Platelet Response to Induction of Hemorrhagic Pancreatitis[1] (35413)

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Experimental hemorrhagic pancreatitis can be regularly produced by a duodenal loop obstruction with the pancreatic ducts intact but the bile excluded (1). McCutcheon and Race (2) and Byrne *et al.* (3) demonstrated that this was due to regurgitation of duodenal contents into the pancreatic ducts. The pancreatitis can be alleviated by instillation of antibiotics in the duodenal loop, suggesting a bacterial nature of the process (4). However, some other effect of antibiotics may be important since Tedesco *et al.* (5) have shown the benefit of antibiotics in germ-free Pfeffer preparations. The exact nature of the pancreopathic factor is not known, but we have evidence that it is the product of a trypsin enhanced reaction between *E. coli* or its endotoxin with some blood protein (6).

The pancreopathic factor induces hemorrhagic pancreatitis by a vascular lesion with resultant thrombosis, hemorrhage, and necrosis and it can be alleviated by heparin (7) and sympathectomy (8). In order to further study the vascular factor, we have investigated the relationship between the systemic platelet count and the development of pancreatitis in our experimental model and have tried to influence the platelet behavior with drugs known to affect platelet aggregation.

Dipyridamole is a known vasodilator with a special affinity for the coronary circulation, however, it may also inhibit platelet aggregation—a function it shares with other vasodilators, such as propranolol and prostoglandin E (9, 10). Acetylsalicylic acid also has an effect on platelet aggregation as does sodium salicylate (11, 12).

As the model preparation, we are using a modification of the Pfeffer system designed to separate pancreatitis and duodenal strangulation. Aliquots of fluid removed from a strangulated duodenal loop, but with the bile excluded, are injected at physiologic pressures into the main pancreatic ducts of dogs with the accessory duct ligated. This produces hemorrhagic pancreatitis to the same degree as the Pfeffer preparation and appears also to be a bacterial reaction, since the addition of tetracycline to the fluid decreases the degree of pancreatitis (13).

Methods. A Pfeffer preparation was performed in mongrel dogs under aseptic conditions by fashioning a blind duodenal loop after ligation of the common duct. Gastrointestinal continuity was restored by pylorojejunostomy.

Twenty-four hr later the contents of the strangulated loop (Pfeffer fluid) were removed under sterile conditions and passed through a sterile sponge to eliminate gross particulate matter. Ten-ml aliquots of Pfeffer fluid were injected into the main pancreatic ducts of normal dogs after their accessory ducts were ligated. The injections were performed with a Harvard infusion pump with the pressures never exceeding 500 mm of water.

The pancreatic duct infusions were carried out on four groups of animals: Group I, 13 control dogs received no therapy; Group II, 9 dogs received dipyridamole intravenously (2 mg/kg of body wt); Group III, 8 dogs received 600 mg of acetylsalicylic acid orally the day before and the morning of the pancreatic duct infusion; Group IV, 6 dogs received 500 mg of sodium salicylate intravenously immediately prior to the pancreatic duct infusion and 1000 mg during the test period.

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TABLE I. Degree of Pancreatitis Produced by Pancreatic Duct Infusion of Pfeffer Fluid in Control and Treated Groups of Dogs.

		Pancreatitis		
	No.	Mild	Severe	p^a
Control	13	6	7	—
Dipyridamole, iv	9	5	4	1.00
Aspirin, oral	8	0	8	0.07
Salicylate, iv	6	1.5	4.5	0.75

[a] p denotes probability values compared to controls.

The pancreatitis which developed in these dogs was studied at autopsy. This was graded from 0 to 4+ depending upon the degree of necrosis and hemorrhage seen on the gross specimen. This is similar to the grading of our other experiments.

Platelet counts were performed: (A) before surgery, (B) preinfusion, and (C) 2 hr and (D) 4 hr postinfusion. The platelet counts were performed on venous blood, using disposable blooddiluting pipettes (Unopette, no. 5808, Boston, Becton, Dickinson and Company, Rutherford, New Jersey), with 1% ammonium oxalate diluent (1%), giving a 100:1 dilution of blood. Counts were done in duplicate on special thin counting chambers (no. 1475 American Optical Company, Buffalo, New York) with high power microscopy using American Optical (B-) phase optics.

Results. The degree of pancreatitis produced in each group can be seen in Table I. The control group yielded severe pancreatitis in 7 of 13 animals. Dipyridamole and intravenous sodium salicylate did not significantly decrease the incidence of severe pancreatitis. Oral acetylsalicylic acid yielded severe hemorrhagic pancreatitis in 100% of the animals.

The platelet counts are recorded in Table II together with the means and standard deviation of each group. In general, there was a marked fall 2 and 4 hr after the pancreatic duct infusion. This fall in platelets was not affected by the salicylates or dipyridamole.

When the platelet counts of animals that developed 3–4+ pancreatitis were compared to those that only developed 0–1+ pancreatitis (Table III), there was little difference noted in the response of the animals.

Discussion. The degree of pancreatitis was not reduced by either the salicylates or dipyridamole. One could have speculated that irrespective of their supposed effect on platelets, the salicylates would have modified the pancreatitis because of their anti-inflammatory action. Quite to the contrary, oral aspirin significantly potentiated the pancreatitis. The mechanism of this is obscure at the present time and requires further study.

The constant fall of platelets following pancreatic duct infusion with strangulated duodenal contents may be due to marrow depression, platelet destruction, or platelet aggregation with sequestration in various organs. The rapid occurrence of the fall would seem to rule out marrow depression and favor either platelet aggregation and/or destruction. Regardless of the mechanism, the salicylates and dipyridamole had no effect on the platelet response to the pancreatic duct infusion.

There was no correlation between the platelet response to the pancreatic duct infusion and the degree of pancreatitis produced by the infusion. Whether the pancreatitis was severe or absent, there was a constant fall of platelets of similar magnitude. This suggests some other determinant factor in the pancre-

TABLE II. Platelet Counts (per mm³) in Dogs with Pancreatic Duct Infusion of Pfeffer Fluid.[a]

	A	B	C	D
Control				
Mean	158,000	164,923	95,538	102,846
SD	55,688	102,745	38,821	53,950
Dipyridamole				
Mean	191,888	212,555	108,777	124,875
SD	99,027	105,446	76,790	58,533
Aspirin, oral				
Mean	150,875	203,750	118,000	77,625
SD	52,845	115,319	112,351	42,885
Sodium salicylate				
Mean	94,830	63,660	51,500	43,660
SD	11,930	21,720	12,560	9,300

[a] A = before surgery; B = preinfusion; C = 2 hr; and D = 4 hr postinfusion.

TABLE III. The Systemic Platelet Response to Pancreatic Duct Infusion with Pfeffer Fluid Related to the Degree of Subsequently Induced Pancreatitis.[a]

No. of dogs	Degree of pancreatitis		A	B	C	D
12	0–1+	Mean	198,000	200,500	123,416	135,416
		SD	88,462	90,483	65,198	54,832
23	3–4+	Mean	133,652	142,521	83,956	73,434
		SD	47,237	88,434	71,108	38,555

[a] A = before surgery, B = preinfusion, C = 2 hr, and D = 4 hr postinfusion. Degree of pancreatitis occurring within 24 hr also shown.

opathic vascular process, either a direct action on coagulation or on the blood vessels themselves.

Summary. The infusion of the pancreatic ducts with obstructed duodenal contents (Pfeffer fluid) produced a constant fall in the systemic platelet counts whether or not the animals subsequently developed severe pancreatitis. This fall in platelet count was not prevented by aspirin, sodium salicylate, or dipyridamole in the doses used nor did these agents have any beneficial effect on the subsequent pancreatitis. Aspirin appeared to aggravate the degree of pancreatitis.

1. Pfeffer, R. B., Stasior, O., and Hinton, J. W., Surg. Forum 8, 248 (1957).

2. McCutcheon, A. D., and Race, D., Ann. Surg. **115**, 523 (1962).

3. Byrne, J. J., Reilly, P. S., and Totounghi, F., Ann. Surg. **195**, 27 (1964).

4. Byrne, J. J., and Joison, J., Amer. J. Surg. **107**, 317 (1964).

5. Tedesco, V. E., Evans, T. E., and Nance, F. C., Rev. Surg. **26**, 375 (1969).

6. Williams, L. F., Jr., and Byrne, J. J., Surgery **64**, 967 (1968).

7. Byrne, J. J., Novogradic, W. E., Wilde, W. L., and Seifert, D. E., Exp. Med. Surg. **23**, 332 (1965).

8. Williams, L. F., Jr., Ashworth, C. J., Jr., Healey, E. H., and Byrne, J. J., Surg. Forum **19**, 366 (1968).

9. Hampton, J. R., Harrison, M. J. G., Hanour, A. J., and Mitchell, J. R. A., Cardiovasc. Res. **1**, 101 (1967).

10. Harrison, M. J. G., *in* "Experimental Biology and Medicine," Vol. 3, pp. 175–180. Karger, Basel (1968).

11. Morris, C. D. W., Lancet **2**, 279 (1967).

12. Breddin, K., "Experimental Biology and Medicine," Vol. 3, pp. 14–23. Karger, Basel (1968).

13. Williams, L. F., Jr., and Byrne, J. J., Surg. Gynecol. Obstet. **124**, 531 (1967).

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