# A Possible Mechanism for Gallstone Pancreatitis: Repeated Short-Term Pancreaticobiliary Duct Obstruction with Exocrine Stimulation in Rats (43534)

TETSUYA HIRANO[1] AND TADAO MANABE
*First Department of Surgery, Faculty of Medicine, Kyoto University, Kyoto 606, Japan*

*Abstract.* **The effects of single and repeated short-term (4 hr) obstruction of pancreaticobiliary duct (PBDO), with or without exocrine stimulation (intraductal hypertension) by cerulein infusion (0.2 μg/kg.hr), on the exocrine pancreas were evaluated in the rat. Single blockage of pancreaticobiliary duct for 4 hr caused a significant rise in serum amylase levels, pancreatic water content, and redistribution of lysosomal enzyme, cathepsin B from the lysosomal fraction to the zymogen fraction, which was considered to mean the colocalization of lysosomal enzymes with pancreatic digestive enzymes in the same subcellular compartment in acinar cells. In addition, the accelerated lysosomal and mitochondrial fragility was observed in the single pancreaticobiliary-duct-obstructed animals. Moreover, the repeated PBDO for 4 hr (2 hr in each obstruction and 1 hr of free flowing of pancreaticobiliary juice between two obstructions) caused more marked changes in almost the all parameters, and the repeated PBDO with intraductal hypertension caused an activation of trypsinogen in the pancreas, making more marked changes in almost the all parameters than the repeated PBDO only group. These results indicate that the present model of repeated PBDO with exocrine stimulation seems to be a pertinent model for gallstone pancreatitis in humans, and that redistribution of lysosomal enzymes and subcellular organellar fragility seem to play an important role in the pathogenesis of pancreatic injuries induced by PBDO, particularly by repeated PBDO with exocrine stimulation, probably via activation of trypsinogen to trypsin by lysosomal enzyme, cathepsin B.** [P.S.E.B.M. 1993, Vol 202]

Both morphologic and biochemical studies have shown that, in the early stage, two forms of experimental pancreatitis—diet-induced (1, 2) and secretagogue-induced (3–6)—share the common attribute of colocalization of digestive enzymes with lysosomal hydrolases inside large cytoplasmic vacuoles (7), and the subcellular fractionation of acinar cells has demonstrated redistribution of lysosomal enzymes from the lysosome-rich to the zymogen granule-rich fraction. Since cathepsin B, a lysosomal enzyme, can activate trypsinogen (8, 9), and trypsin can activate many other pancreatic digestive enzymes, the colocalization of digestive enzymes with lysosomal hydrolases could lead to the activation of intracellular digestive enzymes and result in an important triggering event in the development of acute pancreatitis inside the acinar cells. Thus, lysosomal enzymes seem to play a crucial role in the pathogenesis of acute pancreatitis (10).

On the other hand, gallstone pancreatitis, which is the most common in humans, seems to be triggered by the passing through, or incarceration, of a stone in the terminal bile duct (11–14), which is considered to mean the obstruction of both the pancreatic and biliary duct. Moreover, such a gallstone attack is often repeated in a short period. However, the mechanism whereby pancreaticobiliary duct obstruction may induce pancreatitis has not been clarified.

In this study, we examined the *in vitro* lysosomal and mitochondrial fragility, subcellular distribution of lysosomal enzyme, and possible activation of trypsinogen in the pancreas after single and repeated short-term

[1] To whom requests for reprints should be addressed at First Department of Surgery, Faculty of Medicine, Kyoto University 54 Shogoin-Kawaracho, Sakyoku, Kyoto 606, Japan.

Received March 30, 1992. [P.S.E.B.M. 1993, Vol 202]
Accepted September 15, 1992.

0037-9727/93/2022-0246$3.00/0
Copyright © 1993 by the Society for Experimental Biology and Medicine

(4 hr) obstruction of the pancreaticobiliary duct with or without exocrine stimulation by pancreatic secretagogue.

## Materials and Methods

**Animal Preparation.** Twenty-seven male Wistar rats weighing about 350 g (Shizuoka Experimental Animals, Shizuoka, Japan) were used. The rats were kept in light:dark-cycle regulated (light, 5:00–17:00) and air-conditioned (23 ± 3°C) animal quarters in our institute before the experiments, and were allowed to become acclimatized to the standard laboratory conditions for at least 4 days. They were maintained throughout the study in accordance with the guidelines of the Committee on Animal Care of Kyoto University, and this study was approved by the Committee. Tests were performed after a 16-hr fast, starting at between 8:00 and 9:00 AM to rule out the effects of circadian rhythms on the exocrine pancreas.

Anesthesia was induced by intraperitoneal administration of sodium pentobarbital (30 mg/kg Nembutal; Abbott Co., North Chicago, IL), and maintained by periodic intravenous injection of pentobarbital (10 mg/kg). Animals were kept on a heating pad at 40°C (KN-473; Natsume Seisakusho, Tokyo, Japan) and under overhead lamps to maintain their core body temperature. Before opening the abdomen, a catheter (Medicut 18-gauge catheter; Sherwood Medical Industries, St. Louis, MO) was placed in the superior vena cava via the right external jugular vein.

A midline skin incision was made for laparotomy, and at this point, all the rats were divided into the following four groups (Fig. 1): (i) repeated pancreaticobiliary duct obstruction (R-PBDO) group ($n = 7$; the pancreaticobiliary duct (PBD) just adjacent to the duodenum was ligated by a metal clip for 2 hr, and then the clip was removed for the next 1 hr; after this 1 hr of free flowing of pancreaticobiliary juice, the PBD was again ligated for an additional 2 hr) (ii) single, short-term PBD obstruction (S-PBDO) group ($n = 6$); the PBD was ligated for 4 hr at 1 hr after the induction of laparotomy; (iii) repeated PBD obstruction with exocrine stimulation (R-PBDO + IDH) group ($n = 9$); the R-PBDO was made as in the R-PBDO group, but during the second PBDO, cerulein (Ceosunin injection; Kyowa Hakko Co., Tokyo, Japan) was infused at a dose of 0.2 μg/kg.hr for 2 hr to stimulate the pancreatic juice secretion and to create intraductal hypertension (IDH); and (iv) control laparotomy group ($n = 5$); the PBD near the duodenum was gently manipulated and anesthesia was maintained for 5 hr. All the animals were infused with heparinized (30 IU/ml) saline at a rate of 0.58 ml/hr during the experiments by an infusion pump (truth type B-6; Nakagawaseikodo, Tokyo, Japan).

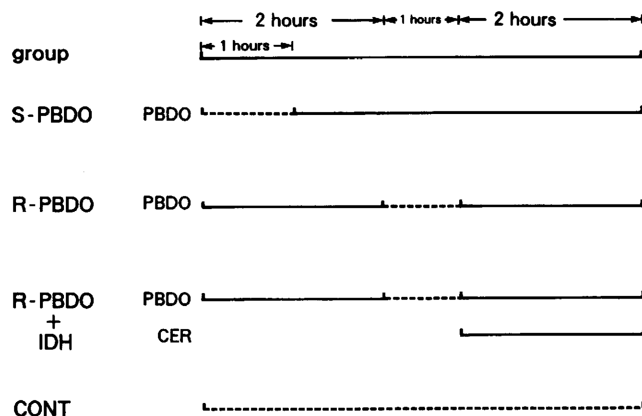


**Figure 1.** Experimental groups. S-PBDO, pancreaticobiliary duct was obstructed for 4 hrs; R-PBDO, pancreaticobiliary duct was obstructed twice (2 hr in each obstruction and 1 hr of free flowing of pancreaticobiliary juice between two obstructions); R-PBDO + IDH, pancreaticobiliary duct was obstructed twice as in the R-PBDO group, but cerulein (0.2 μg/kg.hr) was infused during the second 2-hr obstruction; and the control laparotomized group (CONT).

**Serum Amylase Levels.** Before ligation or manipulation of the pancreaticobiliary duct, 0.3 ml of blood was drawn from the venous catheter to the superior vena cava and after 4 hr (S-PBDO group), or a second 2 hr of ligation (R-PBDO, and R-PBDO + IDH group) or 5 hr of anesthesia (control group), blood was again drawn for the determination of serum amylase levels. After a 0.3-ml blood sample 0.3 ml of lactate-Ringer solution was injected to replace lost blood.

**Pancreatic Water Content.** At selected times after 5-hr experiments in each group, all the rats were painlessly sacrificed by a large dose of pentobarbital. After removal of the pancreas, about 200 mg of the pancreas were used for the quantification of pancreatic edema by comparing the weight immediately after sacrifice (wet weight) with that of the same sample after incubation at 150°C for 48 hr in a dessicator (Sanyo drying oven; Sanyo, Tokyo, Japan) (dry weight). Pancreatic water content was expressed as a percentage of the total wet weight.

**Pancreatic Amylase, Cathepsin B, Trypsinogen, and Trypsin Content.** Another 200 (approximately) mg of the pancreas were used for the determination of pancreatic content of amylase, cathepsin B, trypsinogen, and trypsin. This portion was homogenized in 3 ml of cold phosphate-buffered (pH 7.4) saline containing 0.5% Triton X-100 (Sigma Chemical Co., St. Louis, MO) in a Polytron homogenizer (Brinkmann Instrument, Westbury, NY), and unbroken cell and debris were removed by low speed centrifugation (150 *g* at 4°C for 15 min). Amylase activity, cathepsin B activity, trypsinogen content, and trypsin activity were measured in the resulting supernatant. The DNA concentration was also measured, and pancreatic content of amylase, cathepsin B, trypsinogen, and trypsin were expressed as units/mg of DNA.

**Pancreatic Histologic Examination.** One small portion of the pancreas from the splenic portion was fixed overnight by immersion in phosphate-buffered

(pH 7.4) 10% neutral formalin. After paraffin embedding, sectioning and staining with hematoxylin-eosin, the sections were examined light microscopically by an observer blind to the experiment who graded acinar cell changes, such as interstitial edema, acinar cell vacuolization, inflammatory cell infiltration, and acinar cell necrosis. One section was made in the center of the specimen from each rat, and the whole section was observed.

**Subcellular Distribution of Cathepsin B Activity.** Another part of the pancreas (about 300 mg) was used for subcellular fractionation and for the determination of subcellular distribution of cathepsin B activity in acinar cells. The excised, trimmed, and homogenized rat pancreas was separated into its various subcellular fractions by differential centrifugation. The protocol was originally developed by Tartakoff and Jamieson (15) and further modified for studies of rat tissue (16). Briefly, pancreatic fragments were homogenized in 6 ml of cold 5 m*M* 3-(*N*-morpholino)propanesulfonic acid ([MOPS] Sigma) buffer (pH 6.5) containing 1 m*M* $MgSO_4$ and 250 m*M* sucrose with three up-and-down strokes of a Dounce homogenizer (Wheaton, Millville, NJ). The resulting homogenate was centrifuged (150*g* at 4°C for 10 min) to pellet debris and unbroken cells, which were discarded. The supernatant after this low-speed centrifugation was considered to contain 100% of each of the component measured. This supernatant was centrifuged (1,300*g* at 4°C for 15 min) to obtain a zymogen-granule-rich pellet (1.3 KP), and another supernatant. The resulting supernatant was centrifuged (12,000*g* at 4°C for 12 min) to yield a lysosome- and mitochondria-rich pellet (12 KP) and a supernatant, which was considered to contain a microsomal and soluble fraction (12 KS). The various pellets obtained during fractionation were resuspended in 2 ml of cold (4°C) 5 m*M* MOPS buffer, and cathepsin B activity in each fraction was measured and expressed as a percentage of the total activity as an index of subcellular distribution of lysosomal enzymes in acinar cells.

**Cathepsin B Leakage from Lysosomes and Malate Dehydrogenase Leakage from Mitochondria.** The remaining portions of the pancreas (about 400 mg) were homogenized in cold 5 m*M* MOPS buffer as described as above. This homogenate was centrifuged (150 *g* at 4°C for 10 min) to remove unbroken cells and debris. The resulting supernatant was centrifuged (12,000 *g* at 4°C for 12 min) to pellet the combined zymogen-granule-, lysosome-, and mitochondria-rich fraction. This pellet, arbitrarily considered to contain 100% of the lysosomal and mitochondrial enzyme activity, was suspended in 2 ml of 5 m*M* MOPS buffer and incubated for varying intervals (30, 60, and 90 min) at 25°C in a shaking water bath under room air. The samples were then recentrifuged (12,000 *g* at 4°C for 12 min) to separate the particulate from the soluble lysosomal and mitochondrial enzyme activity, each of which was individually measured after separation of the pellet and supernatant. As a lysosomal enzyme, cathepsin B activity was measured both in the pelleted and soluble fraction. Centrifugation and subsequent measurement of particulate and soluble lysosomal enzyme activity identified the rate and extent of *in vitro* rupture of lysosomal enzyme containing organelles. Soluble cathepsin B activity was expressed as a percentage of the total activity as an index of lysosomal fragility. For the same samples, as a mitochondrial enzyme, malate dehydrogenase (MDH) activity was measured and MDH leakage from mitochondria was expressed in the same way as in the cathepsin B leakage, as an index of mitochondrial fragility.

**Assays.** Amylase activity was measured with blue starch (Shionogi amylase test-A; Shionogi, Osaka, Japan) as the substrate by the method of Irie and coworkers (17). Trypsinogen was activated by purified enterokinase (Sigma) and trypsin activity was measured with *p*-toluenesulfony-L-arginine methylester-HCL (Sigma) as the substrate by the method of Hummel (18), and 1 unit of the activity was calculated from the standard curve made from purified trypsin (Sigma). Cathepsin B activity was measured fluorometrically by the method of McDonald and Ellis (19), with *N*-carbobenzoxy-arginyl-arginine-$\beta$-naphthylamide (Bachem Feinchemikalien AG, Budendorf, Switzerland) as the substrate. MDH activity was measured by the method of Bergmeyer (20), detecting the consumption rate of oxaloacetic acid and reduced diphosphopyridine nucleotide. DNA concentration was measured fluorometrically by the method of Labarca and Paigen (21), with calf thymus DNA (Sigma) as the standard.

**Data Presentation.** The results reported in this study represent the mean ± SE for *n* determinations. Differences between the groups were evaluated by analysis of variance with post hoc comparison using the Tukey procedure, and significant differences were defined as those associated with probability value of less than 0.05. For the histologic changes, the Wilcoxon rank sum test was used.

## Results

**Serum Amylase Levels and Pancreatic Water Content.** Single, 4-hr obstruction of the pancreaticobiliary duct caused a significant hyperamylasemia (18 ± 2 units/ml; before obstruction, 7 ± 2 units/ml) compared with control group (8 ± 2 units/ml), but repeated 4-hr obstruction of the pancreaticobiliary duct caused a more marked and significant rise in serum amylase levels (25 ± 2 units/ml; before R-PBDO, 7 ± 1 units/ml) compared with the S-PBDO group. Moreover, repeated 4-hr obstruction with cerulein stimulation (R-PBDO + IDH) caused the most marked rise in serum amylase levels (38 ± 3 units/ml; before R-PBDO + IDH, 6 ± 2 units/ml) (Fig. 2a).

S-PBDO caused a significant rise in pancreatic

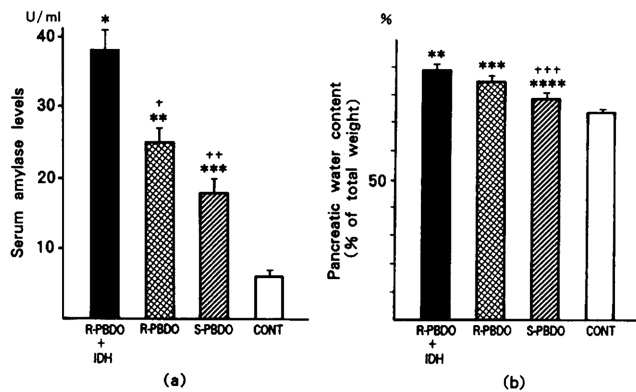


Figure 2. Effect of repeated short-term pancreaticobiliary duct obstruction with exocrine stimulation on (a) serum amylase levels and (b) pancreatic water content in rats. *$P < 0.001$, **$P < 0.01$, ***$P < 0.02$, and ****$P < 0.05$ compared with the control group; $^{+}P < 0.02$, $^{++}P < 0.01$, and $^{+++}P < 0.05$ compared with the R-PBDO + IDH group.

water content (79 ± 2%) compared with the control group (74 ± 1%), but R-PBDO caused a more significant rise (85 ± 2%). Moreover, R-PBDO + IDH caused the most significant rise (89 ± 2%) (Fig. 2b).

**Pancreatic Amylase, Trypsinogen, and Cathepsin B Content.** S-PBDO for 4 hr caused a significant increase in pancreatic amylase (595 ± 52 units/mg of DNA) and trypsinogen content (102 ± 9 units/mg of DNA) compared with the control group (amylase content, 378 ± 29 units/mg of DNA; trypsinogen content, 75 ± 6 units/mg of DNA), suggesting the congestion of pancreatic digestive enzymes induced by PBDO. Repeated PBDO also caused a significant increase in pancreatic amylase content (526 ± 49 units/mg of DNA) compared with the control group. But, on the contrary, R-PBDO with cerulein stimulation caused no significant rise in either pancreatic amylase or trypsinogen content. In regard to the pancreatic cathepsin B content, there were no significant differences among these four groups (Fig. 3, a–c).

**Pancreatic Trypsin Content.** Only in the R-PBDO + IDH group was a small amount of free trypsin activity detected, whereas in the other groups, almost no free activity was detected (Fig. 4).

**Pancreatic Histologic Changes.** Histologic examination after S-PBDO for 4 hr showed a mild but significant interstitial edema, and slight and nonsignificant acinar cell vacuolization compared with the control group. Inflammatory cell infiltration was very slight and not significant in any group, except in the R-PBDO + IDH group. R-PBDO caused more marked and significant histologic changes, such as interstitial edema and acinar cell vacuolization, compared with the S-PBDO group. Moreover, R-PBDO + IDH caused the most significant histologic changes, including focal acinar cell necrosis (Table I).

**Subcellular Distribution of Cathepsin B Activity.** S-PBDO for 4 hr caused a significant increase of cathepsin B activity in the zymogen fraction (1.3 KP, 38 ± 2%) and a significant decrease in the lysosomal fraction (12 KP, 43 ± 2%) compared with the control group (1.3 KP, 24 ± 12%; 12 KP, 59 ± 2%). These changes indicate a marked shift of cathepsin B activity from the lysosomal fraction to the zymogen fraction, suggesting redistribution of cathepsin B in the subcellular fractionation of acinar cells. R-PBDO caused a more marked and significant shift of cathepsin B (1.3 KP, 49 ± 2%; 12 KP, 32 ± 2%) compared with the S-PBDO group. Moreover, R-PBDO + IDH caused the most significant redistribution of cathepsin B (1.3 KP, 54 ± 3%; 12 KP, 27 ± 2%). There were no significant differences in the microsomal and soluble fraction (12 KS) among these four groups (control, 17 ± 2%; S-PBDO, 19 ± 2%; R-PBDO, 20 ± 2%; R-PBDO + IDH, 19 ± 2%) (Fig. 5).

**Cathepsin B Leakage from Lysosomes and MDH Leakage from Mitochondria.** S-PBDO for 4 hr caused a significant increase in cathepsin B leakage from lysosomes, particularly in the prolonged incubation times (≥60 min), compared with the control group, but R-PBDO caused more marked and significant increases in cathepsin B leakage compared with the S-PBDO group, indicating the more accelerated lysosomal fra-

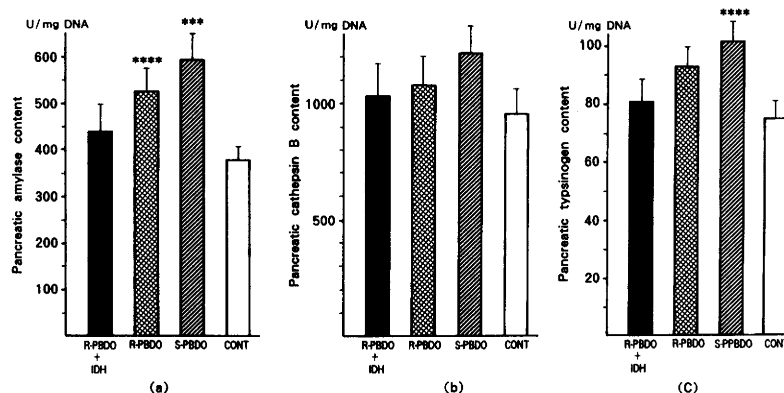


Figure 3. Effect of repeated short-term pancreaticobiliary duct obstruction with exocrine stimulation on (a) pancreatic amylase, (b) cathepsin B, and (c) trypsinogen content in rats. ***$p < 0.02$ and ****$p < 0.05$ compared with CONT group.

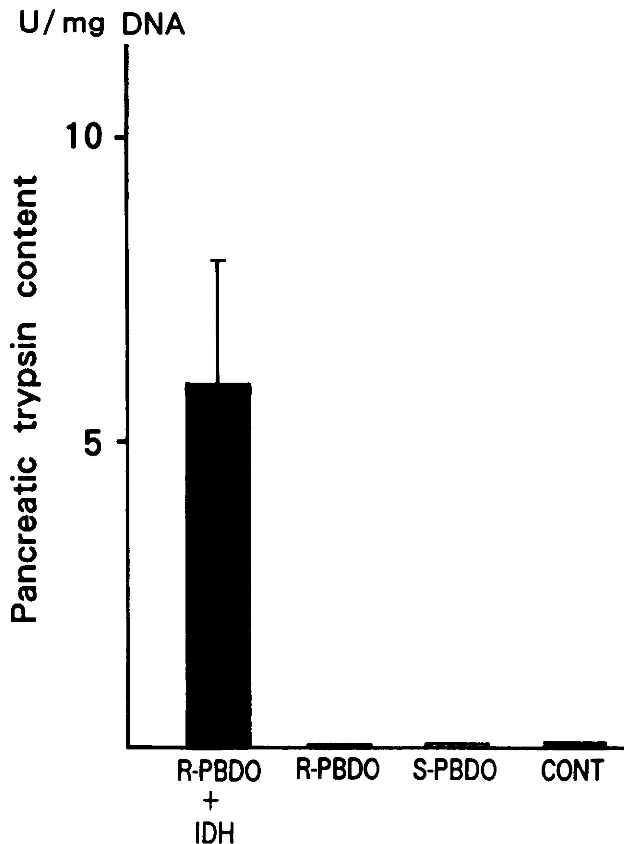


**Figure 4.** Effect of repeated short-term pancreaticobiliary duct obstruction with exocrine stimulation on activation of trypsinogen in the rat pancreas.

gility. Moreover, R-PBDO + IDH caused the most significant cathepsin B leakage. In regard to the MDH leakage from mitochondria, S-PBDO for 4 hr also caused a significant increase, particularly in the prolonged incubation times (≥60 min), as in the lysosomal fragility compared with the control group. R-PBDO also caused more significant increases in the MDH leakage compared with the S-PBDO group. Moreover, R-PBDO + IDH caused the most marked and significant MDH leakage, indicating the most accelerated mitochondrial fragility (Table II).

## Discussion

Gallstone pancreatitis in humans appears to be precipitated by the passage of a stone through, or its incarceration in, the terminal portion of the common bile duct (11, 12). The mechanism whereby such a stone might precipitate acute pancreatitis has been the subject of many studies and continues to be an issue of considerable controversy.

The studies reported here may provide an important clue to the understanding of the triggering events leading to acute pancreatitis in pancreaticobiliary-duct-obstructed animals: common channel theory, since it shows that single, short-term (4 hr) pancreaticobiliary duct obstruction caused the pancreatic injuries, including hyperamylasemia, pancreatic edema, and congestion of digestive enzymes in acinar cells, and also shows that repeated short-term PBDO caused more marked and significant pancreatic injuries compared with the single PBDO group. Moreover, repeated short-term PBDO, where superimposed by the exocrine stimulation, caused the most marked pancreatic injuries, including pancreatic focal acinar cell necrosis and intrapancreatic activation of trypsinogen. Although we have not measured the intraductal pressure after the stimulation of cerulein with pancreaticobiliary duct ligation, this dose of cerulein (0.2 μg/kg.hr) was found to cause a significant increase in both pancreatic juice volume and digestive enzymes (22). Since, in this study, we used a fine metal clip (0.5 mm in diameter) for the

**Table I.** Effect of Short-Term R-PBDO and IDH on Pancreatic Histologic Changes in Rats

| Group[a] | *n* | Pancreatic histologic changes[b] | | | |
|---|---|---|---|---|---|
| | | Interstitial edema | Acinar cell vacuolization | Inflammatory cell infiltration | Acinar cell necrosis |
| R-PBDO + IDH | 9 | ** 3+ (2–3) [2.8 ± 0.1] | ** 3+ (2–3) [2.6 ± 0.2] | **** 1+ (1–2) [1.2 ± 0.1] | **** 1+ (0–1) [1.3 ± 0.2] |
| R-PBDO | 7 | ***, ††† 2+ (1–2) [1.7 ± 0.2] | ***, ††† 2+ (1–2) [1.6 ± 0.2] | 0 (0–1) [0.4 ± 0.2] | 0 (0–1) [0.3 ± 0.2] |
| S-PBDO | 6 | ****, † 1+ (1–2) [1.3 ± 0.2] | † 1+ (0–1) [0.7 ± 0.2] | 0 (0) [0] | 0 (0) [0] |
| CONT | 5 | 0 (0) [0] | 0 (0) [0] | 0 (0) [0] | 0 (0) [0] |

[a] R-PBDO, short-term (2 hr) repeated (twice) PBDO; IDH, cerulein stimulation (0.2 μg/kg·hr for 2 hr); S-PBDO, single 4-hr PBDO; CONT, control laparotomy group.
[b] The values are expressed as the means rounded to the nearest whole numbers. Numbers in parentheses are the ranges of scores; numbers in brackets are means ± SE of scores. ** $P < 0.01$, *** $P < 0.02$, and **** $P < 0.05$ compared with the control group, and † $P < 0.02$ and ††† $P < 0.05$ compared with the R-PBDO + IDH group.

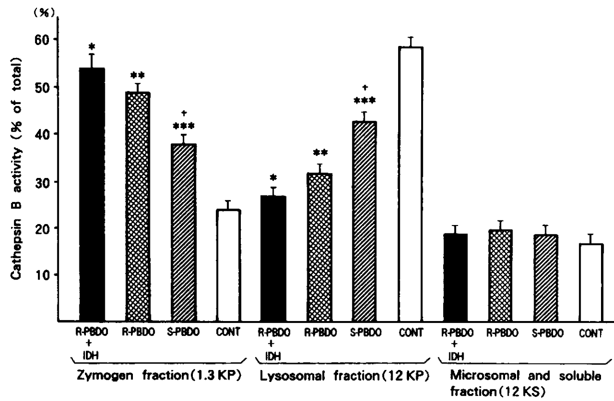


**Figure 5.** Effect of repeated short-term pancreaticobiliary duct obstruction with exocrine stimulation on subcellular distribution of cathepsin B activity in the rat pancreatic acinar cells. Cathepsin B activity in each fraction was expressed as a percentage of the total activity. *$P < 0.001$, **$P < 0.01$, and ***$P < 0.02$ compared with the control group, and $^{+}P < 0.02$ compared with the R-PBDO + IDH group.

ligation of pancreaticobiliary duct and 2-hr ligation with this clip caused no stricture or histologic damages to the pancreaticobiliary duct, removal of the obstructing clip would lead to decompression of the obstructed ductal system.

In both cerulein-induced (3, 4, 6) and diet-induced pancreatitis (1), marked enlargement of zymogen-containing organelles in the cell apex and colocalization of lysosomal hydrolases and digestive enzymes within large cytoplasmic vacuoles have been observed. Although we have not performed immunohistochemical examinations in this study, in both pancreatic duct obstruction (23) and cerulein-induced pancreatitis (24), colocalization of lysosomal hydrolases, such as cathepsin B and D and digestive enzymes such as trypsinogen, has been reported immunohistochemically. In this study, too, subcellular fractionation experiments showed that pancreaticobiliary duct obstruction leads to a redistribution of cathepsin B activity and that, as a result, lysosomal hydrolases become localized in a fraction that is rich in digestive enzymes. The colocalization of these two types of enzymes in our present study is probably the result of a defect in the normal sorting events by which digestive enzymes and lysosomal hydrolases are separated from each other as they pass through the Golgi apparatus (25).

This colocalization could, under appropriate conditions such as exocrine stimulation as in this study, result in the intra-acinar cell activation of potentially dangerous digestive enzymes, since the lysosomal enzyme cathepsin B can activate trypsinogen and trypsin can activate many other digestive enzymes (8, 9). In this study, R-PBDO + IDH induced only a small amount of activation of trypsinogen—about 7% of trypsinogen content.

Another suggestive finding in our present study was that the lysosomal and mitochondrial fragility were accelerated after repeated short-term PBDO compared with single PBDO, although single short-term PBDO also caused a significant increase in the lysosomal and mitochondrial fragility compared with control group. The augumented redistribution of cathepsin B and colocalization of cathepsin B with digestive enzymes in the repeated PBDO animals might have a special clinical importance in the etiology of gallstone pancreatitis. Gallstone attacks are often repeated in a short period. After the first obstruction induced by a gallstone, if the pancreaticobiliary ductal system is obstructed by another stone or another attack and exocrine pancreas is stimulated by food intake, there will be more marked redistribution of lysosomal enzymes and colocalization of lysosomal hydrolases with digestive enzymes in acinar cells compared with a single attack of gallstone. This suggests the more dangerous situation for acinar cells through intracellular activation of trypsinogen or increased subcellular organellar fragility. At present, we cannot explain the mechanism whereby pancreaticobiliary duct obstruction induces the lysosomal and mitochondrial fragility. However, this colocalization phenomenon, and possible intracellular activation of trypsinogen and digestive enzymes, seems to play some role in the pathogenesis of these subcellular organellar fragility.

Although the currently reported studies support

**Table II.** Effect of Short-Term R-PBDO and IDH on Lysosomal and Mitochondrial Fragility in Rats[a]

| Group | n | Cathepsin B leakage from lysosomes (% of the total activity) Incubation time | | | Malate dehydrogenase leakage from mitochondria (% of the total activity) Incubation time | | |
|---|---|---|---|---|---|---|---|
| | | 30 min | 60 min | 90 min | 30 min | 60 min | 90 min |
| R-PBDO + IDH | 9 | 14 ± 2**** | 34 ± 3** | 55 ± 3** | 12 ± 2 | 31 ± 3** | 53 ± 3* |
| R-PBDO | 7 | 11 ± 2 | 27 ± 3*** | 44 ± 3***,††† | 10 ± 2 | 25 ± 2*** | 41 ± 3**,† |
| S-PBDO | 6 | 9 ± 2 | 21 ± 2† | 33 ± 2****,†† | 9 ± 2 | 19 ± 2† | 31 ± 2****,†† |
| CONT | 5 | 7 ± 2 | 16 ± 2 | 28 ± 2 | 7 ± 2 | 16 ± 2 | 25 ± 2 |

[a] For group details, see footnotes to Table I. * $P < 0.001$, ** $P < 0.01$, *** $P < 0.02$, and **** $P < 0.05$ compared with the control group, and † $P < 0.02$, †† $P < 0.01$, and ††† $P < 0.05$ compared with the R-PBDO + IDH group.

the hypothesis that pancreaticobiliary duct obstruction may be important in the pathogenesis of gallstone pancreatitis, it is clear from these as well as other studies that duct obstruction alone is not sufficient to cause the more definite morphologic changes in the pancreas. Clearly, other events must occur, such as intraductal hypertension in this study, or pancreatic ischemia, if the changes induced by duct obstruction are to lead to the injuries seen in more severe pancreatitis. Studies designed to identify and clarify those events are of great importance and the next step to this study, because they are likely to be the ultimate determinants of severity of pancreatitis and to advance our knowledge of the pathogenesis of this disease. Even more important, they may lead to therapeutic advances.

This study was supported by Grant B-03454319 from the Ministry of Education, Science, and Culture of Japan. The authors thank Kimiko Hirano for preparing the manuscript and her excellent assistance.

1. Koike H, Steer ML, Meldolesi J. Pancreatic effects of ethionine: Blockage of exocytosis and appearance of crinophagy and autophagy precede cellular necrosis. Am J Physiol **242:**G297–G307, 1982.
2. Ohshio G, Saluja AK, Leli U, Sengputa A, Steer ML. Esterase inhibitors prevent lysosomal enzyme redistribution in two noninvasive models of experimental pancreatitis. Gastroenterology **96:**853–859, 1989.
3. Watanabe O, Baccino FM, Steer ML, Meldolesi J. Supramaximal caerulein stimulation and ultrastructure of rat pancreatic acinar cell: Early morphological changes during development of experimental pancreatitis. Am J Physiol **246:**G457–G467, 1984.
4. Saluja A, Saito I, Saluja M, Houlihan MJ, Powers RE, Meldolesi J, Steer ML. *In vivo* rat pancreatic acinar cell function during supramaximal stimulation with caerulein. Am J Physiol **249:**G702–G710, 1985.
5. Saluja A, Hashimoto S, Saluja M, Powers RE, Meldolesi J, Steer ML. Subcellular redistribution of lysosomal enzymes during caerulein-induced pancreatitis. Am J Physiol **253:**G508–G516, 1987.
6. Saito I, Hashimoto S, Saluja A, Steer ML, Meldolesi J. Intracellular transport of pancreatic zymogens during caerulein supramaximal stimulation. Am J Physiol **253:**G517–G526, 1987.
7. Steer ML, Meldolesi J. The cell biology of experimental pancreatitis. N Engl J Med **316:**144–150, 1987.
8. Greenbaum LM, Hirshkowitz A. Endogenous cathepsin activation of trypsinogen in extracts of dog pancreas. Proc Soc Exp Biol Med **107:**74–76, 1961.
9. Figarella C, Miszczuk-Jamska B, Barrett AJ. Possible lysosomal activation of pancreatic zymogens. Activation of both human trypsinogen by cathepsin B and spontaneous acid activation of human trypsinogen I. Biol Chem Hoppe-Seyler **369**(suppl):293–298, 1988.
10. Steer ML, Meldolesi J, Figarella C. Pancreatitis. The role of lysosomes. Dig Dis Sci **29:**934–938, 1984.
11. Opie EL. The etiology of acute hemorrhagic pancreatitis. Bull Johns Hopkins Hosp **12:**182–188, 1901.
12. Acosta JL, Ledesma CL. Gallstone migration as a cause of acute pancreatitis. N Engl J Med **290:**484–487, 1974.
13. Armstrong CP, Taylor TV, Jeacock J, Lucas S. The biliary tract in patients with acute gallstone pancreatitis. Br J Surg **72:**551–555, 1985.
14. Frei GJ, Grei VT, Thirlby RC, McClell RN. Biliary pancreatitis. Clinical presentation and surgical management. Am J Surg **151:**170–175, 1986.
15. Tartakoff A, Jamieson JE. Fractionation of guinea pig pancreas. Methods Enzymol **31:**41–59, 1974.
16. DeLisle R, Schulz I, Tyrakowski T, Haase W, Hopfer U. Isolation of stable pancreatic zymogen granules. Am J Physiol **246:**G411–G418, 1984.
17. Irie A, Hunaki K, Bando K, Kawai K. Activation of $\alpha$-amylase in urine. Clin Chim Acta **51:**241–245, 1974.
18. Hummel BC. A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. Can J Biochem **37:**1393–1399, 1959.
19. McDonald JK, Ellis S. On the substrate specificity of cathepsin $B_1$ and $B_2$ including a new fluorogenic substrate for cathepsin $B_1$. Life Sci **17:**126–1276, 1975.
20. Bergmeyer HU. Malate dehydrogenase. In: Bergmeyer HU, Ed. Methods of Enzymatic Analysis. New York: Academic Press, pp485–486, 1971.
21. Labarca C, Paigen K. A simple, rapid and sensitive DNA assay procedure. Anal Biochem **102:**334–352, 1980.
22. Hirano T, Manabe T, Tobe T. Pancreatic lysosomal enzymes secretion via gut-hormone-regulated pathway in rats. Arch Jpn Chir **60:**415–423, 1991.
23. Saluja A, Saluja M, Villa A, Leli U, Rutledge P, Meldolesi J, Steer ML. Pancreatic duct obstruction in rabbit causes digestive zymogen and lysosomal enzyme colocalization. J Clin Invest **84:**1260–1266, 1989.
24. Willemer S, Bialek R, Adler G. Localization of lysosomal and digestive enzymes in cytoplasmic vacuoles in caerulein-pancreatitis. Histochemistry **94:**161–170, 1990.
25. Rosenfeld MG, Kreibich G, Popov D, Kato K, Sabatini DD. Biosynthesis of lysosomal hydrolases; Their synthesis in bound polysomes and the role of co- and post-translational processing in determining their subcellular distribution. J Cell Biol **93:**135–143, 1982.