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Original Paper

A Collection Method and Its Application to Biliopancreatic Exocretional Experiments on Conscious Rats

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Abstract

A biliopancreatic exocrine apparatus was attached to a conscious rat without housing it in a Bollman type cage. The rat was allowed exercise and free access to solid food and water in an individual stainless steel cage in a room lit automatically from 08:00 to 20:00hr. The rat survived for several days with this apparatus and it was possible to collect biliopancreatic juice at any time. The apparatus was constructed using a biliopancreatic silastic fistula, a gastric cannula, a dorsal cover made from a disposable syringe and tubing available at any physiological laboratory.

To test the viability of the apparatus, the rat's pancreas was indirectly stimulated by introducing various nutrients via the gastric tube. In multiple tests, the same nutrient elicited a consistent response from the pancreas but casein stimulated more strongly than starch.

This system using rats can be useful for several other experiments such as observing the pancreatic exocrine response to other stimulants. Moreover, it is useful due to low cost and because the technique is simple, many rats can be prepared simultaneously.

Introduction

The pancreatic secretion in experimental animals has been measured using the following four methods: 1) using acinar cells[1-5], 2) using pancreatic perfusion techniques[6-8], 3) using pancreatic cannulation under anesthesia[9-13] and, 4) using conscious ani-

mals after recovery from a surgical cannulation[14-24]. Methods 1, 2 and 3 can be used relatively simply in all experimental animals. Method 4 can be used in large animals such as dogs as demonstrated by the Thomas and Scott cannulas[25,26], the Cohen cannula[27] and the Herrera cannula[28]. However, it is difficult in small animals such the rat and guinea pig. Moreover, when using rats, Bollman type restraining cages[29] were needed .

The present study was aimed at devising an easier method for conduct biliopancreatic secretion experiments on conscious rats in a better physiological state. This procedure would be useful in conducting various biliopancreatic secretion tests and would have the additional advantage of being cheaper than using dogs.

Methods and procedures

Surgical procedure (Fig. 1)

Male Sprague-Dawley rats fed *ad libitum* on a standard laboratory solid diet (Oriental Kobo, Tokyo) were used in this study. After a 24 hour fasting period, the rat was anesthetized with intraperitoneal Nembutal (40mg/kg BW). Through a median abdominal incision, a small opening was made in the intraduodenal portion of the common biliopancreatic duct. The biliopancreatic duct was cannulated at this opening. The fistula consisted of a 33cm piece of Silastic Medical Grade tubing (o.d. 1.0mm, i.d. 0.5mm, Dow-Corning) to which a 10mm piece of PE50 tubing (Natsume Seisakusho, Tokyo) and a 5mm piece of Silastic tubing were added at both ends. Three rings (Fig. 1A) were added to secure the joints. The beveled tip of the PE50 piece protruding from one end of the fistula was gently inserted into the biliopancreatic duct and pushed 3 millimeters toward the hepatic hilum. And doubly secured with a silk suture. The PE50 at the other end of the fistula was placed in the duodenum just above the Vater ampulla in order to return secretions to the intestine. One end of the gastric cannula was inserted into the squamous portion of the stomach and secured there with a silk suture. A stopper was placed in the other end of the cannula (Fig. 1A). Before closing the abdominal incision,about ten thousands units of Penicillin G (Meiji Seika, Tokyo) were placed into the abdominal cavity. The central portion of the fistula and the stoppered end of the gastric cannula were brought out through the incised point and passed under the skin to exit dorsally about 5cm from the neck. The two tubes were placed in a newly devised housing (Fig. 1B) to protect then from biting and scratching. The housing consisted of ① a portion of a 10ml disposable syringe (Terumo, Tokyo), ② a 4cm piece of vinyl tube (o.d. 5mm, i.d. 4mm), ③ a wire ring and ④ six holes (Fig. 1B). The biliopancreatic fistula and gastric cannula were pulled 2cm from the dorsal side.While making sure that the fistula with ⑤ a connector of PE50 was gently curved, to prevent kinking,the two tubes were covered with ⑥ a colorless vinyl tape. To ensure that the contents of the fistula could be observed, only one layer of tape was used. The taped tubes were then placed in a ⑦ 1.5cm colorless plastic tube (16F: Sanko Plastic, Osaka). To hold the various components together ⑧ a wire was passed through a hole on one side of the syringe, through the plastic tube and the vinyl tape (avoiding the fistula and the cannula), and then out through the syringe on the opposite side. The wire was then slightly bent on the ends. Finally the wire ring (③) and the six holes (④) in the housing were used to attach the unit to the dorsal skin of the rat with fishing vinyl line (Gamakatsu No4, Osaka). The animals were maintained post-oper-

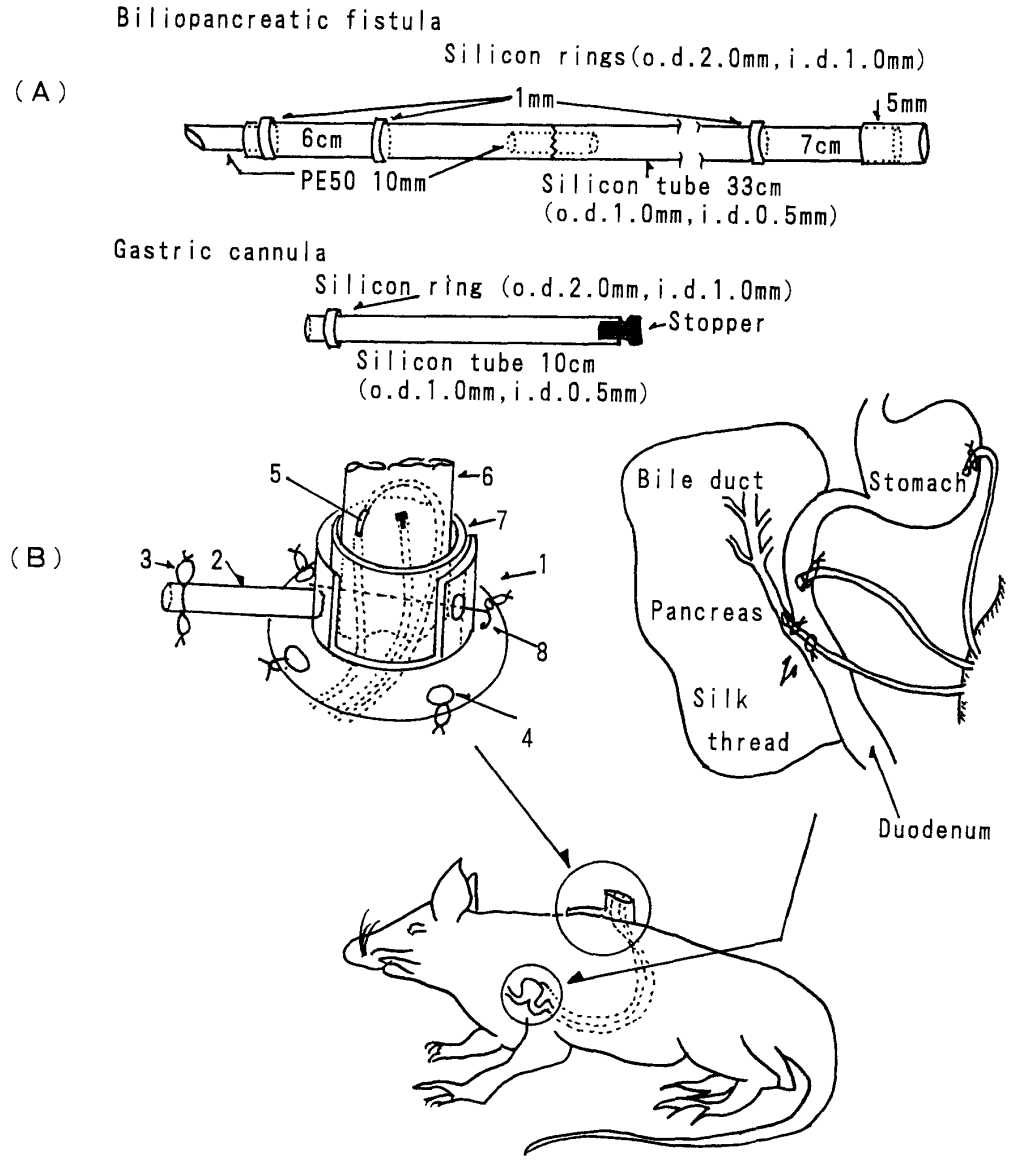

Fig. 1 Schematic representation of the operation method, and both the biliopancreatic and the gastric apparatus.
(A) The biliopancreatic fistula consisted of 33cm Silastic Medical Grade tubing,o.d. 1.0mm, i. d. 0.5mm (Dow-Corning), to which a 10mm piece of PE50 tubing (Natsume Seisakusho, Tokyo) and a 5mm piece of Silastic tubing were added respectively at the both ends, and then three rings were added. The gastric cannula consisted of 10cm Silastic tube the same one as in the fistula with a stopper. The beveled tip of the PE50 piece was gently inserted only 3 millimeters toward the hepatic hilum through the bile duct and doubly secured there with a silk suture. The other end of the fistula was inserted 0.5 millimeters toward jejunum through the duodenum just above the Vater ampulla in order to return the secretions to the intestine. The one end of the gastric cannula was inserted into the squamous portion of the stomach and secured there with a silk suture, and a stopper was attached to the other end of the cannula.
(B) This cover consisted of 1; a cutting of 10ml disposable syringe (Terumo, Tokyo), 2; a 4cm vinyl tube (o. d. 5mm, i.d. 4mm), 3; a stainless steel wire ring and 4; six holes, 5; a 1cm PE50 joiner, 6; colorless vinyl tape, 7; a 1.5cm colorless plastic tube 16F: Sanko Plastic, Osaka), 8; a stainless steel wire. The details are described in the methods.

atively in stainless steel cages and allowed to recover from the anesthesia. These cages allowed the animal to move about (Fig. 2) and to have food and water.

Experimental procedures

Experiment I was done to compare the growth rate of operated and normal rats. Food, but not water, was withheld from 20 rats (B.W. 235±7g) for 24hrs before the operation. The rats were divided into two groups of 10, and surgery was performed on one of the groups, after surgery,which took approximately 30 minutes per rat,the rats, including controls, were fasted an additional 24 hours. The rats were then allowed to eat a standard laboratory solid diet *ad libitum* for 14 days.

Experiment II was done to measure biliopancreatic secretion when nutrients were introduced into the stomach via the gastric cannula. Surgery was performed on 10 rats (B.W. 300±5g) as previously described. Following surgery the rats were fasted for an additional 27 hours. The operate rats were fed *ad libitum* from noon on the first day post operative to 08:00PM on the second day. Then they were fasted until 09:00AM on the third day when the following experiment was carried out. The central connector of the fistula (Fig. 1A-⑤) was opened and a 15cm silicon tube (o.d. 1.0mm, i.d. 0.5mm) was added to make collecting easier. Three basal secretion samples were obtained at 10 minute intervals, collected in tubes placed in ice, and saved for analysis. Then 5ml of 5% nutrient solution was introduced directly into the stomach through the gastric cannula, and 10 biliopancreatic fluid samples were collected at 10 minute intervals. This completed one cycle. The fistula was reconnected and the rats were allowed to eat.This process was repeated six times with each operated rat,

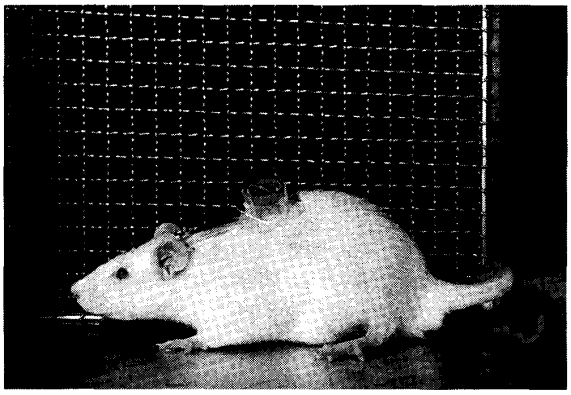

Fig. 2 Photograph of a rat with the biliopancreatic fistula, the gastric cannula and the cover.
The rat was allowed to move freely and to have solid food and water in a stainless steel cage.

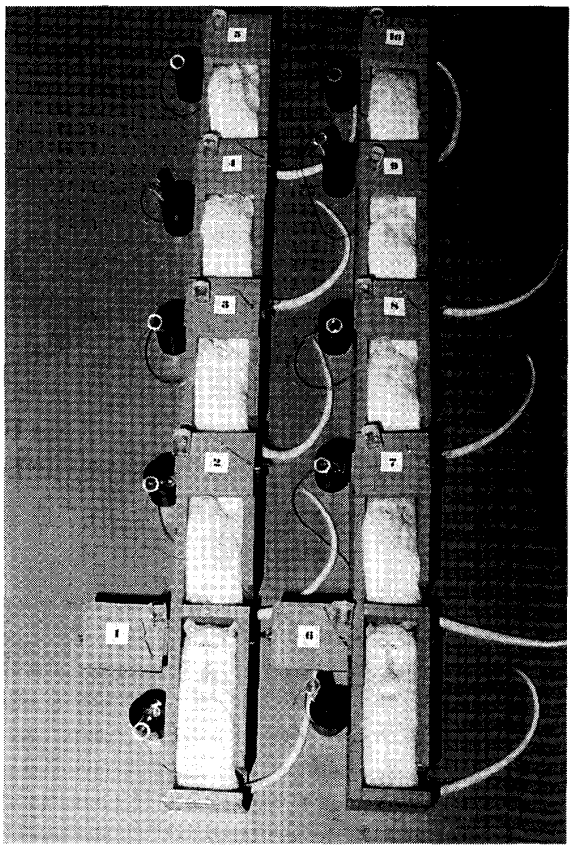

Fig. 3 Photograph of 10 rats in individual boxes when biliopancreatic fluid is collected.
Fluid mixture is able to be collected simply via a 15cm silastic tube (o.d. 1.0mm, i.d.0.5mm) connected with the joiner of the fistula in the cover for several hours.

starch was used in the first three runs and casein was used for the next three.

Assay

The volumes of biliopancreatic juice secreted as well as the protein contents and amylase activities were determined on the samples. The protein content was measured by the Lowry method[30] with bovine serum albumin as the standard. The amylase activity was measured by the Bernfeld method[31], expressed as the amount of maltose produced per minute at 20°C.

Results

Experiment I: Changes of body weight in the surgical and control groups are expressed in Fig. 4. The body weight of both groups

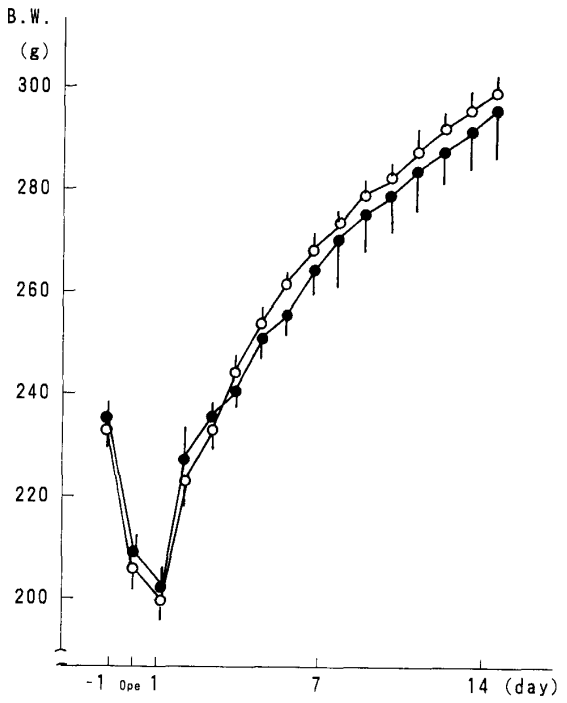

Fig. 4 Correlation of the body weight between the control rats and the operated ones. Rats fed *ad lib* from one day postoperation. ○ normal rat, ● operated rats.

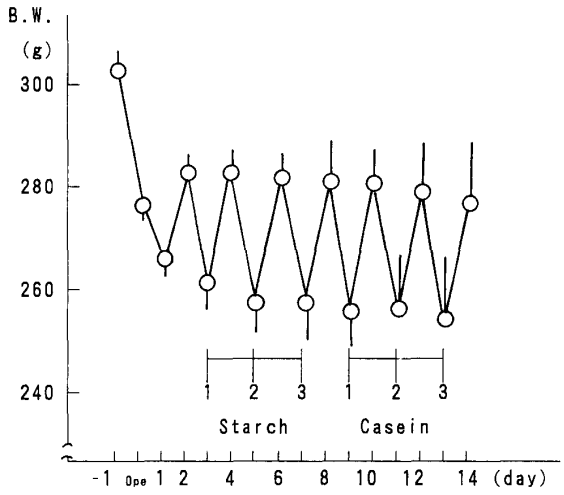

Fig. 6 Changes in body weight accompanied with biliopancreatic collection on conscious rats.
Biliopancreatic juice was collected after the treatment of injection of 5ml Starch solution (5%) via gastric tube on day 3, 5 and 7, or collected after the treatment of injection of 5ml Casein solution (5%) via gastric cannula on day 9, 11 and 13. The details are described in the methods.

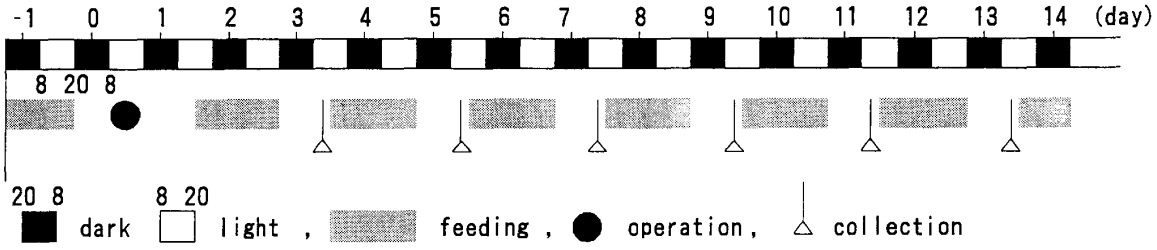

Fig. 5 Schedule of biliopancreatic juice collection on conscious rats. The animal room was lightened automatically from 08:00 to 20:00hr. ■ dark: □ light, ● operation.one study cycle; ▨ fed *ad lib* from 12:00 to 20:00 on the next day. Collection from 09:00 to 11:10. Basal collection of biliopancreatic juice was done for the first three times, and then the collection after the introduction of a stimulant via gastric cannula was done for the next 10 times.

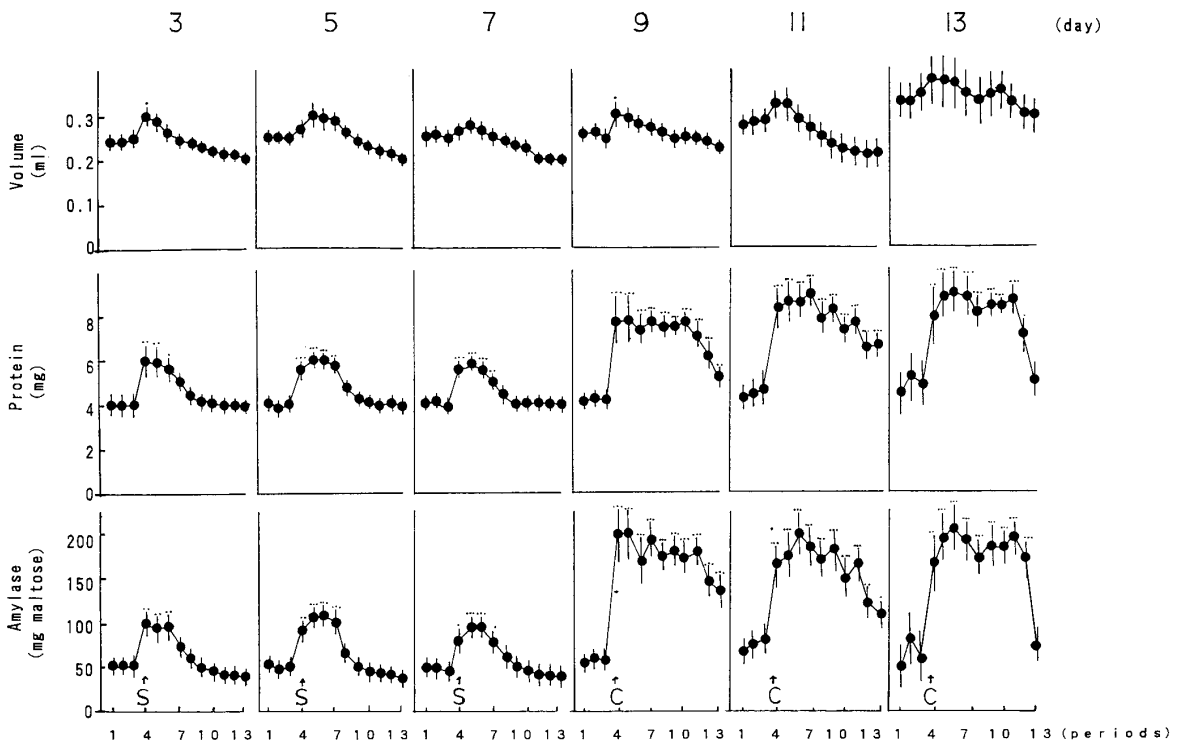

Fig. 7 The values of the volumes, protein contents and the amylase activities of biliopancreatic fluid stimulated by starch or casein administration via the gastric cannula. S; injection of Starch, C; injection of Casein. The details are described in the methods. The values are significantly different from that of basal collections. The deviation levels of 5%(·), 1%(··) and 0.1%(···) are shown in the figure.

decreased one day after surgery since food was withheld. However, the body weight of both groups began to increase on the second day. Food intake was the same in the two groups (data not shown).

Experiment II: As the rats were cycled through the collection process (Fig. 5), the body weight of the rats fluctuated from about 240g to about 270g (Fig. 6). The volume of biliopancreatic juice, the protein contents and the amylase activities were measured as shown in Fig. 7. The protein contents and the amylase activities were higher with casein than with starch, but there was very little increase in the volume of secretion.

Discussion

Biliopancreatic exocretional experiments on conscious rats have been done using a Bollman type restraining cage[16-18]. A rat kept in this cage is considered to be stressed because it cannot move about freely. The procedure used in this study allowed the rat freedom of movement and free access to food and water. In addition, many experimental animals can be prepared easily and quickly. As shown in figure 4, the fistula and the housing did not affect the rats' growth. Moreover, trypsin, chymotrypsin (assayed using specific substrates, BETT and TAME, respectively)[32] and amylase activities in the intestinal contents of operated rats autopsied after 15 days were normal. The intestinal contents were collected by flushing with saline.

Experiment II: Biliopancreatic juice was collected after the rat had been transferred from stainless steel cages to individual boxes

(15×5×6cm) available for 200-300g B.W. rats (Fig. 3). Since it likes darkness,a rat was inactive for several hours in the partially covered box in a lighted room. This means that many collections from the same rat are possible using this practical system.

Although experiment II was done to test the response to the introduction of nutrients into the stomach, it also was possible to observe the response to eating solid food (data not shown). The system is not quite complete. This is because the growth rate and secretion rate deteriorate as time progresses. (Especially compare the basal secretions on the 13th day with previous days in Figs. 4, 6, 7). The reason for this problem was that the fistula became blocked, perhaps by the accumulation of protein and/or bile components of the juice. Therefore, the best data are obtained in the first 10 days after surgery in this procedure. However, this method, using a small animal such as a rat, may serve as a tool for understanding the pancreatic exocrine mechanism.

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