ON THE COLLECTION OF THE ENTIRE EXTERNAL SECRETION OF THE PANCREAS UNDER STERILE CONDITIONS AND THE FATAL EFFECT OF TOTAL LOSS OF PANCREATIC JUICE.

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Though de Graaf first put a cannula into the pancreatic duct and collected pancreatic juice in 1664 (1), our knowledge of this secretion really dates from the epoch-making discoveries of Claude Bernard (1849) (2), who studied in great detail the juice obtained by placing a silver cannula joined to a rubber tube into the large pancreatic duct. He found, and others after him, that such a method yields pancreatic secretion for only a few days, since infection soon leads to the sloughing of the cannula and the rapid closure of the fistula thus left, the secretion either draining through accessory ducts or newly made channels back to the duodenum.

The successful establishment of a more or less permanent pancreatic fistula was performed by Pavlow (3). Bernstein (4) before him had tried to keep an open channel for the flow of pancreatic juice by inserting lead wires into the duct, but this did not succeed. Pavlow transplanted the entire pancreatic duct together with a rhomboidal piece of duodenal mucous membrane surrounding its orifice into the anterior abdominal wall, closing the buttonhole opening in the duodenum with sutures. Heidenhain described a similar method; but he excised the entire circumference of the bowel, including the opening of the duct, anastomosed the cut intestines, and transplanted the duct as Pavlow did.

In either event, the subsequent course of the operation proved troublesome, for the secretion quickly excoriated the edges of the wound and, even with special precautions, frequently led to the digestion of the anterior abdominal wall (5). Though pancreatic juice, as secreted, has little or no proteolytic power, it was soon demonstrated by one of Pavlow's pupils, Schepovalnikow (6), that the inactive trypsinogen was activated by enterokinase, a secretion of the duodenal mucosa surrounding the duct opening. The difficulty was finally obviated by Babkin (7) who excised the offending duodenal tissue. In this way, a tight scar was formed at the opening of the fistula so that, at times when secretion was desired, a metal or glass catheter was introduced and the juice collected, while at other times it was forced to follow other channels back to the duodenum.
Others have modified the technique of Pavlow and his pupils by isolating the pancreatic duct outside the duodenum, ligating, cutting and sewing the distal end into the anterior abdominal wall. It appears to have been first described by Frouin (8) in 1913 and again by Inlow (9) in 1921, but it was really Senn (10) in 1886 who originally performed this operation and collected pancreatic juice in this way. He was able to collect as much as 120 cc. per day but found that the amount secreted quickly diminished and finally ceased.

Still another type of pancreatic fistula has been described by Fodera (11) who placed a T-tube into the pancreatic duct so that the juice could either go on directly to the duodenum or through one arm of the T to the outside. It is similar in principle to the "amphibilous" biliary fistula described by Schiff (12). But it has never been used by others, apparently, and little is known of its merits.

The general objection, however, to all open fistulas for the collection of pancreatic juice is the impossibility of obtaining sterile secretions. Infection leads to marked alterations of the properties of pancreatic juice and moreover travels up into the ducts and brings about inflammatory changes in the gland itself, and frequently in other organs (13). With an open fistula it is difficult or impossible to prevent licking of the secretions by the animal so that one can only guess how much of the juice is returned to the body and how much is lost.

Similar objections apply to the collection of bile through an open biliary fistula. Rous and McMaster (14) in 1923 described an ingenious operation whereby the total bile was collected under sterile conditions for many months. In our studies of the pancreatic juice we have employed this type of intubation, as described in detail below.

**Experimental Procedure.**

Under ether anesthesia and with the most scrupulous aseptic precautions, the duodenum was brought into a right rectus incision and the accessory pancreatic duct located on its dorsal side close to where the common duct ends. This duct was ligated and cut and the dissection continued downward till the large duct was reached.

As pointed out by Hess (15), there may be more than two pancreatic ducts in the dog. If there are, they empty into the duodenum between the two largest ones mentioned above. In dissecting the head of the pancreas away from the duodenum, therefore, we were assured of having cut all of them. During the course of our operations, however, we encountered such additional ducts only a few times, and they were very small.

The large duct was dissected from the surrounding tissue and two small arteries which usually accompany it were tied and cut in order to prevent troublesome bleeding later. The duct was then tied close to the duodenum, from the ventral side, a small incision made and the cannula inserted and tied in place. The duct was cut across between the point of intubation and the duodenum. Omentum was tucked between the head of the pancreas and the gut to insure against reconstitution, as well as to cover the raw areas exposed by the dissection.
The collecting tubes were prepared beforehand and autoclaved twice in small Erlenmeyer flasks. They consisted of two parts, one of soft compressible “hemocytometer” tubing (E and A, Fig. 1), the other of harder and firmer material (C and F, Fig. 1). The former was attached to the cannula and, being soft, conformed more or less readily to the intraabdominal movements without kinking, the latter emerged from the abdomen through a separate opening in the side and, being harder, was not so easily compressed. The two were joined by a curved glass connection. The tubes were placed down into the pelvis before the abdomen was closed, so that they formed a large U before emerging to the outside. The omentum so adheres to this length of tubing that infection does not extend upward and the collecting system may remain uninfected very often for weeks or months in the absence of other complications.

The emerging tube was attached to a T-tube of glass, the other two arms of which were joined to the collecting balloon and the outlet tube all of which had been previously sterilized by autoclaving. All joints were covered with gauze soaked in 5 per cent phenol.

Fig. 1. “Altercursive” intubation of the pancreatic duct. The accessory pancreatic duct (APD) is cut and omentum (O) is tucked between the head of the pancreas (P) and the duodenum (D). Pancreatic juice flows from the main pancreatic duct (PD) through the efferent tube (A–C) into the rubber collecting bag (B) whenever the clamp (R) is closed. When the clamp (S) is closed on the other hand juice flows back through the afferent tube (G–F–E) into the gall bladder (GB) and through the common duct to the duodenum. (See text.)
It was found that the preoperative feeding of the animal was important. All food was withheld for 24 to 48 hours, but, about 5 hours before operation, 200 gm. of finely ground meat was given which acted as a stimulant to pancreatic flow so that when the duct was incised juice poured out profusely thus facilitating intubation and insuring the continuance of secretion afterward without accidental obstruction due to kinking.

Aseptic precautions were maintained particularly during the collection of the juice which was prone to infection by contact or contamination from the air. Collections were made as quickly as possible into sterile containers and the outlet tube carefully covered with several layers of sterile gauze soaked in 5 per cent phenol. The secretion was frequently cultured and the sediment from centrifuged specimens stained for bacteria. When infection occurred, the animal was either killed or used for other special studies.

**EXPERIMENTAL FINDINGS.**

We shall here be concerned with those instances in which the flow of pancreatic juice continued uninterruptedly after operation.

In a good many experiments this did not occur. Frequently, the secretion became infected from contact or contamination from the air during periods of collection. In this event, the flow diminished or even ceased entirely in most cases after the lapse of several days. In other instances obstruction developed due, most commonly, to a kink in the rubber collecting tube, a twist in the bag or to the duct's turning on itself so as to close the opening of the cannula. The findings, when such complications took place, proved of much interest and will be described in subsequent reports.

When obstruction or infection did not occur, the secretion continued to flow profusely and, after 5 to 8 days, quite to our surprise, the animals died with marked asthenia, as described briefly in a previous paper (16). In all, twelve dogs were studied in which this fatal outcome was observed.

It is of interest to note that, according to Babkin (5), Bernard, Pavlow, Heidenhain and others observed hypersecretion followed by death in dogs with an open pancreatic fistula. It was regarded as an exceptional occurrence. In our experiments, where the secretion remained sterile and unobstructed, it was the rule.

By the next day after the operation, which usually consumed about an hour, pancreatic juice was found in the collecting balloon and by the 2nd day, the amount had increased. The dog was healthy, active and eating well. Though the secretion was at first slightly turbid, by the
2nd day it had assumed its normal appearance—colorless, very slightly opalescent and quite odorless. It was alkaline enough to turn phenolphthalein a faint pink and when titrated with acid, bubbles of carbon dioxide were given off. With Töpfer's reagent as an indicator, it would neutralize more than its own volume of $\frac{1}{10}$ HCl, the usual value being $0.11$ to $0.12$ N. It contained a large amount of coagulable protein. Amylase and lipase were present in active form. Casein was very slowly digested unless a drop or two of duodenal extract were added when it was dissolved almost instantly.

For 2 days or 3, the animals thus deprived of the total external secretion of the pancreas appeared normal in every way. But then the appetite rapidly diminished. They ate their daily ration of cooked meat, biscuit and milk but instead of eating heartily in gulps as usual, they took longer and often consumed the meal only after several hours or all day. By the 4th day, food was refused entirely and even milk given by gavage was returned. Large amounts of water were taken but soon it was not tolerated. By the 5th or 6th day vomiting had become marked whether fluid was swallowed or not, in the latter case only mucus and froth appearing. The stools, at first bulky and soft, soon diminished in volume and became scanty and loose, although always well pigmented.

Asthenia was noted early and became a prominent symptom. The animal, though in no distress, became increasingly weak and unsteady, reminding one of the asthenia observed after adrenalectomy. But the temperature was not subnormal and respirations and pulse rate were unchanged. By the 6th to 8th day, the animal died. Convulsions were not observed.

The secretion of pancreatic juice continued up to the very end in amounts dependent somewhat upon the size of the dog but apparently in no relation to the intake of food since the largest amounts were secreted in the later days of the experiment when no food was taken at all (see Table I), and while vomiting was persistent. In Dogs VIII and IX, for example, 475 cc. of secretion were secreted during the 24 hours preceding death.

At autopsy, performed in most instances immediately after death, no gross changes were observed. The peritoneal cavity was everywhere smooth and glistening and the operative wound was healed.
per primam. The long outlet tube was firmly fixed by omentum and no infection anywhere seen. The pancreas seemed somewhat smaller than normal but was soft and pink. The glass cannula was tightly bound to the duct by fibrous adhesions. The liver and biliary system appeared normal. The stomach was small and firmly contracted particularly the pyloric portion. Its mucous membrane was thrown into deep folds, was hyperemic and showed small erosions or ulcerations. Microscopic sections of the pancreas showed small contracted acini surrounded by rather wide clear spaces, in distinct contrast to their plump full appearance in the normal gland. The islets of Langerhans seemed unchanged. The adrenals revealed no abnormality.

Blood changes were observed in many cases and will be reported in detail subsequently. In all a marked dehydration was demonstrable by taking hematocrit readings with the Van Allen tube (17). The proportion of red cells to whole blood often reached 80 per cent, the normal being 40 to 50 per cent. There was a decrease in blood chlorides and an increase in pH. Blood sugar determinations were within

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normal limits.* The urine was repeatedly examined for reducing bodies but none found.

"Altercursive" Intubation of the Pancreatic Duct.

For the permanent study of the total pancreatic juice, it was necessary to provide for the return of the secretion to the duodenum at will so that the fatal effect of its continued loss might be avoided and still its drainage at times of observation be possible. Such an experimental animal can be prepared by using two tubes instead of one, the second tube being used to return the outflowing secretion through a cannula intubated into the duodenal end of the severed duct.

In the case of bile such a method has already been described by McMaster and Elman (18), and successfully used by them in many experimental studies. The same technique was employed for the pancreatic juice. In this way several dogs were prepared and kept alive for several weeks, and in one case for 2 months, by alternately shifting the secretion to the bag and to the duodenum. If, at any time, the juice were allowed to flow off continuously, the same fatal course followed as described above. On the other hand, two dogs, almost moribund from continued loss of pancreatic juice, were quickly restored to normal by merely turning the flow back to the duodenum.

There are difficulties, however, with the type of altercursive intubation mentioned above, as applied to the pancreatic duct. Frequently, the main duct is so short that there is not enough tissue for the insertion of two cannulas. Attempts to use the accessory duct to return the secretion failed because of the small size of this channel. The most serious obstacle, however, was the difficulty in maintaining sterility of the secretion for very long periods of time. Perhaps it was due to the shortness of the duct which allowed intestinal bacteria to penetrate. In the case of the common duct there appears to be a sufficient protection due probably to its length and the effectiveness of the sphincter of Oddi. Thus, McMaster and Elman (18) found that, with an altercursive intubation of the common duct, the bile remained sterile for months.

* These determinations were made by Dr. Ronzoni.
EXTERNAL SECRETION OF PANCREAS

It is likely that the difficulty in the case of pancreatic juice lies in the fact that fecal contamination is more readily possible. We have noted that fecal contents are always present in the stomach of dogs draining the total pancreatic juice and it is therefore easy to see how such infective material could easily ascend the short length of duct and reach the collecting tubes.

Another attempt was made by returning the pancreatic juice through the biliary system (see Fig. 1). That is, the tube by which the secretion is to be carried back to the duodenum was inserted into the gall bladder or into the cystic duct after removal of the gall bladder. Two successful instances were possible, no ill effects apparently following the admixture of bile and sterile pancreatic juice. More experiments must be performed, however.

DISCUSSION.

It has been possible by appropriate intubation of the pancreatic duct, by a method described above, to obtain for study the entire external secretion of the pancreas under sterile conditions. The secretion thus obtained is abundant in amount, slightly opalescent in appearance and without odor. It is decidedly alkaline in reaction and contains a large amount of protein, and active lipolytic and amylolytic ferment. Trypsin is present but in the inactive form so that it exerts little proteolytic action. On the addition of a drop or two of duodenal extract it digests protein rapidly.

The fact that such drainage of the total pancreatic juice uniformly leads, in absence of infection or obstruction, to the death of the animal in 5 to 8 days brings up a number of important questions in the mechanism of the external secretion of the pancreas and concerning its relation to the stomach.

The stimulus to this seemingly intense secretion must be one quite apart from the ingestion of food since it continues long after food is refused. The acid gastric juice immediately suggests itself since it is normally a most powerful stimulant of pancreatic secretion. Indeed, we may imagine the gastric acidity as constantly effective under the conditions of the experiment, even in the absence of food, if we assume that the regurgitation of alkaline pancreatic juice into the stomach normally occurs as a regulator of gastric acidity. The drain-
age of the pancreatic juice to the outside thus gives rise to the unop-
posed acid secretion and a vicious circle is formed quickly leading
to the death of the animal since, by the vomiting, the loss of gastric
juice is added to that of the pancreatic juice.

From the blood findings mentioned above and from the fact that
death occurs on the 6th to 8th day—about the duration of life after
acute pyloric obstruction—a possible analogy between the two con-
ditions may exist. The persistent vomiting in itself may be respon-
sible for these findings since Gamble (19) has shown that loss of fixed
base in the vomitus brings about the alkalosis and lowered blood
chlorides. That the death may be analogous to that following the
establishment of a duodenal fistula seems possible. Hartmann (20)
has shown that the loss of fixed base in this condition leads to the same
blood findings as that following persistent vomiting. The absence
of glycosuria and abnormal blood sugar values would seem to rule out
any involvement of the internal secretion of the pancreas.

The reason for the persistent vomiting may be connected with the
elimination of the regurgitation of the alkaline pancreatic juice into
the stomach, which is claimed to be a normal and from our findings
might be an indispensable phenomenon. We have frequently noted
in our animals that the giving of 0.5 per cent acid solutions into the
stomach after the 3rd or 4th day of drainage would promptly result in
vomiting even though tap water would be tolerated. Boldyreff (21),
in similar experiments noted “the toxic effect of acid solutions in the
stomach” of dogs draining pancreatic juice. The prompt restoration
of a moribund animal to a practically normal condition by the simple
procedure of turning pancreatic juice back into the duodenum sug-
ests the possibility of the presence in the juice of a substance neces-
sary for life.

SUMMARY.

It has been possible by appropriate intubation of the main pan-
creatic duct of the dog to collect the total external secretion of the
pancreas under sterile conditions. When all of the secretion is thus
collected exitus occurs in a characteristic way in about a week with
anorexia, gastric irritability, vomiting and asthenia. The significance
of this finding has been briefly discussed.
A method is also described for the “altercursive” intubation of the pancreas whereby the secretion may be collected or allowed to flow back to the duodenum at will.

To Dr. Evarts A. Graham we wish to express our thanks for his helpful criticisms and advice.

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