The Effect of Atropine and Duct Decompression on the Evolution of Diazinon®-Induced Acute Canine Pancreatitis

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Three groups of eight dogs each were studied to evaluate the early evolution of the hyperamylasemia, hyperlipasemia, and acinar cell pathology at the light and electron microscopic levels during acute Diazinon®-induced pancreatitis. Two more groups of five dogs each were evaluated for the effects of cholinergic receptor blockade with atropine and ductal decompression on the evolution of serum enzyme changes and acinar cell pathology. Group I dogs received a secretin infusion of 2 units/kg/hr, and a Diazinon infusion of 75 mg/kg, and demonstrated significant increases in serum amylase and lipase at one, two, and three hours. Light microscopy revealed acinar cell vacuolization and progressive interstitial edema. Electron microscopy revealed the formation of large intracytoplasmic vacuoles filled with flocculent material, the fusion of these vacuoles with basolateral membrane, and the formation of interstitial edema. In both Group II dogs (that received secretin alone) and Group III dogs (that received atropine, 200 μg/kg IV prior to secretin and Diazinon), the serum enzyme levels and histologic results were normal. In Group IV dogs, pancreatic duct cannulation to prevent hypertension prevented the hyperamylasemia and hyperlipasemia, but not the acinar cell vacuolization and interstitial edema. This model for acute interstitial pancreatitis is apparently cholinergic-receptor mediated, the serum enzyme elevations are due primarily to ductal hypertension, and the acinar cell pathology is primarily due to cholinergic stimulation and occurs independent of ductal hypertension.

ACUTE PANCREATITIS can be caused by alcoholism, cholelithiasis, and hyperparathyroidism, as well as by many drugs. None of the causes is well understood, although experimental and clinical evidence suggests that pancreatic ductal obstruction, in association with pancreatic exocrine stimulation, may be a common mechanism in some of these conditions.[1] We previously reported acute pancreatitis and pseudocyst formation in a 19-year-old female who had accidentally ingested a cholinesterase inhibitor insecticide.[2]

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Cholinesterase inhibitors depress cholinesterase activity and cause accumulation of acetylcholine on postsynaptic receptors. At least two classes of enzymes are responsible for the total cholinesterase activity of whole blood—a membrane-bound cholinesterase, acetylcholinesterase (AChE, EC 3.1.1.7.) in the erythrocyte, and a soluble enzyme, pseudocholinesterase or butyrylcholinesterase (BuChE, EC 3.1.1.8.) in the serum.[3] In tissues that contain these two forms of cholinesterase, AChE is predominant in regulating neuromuscular activity and autonomic ganglion transmission,[4] while the function of BuChE is unclear. The proposed functions of BuChE include destruction of butyrylcholine produced by fatty acid metabolism, regulation of choline metabolism, lipid metabolism, and regulation of membrane permeability.[5]

Canine pancreatic juice and acinar cells contain BuChE activity.[6] Following intoxication with the cholinesterase inhibitor Diazinon® (Ciba Geigy Corporation, Greensboro, NC), we demonstrated stimulation of pancreatic exocrine flow in dogs.[2] In doses of up to 25 mg/kg, Diazinon also caused a dose-related increase in pancreatic intraductal pressure,[7] which correlated with decreased serum BuChE activity.[8] Thus, intoxication with Diazinon results in pancreatic ductal hypertension and stimulation of exocrine secretion, the two conditions that are classically considered to predispose to pancreatitis. Work in our laboratory has shown, in fact, that a sublethal dose of Diazinon, 75 mg/kg, in combination with a secretin infusion, 2 units/kg/hr, caused acute pancreatitis with interstitial edema, acinar cell vacuolization, hyperamylasemia, and hyperlipasemia.[2]

The present study was undertaken to define the early development of the serum amylase and lipase changes as related to acinar cell pathology at the light and elec-

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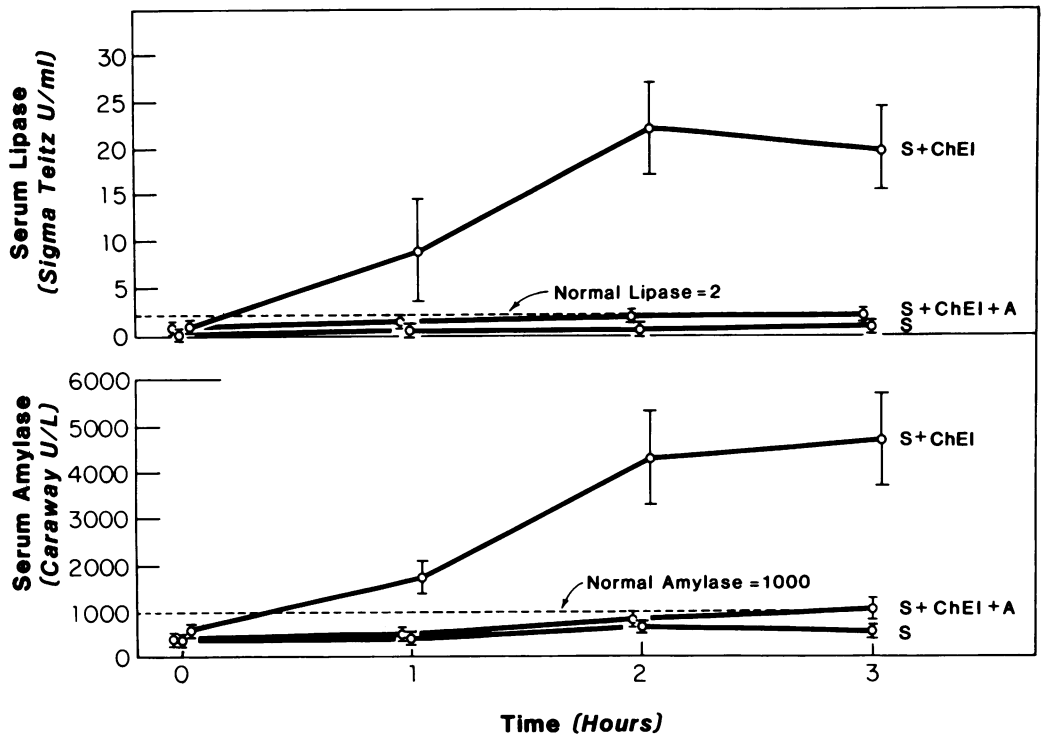

FIG. 1. In Group I secretin and cholinesterase inhibitor (S + ChEI) resulted in a significant increase in serum amylase and lipase. Secretin infusions alone, in Group II, caused no significant increase in either enzyme. Cholinergic blockade with atropine (S + ChEI + A), in Group III, prevented hyperamylasemia and hyperlipasemia.

tron microscopic levels during Diazinon intoxication. In addition, we studied the effects of cholinergic receptor blockade with atropine and the effect of pancreatic ductal decompression on serum enzymes and acinar cells. Finally, with a histochemical technique, we determined the nature and distribution of the cholinesterases in the canine pancreas and the effect of Diazinon on the activity of these cholinesterases.

Methods

Receptor Blockade Study

Twenty-four dogs were divided into three groups of eight. They were anesthetized with alpha-chloralose, 100 mg/kg, and received a continuous IV secretin (The Boots Company, Ltd., Nottingham, England) (Crick, Harper and Raper Units) infusion (S), 2 units/kg/hr, throughout the experiment. Group I received an IV bolus dose of the cholinesterase inhibitor Diazinon, 75 mg/kg, at time 0. Blood was drawn at zero, one, two, and three hours for serum amylase and lipase determinations in five dogs in this group. At three hours, the end of the experiment, a pancreatic biopsy was taken, which was fixed in 10% neutral buffered formalin for light microscopy. To study the early course of the pathologic changes, three of the remaining dogs in this group underwent a laparotomy for biopsy of the pancreas at zero, one, two, and three hours. Aliquots of pancreatic tissue fragments were frozen at −70 C for cholinesterase histochemistry by the method of Karnovsky and Roots,[9] as previously described.[8] Other fragments were fixed in 10% neutral buffered formalin for light microscopy, or in 5% glutaraldehyde for electron microscopy. Tissue collected for electron microscopy was postfixed in 2.5% osmic acid, dehydrated in graded series of alcohol to propylene oxide, embedded in epon 812, sectioned at 600 A, stained with uranyl acetate and lead citrate, and viewed in an RCA EMU-4 electron microscope.

Group II, serving as controls, received secretin infusion, 2 units/kg hr IV. Group III received atropine, 200 μg/kg, given as an IV bolus at time 0 just prior to receiving Diazinon, 75 mg/kg.

Duct Decompression Study

Ten dogs were divided into two groups of five (Groups IV and V) in the ductal decompression study. In both groups, a laparotomy under chloralose anesthesia was performed, and through a duodenotomy, the major pancreatic ampulla was cannulated with PE 60 tubing (ID 0.76 mm, OD 1.22 mm) for duct decompression and

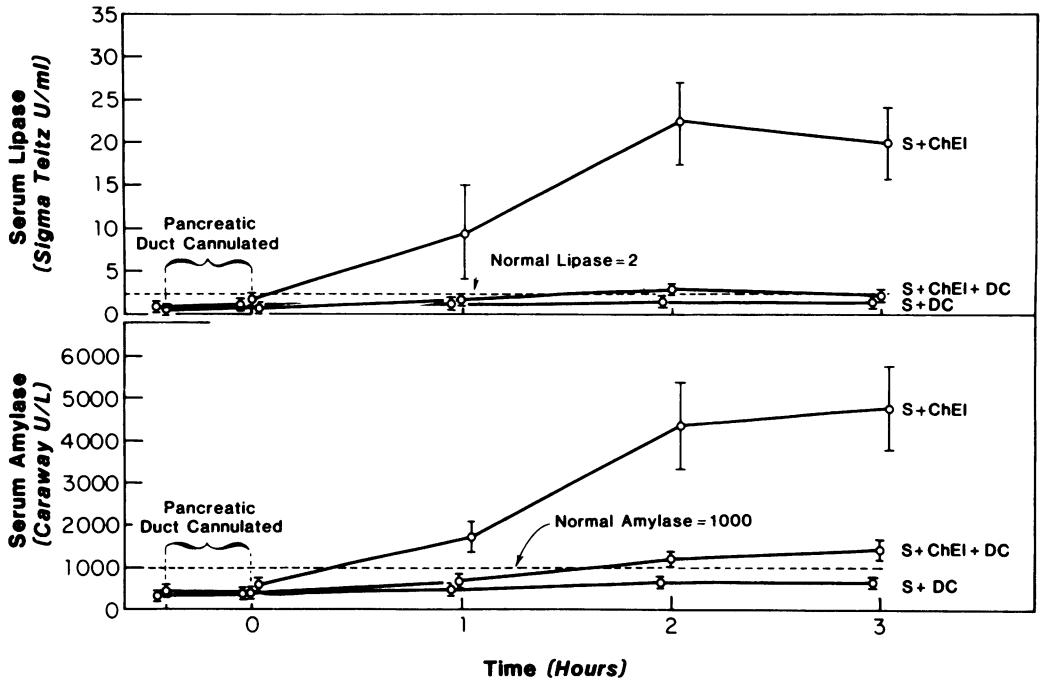

FIG. 2. In Group IV, pancreatic duct cannulation, prior to the administration of secretin and cholinesterase inhibitor (S + ChEI + DC), prevented the hyperamylasemia and hyperlipasemia seen in Group I with secretin and cholinesterase inhibitor administration (S + ChEI). In Group V duct cannulation and secretin administration (S + DC) caused no significant increase in the serum enzymes.

external diversion of the pancreatic juice by gravity drainage. The distal end of the cannula was positioned 30 cm below the level of the operating table. Group IV received a continuous IV secretin infusion of 2 units/kg/hr, and Diazinon, 75 mg/kg at time 0. Venous blood was obtained for amylase and lipase determinations prior to cannulation; 15 minutes following cannulation; and at one, two, and three hours after Diazinon administration. A pancreatic biopsy was obtained at three hours for light microscopic examination. Group V served as controls; after cannulation, only IV secretin was given. A two-tailed t-test was used for all statistical analyses of enzyme values, and significance was established at the $p < 0.05$ level.

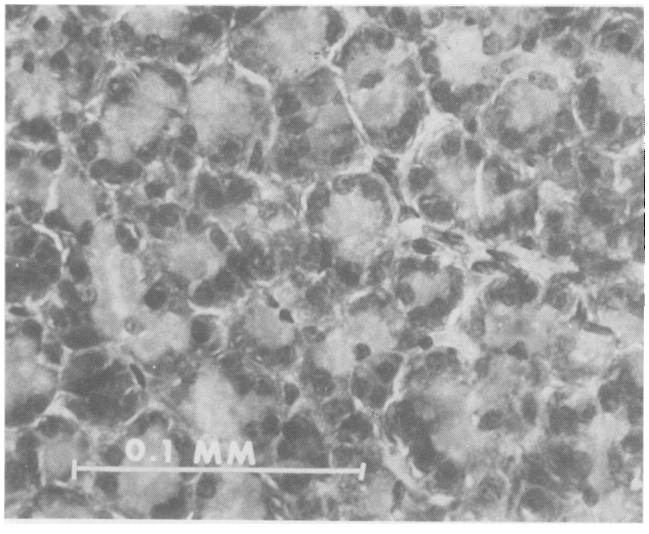

FIG. 3a. The microscopic appearance of the normal control canine pancreas from Group I. (H & E)

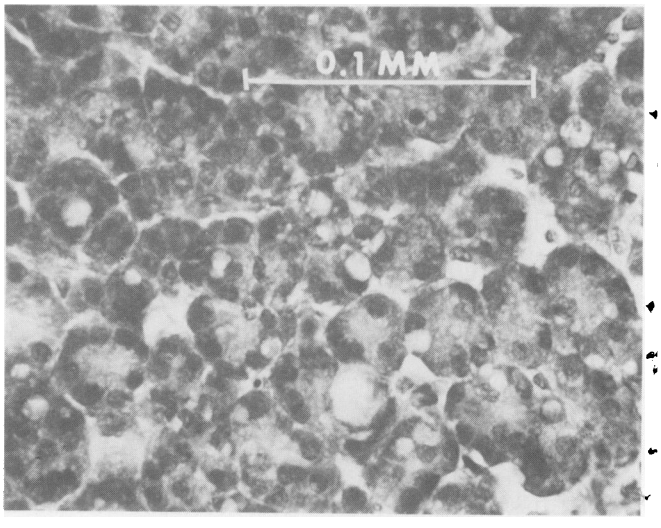

FIG. 3b. Same pancreas three hours following secretin and Diazinon administration. A marked degree of acinar cell vacuolization is present. (H & E)

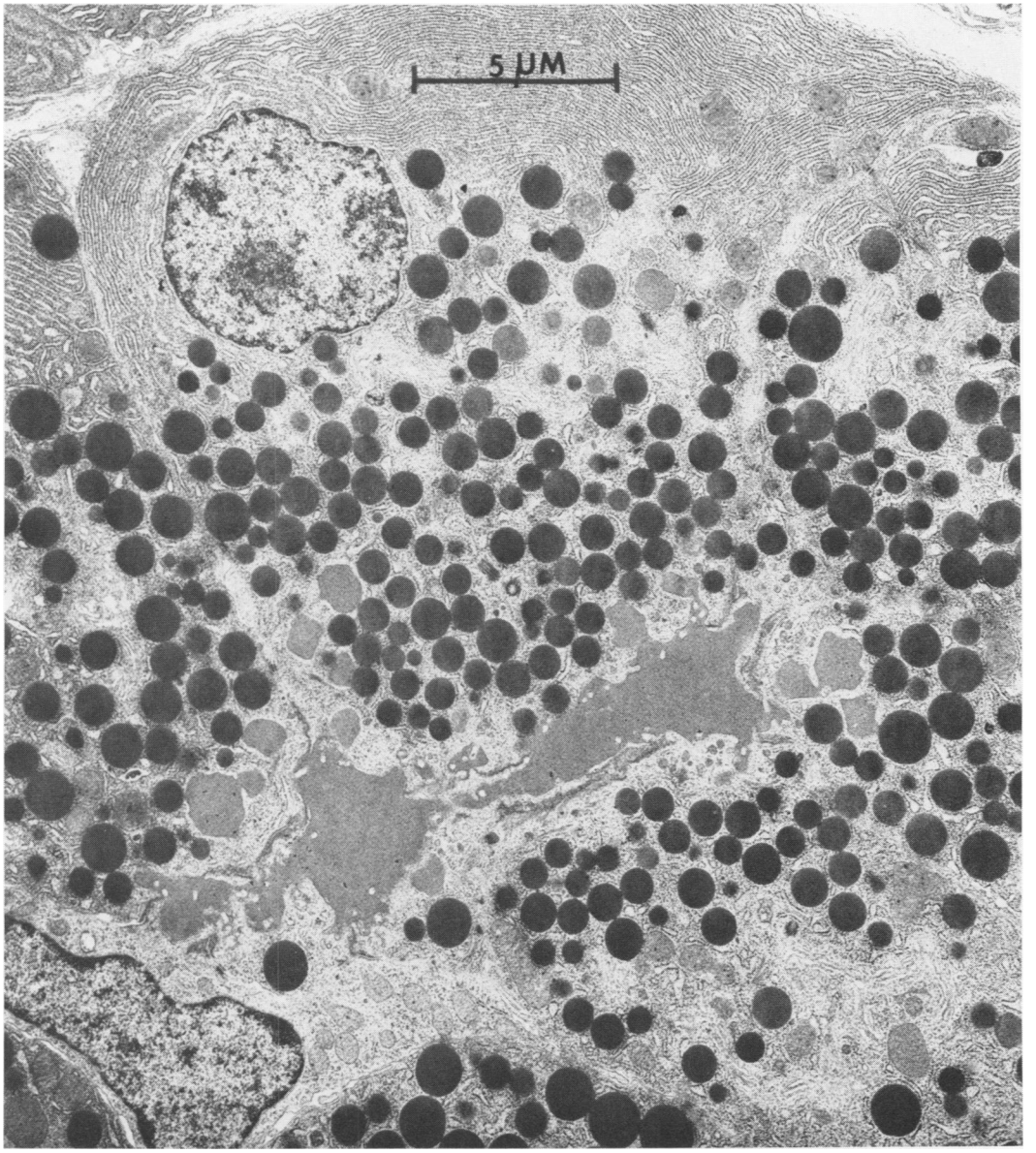

FIG. 4. The normal appearance of acinar cells from control biopsy prepared for EM examination.

Results

Serum Enzyme Changes—Receptor Blockade Study

In dogs receiving Diazinon and secretin (Group I), there was a progressive and significant increase in the serum amylase from a control level of 607 ± 106 Caraway units/l (mean ± SEM) to 4700 ± 1020 at three hours (see Fig. 1). Serum lipase also increased concomitantly from a control of 0.23 ± 0.01 Sigma Teitz units/ml to 19.8 ± 4.8 over the same time interval. In controls receiving secretin infusion only (Group II), there was no significant increase in the serum amylase from a control level of 464 ± 75 to 510 ± 108 at three hours (Fig. 1). Serum lipase was also not significantly increased from the control level. In Group III, which received cholinergic receptor blockade with atropine prior to secretin and Diazinon administration, there was a slight but not significant increase in serum amylase from a control of 469 ± 77 to 1010 ± 240 at three hours (Fig. 1). Serum lipase was also not significantly increased from a control of 0.64 ± 0.28 to 1.7 ± 1.0 at three hours. The amylase and lipase levels were not significantly different between Group II and Group III.

Serum Enzyme Changes—Duct Decompression Study

In Group IV, the ductal cannulation procedure caused no significant or immediate change in the serum

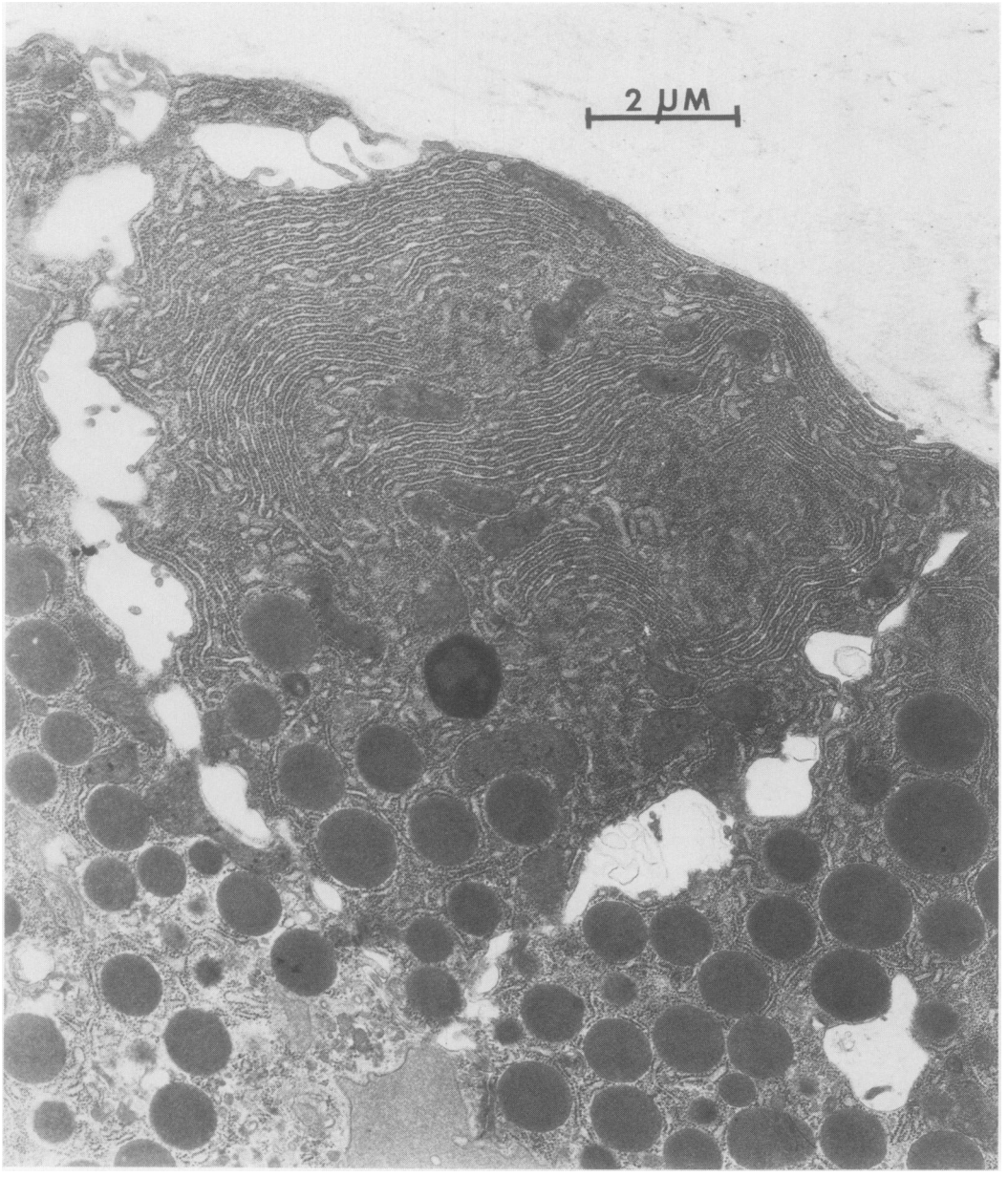

FIG. 5. In Group I, one hour after the administration of secretin and Diazinon (S + ChEI), there is accumulation of fluid between acinar cells, and interstitial edema.

amylase or lipase (see Fig. 2). After secretin and Diazinon administration, however, there was a gradual rise in serum amylase from a control of 468 ± 69 to 1330 ± 283 at three hours. This increase attained statistical significance at two and three hours. Serum lipase levels also increased from a control of 0.36 ± 0.14 to 1.7 ± 0.5 at three hours, but this change was significant only at two hours. Amylase and lipase increases in Group IV were significantly less than those in Group I. Group IV levels were not significantly different from levels in the cannulated group, which received secretin alone. Thus, ductal decompression did ameliorate the hyperamylasemia and hyperlipasemia seen without decompression. In Group V, there were no changes in either amylase or lipase levels after duct decompression and secretin administration.

Histologic Changes—Receptor Blockade Study

Interstitial edema was noted at 30 minutes in the three dogs in Group I who underwent serial biopsies. By three hours, the gland was enlarged and boggy, and the lobules were separated by a colorless, jellylike fluid. In the five remaining dogs the same degree of gross interstitial edema was present at three hours.

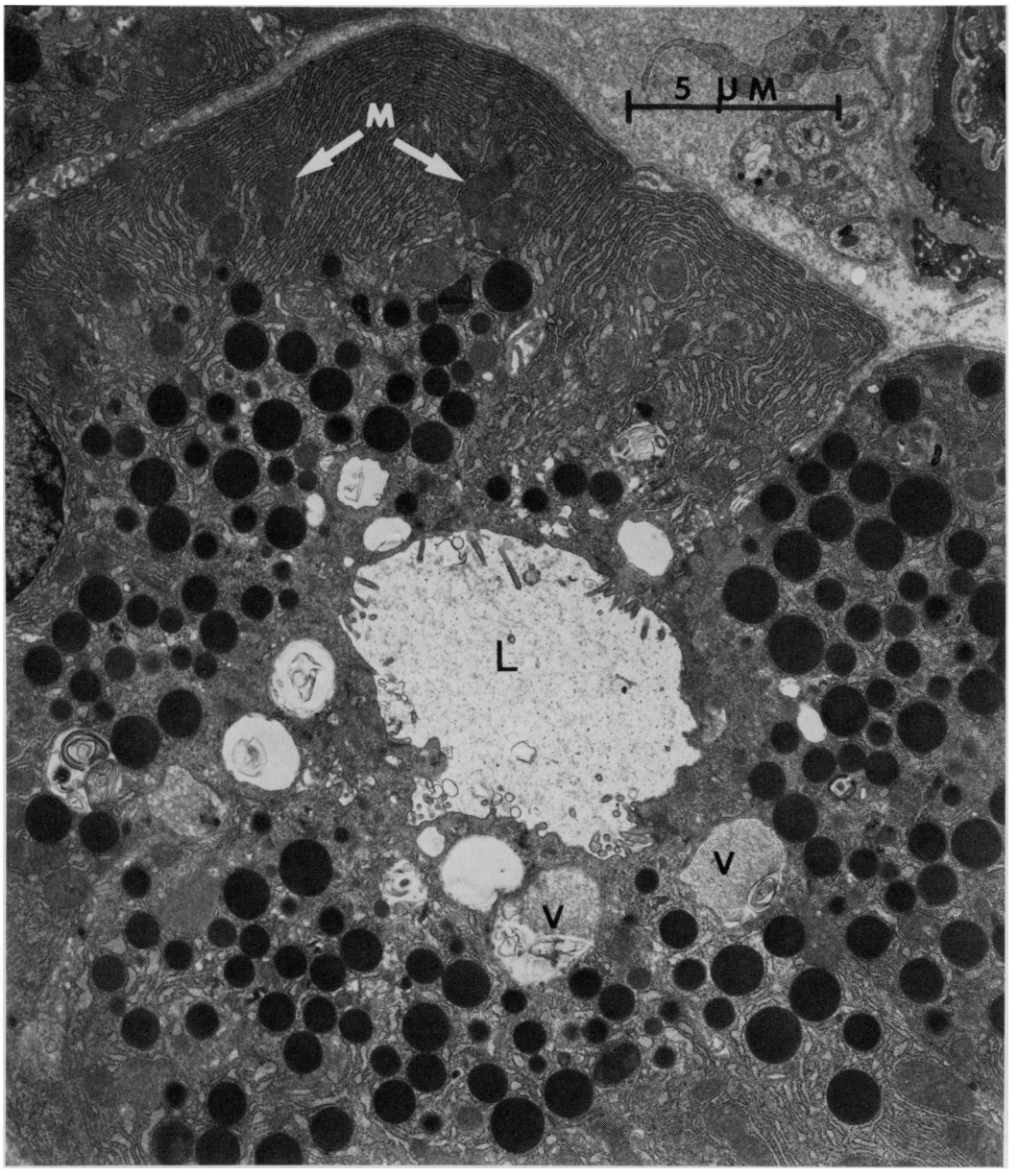

FIG. 6. In Group I, at two hours following secretin and Diazinon administration (S + ChEI) large intracytoplasmic lakes are forming. These vacuoles (V) are filled with flocculent material. The acinar lumen (L) is dilated. Mitochondria (M) show little or no pathologic changes.

The serial biopsies revealed progressive development of diffuse acinar cell vacuolization, interstitial edema, and loss of apical eosinophilia (Figs. 3a, 3b). Within three hours all eight dogs had a similar degree of diffuse vacuolization, edema, and zymogen loss in acinar cells.

Control biopsies prepared for electron microscopy (Fig. 4) were compared with biopsy samples taken after Diazinon administration. Dilation of the acinar lumens was seen within one hour, and accumulation of fluid between the acinar cells occurred with distension of the lateral intracellular spaces (Fig. 5). Villouslike projections became visible on the lateral acinar cell membranes. The fine structure of the intracellular organelles remained essentially unchanged. At two hours, however, distension of the Golgi saccules was noted (Fig. 6). These spaces filled with flocculent material and fused to form large intracytoplasmic vacuoles. At three hours, the acinar cells were filled with large intracytoplasmic lakes, which displaced the cytoplasm and bulged into the interstitial space (Fig. 7). Occasionally the acinar cell basal membranes were avulsed inward and separated from the basement membrane, which

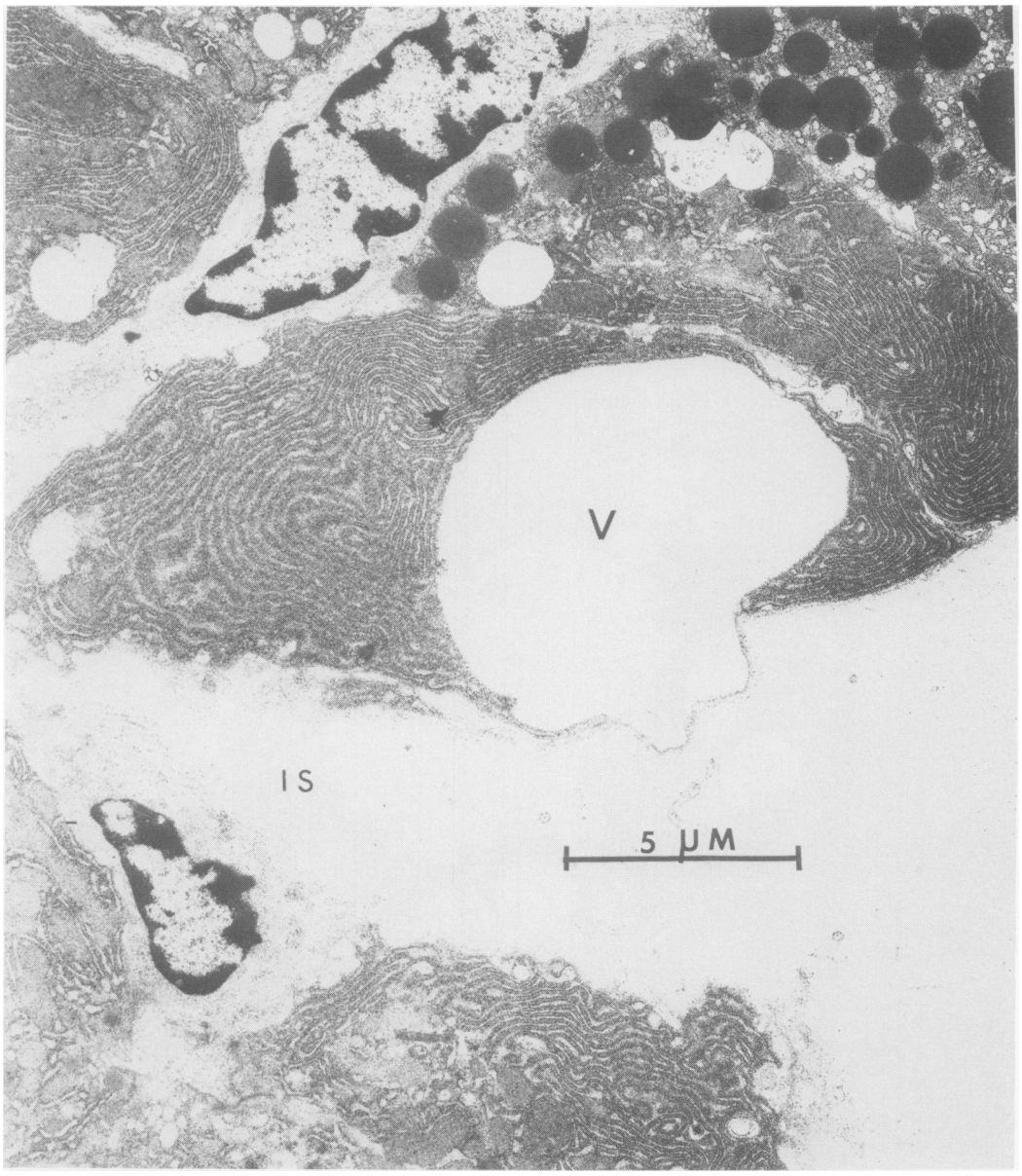

FIG. 7. At three hours, large intracytoplasmic vacuoles (V) appear to burst into the interstitial space (IS). A large amount of interstitial edema fluid is present.

surrounds the acinus, and the basolateral spaces were filled by flocculent material and membrane debris (Fig. 8). There was also a marked reduction in the number of zymogen granules in the cell.

There was no gross, light microscopic (Fig. 9), or electron microscopic evidence of pancreatic changes in the control dogs (Group II), or in those who had received atropine pretreatment (Group III).

Histologic Changes—Duct Decompression Study

Gross interstitial edema was present in Group IV animals, despite ductal cannulation to prevent ductal hypertension. Light microscopic examination at three hours showed marked acinar cell vacuolization and edema indistinguishable from the lesions noted in Group I (Fig. 10). No gross edema was noted in the control dogs of Group V, who underwent ductal cannulation and secretin infusion without Diazinon, and biopsies taken at three hours were normal.

Cholinesterase Histochemistry

The histochemical sections from the control animals revealed that AChE activity was confined to the intrin-

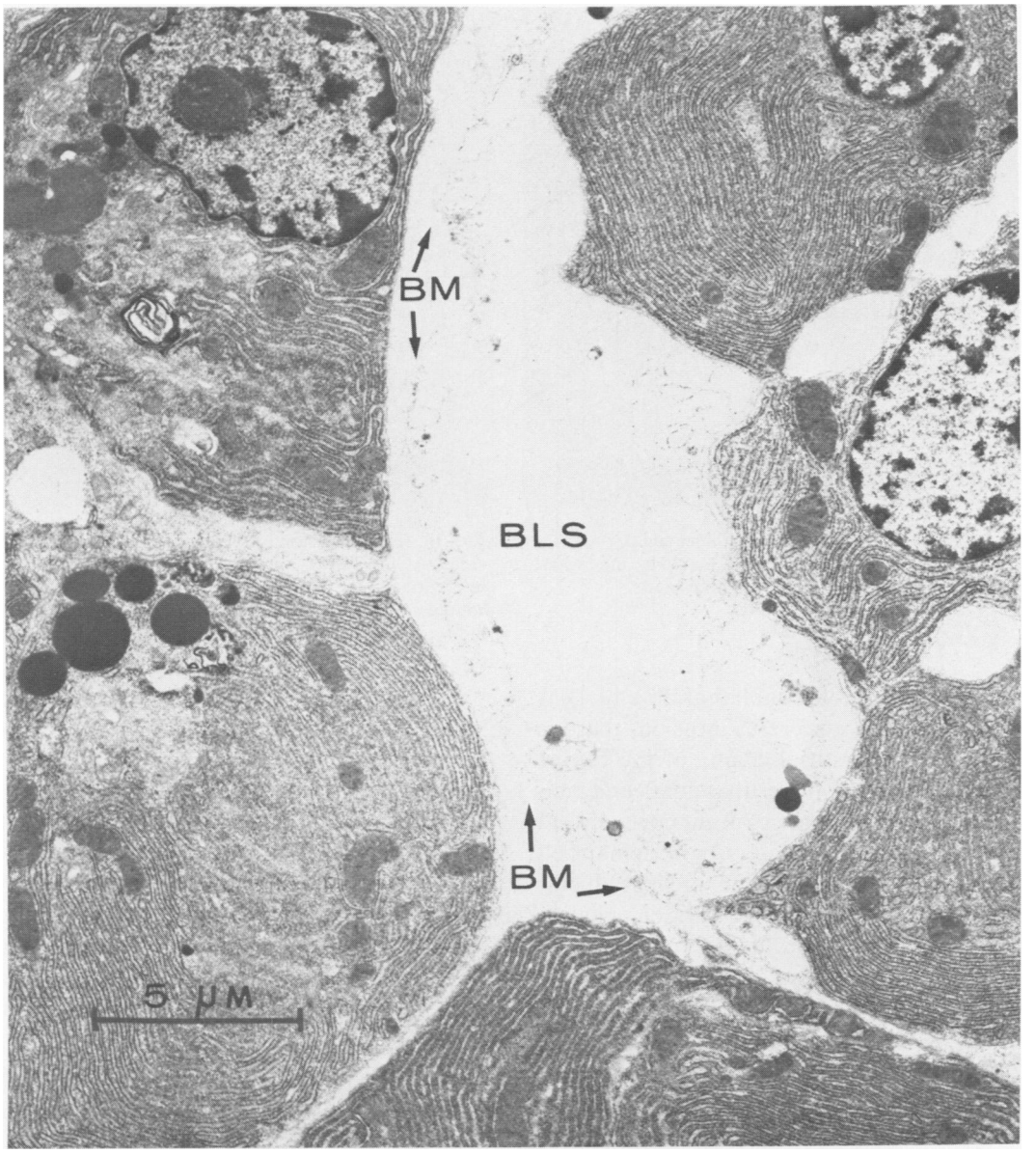

FIG. 8. At three hours, the accumulation of flocculent material and membrane debris in the basolateral space (BLS) has caused the avulsion of the basement membrane (BM) away from the acinar cells.

sic pancreatic nerves and ganglia. The nerve trunks primarily followed the vascular pattern, and large arteries were often accompanied by nerve trunks coursing in the perivascular interstitial space; fibers then branched out and spread into the exocrine parenchyma. There was no AChE activity associated with the acinar cells (Fig. 11). The acinar cells, however, contain abundant BuChE activity, which was most prominent in the zymogen granules. BuChE activity was also present in the intrinsic nerve trunks and ganglia in the same distribution as AChE (Fig. 12). Neither AChE nor BuChE activity was present in ductal mucosa or islet cells.

The biopsy samples taken from Group II, the controls, revealed no qualitative change in the distribution and intensity of acinar cell and axonal cholinesterase activity under secretin stimulation. In both groups (I and III) that received Diazinon, however, a marked reduction of acinar cell and axonal BuChE activity was seen at one hour. By two hours, there was complete absence of BuChE activity. The axonal AChE activity was preserved throughout the experiment (Fig. 13). This confirms our previous observation[8] that Diazinon, at doses of 25–75 mg/kg, selectively inhibits BuChE with the preservation of histochemically identifiable AChE activity. Table 1 summarizes the experimental results.

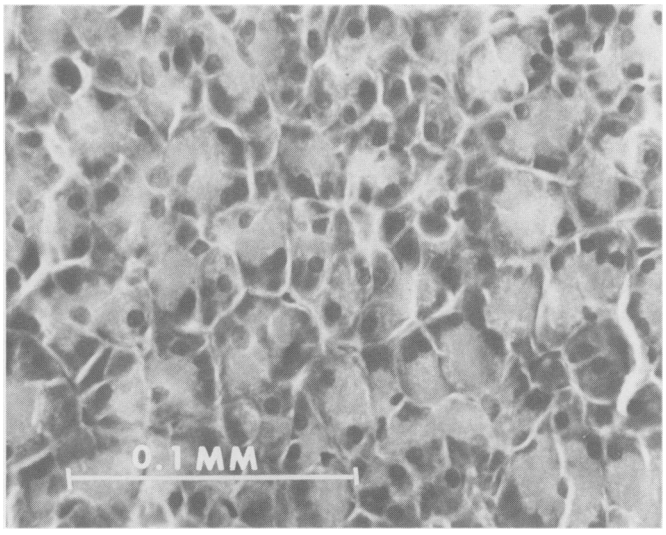

FIG. 9. In Group III, with cholinergic receptor blockade prior to secretin and Diazinon administration (S + ChEI + A) the interstitial edema and acinar vacuolization are absent at three hours.

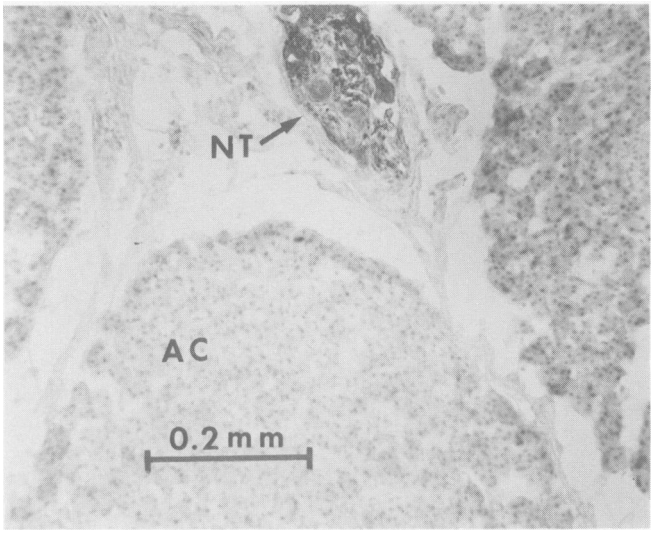

FIG. 11. Cholinesterase histochemistry reveals that in the canine pancreas, AChE activity is found in nerve trunks (NT) coursing in the interstitial space but not in the acinar cells (AC). The nuclei are counterstained with hematoxylin. (Substrate: acetylthiocholine. Inhibitor: iso-OMPA.)

Discussion

After the administration of a sublethal dose of Diazinon in combination with a secretin infusion, there is a rapid onset of acute interstitial edema of the canine pancreas, hyperamylasemia, hyperlipasemia, and acinar cell vacuolization. These changes occur concomitant with a selective reduction in the activity of acinar cell BuChE activity. Duct cannulation to prevent ductal hypertension provides significant protection against acute hyperlipasemia and hyperamylasemia, but does not prevent interstitial edema or acinar cell vacuolization. Cholinergic receptor blockade with atropine, however, provides protection, as evidenced by stable serum enzyme levels and absent structural changes.

Our histochemical observations confirm the work of Hebb and Hill,[6] who described BuChE activity in the canine acinar cells. They reported, however, that secretin injections caused a marked reduction in acinar cell BuChE activity, whereas we found no qualitatively detectable reduction in acinar cell cholinesterase activity following secretin infusion. This discrepancy is hard to explain, but they used a different secretin prepara-

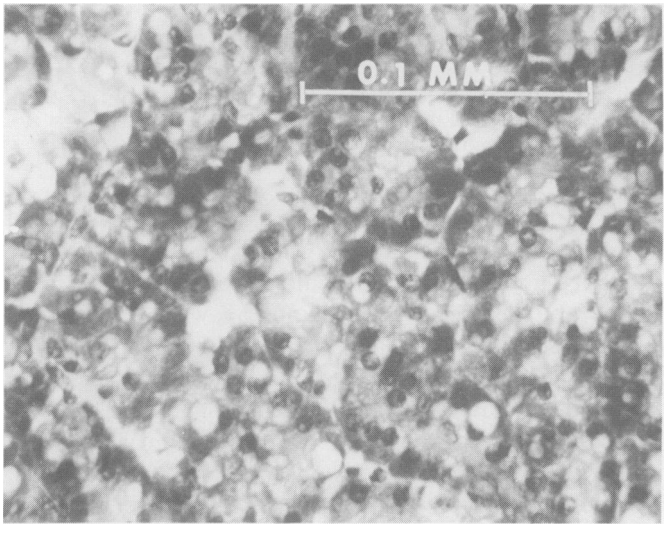

FIG. 10. In Group IV, duct cannulation prior to secretin and Diazinon (S + ChEI + DC) did not prevent acinar cell vacuolization and interstitial edema at three hours. (H & E)

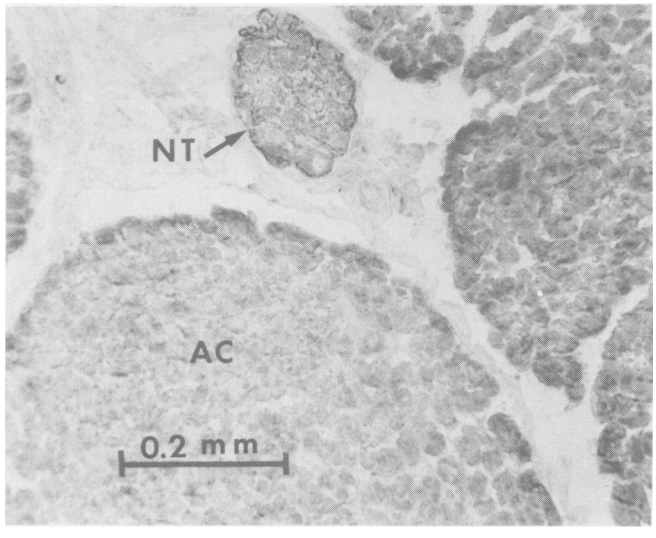

FIG. 12. Cholinesterase histochemistry reveals that BuChE activity is found in the nerve trunks (NT) and the acinar cells (AC). (Substrate: butyrylthiocholine. Inhibitor: none.)

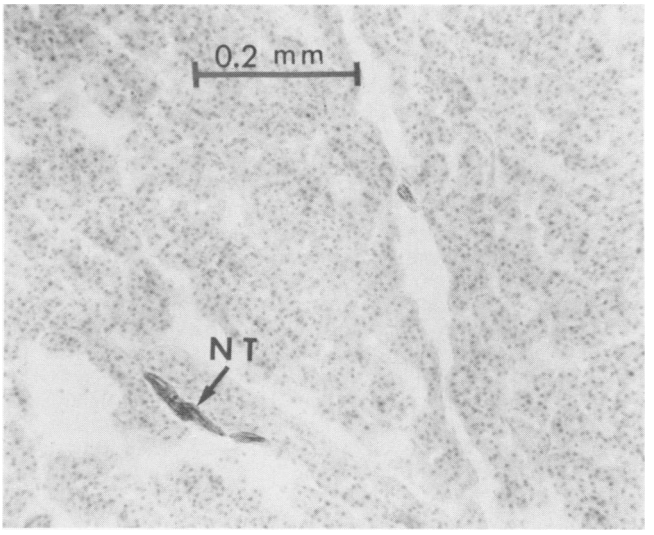

FIG. 13. Histochemical staining for total cholinesterase activity one hour following Diazinon and secretin indicates that BuChE activity is markedly decreased or absent; however, AChE activity in nerve trunk (NT) is preserved. Thus, Diazinon selectively inhibits canine acinar cell BuChE. (Substrate: acetylthiocholine. *In Vivo* Inhibitor: none.)

tion. The addition of Diazinon, however, caused complete and rapid inhibition of acinar cell BuChE activity.

Our histologic data confirm the results of Villaret, Justin-Besancon, and Even[10] and Leblond and Sergeyeva.[11] They observed pancreatic acinar cell vacuolization in rats at the light microscopic level following cholinergic stimulation with acetylcholine. Our ultrastructural observations also confirm the observations of Lampel and Kern.[12] They used high-dose cerulein stimulation in rats and observed progressive acinar cell vacuolization, the emptying of these vacuoles into the basolateral space, and subsequent interstitial edema. They suggested that the vacuolar changes are the result of disruption of the cellular mechanisms of protein synthesis, intracellular transport, packaging, and granular discharge[13] that occur as a result of intense peptidergic stimulation of the acinar cells. Our ultrastructural data could be interpreted as showing that intense cholinergic stimulation of the acinar cells also causes the vacuolar transport of digestive enzymes directly from the cells into the interstitial space. In addition, cholinergic receptor blockade with atropine provides complete protection against the acinar cell vacuolization and serum enzyme elevations.

Our data support the hypothesis that BuChE is important in down-regulating the acinar cell response to acetylcholine in the canine pancreatic acinar cell. If BuChE is the only cholinesterase present in the juxta-receptor area of the acinar cell, then inhibition of BuChE would allow the cholinergic receptors to be flooded with endogenous acetylcholine and result in intense cholinergic stimulation. Although the precise intracellular mechanism is unknown, the cholinergic stimulation that results from the loss of the juxta-receptor enzyme for hydrolysis of acetylcholine may place maximal demands upon the intracellular processes of energy supply, membrane synthesis, and protein packaging. The protein packaged within the zymogen granules is normally osmotically inactive.[14] This prevents the influx of water that would occur if high concentration of osmotically active protein were to be stored within the cell. The vacuolar changes we observed may be the consequence of insufficient energy for protein packaging or of insufficient membrane with which to package the protein into zymogen granules, with the resultant accumulation of osmotically active protein and water influx.

Pancreatic ductal cannulation prevents the hyperamylasemia and hyperlipasemia in this model of pancreatitis. Ductal decompression, however, does not prevent the acinar cell vacuolization and interstitial edema. There may be two mechanisms by which digestive enzymes gain access to the interstitial space. First, there is a ductal pressure-induced leak mechanism, which is caused by either functional or anatomic disruption of

TABLE 1. *Summary of Experimental Results*

	Group/Treatment	Gross Edema	Acinar Cell Vacuoles	Serum Amylase and Lipase	Cholinesterase Histochemistry
I	S + ChEI	+	+	Elevated	AChE normal, BuChE absent
II	S	0	0	Normal	AChE normal, BuChE normal
III	S + ChEI + A	0	0	Normal	AChE normal, BuChE absent
IV	S + ChEI + DC	+	+	Slight increase	Not done
V	S + DC	0	0	Normal	Not done

S = Secretin infusion, 2 U/kg/hr; ChEI = Diazinon, 75 mg/kg; A = Atropine, 200 μgm/kg; DC = pancreatic duct cannulation.

the acinar "tight junctions," as described by Oliver and Hand,[15] and Saito and Kanno.[16] This allows digestive enzymes to leak from the duct lumen into the interstitial space. A second mechanism is a vacuolar transport process, described by Lampel and Kern,[12] which carries digestive enzymes directly from the acinar cells into the interstitial space. Although the ductal pressure mechanism is significantly reduced by ductal decompression, the vacuolar transport mechanism seems to continue unabated and could explain the slight serum enzyme elevation that we observed.

Our data clearly suggest that acute interstitial pancreatitis can result from a reduction in the activity of a regulatory enzyme (BuChE) that is normally present within the canine acinar cell, and that these acute changes can be prevented by blockade of the cholinergic receptor. In this model, ductal decompression ameliorates the serum enzyme changes but does not prevent pathologic events in acinar cells.

Acknowledgments

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