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Significance of Thromboxane A_2 and Prostaglandin I_2 in Acute Necrotizing Pancreatitis in Rats

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Plasma thromboxane concentrations were found to be significantly elevated in acute necrotizing pancreatitis in rats, whereas prostaglandin I_2 levels were not. The significance of these alterations was investigated. Pancreatitis was induced by injecting 5% sodium taurocholate into the pancreatic duct. Iloprost (ZK 36374, a stable analog of prostaglandin I_2, 25 ng/kg body weight) decreased the mortality rate from 100% to 50%. When treatment with iloprost was combined with simultaneous administration of either Sibelium (flunarizine R 14 950, 0.2 mg/kg body weight) or dazmegrel (UK 38 485, 50 mg/kg body weight) an additional decrease in the mortality rate was recorded. Dazmegrel is a selective thromboxane A_2 synthetase inhibitor and flunarizine (a calcium entry blocker) also inhibits the effects of elevated thromboxane A_2 levels. With flunarizine and iloprost the mortality rate was 40% ($P < 0.05$); with dazmegrel and iloprost it was 10% ($P < 0.01$). The results of the present study suggest that thromboxane A_2 and prostaglandin I_2 play a role in the course of acute necrotizing pancreatitis.

KEY WORDS: eicosanoids; dazmegrel; flunarizine; iloprost; pancreatitis; rats.

Prostaglandins play an important role in the pathophysiology of several diseases (1). They possess potent and diverse biological activities. Thromboxane A_2 (TXA_2) is a vasoconstrictor and stimulates platelet aggregation (1). Prostaglandin I_2 (PGI_2) is a vasodilator and the most potent naturally occurring inhibitor of platelet aggregation yet discovered (2). These differences in biological activities have led to the development of new concepts in vascular and cellular homeostasis (3).

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Recently we demonstrated that the plasma levels of thromboxane B_2, the stable end-product of TXA_2, were elevated in acute necrotizing pancreatitis (4, 5). The TXB_2 levels tend to rise dramatically compared with those of PGE_2 and I_2 (4). Simple inhibition of TXA_2 synthesis and effects, however, did not alter survival time significantly (4), but with simultaneous administration of PGE_2 a significant amelioration was encountered (6).

In the present study we investigated the effects of iloprost (ZK 36 374, a stable PGI_2 derivate) (7–9) on the survival time of rats with acute pancreatitis. Iloprost was administered with and without simultaneous inhibition of TXA_2 synthesis. The synthesis of TXA_2 was inhibited by dazmegrel (10) and flunarizine. Flunarizine, a calcium entry blocker, decreases TXA_2 formation (5) and also inhibits the effects of raised TXA_2 concentrations (11).

MATERIALS AND METHODS

Inbred male Wag/Rij rats, weighing 200–250 g, were used. Acute necrotizing pancreatitis (ANP) was induced by the retrograde injection of 5% sodium taurocholate (0.1 ml/100 g body weight) into the pancreatic duct as previously described (5).

Iloprost (ZK 36 374) vials containing 1 ml of injection fluid No. SH L 401 A, were a gift from Schering AG, Berlin, West Germany. Final dilutions in physiological saline (10 ng/ml) were prepared on the day of injection. Test animals were injected subcutaneously with iloprost at the time of induction of ANP (zero time) and 1, 2, 3, 6, and 9 hr after induction in a dose of 25 ng/kg body weight. Control animals received 0.9% NaCl instead of iloprost.

Dilutions of dazmegrel (UK No. 38485, lot No. R22; Pfizer Central Research Laboratories, Sandwich, England) in 0.1 N NaOH (12.5 mg/ml) were prepared on the day of administration. Experimental animals received a dose of 50 mg/kg body weight dazmegrel via a gastric tube 1 hr prior to the induction of ANP and 12 hr later.

Flunarizine was a gift of Janssens Pharmaceuticals, Goirle, The Netherlands. Dilutions in 0.9% NaCl (0.1 mg/ml) were prepared on the day of injection. Experimental animals were given flunarizine intravenously at the time of the induction of ANP in a dose of 0.2 mg/kg body weight.

The rats were randomly assigned to one of four groups. Acute pancreatitis was induced in all groups: group 1 (eight rats), saline; group 2 (eight rats), iloprost; group 3 (10 rats), iloprost + dazmegrel; group 4 (10 rats), iloprost + flunarizine. Survival time was recorded and survivors were sacrificed after a 72-hr observation period. Ascites was measured at autopsy by weighing cotton rolls saturated with the ascitic fluid. Fat necrosis was scored. The following scoring system was used: 0, scattered; 1, mild; 2, moderate; 3, severe. The pancreas was removed, fixed in 4% buffered formalin, and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin. The sections of the pancreas were assessed by a pathologist who was unaware of the treatment group. The amount of acinar necrosis was scored (0, <10%; 1, 10–25%, 2, 25–50%; 3, >50%), as well as the interlobular edema, the inflammatory response, and the severity of hemorrhages (0, scattered; 1, mild; 2, moderate; 3, severe). The amounts of ascitic fluid for the different groups were compared using the Mann-Whitney U test, the histological scores using the chi-square test for $2 \times r$ tables, and the survival data using Fischer's test for 2×2 tables.

RESULTS

The effect of different treatments on the mortality rate for rats with acute necrotizing pancreatitis is shown in Table 1. Saline-treated animals exhibited a 100% mortality at 36 hr whereas treatment with iloprost (25 ng/kg body weight) reduced mortality to 50%. The best results were obtained after treatment of the animals with flunarizine and dazmegrel. Ascites was found in all animals that died. The largest amounts of ascitic fluid were found in the first 36 hr. The amount decreased significantly after treatment with the drugs (Table 2). Hydrothorax developed in some of the animals. In several cases a distended stomach with a paralytic ileus of the proximal bowel was found. Fat necrosis was seen in all animals that died of the disease within 36 hr. The necrosis was less pronounced in the animals that were treated with the drugs. The amount of necrosis tended to increase with time. A significantly lower amount of necrosis was found in animals treated with iloprost in combination with either flunarizine or dazmegrel (Table 3).

TABLE 1. MORTALITY*

Group	Time period (hr) 0–36	36–72	0–72
ANP	100	0	100
ANP + iloprost	50	0	50
ANP + iloprost + F	40*‡	0	40*‡
ANP + iloprost + D	10†‡	10	20†‡

*The mortality rate is expressed as the percentage of the animals that died in the several time periods. ANP = acute necrotizing pancreatitis: D= dazmegrel; F = flunarizine.
*$P < 0.05$ statistically significant from ANP group.
†$P < 0.01$ statistically significant from ANP group.
‡Not statistically significant from ANP + iloprost group (chi-square tests for 2×2 table).

Light microscopy studies of the pancreas (Figures 1 and 2) showed an inflammatory infiltrate with areas of acinar necrosis. The inflammatory infiltration became the most severe at 72 hr. Interlobular edema developed in all animals. No significant differences in inflammatory response, edema, and hemorrhages were seen between the several groups (Table 3).

Acinar necrosis apparently continued, since animals that survived for 72 hr exhibited a larger amount of necrosis than those that died within 36 hr. Compared to the saline group, the acinar necro-

TABLE 2. AMOUNT OF ASCITIC FLUID*

Group	Amount (g, mean ± SD)
ANP	6.4 ± 1.5 (8)
ANP + iloprost	3.3 ± 1.0 (8)†
ANP + iloprost + F	2.0 ± 0.8 (10)†
ANP + iloprost + D	1.3 ± 0.8 (10)†

*The values are given in grams of ascites fluid and expressed as means ± SD. The numbers of animals are indicated in parenthesis. ANP = acute necrotizing pancreatitis. D = dazmegrel; F = flunarizine.
†$P < 0.01$ statistically significant from ANP group (Mann-Whitney U test).

TABLE 3. HISTOLOGICAL SCORES*

Group	Scores			
	Fat necrosis	*Inflammatory response*	*Interlobular edema*	*Hemorrhage*
ANP (8)	2.3 ± 0.7	1.0 ± 0.6	2.0 ± 0.8	1.0 ± 0.5
ANP + ilo (7)	1.5 ± 0.8	1.6 ± 0.8	1.3 ± 1.1	0.6 ± 0.5
ANP + ilo + F (10)	0.4 ± 0.5†‡	1.8 ± 0.7	1.0 ± 1.0	0.5 ± 0.5
ANP + ilo + D (9)	0.7 ± 0.5†	1.9 ± 1.0	0.9 ± 0.8	0.6 ± 0.7

*The histological scores are given as means ± SD. The numbers of animals are indicated in parenthesis. Score 0 = scattered, 1 = mild, 2 = moderate, 3 = severe. ANP = acute necrotizing pancreatitis, ilo - iloprost, F = flunarizine, D = dazmegrel. Unless indicated otherwise scores are not statiscally significant.
†$P < 0.01$ statistically significant from ANP group.
‡$P < 0.05$ statistically significant from ANP + ilo group.

sis was less pronounced in the groups treated with drugs. The lowest amount of necrosis was found in the dazmegrel/iloprost group (Table 4).

DISCUSSION

Thromboxane A_2 and prostaglandin I_2 are products of arachidonic acid with different and, in many respects, opposite biological activities. The interaction between platelet and vessel wall is responsible for eicosanoid production (3). TXA_2 is produced in response to ischemia in particular (12). The main effect of TXA_2 on the circulation is constriction of blood vessels and stimulation of platelet aggregation with formation of microthrombi. As such it plays an important role in the macro- and microcirculation of various organs. PGI_2 is a strong vasodilatory agent that dilates all vascular beds with cytoprotective properties in the splanchnic area (2). A precise balance between TXA_2 and PGI_2 seems to be important for vascular and cellular homeostasis (3).

Pancreatic blood flow decreases profoundly during the first hours of acute necrotizing pancreatitis (13, 14). Edema, poor vascular filling, and spastic changes in the lobular vessels have been observed (14). Eicosanoids may, in part, be responsible for these changes.

TXA_2 is generated (fourfold increase) in rats with acute pancreatitis (4, 5, 15), although not in dogs (16). In these rats thromboxane levels are reduced by treatment with inhibitors of thromboxane synthesis of flunarizine (5). This inhibition tends to improve survival time in ANP (5). Plasma 6-keto-$PGF_{1\alpha}$ (the bioconversion product of PGI_2) levels are increased to a much lesser extent (60% increase) (4, 15). PGI_2 is an inhibitor of TXA_2 production (2). The small increase in 6-keto $PGF_{1\alpha}$ levels may be the reflection of an insufficient response to the pathological stimuli.

From the present series of experiments it is clear that TXA_2 and PGI_2 play a role in acute pancreatitis in rats. Administration of iloprost significantly improves the survival rate when TXA_2 is inhibited. Iloprost is easily absorbed into the general circulation and its pharmacological profile is well known (7–9). The interaction between prostanoids is in accordance with the opinion that one prostanoid may be the pharmacological inhibitor of the other and that their balance may be of more importance than the individual plasma levels (3, 6, 17). Other prostanoid mediators may play a more or less significant role as well (6). When prostaglandin synthesis is completely blocked by indomethacin (1 mg/kg), the course of ANP is not ameliorated at all (5), although no agreement exists (18–20).

The mode of action of prostaglandin I_2 and thromboxane A_2 in ANP is not clear. Their balance may protect the pancreas from ischemic damage. PGI_2 may reduce the formation of activated pancreatic enzymes and their release into the systemic circulation, because PGI_2 is known to reduce the formation and release of lysosomal hydrolases, also in infarcted areas (2). Lysosomal hydrolases are thought to play a role in the intracellular activation process of proteolytic enzymes (21, 22). PGI_2 exhibits a cytoprotective effect on the pancreatic lysosomes in less advanced acute pancreatitis in dogs (23). This cytoprotective effect may be a consequence of local vascular effects of PGI_2, because lysosomes are sensitive to stimuli such as ischemia, hypoxia, and acidosis (24). Histological examination of the pancreas showed that there was still considerable necrosis in treated animals, although there was a trend towards less severe histo-

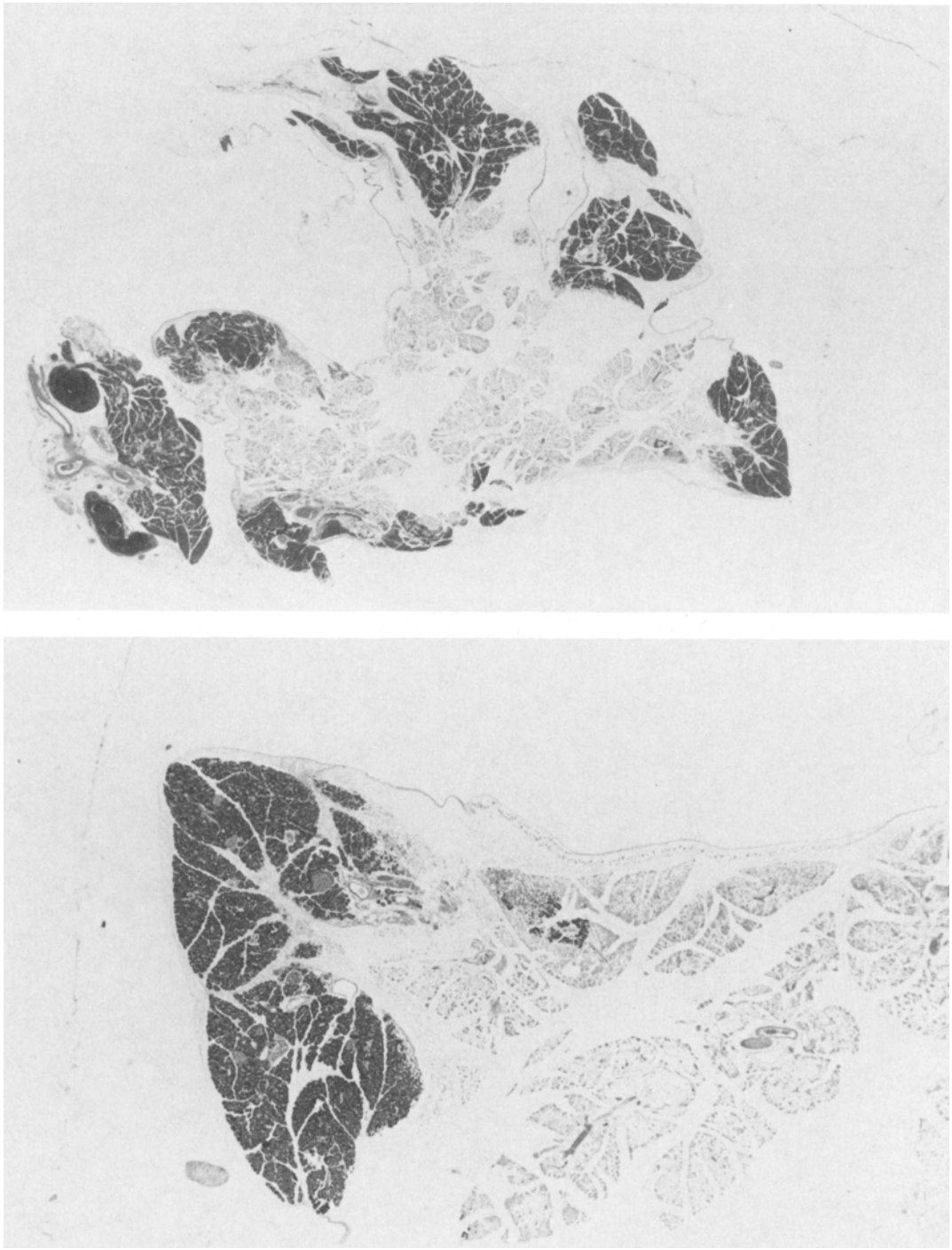

Fig 1. Large areas of coagulative necrosis 12 hr after induction of ANP. The inflammatory response is almost absent.

logical scores (Table 3 and 4). Relatively, the smallest percentage necrosis was seen in dazmegrel/iloprost-pretreated animals (Table 4), which may point to cytoprotection as well.

Local lesions of the pancreas are not exclusive factors of survival, because animals that were sacrificed at 72 hr exhibited more necrosis and inflammatory reaction than animals that died within 24 hr.

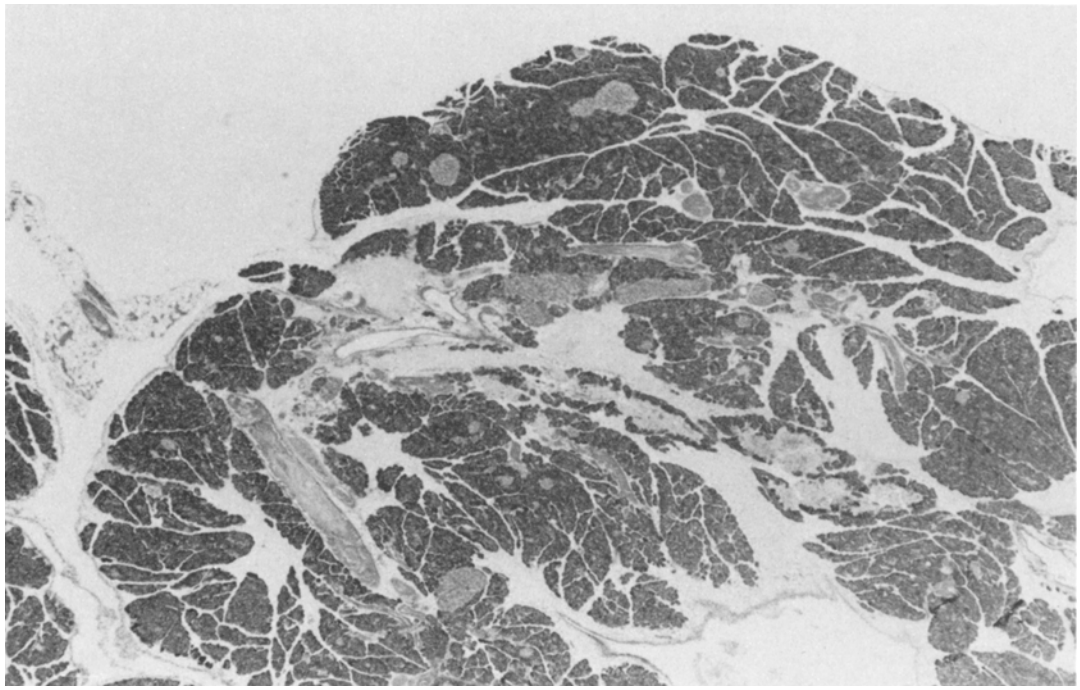

Fig 2A. Small areas of coagulative necrosis 24 hr after induction of ANP and treatment with iloprost. The inflammatory response is mild. Edema is present.

It is possible that the balance between TXA_2 and PGI_2 is important for maintenance of a sufficient blood flow to vital organs until the large fluid loss into the peritoneal cavity stops. A lower amount of

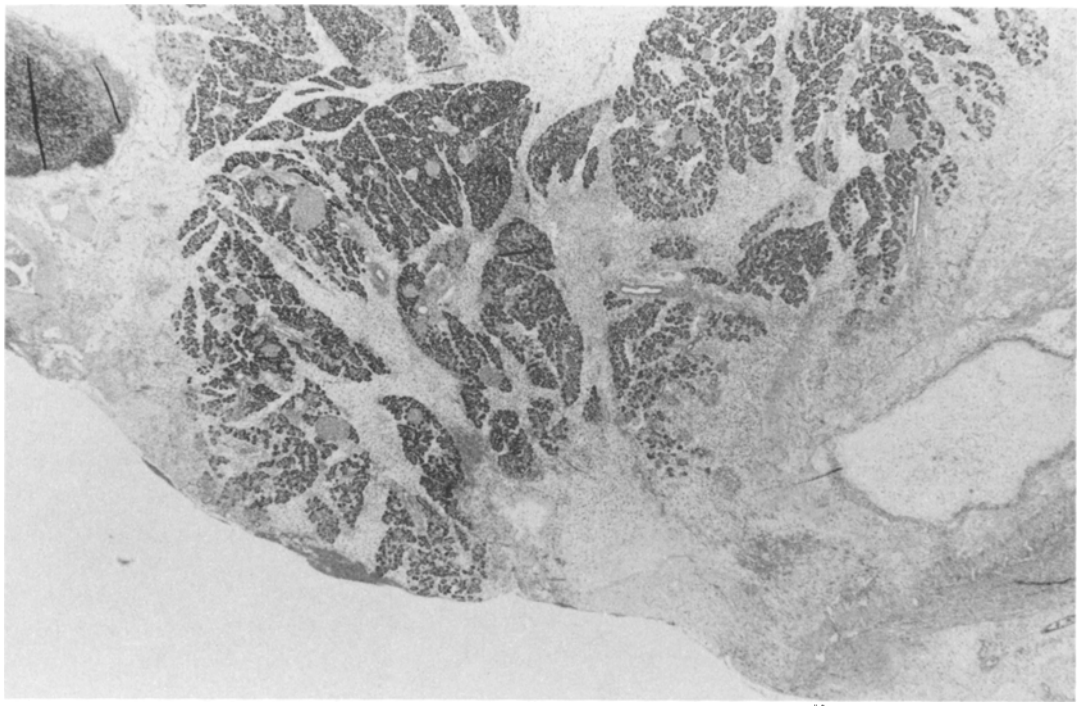

Fig 2B. The pancreas 72 hr after induction of ANP and administration of iloprost and Flunarizine. Areas of necrosis with a clear inflammatory response.

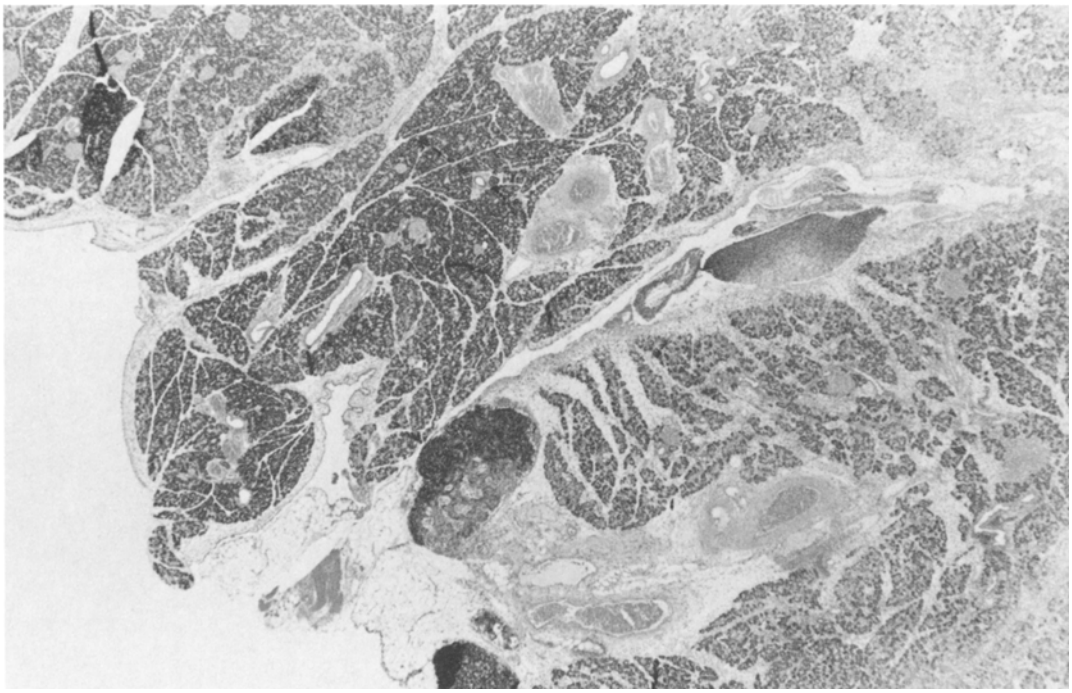

Fig 2C. The pancreas 72 hr after induction of ANP and treatment with iloprost and dazmegrel. Areas of acinar necrosis, normal architecture of peripancreatic fat tissue, mild inflammatory response.

ascitic fluid had accumulated in pretreated animals. Absorption of toxic ascitic fluid into the general circulation is important for multiple organ failure. A protective effect of PGI_2 on hepatic, renal and pulmonary lysosomes is reported to occur during acute pancreatitis in dogs (25–27). These organs are frequently damaged in the course of ANP.

In summary, the results of this study indicate that thromboxane A_2 and prostaglandin I_2 play a role in acute necrotizing pancreatitis. The present findings have yet to be tested in a clinical setting. It is promising that the bile-induced model in rats resembles acute pancreatitis in man (28); the behavior of eicosanoids is also comparable (29).

TABLE 4. AMOUNT OF ACINAR NECROSIS*

	Scores		
Group	*Nonsurvivors*	*Survivors*	*All animals*
ANP	3.6 ± 0.5 (8)	(0)	3.6 ± 0.5 (8)
ANP + ilo	2.7 ± 1.1 (3)	3.5 ± 1.0 (4)	3.1 ± 1.1 (7)
ANP + ilo + F	2.8 ± 1.0 (4)	3.3 ± 1.0 (6)	3.1 ± 1.0 (10)
ANP + ilo + D	2.5 ± 0.7 (2)†	3.1 ± 0.9 (7)	2.9 ± 0.9 (9)†

*The histological score of acinar necrosis is given as means ± SD. The numbers of animals are indicated in parentheses. Score 0 = <10% of the pancreas, 1 = 10–25%, 2 = 25–50%, 3 = >50%. ANP = acute necrotizing pancreatitis, ilo = iloprost, F = flunarizine, D = dazmegrel.

‡$P < 0.01$ not statistically significant from ANP group (chi-square test for $2 \times r$ tables).

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