Pancreatitis as a Complication of Anticholinesterase Insecticide Intoxication

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Severe pancreatitis and a pseudocyst occurred in a patient following accidental ingestion of an anticholinesterase insecticide, a substance not previously known to produce pancreatitis. Experiments were done to elucidate the mechanism. In one group of dogs the pancreatic duct was perfused and intraductal pressures were measured. The cholinesterase inhibitor 0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate (25 mg/kg) caused a significant increase in the mean intraductal pressure from 12 ± 2.4 to 27.8 ± 5.9 cm saline. In a second group of dogs pancreatic secretory rates were measured. Anticholinesterase (75 mg/kg) in combination with secretin infusion (1 U/kg/hr) caused a significant increase in the secretin stimulated flow rate from 0.13 to 0.56 cc/min. Atropine (75 μg/kg) abolished the anticholinesterase induced pressure and secretory rate increases. In a third group of dogs administration of cholinesterase inhibitor 75 mg/kg and secretin infusion 2 U/kg/hr resulted in acute pancreatic interstitial edema, acinar cell vacuolization, hyperamylasemia and hyperlipasemia. These results suggest that occurrence of pancreatitis as a complication of anticholinesterase insecticide intoxication is the result of hypersecretion and pharmacologic ductal obstruction.

THE USE OF ANTICHOLINESTERASE insecticides has become widespread: over 220 million pounds are manufactured annually in the U.S.[9] There is an increased opportunity for accidental human intoxication among farmers and insecticide workers. In 1975 at the Hennepin County Poison Control Center, 4% of a total of 9,111 inquiries were related to problems of human anticholinesterase intoxication.* Irreversible anticholinesterase intoxication causes accumulation of acetylcholine at postsynaptic receptors and results in profound sustained stimulation of skeletal muscle, the central nervous system, and the autonomic nervous system. Clinically, intoxication with the lipid soluble cholinesterase inhibitors results in preponderance of parasympathetic symptoms, including bradycardia, salivation, lacrimination, and diarrhea.[4] The parasympathetic stimulation of the pancreas with acetylcholine, pilocarpine, or vagal stimulation causes augmentation of the secretory flow and increased intraductal pressure. The effects of a more profound and sustained parasympathetic stimulation by irreversible cholinesterase inhibitors on the normal pancreas have never been systematically studied.

The following study was prompted by the occurrence of pancreatitis in a previously healthy young woman who accidentally ingested an anticholinesterase. The experiments reported herein were performed to determine if there was any causal relation between anticholinesterase intoxication and pancreatitis.

Case Report

On January 30, 1977, a 19-year-old woman, previously in good health, became acutely ill shortly after preparing pancakes for her family's breakfast. She developed nausea, vomiting, salivation, sweating, and was taken to a local hospital. There she had a cardiopulmonary arrest but was resusitated. The initial history suggested an inadvertant inhalation of fuel oil because a pail of kerosene had been recently placed on a kitchen heater. After successful resusitation, she was transferred to the University of Minnesota Medical Center.

She arrived unresponsive with muscle fasciculations. Blood pressure was 64/0. The pulse was 46. Spontaneous respirations were not present. Pupils were pin point. Profuse salivary and bronchial secretions were noted. Rales were present bilaterally. The roentgenogram of the chest showed bilateral infiltrates suggesting interstitial pneumonia. The abdominal examination was negative. She was treated supportively on a respirator, and given Gentamycin and Penicillin for presumed chemical pneumonitis. Her clinical course was characterized by persistent lethargy, spiking temperatures to 40° and hyperamylasemia (Fig. 1). Cultures of blood, cerebral spinal fluid and urine were negative. On the ninth day, abdominal distention was noted and an epigastric mass was palpated. Ultrasound examination revealed an 8 cm cystic lesion in the region of the pancreas. An upper gastrointestinal series confirmed the location of the mass. On the tenth hospital day, the medical examiner's investigation revealed the toxic agent to be the anticholinesterase, O-ethyl-S-phenylethylphospheno dithioate which had been stored in the kitchen and was inadvertantly used as flour in making the pancakes. The specific therapeutic drugs atropine or pralidoxime were unfortunately not given because of the delay in obtaining the history of anticholinesterase intoxication.

On the twelfth hospital day a 10 cm diameter pancreatic pseudo-

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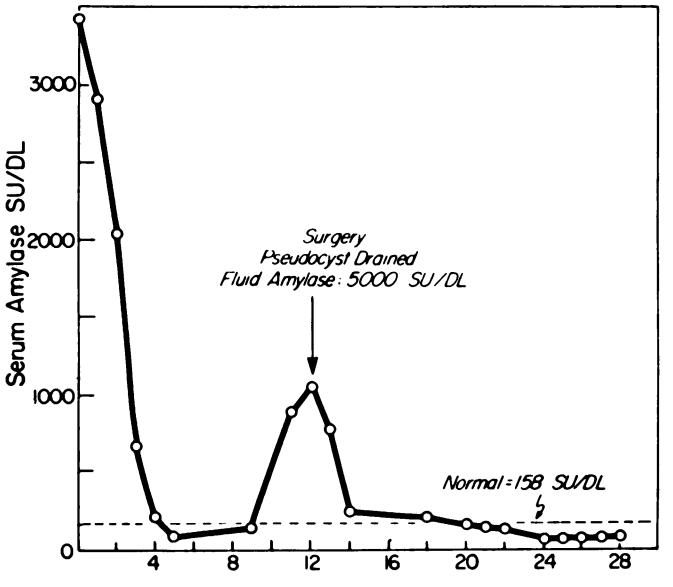


FIG. 1. Time course of serum amylase in patient accidentally ingesting an anticholinesterase insecticide. A pseudocyst was externally drained on the twelfth hospital day.

cyst was externally drained. The fluid contained 5,000 Somogyi U/dl of amylase. The patient had a protracted postoperative course due to a left plural effusion and persistent pancreatic fistula drainage. She was eventually discharged from the hospital two months postop., asymptomatic, eating well, with normal serum and urine amylase, and has remained well for the past 15 months.

Methods: Canine Experiment

Pancreatic Intraductal Pressure Measurements

Six mongrel dogs, 15–18 kg were anesthetized with Pentobarbital 20 mg/kg and through laparotomy, the tail of the ventral pancreas was transected and cannulated with a polyethylene catheter ID-0.584 mm, OD-0.965 mm, which was secured in place with ligature. The pancreatic duct was perfused with normal saline at 37° through the catheter at a constant flow of 0.764 cc/min by means of a Harvard pump. The pressure at the catheter tip was measured by a Stathem pressure transducer and recorded continuously by a Sanborn recorder (Fig. 2). After a 45 minute control period, the dogs received an IV injection of the anticholinesterase 0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate* 25 mg/kg. Finally, 45 minutes following the anticholinesterase injection, atropine 75 μg/kg IV was given. Pancreatic intraductal pressure was tabulated every five minutes. In the calculation of the time averaged pressure, the first 15 minutes of each test period was deleted to allow for equilibration.

Pancreatic Secretory Flow Rate

In five other anesthetized mongrel dogs, the major pancreatic duct was cannulated through a duodenotomy, the cannula was secured by a ligature, the minor pancreatic duct was ligated, and the pancreatic juice was collected by gravity into a small reservoir connected to a pressure transducer. The transducer output was recorded continuously on a Sanborn recorder. Suitable calibration of the pressure versus reservoir volume permitted continuous monitoring of secretory flow rate. The pylorus was clamped to prevent endogenous release of Secretin (Fig. 3). Secretin† in low dose of one U/kg/hr was infused IV for one hour. Then while the Secretin infusion was continued, a total of 75 mg/kg of 0,0-diethyl-0-(2-isopropyl-6-methyl-4-primidinyl)phosphorothioate was given IV in three divided doses, five minutes apart. The Secretin infusion was discontinued 45 minutes

* Supplied by Ciba Geigy Corporation, Greensboro, North Carolina.

† Boots Company, LTD, Nottingham, England.

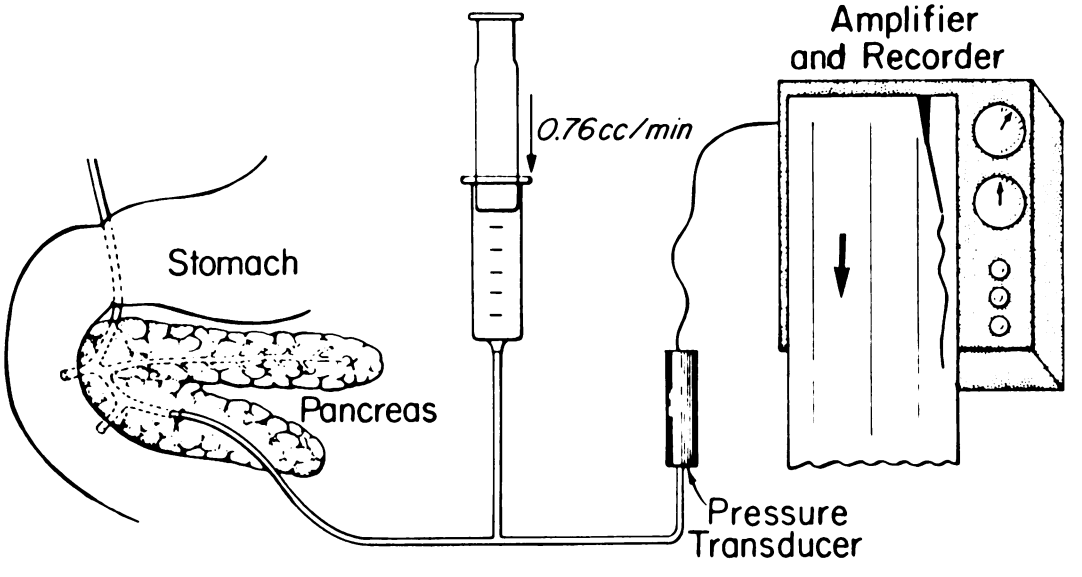


FIG. 2. Schematic drawing of apparatus for measuring and recording pancreatic intraductal pressure.

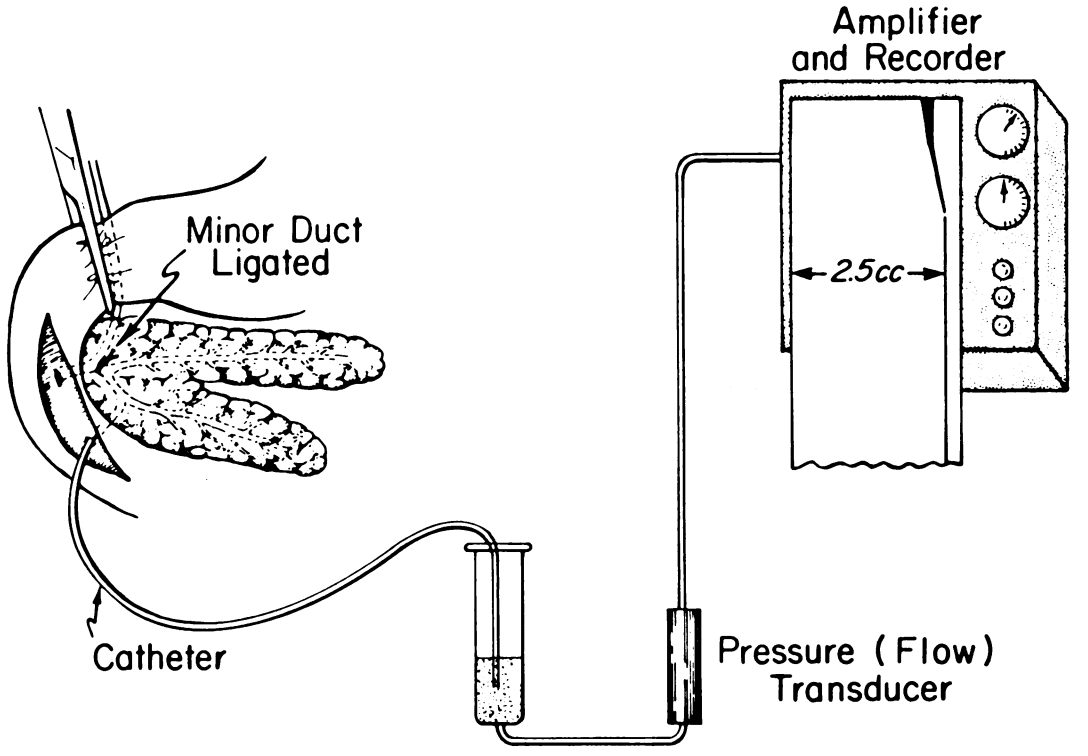


FIG. 3. Schematic drawing of apparatus for measuring and recording pancreatic secretory flow rate.

after the anticholinesterase was given. Atropine was given 75 μg/kg, 45 minutes after discontinuing Secretin. Flow rates were tabulated at five minute intervals. The first 15 minutes of each test period was deleted, and the mean flow for the remainder of the test period was determined.

Canine Pancreatitis

Nonoperative studies of anticholinesterase intoxication in 12 healthy mongrel dogs were carried out. The dogs were divided into four groups.

Group I, five dogs, were given Secretin IV 2, U/kg/hr for one hour. Fifteen minutes later anticholinesterase 75 mg/kg was given IV in three divided doses, five minutes apart. Blood samples were obtained before and two hours after the start of the Secretin infusion and then again every 24 hours for amylase and lipase determinations. These dogs were sacrificed at 72 hours.

Group II, three dogs underwent the same procedure as Group I but were sacrificed two hours after the start of the Secretin infusion.

Group III, three dogs underwent the same procedure as in Group I, except anticholinesterase injections were omitted. These dogs were sacrificed at 72 hours.

Group IV, two dogs were treated exactly as Group II, except the anticholinesterase injections were omitted. These dogs were sacrificed at two hours.

At the time of sacrifice, the gross appearance of the pancreas was noted and sections of the pancreas were taken for microscopic study with hematoxylin and eosin staining. In the analysis of serum enzyme data, Group I and II were considered together and Group III and IV were considered together. The two tailed-t-test of the differences of the mean was used for all statistical analyses.

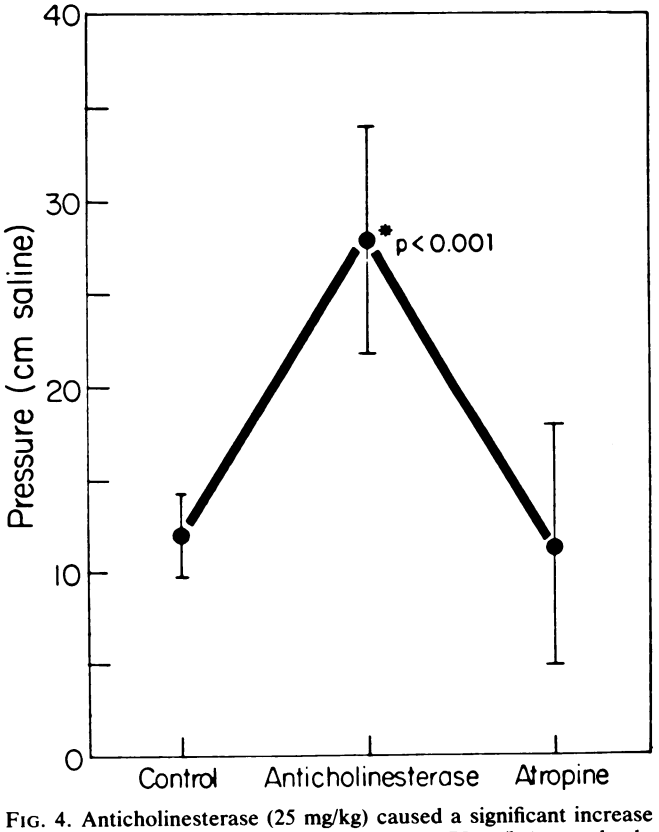


FIG. 4. Anticholinesterase (25 mg/kg) caused a significant increase in pancreatic intraductal pressure. Atropine (75 μg/kg) completely blocked the anticholinesterase effect.

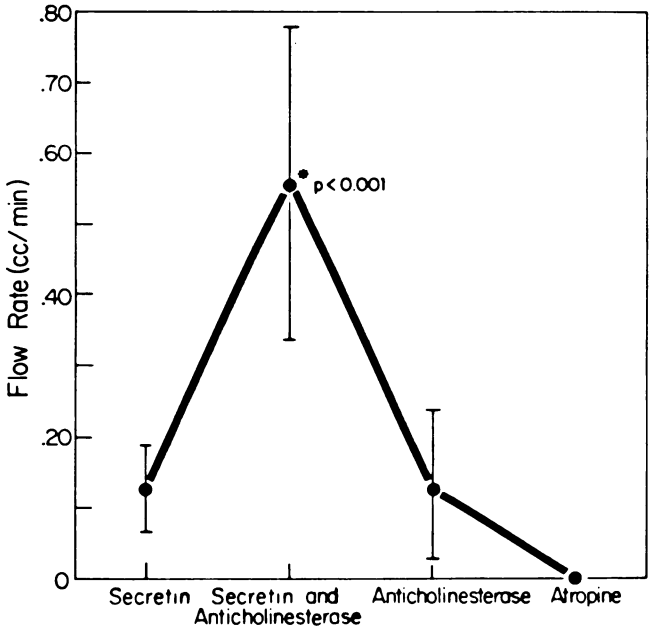


FIG. 5. Anticholinesterase (75 mg/kg) in addition to a low dose Secretin infusion caused a significant increase in mean secretory flow rate. Anticholinesterase alone resulted in a mean flow rate not significantly different from the Secretin stimulated rate. Atropine (75 μg/kg) completely blocked the anticholinesterase.

Results

Pancreatic Intraductal Pressure

Figure 4 shows the results in six dogs who underwent cannulation and perfusion of the pancreatic duct. The mean control pressure 12.0 ± 2.4 (mean ± S.D.) cm saline. Following anticholinesterase administration the mean pressure was significantly increased to 27.8 ± 5.9 cm saline $p < 0.001$. When atropine was given the mean pressure returned to control levels, 11.3 ± 6.5 cm saline within ten minutes in all dogs.

Pancreatic Secretory Flow Rate

In these anesthetized dogs there was no flow in the basal unstimulated state. A Secretin infusion of one U/kg/hr resulted in a mean flow rate in five dogs of 0.13 ± 0.07 cc/min (Fig. 5). When anticholinesterase 75 mg/kg was added there was a significant $p < 0.001$ increase in the mean flow rate to 0.56 ± 0.23 cc/min. Anticholinesterase without Secretin stimulation resulted in flow rates not significantly different from the Secretin stimulated rate 0.14 ± 0.11 cc/min. Atropine 75 μgm/kg completely inhibited the anticholinesterase stimulated flow.

Canine Pancreatitis

Of the five dogs in Group I receiving anticholinesterase and Secretin, two died. All eight dogs (Group I and II) which received IV bolus doses of anticholinesterase in addition to Secretin developed acute hyperamylasemia and hyperlipasemia. The mean serum amylase level increased to 11,580 ± 7,040 SU/dl at two hours. This was significantly greater ($p < 0.02$) than the mean control (preinfusion) level of 1610 ± 736 SU/dl (Fig. 6). The subsequent amylase levels at one, two and three days were not significantly increased over the control.

The mean serum lipase levels were significantly increased over control level of 0.41 ± 0.15 STU/cc at two hours and one day to 8.75 ± 3.14 STU/cc ($p < 0.001$) and 2.13 ± 0.76 STU/cc ($p < 0.02$) respectively (Fig. 7). Subsequent lipase levels were not significantly increased over the control. There were no significant serum enzyme elevations in the five dogs receiving Secretin alone, Group III and IV.

In the three surviving dogs in Group I receiving Secretin and anticholinesterase, the pancreata were grossly and histologically normal at 72 hours (Fig. 8a). In the one dog in Group I that died in two days, the pancreas was grossly normal, but microscopically showed mild lymphocytic infiltrate. In the dog that died after one day, postmortem autolytic changes precluded meaningful histological evaluation.

Gross examination of the dogs in Group II (receiving Secretin and anticholinesterase) revealed marked colorless jell-like interstitial edema at two hours. Microscopic examination revealed a marked degree of acinar cell vacuolization with the appearance of vacuoles in the lateral and basilar portions of the cells displacing the nuclei. Occasionally vacuoles were seen to be bulging into the interstitial space. There was no inflammatory cellular infiltrate (Fig. 8b).

In the dogs receiving only a Secretin infusion

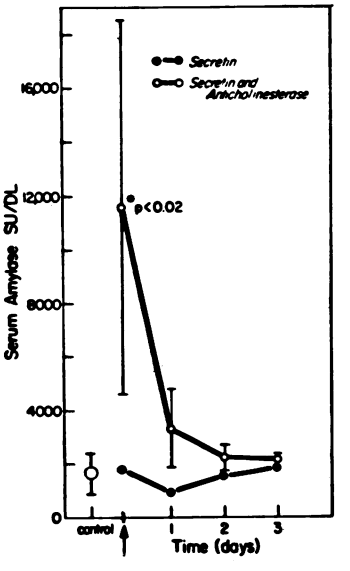


FIG. 6. Significant acute hyperamylasemia occurred in dogs receiving Secretin and anticholinesterase (Groups I and II). Control dogs (Groups III and IV) receiving Secretin alone showed no enzyme elevation.

(Group III and IV) the pancreata were grossly and histologically normal at two hours and 72 hours.

Comment

The causes of acute pancreatitis are legion, and not all are well understood. Alcoholism, cholelithiasis, hyperparathyroidism, hyperlipidemia, direct trauma, tumors and a growing list of drugs can cause pancreatitis.[2]

There is experimental and clinical evidence that some form of pancreatic ductal obstruction or disruption in association with pancreatic exocrine stimulation is a common mechanism though not the exclusive mechanism in many of these conditions. The cholinergic effects of drugs like acethylcholine, pilocarpine, or urecholine can cause very mild elevations of pancreatic ductal pressures and augmentation of pancreatic secretory flow rate. Menguy,[8] using pilocarpine in doses sufficient to cause hypotension, observed intraductal pressure rise of 2 cm H_2O for less than ten minutes. Anrep[1] noted that peripheral electrical vagal stimulation caused an increase in volume of the pancreas associated with stimulation of pancreatic secretion. These findings were consistent with intra-

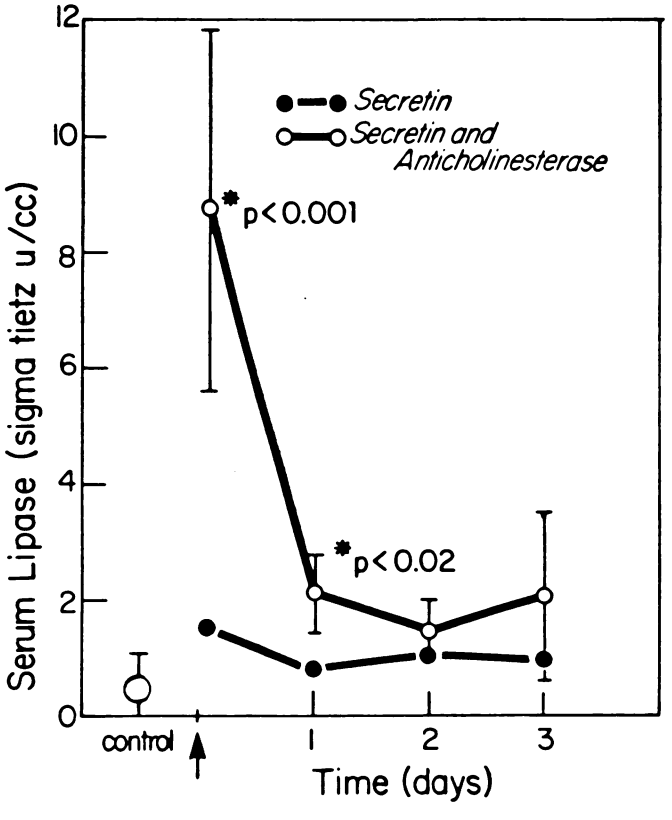
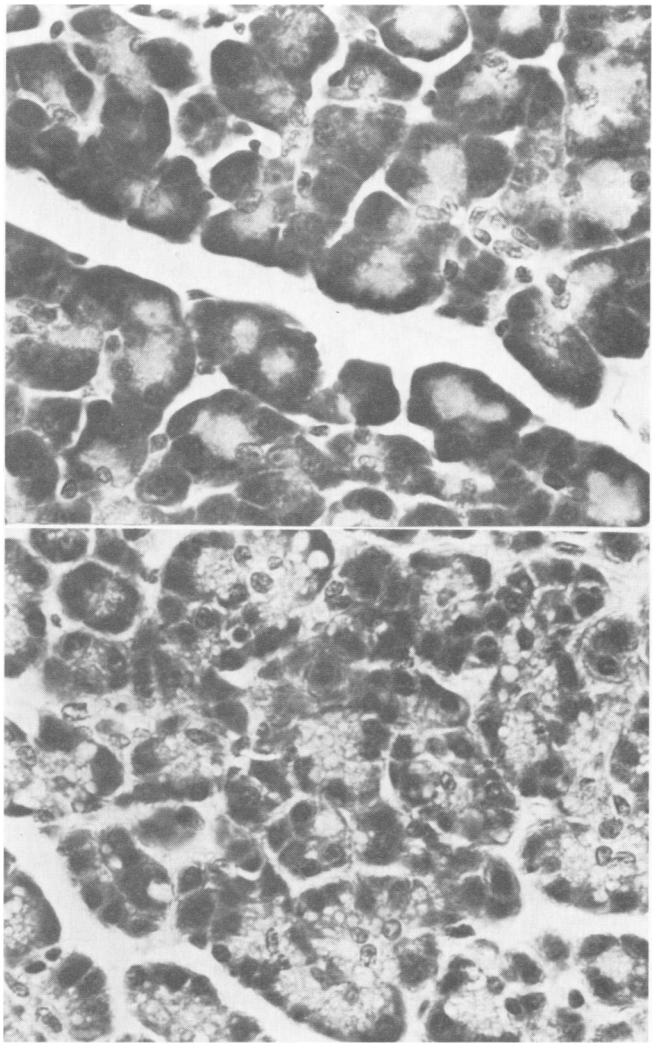


FIG. 7. Significant acute hyperlipasemia occurred in dogs receiving Secretin and anticholinesterase (Groups I and II). Control dogs (Groups III and IV) showed no enzyme elevations.



FIGS. 8a and b. (a, top) Photomicrograph of normal canine pancreas (b, bottom) Typical microscopic appearance of canine pancreas two hours following Secretin infusion and anticholinesterase. A marked degree of acinar cell vacuolization is present. (H & E. ×580).

pancreatic retention of juice secondary to increased ampullary tone, but the pressure changes were not quantitated.

Studies by Earl et al.,[3] were focused upon the effects of more profound cholinergic stimulation produced by organophosphate anticholinesterases. In chronic anticholinesterase intoxication studies in pigs and dogs, they found persistent serum amylase elevation in 20% of the animals and observed gross pancreatitis in one dog.

The results from the present study in dogs indicate the anticholinesterase 0,0 - diethyl - 0 - (2 - isopropyl - 6 - methyl-4-pyrimidinyl)phosphorothioate causes a marked increase in the canine pancreatic intraductal pressure, presumably through ampullary or ductal spasm. In fact, pressure levels were reached which, according to

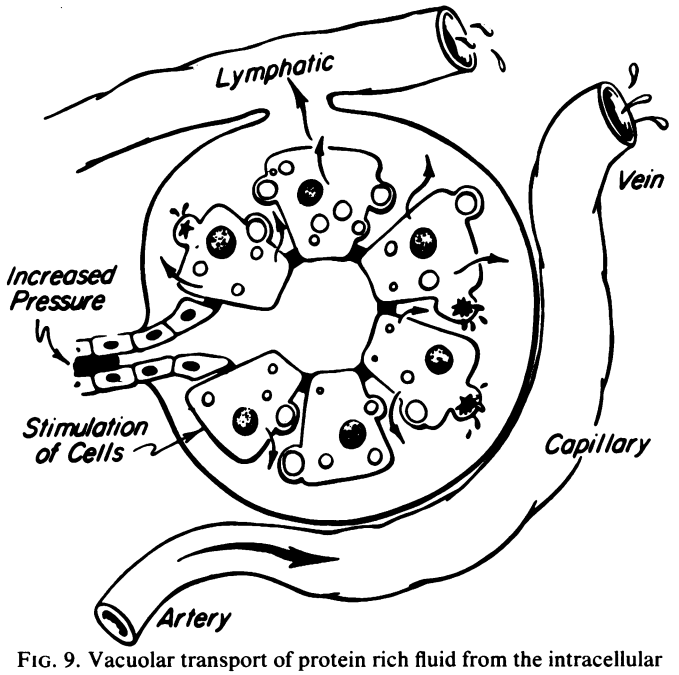


FIG. 9. Vacuolar transport of protein rich fluid from the intracellular space to the interstitium is the proposed mechanism for generation of interstitial edema.

Herring and Simpson,[5] can result in interstitial extravasation of pancreatic fluid. The increased intraductal pressure is associated with an augmentation of exocrine flow rate. The secretagogue effect may be the result of a direct cholinergic stimulation of the pancreatic acinar and ductal cells or a result of cholinergically released endogenous Gastrin, Secretin, or Cholecystokinin-Pancreaozymin (a pyloric clamp prevented endogenous Secretin release secondary to duodenal acidification).

Histologic examination of the pancreata two hours after anticholinesterase and Secretin administration revealed a marked degree of pancreatic acinar cell vacuolization. Studies by Villaret[10] and LeBland and Sergeyeva[7] have also revealed pancreatic acinar cell vacuolization following cholinergic stimulation with acetylcholine. These vacuoles appear to be emptying their contents into the pancreatic interstitial space, rather than the acinar lumen. There is also considerable gross interstitial edema. Such a mechanism is capable of producing interstitial pancreatitis, as shown by Lampel in an elegant serial electron micrographic study of caerulein stimulation in rats.[6] Presumably, these vacuoles contain pancreatic enzymes. Enzymes then are drained from the interstitial space, via lymphatics and venous blood, resulting in the hyperamylasemia and hyperlipasemia which we observed. The vacuolar transport of protein rich fluid from the cell back into the interstitial space increases both the interstitial colloid osmotic pressure and the lymphatic fluid load, resulting in interstitial edema. This situation is represented schematically in Figure 9. These morphologic changes may simply be the result of increased hydrostatic pressures in the acinar lumen. Using a controlled ductal injection technique, Herring and Simpson[5] demonstrated the extravasation of carmine gelatine from the pancreatic acinar lumen through the acinar cells into the interstitial space. However, Lampel[6] has suggested that acinar cell vacuolization is due to a disruption of the intracellular mechanism of protein synthesis, intracellular transport, packaging, and granule discharge, as a direct result of excessive stimulation of the acinar cell.

The experimental data supports the view that this organophosphate anticholinesterase causes a functional ductal obstruction at the same time as stimulation of pancreatic exocrine secretion. There is pancreatic interstitial edema, acinar cell vacuolization hyperamylasemia and hyperlipasemia. In this canine model, interstitial pancreatitis is transient, and frank pancreatic necrosis or hemorrhagic pancreatitis was not observed.

To our knowledge, this study includes the first report of clinical pancreatitis complicating anticholinesterase insecticide intoxication. Certainly, the cardiopulmonary arrest sustained by the patient could have added hypoxic and ischemic insult to the pancreas and may have been a strong contributing factor in the development of overt pancreatic necrosis and the pseudocyst. It should be noted that pharmacologic doses of atropine inhibit the muscarinic effects of this anticholinesterase. The therapeutic benefits of atropine should be borne in mind in cases of suspected anticholinesterase insecticide intoxication.

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