treatment. In pancreatic acinar cells, the formation of autophagic vacuoles appears to be a common pattern of reaction since these vacuoles have been observed after the administration of several toxic agents [3,6–8,26] and in some deficiency states.[9,10] The contents of these autophagic vacuoles were almost always acinar cell cytoplasmic organelles which showed varying degrees of degradative alteration. The change probably represents sequestration of damaged cytoplasm as a sort of cellular refuse-disposal system, as suggested by Hruban *et al.*[26] and Swift and Hruban [27] in their concept of focal cytoplasmic degradation.

Increased cytoplasmic lipid deposits, similar to those noted after puromycin administration, have been reported in the pancreas of rats following lysine deficiency,[9] protein deficiency,[10] and ethionine administration,[6] and its possible significance has been discussed.[9] In the case of puromycin, we feel that this might reflect an altered metabolic balance resulting from inhibition of lipoprotein synthesis.

The occurrence of cytoplasmic inclusions in the centroacinar cells deserves comment. Although centroacinar cells are located in the acini of the pancreas, their morphologic appearance differs markedly from that of the acinar cells. They are considered to be homologous to the duct cells, and their major function is participation in the transport of the secretory products.[19] The inclusions of the centroacinar cells contained, in addition to unidentifiable membranous debris, clearly recognizable acinar cell or-

ganelles in various stages of degradation. Herman and Fitzgerald[6] apparently observed similar changes in the centroacinar cells of ethionine-treated rats. These observations indicate that centroacinar cells have phagocytic properties, an activity not heretofore documented for these cells. Conceivably, this activity may have functional significance in pancreatic physiology.

Although a variety of agents and conditions lead to pancreatic acinar cell damage, the puromycin-induced lesion is particularly interesting from the pathogenetic viewpoint. Puromycin is generally considered to inhibit protein synthesis by substituting for transfer RNA at the site of peptide bond synthesis on the ribosome.[28] This leads to premature termination of polypeptide synthesis and release of incomplete peptide chains or proteins. Thus, at least 2 possible hypotheses for the molecular pathogenesis of pancreatic acinar cell degeneration induced by puromycin may be formulated[11]—i.e., that cell death is the result of (1) inhibition of protein synthesis with the depletion of 1 or more labile proteins essential for cell function, or (2) the production of abnormal peptides or incomplete proteins which are toxic to the cell.

The results of this study offer little evidence for or against either hypothesis; however, the first appears less likely at this time, since the virtually complete inhibition of protein synthesis in the pancreas by cycloheximide does not cause pancreatic necrosis,[29] and produces few, if any, of the ultrastructural changes seen with puromycin (unpublished observations). This negative evidence would favor placing more emphasis on alternate hypotheses at this time. The known pharmacologic potency of a variety of small peptides of 8–12 amino acids such as vasopressin, oxytocin, bradykinin, and angiotensin, and the probable release of peptides into the cisternae of the endoplasmic reticulum by puromycin[24] suggests that evaluation of the second hypothesis might offer a rational basis for an analysis in depth of the pathogenesis of cell death induced by puromycin.

## Summary

Induction of pancreatic acinar cell necrosis within 24 hr. following repeated injections of puromycin has been reported previously. Progression of the puromycin-induced changes in organelles of the acinar cells was studied by electron microscopy during the interval of 4–24 hr. after initiation of injury. The granular endoplasmic reticulum was greatly altered, showing vesiculation, cisternal dilatation, and formation of intracisternal granules. Disruption of cell structure was further manifested by the appearance of groups of amorphous granules in the cytoplasm. An increase

in the number and size of autophagic vacuoles was a progressive change during the course of treatment. Mitochondria, Golgi complexes, and nuclei were less consistently involved. Possible implications of the ultrastructural changes are discussed in relation to the pathogenesis of puromycin-induced acinar cell damage and in light of the known effects of puromycin on protein synthesis.

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[ *Illustrations follow* ]

## Legends for Figures

All electron micrographs were taken from sections stained with lead hydroxide.

**Fig. 1.** Portions of pancreatic acinar cells and an acinar lumen (al) from fasted, saline-injected control rat. Rough endoplasmic reticulum (er) has regular arrangement with elongated cisternae of relatively uniform width. Zymogen granules (z) are abundant. Less-dense prozymogen granules (pz) are seen near Golgi structures (G). Small lipid deposit (lip) is present at base of 1 cell. Mitochondria (m), nuclei (N), and nucleolus (ncl) are also evident. × 11,000.

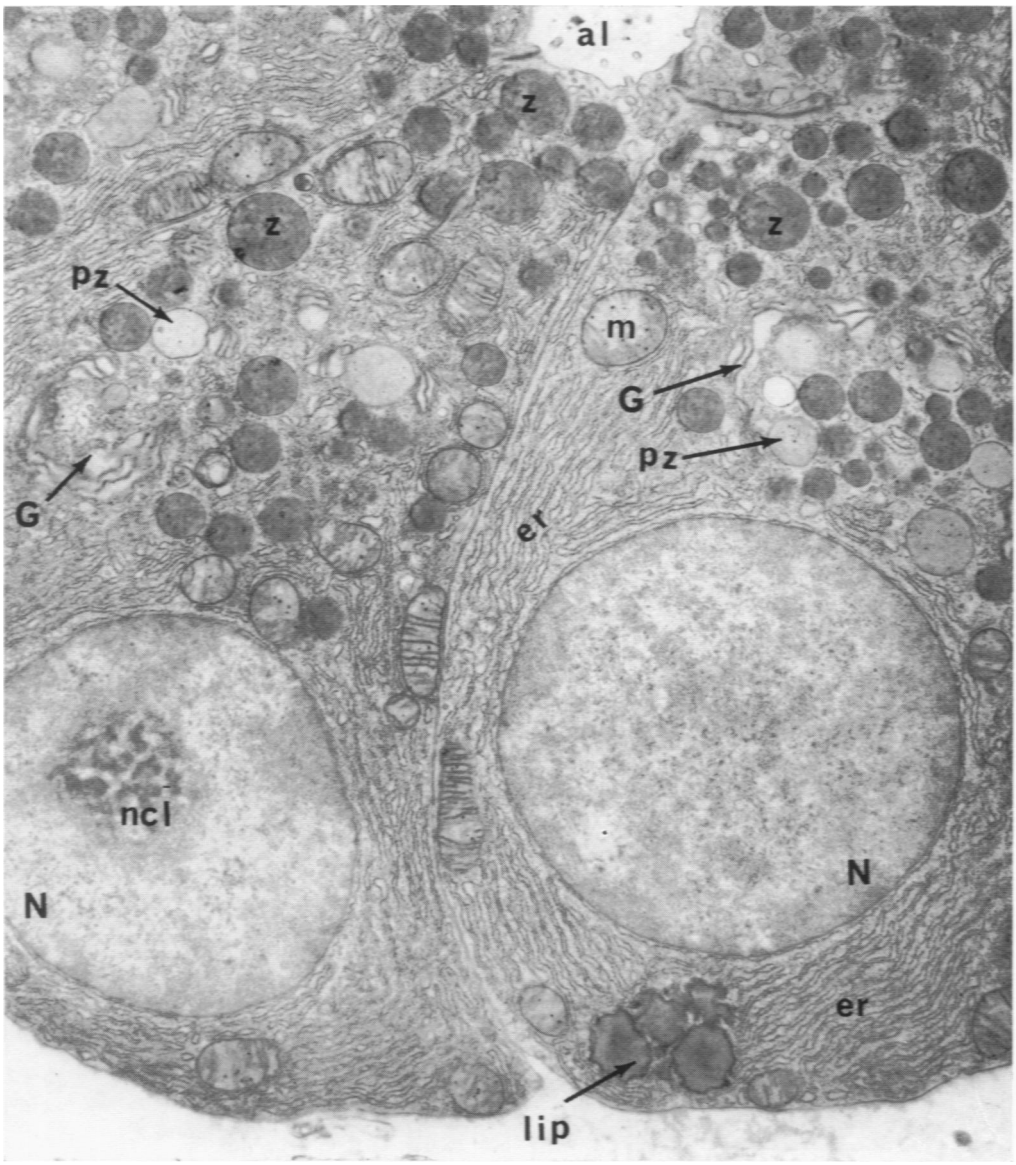
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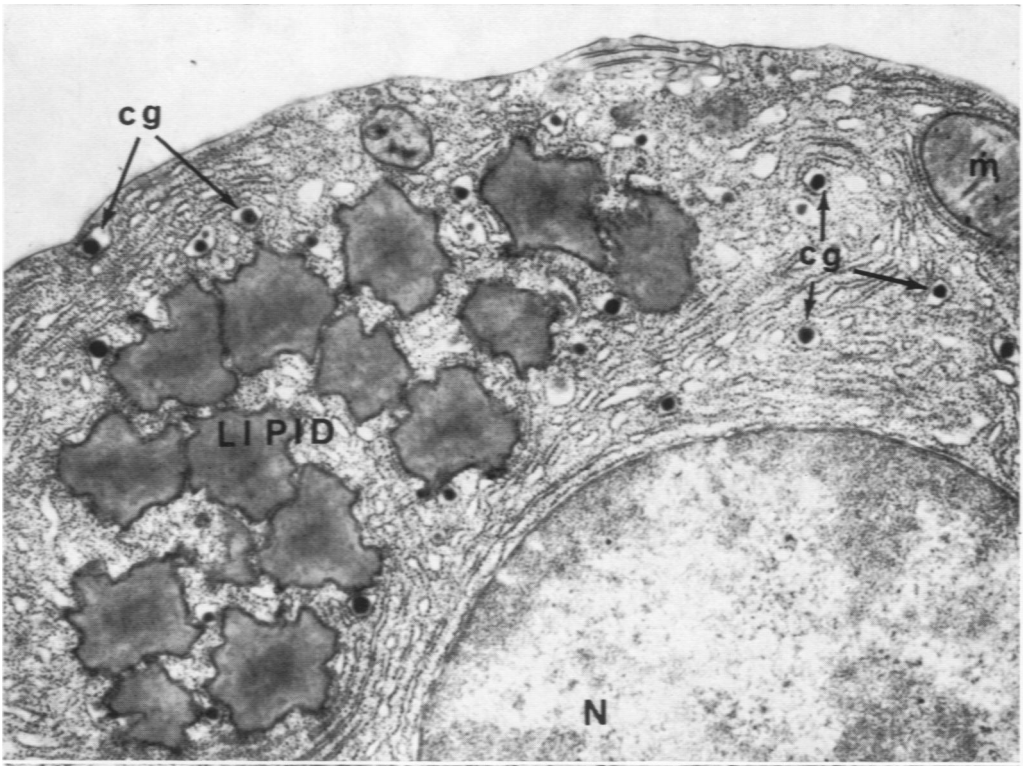
**Fig. 2.** Basilar portion of pancreatic acinar cell from rat killed after 6 hr. of subcutaneous puromycin treatment. Numerous lipid deposits are obvious; granules (cg) are present in cisternal space of rough endoplasmic reticulum. Mitochondrion (m) and nucleus (N) are also present. × 15,000.

**Fig. 3.** Paranuclear portion of acinar cell from rat killed after 6 hr. of puromycin treatment, showing intracisternal granules (cg), cytoplasmic granular lesions (double arrows), small autophagic vacuoles (av), mitochondria (m), and nucleus (N). There is vesiculation of some portions of granular reticulum. Small Golgi vesicles (Gv) are prominent. × 19,500.

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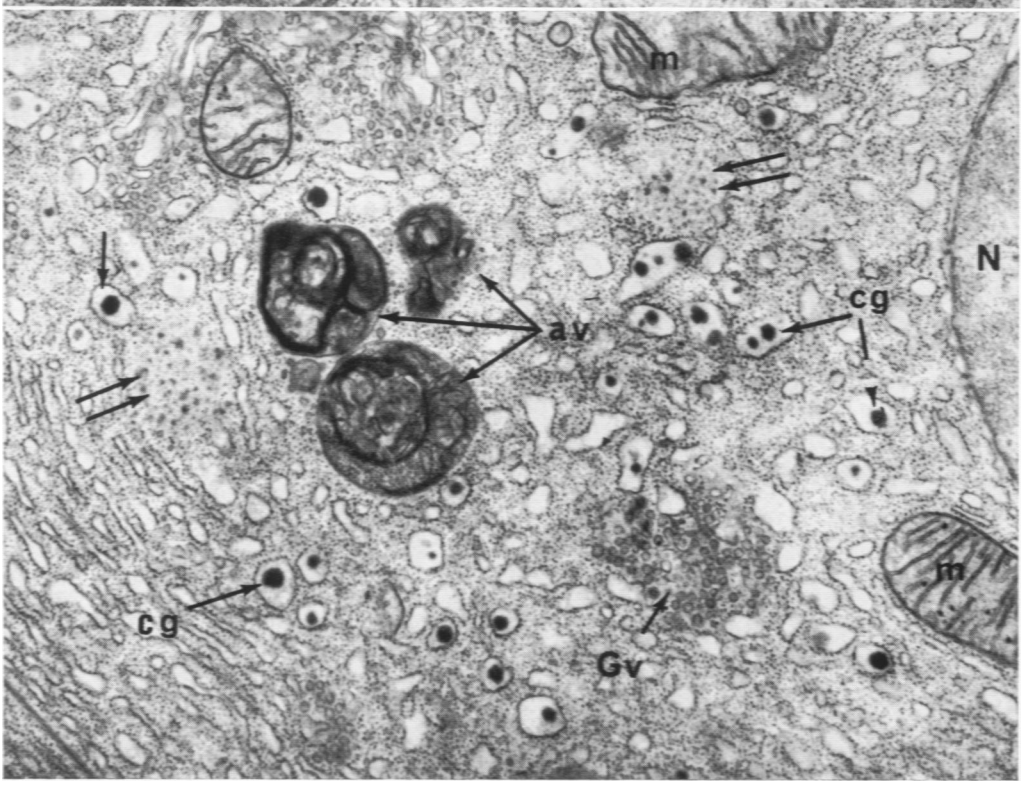


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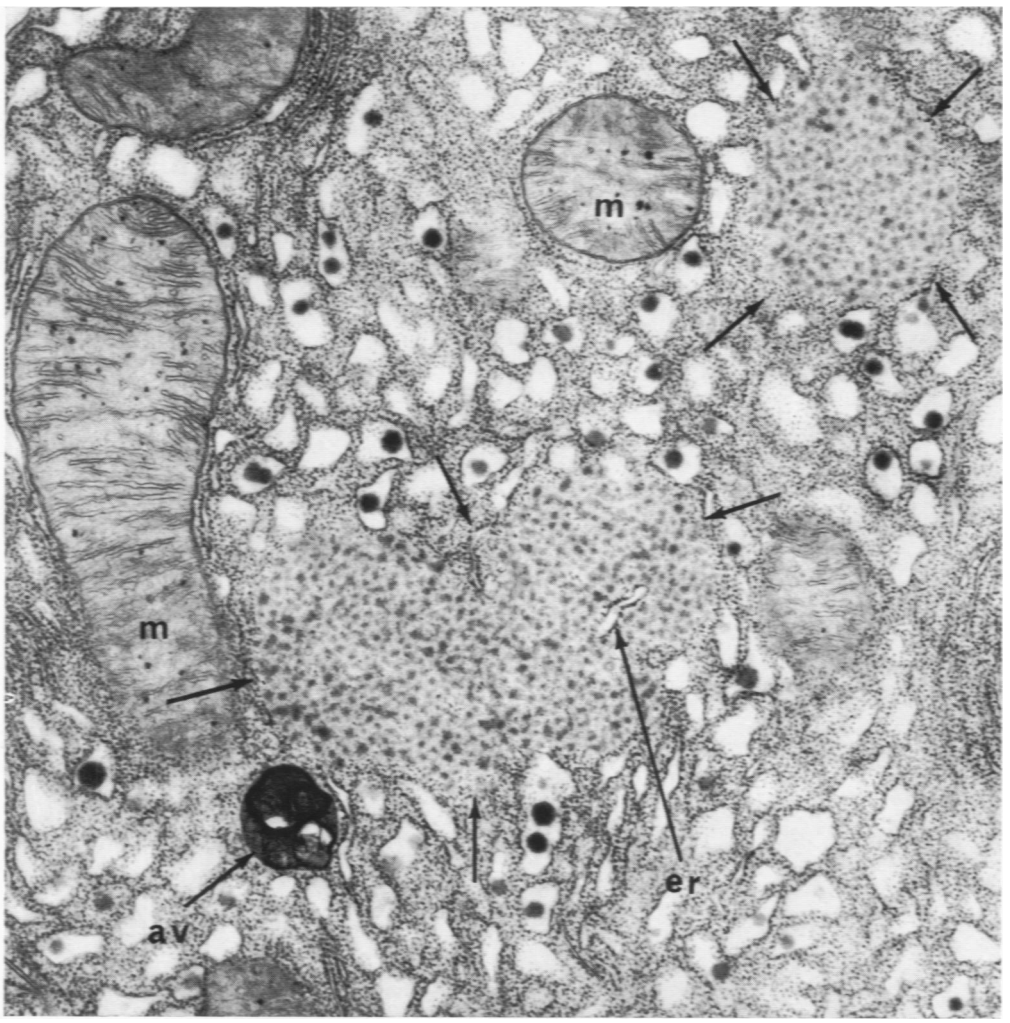
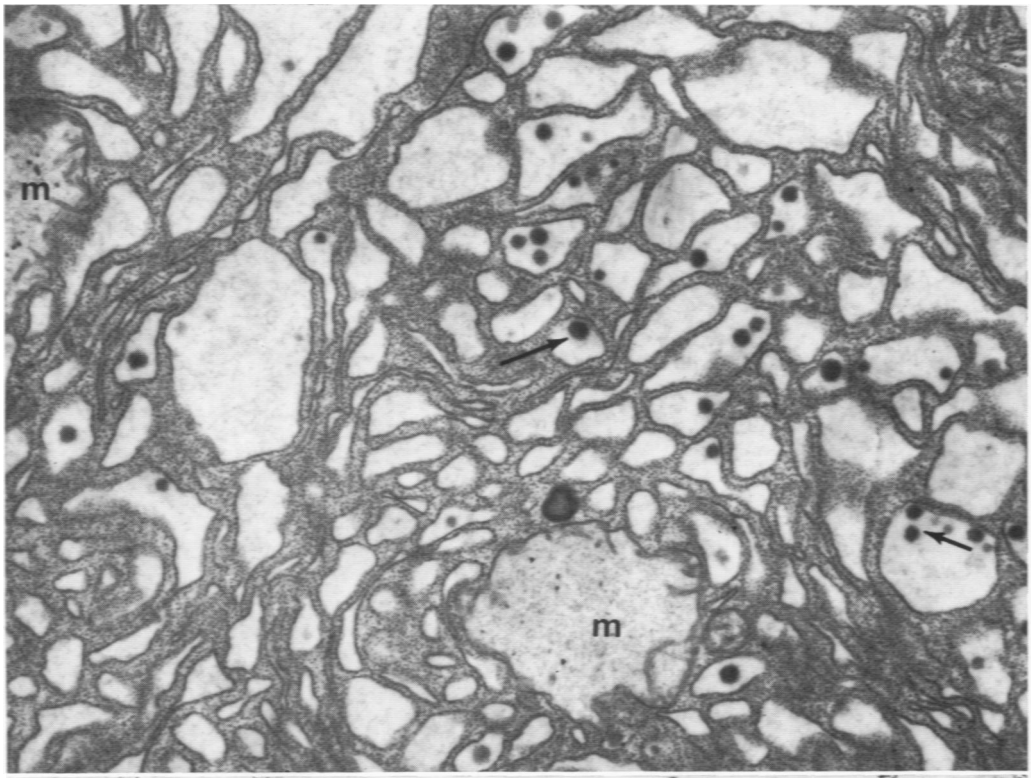



**Fig. 4.** Portion of acinar cell cytoplasm from rat killed 10 hr. after first of 8 intraperitoneal injections of puromycin. Two large areas of cytoplasmic granules (unlettered arrows) lie in area of vesiculated granular endoplasmic reticulum containing intracisternal granules. Remnant of endoplasmic reticulum (er) lies within 1 of the granular lesions. Mitochondria (m), autophagic vacuole (av). × 22,000. **Fig. 5.** Portion of acinar cell cytoplasm from rat killed 10 hr. after first of 8 subcutaneous injections of puromycin. Cisternal spaces of granular endoplasmic reticulum are dilated to varying degrees, and granules are present in many such spaces (arrows). Mitochondrion (m) shows irregular outer membrane and partial loss of cristae. × 17,000. **Fig. 6.** Portions of 2 acinar cells from rat killed 24 hr. after first of 6 hourly subcutaneous injections of puromycin. One cell has greatly dilated cisternal spaces containing a few granules (arrows). Cell membrane (cm), nucleus (N), nucleolus (ncl). × 12,000.

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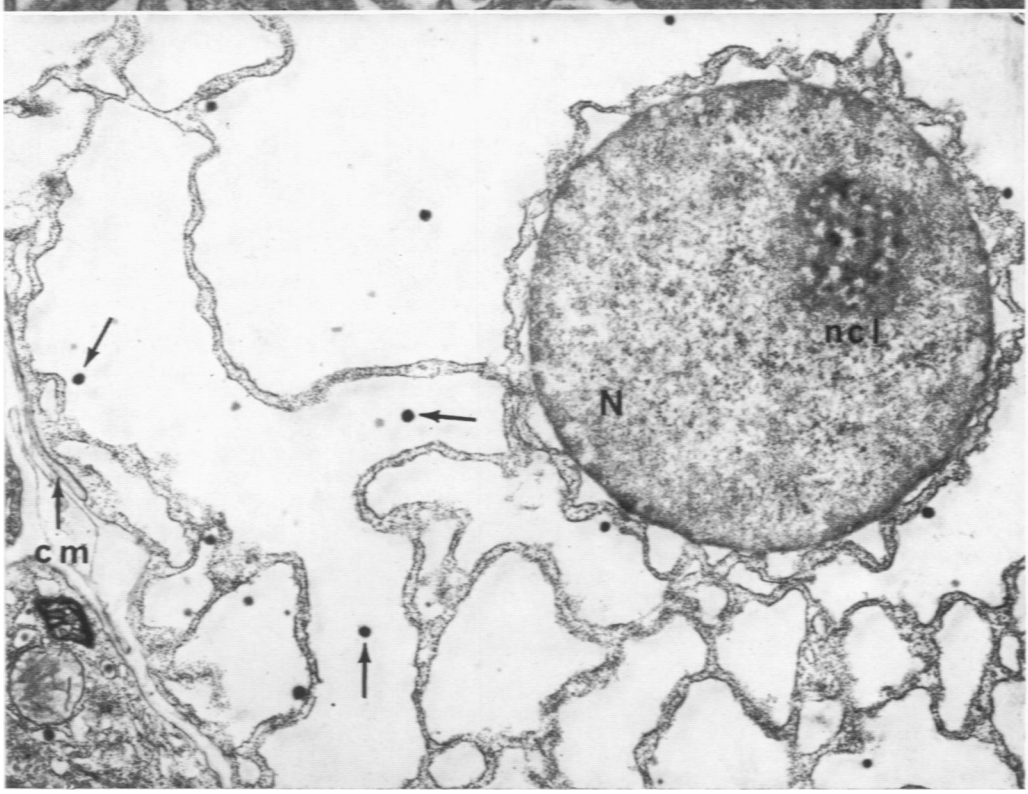


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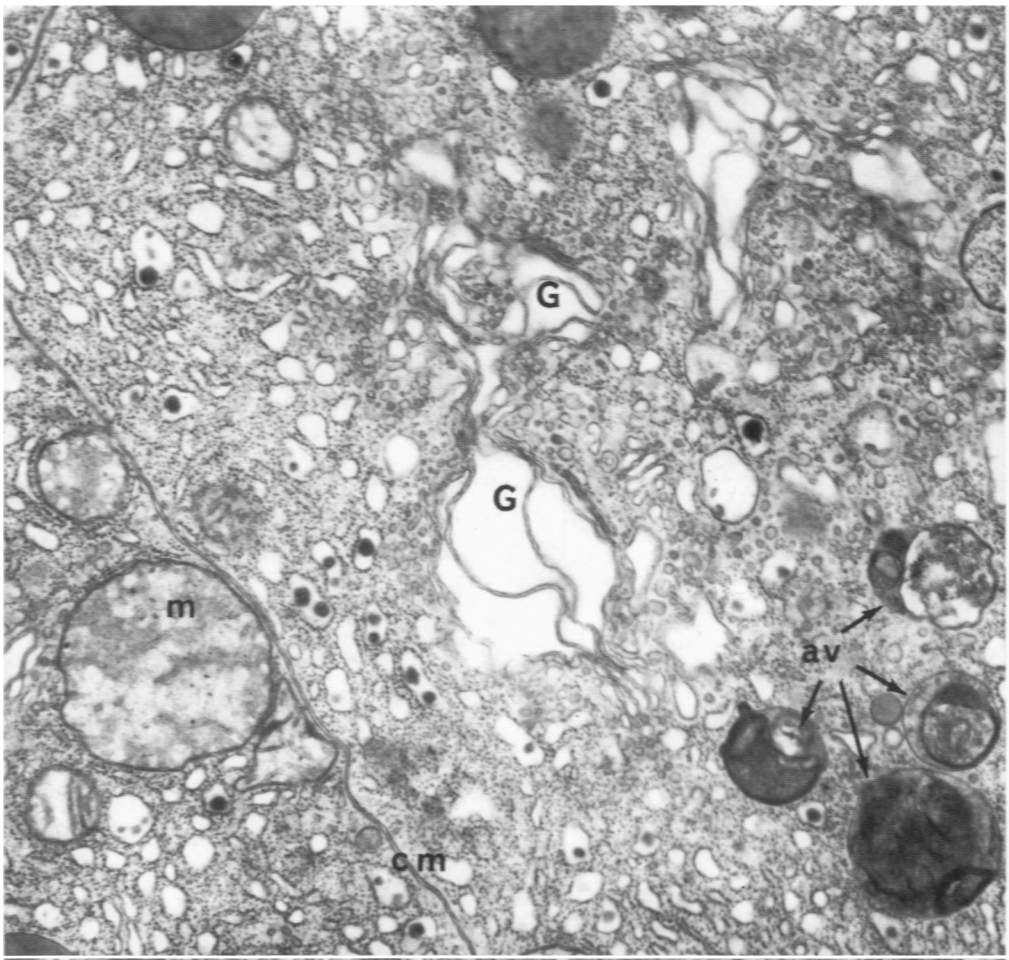
**Fig. 7.** Portions of 2 acinar cells from rat killed after 6 hr. of subcutaneous puromycin treatment. Dilated Golgi cisternae (G) are surrounded by numerous small smooth vesicles. Several small autophagic vacuoles (av) are present. One mitochondrion (m) appears swollen and shows smudging of the matrix and loss of cristae. Cell membrane (cm). × 21,000.

**Fig. 8.** Portion of acinar cell with 2 nuclei from rat killed 10 hr. after first of 8 subcutaneous injections of puromycin. Nuclei (N) show peripheral condensation of chromatin (chr). Nucleolar (ncl) components have aggregated into a mass of increased density. Cytoplasm contains intracisternal granules, a granular lesion (double arrows), and an autophagic vacuole. × 8700.

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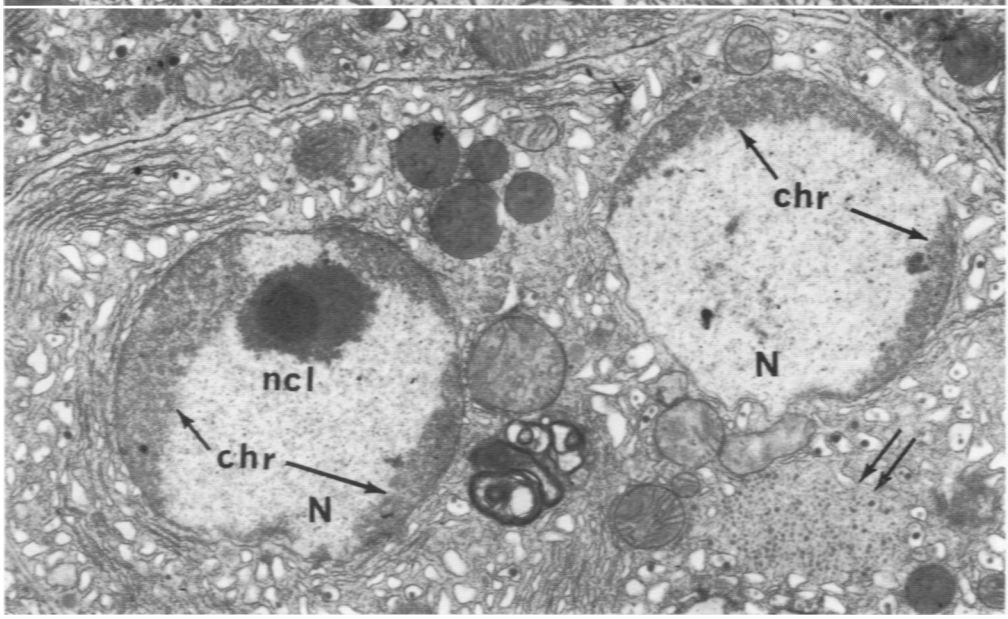


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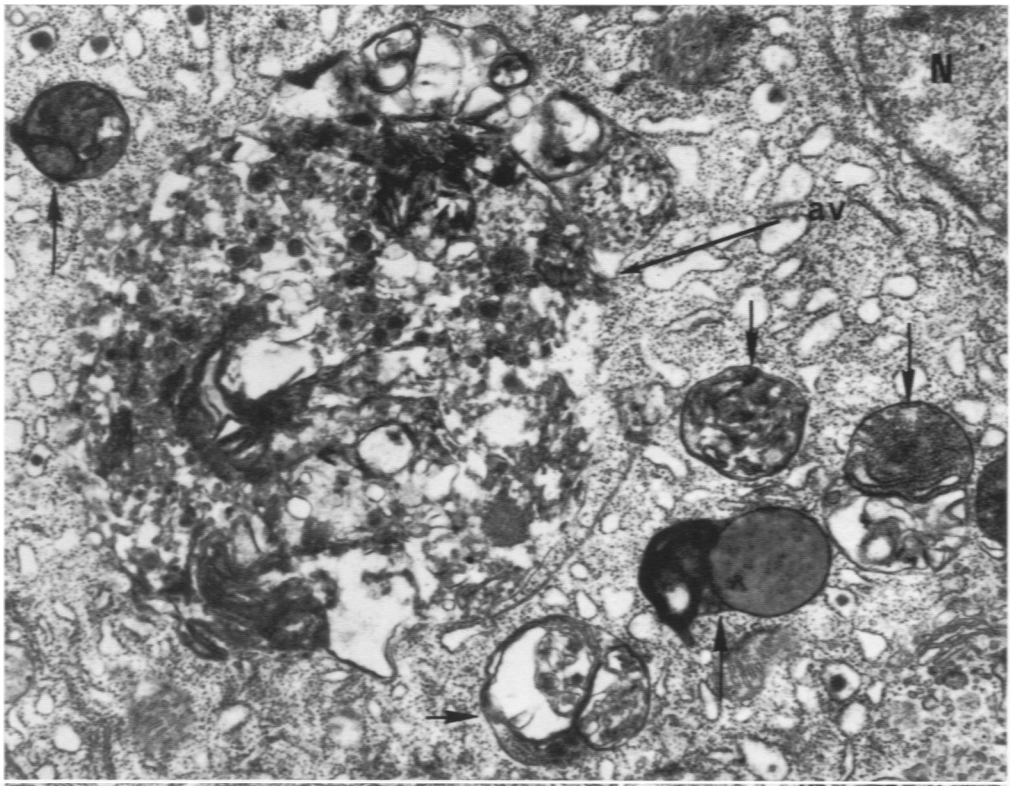
**Fig. 9.** Portions of acinar cell from rat killed 10 hr. after first of 8 subcutaneous injections of puromycin. One large (av) and 5 smaller autophagic vacuoles (arrows) are evident. Zymogen granule, portions of granular endoplasmic reticulum, and cisternal granules can be identified within various vacuoles. Nucleus (N). × 19,500.

**Fig. 10.** Portion of acinar cell from rat killed 10 hr. after first of 8 intraperitoneal injections of puromycin. An autophagic vacuole contains numerous zymogen granules (z) and membranous debris. × 15,600.

**Fig. 11.** Portion of acinar cell from same rat as shown in Fig 10, with 5 autophagic vacuoles containing debris, which is largely unidentifiable. × 14,000.

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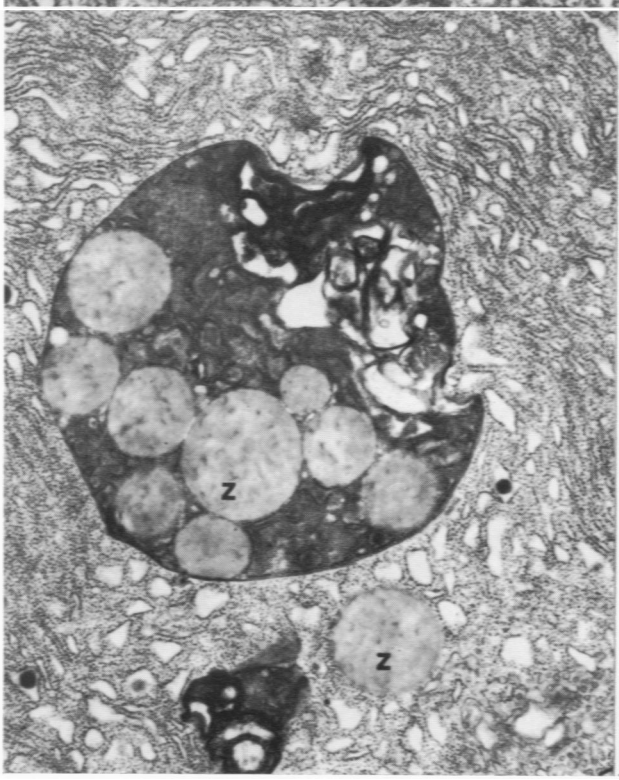


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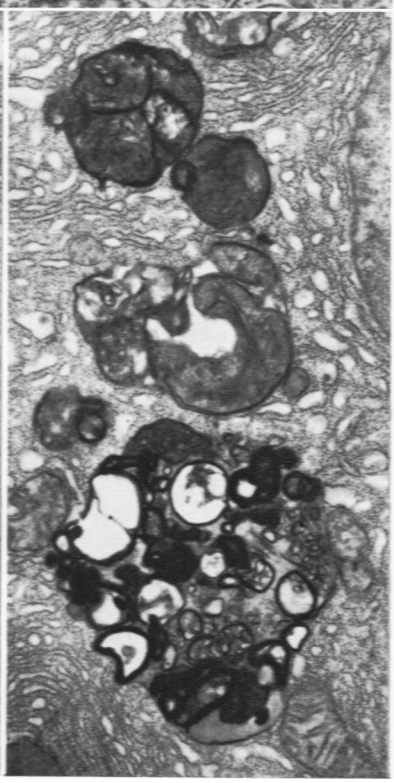

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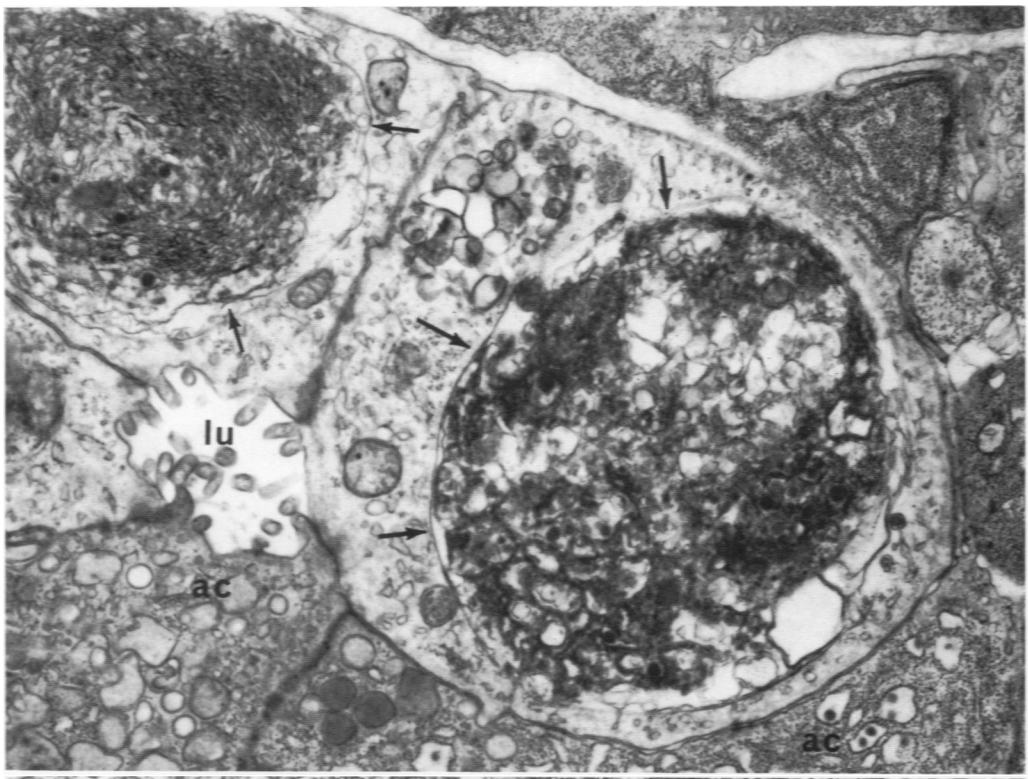
**Fig. 12.** Portions of several acinar cells (ac) and 3 centroacinar cells enclosing a lumen (lu) from rat killed 24 hr. after first of 6 hourly subcutaneous injections of puromycin. Two centroacinar cells contain inclusions (arrows) containing membranous debris and dense granules having appearance of intracisternal granules of acinar cells. × 17,000.

**Fig. 13.** Portion of acinar cell from same rat as shown in Fig. 12. Field is largely occupied by cytoplasmic debris which we interpret as lying within a large autophagic vacuole separated from viable cytoplasm by double membrane (arrow). × 18,000.

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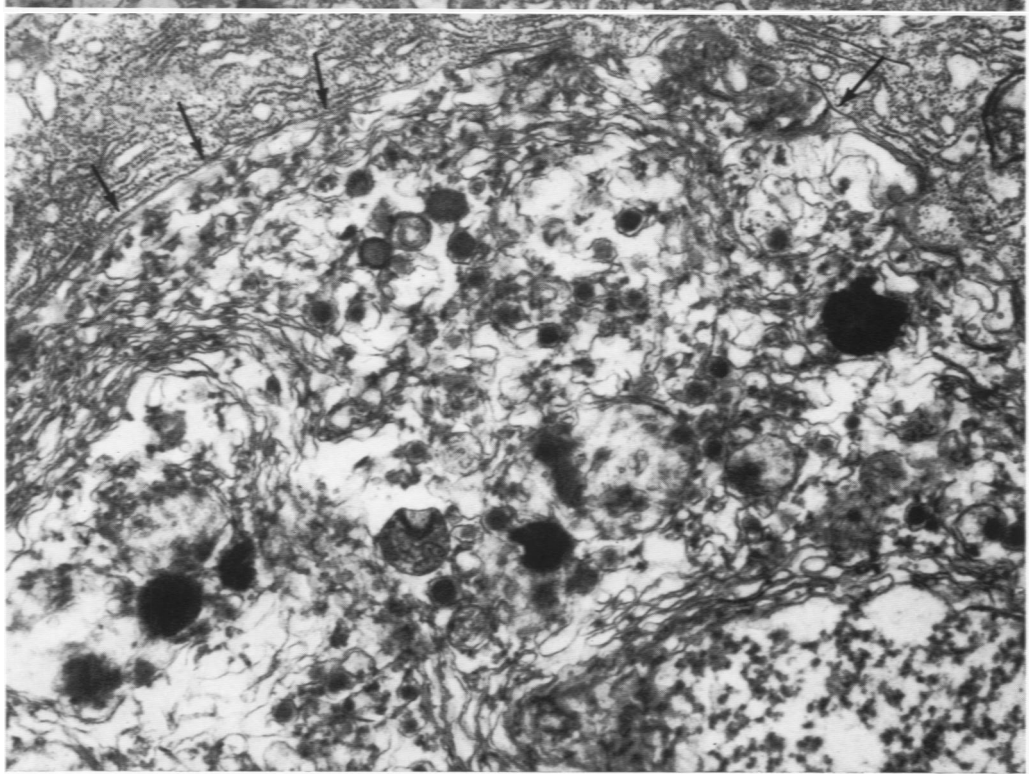

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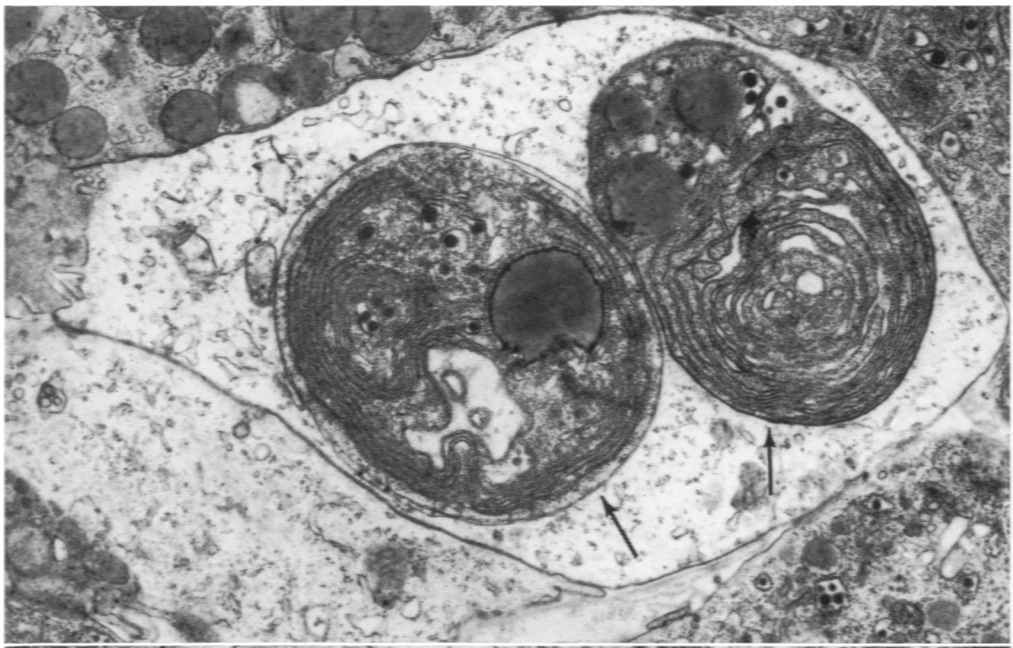
**Fig. 14.** Centroacinar cell from rat killed 10 hr. after first of 8 subcutaneous injections of puromycin. Cell contains 2 membrane-bound inclusions (arrows) with readily identifiable acinar cell organelles including rough endoplasmic reticulum and zymogen granules. × 13,000.

**Fig. 15.** Portion of necrotic acinar cell from rat killed 24 hr. after first of 6 hourly subcutaneous injections of puromycin. Cytoplasmic organelles show varying degrees of degradation; nucleus (N) appears to be severely altered. Dilated cisternae with intracisternal granules (cg) are seen at left. Several altered mitochondria (m) contain electron-dense material in the matrix. × 18,000.



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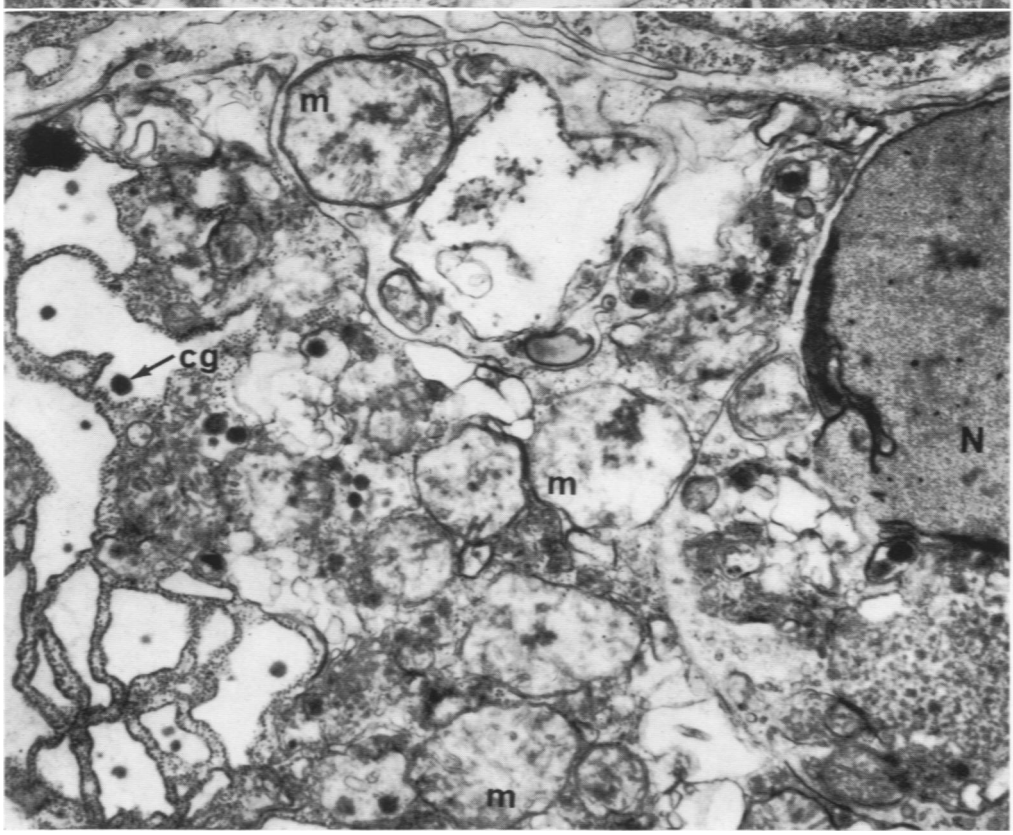


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