

Biochemical Changes in a Porcine Model of Acute Pancreatitis

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Pancreatitis was induced in 11 miniature pigs by infusing a bile salt-trypsin solution into the pancreatic duct. Seven animals served as sham-operated controls. Serum ionized calcium, total calcium, albumin, total protein, inorganic phosphorus, urea nitrogen, magnesium, insulin, glucagon, and hematocrit were determined every six to 12 h over a period of one week in both test and control animals. We observed significant decreases in ionized and total calcium, modest decreases in albumin, and significant increases in the inorganic phosphorus, urea nitrogen, and hematocrit in the pancreatic pigs. The latter two findings were consistent with early acute hypovolemia. Glucagon and insulin appeared to play no role in the hypocalcemia. Glucagon concentrations increased to the same degree in both test and control animals, probably as a result of the stress of being handled and operated on. The highest concentrations of inorganic phosphorus and the lowest concentrations of both ionized and total calcium were seen 18 h after the induction of pancreatitis in the test animals. These findings suggest that parathyrin (parathormone) was not being secreted in adequate amounts, or that the target organs were unresponsive to parathyrin.

Additional Keyphrases: *parathyrin · hypocalcemia · animal models of disease · biochemical changes in pancreatitis*

Hypocalcemia and hyperamylasemia are the commonest biochemical abnormalities associated with acute pancreatitis, and profound hypocalcemia is a grave prognostic sign. Many investigators have explored the mechanism by which hypocalcemia is produced. Deposition of calcium soaps in the area of the pancreas has been implicated (1), but the deposits were found to be too small (2). Hypersecretion of glucagon was thought to be responsible (3), because glucagon has been shown to be a hypocalcemic agent (4, 5) or possibly to stimulate the secretion of calcitonin (6), but calcitonin was found not to be increased in pancreatitis (7, 8). Magnesium deficiency has been considered a cause of hypocalcemia (9, 10), but it has been shown to be slight or nonexistent in human acute pancreatitis (8, 11).

Imrie et al. (12) suggested that the hypocalcemia of pancreatitis reflects concomitant hypoalbuminemia, stating that some patients are actually normocalcemic when apparently low calcium values are mathematically adjusted for low al-

bumin values. Peoples et al. (13) showed that hypocalcemia in pancreatic pigs can be corrected by administration of plasma and calcium, and attributed the hypocalcemia to exudation of plasma from the vascular compartment.

The role of parathyrin (parathyroid hormone) has been evaluated (7, 8, 11). In one study (7), 75% of hypocalcemic patients had undetectable concentrations of parathyrin, and the hypocalcemia was considered to be due to hypoparathyroidism. In a second study (8), in which five of 11 patients had undetectable concentrations of parathyrin and the others had only modest increases, hypocalcemia was attributed to extraskelatal sequestration of calcium or to some as yet unidentified defect of bone metabolism, or both. Robertson et al. (11) found seven of eight patients had normal values for parathyrin despite abnormally low ionized calcium concentrations in all patients and abnormally low total calcium concentrations in seven of nine patients. Why hypocalcemia persists in pancreatitis in the presence of normal parathyrin concentrations remains to be explained.

Jones et al. (14) showed that dogs in shock have a decreased ability of the second messenger system (cyclic AMP-adenylate cyclase) to respond to the hormonal stimulation of epinephrine, due not to improper delivery of hormone but rather to failure of adenylyl cyclase (EC 4.6.1.1) to increase in response to hormonal stimulation. It is tempting to speculate that pancreatitis is a form of biochemical shock in which there is adequate hormonal stimulation by parathyrin, but end-organ unresponsiveness.

It was our purpose to carry out a one-week, well-controlled study of pancreatitis in pigs. To define any hypocalcemia, we decided to determine ionized and total calcium, albumin, total protein, and inorganic phosphorus. We wanted to measure glucagon and insulin to see if they played any role in the hypocalcemia in view of earlier reports. We intended to follow changes in urea nitrogen and hematocrit to determine what role plasma volume shifts might play in the hypocalcemia. To rule out dilutional effects, we decided not to administer intravenous fluids to the animals, but rather to let the pancreatitis run its course untreated.

Material and Methods

Design of animal studies. Eighteen Pitman-Moore miniature pigs, weighing between 18 and 28 kg and about five to six months old, were divided into two groups, 11 test animals and seven controls. All animals were maintained on a diet of normal fattening meal equal to 5% of the body weight per day plus water *ad libitum* for at least two weeks before the experiments.

The pigs were given only water 18 to 24 h before operation. Two to three animals were operated on per day under sterile conditions. Atropine sulfate, 0.04 mg/kg, and acepromazine, 1.1 mg/kg, were given intramuscularly preoperatively, and anesthesia was induced with intravenous sodium pentobarbital, 12.5 mg/kg. An endotracheal tube was inserted and

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general anesthesia was maintained with a mixture of halothane, 1 to 2%, in nitrous oxide. A silicone rubber catheter was inserted into the internal carotid artery, exteriorized behind the ear, and filled with the minimal amount of sodium heparin (Lipo-Hepin, Riker; 1000 units/ml) required to maintain patency. A baseline blood sample was obtained at this time, before any further procedures were carried out.

A solution of sodium taurocholate (60 g/liter) and trypsin (160 000 U in 20 ml of phosphate buffer, 0.1 mol/liter, pH 7.6) was prepared freshly before each procedure and filtered through a 0.45- μ m filter (Millipore Corp., Bedford, Mass. 01730). A midline incision was made, the duodenum opened, and a small metal cannula was placed transduodenally into the pancreatic duct. The 20 ml of bile salt-trypsin solution was infused at a pressure of 40 cm of water into each test animal according to the method described by Elliot et al. (15) as modified by Carey (16). The time at which the infusion was completed is designated "time zero." The metal cannula was then removed and the duodenum and abdomen were closed. The control animals were subjected to the same surgical procedures except that the bile salt-trypsin solution was not infused. All animals were given 0.5 g of sodium cephalothin (Keflin, Lilly) twice a day by intramuscular injection, starting with the day of surgery, because we believed that the site of the exteriorized catheter was prone to infection.

Blood samples were obtained from the catheter every 6 h during the first 48 h and twice daily thereafter for a total of seven days. About 13 ml of blood was collected each time. Three milliliters of blood was placed at once into a cold test tube containing 3.6 mg of Na₂EDTA and 1500 units of aprotinin (Trasylol, F.B.A. Pharmaceuticals), the tube was centrifuged at 4 °C for 15 min at 1000 \times g, and the supernatant fluid was taken off and frozen at -20 °C until the glucagon and insulin assays could be performed. The rest of the blood was transferred at once to a 10 ml red-top Vacutainer Tube (Becton-Dickinson), kept closed at 4 °C for 6 h, and then centrifuged closed for 15 min at 1000 \times g. After centrifugation, 1 ml of serum was drawn from the closed Vacutainer Tube into a syringe and stored closed at 4 °C for up to 48 h until the ionized calcium analysis was performed. The rest of the serum was frozen until the other analyses were done.

All pigs were allowed water *ad libitum* after one day, and food up to 5% of body weight two days after operation. No infusions were given. One animal died during operation, and two others died before the end of seven days. Data from these animals are not presented here. Surviving test animals were killed and autopsied after seven days.

Analytical methods. Serum calcium and magnesium were determined by atomic absorption (17), ionized calcium was measured potentiometrically with an Orion 98-20 calcium electrode (18), and phosphorus was determined with a kit method (Hycel No. 198A). Blood urea nitrogen was determined with a kit (Boehringer Mannheim No. 1594) and the hematocrit was determined with a standard microhematocrit technique, in heparinized capillary tubes.

Serum pancreatic glucagon was determined by radioimmunoassay by Unger's method. Glycine buffer (0.2 mol/liter, pH 8.8) containing, per liter, 2.5 g of normal human serum albumin (Parke-Davis) and 10 ml of normal sheep serum (Grand Island Biological Co.), was used as assay diluent. The incubation mixture was prepared by consecutive additions of 0.7 ml of assay diluent, 0.1 ml of aprotinin, 0.2 ml of either plasma sample or buffered standard, 0.1 ml (15 pg) of ¹²⁵I-labeled glucagon (Nuclear Medical Laboratory), and 0.1 ml of antiserum 30K, giving a final antibody dilution of 1:40 000 in a final reaction mixture of 1.2 ml. After incubation at 4 °C for 4 days, free and antibody-bound glucagon were separated by adding 0.5 ml of a suspension of, per liter, 2.5 g of dextran

(Dextran T70; Pharmacia Fine Chemicals, Inc., Piscataway, N.J. 08854) and 5 g of charcoal (radioimmunoassay grade; Schwarz/Mann, Orangeburg, N.Y. 10962). The tubes were kept at 4 °C for 45 min and then centrifuged at 4 °C for 15 min at approximately 1000 \times g. The supernatant fraction was decanted carefully and discarded. Tubes containing the charcoal pellet were counted in a gamma scintillation counter for 5 min each. Standards were run in triplicate, and plasma samples were run in duplicate.

Total protein was determined with biuret (19), albumin with bromocresol green (20), amylase with Amylochrome (Roche), and insulin with the Phadebas (Pharmacia) solid-phase radioimmunoassay method.

Results

Induction of pancreatitis was successful, as judged by observed amylase activities. Serum amylase activity increased promptly 18 h after operation in the test group to 15-fold the baseline values, which was significantly greater than the threefold elevation seen in the controls ($P < 0.01$). Amylase activities were higher at all times in the test group for the first 72 h after induction of pancreatitis. Ionized calcium values dropped sharply after operation in the test group as compared with the controls (Figure 1). Changes in total serum calcium closely paralleled changes in ionized calcium. The lowest value for mean total calcium was temporally coincident with the lowest mean value for ionized calcium (Figure 2). Although values for total calcium also declined in the controls, lower mean values were observed in the test animals at all time intervals.

Inorganic phosphorus increased dramatically in the test animals and dropped slightly in the controls (Figure 3). Urea nitrogen increased in the test animals, but was unchanged in the control group (Figure 4), and a similar observation was made for the hematocrit in the two groups (Figure 5). Albumin dropped in the test group over time (Figure 6), while total protein concentration showed only a slight decline in the test group as compared to the controls. There was no significant difference in the total protein between the test animals and the controls during the first 72 h. Insulin values were not significantly different in the two groups throughout the one-week study. Glucagon increased in both groups to similar values in the first 48 h and declined slowly thereafter (Figure 7). Serum magnesium was significantly higher in the test group for the first 48 h (Figure 8).

Autopsy studies of all animals in the test group showed extensive pancreatic necrosis. Necrosis of peri-pancreatic fat tissue was also a prominent feature. The microscopic appearance of the pancreas was characterized by nearly total dissolution of the pancreatic parenchyma, with interstitial hemorrhage. Pancreatic pseudocysts containing about 500 ml of fluid were found in two of the test pigs. Gross and microscopic appearance of the pancreas of the control animals was judged normal.

Discussion

Acute pancreatitis in humans can be life-threatening. The prognosis is especially grave in the presence of acute hypocalcemia. We believe our animal model of pancreatitis mimics the disease as seen in humans reasonably well. We were able to produce the hyperamylasemia and severe hypocalcemia in pigs, two findings which are nearly always present in the acute disease in humans.

The hypocalcemia we observed at 18 h in the pancreatic pigs was severe. We did not give the pigs intravenous fluids after surgery, so the cause of the hypocalcemia was not plasma dilution. Patients with pancreatitis are commonly given intravenous fluids, and therefore the hypocalcemia seen in hu-

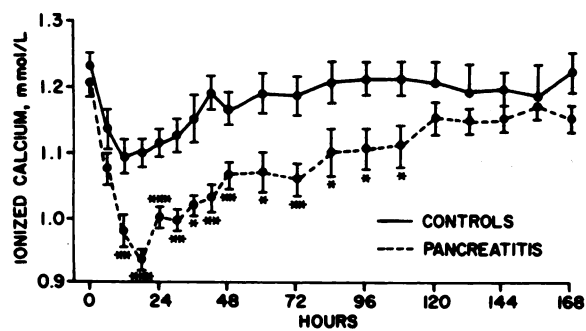


Fig. 1.

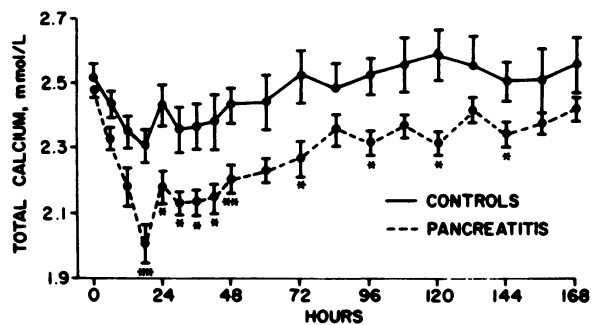


Fig. 2.

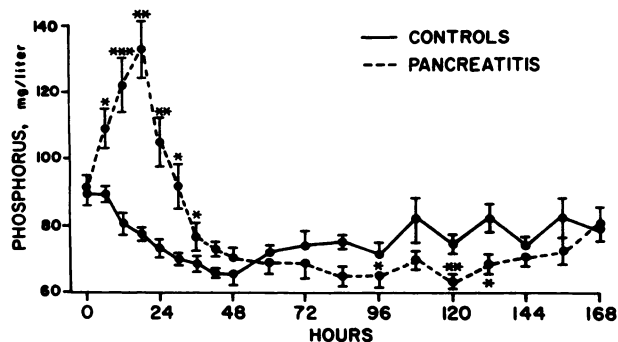


Fig. 3.

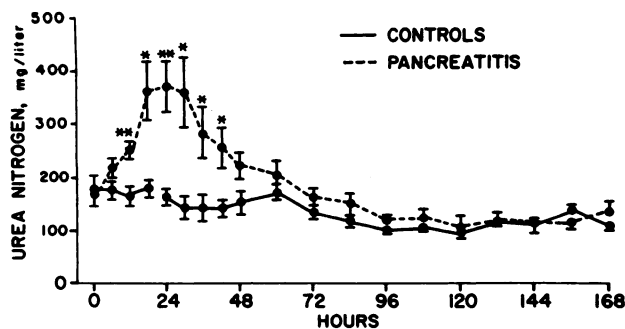


Fig. 4.

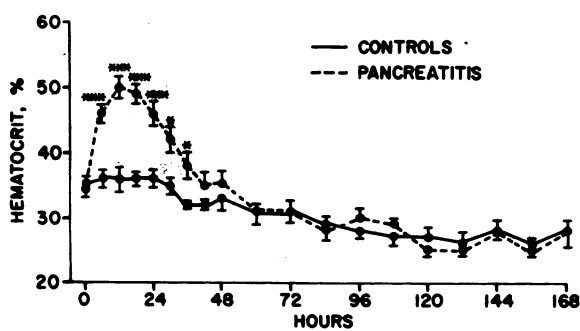


Fig. 5.

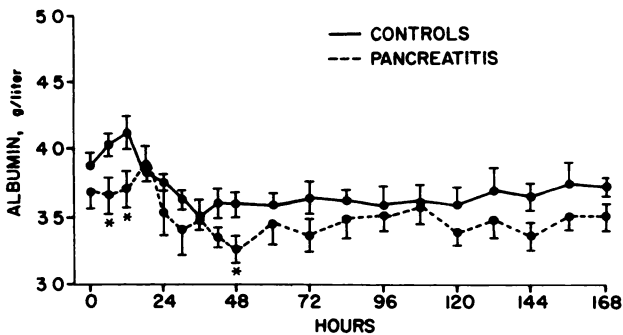


Fig. 6.

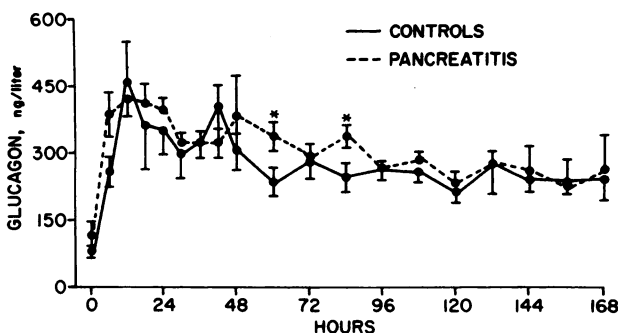


Fig. 7.

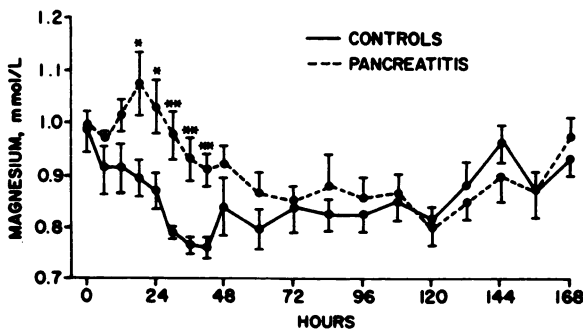


Fig. 8.

Figs. 1-8. Concentration of analyte vs. time in hours for the eight pancreatic test animals and the seven controls. Vertical bars represent one standard error. Induction of pancreatitis or sham operation occurred at zero hours. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

mans may in part be a dilution effect. Ionized calcium determinations did not give any additional information that was not already evident from the total calcium estimations. Since the changes in total and ionized serum calcium values paralleled each other, it appears that total serum calcium determinations were adequate in estimating the hypocalcemia in our model.

The changes we saw in serum albumin were too small to

explain the hypocalcemia. Also, the albumin concentrations in the test and the control animals were the same at the point of maximum hypocalcemia in the test animals.

Our test animals became hypovolemic after surgery, as indicated by the hematocrit, which reached a peak at 12 h. The hematocrit started to return to baseline values after the animals began to drink water. It is also likely that there was extravasation of fluid into the retroperitoneal space, and the

animals took about 24 h to compensate for the fluid loss. The hypovolemia apparently resulted in a decreased perfusion of the kidneys as reflected in the increase in urea nitrogen in the test animals during the first 48 h.

Glucagon values were increased in both groups to about the same extent, hence the differences in calcemia in the test and the control animals cannot be attributed to glucagon. Nevertheless, it is possible that the higher concentrations of glucagon exerted a mild hypocalcemic effect in both groups. It is unlikely that calcitonin had any effect, because the concentrations of glucagon were considerably below those required for the release of calcitonin (21). The increase in glucagon in both groups probably occurred as a result of the stress of handling and operation. Reports of adrenergic effects on pancreatic alpha cell activity (22) suggest a possible mediation of increased glucagon by catecholamines.

The cause of the hypocalcemia remains unresolved. The nadir for both ionized and total calcium at 18 h coincided with the highest concentrations of inorganic phosphorus in the test animals. These findings are consistent with the presence of an absolute or functional hypoparathyroidism. Possible explanations for these changes are impaired synthesis or impaired release of parathyrin, or the failure of target organs to respond to the hormone. Further studies into the role that parathyrin might play in acute pancreatitis are certainly warranted.

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