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Dogs with a Gastric Fistula

A Simple but Advanced Model for Simultaneous Recording of Secretion, Motility, Blood Flow and Output of Hormones

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Introduction

Parameters of gastroduodenal function (motility, secretion of acid and pepsin, output of peptides, blood flow) can be measured both *in vitro* and *in vivo*. Contradictory results may be obtained with experiments *in vitro* and *in vivo*. As an example; β-adrenoceptor agonists induced inhibition of castric acid secretion *in vivo* (*Bech* 1989) and stimulation *in vitro* (*Canfield & Price* 1984). The different functions are interdependent in a most complex way and susceptible to influence from external sources (*Schurizek* 1989) in an unpredictable way. An overview of the functions, therefore, can only be obtained by measuring the parameters simultaneously in the same *in vivo* model.

In 1980 we established a model which made it possible to perform simultaneous measurements of gastric secretion, blood flow, antro-duodenal motility and selective gastric output of hormones in conscious dogs. The purpose of this report is to describe this model with subsequent developments and the difficulties encountered during its use.

Figure 1. Dog with gastric fistula equipped with a collecting bottle. In addition, a leg vein and the portal vein have been cannulated.

Materials and methods

Dogs (Beagle) with a gastric fistula and a catheter in the Portal vein were used (Fig. 1). The dogs were habituated to the laboratory conditions. Surgery and the subsequent experimentation were carried out at the age of 8–12 months. Laparotomy was done under general anaesthesia whereas the experiments were performed without any sedation or anaesthesia. The dogs were fed with Science Diet, Canine Maintenance® (Hills). They were fasted 12 hours prior to operation and experiments but allowed free access to water.

Laparotomy

The dogs were premedicated with acepromazin (Plegicil®, Pharmacia) 1 mg/kg i.m. A foreleg vein was cannulated for infusion of saline and induction of sedation with pentobarbital (Mebumal®, SAD) 25 mg/kg. After oro-tracheal intubation anaesthesia was maintained with Halothane (Halocarbon Laboratories) and Enfluran (Efrane®, Abbot) administered with a Komesaroff anaesthetic machine. Spontaneous respiration and electrocardiogram were recorded. Analgesia was induced per- and postoperatively by Buprenorphine (Temgesic®, Reckitt & Colman) 0.3 mg i.m.

Two inches to the left of the spine on the posterior part of the body a dorsal subcutaneous pocket was prepared for the reservoir body of the catheter (Vascular-Access-Port®, Norfolk Medical Products), which was fixed with a 2-0 absorbable suture. The catheter was cannulated and was passed subcutaneously and anteriorly into the abdominal cavity via a ventral midline laparotomy. The splenic vein was cannulated by the catheter and ligated peripherally. The spleen was removed without damaging the gastric innervation. In some of the dogs the left gastric artery was also cannulated (*Johansen & Bech* 1991). A cannula (*Thomas* – see later) was implanted at the major curvature of the stomach near the delimination between corpus and antrum (Fig. 2). Three cm

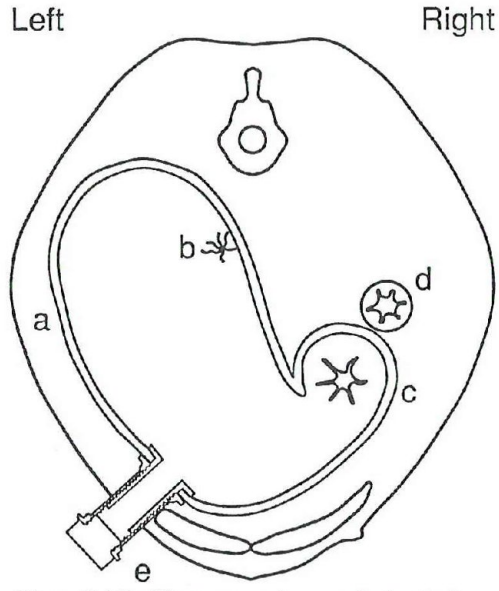

Figure 2. The Thomas gastric cannula in situ in a dog (posterior aspect). Greater curvature of the stomach (a), cardia (b), pylorus (c), duodenum (d) and fistula (e).

of the greater curvature was cleared for the small branches of the left gastroepiploic artery. A string purse suture was made around a 2.5 cm long incision in the major curvature. The wall of the stomach was placed between the fixed flange and the adjustable ring of the cannula. The string purse suture was tightened up and the wall of the stomach fixed with 3-0 suture (Dexon®) to the holes in the adjustable ring. An oval incision was made in the skin over the lateral part of the left rectus muscle near the sternal curvature, and the left cranial abdominal nipple was removed. A cross shaped incision was made in the external rectal fascia. The rectal muscle and internal fascia was penetrated bluntly. The wound was dilated until the diameter of the opening was equivalent to the diameter of the cannula. The canula was pulled through the abdominal wall from inside.

The muscle incision was closed with 2-0 suture (Dexon®), the skin with 3-0 suture

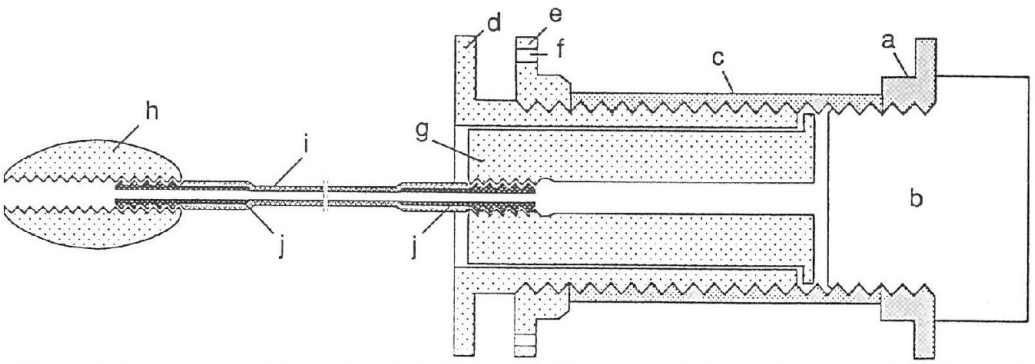

Figure 3. The catheter guide introduced via the modified Thomas gastric cannula. A ring of stainless steel (a) protects the plastic plug (b) against dog bite. A tube of stainless steel (c) penetrates the abdominal wall. The wall of the stomach is located between the fixed flange (d) and the adjustable ring (e) both made of polyvenylchloride. Eight holes (f) used for fixation of the stomach. The catheter guide consists of a fixed part made of polycarbonate (g), a mobile polyvinylchloride pearl (h) and a 14 cm long silicone tube (i). The two tube fittings (j) are made of stainless steel. The lumen of the catheter guide is 2 mm and is sufficient for the passage of a guide wire.

(Vicryl®) and the cannula was kept tight to the abdominal wall for a week with a bandage. Metronidazol (SAD) 500 mg i.v. and Ampicillin (Anhypen®, Gist-Brocades) 1 g i.v. was given twice a day for two days starting preoperatively.

The cannula

A modified Thomas cannula (*Thomas* 1941) was made of acidproof stainless steel and polyvinylchloride (Fig. 3). The outer opening was closed with a plastic plug and protected against dog bite with a ring of stainless steel. The cannula could be joined to a plastic bottle for sampling of gastric secretion, and to a device for introduction of an intraluminal catheter for monitoring the motility in the antrum and duodenum.

Portal vein catheter

To maintain patency, the catheter was flushed daily with 10 ml heparin 50 IE/ml and locked with 1 ml heparin 5000 IE/ml. Saline with heparin 25–50 IE/ml was infused at a rate of 0.25–0.5 ml/min in the catheter during experiments. It was required that aspiration of blood for sampling could take place unimpeded at any time and any interval. Flushing with saline could often open a catheter in case impeded aspiration. Fibrinolytics, Urokinase 5.500 IU (Leo) or Streptokinase 100.000 IU (Streptase®, Behring) were infused i.v. in case of severe difficulties with aspiration from the catheter. The use of the catheter was abandoned if fibrinolytics and flushing were unsuccessful. A similar technique was used with the catheters placed intraarterially.

Manometry

A catheter guide (Fig. 3), consisting of a fixed part located in the cannula and a mobile plastic pearl, was developed and used in order to direct the manometry catheter (S-5-5-5-5-5 Mui Scientific) into the duodenum. The oval plastic pearl with a diameter of 12 mm was placed in the stomach and propelled through the pylorus into the duodenum by the inherent peristalsis. When correctly positioned, the plastic pearl of the catheter guide was located on the right side of the spine with the thread pointing to the rear (Fig. 4). The pearl makes it possible to introduce a guide wire followed by the manometry catheter into duodenum. The installation process was monitored by fluoroscopy.

The catheter was perfused by water through

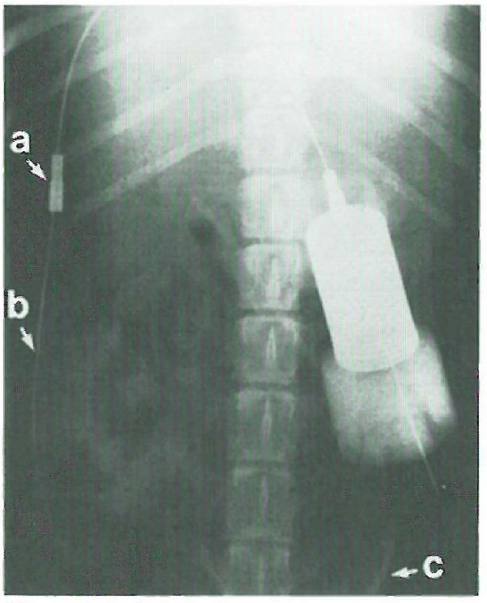

Figure 4. Catheter guide with a correctly positioned and directed plastic pearl in the duodenum (a) allows the passage of a guide wire (b). Portal vein catheter (c).

a pneumo-hydraulic system with the pressure transducers located outside the dog. The analogous signals from the transducers were amplified and converted to a digital signal (12-bit) by an A/D-converter Micronix Z80-403 (Microtecnic). The signals from eight transducers (2 dogs) were sampled at 10 Hz and read into an IBM compatible personal computer (Unisys PW^2 300/10 Model 3). The data were immediately visualized on the screen, collected in a buffer and then stored on the hard-disc. Analyses of the number of contractions, duration, amplitude as well as the area under the curve for each contraction were later performed.

Acid secretion

The secretion volume was measured to the nearest 0.5 ml and a 1 ml aliquot of gastric juice was titrated to pH 7.0 with 0.1 mmol/l NaOH by an autotitrator (TTA81 Autopipetting station, Radiometer). Acid secretion was expressed as mmol H^+/min.

Pepsin secretion

The pepsin content was determined immediately after the experiments by means of the hemoglobin substrate method (6). 0.1 ml of the gastric juice was diluted to 5 ml with 0.01 N HCl, 0,5 ml of this solution was mixed with a hemoglobin solution (2.0 ml haemoglobin 1.55 mmol/l and 0.5 ml 0.3 N HCl). The reaction was performed in a water bath (25° C) and was stopped after exactly 10 min with 5 ml 0.3 N Trichloroacetic acid (Sigma). The content was filtered and analyzed at 280 nm on a Beckman spectrophotometer. Crystalline pepsin from pig stomach (Koch-Light Ltd) with a known activity was used to produce a standard curve. The pepsin secretion was expressed as units $\times 10^3$/min.

Peptides/hormones

Various peptides and hormones could be measured in samples of gastric and duodenal juice, portal and peripheral venous blood. Handling technique depended on the method of analysis.

Mucosal blood flow

Neutral red clearance was used to determine relative changes in gastric mucosal blood flow (*McIssac et al.* 1980, *Hovendal* 1985). Neutral red was infused intravenously (forelimb) 3.5 mg as bolus and 0.6 mg/kg/h as constant infusion. Peripheral venous blood samples were withdrawn from the other forelimb. Neutral red was extracted from samples with gastric juice (1.0 ml) and peripheral blood (3 ml) with diethylether (10 ml). The neutral red could then be extracted from the ether with 1 ml 0.1 mol/l HCl and measured with a Beckman spectrophotometer at 540 nm. The clearance of neutral red from gastric mucosal blood to gastric juice in ml/min is: (Gastric juice/Blood concentration of neutral red) × (Volume of gastric juice/Sampling time). The relation between gastric mucosal blood flow and gastric acid or pepsin secretion was expressed as a ratio.

Pitfalls

The model has been used over a period of 12 years. A few dogs failed to thrive in the setup but autopsy did not reveal the cause. Histological examination of stomach and duodenum did not reveal any signs of ulcer or tumor.

The cannula

It was important to implant the cannula at the lowest possible location of the stomach for obtaining optimal sampling ratio of gastric secretion (Fig. 1). The cannula caused local inflammation and fibrosis, which caused the stomach to adhere to the abdominal wall.

The plug should be tight-fitting in the cannula between experiments as an open fistula caused dehydration. In addition the cannula could withdraw into the stomach, which could be overcomed by sedation of the dog, retrieving and replacing the cannula.

Portal vein catheter

The catheters were usable for unimpeded aspiration from a few days until 4 1/2 months. Both Urokinase and Streptase were able to reopen a blocked catheter for aspiration. Repeated injection of Urokinase took place without problems. Two cases of repeated injection of Streptase resulted in anaphylaxia which was counteracted by an i.v. injection of epinephrine.

Manometry

Sometimes the silicone tube passed the pylorus before the plastic pearl, which could be seen at fluoroscopy. In this case it was impossible, because of kinking, to lead the guide wire through the silicone tube (Fig. 5). A short firm pull in the silicone tube could often reverse the pearl to the correct direction in the duodenum and allow installation of the manometry catheter. If the reversion of the pearl was too difficult or associated with discomfort for the dog, the silicone tube was cut and allowed to pass with the stools. Silicone is a very suitable material for the catheter guide tube as it is resistant to the harsh environment of the stomach, whereas other materials became uncomplicant and broke after a few days.

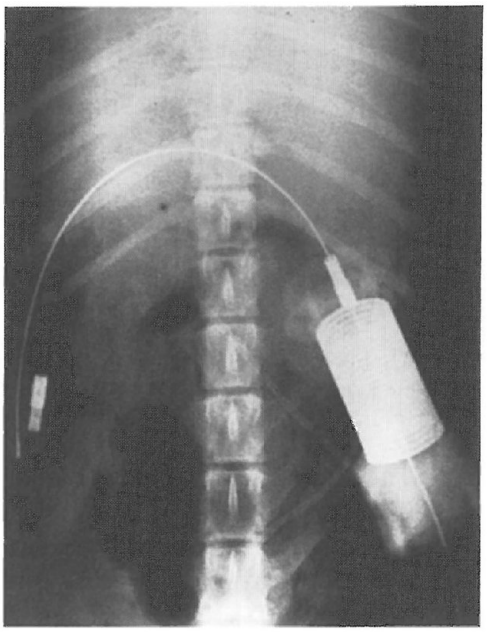

Figure 5. Catheter guide with a correctly positioned, but wrongly directed plastic pearl in duodenum. Kinking hinders the passage of the guide wire.

Precipitation of air in transducers or catheter resulted in an unacceptable damping. This could be minimized by a pneumo/hydraulic perfusion system with air separated from the water by a diaphragm. Flushing the transducers with detergent before connection to the catheters facilitate the removal of air bubbles.

All the perfused water flowing through the antral transducer and a unknown part from the pyloric transducer will mix up with the gastric secretion. The dilution will change the acidity of the secretion but not the output of acid or pepsin. Simultaneous registration of gastric secretion and motility requires a micro-tip strain gauge transducer system for the recording of motility.

Acid secretion

Acid secretion was analyzed immediately after the experiments, but the samples could be stored for several days without loss of acid. The samples had to be stirred before an aliquot could be taken.

Pepsin secretion

Analyses of pepsin should be performed immediately after the experiments in order to minimize the degradation of pepsin.

Peptides/hormones

Immediate stabilization of the samples was necessary in order to avoid degradation.

Discussion

The dog was used as experimental animal because it has gastric functions very similar to those in humans. The mechanisms of the gastric secretion have been examined more extensively in dogs than in any other species (*Grossman* 1981). The stomach of a 20 kg dog is about the same size as that of a 70-kg man and has the same maximum capacity to secrete acid (*Marks et al.* 1960). Dog and man (*Sarna* 1985) but not the cat (*Bendeck* 1986) have similar cyclic patterns of gastrointestinal motor activity.

Gastrointestinal peptides and hormones released from the gastrointestinal tract are often metabolized in the liver and the lungs with serotonin (5-Hydroxytryptamine) (*Davis & Wang* 1965) as an example. Furthermore, they will be considerably diluted during the passage from the liver to a peripheral vein. Blood samples from the portal vein will, therefore, be optimal for monitoring the release of gastrointestinal peptides and hormones.

Gastric mucosal blood flow could be determined with different methods among which the clearance technique is the most easy to establish (*Curwain & Holton* 1973, *Jacobson et al.* 1966). We chosed neutral red clearance because aminopyrine accumulates in the parietal cells (*Soll* 1981).

Infusions into gastric arteries (*Johansen* 1991) with simultaneous blood sampling from the portal vein made it possible to establish an isolated perfused stomach in conscious dogs.

By meticulous care of the catheter it was possible to preserve open access for several months.

The installation of the manometry catheter is often uncomplicated when a catheter guide is used and it does not causes any unpleasant experiences in the dog. The cannula made it possible to place and fix the manometry catheter exactly in the position wanted, and vomiting could not dislocate the catheter.

More than 95 % of the gastric secretion can be sampled via the cannula (*Ørnsholt et al.* 1979).

Gastrointestinal motility can be recorded at fixed locations by means of extraluminally implanted strain gauges (*Ehrlein* 1980), electrodes and miniature ultrasonic transducers (*Mandrek* 1991). The cannula gives access to record motility intraluminally from different locations in the upper gastrointestinal tract by means of manometry-catheters, sleeves (*Dent* 1975) and impedance planimetry (*Gregersen & Djurhuus* 1991). The location of the pylorus can be determined by the presence of isolated pyloric pressure waves or by a change in the transintestinal potential difference from the antrum to the duodenum (*Houghton* 1988). Using the cannula as entrance to the gastrointestinal tract makes it possible to use the same dog for different kinds of motility recordings at different locations without any anaesthesia or sedation. Devices for intraluminal motility recordings can disturb the cyclic pattern of gastrointestinal motor activity if the catheter is to rigid or the passage of pylorus has been done with the use of force. A stiff catheter causes persistent regular contractions in the antrum and simultaneous relaxation of pylorus and duodenum. Transitory irregular contractions in antrum, pylorus and duodenum are seen after a traumatic passage of pylorus.

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Summary
The conscious dog with a gastric cannula and permanent catheters in the portal vein and gastric arteries can be used for chronic simultaneous investigations of gastrointestinal functions. We have used the model for the recording of gastric secretion, blood flow, output of hormones and antroduodenal motility. The procedures for surgery, measurements, maintenance and pitfalls of the model are described.

Sammendrag
Vågne hunde med en gastrisk kanyle og permanente katetre i vena porta og gastriske arterier kan bruges til kroniske simultane undersøgelser af gastrointestinale funktioner. Vi har brugt modellen til registrering af gastrisk sekretion, blood flow, frigivelse af hormoner og antroduodenal motilitet. Den kirurgiske procedure, målemetoder, modellens vedligeholdelse og faldgruber er beskrevet.

Yhteenveto / K. Pelkonen
Tajuista koiraa, jolla on mahaletku ja pysyvät katetrit maksan porttilaskimossa ja vatsan valtimoissa, voidaan käyttää jatkuvaan samanaikaiseen ruuansulatuskanavan useiden toimintojen seuraamiseen. Tässä seurattiin koiralla mahalaukun eritystoimintaa, verenvirtausta, hormonieritystä ja ohutsuolen liikkeitä. Artikkeli kuvaa käytettyä kirurgiaa, mittausmenetelmiä, ylläpitoa ja koemallin virhelähteitä.

References
Bech K: Autonomic control of secretion of gastric acid and pepsin. J. Autonom. Pharmac. 1989, *9,* 419–428.

Berstad A: A modified hemoglobin substrate method for the estimation of pepsin in gastric juice. Scand. J. Gastroent. 1970, *5,* 343–348.

Canfield SP & C Price: The effect of sympathomimetic agents on gastric acid secretion in rats. Scand. J. Gastroenterol. 1984, *19 (Suppl. 89),* 23–26.

Curwain BP & P Holton: The measurement of dog gastric mucosal blood flow by radioactive aniline clearance compared with aminopyrine clearance. J. Physiol. 1973, *229,* 115–131.

Davis RB & V Wang: Rapid pulmonary removal of 5-hydroxytryptamine in the intact dog. Proc. Soc. Exp. Biol. Med. 1965, *118,* 797–800.

Dent J: A new technique for continuous sphincter pressure measurement. Gastroenterology 1976, *71,* 263–267.

Ehrlein HJ: Dehnungsmessstreifen zur registrerung der magen-darm-motorik bei wachen versuchstieren. Z. Gastroenterol. 1980, *18,* 191–197.

Gregersen H & JC Djurhuus: Impedance planimetry: A new approach to biomechanical intestinal wall properties. Dig. Dis. 1991, *9,* 332–340.

Grossmann MI: Regulation of gastric and acid secretion. In: Physiology of the gastrointestinal tract. pp 659–671 (Johnson LR ed.). Raven Press, New York 1981.

Houghton LA: Relationship of the motor activity of antrum, pylorus, and duodenum to gastric emptying of a solid-liquid mixed meal. Gastroenterology 1988, *94,* 1285–1291.

Hovendal CP: Adrenergic influence on canine gastric acid secretion and gastric mucosal blood flow before and after parietal cell vagotomy. Thesis, Odense University, Odense 1985, 1–64.

Jacobson ED, RH Linford & MI Grossmann: Gastric mucosal blood flow measured by aminopyrine clearance. J. Clin. Invest. 1966, *45,* 1–13.

Johansen B & K Bech: Intra-arterial versus intravenous administration of gastric secretory inhibitors in conscious dogs. Eur. Surg. Res. 1991, *23,* 51–57.

Mandrek K: Diameter and wall thickness recording of canine pylorus with implantable miniature ultrasonic transducers. Dig. Dis. 1991, *9,* 325–331.

Marks IV, SA Komarov & H. Shay: Maximal acid secretory response to histamine and its relation to parietal cell mass in the dog. Am. J. Physiol. 1960, *199,* 579–588.

Melsaac RL, LP Fielding, SE Knight, CD Rennie & RA Hittinger: Neutral red clearance as an estimate of gastric mucosal blood flow in man; validation and application of a new technique. Scand. J. Gastroent. 1980, *15 (Suppl. 63),* 131–140.

Sarna SK: Cyclic motor activity; migrating motor complex. Gastroenterology 1985, *89,* 894–913.

Schurizek BA, LHO Willacy, K Kraglund, F Andreasen & B Juhl: Effects of general anaesthesthesia with halothane on antroduodenal motility, pH and gastric emptying rate in man. Br. J. Anaesth. 1989, *62,* 129–137.

Soll AH: Physiology of isolated canine parietal cells: Receptors and effectors regulating function. In: Physiology of the gastrointestinal tract. pp 673–691 (Johnson LR ed.). Raven Press, New York 1981.

Thomas JE: An improved cannula for gastric and intestinal fistulas. Proc. Soc. Exp. Biol. Med. 1941, *46,* 260–261.

Ørnsholt J, J Marqversen & E Amdrup: Gastroduodenostomy without vagotomy. J. Surg. Resch. 1979, *26,* 163–166.