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Sequential Ultrastructural Changes of the Pancreas in Zinc Toxicosis in Ducklings

Evelyn A. Kazacos and John F. Van Vleet
From the Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana

The sequential ultrastructural alterations of the pancreas in zinc toxicosis were examined in ducklings fed 2500 ppm Zn (as $ZnSO_4$) for 56 days. From days 3 to 17, acinar cells had cytoplasmic vacuoles that contained electron-dense, zymogen-like material and increased autophagocytosis. Other changes were swollen mitochondria and dilatation, vesiculation, degranulation and intracisternal sequestration of rough endoplasmic reticulum. Apoptosis was the predominant form of cell deletion. By day 10, acinar cellular atrophy and interstitial fibrosis were noted. Islets appeared normal. After day 19, the pancreas consisted of ductlike structures embedded in fibrous connective tissue with a minimal inflammatory cell response. These ductlike structures were lined by attenuated to cuboidal, atrophic acinar cells. Many cells contained granular, electron-dense cytoplasmic debris that served as a marker of previous cell damage. This ultrastructural study provides support for a previously proposed theory that ductlike structures (tubular complexes) arise by atrophy and dedifferentiation of acinar cells. (Am J Pathol 1989, 134:581–595)

The toxic effects of zinc have been reported in numerous animal species[1,2] and in humans,[1] but zinc is considered a relatively nontoxic element in comparison with other metals.[1] Pancreatic alterations were observed in experimentally-produced zinc toxicosis in cats,[3] sheep,[4] calves,[4] chickens,[5,6] and ducklings,[7] and in naturally occurring cases of zinc toxicosis in sheep[4] and calves.[4] In human cases of zinc toxicosis, elevated plasma amylase or lipase activity suggested pancreatic derangement.[1,8]

In animals, descriptions of the light microscopic changes in the pancreas were limited to chronically affected organs and were similar in the various animal species.[3–7] Changes within acinar cells consisted of loss of zymogen granules, loss of basophilic staining, cytoplasmic vacuolation, cellular atrophy, and necrosis of individual acinar cells. In sheep,[4] calves,[4] and cats,[3] an inflammatory cell infiltrate was present in the interstitium and, in sheep[4] and chickens,[6] islet changes were reported. Ductlike structures embedded in abundant fibrous connective tissue characterized the end-stage pancreatic lesion of zinc toxicosis. Similar ductlike structures have been reported in a number of diseases of the pancreas;[9–25] however, the origin of these ductlike structures is in question.[9]

The ultrastructural alterations in the pancreas of chickens with zinc toxicosis consisted of dilated rough endoplasmic reticulum (RER), fewer zymogen granules, and increased numbers of small, electron-dense granules and autophagic vacuoles.[26] These observations, however, were limited to an advanced stage of pancreatic injury.

The purpose of the present study was to characterize the acute and chronic sequential ultrastructural alterations of the pancreas in zinc toxicosis in ducklings, compare these findings with those reported in other models of pancreatic injury, and determine the morphogenesis of the ductlike structures.

Materials and Methods

Three hundred sixty, one-day-old, male White Pekin ducklings (Maple Leaf Farms, Inc., Milford, IN) were randomly divided into three groups and raised in pens on the floor. After acclimation to these environmental conditions for 2 days, the ducklings were placed on the experimental rations. The control group (N = 109) was fed *ad libitum* a commercial duck ration (Sauders Feed, Grabill, IN) supplemented with 200 I.U. vitamin E (Rovimix E 50%, Hoffman La-Roche, Nutley, NJ) per kg feed (CR + E) to prevent nutritional myopathy. The zinc group (N = 139) was fed *ad libitum* CR + E plus 2500 ppm Zn as $ZnSO_4 \cdot H_2O$ (Sargent-Welch, Skokie, IL). The inanition control group (N = 112) was fed measured amounts of CR + E based on the lowest average daily feed intake of ducklings in the zinc group. Six or eight ducklings from each of the 3

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Address reprint requests to Dr. Evelyn A. Kazacos, Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907.

treatment groups were randomly selected for necropsy on days 0, 3, 5, 7, 10, 12, 14, 17, 19, 21, 24, 26, 28, 35, 42, 49, and 56. Ducklings were killed by cervical dislocation. The pancreas was quickly removed and 2–3 mm thick slices from the middle of the dorsal and ventral lobes were placed in ice-cold 3% glutaraldehyde in 0.1 M phosphate buffer. These slices were minced to 1 cu mm and fixed overnight at 5 C. The tissue samples were transferred to a sucrose-buffer storage solution until processed for electron microscopy. The remaining portion of pancreas was fixed in 10% neutral buffered formalin, processed routinely for light microscopy, and stained with hematoxylin and eosin (H & E). Based on the findings from the microscopic evaluation of paraffin-embedded sections, pancreata from selected ducklings were chosen for further processing for electron microscopy. The sections of pancreas stored in the sucrose-buffer solution were rinsed overnight in phosphate buffer solution. They were postfixed in 1% osmium tetroxide, dehydrated in a graded series of alcohol solutions, *en bloc* stained with 2% aqueous uranyl acetate, and embedded in an epoxy resin (Poly/Bed, Polysciences Inc., Warrington, PA). One-micron thick sections of pancreata from 9 control ducklings (4 day 0, 1 day 3, 1 day 7, 1 day 14, 1 day 28, 1 day 56), 6 inanition control ducklings (2 day 7, 1 day 14, 1 day 17, 1 day 24, 1 day 56), and 30 zinc-fed ducklings (4 day 3, 2 day 5, 3 day 7, 3 day 10, 2 day 12, 2 day 14, 3 day 17, 3 day 19, 1 day 21, 3 day 24, 1 day 28, 1 day 35, 2 day 42) were stained with methylene blue-azure II for microscopic study. From these sections, samples were selected from 8 control ducklings (3 day 0, 1 day 3, 1 day 7, 1 day 14, 1 day 28, 1 day 56), 3 inanition control ducklings (1 day 7, 1 day 14, 1 day 56), and 21 zinc-fed ducklings (4 day 3, 2 day 5, 3 day 7, 2 day 10, 2 day 12, 2 day 14, 2 day 17, 1 day 19, 1 day 24, 1 day 35, 1 day 42) for electron microscopy. The tissue samples were thin-sectioned, stained with uranyl acetate and lead citrate and examined in the electron microscope (JEOL/100 CX, JEOL USA, Electron Optics, Medford, MA).

Results

Control Ducklings

The ultrastructural features of the pancreas from control ducklings were similar to those described previously in various avian[27] and mammalian species.[28,29] Ductal cells of the normal pancreas could easily be distinguished from acinar cells (Figure 1). Ductal cells were more electron dense and had fewer cytoplasmic organelles, longer and more numerous microvilli, and more prominent bundles of fine filaments. The lateral plasma membrane had numerous deep interdigitations as compared to the straight borders of adjacent acinar cells. The nuclear membrane of ductal cells was often convoluted. These ultrastructural features of duct cells were important in distinguishing them from atrophic acinar cells in the chronic lesions of zinc toxicosis. The only change noted in acinar cells of control pancreata was an increase in the number of zymogen granules over the 56-day experimental period. No changes were noted in ductal or islet cells. The ultrastructural features of pancreata from the inanition control ducklings were similar to those of the control group.

Zn-Fed Ducklings, Day 3

The semi-thin, plastic-embedded sections from all four ducklings revealed mild to marked cytoplasmic degenerative changes that were multifocally distributed throughout the tissue specimens. Ultrastructurally, acinar cells in the most severely affected pancreas had multiple vacuoles that contained electron-dense, nonhomogeneous, zymogenlike material or flocculent, electron-lucent material (Figures 2, 3). Some of these vacuoles also contained membranous debris or fragments of RER. Some cells had normal-appearing zymogen granules, whereas others had numerous, small, electron-dense granules. These smaller granules often were associated with the vacuoles containing zymogenlike material. Scattered cells had large, irregularly-shaped, apical vacuoles, some of which were continuous with the acinar lumen and contained flocculent material that was similar to the secretory material present within the lumen. These latter structures were consistent with exocytic vacuoles. Autophagic vacuoles consisting of small fragments of RER were also noted. The mitochondria of affected cells were swollen, electron-lucent, and had loss of cristae. Alterations in the RER included dilatation, vesiculation, degranulation, and a decrease in the number of membranous arrays. Most acinar cells were affected but to variable degrees. Acinar lumina were small and contained a scant amount of granular material. The interacinar septa contained fragments of acinar cells and granular, electron-dense material that was consistent with edema fluid. In the other three ducklings, few, scattered acinar cells had large exocytic and autophagic vacuoles.

Zn-Fed Ducklings, Days 5 and 7

On days 5 and 7, acinar cell changes (Figure 4) were marked, diffuse, and similar to those described above. In addition, increased numbers of acinar cells contained numerous, small, variably-shaped vacuoles that contained homogeneous, electron-dense material or small fragments of RER. Apoptotic bodies were within cells or acinar lumina (Figure 5). A few acinar cells contained phagocytosed apoptotic bodies consisting of dense

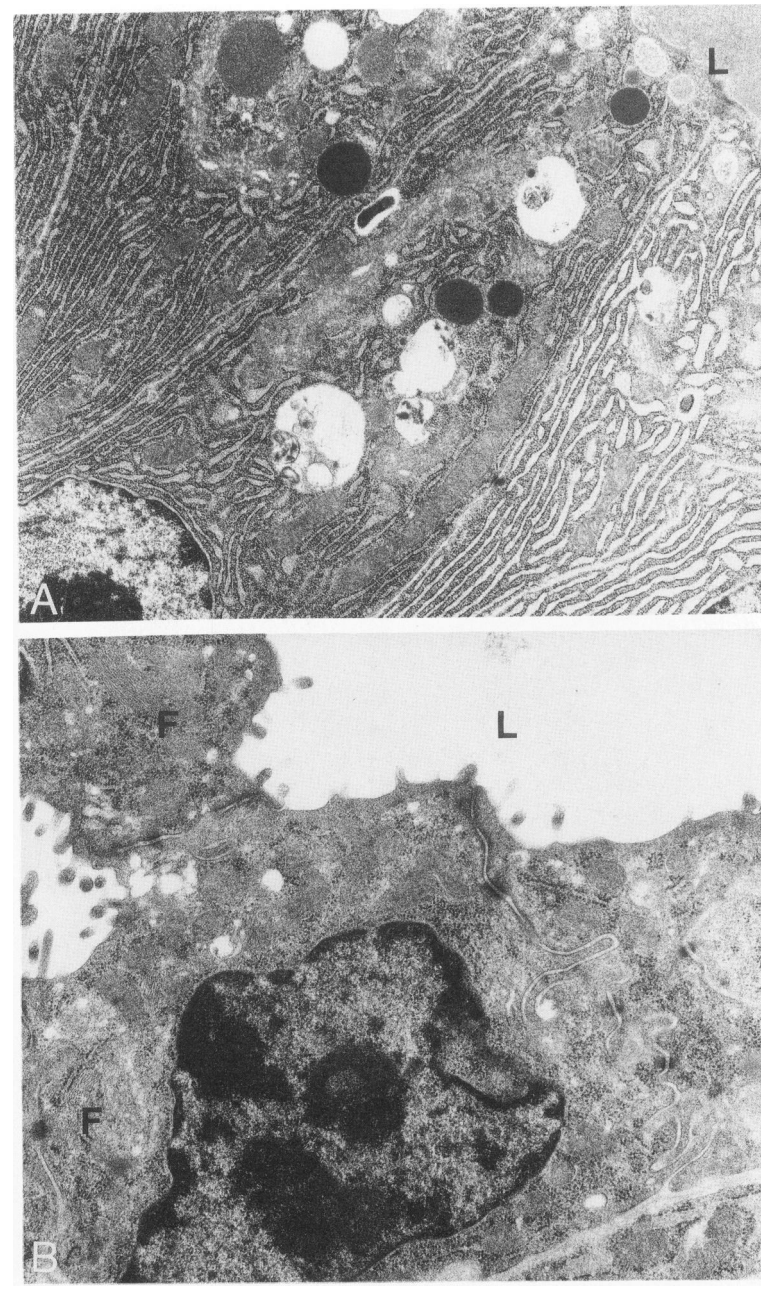

Figure 1A. *A high magnification of normal acinar cells from a control duckling. Note the round, basally-located nucleus and the straight lateral plasma membranes. The cytoplasm contains abundant RER, a few zymogen granules, rod-shaped mitochondria, and several autophagic vacuoles (×8800). L, lumen.* **B:** *A high magnification of normal intralobular duct cells from a control duckling. The ductal cell possesses an irregularly-shaped nucleus, few cytoplasmic organelles and bundles of fine filaments (F). Note the prominent interdigitations of the lateral plasma membranes (×16,000). L, lumen.*

whorls of RER, intact zymogen granules and condensed nuclear material. Phagosomes containing degenerating apoptotic bodies were also noted within acinar cells. Occasional acinar lumina contained apoptotic bodies consisting of membrane-bound blebs of cytoplasm and intact organelles. Interstitial cells contained apoptotic bodies, membrane debris or zymogenlike material.

Zn-Fed Ducklings, Day 10

By day 10, acinar cells were atrophic and had severe degenerative changes consisting primarily of cytoplasmic vacuolation. The contents of these membrane-bound vacuoles were variable and included zymogenlike material, electron-dense coarse granular material, whorls of RER, and recognizable zymogen granules. Numerous small vacuoles that were empty or contained homogeneous electron-dense material were located in the apical cytoplasm or adjacent to the Golgi complex. Normal-appearing zymogen granules could still be identified. The RER had a number of alterations. In a few cells, the RER formed "fingerprintlike" whorls around mitochondria. In other cells, the RER was vesiculated and dilated. Some of the dilated cisternae contained electron-dense intracisternal granules, fine granular material or invaginated portions of RER. There

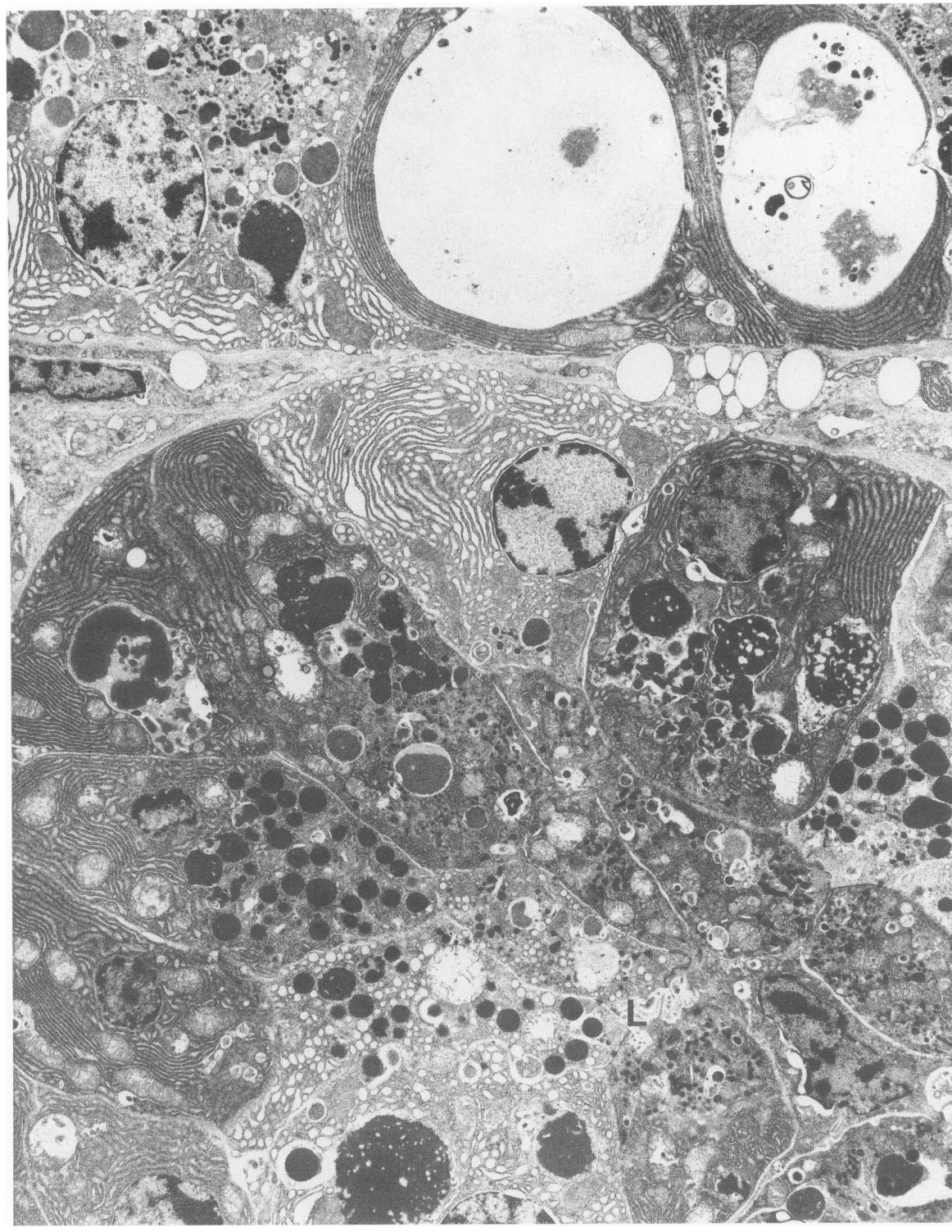

Figure 2. *Pancreatic acini from a duckling fed zinc for 3 days, showing cellular vacuolar degeneration. Many cells have vacuoles that contain nonhomogeneous, zymogenlike material. Some vacuoles contain little granular and membranous debris (top). Dilatation and vesiculation of the RER and swollen mitochondria are present. The acinar lumen (L) is empty (×4400).*

was evidence of intracisternal sequestration. Nuclear changes were minimal and consisted of multifocal dilatation of the nuclear envelope. Some acinar lumina contained granular and membranous debris or blebs of cytoplasm with intact organelles. Small apoptotic bodies consisting of membrane-bound whorls of RER and zymogen granules or mitochondria were located in the intercellular space between acinar cells (Figure 5). Acinar cells and an occa-

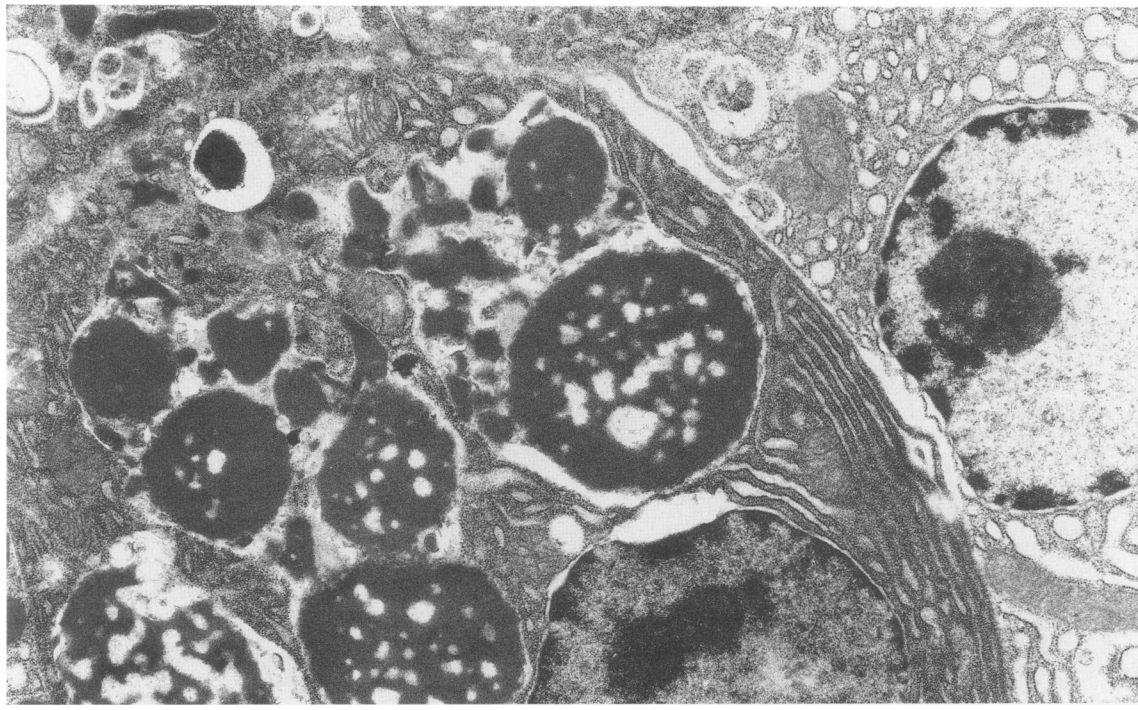

Figure 3. *Higher magnification of a portion of the acinus in Figure 3. Large cytoplasmic vacuoles contain zymogenlike material. The adjacent cell has dilatation and degranulation of the RER and abundant free ribosomes (×11,000).*

sional centroacinar cell contained apoptotic bodies in various stages of degeneration.

The interstitium had granular, electron-dense material, increased numbers of collagen fibers and fibroblasts, and a few macrophages that contained apoptotic bodies of acinar cell origin within their cytoplasm. Capillary endothelial cells were swollen, had numerous apical processes, and increased numbers of caveolae.

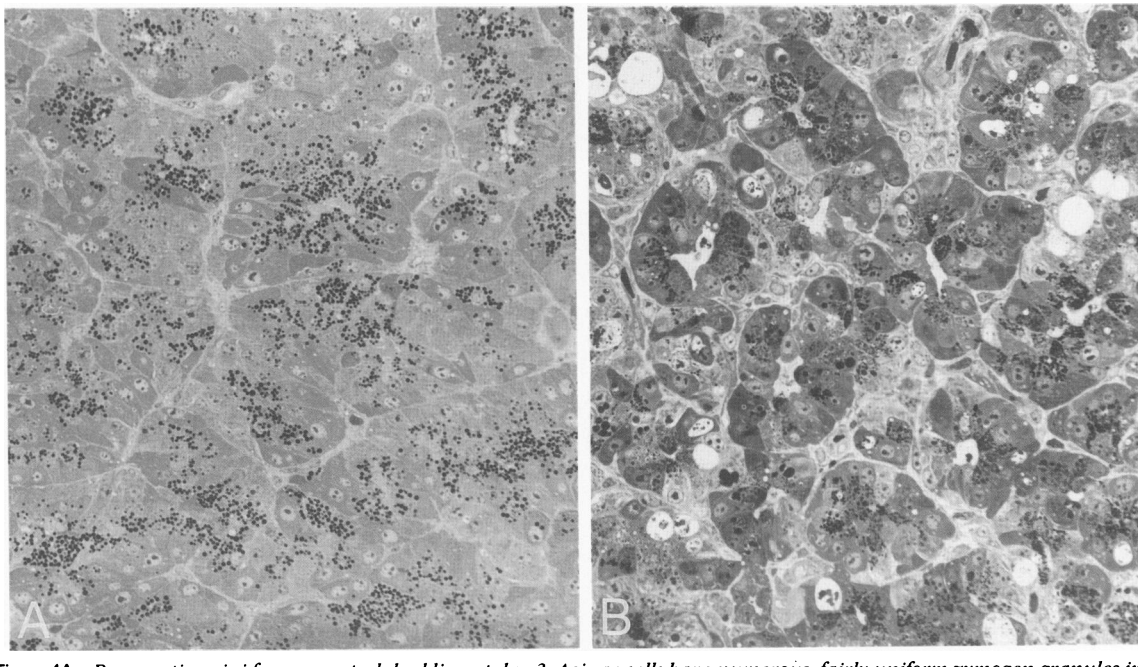

Figure 4A. *Pancreatic acini from a control duckling at day 3. Acinar cells have numerous, fairly uniform zymogen granules in the apical cytoplasm. Plastic-embedded section, methylene blue-azure II; ×400.* **B:** *Pancreatic acini from a duckling fed zinc for 5 days. Note uniform involvement of acini within the field. Acinar cells contain vacuoles that are empty or contain dense-staining material. Some cells still have normal zymogen granules. Plastic-embedded section, methylene blue-azure II; ×400.*

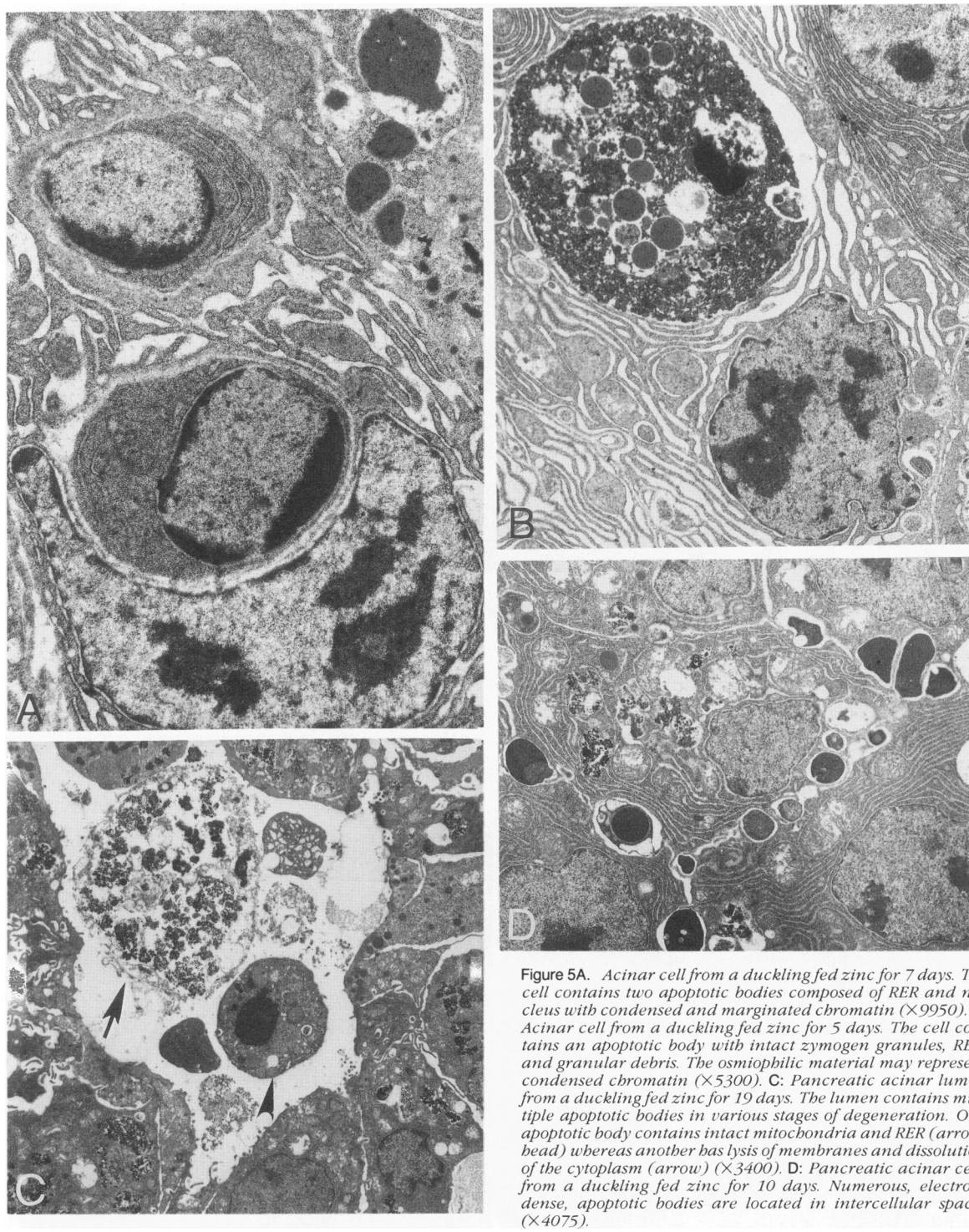

Figure 5A. *Acinar cell from a duckling fed zinc for 7 days. The cell contains two apoptotic bodies composed of RER and nucleus with condensed and marginated chromatin (×9950).* B: *Acinar cell from a duckling fed zinc for 5 days. The cell contains an apoptotic body with intact zymogen granules, RER, and granular debris. The osmiophilic material may represent condensed chromatin (×5300).* C: *Pancreatic acinar lumen from a duckling fed zinc for 19 days. The lumen contains multiple apoptotic bodies in various stages of degeneration. One apoptotic body contains intact mitochondria and RER (arrowhead) whereas another has lysis of membranes and dissolution of the cytoplasm (arrow) (×3400).* D: *Pancreatic acinar cells from a duckling fed zinc for 10 days. Numerous, electron-dense, apoptotic bodies are located in intercellular spaces (×4075).*

Ultrastructural alterations within ductal epithelial cells were minimal. In some sections of affected pancreata, ductal cells stained less electron-dense than normal ductal cells. Most ductal cells had a few empty cytoplasmic vacuoles. Ducts with these relatively mild changes were located adjacent to severely degenerated acinar cells. Islet cells were ultrastructurally normal.

Zn-Fed Ducklings, Days 12 to 17

The principal alteration during this time period was progression of the degenerative and atrophic changes within acinar cells as described above. Apoptotic bodies were occasionally observed. At day 17, altered acinar cells could be categorized into one of the following five pat-

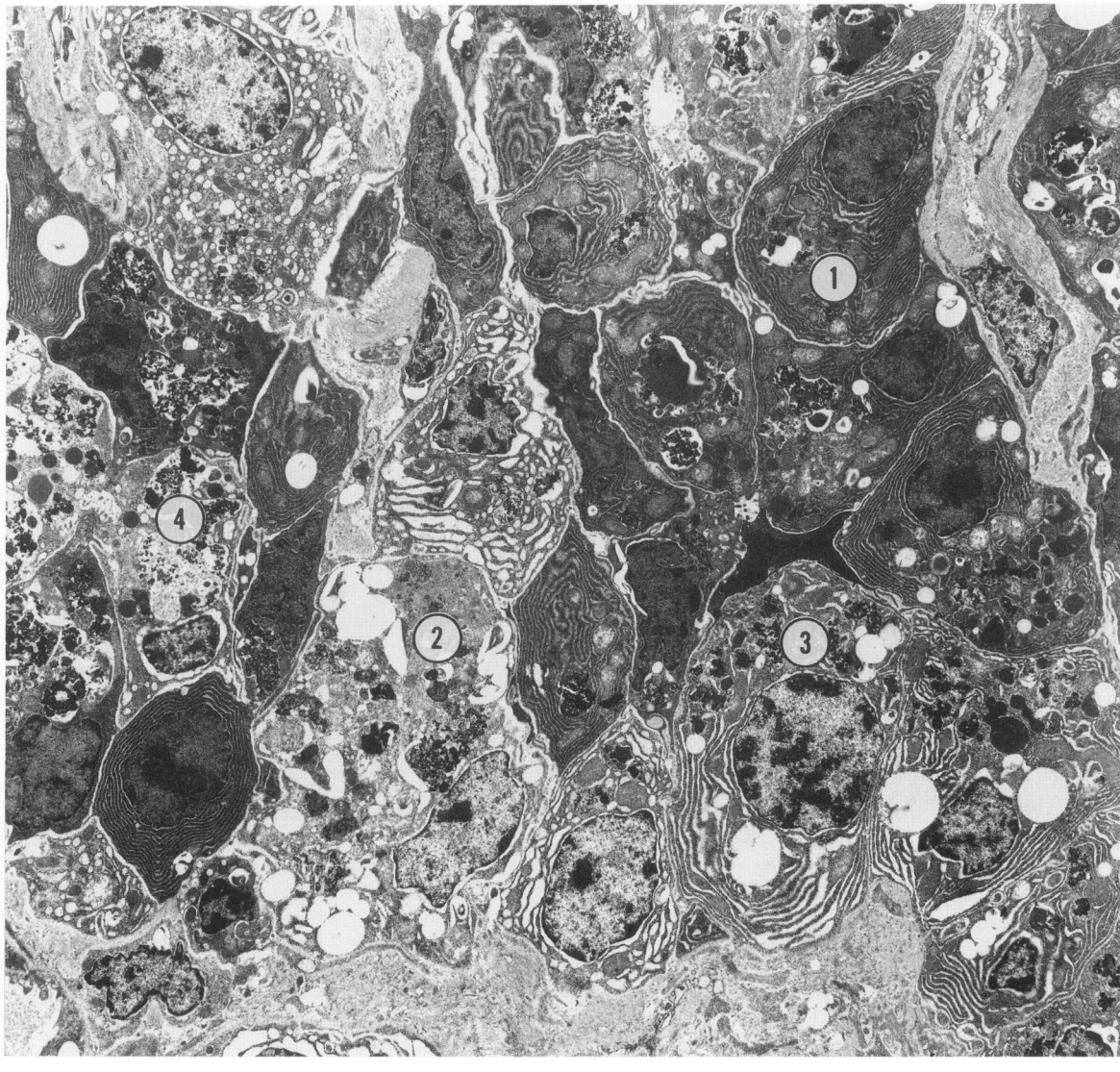

Figure 6. *A section through several pancreatic acini from a duckling fed zinc for 17 days. Acinar cells are atrophic and contain a moderate amount of RER. Vesiculation and dilatation of the RER are observed in some cells. Cytoplasmic vacuoles contain electron-dense, granular debris. A cell with electron-dense cytoplasm and nucleus is present. Cell types 1 to 4, as described in the text, can be identified. The interstitium (bottom) has numerous collagen fibers and fibroblasts (×3650).*

terns based on the ultrastructural features of their degenerative changes (Figure 6). 1) Type 1. This dense cell contained closely packed arrays of RER that had granular electron-dense material within the cisternae. Abundant mitochondria and some phagosomes also were present. The nucleus was generally electron-dense but clumps of heterochromatin could still be distinguished. 2) Type 2. This pale cell had electron-lucent cytoplasm that contained little RER. Vesiculation and degranulation of the remaining RER and phagosomes were present. The nucleus was electron-lucent and had heterochromatin. 3) Type 3. This intermediate cell had moderate amounts of RER, phagosomes, and electron-lucent nucleus. The cisternae of the RER were moderately dilated and empty. Cells of the intermediate type were the most abundant. 4) Type 4. Dying acinar cells were identified by their markedly condensed cytoplasm and nucleus or severe cytoplasmic autophagy. In cells with the latter change, the cytoplasm consisted primarily of phagocytic vacuoles and only a thin rim of cytoplasm could be identified. 5) Type 5. Acinar cells with large, euchromatic nuclei and electron-lucent cytoplasm were noted between days 10 and 19 (Figure 7). The cytoplasm of this cell type contained RER with dilated cisternae that contained a slight amount of granular material or invaginated portions of RER. Some cisternae were empty. The nuclei of these cells usually had prominent nucleoli.

The interstitial changes (Figure 6) were more prominent than in the acute lesions and consisted of thickening of the interacinar septa due to increased numbers of fi-

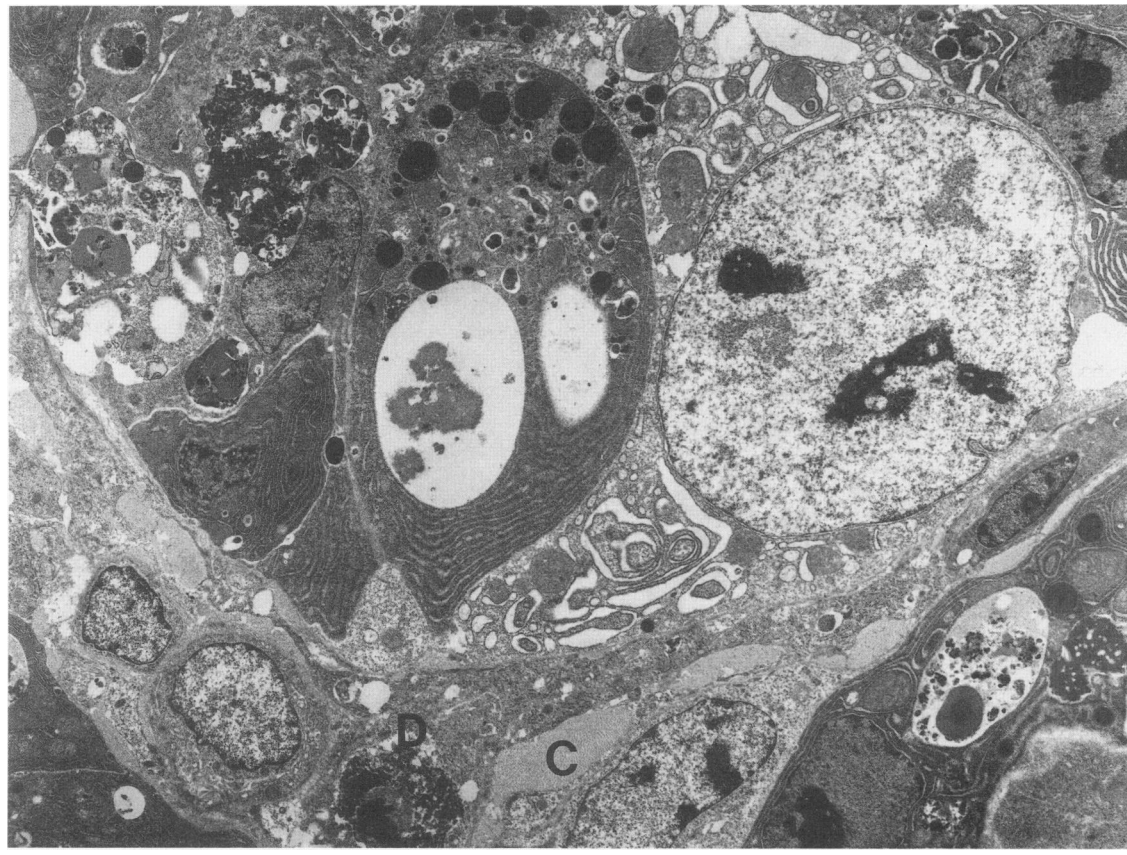

Figure 7. *Pancreatic acinar cells from a duckling fed Zn for 10 days. The cell to the right has a large euchromatic nucleus and electron-lucent cytoplasm. The RER is dilated and vesiculated and has intracisternal sequestration. Adjacent degenerative acinar cells contain autophagic vacuoles. The interstitium (bottom) contains abundant collagen fibers (C), a few fibroblasts, and macrophages with cell debris (D) (×4000).*

broblasts and deposition of collagen fibers. A minimal inflammatory cell infiltrate consisted primarily of macrophages with acinar cell debris and few heterophils and mast cells.

Zn-Fed Ducklings, Days 19 to 56

From days 19 to 56, chronically-injured pancreata had atrophic, ductlike structures embedded in variable amounts of fibrous connective tissue (Figure 8). Ultrastructurally, acinar cells were markedly atrophic and had residual degenerative changes. Longitudinal sections through branching, ductlike acini (Figure 9) revealed that the degree of cellular atrophy was variable. Some acinar cells were attenuated whereas others were low cuboidal. The five cell variants described above were still present in the chronically-injured pancreata of ducklings fed Zn for up to 56 days. Atrophic acinar cells had ultrastructural features of ductal cells, such as convoluted nuclei and numerous interdigitations of the lateral plasma membranes; however, these atrophic cells lacked the bundles of fine filaments characteristic of ductal cells. These altered cells were identified as acinar cells by the presence of moderate amounts of RER, occasional zymogen granules, and electron-dense granular debris within vacuoles and free in the cytoplasm (Figure 10). Apoptotic bodies were rarely observed.

The interstitium (Figures 9, 10) contained irregularly-arranged bundles of collagen fibers and numerous polygonal to elongate fibroblasts that often formed whorls around atrophic acini. Fibroblasts and macrophages contained acinar cell debris within their cytoplasm. Intraepithelial mononuclear phagocytes containing electron-dense debris were occasionally noted and one was caught in the process of traversing the basement membrane (Figure 11). Ductal epithelium appeared normal; however, the lumen of a few ducts and ductules contained cellular debris and was partially occluded by this material (Figure 12). There were no significant alterations within islet cells.

Discussion

Morphology of the Acute Lesion, Days 3 to 14

In the present study, no ultrastructural changes were noted in the inanition control ducklings that were fed a

semistarvation diet. Similar findings were noted in rats with reduced caloric intake,[30] but decreased numbers of zymogen granules, dilated RER, and a slight increase in the number of autophagic vacuoles were noted in semi-starved rabbits.[31] Mild to severe ultrastructural alterations have been reported in protein-deficient[30–34] and starved[30,35] animals and in human kwashiorkor.[36] The reason for differences in the degree of injury in pancreatic acinar cells under conditions of semi-starvation, starvation, and protein deficiency are unknown, but may be due to the combined effects of significant protein loss from pancreatic secretion and decreased protein intake.[37]

Vacuoles that contained electron-dense, nonhomogeneous, zymogenlike material or granular, electron-lucent material were a distinctive feature in degenerated acinar cells of Zn-fed ducklings at day 3 and throughout most of the acute stage of injury. Structures with similar ultrastructural morphology were noted in acute hemorrhagic pancreatic necrosis (AHPN) in mice,[38] in pancreatitis due to suprastimulation,[39,40] and in cultured pancreatic explants.[41] In these three experimental conditions, cytochemical analyses indicated that these vacuoles contained both amylase, a digestive enzyme, and acid phosphatase, a lysosomal enzyme.[38,39,41] Such vacuoles may be significant in the pathogenesis of acute pancreatitis.[42] Intraparenchymal activation of digestive proenzymes is generally accepted as the inciting cause of pancreatitis, but the site of this activation is unknown. Steer and coworkers[42] proposed that intracellular activation of digestive proenzymes by lysosomal enzymes occurred within cytoplasmic vacuoles and initiated the autodigestive process that accounted for the cellular changes observed in two forms of experimental pancreatitis. A similar sequence of events might explain acinar cell injury in zinc toxicosis but cytochemical analyses of the cytoplasmic vacuoles are needed to support this hypothesis.

Alterations in mitochondria and RER were noted in our Zn-fed ducklings throughout the experimental period and were reflective of sublethal injury to cells.[43] Mitochondrial changes appeared to be reversible because acinar cells of chronically-injured pancreata of Zn-fed ducklings had numerous mitochondria. Alternatively, a few mitochondria may have survived acute damage and later divided. Apparently mitochondrial function was not appreciably altered because autophagocytosis and heterophagocytosis, which require energy,[43] were also observed in these cells.

Intracisternal sequestration of the RER and increased autophagocytosis were noted in many acinar cells of our Zn-fed ducklings. These changes are also a feature of kwashiorkor in humans[36] and a number of other pathologic conditions.[44] Intracisternal sequestration and autophagocytosis may be responsible for elimination and degradation of excess membranes and organelles in acinar

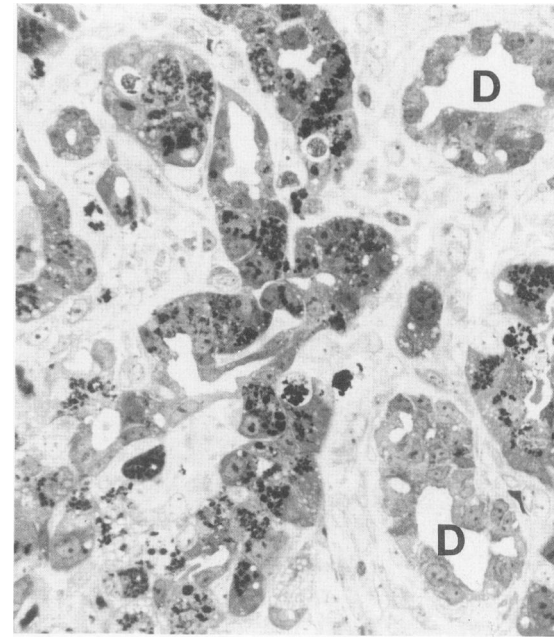

Figure 8. *Pancreatic tissue from a duckling fed Zn for 24 days. Two intralobular ducts (D) are adjacent to branching, atrophic, ductlike acini. The densely-stained cytoplasmic debris distinguishes acinar cells from ductal cells. The interstitium is moderately fibrotic. Plastic-embedded section, methylene blue-azure II; ×520).*

cell atrophy.[36,44] The findings of the present study are in agreement with that proposal.

The severity of acute cellular injury in Zn-fed ducklings varied between acini and within an acinus. This variation may be attributed to differences in zinc uptake or the metabolic state of acinar cells. Marked differences in the degree of cellular injury between adjacent acinar cells were noted in acute pancreatitis[45] and puromycin toxicosis.[46] In contrast, variation in the degree of cellular injury between acini but not within acini was noted in a study on experimental ductal occlusion.[47]

Apoptosis was the predominant form of cell death in the pancreas of ducklings with zinc toxicosis. The morphologic and biochemical features of apoptosis have been reviewed previously.[48,49] In summary, apoptosis is controlled, programmed cell death that is accompanied by little or no inflammation. The nuclear and cytoplasmic changes of apoptosis are distinctive and different from those observed in necrosis. The first stage of apoptosis consists of margination and compaction of chromatin, condensation of cytoplasm, and formation of membrane-bound blebs of cytoplasm containing intact organelles. This process is rapid and can easily be missed with infrequent sampling. The second stage is longer and consists of phagocytosis and subsequent degradation of these apoptotic bodies. Ingested apoptotic bodies can be confused with autophagosomes, but are definitively identified by the presence of nuclear, as well as cytoplasmic material. Because of these difficulties in identification, the de-

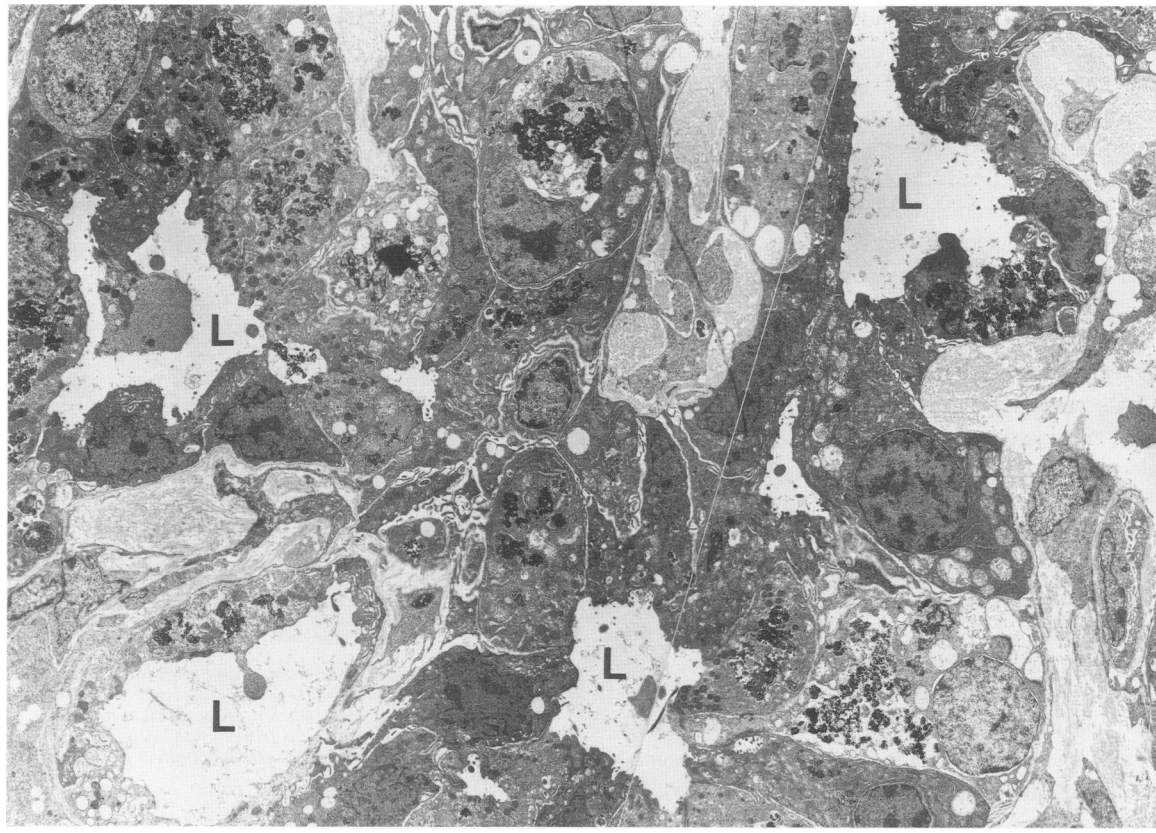

Figure 9. *A montage of a longitudinal section through a branching, ductlike, atrophic pancreatic acinus from a duckling fed Zn for 19 days. Acinar cells are attenuated or low cuboidal and contain electron-dense, granular debris. Various cell types, as described in the text, are present. The interstitium has abundant collagen fibers and fibroblasts (×2450). L, lumen.*

gree of apoptosis is usually greater than that observed in micrographs. Apoptosis is responsible for cell deletion during embryonic development, normal involution, pathologic atrophy and regression of hyperplastic organs. Radiation and radiomimetic drugs cause increased apoptosis in target organs. Apoptosis also accounts for atrophy after injury, such as mild ischemia in the liver, and may be a means of removing cells with damaged DNA or cellular organelles.[48,49] Little is known about the biochemical events associated with apoptosis, but activation of nonlysosomal endonucleases and subsequent cleavage of double-strand DNA may be involved.[49]

Apoptosis in the pancreas has been described in only a few studies. Apoptosis was the principal mode of cell death responsible for involution of the hypertrophied pancreas of rats fed raw soya flour.[50] Cell deletion after ductal ligation of the rat pancreas was attributed to apoptosis.[51] A plant nitrile, 1-cyano-2-hydroxy-3-butene, produced pancreatic lesions consisting of dilatation of the RER and apoptosis.[52] Considering the difficulty in identifying the apoptotic process, it is possible that apoptosis of pancreatic acinar cells occurs more commonly than is reported in the literature and in a wider variety of toxic pancreatic injury.

In our study of zinc toxicosis, apoptosis was the principal means of cell deletion and most frequently observed between days 3 and 17. This process occurred concomitantly with acinar cell injury and may have been a way of removing damaged cells. Apoptotic bodies appeared to be released into acinar lumina and the intercellular space where they were phagocytosed primarily by acinar cells. Apoptotic bodies that had been phagocytosed often were difficult to differentiate from autophagosomes, but a few apoptotic bodies clearly contained nuclear material. Apoptotic bodies remaining within lumina became autolyzed. A few centroacinar cells contained apoptotic bodies of acinar cell origin. Phagocytic capabilities of centroacinar cells, which normally have a secretory function,[53] also have been reported in puromycin toxicosis.[46]

A similar apoptotic process was reported in experimental ductal ligation in rats; in that study, however, apoptotic bodies were preferentially removed by intraepithelial mononuclear phagocytes.[51] In our study, intraepithelial mononuclear phagocytes that contained cellular debris were noted occasionally in the basilar portion of the acinus during the chronic stage of the lesion, but preferential ingestion of apoptotic bodies by this cell type was not obvious.

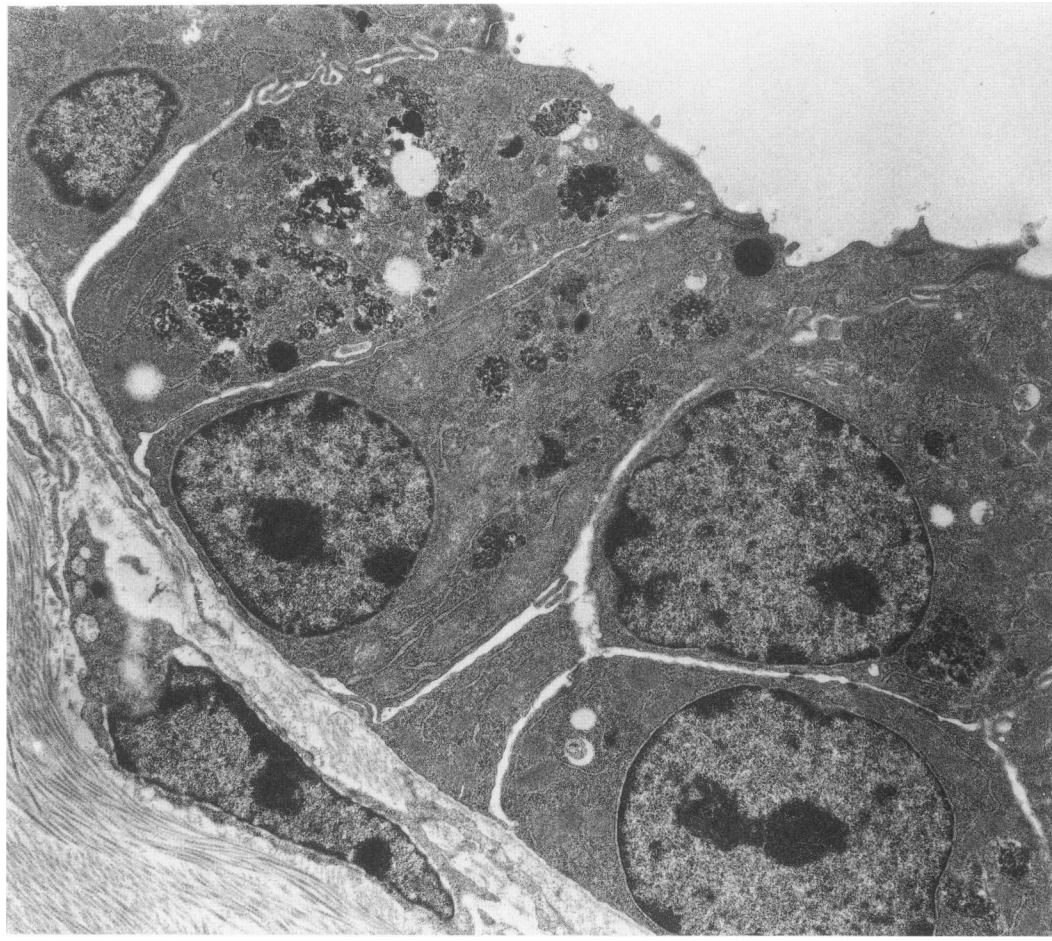

Figure 10. *A high magnification of an atrophic, ductlike acinus from a duckling fed Zn for 42 days. Cells have prominent interdigitation of lateral plasma membranes. Zymogen granules, moderate amounts of RER and mitochondria, and cytoplasmic debris identify these cells as acinar cells. A fibroblast and numerous collagen fibers are in the interstitium (bottom-left) (×7800).*

Apoptotic bodies and degenerating cellular debris often were observed in interstitial cells, but determining how this acinar cell debris reached the interstitial space was difficult. Movement of mononuclear phagocytes between the interstitium and acinus could be one means by which acinar cell debris reached the interstitium. One such phagocyte was caught in the process of penetrating the acinar basement membrane. Passage of cell debris through a damaged basal lamina is another possible mechanism.

The interstitial changes in the pancreas of ducklings with acute zinc toxicosis were not as striking as the parenchymal changes. Edema of the interacinar septa may have been related to the alterations in the capillary endothelium. The lack of a significant inflammatory cell response was a distinctive feature in our study. In contrast, a marked inflammatory response was characteristic of acute pancreatitis,[54] AHPN,[55] and pancreatitis due to suprastimulation.[40] In these conditions, the inflammatory response was secondary to massive release of digestive enzymes into the interstitium and cell necrosis.[40,54,55] A critical factor in the pathogenesis of the severe interstitial lesions of AHPN was accumulation of zymogen granules within the acinar cells.[56] A similar mechanism accounted for more severe lesions of pancreatitis in well-nourished animals than in animals with depleted stores of zymogen granules.[57] In the present experiment, the lack of a significant pancreatic inflammatory response may be related to an initial low concentration of digestive enzymes, controlled release of lysosomal and digestive enzymes, or apoptosis.

Morphology of the Chronic Lesion, Days 17 to 56

Five acinar cell variants were observed in the chronic stage of zinc toxicosis. The ultrastructural differences between these cell types probably reflected both the degree of cell injury and the variable response of cells to injury. The dense cell type represented a cell that had undergone reversible damage and was now maintained in a

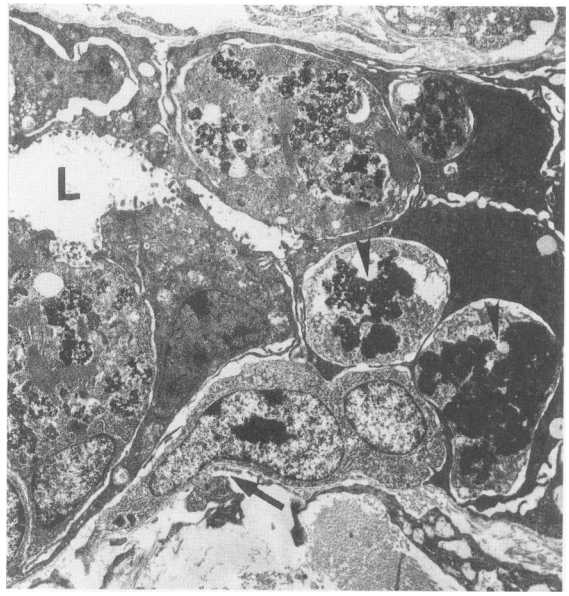

Figure 11. *Pancreatic acinus from a duckling fed zinc for 19 days. An intraepithelial macrophage located in the basal portion of the acinus has a cytoplasmic process penetrating the basal lamina (arrow). Electron-dense debris (arrowhead) is located presumably within other cytoplasmic processes of this same cell (×14,000). L, lumen.*

different homeostatic state characterized by cytoplasmic atrophy and retention of relatively abundant RER and mitochondria. At the other extreme were irreversibly damaged cells that were undergoing autophagy or marked condensation. Between these two extremes were the remaining cell variants with mild to moderate cell injury that

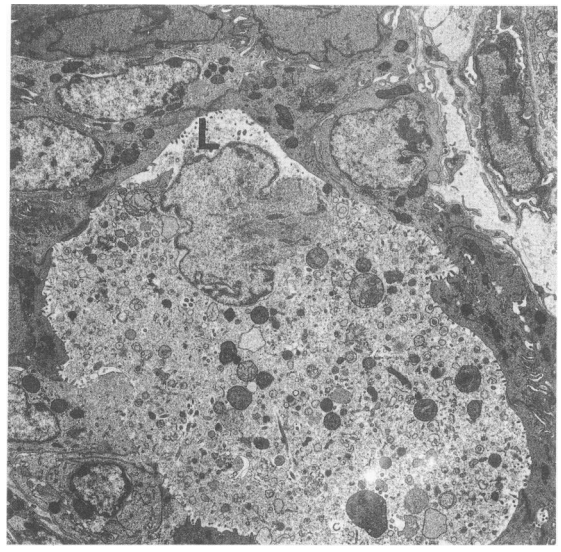

Figure 12. *Pancreatic ductule from a duckling fed zinc for 14 days. Ductular lumen is partially occluded by a swollen, autolyzed acinar cell. The cell membrane has lysed but membranes of mitochondria, RER, and the nucleus are intact. Some mitochondria are swollen (×2950). L, unoccluded portion of lumen.*

may have represented cells in transition to one of the above described end stages. The chronic stage of zinc toxicosis consisted of pancreatic atrophy due to decreased numbers and decreased size of acinar cells. Apoptosis was the predominant means of cell deletion, although progressive autophagy was noted in a few acinar cells. Progressive autophagy accounted for loss of cytoplasmic organelles and reduced cell size.

Chronically-injured pancreata had ductlike acini that were lined by attenuated to low cuboidal cells with features of ductal cells, such as convoluted nuclei and numerous interdigitations of the lateral plasma membranes. These cells were identified as acinar cells based on moderate amounts of RER, occasional zymogen granules, and electron-dense, granular debris within vacuoles and free in the cytoplasm. This debris served as a distinctive marker of previous cell damage.

Ductlike structures (pseudoductules, tubular complexes)[9] have been observed in a number of diseases of the pancreas and experimental models of pancreatic injury, including vitamin E-selenium deficiency,[10] pancreatitis,[11-13] ductal ligation,[14,15] uremia,[16] cystic fibrosis,[17] ethionine[18] and methionine[19] toxicoses, and pancreatic adenocarcinoma.[20-25] The question regarding the morphogenesis of these structures is unresolved. Several workers proposed that these ductlike structures originated from proliferating centroacinar/ductal cells;[10,11,15,25] however, Zeligs et al[14] were unable to demonstrate, by autoradiographic techniques, significant mitotic activity within ductal cells after pancreatic injury due to ductal ligation.

A new model of normal pancreatic architecture has been proposed that elucidates the role of acinar cells in the formation of tubular complexes in pancreatic injury.[9] The pancreas has classically been described as a tubuloalveolar gland where acini are arranged in a grape-like fashion at the ends of branching ducts (Figure 13). Studies in the rat,[58,59] dog,[60] and humans[9] revealed that acini were arranged as a system of curved, branching, variable-sized, anastomosing tubules, some of which ended as blind sacs. This anastomosing network of tubules was connected to the duct system (Figure 13). Bockman et al[9] proposed that tubular complexes arose by progressive atrophy and dedifferentiation of acinar cells. Such regressive changes within acinar cells that are normally arranged as a branching network could readily account for the appearance of branching, ductlike structures in the injured pancreas.

More recently, Pour[25] demonstrated that tubular complexes (pseudoductules) produced in Syrian golden hamsters by N-Nitrosobis(2-oxopropyl)amine originated from proliferating centroacinar cells. He speculated that tubular complexes may, in fact, develop by more than one mechanism. Tubular complex formation attributed to acinar cell atrophy and dedifferentiation may represent an adaptive

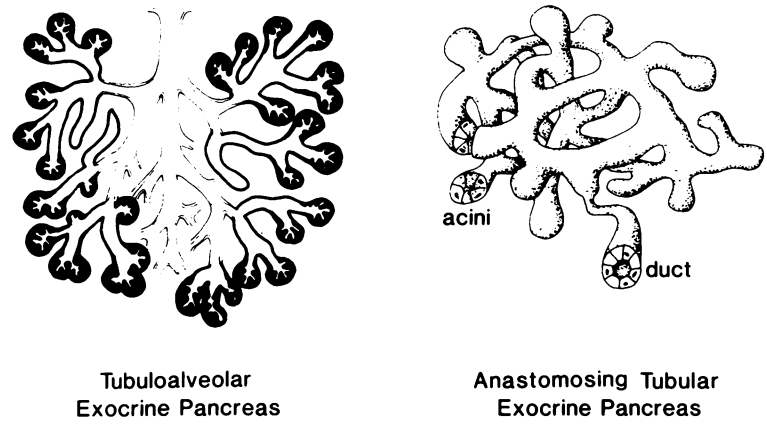

Figure 13. *Left: The classical view of exocrine pancreatic architecture. Glandular cells (dark lines) are arranged as tubules or acini at the ends of branching ducts (open lines). Adapted with permission from Bloom and Fawcett.*[71] *Right: A new model of pancreatic architecture. Acinar cells are arranged in a network of curved, variable-sized, anastomosing tubules. Redrawn with permission from Bockman*[59].

response of acinar cells to injury, whereas centroacinar/ductal cell proliferation may be a response to irreversible damage.[25]

Several ultrastructural studies support the proposal that tubular complexes arise from atrophic, dedifferentiated acinar cells. Ultrastructural evaluation of pancreatic ductlike structures associated with uremia,[16] chronic pancreatitis,[12] ethionine[18] and methionine[19] toxicoses, and cystic fibrosis[17] indicated that these ductlike structures were lined by atrophic acinar cells. Similarly, tubular complexes in cerulein- and oleic acid-induced pancreatitis consisted of degenerating and atrophic acinar cells.[13] Ultrastructural studies in pancreatic carcinogenesis[20,21] revealed that the ductlike structures (tubular complexes) were lined by acinar cells and cells in various transitional stages between acinar and ductal cells. Sequential ultrastructural studies of pancreatic adenocarcinoma induced by N-nitroso-bis(2-hydroxypropyl)amine (BHP) revealed that a controlled process of progressive autophagy resulted in atrophic acinar cells with few cytoplasmic organelles and ultrastructural similarities to centroacinar and ductal cells.[22-24] The ultrastructural findings of zinc-induced pancreatic injury, presented here, provide further support for the theory of Bockman et al[9] that tubular complexes arise by progressive atrophy and dedifferentiation of acinar cells.

Pathogenesis of the Lesion

The biochemical basis for the acinar cell alterations in zinc toxicosis cannot be determined from the present study; however, several direct and indirect mechanisms may be considered. Zinc may interact with important divalent cations, such as calcium or copper. Calcium plays a pivotal role in stimulus-secretion coupling in acinar cells[53] and this role may be altered by zinc. In other cell types, calcium-dependent cellular events are inhibited by zinc.[61] Zinc–copper interactions are also well documented[1,2] and pancreatic acinar cell degeneration and atrophy are a feature of experimental copper deficiency.[62,63] The role of cation interactions in the pathogenesis of the pancreatic lesions described in the present study is unknown.

Zinc-induced membrane changes may be responsible for the cellular alterations in the pancreas. Zinc has a stabilizing effect on membranes due to oxidation of sulfhydryl groups, inhibition of membrane-bound enzymes, inhibition of lipid peroxidation, interference with calcium transport and alterations in biomembrane structure and fluidity.[64] It is possible that these same biochemical mechanisms may have a deleterious effect on biomembranes.

An indirect effect of zinc on the pancreas, such as zinc-induced release of secretagogues, may result in pancreatic alterations. Physiologic studies in dogs revealed that intraduodenal or intravenous zinc infusion stimulated release of cholecystokinin (CCK) and increased pancreatic secretion.[65] The early ultrastructural changes of zinc toxicosis in ducklings were similar to those induced by high levels of a CCK-analog.[39,40] Zinc-induced stimulation of CCK or other neurohumoral factors may play a role in the pathogenesis of zinc toxicosis.

Zinc-induced release of corticosteroids was also considered as a mechanism for the pancreatic changes. Pancreatic atrophy and ectasia have been reported in rabbits, rats and humans given high doses of corticosteroids.[66] Ultrastructural studies of pancreatic acinar cells from mice given a synthetic glucocorticoid revealed loss of zymogen granules, dilatation of the RER, swelling of mitochondria and increased autophagocytosis.[67]

Glucocorticoids had a direct effect, *in vitro,* on acinar cell morphology, amylase content, and responsiveness to CCK.[68] The interactions of corticosteroids and zinc have not been extensively studied but some experiments suggest that zinc may affect adrenal gland structure[69] and function.[70]

In summary, a toxic concentration of zinc produced progressive ultrastructural alterations in the pancreas of ducklings. The changes were limited to acinar cells and consisted of cytoplasmic vacuoles with zymogenlike ma-

terial and increased autophagocytosis. Cell deletion by apoptosis and progressive loss of cytoplasmic organelles by autophagy resulted in formation of atrophic acini with ductlike features (tubular complexes). Zinc-induced pancreatic injury could prove to be a useful experimental model for biochemical characterization and modulation of the response of acinar cells to injury.

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