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Intestinal Cannulation: Model for Study of the Midgut of the Pig

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Purpose: **To develop a pig model that would enable repeated biopsy specimen collection and endoscopic monitoring of the gut. This would increase precision of the experiment and reduce the number of experimental animals required.**

Methods: **Six 10-week-old Yorkshire pigs underwent surgery, and a cannula was inserted in the cecum. Two pigs served as non-operated controls. The health status of the animals was monitored by clinical, hematologic, and biochemical examinations and by studies of gut motility and microbial flora. The experimental period lasted for eight weeks and approximately 45 biopsy specimens were obtained from each animal.**

Results: **Repeated endoscopy was performed and biopsy specimens were taken. Adverse effects on the animal's health were not apparent, and differences were not evident in transit time of digesta or in diversity of the gut microbial flora. After surgery there was a transient increase in the concentrations of haptoglobin, serum amyloid A, and plasma cortisol, and in body temperature and white blood cell count.**

Conclusions: **It is possible to use an intestinal cannula in the cecum both for endoscopy and biopsy specimen collection. The procedures did not influence health status of the pigs, nor alter gut function. The method will be useful in experimental infection studies as well as in other physiologic investigations.**

Experimental animal models are important in the study of infectious diseases and their treatment and prevention. In general, an infection is induced in several animals, which are sacrificed at different times after challenge in order to monitor the development of the disease (1, 2). This procedure requires a considerable number of animals, and the interaction between the microorganism and its host is difficult to follow. An experimental model that allows repeated measurements in each individual would increase precision of the experiment and reduce the number of animals required.

In many species, endoscopy is used to diagnose and study diseases of the intestinal tract. In pigs, many important diseases are located in the midgut (i.e., ileum, cecum and proximal portion of the colon), and use of endoscopy has, therefore, been hampered by length of the gut, which may approach 27 m (3). Intestinal cannulas have been used since the nineteenth century to study the physiology and nutrition of animals (4). Only a few studies have addressed the possibility of taking tissue specimens through the cannula (5-7), and in those studies, the specimens were taken blindly. Use of a fiberscope would probably improve the results.

Various cannulas have been developed and are frequently used in experimental research (8-14). In the study reported here, the postvalvular T-cecum (PVTC) cannula was chosen because of its suitable location (10, 13, 15). However, the possible systemic effects of the cannula need to be studied thoroughly. Gut motility and the balance of the gut flora may be altered by the partial resection of the cecum and by presence of the cannula. The transit time of digesta can be studied by use of various feed markers, and inert markers, such as titanium dioxide (TiO_2), preclude the necessity of complete collection of digesta (16-18). Apart from determination of some of those commonly found, specific pathogenic microorganisms, such as *Lawsonia intracellularis* and *Brachyspira hyodysenteriae*, the ecologic balance of the gut has been studied by monitoring the phenotypic diversity of the coliform flora (19, 20).

Further, the potential interaction between the surgical inflammatory response and establishment of experimental induced infection must be considered, as well as the possibility of adverse effects, such as secondary infections. In addition to clinical health examination and conventional hematologic examination, measurement of acute phase reactant proteins (APRP) can be useful as an indication of the inflammatory response (21, 22). In pigs, haptoglobin (Hp) is the best studied APRP, but C-reactive protein (CRP), major acute phase protein (pig-MAP), and serum amyloid A (SAA) also are valuable (23-25).

In experimental surgery of swine, halothane is a commonly used inhalation anesthetic, but it may induce centrolobular liver necrosis (26, 27). This can be reflected by increased serum activities of liver-specific enzymes, such as glutamate dehydrogenase, GD (28, 29), which is thus of interest to monitor when repeated anesthesia is applied. In addition, surgery and other stressors are known to cause a transient increase in the cortisol concentration, and should be monitored (30-32).

The aim of the study reported here was to develop a pig model in which intestinal cannulation would enable repeated endoscopic biopsy specimen acquisition and monitoring of the gut, which would be useful in physiologic and experimental infection studies. Further, the influence of the procedure on the animal's clinical state of health, various blood parameters and inflammatory markers, transit time of the digesta, and gut flora was

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investigated. On termination of the experiment, complete necropsy was performed.

Materials and Methods

Animals. Eight 10-week-old, clinically healthy Yorkshire pigs (4 sows, 4 barrows) of 25-kg body weight were obtained from the University research farm (Funbo-Lövsta Research Centre, Uppsala, Sweden). The animals were kept indoors on straw in individual pens (approx. 3.5 m^2 each) within sight and sound of each other. They were fed a commercial finisher diet without growth promoters (Singel Flex, Odal, Sweden) and had access to water ad libitum. Prior to endoscopy, the feed was withheld for 18 h, and the straw was replaced with a synthetic fiber-fur blanket. To ensure an optimally comfortable temperature of 20°C, infrared lamps were provided from the day of surgery onward.

Experimental design. The experimental design was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden. Clinical examination, including measurements of body temperature and of heart and respiratory rates, was performed daily, without restraint and without causing stress to the animal. The animals were weighed once a week, and daily weight gain (DWG) was calculated. The protocol ran for eight weeks, initiated by a two-week acclimatization period. Six pigs underwent surgery, and two pigs served as non-operated controls. After a two-week postoperative recovery period, the experimental pigs were subjected to endoscopy on four or six occasions during a two-week period, and finally, twice during the subsequent two weeks.

The day of surgery was designated as day 0. Blood samples were collected at 10:30 a.m. on days 0, 1, 2, 4, 7, 10, and 14, and at slaughter. An additional sample was taken after the intense endoscopy period (day 23 or 24). All blood analyses were performed at the Department of Clinical Chemistry at the Swedish University of Agricultural Sciences.

Retention time of the digesta was measured on day 29. Fecal samples were collected once a week for analysis of the diversity of the coliform gut flora and for microbial analyses concerning the pathogens *Brachyspira hyodysenteriae* and *B. pilosicoli*. A tissue specimen was collected at necropsy and analyzed for presence of *Lawsonia intracellularis*. At the end of the trial, the pigs were euthanized and necropsy was performed.

The PVTC cannula. The cannula was made of silicon rubber and consisted of two similar flanges from which barrels protruded (15). The inner diameters of the flanges were 22 and 30 mm and the outer diameters were 28 and 35 mm, respectively. The cannula was closed with a plug made of polyvinyl chloride (PVC) plastic and fixed in position with self-tightening nylon clips.

Preoperative care and anesthesia. Food was withheld from the animals overnight before surgery, but they had ad libitum access to water. Fifteen minutes prior to anesthesia induction, atropine sulfate (Atropin, NM Pharma AB, Stockholm, Sweden) was given intramuscularly (i.m.) at a dosage of 0.05 mg/kg of body weight. Anesthesia was then induced by i.m. administration of a combination of medetomidine (Domitor vet 1 mg/ml, Orion Pharma AB, Stockholm, Sweden) and tiletamine and zolazepam (Zoletil 250+250 mg, Reading, Carros, France). The mixture contained 1 mg of Domitor/ml and 100 mg of Zoletil/ml, and it was given at a dosage of 0.05 ml/kg (33). The animals were intubated, and general anesthesia was maintained by inhalation of halothane vaporized in oxygen, with nitrous oxide added initially (34).

Surgery and postoperative care. The surgery was performed under aseptic conditions according to the method described by van Leeuwen and co-workers (15). The pigs were positioned on their left side. The hair over the surgical area was shaved, and the skin was washed with Hibitane soap and disinfected with Hibitane ethanol (Astra Zeneca, Gothenburg, Sweden), then draped. Four centimeters below the transverse processes, a 10-cm incision was made, 2 cm behind and parallel to the last rib. The cecum was located, the ileocecal ligament was transsected, and vessels were ligated with 4-0 polydiaxanone (Vicryl, Ethicon GmbH, Norderstedt, Germany).

Partial cecectomy was performed. An intestinal clamp was applied across the corpus opposite to the papilla ilealis, leaving 5 cm of the cecum intact. A purse-string suture (Vicryl 0) was placed 0.5 cm below the clamp, and the surrounding abdominal cavity was packed with gauze. The cecum was transsected between the clamp and the suture, and ingesta was evacuated.

The flange of one part of the cannula was inserted in the remaining part of the cecum. The suture was tightened without inverting the mucosa to further secure the cecal wall around the barrel protruding from the gut. A second purse-string suture, 0.5 cm below the first one, served to further tighten and secure the cannula. A stab incision was made through the skin 3 cm caudal to the laparotomy, and the muscles were dissected bluntly in the direction of the fibers. The barrel of the cannula, packed with gauze, was exteriorized through the incision. The laparotomy was closed by continuous sutures with Vicryl 2-0 or 0 in the peritoneum and the two muscular layers, and by single 2-0 supramid or 2-0 monofil steel sutures in the skin. The outer part of the cannula was attached, the plug was inserted, and all parts were fixed in position with nylon clips.

Every 15 min during surgery, measurements were made of rectal temperature, heart and respiratory rates, and arterial blood pressure; an electrocardiogram (ECG) was recorded, and the tonus of the jaw, corneal reflex, and reaction to pain stimuli were examined. In two pigs, arterial blood gas tensions were measured invasively through an indwelling catheter in the saphenous artery. Pain stimuli were induced by pinching the coronary band and tail by use of a forceps.

Analgesia after surgery was achieved by epidural administration of medetomidine at a dosage of 0.015 ml/kg (35, 36). The pigs were covered with a blanket until consciousness was regained. The wound and adjacent skin were lubricated daily with zinc oxide ointment (Natusan, Johnson & Johnson, Maidenhead, UK). Antibiotics were not given.

Hematologic and clinical biochemical analyses. Blood samples (5 ml) were collected from the jugular vein in vacuum tubes containing EDTA and were analyzed immediately for total and differential WBC counts, using an electronic cell counter validated for porcine blood (Cell-Dyn 3500, Abbott, Wiesbaden, Germany). Sera, extracted from samples without additives, were stored at –80°C until analyzed.

Haptoglobin was measured by use of a commercial kit (Tridelta Phase range Haptoglobin Assay, Tridelta Development Limited, Greystones, Wicklow, Ireland), which measures the peroxidase activity of haptoglobin-methemoglobin complexes (37). The analysis were performed by use of an automated procedure (Cobas MIRA, Roche Diagnostics, Basel, Switzerland). The SAA was assayed by use of a commercially available solid-phase sandwich enzyme-linked immunosorbent assay based on monoclonal antibodies specific for SAA (Tridelta Phase range SAA kit,

Tridelta Development Limited, Greystones, Wicklow, Ireland). Samples were diluted 1:500. The working range of the assay was 15.6 to 1,000 g/L, taking into account the dilution factor, and the intra- and interassay coefficients of variation were 20%.

Serum cortisol concentration was determined by use of a solid-phase radioimmunoassay kit (Coat-A-Count, Diagnostic Products Co, Los Angeles, Calif.), according to the recommendations of the manufacturer. Serial dilutions of porcine serum and serum with high concentrations of cortisol produced displacement curves parallel to the standard curve. The detection limit of the assay was 7 ± 3 nmol/L. The intra-assay coefficients of variation for three control samples were 8% (40 nmol/L), 5% (85 nmol/L), and 5% (524 nmol/L). The corresponding interassay coefficients of variation were 9, 10, and 12%, respectively.

Serum samples obtained on day 23 or 24 were analyzed for activities of creatine kinase (CK), γ-glutamyltransferase (γ-GT), and GD. The analyses were performed by use of automated equipment (Cobas MIRA, Roche Diagnostics, Basel, Switzerland) (38, 39).

Gut motility and microbial flora. Retention time of the digesta was calculated from the concentration of TiO_2 in the feces at various times after its addition to the feed. Fecal samples (50 to 100 g) were collected from the pen floor every second hour for 16 h, then every fourth hour for a total period of 60 h. The samples were freeze-dried, ashed, and dissolved in sulfuric acid; hydrogen peroxide was then added, and absorbance at 410 nm was read (modified from Short, 40).

Fractional outflow rates were estimated from the decrease in marker concentration (41, 42), using a two-compartment model $y= a(b/c-b)(e^{-b\,(x-d)} – e^{\,c\,(x-d)})$, where y denotes the concentration of marker in the feces at time x; a is the scaling factor; b is the fractional rate constant for the first compartment; c is the fractional rate constant for the second compartment; x is time; and d is the lag phase. Mean retention time (MRT_{tot}) was calculated as (1/b + 1/c + d) (42, 43). The coefficient of determination (R^2) was 0.88 - 0.96.

The phenotypic diversity of the coliform flora indicates the stability of the total gut flora (20), and was estimated in fecal samples collected from the cannula and from the rectum in three experimental pigs and from the rectum in the two control pigs. This method is based on a biochemical fingerprinting scheme, and reflects the coliform flora. The results are given on a scale from zero to one, where zero corresponds to no diversity of the coliform flora (20, 44). The first and last rectal fecal samples were analyzed for the presence of *Brachyspira* spp. (45), and ileal tissue specimens were analyzed by use of the pol0ymerase chain reaction (PCR) technique for the presence of the microbial gut pathogen *Lawsonia intracellularis* (46, 47).

Endoscopic and biopsy specimens. Endoscopy with biopsy specimen collection was performed on days 14, 16, 18, 21, 23, 25, 28, and 35 in three of the pigs (No. 1, 3, and 6), and on days 14, 17, 21, 24, 28, and 35 in the other three pigs (No. 2, 4, and 7). The pigs were premedicated and anesthetized as described previously. An enema of 8 to 10 L of body-tempered water was given through the cannula, and endoscopy was performed immediately after. A videocolonoscope with diameter of 10 mm and length of 1 m (Fujinon videocoloscope EC-300 HL, Fujinon, Tokyo, Japan) and a biopsy forceps 2.2-mm thick and 230-cm long, fenestrated, with an oval spoon-shaped mouth, with or without a spike (MTW, Wesel, Germany), were used.

Biopsy specimens were taken from the ileum, cecum, and proximal portion of the colon. The ileum and colon biopsy specimens were taken 40, 30, 25, and 20 cm from their origin in the cecum. A total of about 6 biopsy specimens were taken on each occasion. The specimens were mounted on blotting paper, fixed in buffered 10% formalin, embedded in paraffin, cut into 4-μm-thick sections, and stained with hematoxylin and eosin. Specimens were graded as "good" (i.e., they had full mucosal thickness, good sample orientation and no artifacts), "fair," or as being of poor quality.

Necropsy. Complete necropsy was performed within 60 min after euthanasia. Specimens for microscopic investigation were taken from the heart, lung, trachea, liver, spleen, mesenteric lymph nodes, intestine, and skin adjacent to the cannula. The intestinal specimens were taken from the papilla ilealis and from the ileum and colon, approximately 10 and 30 cm, respectively, from the origin of the cecum. The samples were fixed in buffered 10% formalin and embedded in paraffin after conventional processing, cut into 4-μm-thick sections, and stained with hematoxylin and eosin.

Results

Pig 7 developed ileus during the second endoscopic procedure, and was excluded from the trial. This pig had high GD activity prior to surgery (41 nkat/L) and performed poorly after surgery (DWG of 0.3). At the time of euthanasia, GD activity had further increased (58 nkat/L), as did SAA (44.9 μg/ml) and Hp (3.3 g/L) values. Necropsy of this pig revealed multiple abscesses in the mesentery and omentum, neutrophilic infiltrations in the mesenteric lymph nodes and spleen, and fibrinous serositis in the abdomen.

Observations during surgery. The operation generally lasted for two hours, and the first attempts of the pigs to rise were observed 1 to 3 h later. During anesthesia, rectal temperature decreased to 37.6 to 38.0°C, then further to a mean value of 36.4°C, with the lowest temperature being 33.9°C (pig 1). Heart rate decreased from between 90 and 156 beats/min to between 70 and 80 beats/min, and respiratory rate decreased from between 55 and 60 breaths/min to between 30 and 50 breaths/min. Mean arterial blood pressure was 68 to 98 mm Hg, and oxygen saturation was satisfactory (pO_2, 54.8 to 71.1 kPa).

Clinical examination. The experimental pigs appeared depressed for two days after surgery, but from day three onward, their behavior, appetite, and fecal consistency had returned to normal. Two days after surgery, five of the six experimental pigs had an increase in rectal temperature (39.4 to 40.1°C), to a slightly higher value than that in the two control pigs (39.1 and 39.2°C). During the course of the experiment rectal temperature and heart (60 to 170 beats/min) and respiratory (12 to 44 breaths/min) rates, varied in all pigs, but difference was not observed between the groups (Fig. 1).

Two weeks after surgery, three pigs developed localized abscesses in a few skin stitches. These were cleaned with 3 % hydrogen peroxide, and they healed without further complications. Two pigs removed the cannula several times while rubbing their back against the pen walls. The pigs were sedated and the cannulas were replaced. Control pig 5 had intermittent diarrhea during the entire experiment, but did not develop other signs of illness.

Difference in DWG between the experimental (0.4 to 0.5 kg) and control (0.4 kg) pigs was not evident after surgery. During the intense endoscopy period, when food was withheld from the experimental pigs before endoscopy, DWG was 0.3 to 0.6 kg and was 0.7 to 0.9 kg in the controls.

Hematologic and clinical biochemical variables. The WBC

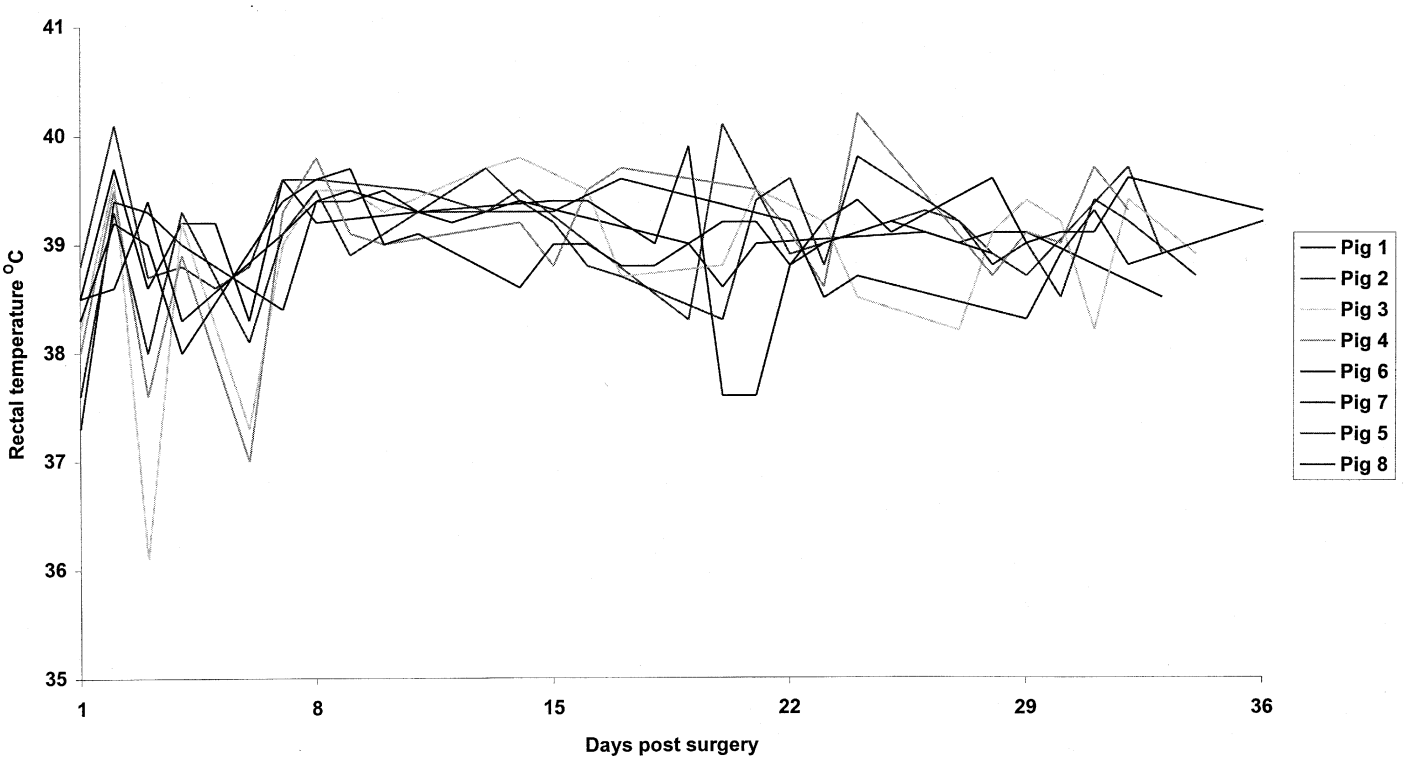

Figure 1. Rectal temperature in the six experimental pigs and two control pigs (5 and 8). Large interindividual variation was seen, and conclusions concerning postsurgical infections and a surgical inflammatory response could not be drawn.

count in four experimental pigs increased during the first or second day after surgery, and in one control pig, mainly due to an increase in the number of neutrophils (12.0 to 26.2 $\times 10^9$ cells/L). Normal neutrophil concentrations (< 7.0 $\times$ 10^9cells/L) were regained on the second or fourth day. A 13- to 78-fold increase in the SAA value was seen in the experimental pigs in the first two days after surgery (Fig. 2), but from day 4 onward, the values were at baseline. Haptoglobin concentration increased 2- to 20-fold on the first day and peaked between days 2 and 4 (to 2.0 to 4.0 g/L) in experimental pigs. Thereafter, the values continuously decreased, and on day 10, they were similar to control values (Fig. 2).

Cortisol concentration increased on the day of surgery, with the highest value in pig 4 (536 nmol/L). From day 1, the values decreased, and on day 4, all values were < 200 nmol/L. Values in control pigs ranged from 48 to 269 nmol/L throughout the experiment.

Glutamate dehydrogenase activity was < 25 nkat/L in all pigs, except pig 3 which had somewhat increased activity (39 nkat/L) on day 24. The CK and γGT values were below the upper reference limits (130 μmol/L and 0.6 μkat/L, respectively) in all pigs except for control pig 5 (191μmol/L and 0.8 μkat/L, respectively).

Gut motility and microbial flora. Difference in mean retention time of digesta was not found between the two groups, with values varying from 24.5 h (control pig 5) to 47.1 h (experimental pig 6). Pigs 3, 4, and 8 had MRT_{tot} of 32.4 to 37.0 h.

There was no difference in the pattern of diversity of the coliform flora between experimental and the control pigs. In experimental pigs, the median values for the cannula samples ranged from 0.29 to 0.81 and the median values for the rectal samples ranged from 0.39 to 0.68. In control pigs, diversity ranged from 0.42 to 0.76 (Fig. 3). All fecal and tissue specimens were culture negative for *Brachyspira* spp. and *Lawsonia intracellularis*.

Biopsy specimens. The quality of the biopsy specimens varied. Sixteen percent were considered "good" (Fig. 4), and 41% were "fair", whereas 43% were of poor quality. The main faults were: specimens were too superficial, poorly oriented, or compressed or extended. Most specimens were obtained from the colon. It was difficult to penetrate through the papilla ilealis with the endoscope, and ileal specimens were obtained from only three pigs. These biopsy specimens were generally good.

Necropsy. All animals were in good nutritional condition. Nothing pathologic was observed in the control animals. In all experimental animals, the cecal fistulas were healed, with normal scar tissue and minimal inflammatory cell infiltration. In pigs 1 and 2, there was a subcutaneous abscess (2 $\times$ 1 cm) in close proximity to the cannula, containing remnants of suture material. In most of the animals were small focal fibrous adhesions between intestinal loops, without any visible inflammatory reactions. Small foci of necrosis with a deep fibrinopurulent reaction and bacteria were found in the colon in two of the animals. Pig 4 had two liver abscesses, 2 to 3 cm in diameter.

Discussion

The results of the study reported here indicate that intestinal cannulation in combination with consecutive biopsy may be a useful experimental model in studies of the porcine midgut. The cannulation technique makes it possible to inspect the intestinal mucosa in live animals through a fiberscope catheter. It is also possible to take repeated biopsy specimens and other samples from each animal.

The animals were healthy, as judged by results of clinical examinations and various measurements, and further confirmed by the findings at necropsy. The pigs tolerated the anesthesia

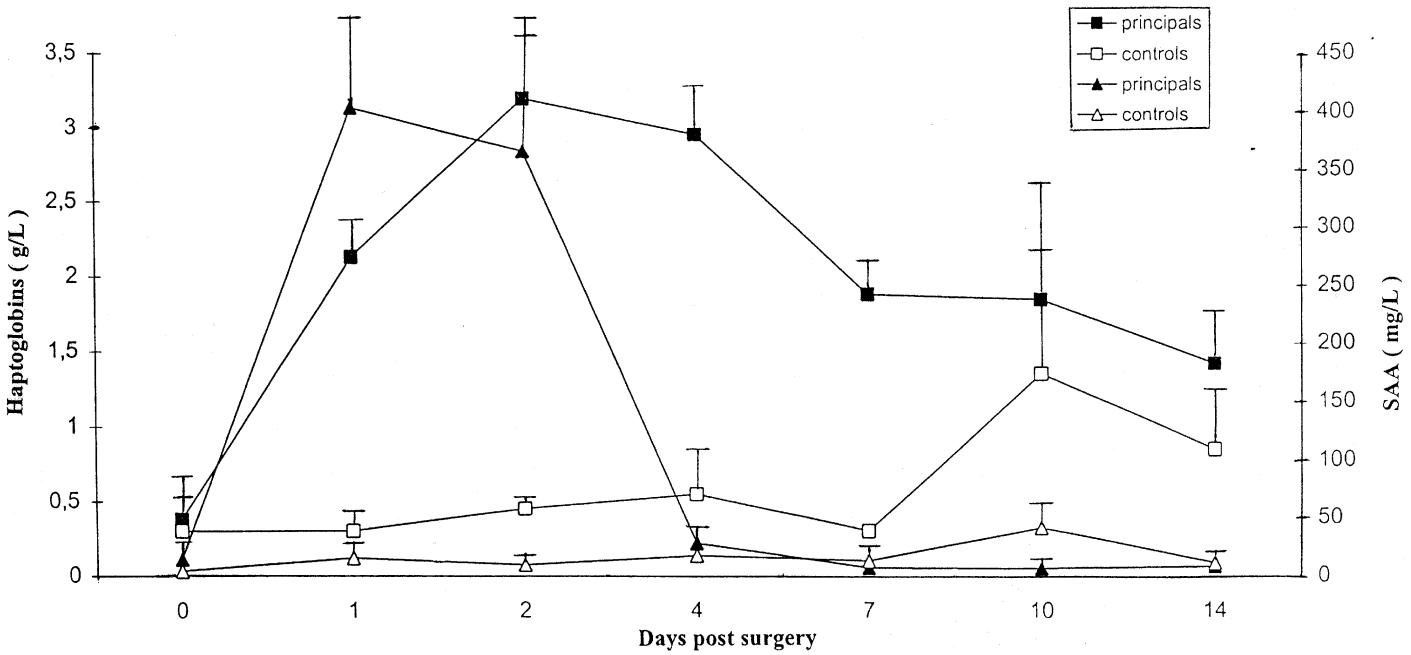

Figure 2. Mean (±SD) values for the acute phase reactant proteins, haptoglobin (■, □) and serum amyloid A (SAA; ▲, △) in experimental (n = 6) and control (n = 2) pigs, in response to surgery. The samples were collected prior to surgery on day zero, which denotes the day of surgery.

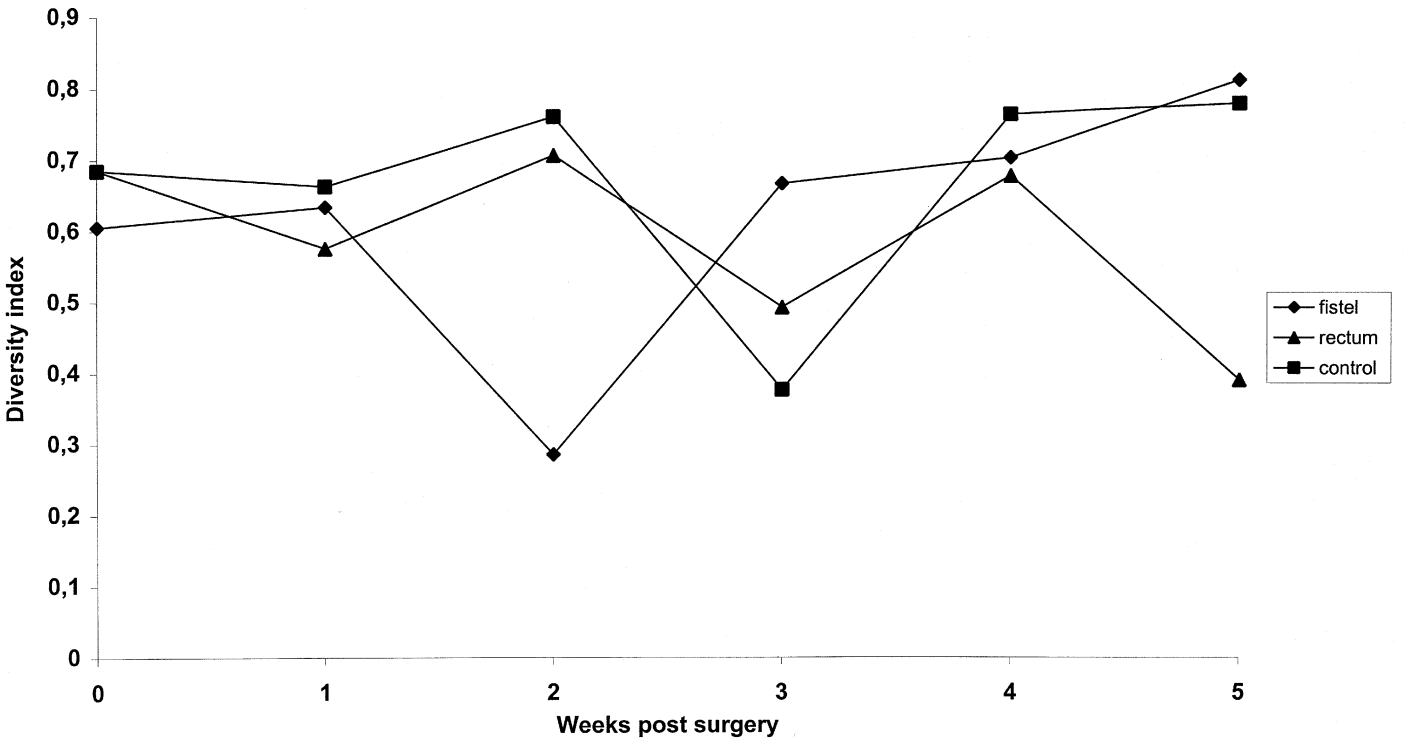

Figure 3. Analysis of the phenotypic diversity of the coliform flora indicating the stability of the gut flora. Results are shown as median values. Consistent change was not seen, either when fecal samples from the cannula were compared with those from the rectum of the experimental pigs (n = 3), or when rectal fecal samples were compared between the experimental and control (n = 2) pigs.

and operation well, and three days after surgery, their general appearance and behavior had returned to normal. On day four, the APRP, WBC count, and cortisol concentration were decreasing or normal. The experimental pigs had slightly high rectal temperature on the second day, which was considered surgical fever (48, 49). The large variations in recorded rectal temperature (Fig. 1) also have been observed in other studies and might be dependent on the activity of the pig (50, 51). The DWG was in accordance with that in pigs of commercial Swedish herds, and difference was not found between experimental and control animals before the endoscopy period. The first pigs developed local infections in some suture lines. Fewer skin sutures enabled better drainage and, together with the use of monofil steel, such infection was further prevented.

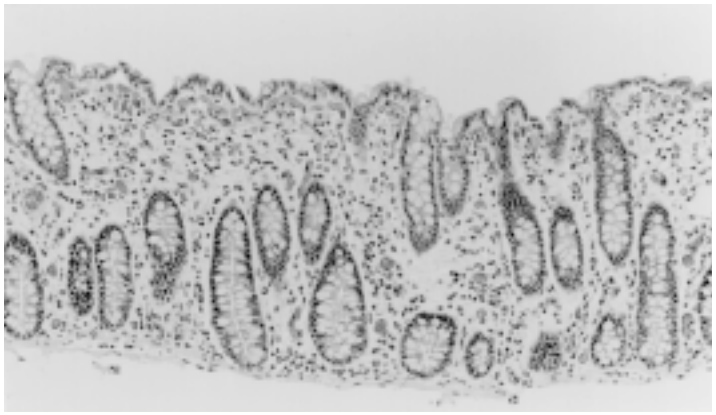

Figure 4. Photomicrograph of a section of a colon biopsy specimen considered "good," with full thickness of the lamina propria, resting on a superficial fragment of the lamina muscularis mucosae at bottom. H&E stain; x 120.

The quality of the biopsy specimens varied, but improved with time and experience. It was occasionally difficult to empty the colon sufficiently to get a sharp picture in the endoscope. A complementary study aimed at improving emptying of the gut and the biopsy technique was later performed and 22 further biopsy specimens were taken (data not shown); use of Phosporal (E-C de Witt, Cheshire, England) given orally, instead of enemas, improved gut emptying. Also, use of a forceps with a central spike improved the quality of the specimens. The biopsy specimen should only be allowed to dry briefly before being put into formalin. Good orientation of the specimen is achieved if it is mounted, but it should be removed from the paper before preparation. To ensure collection of a fair specimen from each location, several can be taken on the same occasion.

The PVTC cannula allows total collection of ingesta from the ileum (10), and its location in close proximity to the ileum and colon is an advantage, but partial resection of the cecum might interfere with function of the intestines. In this study, the papilla ilealis was difficult to penetrate with the fiberscope catheter in some pigs. Thus, a simple T-cannula, causing less alteration in the tissue and located in the ileum, might be an alternative for the study of ileal diseases. The PVTC cannula itself did not seem to cause any inconvenience to the animals, but its external part could be smaller and, thus, more difficult to rub off.

Large interindividual variation was observed in food transit time and microbial diversity. As expected, pig 5, which had intermittent diarrhea, had the fastest MRT_{tot}. The manipulations in connection with surgery and endoscopy did not influence the phenotypic diversity of the intestinal flora, as judged either by comparison between the diversity near the cannula with that in the rectum, or by comparison of the rectal samples between experimental and control pigs (Fig. 3).

The neutrophil count increased after surgery, in conformity with the report by Dalin and co-workers (32), whereas the total WBC count varied among all pigs. In this study, the WBC count increased the day after surgery, but Freischlag (48) did not find any association between degree of leukocytosis and incidence of infection in humans with postoperative fever. It is well known that an increase in the neutrophil count is related to an increase in cortisol concentration (52). In this study, the concentration of APRPs increased in response to surgery; the SAA value increased earlier than did Hp concentration, and decreased more rapidly (Fig. 2). The Hp response corresponded well to that in other studies of aseptic inflammation in pigs (23, 24), but contrasted to that in an infection model where a higher increase and a prolonged normalization period was seen (25). In that study, the SAA value peaked two days after inoculation, but it was not quantified. In the study by Dalin and co-workers (32), cortisol concentration peaked on the day of surgery, with a return to baseline on the following day. In this study, there was still a slight increase the day after surgery, but thereafter, difference was not seen between the groups, and, thus, the surgical alterations were considered not to have caused any persistent stress to the animals.

Premedication and the combination of zolazepam, tiletamine, and an α_2-agonist results in smooth induction of anesthesia, permits intubation and, similar to addition of nitrous oxide, decreases the requirement of inhalant anesthetic (26, 33, 34). However, tachycardia, tachypnea, and decreased body temperature are well-documented side effects (33, 53). The low rectal temperature in the first pigs could also have been due to the cold metal operating table; thus, the remaining pigs were placed on a fiber-fur blanket covered with a plastic sheet during surgery.

Halothane has several advantages, but may induce hepatocyte necrosis (28, 29). Glutamate dehydrogenase was chosen as a marker for necrosis, since the commonly studied alkaline phosphatase and alanine transaminase enzymes are difficult to assess in swine (29, 54). The slightly increased GD activity in two of the pigs is, however, difficult to interpret since there were no obvious morphologic changes in the liver, but may reflect mild liver damage. In healthy pigs, this enzyme activity is considered to be close to the detection limit (20 nkat/l), whereas in pigs with experimentally induced necrosis, it approaches 100 nkat/l (H. Holst, personal communication).

Epidural injections of medetomidine are generally reported to result in good analgesia (35, 36). In this study, a response to pain stimuli was observed as early as one hour after the epidural administration, as was also reported by Ko and co-workers (55), indicating that pigs may differ in this respect. An epidural administration of, for instance, xylazine or opioids might be a preferable alternative.

Five pigs underwent the endoscopic procedure on a total of 36 occasions without complications. One pig, however, developed ileus during endoscopy. Autonomic reflexes that inhibit gut motility, caused by stimulation of the peritoneum (56), inadequate emptying of the gut (26), or decreased motility due to anticholinergic agents and α_2-agonists (56, 57) might be the cause. However, necropsy revealed small abscesses in the mesentery and omentum and a fibrinous serositis, which in this pig, is a more probable explanation. The abscesses could have been caused by abdominal contamination when ingesta were evacuated, despite absence of postoperative clinical signs of infection. The cause of the small focal ulcerative lesions in the colon of two of the pigs is not known, but they did apparently not affect the health of the pigs.

In conclusion, it is possible to use an intestinal cannula for endoscopy and biopsy. This will be a useful tool in studying experimentally induced infections as well as in other physiologic studies. The procedures did not influence the health status of the animals, nor alter the function of the gut. The Hp concentration and SAA values indicated a transient inflammatory re-

sponse to surgery and seem to be suitable parameters for monitoring the inflammatory response.

Acknowledgments

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