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A NON-SURGICAL METHOD FOR COLLECTING PANCREATIC JUICE IN DOGS[1])

By

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Dogs have been used extensively for studies of the pancreatic function. The early experiments by *Claude Bernard* (1850) who collected juice by cannulating the pancreatic duct of dogs are well known. He observed the presence of protein-splitting, starch-splitting, and fat-splitting substances.

Numerous reports have subsequently been published on the composition and volume of pancreatic juice which has been obtained by performing pancreatic duct fistulas in dogs (cf. 6).

It is known that pancreatic disorders such as pancreatitis, fibrosis, and atrophy occur spontaneously in dogs (2, 3, 7, 9). Few methods for clinical diagnosis of such disorders in dogs have been devised, however. The inability of the damaged gland to maintain a normal fat absorption was studied by the vitamin-A-absorption test (3) and by feeding triolein and oleic acid, the acid being tagged with radio-iodine (2). Similarly tagged serum albumin was shown to be uncompletely absorbed in a dog suffering from pancreatic atrophy (2).

In human medicine the examination of pancreatic juice collected by suction via the oesophagus is a valuable aid to clinical diagnosis. The basic work by *Ågren & Lagerlöf* (10) has been followed by several other reports using this technique.

So far this method does not seem to have been successfully applied to dogs.

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MATERIAL AND METHODS

Animals. The animals used in the present study were 26 experimental dogs, and 19 sick dogs which had been admitted to the medical department of the veterinary college, because they suffered from gastrointestinal diseases. The body-weight ranged from 5 to 35 kg. Mongrels as well as pure breeds were used. The ages of the dogs were between 5 months and 13 years. Both sexes are represented.

Technique. Application of tubes. The dog is starved for at least 6 hours. Acepromazine ("Plegicil" ®, Pharmacia), is given intramuscularly as a prenarcotic, 0.3 mg. per kg. body-weight. This is followed about 15 minutes later by thiomebumal sodium (Intraval-natrium ®, May & Baker), 10—20 mg. per kg. body-weight, intravenously. Surgical narcosis is necessary.

Three plastic tubes are used. One (tube A, "Portex 4 E") has an outer diameter of 3 mm. The tip of tube A is provided with a solid metal cylinder (diameter 2.5 mm., length 12 mm.), on which is applied a spherical or oval rubber ball (diameter 6 mm.) with a rough surface (Fig. 1). The tube is perforated by 10—12 holes,

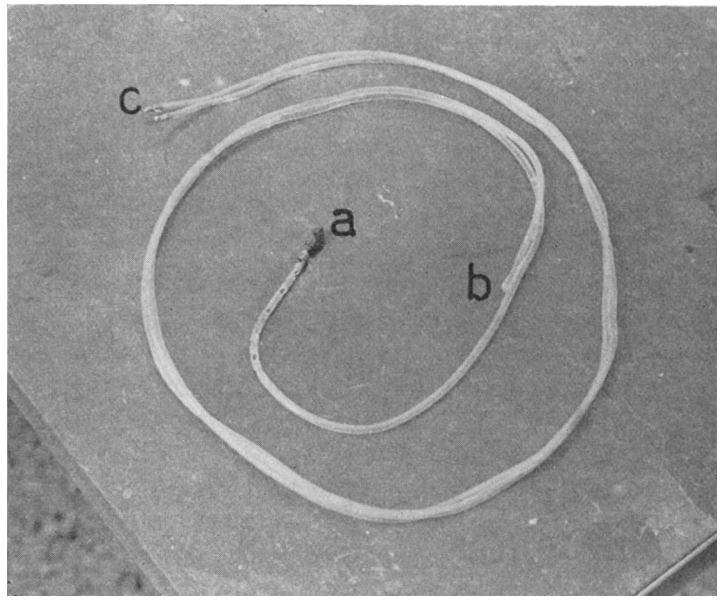

Fig. 1. Double tube (A).
a. rubber ball. — b. opening of sub-tube for evacuation of stomach fluid. — c. metal cylinders for attachment to tube C.

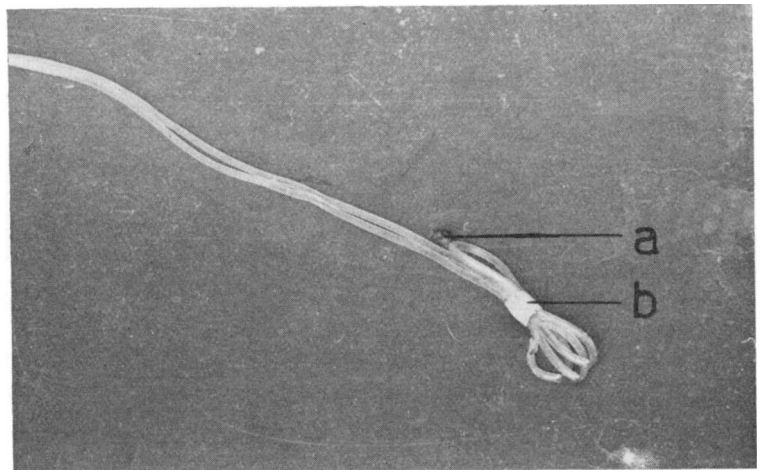

Fig. 2. Coil of tube A.
a. rubber ball. — b. gelatine capsule cuff.

diameter 2 mm., over a distance of 6 cm. proximate to the rubber ball. The length of tube A varies according to the size of the dog, from 100 to 150 cm. During the later course of the experiments a double tube A (MLT — B) was substituted for the single one. It consists of two equal-sized tubes, attached longitudinally to each other with a total breadth of 4.2 mm. At the aboral end the two "sub-tubes" are separated from each other and one is cut off so that roughly two-thirds of the coil are produced by one sub-tube only. When the latter has reached its duodenal position, the opening of the other one is still in the stomach. Through continuous sucking stomach fluid content can thus be evacuated.

Tube A is coiled at its aboral end as shown in Fig. 2. The length of the coil depends upon the size of the dog and varies between 15 and 20 cm. A gelatine capsule 12 mm. in diameter is cut off at both ends providing a cylinder 13 mm. long. This is placed like a cuff on the coil of tube A. It will hold the coil together while it is brought through the oesophagus.

The metal-rubber piece with adjacent parts of the coil of tube A is inserted into the lumen of tube B (length 90 cm., diameter 13 mm.) through its lower (aboral) opening so that the tip of tube B touches the gelatine cylinder (Fig. 3). From the upper (oral) end of tube B a flexible metal mandrin is inserted (Fig. 4). Both tubes are covered with an appropriate lubricant.

The jaws of the dog, which is lying on its left side, are sepa-

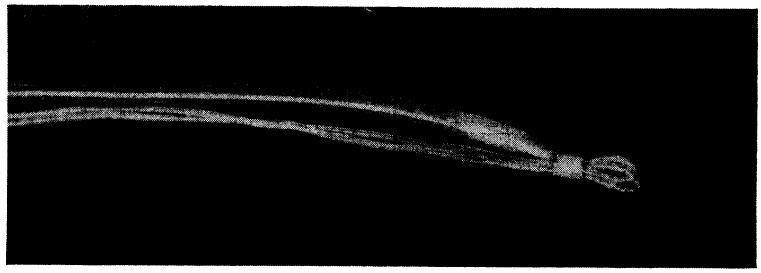

Fig. 3. Coil of tube A inserted into tube B.

rated by a mouth clamp. An assistant pulls the tongue of the dog forwards and to the side, thus making the pharynx visible. The tubes are introduced into the pharynx, conveniently supported by a spatula and passed through the oesophagus well down into the stomach. The coiled part of tube A is pushed out of tube B by means of the mandrin. Tube B is slowly withdrawn and tube A is meanwhile fixed in its position. A third tube (C, 40 cm. long, the same diameter and quality as tube A), the tip of which is formed into a cone by melting and which may be supported by a flexible metal mandrin, is then inserted through the right nostril into the nasal cavity until it becomes visible behind the

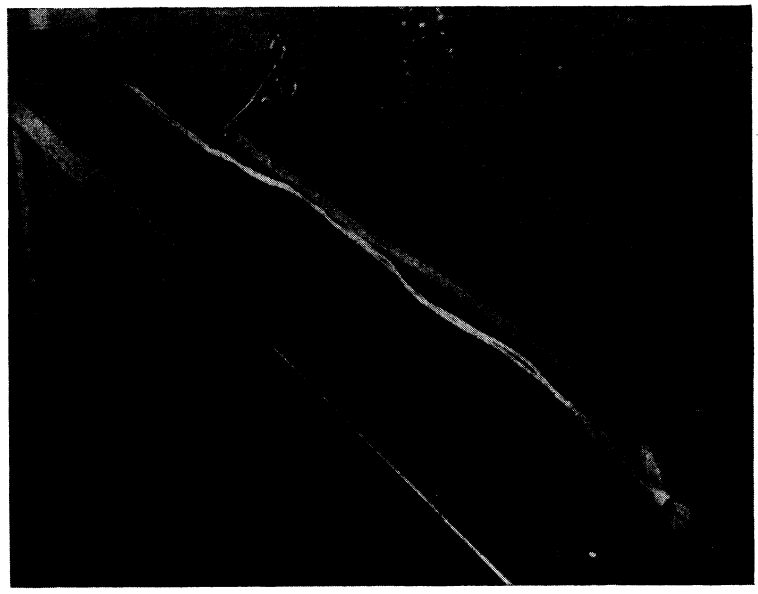

Fig. 4. Tubes A and B (with metal mandrin) ready for insertion.

soft palate. There it is grasped by a clamp and pulled out through the mouth. After the cone has been cut off, the tube end is fixed to the tip of tube A by a small metal cylinder (or two when double tubes are used), 5 mm. in length, which is firmly pushed into the lumen of the adjacent tube end (or tube ends) (Fig. 5). By pulling the nostril end of tube C, tube A is brought out through the nasal cavity. It is then separated from tube C and attached to a supporting copper tube which is formed after the shape of the head, running from the nostril over the nose and forehead. The copper tube is firmly fixed to the head by adhesive tape. The dog is provided with a muzle of metal wire and a funnel-shaped collar to prevent it from removing the tubes (Fig. 6).

The whole operation is completed within 30 minutes and the dog wakes up 20—30 minutes later. It is still under some sedative influence of the acepromazine, however. After a couple of hours it is given free access to water but not to food.

The first X-ray control of the position of the aboral end of tube A is done after 15—20 hours. At this time the tube has reached its final duodenal position in about 50 per cent of the dogs. If the tube is still in the stomach, usually in the vicinity of the pylorus, the next checking is done after a further 6—8

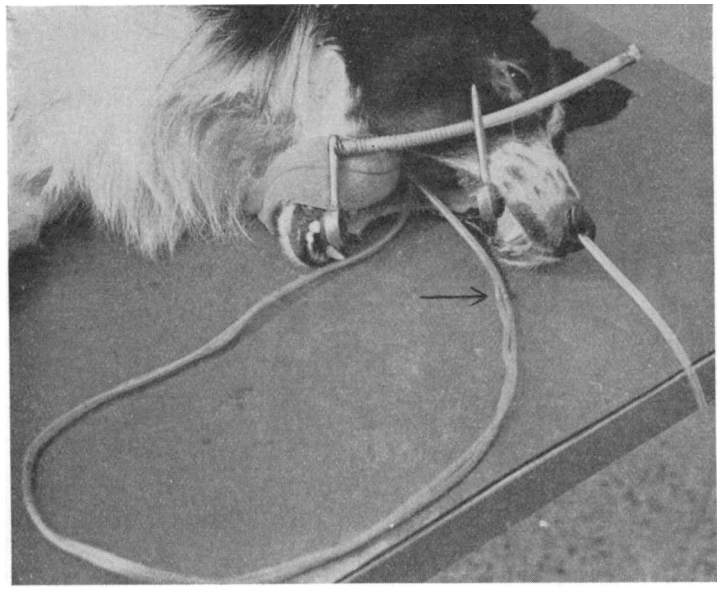

Fig. 5. Tube C is attached to tube A as shown by the arrow.

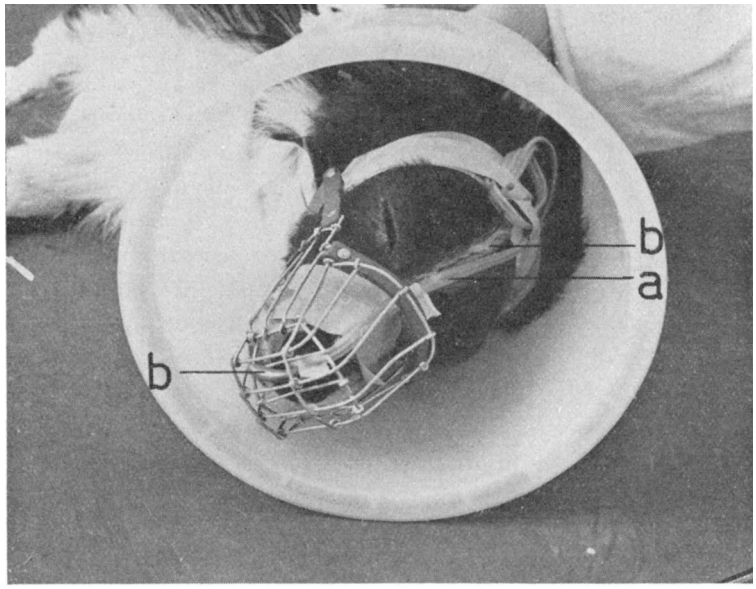

Fig. 6. Tube A in its final position on the head of the dog.
a. tube A. — b. supporting copper tube.

hours. Now the tube is in the desired position in another 25—30 per cent of the dogs.

At these checkings it may be found that the coiled part of tube A has been made too short so that its end cannot reach its proper position although the tube is stretched in its whole length. When this is the case the tube is pushed down sufficiently by manual operation of the free tube at the nostril. The pylorus passage of the tube may also be stimulated be feeding some small pieces of food.

In dogs where the tube has not yet passed through the pylorus, a last checking is done about 40 hours after it was brought into the stomach. By this time the tube may still be in the stomach in roughly 5 per cent of the animals. Owing to the influence of the stomach content the aboral tube end may now have become stiff and hard. It is therefore withdrawn and after a day or two a new tube is inserted.

Collection of duodenal content. The draining system of the pancreas was elaborately studied by *Nielsen & Bishop* (4). In the majority of dogs there are two duct openings in the duodenum. The smaller duct (Wirsung) opens 3—7 cm. from the pyloric

sphincter, usually together with the bile duct on or near the major duodenal papilla. The larger duct (Santorini) opens on the minor duodenal papilla which is situated 8—54 mm. caudal to the major papilla. Consequently the most aboral opening is at a distance of 12—13 cm. from the pylorus.

Suction is done with the tip of the tube situated 12—16 cm. from the pylorus as estimated on the X-ray film. In cases where the end of the tube is found more caudal the tube is carefully withdrawn until the end is in the correct position.

A 20 ml. syringe is applied to the free end of the tube and sucking is started for a first 20-minute period. The vacuum pressure is varied with short intervals in order to evacuate a maximal volume of fluid. It may be useful to inject a small volume of air now and then in order to clear the tube from coarse particles, which may block the entrance openings of the distal end.

The fluid collected over 20 minutes is emptied intermittently into a stoppered flask which is placed in ice water. The duodenal fluid is collected under a layer of liquid paraffin. The pH value of each sub-fraction is checked with indicator paper ("Universal", covering a pH range from 1 to 10). A pH of 6 or lower indicates that stomach content has been emptied into the duodenum and the specimen should be discarded.

Immediately after the first suction period a venous blood sample for determination of serum-amylase is drawn. Through the same cannula secretin ("Vitrum") is injected slowly at an amount of 1 clinical unit per kg. body-weight. This done, suction is again performed for another two 20-minute periods and the fluid volumes are collected separately as described. At the end of the last period a second blood sample is drawn for determination of serum-amylase.

In the majority of dogs about 1.5—4 ml. of duodenal content per kg. body-weight are collected during the three suction periods. Mean value for experimental dogs ($n = 26$) was 2.84 ml. with standard deviation 0.79. The largest volume is obtained during the second period.

Amylase content was determined by the method of *Smith & Roe* (5) and bicarbonate by titration with H_2SO_4 and NaOH (11). Results of the determinations will be published later.

In order to estimate the accuracy of the method a comparison was made with collection of juice through fistulas. Three dogs were operated upon by the method of *Archambeau et al.* (1). Two

fistulas are made. One leads to a duodenal pouch, where the duct of Santorini opens. The duct of Wirsung is ligated. The other fistula leads to the duodenum, which is connected with the pylorus. In the intervals between the experiments the steel tubes in the fistulas are connected by a rubber tube allowing pancreatic juice from the pouch to flow into the duodenum. After healing of the acute operation trauma the dogs were able to eat normal food and seemed to be in perfect health. Juice was collected shortly before the operation, and after the operation, when the dogs had been able to eat normally for 10, 8, and 3 days, respectively. At the second collection, juice was sucked from the duodenal-pouch fistula and from the duodenal fistula simultaneously. In all other respects, the conditions were the same at both experiments. In one dog (B_4) the non-surgical method was also used after the operation. The results are set out in Table 1.

DISCUSSSION

In man it is possible to operate the tube actively via the stomach into the duodenum. I have not been able to do this in dogs. In collecting pancreatic juice in dogs the tube has to be carried passively by the peristaltic movements of the stomach through the pylorus to its proper position. This requires a fairly long time, during which the "unwilling" patient must be prevented from removing the tube or damaging its free end. Therefore the tube must not pass through the mouth cavity, where it might be crushed by the teeth. Fixation of the jaws in an open position by means of a clamp for a day or more makes the dogs feel uneasy and they try in different ways to remove the clamp. Passage through the nasal cavity offers a possibility of bypassing the mouth. Dogs will accept a partial blocking of one nostril without objections. It is, however, necessary to have the free part of the tube in close contact with the nose and forehead. Otherwise the dog will easily scratch it off.

The use of a single tube without continuous evacuation of the stomach, obviously raises the question to what extent stomach content is admixed to the duodenal fluid that is collected. Regular checking of the pH-value of each sub-fraction seems to be a fairly reliable guarantee against this possibility. Discarding of subfractions because of a low pH was not necessary more often than in 1 dog of 4 or 5. It is important to prevent the dog from smelling

Table 1. Volume (ml.) and amylase content (units) of juice collected by non-surgical and surgical methods in 3 dogs, B_3, B_4 and B_5. The operation was made on day 0. I, II, and III are the three 20-minute periods of collections. The double tube was used.

	Non-surgical method				Surgical method								Non-surgical method in operated dogs			
					Duodenal pouch fistula				Duodenal fistula							
Day	— 1				+ 13											
	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total
Volume	6	12	10	28	5	13	6	24	< 1	< 1	< 1	< 3				
Amylase	4240	12300	8112	24650	6250	9300	5360	20910	≃ 0.1	≃ 0.2	≃ 0.1	≃ 0.4				
B_3 ♂ 13 kg.																
Day	— 17				+ 17								+ 18			
Volume	25	10	0	35	13	20	8	41	2	< 1	< 1	< 4	10	22	10	42
Amylase	23700	8200	0	31900	14200	20200	12550	46950	106	< 8	< 14	≃ 128	10550	17900	4670	33120
B_4 ♂ 15 kg.																
Day	— 2				+ 7											
Volume	5	18	2	25	1.5	14	7	22.5	3	1	< 1	< 5				
Amylase	9400	10000	1100	20500	2620	12400	10300	25320	12	4	< 2	≃ 18				
B_5 ♀ 15 kg.																

food of any kind, seeing food, or licking blood spots, etc., because this will initiate production of gastric juice.

Admixture of stomach content also stimulates the pancreas to increased activity and therefore interferes with the standardized procedure of secretin administration. A low pH also inactivates the amylase activity and liberates bicarbonate from the specimen. The use of a double tube with continuous evacuation of the stomach is therefore preferable. In the early course of the experiments suitable double tubes were not available. A single tube, when used with the precautions described, and a double tube will give values of amylase activity, bicarbonate amount, and volume of juice, which are well comparable.

In small dogs, i. e. roughly those weighing less than 8 kg. the insertion of the tube was difficult because of their narrow nasal lumina.

In 2 dogs it was not possible to bring the tube into its proper duodenal position. Despite several attempts the tube did not pass through the pylorus. These dogs were of middle-size and no anatomical abnormalities of their digestive tracts could be established clinically. The explanation of the failure is therefore so far unknown.

The results gained in dogs that were operated upon show that there is good agreement between values obtained by the non-surgical and the surgical methods. The quantity of juice obtained over the three suction periods before the operation, and, in dog B_4 via the stomach-duodenum tube after the operation as well, compares well with the quantity collected via the duodenal-pouch fistula. This indicates that the suction in non-operated dogs fairly completely emptied the part of the duodenum where the tube was situated. The volumes obtained through the duodenal fistula is of a low order of magnitude. This fluid can be expected to represent stomach juice which may "leak" into the duodenum in spite of the evacuation of the stomach, juice produced by the duodenal glands, and bile. The volume amounts to 11, 9, and 18 per cent, respectively, of the total quantity collected through both fistulas. The question also arises whether the duct of Wirsung was successfully ligated during the operation. The very low amylase content of the fluid collected through the duodenal fistula indicates that the ligation was adequate.

No special effort was made to keep the operated dogs alive for a long time. Two of them died suddenly, B_4 and B_5 25 days

after the operation. The cause of death was in both cases peritonitis, probably due to leakage between the intestinal wall and the cannula. A functioning duct of Wirsung could not be established at postportem examination. Dog B_3 is still alive 135 days after the operation and is in good health.

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SUMMARY

A non-surgical method for obtaining pancreatic juice in dogs is described. A tube, preferably double for simultaneous suction of duodenal and stomach fluid, is inserted into the stomach. The upper part passes through the nasal cavity and one of the nostrils. The most aboral end is passively carried into the duodenum. The proper position of

this end is checked by X-ray. Suction is performed under standardized conditions without and with the use of secretin stimulation. The values obtained with this method and those obtained by collection of juice via fistulas in 3 operated dogs are in fairly good agreement.

ZUSAMMENFASSUNG

Eine nicht-chirurgische Methode für Einsammlung von Pankreassaft in Hunden.

Es wird eine Sonde, am besten eine doppelte für gleichzeitige Aufsaugung von Duodenal- und Mageninhalt, in den Magen hineingeführt. Ihr oberer Teil geht durch die Nasenhöhle und einer der Nasenöffnungen. Das aborale Ende wird passiv zum Duodenum gebracht und ihre Lage wird mit Röntgen kontrolliert. Die Aufsaugung geschiet unter standardizierten Verhältnissen ohne oder mit Anwendung von Sekretinstimulation. In drei Hunden, wo Fisteln angelegt wurden, gab die chirurgische und die nicht chirurgische Methode gut übereinstimmende Resultate.

SAMMANFATTNING

En icke-kirurgisk metod för samling av pankreassaft hos hundar.

En sond, helst dubbel för samtidig uppsugning av duodenal- och ventrikelinnehåll, nedföres i ventrikeln. Dess övre del passerar näshålan och en av näsöppningarna. Den aborala ändan föres passivt till duodenum och dess position kontrolleras med röntgen.

Uppsugning sker under standardiserade förhållanden utan resp. med användning av sekretinstimulering. Hos 3 hundar på vilka fistlar anlades gav den kirurgiska och den icke-kirurgiska metoden väl överensstämmande resultat.

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