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Pancreatitis in the Rat. (30392)

T. ARAI* (Introduced by H. Necheles)

Department of Gastroenterology Research, Medical Research Institute, Michael Reese Hospital and Medical Center, Chicago, Ill.

Most experimental work on pancreatitis has been done in the dog, usually with bile injected under pressure into the major pancreatic duct. The rat, a smaller and more economical animal, has many pancreatic ducts which open into the lower part of the common bile duct, simulating a common channel where biliary and pancreatic secretions can mix. However, earlier efforts to produce pancreatitis in the rat by ligation of the lower common bile duct were unsuccessful. Baggenstoss(1), Edlund(2), Nestel(3), and others reported that simple occlusion of the common bile duct at the duodenum of the rat produced edema of the pancreas and fat necrosis, but no parenchymal hemorrhagic necrosis.

We have found earlier(4) that in the rat ligation of the common bile duct at the duodenum produced parenchymatous necrosis, hemorrhage and fat necrosis within a few days. We have now established this on a larger series of animals.

Methods. Sprague-Dawley male and female rats with average weight of 222 g, fasted for 24 or 48 hours (with water available), were anesthetized with ether. In one group (Group A, 45 animals; Tables I and II, Group A-1, 28; Group A-2, 12; Group A-3, 5 animals), the common bile duct was ligated on the duodenum. It was important to employ fine silk sutures (size 5-0), because coarse thread may permit leakage. Blood vessels on and near the duct were excluded from the ligatures. Groups A-2 and A-3 received secretin Vitrum i.v. After 3-10 days, the contents of the dilated duct were aspirated and examined for enzymes; since the amount of fluid from a single rat was small, fluids obtained from 2-5 animals were pooled. Trypsin and trypsinogen were estimated by the method of Anson(5) and amylase by the method of Somogyi(6). Most animals died (38), and those in critical condition were killed. All animals were autopsied immediately; those that died during the night were discarded. Tissue sections from parts of the pancreas were stained with H.E. In 5 rats (Group B), the bile duct was ligated above and below the entrance of the pancreatic ducts. Pancreatic secretion which accumulated in the interligature segment was used for assay of enzymes. Histological examinations of 5 parts of the pancreas, and of the liver and omentum, were performed; classification of pancreatic pathology is based on histologic and gross observations.

In another group, the common duct was cut above and below the entrance of the pancreatic ducts, and plastic tubing was introduced into each end, thus permitting pure biliary and pancreatic secretion to be obtained or to determine secretory pressures.

* Research Fellow from Department of Surgery, Nagoya University School of Medicine, Japan. Supported by a grant from the Hartford Foundation. The Department is supported in part by the Michael Reese Research Foundation.

TABLE I. Pancreatitis in the Rat by Ligation of Bile and/or Pancreatic Ducts.

Group	No. of rats	Secretin, i.v.	No. of days p.o.	Autopsy findings: Gross		Autopsy findings: Microscop. (pancreas)	
A-1	28 (5 ♀, 23 ♂)	None	5.9 (1-10)	Ascites	16/28	Parenchym. necrosis	27/28
				Fat necrosis	20/28		
				Focal liver necrosis	7/28	Hemorrhage	26/28
				Dilated common duct	23/28	Edema	28/28
A-2	7 ♂	1 × 10 u	3.2 (1-5)	Ascites	4/7	Parenchym. necrosis	7/7
				Fat necrosis	7/7		
				Focal liver necrosis	3/7	Hemorrhage	7/7
				Dilated common duct	4/7	Edema	7/7
A-3	5 ♂	2 × 10 u	5.5 (5-6)	Ascites	4/5	Parenchym. necrosis	5/5
				Fat necrosis	5/5		
				Focal liver necrosis	1/5	Hemorrhage	5/5
				Dilated common duct	5/5	Edema	5/5
B	5 ♂	None	4 (Sacrificed)	Ascites	1/5	Parenchym. necrosis	0/5
				Fat necrosis	2/5		
				Focal liver necrosis	3/5	Hemorrhage	0/5
				Dilated common duct	5/5	Edema	5/5

Group A-1-3—Ligation of common bile duct on duodenum.
Group B—Ligation of common bile duct above and below entrances of pancreatic ducts.

These animals were kept in restraining cages, with drinking water available. Secretory pressures were recorded on a Type R Dynograph by way of P23Db Statham pressure transducers. Secretions were collected in tubes kept at 1°C for determination of enzymes.

Most rats received antibiotics i.m. after operation.

Results. Table I. Group A. In 39 out of 40 animals (97.5%) with the common bile duct ligated at the duodenum, hemorrhagic and necrotizing pancreatitis was observed within 1 to 10 days; bile stained peritoneal fluid was found in 24 of them; fat necrosis, often widespread in abdomen and chest was found in 32. Secretin i.v. increased the severity of pancreatitis in all groups.

Group B. The common bile duct was ligated above and below the entrance of the pancreatic ducts, so that stagnation and increased pressure of pancreatic secretion occurred. Four days postoperatively, the rats were sacrificed; edema of the pancreas was found in all rats, but no pancreatic hemorrhage or necrosis. Therefore, stasis and increased pressure in the pancreatic ducts does not produce hemorrhagic pancreatic necrosis. In Group A, 24% of the rats had died with hemorrhagic and necrotizing pancreatitis within 4 days.

Table II. The pancreatic secretion of the normal rat contains small amounts of free trypsin and considerable amounts of trypsinogen and amylase. Serum amylase content was found to be quite variable.

Group A. Determination of trypsin and trypsinogen in the fluid of the dilated common duct and in 2 ascitic fluids revealed no free trypsin and no or only traces of trypsinogen (less than 4 units).

Group B. In this series of rats the common bile duct was ligated above and below the entrance of the pancreatic ducts, permitting pure pancreatic secretion to accumulate. Four days later, this fluid was aspirated from a number of rats (2 to 5) and pooled. Trypsinogen was activated with enterokinase and trypsin was determined; none or very little was found. Trypsin and trypsinogen were absent in the ascitic fluid.

Pressures in the biliary and pancreatic ducts after their occlusion by pressure transducers rose, often interrupted by a drop, with the final pressure up to 300 mm of water in the bile duct and 200 mm of water in the pancreatic ducts. Maximum pressures were attained between 30 minutes and 2 hours, sooner in the bile duct than in the pancreatic duct. In 3 rats, the pressure in the common duct intubated at the duodenum rose to 220 mm water within 3 hours.

TABLE II. Trypsin, Trypsinogen, and Amylase in the Rat With and Without Pancreatitis.

Specimen	No. of rats and sex	Secretin, i.v.	Free trypsin, u/ml	Trypsin (activ. trypsinogen), u/ml	Amylase u/100 ml	Days after operation
			NORMAL			
Pancreatic secretion	18 (16♀, 2♂)	None	.23* (0-.6)†	35.9 (21.4-49.6)	26,400 (8)‡ (21,000-35,700)	
	12 (6♀, 6♂)	1 × 10 u	2.43 (0-5.1)	63.2 (45.1-90.6)	14,800 (1)	7
Serum	10 (8♀, 2♂)	None			7,892 (10) (2,580-11,526)	
			GROUP A: Hemorrhagic necrotizing pancreatitis			
Pancreatic secretion and bile	11 (5♀, 6♂)	None	0 (0-0)	2.5 (0-4)	312 (6) (174-450)	5.4 (3-10)
	9 (5♀, 4♂)	1 × 10 u	0 (0-0)	.4 (0-4)	14,414 (7) (14,200-14,500)	6.4 (5-7)
Serum	11 (6♀, 5♂)	None			4,587 (11) (3,855-5,225)	4.9 (3-8)
	6 (3♀, 3♂)				3,772 (6) (1,064-4,718)	6.3 (5-7)
Ascitic fluid	1♀	None	0	0	118	8
	1♂	1 × 10 u	0	0	213	8
			GROUP B: Pancreatic edema (pooled specimen)			
Pancr. secr. (intraductal fluid)	5♂	None	—	5.1	—	4
Serum	5♂	None			3,369 (5)	4
Ascitic fluid	1♂	None	0	0	59 (5)	4

* Average † Range ‡ No. of animals

Discussion. There is consensus that the presence of bile in the ducts of the pancreas does not produce pancreatitis unless it is under considerable pressure so that ductules break. Likewise, bile mixed with trypsin is considered not to be able to produce pancreatitis itself. Pancreatic elastase, bacterial toxins, and blood incubated with pancreatic enzymes, supposedly lower permeability of the canaliculi and of the walls of blood vessels of the pancreas. Thus, hemorrhagic pancreatitis obtained in our experiments may be caused by these factors, in addition to high secretory pressures in the obstructed common bile duct and breaking of pancreatic canaliculi. The absence of or very low values of trypsin and trypsinogen in the obstructed common bile duct and in the peritoneal fluid remains to be investigated. We are unable to explain why Block *et al*(1) and Edlund *et al*(2), using procedures similar to those we used, obtained only pancreatic edema, while we obtained hemorrhage and necrosis in most experiments.

Conclusions and summary. We have demonstrated that ligation of the common bile duct in the rat is followed within a few days by necrotizing hemorrhagic pancreatitis, considerable edema of the gland, fat necrosis, and often relatively large amounts of yellow ascitic fluid. The mixture of pancreatic and biliary secretion accumulated in the obstructed common duct as well as the ascitic fluid contained little or no trypsinogen and no free trypsin. Values of enzyme concentrations in pancreatic secretion of the normal rat are reported.

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Mechanisms of Inflammation. I. Laser-Induced Thrombosis, a Morphologic Analysis.* (30393)

LESTER GRANT,† AND FREDERICK F. BECKER† (Introduced by Lewis Thomas)
Departments of Medicine and Pathology, New York University School of Medicine, New York City

It is well recognized that thrombosis in small blood vessels plays a significant role in the development of inflammatory states (1-4). The adherence of the thrombus to the vessel wall involves platelet aggregation, among other things, and the sticking of formed elements such as white cells and platelets(4) to each other and to the endothelial cell.

Recently developed laser systems(5-10) now permit a unique approach to the study of thrombosis and thromboembolism, enabling the investigator to analyze, *in vivo*, in a highly reproducible way, the formation, propagation and dissolution of microthrombi (10,11). In contrast to virtually all previously used experimental models, it is possible with a laser instrument to produce microthrombi in single vessels. One can arrange the experimental system in such a way as to exclude, apparently, extravascular factors which may influence the development of such thrombi. Under these circumstances, discrete intravascular injuries yield white cell and platelet adherence to the endothelial walls of small blood vessels. Such capillary preparations as mesenteries(10) permit short-term observations of such lesions but the rabbit ear chamber, equipped with a transparent coverslip, is an ideal preparation for long-term study of laser injury and the resolution of the lesion(11). Lesions can be observed for minutes, hours, days or weeks with the coverslip of the chamber protecting the capillary bed from extraneous stimuli, yet allowing a constant evaluation of the status of the injury site.

This communication deals with the morphology of a lesion produced by a laser beam in an ear chamber under circumstances where the injury to the blood vessel was of a sufficiently limited nature to permit recovery. Future communications will deal with the dynamic aspects of this system.

Materials and methods. Rabbits used were 2 kg male English half-lops, obtained from Bar F Rabbitry, Fullerton, Md. Ear chambers were similar in design to those described by Sanders *et al*(12) and Grant *et al*(13) except for certain modifications in coverslip support. The principle of the chamber was essentially that of Sandison(14). Connective tissue in the ear chamber is made up of subcutaneous areolar tissue and is 30 to 50 μ in thickness. The laser system used was a Ruby Minilaser 103, purchased from TRG, Inc., Melville, N. Y., adapted for use with a Leitz Ortholux microscope. The laser beam was projected by the use of a prism through the $\times$50 objective of the microscope to the target vessel. After preliminary testing of the system by production of more than 100 lesions, it was determined that a satisfactory injury in a venule could be achieved by setting the Variac on the power supply at intensities from 150 to 200 joules input. Under conditions of the experiment, the diameter of the lesion produced immediately after the stimulus *in vivo* was approximately 5 to 20 μ, which correlated well with diameters of color alterations produced by this laser beam on glass slides coated with a green dye.

Ten minutes before application of the laser beam to the ear chamber vessel, the rabbit received 5 cc of Pelikan ink in gelatin, pre-

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† Career Scientist, New York City Public Health Research Council.