

# Morphological Changes in the Endocrine and Exocrine Pancreas of Rats after Experimental Obstructive Jaundice

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**ABSTRACT.** Obstructive jaundice causes multiple malfunctions in various organs including the pancreas. To establish how such malfunctions occur, we experimentally induced obstructive jaundice through bile duct ligation (BDL) using rats, measured serum bilirubin, amylase and insulin levels, and examined histological, immunohistochemical and cytological changes in the pancreas at 3 days, 1 week, and 4 weeks after the BDL. Morphometrical analysis was also conducted. Serum amylase levels steeply increased at 3 days, and then decreased at 1 and 4 weeks after the BDL to lower than the control level. In contrast, the number of zymogen granules decreased at 3 days after the BDL, then increased and eventually surpassed the control group at 4 weeks after the BDL. On the other hand, serum insulin levels dramatically decreased at 3 days after the BDL but recovered to a level close to that of the control group at 1 week after the BDL. At 4 weeks after the BDL, however, the serum insulin levels again showed a marked decline. Slight decrease in insulin immunoreactivity and number of insulin granules were observed at 4 weeks after the BDL. Cholecystokinin receptors (CCK-R) were expressed in both acinar and islet cells; their immunoreactivity significantly decreased in the acinar cells at 4 weeks after the BDL. Our results suggest that CCK may play a role in regulating changes in the pancreas after obstructive jaundice.

**KEY WORDS:** amylase, bile duct ligation, insulin, obstructive jaundice, pancreas.

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It has been reported that pancreatic disorders such as pancreatic cancer and pancreatitis can cause liver damage by physical pressing of the common bile duct to lead to the obstructive jaundice [23]. Although it has been reported that obstructive jaundice can cause pancreatic hyperplasia [2, 3], neither the precise morphological changes nor the influence of liver impairment to the pancreas are fully understood. In the present study, we aim to examine changes in secretory activity and histological and cytological features in both the exocrine and endocrine pancreas using rats which were experimentally induced obstructive jaundice.

The question is, although there must be multiple factors involved in pancreatic disorders after obstructive jaundice, what could be the most prominent factor? One possible candidate is cholecystokinin (CCK). CCK is a hormone mainly secreted by the duodenum and the proximal jejunum and plays an important role in the secretion of pancreatic enzymes such as amylase. It has also been reported that rats with obstructive jaundice showed elevated serum concentrations of cholecystokinin (CCK) [20, 25]. Therefore, we also explored a possible mechanism by immunohistochemistry for CCK receptors. Since CCK-A receptor (CCK-AR) and CCK-B receptor (CCK-BR) is mainly distributed in the gastrointestinal system [24] and the nervous system [30, 31], respectively, we focused on CCK-AR in the present study.

## MATERIALS AND METHODS

**Animals:** Nineteen male Wistar rats, 6 weeks of age,

weighing between 170 and 190 g, were purchased from Japan Charles River (Kanagawa, Japan) and used as experimental animals. All the rats were kept in the Kitasato Animal Facility, where the room temperature (RT) was maintained at  $21 \pm 1^\circ\text{C}$ ,  $50 \pm 5\%$  in humidity, in alternating 12 hr cycles of lightness and darkness. The rats were fed rat pellet food and given water *ad libitum*.

**Experimental operations:** The 19 rats were categorized into two groups: an experimental group of 13 rats and a control group of 6 rats. Under deep anesthesia with diethyl ether inhalation, the abdominal cavity was cut open and the common bile duct was tightly ligated with suture. The pancreatic duct was kept intact. The 13 rats of the experimental group were further divided into three subgroups: 4 rats were euthanized at 3 days after the bile duct ligation (BDL) (labeled the 3-day BDL group); 4 rats were euthanized at 1 week post BDL (1-week BDL group); and 5 rats were euthanized at 4 weeks post BDL (4-week BDL group). The 6 rats in the control group were given sham operations as follows: each rat was deeply anesthetized with diethyl ether inhalation and its abdominal cavity was cut open, and the bile duct was touched with a pair of forceps (not ligated). Three rats of the control group were euthanized 1 week after the sham operation, and the remaining 3 were euthanized 4 weeks after the sham operation.

All rats were deprived of food from 6 PM to 9 AM pm on the day prior to euthanasia. All rats were deeply anesthetized with ether at 9 AM on the day of euthanasia. First, the blood was taken (for later analysis) from the jugular vein with a syringe containing heparin. The rats were then euthanized by exsanguination through the abdominal aorta under deep anesthesia. The pancreas was removed from the rats and placed in a fixative for histological and immunohis-

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tochemical observations or in a fixative for transmission electron microscopic observations. All experimental procedures were approved in advance by the Kitasato University Animal Rights Committee.

**Biochemical analysis of the serum:** The blood was centrifuged at 4°C, 6,500 rpm, for 5 min and processed for measurements of serum concentrations of three hormones: amylase, bilirubin and insulin. The amylase and bilirubin concentrations were measured with an Olympus AU 400 clinical chemistry analyzer in the Clinical Examination Laboratory of the Animal Hospital in Kitasato University Veterinary School. The insulin concentration was measured with Eu-labeled competitive binding assay in the High-Tech Research Center of Kitasato University Veterinary School. The results were statistically analyzed using one-way ANOVA followed by *Tukey's post hoc test*. Their averages and standard deviations were also calculated.

**Histological and immunohistochemical procedures:** The pancreases were fixed in a 10% neutral buffered formalin for 24 hr, washed in tap water, dehydrated in an ascending series of ethanol, cleared in xylene, and embedded in paraffin. Paraffin sections, 5  $\mu$ m in thickness, were cut with a microtome. Paraffin sections for histological observations were deparaffinized, stained with hematoxylin and eosin (HE) and observed with a bright field microscope (Olympus, Tokyo, Japan).

Paraffin sections for immunohistochemistry were deparaffinized, incubated in a solution of 99.7% methanol and 0.3% hydrogen peroxide to block endogenous proteinase activity, and then divided into two groups: the sections for the observation of insulin and CCK-AR, and the sections for the observation of glucose transporter 2 (GLUT2). The sections for the observation of insulin and CCK-AR were incubated in 10% normal goat serum in order to block non-specific binding, and then incubated with either an anti-insulin antibody (Zymed Laboratories, Inc., San Francisco, CA, U.S.A.) or an anti-CCK-AR antibody (Everest Biotech Ltd., Oxfordshire, OX25 5HD, U.K.) at 4°C overnight. Then, these sections were rinsed in 10 mM phosphate buffered saline (PBS) (pH 7.4), 3 times, for 5 min each, and further incubated with a secondary antibody (biotinylated goat anti-goat IgG antibody; Nichirei Biosciences Inc., Tokyo, Japan) for 1 hr at RT. These sections were rinsed in PBS again and incubated with peroxidase-labeled Streptavidin (Nichirei Biosciences Inc.) for 40 min at RT. The sections for the observation of GLUT2 were microwaved to retrieve the antigen, incubated with an anti-GLUT2 antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, U.S.A.), rinsed in PBS, and further incubated with "Simple-Stain MAX-PO (R) for Goats" (Nichirei Biosciences Inc.). Between each step, all sections were rinsed with PBS 3 times, for 5 min each. Finally, all sections were incubated in 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Nichirei Biosciences Inc.) and observed with the same bright field light microscope as the histological observations. As control, the primary antibodies were replaced with PBS. All control reactions tested were negative.

**Transmission electron microscopic procedures:** The pancreas was fixed in a solution of 1.5% paraformaldehyde, 5% glutaraldehyde, and 0.1 M phosphate buffer (pH 7.4) (PB) in 4°C, overnight. After rinsing in PB, the tissue was post-fixed in chilled 1% tetra-osmium in PB for 1 hr, dehydrated in an ascending series of ethanol, cleared in N-butyl glycidyl ether (QY-1), and embedded in epoxy resin. Semi-thin sections were stained with toluidine blue, and ultra-thin sections were cut with an Ultracut N (Reichert-Nissei, Vienna, Austria) and examined with a transmission electron microscope (H-7650, Hitachi, Tokyo, Japan).

**Morphometrical analysis:** In the exocrine pancreas, the number of zymogen granules per acinar cell was estimated on the basis of analysis of transmission electron microscopic images from a minimum of 13 sample sections from each group. In addition, the average diameter of the zymogen granules was estimated with an image analyzing software (Motic Image Plus 2.0 S, Shimazu Co., Tokyo, Japan) on transmission electron microscopic images from a minimum of 13 sample sections from each group. In the endocrine pancreas, the number of cells per pancreatic islet was estimated based on analysis of histological images of HE-stained sections from each of the groups. Calculations were based on an analysis of a minimum of 50 islets per group. All of the results were statistically analyzed using one-way ANOVA followed by *Tukey's post hoc test*.

## RESULTS

### 1. Biochemical analysis of the serum

**Serum bilirubin concentration:** Serum bilirubin concentration was  $0.16 \pm 0.02$  mg/dl in the control group,  $6.88 \pm 0.91$  mg/dl in the 3-day BDL group,  $5.31 \pm 3.12$  mg/dl in the 1-week BDL group, and  $2.25 \pm 2.70$  mg/dl in the 4-week BDL group (Fig. 1a). The bilirubin concentration of the 3-day and 1-week BDL groups increased dramatically—more than 30 times higher than that of the control group ( $P < 0.05$  each). Then, the bilirubin concentration decreased in 4-week BDL groups, although it remained at much higher levels than that of the control group. The bilirubin concentration of the 4-week BDL group was significantly lower than that of the 3-day BDL group ( $P < 0.05$ ).

**Serum amylase concentration:** The serum amylase concentration was  $1,720 \pm 15$  IU/l in the control group,  $2,148 \pm 750$  IU/l in the 3-day BDL group,  $1,252 \pm 178$  IU/l in the 1-week BDL group, and  $1,280 \pm 99$  IU/l in the 4-week BDL group (Fig. 1b). The amylase concentration of the 3-day BDL group was higher than that of the control group, although it was not statistically significant. The amylase concentrations of the 1- and 4-week BDL groups were significantly lower than that of the 3-day BDL group ( $P < 0.05$  each).

**Serum insulin concentration:** The serum insulin concentration was  $4.83 \pm 0.50$   $\mu$ U/ml in the control group,  $1.50 \pm 0.21$   $\mu$ U/ml in the 3-day BDL group,  $3.95 \pm 0.38$   $\mu$ U/ml in the 1-week BDL group, and  $2.87 \pm 0.28$   $\mu$ U/ml in the 4-week BDL group (Fig. 1c). The insulin concentration of all

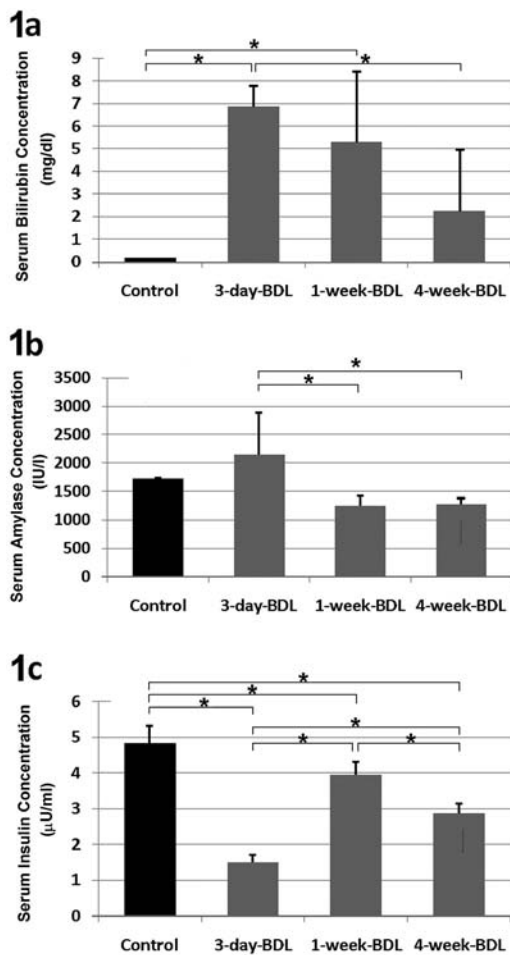


Fig. 1. Serum concentrations of bilirubin (a), amylase (b) and insulin (c) in the control, 3 days after the bile duct ligation (BDL), 1 week after the BDL, and 4 weeks after the BDL. Average  $\pm$  SD. \*  $P < 0.05$ . a. Serum bilirubin concentration is only faint in the control group, but in the 3-day BDL group, it rises dramatically and reaches a level more than 30 times higher than that of the control group ( $P < 0.05$ ). Then, it gradually decreases over time. b. The serum amylase concentration of the 3-day BDL group is higher than that of the control group; although it is not statistically significant. The amylase concentrations of the 1- and 4-week BDL groups are significantly lower than that of the 3-day BDL group ( $P < 0.05$  each). c. The insulin concentration of the 3-day BDL group decreases significantly from the control group and is lower than any other group (all:  $P < 0.05$ ). The serum insulin concentration of all three BDL groups remains lower than that of the control group ( $P < 0.05$ ).

three BDL groups significantly decreased from the control group ( $P < 0.05$  each). The insulin concentration of the 1-week BDL group was significantly higher than that of the 3 day and 4-week BDL groups ( $P < 0.05$  each).

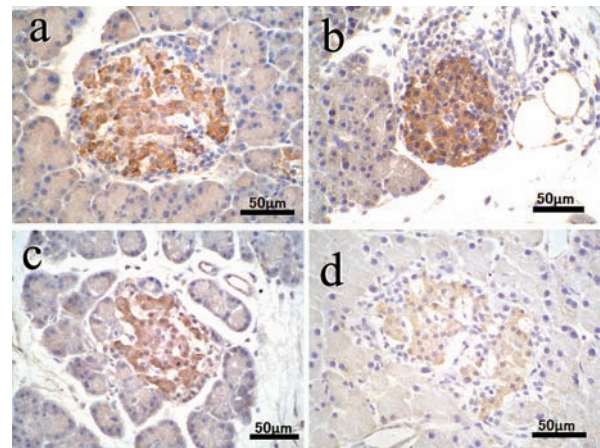


Fig. 2. The pancreas immuno-stained with an antibody against cholecystikinin-type A receptor (CCK-AR). Control (a), 3-day BDL (b), 1-week BDL (c), and 4-week BDL (d). CCK-AR is intensely immunopositive in the pancreatic islet cells and weakly immunopositive in acinar cells in the exocrine pancreas. The immuno-reactivity of CCK-AR in the control, 3-day and 1-week BDL groups are almost the identical (a–c), while that in the 4-week BDL group is much weaker in immunoreactivity than the control group (d).

## 2. The exocrine pancreas

**Histology:** A variety of changes were observed in comparison of the control and BDL groups with respect to the lumen of the acini, intercalated and interlobular ducts, as well as the acinar cells. The lumen of the acini, intercalated and interlobular ducts were expanded greatly in the 3-day BDL group, and slightly in 1-week BDL group, as compared to the control group. The lumen in the 4-week BDL group was unchanged as compared to the control group. On the other hand, the number of zymogen granules in acinar cells in HE stained sections in the 3-day BDL group was noticeably smaller than that of the control group. In the 1-week BDL group, enlargement of the acinar cells was observed in the majority of the regions of the pancreas as compared with the control group. In addition, in the 3-day and 1-week BDL groups, the acinar cells were only weakly stained with eosin. In the 4-week BDL group, there was a conspicuous enlargement of the acinar cells as compared to the control group.

**Immunohistochemistry:** No immunopositive reactions were detected in the exocrine pancreas in the sections immunostained with either anti-insulin antibody (Fig. 5) or anti-GLUT2 antibody (Fig. 6). Weak reactions, however, were observed in the sections incubated with anti-CCK-AR antibody; the immuno-deposits were diffusely distributed in the cytoplasm of acinar cells (Fig. 2).

**Transmission electron microscope:** In the control group, acinar cells had a nucleus at the base of the cell, and had well-developed rough endoplasmic reticulum (rER) in the perikarya and in the basal cytoplasm (Fig. 3a). The acinar cells had many electron dense zymogen granules in the api-

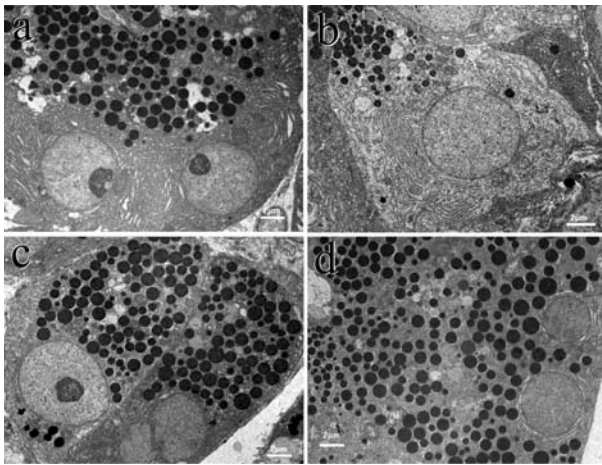


Fig. 3. Transmission electron microscopic sections of acinar cells taken from the control (a), 3-day BDL (b), 1-week BDL (c), and 4-week BDL (d). Many electron dense zymogen granules are located in the apical cytoplasm in the control group (a). The number of zymogen granules dramatically decreases at 3 days after the BDL (b). The zymogen granule number recovers, increases and even reaches the basal cytoplasm at 1 and 4 weeks after the BDL (c, d).

cal cytoplasm. Organelles, such as mitochondria, were widely distributed in the cytoplasm. Lysosomes were also found in the cytoplasm, but in very small numbers. There were  $49 \pm 10$  zymogen granules per TEM section taken from the approximate center of the nucleus of the acinar cell (Fig. 4a).

In the 3-day BDL group, zymogen granules greatly decreased and were found only in the vicinity of the apical luminal surface (Fig. 3b). Decrease in zymogen granules resulted in a smaller cross-section area of acinar cells themselves. Although densities and distributions of mitochondria and rER were the same as in the control group, some of the cisterns of rER were enlarged in the perikarya and basal cytoplasm. Zymogen granules decreased to  $32 \pm 16$  granules per TEM acinar cell section observed (Fig. 4a).

In the 1-week BDL group, the majority of acinar cells had a nucleus at the base of the cell and a minority had a nucleus in the vicinity of the center of the cell (Fig. 3c). The densities and distributions of mitochondria and rER were unchanged; in contrast, the number of lysosomes increased in the basal cytoplasm. The cells underwent atrophic changes, resulting in an increase in the electron density of the cytoplasm and a breakdown of some organelles. Zymogen granules were double of the previous 3-day BDL group— $69 \pm 29$  granules per TEM acinar cell section (Fig. 4a).

In the 4-week BDL, zymogen granules increased and the area where the granules were distributed expanded correspondingly to the point where they were found in the basal cytoplasm (Fig. 3d). The rER were restrictedly localized in the basal cytoplasm. No increase in lysosomes was noted in the basal cytoplasm. The number of zymogen granules

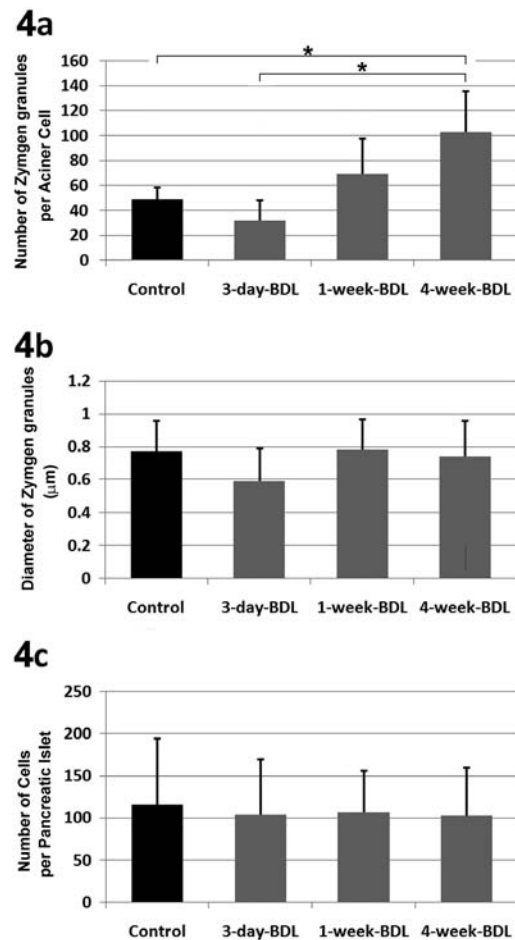


Fig. 4. Morphometrical changes in the number of zymogen granules per TEM section taken from the middle of an acinar cell (a), the diameter of zymogen granules (b), and the number of cells per pancreatic islet (c). Average  $\pm$  SD. Control, 3 days, 1 week and 4 weeks after the BDL. \*  $P < 0.05$ . a. The number of zymogen granules per acinar cell decreases in the 3-day BDL group from the control group, then gradually increases through the 1- and 4-week BDL groups. The zymogen granule numbers in the 4-week BDL group is significantly higher than that of the control and 3-day BDL groups ( $P < 0.05$  each). b. The diameter of zymogen granules show no significant difference among all groups tested. c. The cell numbers per islet are similar to each other in all groups tested, showing no significant differences among all experimental and control groups.

increased significantly compared to those in the control and 3-day BDL groups ( $P < 0.05$  each) and reached  $103 \pm 33$  granules per TEM section of an acinar cell, which was more than double the number in the control group (Fig. 4a). The diameter of the granules in all groups showed no significant difference to each other (Fig. 4b).

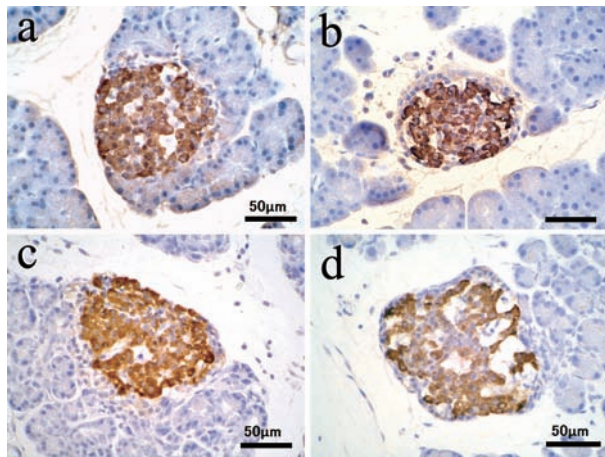


Fig. 5. The pancreas immuno-stained with an antibody against insulin. Control (a), 3-day BDL (b), 1-week BDL (c), and 4-week BDL (d). Dark brown immuno-positive deposits are localized in the pancreatic islet. The intensity of insulin immunoreactions is the same in the control group as it is in the 3-day and 1-week BDL groups (a-c). In the 4-week BDL group, however, the intensity slightly falls (d).

### 3. The endocrine pancreas

**Histology:** The pancreatic islets in the 3-day and 1-week BDL groups were unchanged in histological features and staining. In the 4-week BDL group, however, the intensity of eosin staining was slightly reduced, and the intercellular space widened. The number of cells per islet in HE-stained sections did not show any significant differences among the groups tested:  $116 \pm 78$  cells/islet in the control group,  $104 \pm 66$  in the 3-day BDL,  $107 \pm 49$  in the 1-week BDL, and  $103 \pm 57$  cells/islet in the 4-week BDL group (Fig. 4c).

**Immunohistochemistry:** Insulin was detected in cells located in the central area of the pancreatic islets. The intensity of insulin immunoreactions was the same among the control, 3-day and 1-week BDL groups. In the 4-week BDL group, however, the immunoreactions were slightly reduced in intensity and wider intercellular space was noted (Fig. 5). GLUT2 was localized in islet cells and showed similar immunoreactivity in experimental and control groups (Fig. 6). CCK-AR was intensely immunopositive in the islet cells and weakly positive in acinar cells in all of the groups tested. The degrees of intensity of immuno-reactions of CCK-AR in the 3-day and 1-week BDL groups were similar to those in the control group, while that in the 4-week BDL was noticeably weaker than the other groups (Fig. 2).

**Transmission electron microscope:** In all groups, numerous insulin granules, which were moderately electron-dense, filled the cytoplasm of  $\beta$  cells. Although  $\beta$  cells appeared similar among the control, 3-day and 1-week BDL groups (Fig. 7a-c), the islet cells shrank in the 4-week BDL group displaying many vacuoles and fewer organelles. Wider intercellular spaces and slightly fewer insulin granules were noted (Fig. 7d).

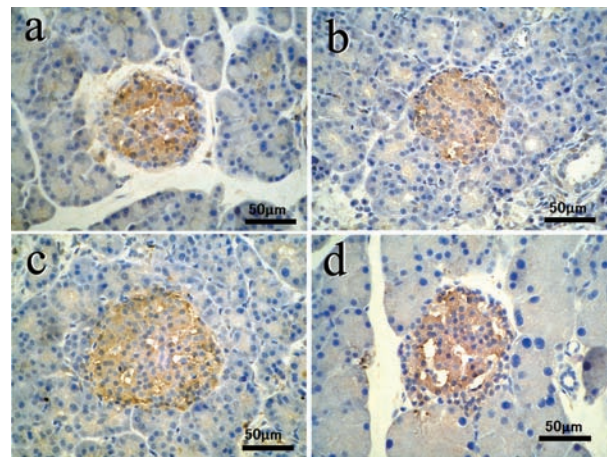


Fig. 6. The pancreas immuno-stained with an antibody against glucose transporter 2 (GLUT2). Control (a), 3-day BDL (b), 1-week BDL (c), and 4-week BDL (d). Dark brown immunopositive deposits are localized in pancreatic islets but not in the acinar cells. All pancreatic islets show similar intensity in the control and all experimental groups.

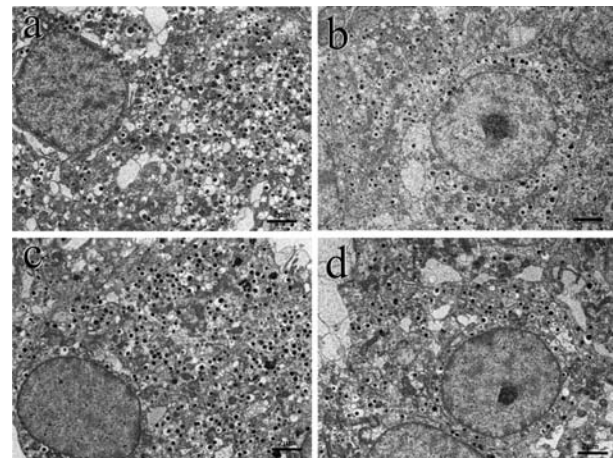


Fig. 7. Transmission electron microscopic sections of beta cells taken from the control (a), 3-day BDL (b), 1-week BDL (c), and 4-week BDL (d). Numerous insulin granules with moderate electron density are located in the cytoplasm in all groups tested. Although islet cells look similar to each other among the control, 3-day and 1-week BDL groups (a-c), the islet cells shrink in the 4-week BDL group with vacuoles and fewer organelles, resulting in wider intercellular spaces and fewer insulin granules (d).

## DISCUSSION

The present study demonstrates that experimentally induced obstructive jaundice causes histological and cytological changes in both the exocrine and endocrine pancreas, as well as changes in serum amylase and insulin concentrations. In order to verify the obstructive jaundice, we measured serum bilirubin concentration and confirmed its elevation to 30 times higher than in control groups after

ligation of the bile duct. This confirmation is in concert with findings of obstructive jaundice causing increase of serum bilirubin levels in other studies [17, 21].

### 1. The exocrine pancreas

Our results revealed post-BDL changes in the serum amylase concentrations in all the experimental groups. At 3 days after the BDL, serum amylase concentrations increased at first, while the number and diameter of zymogen granules in acinar cells greatly decreased. Our electron microscopic observations revealed that zymogen granules were almost depleted and only a few remained in the apical cytoplasm of acinar cells. In contrast, at 1- to 4-weeks after the BDL, serum amylase concentrations decreased (lower than control and 3-day BDL groups), while zymogen granules increased in number, accumulating in the cytoplasm and spreading to the basal cytoplasm. Increased eosinophilic cytoplasm was observed in the HE sections. Since zymogen granules are intensely eosinophilic, the increased staining for eosin in HE sections and accumulation of zymogen granules observed by electron microscopy indicate identical results; i.e., zymogen granules were newly synthesized and accumulated in the cytoplasm.

Our results show an initial increase in amylase after the BDL, but the increase was transit, and it fell below control levels throughout the 1- and 4-week BDL groups. Since secretory activity of the pancreas is reportedly linked to the serum amylase level [5, 9], we assume the transit increase in the serum amylase concentration is related to the transit enhancement of secretory activity in the pancreas. Increased serum amylase levels are seen in pancreatic disorders as well, such as pancreatitis and reflux of pancreatic fluid caused by gallstones [8]. In the present study, we ligated the common bile duct close to the liver but left the pancreatic duct intact, so reflux of pancreatic fluid is unlikely to have taken place.

One of the suggested factors affecting pancreatic secretory activity is CCK [7, 11, 27]. CCK is a hormone synthesized mainly in the duodenum and proximal jejunum and binds to its receptor, CCK-AR, in the digestive system and CCK-BR in the nervous and urinary systems. In the pancreas, CCK-AR is localized on acinar cell membranes [11, 32] and islet cell membranes [18]. When a CCK-AR antagonist is administered, the secretion of pancreatic fluid from zymogen granules is blocked, resulting in restrained serum amylase concentrations [4]. It has been reported that the serum CCK concentration increases when obstructive jaundice occurs [15, 26], so the histological and cytological changes in the pancreas after obstructive jaundice might be connected to high CCK levels. At first, CCK induces the release of amylase. However, persistently high serum CCK concentrations induce a down-regulation of its receptor, CCK-R [20]. High CCKemia is reported to reduce translational efficiency of amylase mRNA [10]. Moreover, high CCKemia is also reported to down-regulate CCK-R [10].

In the present study, lack of bile causes abnormally high concentration of fat shortly after the BDL, which may stim-

ulate CCK release from intestinal epithelium into the blood. The CCK binds to its receptor, CCK-AR, on pancreatic acinar cells and enhances pancreatic fluid secretion from zymogen granules, resulting in depletion of the granules. Our results show decrease in zymogen granules and expansion of the acinar lumen and the pancreatic duct lumen in the 3-day and 1-week BDL groups. Abrupt increase of pancreatic fluid might have caused the expansion of the lumen and pancreatic ducts. Then, at 1 to 4 weeks after the BDL, the granules were re-synthesized and accumulated in the acinar cells. At this stage, the release of zymogen granules did not occur and the newly synthesized granules were accumulated in the cytoplasm. Our immunohistochemical observation using anti-CCK receptor antibody revealed the decreased immunoreactivity in the 4-week BDL group. This result supports the study by Ohlsson *et al.* that persistent high serum CCK may down-regulated its receptor [20]. Our results suggest down-regulation of CCK receptor occurred in the 4-week BDL group. The down-regulation of CCK-AR may no longer induce secretion of zymogen granules, resulting in accumulations of zymogen granules in the acinar cells and a decrease in the serum amylase concentrations. In addition, if CCK directly suppresses synthesis of amylase mRNA as Hara *et al.* suggested [10], it would reduce serum amylase concentrations as well. As expected, our results showed decreased serum amylase levels in the 1- and 4-week BDL groups.

### 2. The endocrine pancreas

The present study demonstrates that experimental obstructive jaundice can cause changes not only in the exocrine pancreas but also in the endocrine pancreas. Serum insulin steeply decreased in the 3-day BDL group compared to the control group, then recovered in the 1- and 4-week BDL groups to levels closer to the control group. Our results in the second phase are consistent with previous research [6], which measured serum insulin concentrations at 4, 7 and 14 days after BDL and reported that the insulin concentration at 4 days after the BDL declined at first, and then progressively increased. Two possible factors can be suggested as affecting the decrease in serum insulin concentrations: (1) Decrease in the number of  $\beta$  cells, or (2) Decrease in the secretory activity of  $\beta$  cells.

In order to verify the first hypothesis, we counted the islet cell numbers in the present study; however, no significant differences in islet cell numbers was found in any group tested. In HE-stained sections, significant degeneration was found neither in the 3-day nor the 1-week BDL groups, but enlarged intercellular space and small vacuolar degeneration were observed in the islet cell cytoplasm in the 4-week BDL group. Immunohistochemical study using anti-insulin antibody as well as electron microscopic observations showed no significant differences in the control, 3-day and 1-week BDL groups but revealed a decrease in insulin granules in the 4-week BDL group. These findings suggest the decrease in serum insulin concentrations might be derived from the decrease in insulin synthesis.

There must be multiple factors affecting the secretory activity of islet cells. One of the potential molecules involved in secretory activities of  $\beta$  cells is the glucose transporter, GLUT2 [28]. Glucose transported by GLUT2 induces synthesis and secretion of insulin [19]. We immunohistochemically investigated GLUT2 reactivity, but found no significant differences among the control and experimental groups. This observation suggests that glucose transported by GLUT2 is not directly responsible for the decrease in insulin secretion after obstructive jaundice. Similarly, glucose transported by GLUT2 is not directly related to the decrease in insulin secretion after acute pancreatitis caused by functional impairments of  $\beta$  cells [1].

Another candidate molecule suggested both *in vivo* and *in vitro* as regulating insulin secretion after jaundice is CCK [13, 29]. CCK binds to its receptor, CCK-AR, in both the exocrine and endocrine pancreas. Kuroda *et al.* (1993) examined canine insulin and glucagon releases in isolated and perfused pancreas tissue and reported that the dogs with jaundice released only a smaller amount of insulin and glucagon in response to CCK than the dogs without jaundice [13]. As in the exocrine pancreas, the down-regulation of CCK-AR may also occur in the endocrine pancreas to result in a reduced response to CCK in insulin secretion by  $\beta$  cells. Our results confirmed the presence of CCK-AR immunoreactivity in the majority of  $\beta$  cells as previously reported [12, 14, 22], and that the immunoreactivity is weaker in the 4-week BDL group than in all the other groups. The results suggest that CCK stimulated the release of insulin from  $\beta$  cells and caused elevated serum insulin levels at 3 days after the BDL. However, our rats were exposed to high CCK for a week or more, and a down-regulation of CCK-AR might have taken place not only in the endocrine pancreas but also in the exocrine pancreas. Morphometric results showed no significant difference in islet cell numbers in all groups tested. As a result, we speculate that the reduced insulin concentrations may be induced by both CCK-AR down-regulation and decreased synthesis of insulin, but not induced by a decrease in either GLUT2 or  $\beta$  cell numbers.

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