An animal model for the study of pancreatico-biliary and duodenal secretion

by *Per Svendsen*[1], *Mark Ainsworth*[2] and *Henrik Glad Sørensen*[2],
[1] Biomedical Laboratory, Odense University, 55, Campusvej, DK-5230 Odense M, Denmark,
[2] ClinicaL Institute, Department of Internal Medicine, Odense University Hospital.

INTRODUCTION

Ulcers in the duodenum are believed to result from an imbalance between aggressive and defensive factors, but the cause and nature of this imbalance has not been established. Previous studies have focused on the aggressive factors, i. e. acid and pepsin, but so far no general increase in aggressive factors has been demonstrated in patients with duodenal ulcer disease (*Soll* 1989). Clearly the ability of the duodenum to dispose of the acid, and the ability of the mucosa to withstand acid and pepsin must be of importance (*Flemstrøm & Turnberg* 1984). Acid disappears from the duodenum mainly as a result of neutralization by bicarbonate (*Wormsley* 1969, *Winship & Robinson* 1974 and *Dorricott et al.* 1975).

The pancreas is generally considered to be the most important source of duodenal bicarbonate. The pancreatic bicarbonate mechanism is believed to be of major importance in neutralizing gastric acid in the duodenum (*Schaffalitzky de Muckadell et al.* 1977, *Schaffalitzky de Muckadell et al.* 1979a, b). However, the liver and the duodenal mucosa also secrete bicarbonate in response to duodenal acidification (*Konturek* 1971 and *Isenberg et al.* 1986), and studies in primates have shown that infusion of the hormone secretion, which is normally released from the duodenum during duodenal acidification, causes an increase in both pancreatic and hepatic bicarbonate secretion (*Gardiner & Small* 1976). Furthermore studies in sheep indicate that the liver may be of greater importance to the neutralization of acid in the duodenum than the pancreas (*Caple & Heath* 1972).

The relative importance of the different sources of bicarbonate to the neutralization of acid in the duodenum as well as the interaction between the different sources of bicarbonate remain largely unknown. Answers to these questions could be of importance to our understanding of the pathogenesis of duodenal ulcer. As simultaneous measurements of all the duodenal bicarbonate sources are extremely difficult in human beings, an animal model has been developed for the study of pancreatico-biliary and duodenal secretion, and acid neutralization in the duodenum.

MATERIALS AND METHODS

Animals:

The experiments were performed on female Danish Landrace pigs weighing from 23 to 35 kg. The animals originated from an SPF breeding unit, and were kept in the laboratory for at least 48 hours before being used. They were fed commercial pig feed, and wood shavings were provided as bedding. Feed was withheld for 12 hours before the experiments, whereas water was available at all times. The experiments were performed under general anaesthesia and the total experimental time was 6 hours, after which the animals were euthanized by an overdose of pentobarbital.

A total of 125 experiments has been performed.

Anaesthesia and surveillance:

The anaesthesia procedure has previously been described in detail (*Svendsen et al.* 1990). In short, the pigs were pretreated with azaperone (SEDAPERONE VET 4 %, Janssenpharma A/S) 2 mg/kg i. m. and metomidate hydrochloride (HYPNODIL VET 5 %, Janssenpharm A/S) 2.5 mg/kg i. p. After 15

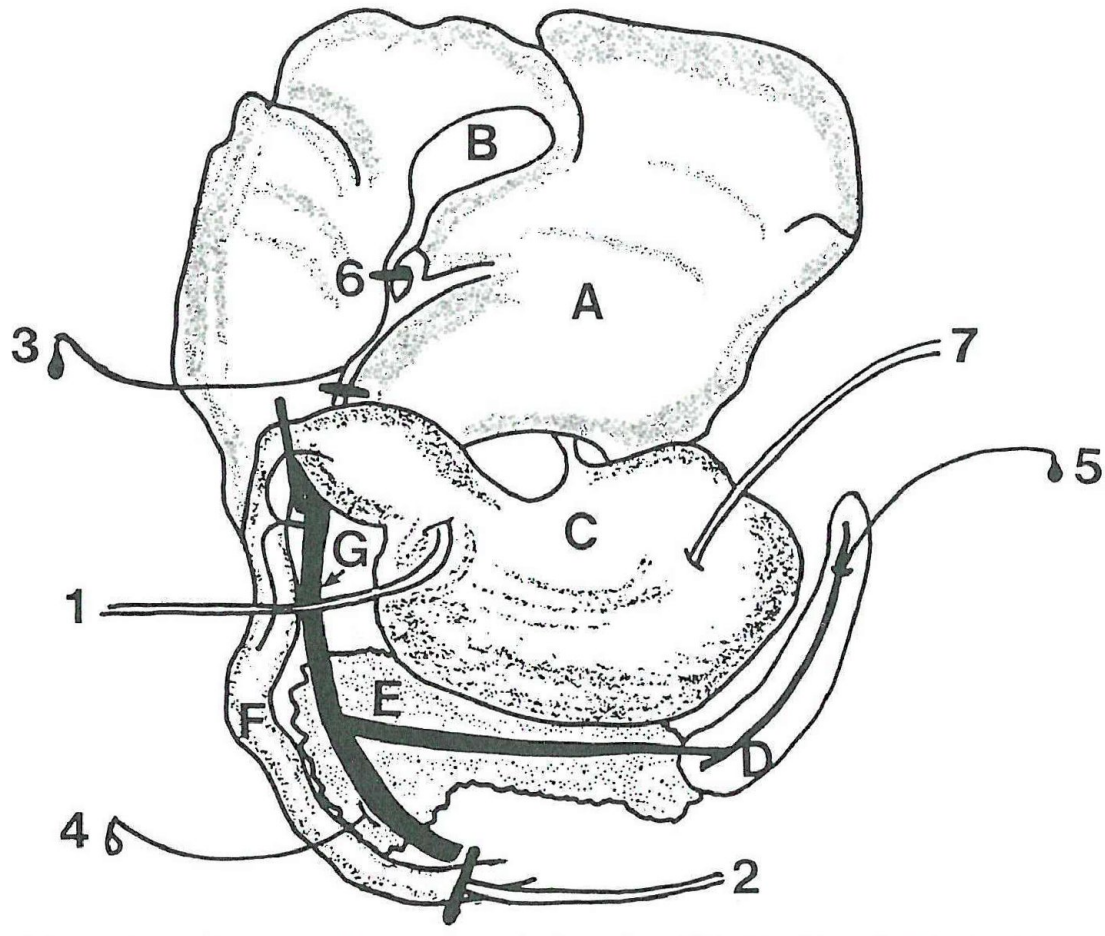

Figure 1: Illustration of the surgical preparation. A: liver, B: gall bladder, C: ventricle, D: spleen, E: pancreas, F: duodenum, G: portal vein. 1: afferent duodenal catheter, 2: efferent duodenal catheter, 3: choledochal catheter, 4: pancreatic duct catheter, 5: portal vein catheter, 6: ligation of the cystic duct, 7: gastric catheter.

min the animals were deeply sedated, and the marginal ear vein was easily cannulated using a Venflon catheter Gauge 20. Additional HYPNODIL VET was given i. v. until laryngeal reflexes were absent. The trachea was intubated, and anaesthesia maintained with halothane (HALOTAN, Halocarbon) 2 % in oxygen (60 %) using artificial ventilation (150 ml/min/kg). When surgery was completed HALOTAN was replaced by 0.25 % (W/V) alpha-chloralose solution 100 mg/kg i. v. to secure minimal respiratory and cardiovascular disturbance.

Electrodes were placed on the skin over the sternum for continuous recording of electrocardiogramme and pulse rate.

A transverse incision was made over the femoral canal, and the femoral artery was exposed. A catheter (Viggo Secalon Gauge 16 × 200 mm) was introduced into the artery using the Seldinger technique. The catheter was connected to a strain gauge and amplifier for continuous recording of arterial pressure (S & W, Denmark), and for sampling of blood for arterial blood gas and pH analysis (BMS 2MK Blood Micro System, Radiometer, Denmark). A longitudinal incision was made in the jugular fossa, and the external jugular vein was exposed and cannulated using a Bardicath Gauge 14 catheter. The venous catheter allowed sampling of venous blood, infusion of anaesthetics and test drugs, and recording of the central venous blood pressure.

A thermometer was placed in the rectum, al-

lowing continuous recording of the body temperature.

Surgical procedure:
A 40 cm long ventral midline incision was made, beginning at the xiphoid process. The wound edges were retracted to allow maximal access to the abdominal organs. An illustration of the preparation is given in fig. 1.
The cystic duct was ligated first in order to exclude the content of the gall bladder and exclusively allow sampling of secretion from the hepatic ducts. The cystic duct was located on the visceral surface of the liver extending from the gall bladder to the junction with hepatic duct. In most animals the cystic duct was in close contact with the hepatic surface and the cystic artery, while in a few animals the duct was found loosely attached to the liver in a small peritoneal plica. Using a pair of Metzenbaum scissors, the peritonaeum was separated over the cystic duct, and the duct was separated from the surface of the liver and from the cystic artery using a fine artery forceps. A 4–0 ligature (Dagrofil) was placed around the cystic duct.
To reduce the volume of the stomach, the content was evacuated. A purse string suture was placed in the serosa of the gastric body, an incision made in the centre, and a stomach tube introduced into the stomach. By applying a slight vacuum the gastric content was removed.
A second purse string suture was placed in the gastric serosa near the pylorus, a central incision made and a Foley catheter No 18 introduced into the stomach. By palpation the catheter was guided through the pylorus into the duodenum, and the balloon inflated. A double ligature was placed around the duodenum just caudal to the pylorus and secured tightly around the catheter. If the experiment required flow of gall to the duodenum, the ligature was placed around the pylorus cranial to the entrance of the choledocal duct. Via this catheter solutions of different composition could be infused into the duodenum.
The plica duodenomesocolica (Treit's ligament) was located, and a purse string suture placed as distal as possible. A second Foley catheter No. 18 was placed in the duodenum and secured by a ligature cranial to the purse string suture. This catheter served as a drainage for the solutions infused via the pyloric catheter.
The choledochal duct was located as an extension to the cystic duct proceding towards the duodenum immediately caudal to the pylorus. The choledochal duct was closely associated with the portal vein. The peritonaeum of the duct was with a pair of Metzembaum scissors, and the duct dissected from the portal vein using a curved artery forceps. Two ligatures were placed round the choledochal duct, and the distal one was tied. A small opening was made in the bile duct using a pair of microscissors, and a catheter (baby feeding tube, ch 8) was introduced and advanced until the tip was located just below the entrance of the cystic duct.
The pig has only one pancreatic duct, which opens directly into the duodenum close to the caudal end of the gland. The duct is small and difficult to locate. In some pigs it was visible by translumination, in others it could be palpated. Once the duct was located, the peritonaeum was carefully cut with a pair of dissection scissors, and the duct isolated with a small curved artery forceps. A ligature was tied close to the duodenal wall, and another ligature was kept in position around the duct a few mm from the first one. using a pair of microscissors a small opening was made in the duct and a catheter (baby feeding tube, ch 5) was passed into the duct. The second ligature was tied to prevent pancreatic juice from entering the peritoneal cavity. The use of magnifying spectacles was helpful when cannulating the pancreatic duct.
The final step in the preparation of the model was the placement of a catheter in the portal vein. The visceral side of the spleen was exposed and the splenic vein dissected and ligated 5–10 cm from the apex. A second ligature was placed a few mm proximal to the first, and a small opening cut in the vein with a

pair of microscissors. A guide wire was introduced into the vein, and a catheter (Cordis, French 5) passed over it into the vein. The catheter was slowly introduced and guided past the gastroepiploic vein through the splenic vein to the portal vein.

RESULTS

In the 125 experiments performed so far, we have experienced a variety of small problems or complications that could be coped with immediately without any major significance to the success of the experiment. We did, however, experience some major complications that forced us to discontinue or to discard the experiments. In order to evaluate the model and in order to help other researchers who wish to use this model, a complete description of both the major and the minor complications that we have experienced is given.

Major complications:

Due to major complications 7 of the 125 experiments were discarded:

Three experiments were discontinued because of signs of shock. The shock was not due to hypovolaemia as central venous pressure was normal and saline infusion showed no effect. The precise cause of the condition was not be established.

One experiment was discontinued because of metabolic acidosis. The disturbance might possibly have been corrected by infusion of sodium bicarbonate, but as infusion of bicarbonate could influence bicarbonate secretion, this was not done.

One experiment could not be completed because of an anatomical abnormality of the biliary tree. In this animal the right hepatic duct communicated directly with the gall bladder.

One experiment could not be completed as it was not possible to locate the pancreatic duct.

The results of one of the experiments were discarded after completion of the experiment as it was discovered that gastric acid had leaked from the stomach into the duodenum.

Minor complications:

Ligation of the cystic duct was usually a fairly simple procedure, but two complications have been recorded. The most common one was accidental rupture of the duct during dissection, and contamination of the peritonaeum with bile. The rupture could, however, be repaired by placing a second ligature. The anatomy of the bile duct system showed some variation. In some animals the cystic duct was very short, and accidental ligation of the duct below the entry of the right hepatic duct did occur. If unnoticed, this complication would cause stasis in part of the liver and reduce the flow of gall to the common bile duct. Careful removal and replacement of the ligature may, however, succeed if performed without delay.

Catheterization of the choledochal duct was usually performed without difficulty. A complication sometimes occurred if the tip of the catheter bypassed the hepatic duct and entered into the ligated cystic duct, thus preventing gall from being collected. The complication was easily recognized when a secretin injection failed to produce gall from the catheter. Careful partial withdrawal of the catheter to a position with the tip below the entrance of the cystic duct could always correct the complication. Rupture of the choledochal duct or insufficient ligation of the duct catheter would cause a loss of gall to the peritoneal cavity, thus reducing the volume collected. Replacing the catheter and the ligature was sufficient to correct the condition.

Dissection of the pancreatic duct often caused difficulties, simply because the duct is not directly visible. In one animal the pancreatic duct was accidentally transsected, and placement of the catheter was only possible with great difficulty using microinstruments. A common complication was occlusion of the pancreatic catheter by a small

blood clot. Careful suction with a 1 ml syringe was always sufficient to remove the obstruction. The most common complication when cannulating the splenic vein was bleeding during dissection. Compression was usually sufficient to stop such bleeding. If not the splenic vein was ligated, and cannulation performed further proximal.

DISCUSSION

The pig was chosen as the experimental animal for the development of this model for a number of reasons. Being an omnivore, the natural diet of the pig is similar to that of man, and the digestive processes in the ventricle and small intestine are comparable. The digestive physiology of the more commonly used laboratory animals like rabbits and rats differs from that of man, for instance the sensitivity to gastric hormones and neuropeptides is much lower (*Jansen & Lamers* 1983 and *Nakano et al.* 1988).

The pancreas of the pig has only one duct, which opens directly into the duodenum, allowing direct sampling of pancreatic secretion from the entire gland. Other commonly used laboratory animals are less acceptable for this type of investigation. The dog has two or occasionally three ducts entering the duodenum, and a minor duct entering into the choledochal duct. In cats a single pancreatic duct opens into the choledochal duct. The single pancreatic duct of the rabbit does open into the duodenum well separated from the opening of the bile duct, but cannulation is only possible using duodenotomy.

Important conditions for the successful application of the experimental model are stable cardiovascular, respiratory, and metabolic conditions. The anaesthetic technique, therefore is critical, and continuous recordings and corrections of the arterial blood pressure, heart function, arterial pCO_2, acid base balance, and body temperature are necessary. The alpha-chloralose anaesthesia combined with artificial ventilation has proved to be satisfactory in relation to the above-mentioned parameters. The preparation of the model requires some surgical experience. The failure rate, however, has been low (approximately 6 %), and mainly due to unclarified pathophysiological conditions and anatomical abnormalities. Only two failures caused by technical problems were experienced.

The surgical preparation required two surgeons, and one technician was needed to perform blood gas analyses. The staff requirements during the experimental phase depend on the type of experiment. In our investigations three persons were employed for approximately six hours, performing the experiment and preparing the samples. In addition, time for analysis of the samples was required.

This experimental model was developed for the study of the quantitative contributions of bicarbonate from the liver, the pancreas and the duodenal mucosa to acid neutralization in the duodenum, and as such it has proved valuable (*Ainsworth et al.* 1990a, b). However, a variety of other physiological, pharmacological and pathophysiological problems can be studied using the model. The influence of stimuli to the duodenal mucosa, like acid, proteins, amino acids, lipids, fatty acids, ethanol etc, on the release of gastrointestinal hormones and neuropeptides, and the concomitant pancreatic and hepatic secretion can be examined. The effects of hormones, neuropeptides and drugs on the pancreatico-biliary and duodenal secretion can be examined after peripheral as well as portal venous injection.

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Summary

A description is given of the surgical preparation of a porcine model for the simultaneous study of the secretory activity of the liver, the pancreas, and the duodenal mucosa. Special emphasis has

been given to major and minor complications. The various applications of the model are evaluated.

Sammendrag
Der gives en beskrivelse af den operative teknik ved fremstillingen af en porcin model til studiet af sekretionen fra lever, pancreas og duodenalmucosa. Der lægges specielt vægt på beskrivelsen af tekniske problemer. Modellens forskellige anvendelsesmuligheder evalueres.

Yhteenveto / K. Pelkonen
Artikkeli kuvaa kirurgisen menetelmän, jonka avulla voidaan tutkia samanaikaisesti sian maksen, haiman ja pohjukaissuolen limakalvon erityistoimintaa. Erityistä huomiota kiinnitetään komplikaatioihin. Artikkelissa arvoidaan myös menetelmän sovellusmahdollisuuksia.

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