Techniques for Measuring Blood Flow in Splanchnic Tissues of Cattle

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ABSTRACT

A useful approach of the study of nutrient absorption and metabolism is in vivo measurement of blood flow across portal-drained viscera and liver, and flux of bloodborne metabolites. Successful application of the approach requires correct placement of chronic catheters in appropriate blood vessels. Additionally, catheters must stay patent long enough to allow the animal to recover from surgery and to complete an experimental protocol. This paper describes surgical techniques to install chronic catheters in mesenteric veins, the hepatic portal vein, and an hepatic vein of cattle. Techniques for access to arterial blood are described also. Materials, equipment, and supplies required for surgery, blood sampling, and blood flow determination are described. Commercial sources of supplies are suggested. Blood flow is measured by downstream dilution of para-aminohippurate, which is infused into a mesenteric vein. Examples of blood flow data for three types of cattle are provided.

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INTRODUCTION

Techniques that we will describe are an extension of efforts with ruminants that began with the classical work of Barcroft et al. (1), which established their potential value for measuring splanchnic tissue metabolism. Schambye et al. (19, 20) explored various surgical techniques and coupled measurement of hepatic portal blood flow and blood metabolite concentration differences in vivo in sheep. Katz and Bergman (11, 12) described procedures for portal-drained viscera and hepatic tissues of sheep. Connor and Fries (3) and Fries and Connor (6) published the first procedures for cattle, followed by Carr and Jacobson (2), McGilliard and Thorp (16), and McGilliard et al. (17). Symonds and Baird (23) first published procedures for dairy cows, which included measures of hepatic metabolism. Dougherty (4) and Hecker (8) are sources of information on surgical procedures and general catheterization techniques for farm animals. Thus, over the course of four decades researchers in the area of ruminant nutrition and digestive physiology have developed, adapted, and implemented surgical techniques and measurement methodology that serve as reference and motivation for our efforts.

The objectives of our approaches center on quantitation of blood flow and metabolite flux across splanchnic tissues of cattle. The first objective was to place tips of chronic catheters in correct locations within appropriate blood vessels. The second objective was complete recovery of the cattle from surgery, with no adverse effects on tissue or organ function. The third objective was to maintain catheter patency for as long as possible to allow completion of
studies that last months instead of days or weeks.

MATERIALS AND METHODS

Instruments, Equipment, and Facilities

Surgery. Usual supplies and instruments required for surgery are listed in Appendix 1. All materials are sterile at surgery, either in commercial packets as received, by steam sterilization, by ethylene oxide, or by "cold" sterilization in a 1:200 dilution of Roccal™-D (Sterling Drug, Inc., New York, NY 10016) before surgery. Surgeons wear caps and clean apparel, scrub arms and hands with antiseptic soap before surgery, and wear sterile surgical gloves.

Special instrumentation or equipment requirements are few, but these are important to facilitate procedures (Appendix 1). A large Burford Finocchiato rib retractor is required if access to viscera is by rib resection. If a paracostal incision is used to access viscera, posts attached to the surgery table with a crossbar positioned over the rib cage and a hook and chain suitable for gripping the last rib at the incision and holding the rib cage in an elevated position facilitate access to the liver. A trocar or stylette is needed for exteriorization of catheters on the paralumbar shelf. We prefer a stylette, 45-cm long with a beveled point and an eyelet for exteriorization of single catheters because it minimizes trauma associated with exteriorization (A. D. McGilliard, personal communication).

Real-time ultrasound with a probe designed for manipulation in the visceral cavity greatly enhances hepatic catheterization and provides assurance of proper location of the catheter tip (22).

Surgical facilities center on a table adequate for large animals, equipment for respiration anesthesia, and facilities for postsurgical recovery. At least one, and preferably two, mobile, high intensity surgical lamps are needed for illumination. We use a small hand-held lamp as well for illumination under the rib cage or other areas. An automobile inner tube or other inflatable apparatus is needed to elevate the front shoulder during surgery to distribute weight and minimize pressure trauma to the radial nerve. A suction apparatus is needed to remove blood and rinse solution during surgery. An udder harness for lactating cows is fitted before surgery to minimize possibility of udder trauma during sedation and after surgery.

Catheters. Description of catheters and sources of catheter materials are listed in Appendix 2. Portal vein and hepatic vein catheters are teflon sheathed in silicone rubber tubing except for the portion exposed to blood. The silicone is swelled by soaking in toluene for 1 h, then slipped over the teflon. A steel wire anchored to one end of the teflon and long enough to pass through two lengths of the catheter facilitates transfer of the silicone over the teflon (G. Varga, personal communication). The wire is inserted through the teflon and the silicone and serves as a stabilizer and fulcrum for moving the silicone. As toluene evaporates (in a properly ventilated hood), the silicone shrinks around the teflon and provides an exterior sheath to which sutures can be tied during surgery and is conducive to healing of the exteriorization wound. Three cuffs are spaced evenly over 20 cm, beginning 1 cm from the end of the sheath. Cuffs are 1-cm sections of the same silicone rubber used to sheath the teflon. They are spread with forceps and slipped over the sheath to the desired location. The tip of the teflon catheter is polished (A. D. McGilliard, personal communication) by inserting a tubing adapter into the tip then buffing with a felt wheel and polishing compound (jeweler's rouge) in an electric hobby drill.

Catheters for the mesenteric veins, mesenteric artery and iliac artery are made of Tygon® and treated with 2% TDMAC-heparin complex (Appendix 2). Treatment consists of filling the catheter and soaking the portion to be exposed to blood for 1 to 2 min, then drying with air suction. Catheters ideally should dry at room temperature overnight before sterilization. Cuffs (.5 to 1 cm) are Tygon® of the same or slightly larger diameter, split longitudinally and cemented in place by a drop of cyclohexanone (13).

All catheters are fitted with tubing adapters of appropriate size and sealed between sampling sessions with plastic luer-lock caps. Three-way valves are used during surgery and sampling sessions. One port is used consistently for withdrawing blood, and the other port is used for infusion of heparinized, sterile saline.

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Physiological (0.15 M) sterile saline containing 20 IU heparin/ml is used for flushing catheters during surgery and sampling sessions. Similar saline containing 200 IU heparin, 0.01 ml procaine penicillin G, and 0.01 ml benzyl alcohol/ml saline is infused into catheters after surgery and between sampling sessions.

Sterile plastic syringes are used during surgery and sampling. A range of sizes (3 to 20 ml) is appropriate, but 10 ml is the most useful. Smaller syringes provide a better "feel" of how the catheter tip is functioning in the vessel.

Animal Preparation and Postsurgery Care. Except for lactating cows, feed is last offered 48 h and water 24 h before surgery. Lactating cows are fed restricted rations the day before surgery; feed is last offered 18 h and water 3 to 4 h before surgery. Udders are not emptied completely the milking before surgery.

Animals are cleaned (washed if possible) and hair in surgical fields is clipped the day before surgery. Before surgery a temporary jugular catheter is installed and an udder harness is fitted to lactating cows. Animals are sedated for insertion of an endotracheal tube with i.v. infusion of 5% thiamylal sodium, then maintained under general anesthesia in left lateral recumbency by respiration anesthesia (1 to 5% halothane, 4 to 6 L O2/min; VML, Fraser Harlake, 145 Mid County Drive, Orchard Park, NY 14217). The inner tube is placed under the left shoulder and positioned so that the point of the shoulder does not touch the table. Forelegs can be extended and retained forward to further reduce chance of radial nerve trauma. Intravenous drip of Normosol®-R (CEVA) is begun after the animal is positioned and continued until 2 to 4 L have been administered, depending on blood loss during surgery. For lactating cows, the i.v. drip is fortified with 500 ml of calcium carbonate (23%, wt/vol).

In general, the postsurgical diet is one to which animals were accustomed before surgery. Hay may be offered during recovery to induce resumption of digestive function. Usual antibiotic therapy is penicillin G procaine, 6600 units/kg live weight daily, given i.m. for 3 d. Further therapy usually is not necessary, but general veterinary practices are followed in response to symptoms observed. For several days after surgery plasma Ca, Mg, and glucose concentrations are monitored in lactating cows, and a sterile, aqueous solution containing 150 g dextrose, 20 g Ca, 5.5 g Mg, and 12 g P/L is infused i.v. if Ca concentrations fall below 70 mg/L, Mg concentrations fall below 15 mg/L, or glucose concentrations fall below 2.5 mM.

Surgical Techniques

Preparation and Access to Vessels. The animal is under general anesthesia and in left lateral recumbency. An area on the right side between the tuber coxae and the 8 and 9th rib, dorsal to the midline, ventral to the flank fold is scrubbed with surgical soap, rinsed, swabbed to remove loose hair or scurf, and rinsed again with 70% (vol/vol) ethyl alcohol. The right side is appropriately draped and a paracostal incision 30 to 40 cm is made perpendicular to the spine from the lumbar shelf 4 to 6 cm caudal to the last rib through skin and muscles. The peritoneum is raised with forceps to avoid cutting viscera, then opened the length of the incision. Bleeding from transected vessels is stopped by clamping or suturing. Both sides of the incision are covered with towels soaked in saline and Furacin®. Liver, gall bladder, duodenum, rumen, and right kidney capsule can be seen.

Distension of the rumen may hinder access to catheterization sites. The rumen can be deflated by puncture with a 12 to 14-gauge needle attached to 45 cm of tubing, holding the needle until the rumen deflates, then removing the needle in gauze. No incision or suture is required, and the procedure can be repeated if needed.

Starting at the gall bladder, the insertion of the lesser omentum is separated by blunt dissection from the liver dorsally for 10 to 15 cm. The liver is retracted by hand or with a ribbon retractor. The hepatic portal vein joins the liver 2 to 6 cm medial to the omental insertion, dorsal to the gall bladder (Figure 1). Landmarks are the pancreas, which covers the lateral surface of the vein, and a lymph node 1 to 3-cm wide, which is attached to the lateral surface of the vein at the liver. The lymph node is removed by digital blunt dissection and the portal vein just caudal to the liver is freed of surrounding pancreas and connective tissue. The portal vein has a tough wall and will tolerate gentle blunt dissection. Much of the dissection...
Hepatic Vein Catheterization. This procedure is patterned after the Seldinger (21) technique. The diaphragmatic surface of the liver is punctured with a 14-gauge, 5-cm long hypodermic needle over an hepatic vein, usually 2 to 6 cm from the lateral edge of the liver (Figure 1). Sterile, used needles or needles that have been purposely dulled provide a better “feel” of puncture than do new needles. Real-time ultrasound allows the surgeon to see the needle and watch it puncture the hepatic vein 2 to 4 cm from the lateral surface. Small teflon tubing (0.9 mm i.d., <1.2 mm o.d.) at least 30 cm longer than the hepatic catheter is inserted through the needle and into the hepatic vein. This step is difficult and requires extreme patience and gentle touch; the tubing simply cannot be forced into the vein, and several attempts (punctures) may be required before success. Once in a hepatic vein, the tubing inserts without resistance and 40 to 60 cm are inserted before the needle is removed completely from the tubing and a tubing adapter inserted in the exterior end of the tubing. Blood withdrawal by syringe should be possible. Checks for correct catheter placement include injection of an air bubble in the tubing to observe its motion in response to respiration and heart beat (the tip should be in the thoracic cavity, close to the heart), palpation of the portal vein (if the tube is palpated in the portal vein, it is not placed correctly), or visual appraisal with ultrasound.

After ascertaining that the tubing is in a hepatic vein, the tubing adapter is removed and the permanent catheter is threaded over the small tubing until the tip approaches the liver. The small tubing is slowly withdrawn from the liver, keeping the tip of the permanent catheter as close as possible to the liver. When the exterior end of the small tubing can be grasped at the exterior end of the permanent catheter, the permanent catheter is inserted into the liver about 75% of its entire length and the small tubing is withdrawn completely. A tubing adapter is inserted in the catheter and checked for patency. By prior decision or visual appraisal with ultrasound the catheter is withdrawn slowly until the tip resides in the liver 2 to 4 cm from the vena cava and is in a patent location. Cuffs, visible outside the liver, are used to suture the catheter to the liver with 0 nonresorbable suture swaged to an atraumatic

Figure 1. Schematic view of bovine intestine, liver, and mesenteric veins. a. Diaphragm, b. liver, c. gall bladder, d. portal vein, e. splenic vein, f. jejunum, g. large intestine, h. cecum, and i. major branches of the mesenteric arcade. H. Sites for insertion of an hepatic catheter, M. sites for insertion of a mesenteric catheter, P. site for insertion of a portal catheter, and T. tip locations for mesenteric catheters.

is done by feel, because it is difficult to see and dissect simultaneously. When complete, 2 to 4 cm of the lateral surface of the portal vein is visible with retraction and the gastroduodenal vein may be seen on the lateral surface. A finger can be inserted completely around the medial surface of the portal vein immediately caudal to the liver, and the portal vein can be squeezed flat between a thumb and forefinger. Diameter of the vein (if it were round) is 2 to 4 cm.

The liver is palpated cranial to the gall bladder and in the area of the umbilical fissure to feel indentations corresponding to hepatic vessels (Figure 1). Real-time ultrasound (22) is extremely useful to scan the liver and identify the vena cava and hepatic vasculature. Fissures on the visceral surface of the left lateral lobe generally lie over branches of the portal vein. Necropsy and inspection and dissection of livers before attempting catheterization in live animals provide valuable insight and familiarity with liver surgical anatomy.
half circle needle. The procedure is to tie an overhand knot loosely in parenchyma, place the catheter on the knot, then secure it with a square knot. One of three or four such sutures should be tied to the insertion of the gall bladder to the liver which provides a firm anchor (T. Avery, personal communication). The catheter is filled with heparinized saline and placed aside.

**Portal Vein Catheterization.** Juxtaposition of the portal vein and portal catheter is visualized by inspection of the vein; the objective is to locate the tip within the liver, at least 5 cm from the porta hepatis (7). Insertion and anchor suture sites are selected on the wall of the portal vein. Number 2 nonresorbable suture swaged to a large atraumatic half circle needle is passed through a 7-mm bite of the lateral wall of the portal vein and one arm of the suture is firmly tied around the silicone sheath of the portal catheter. This is the primary anchor, and placement of the suture determines the ultimate location of the tip. As with hepatic vein catheterization, this procedure is essentially the Seldinger (21) technique. The tip of a .96-mm wire guide is placed in a 15-gauge, 5-cm needle, and the lateral surface of the portal vein is punctured cranial to the anchor suture. The guide is then threaded into the vein while digital palpation with a thumb and forefinger directs the guide toward the porta hepatis. The guide is retained by squeezing the vein, the needle is removed completely from the guide, and the portal catheter is inserted into the vein by threading it over the guide. Traction is then placed on the free arm of the anchor suture until the catheter can be fixed in position by that suture. The guide is removed and a tubing adapter inserted into the exterior end of the catheter. Location of the tip is checked by palpation and determining free patency with a syringe. The catheter should course along the ventral surface of the portal vein toward the left lateral location of the liver; if it courses toward the central or caudal dorsal lobe, it should be carefully withdrawn until the tip can be palpated within the vein, then redirected toward the left lateral position during reinsertion. This location optimizes patency after surgery. When the tip is toward the left lateral location and the catheter is patent, the anchor suture is tied carefully to avoid tearing the portal vein. A second suture is placed between the insertion and the liver by puncturing the vessel as medial as possible, retaining the catheter within the vein and suture, exiting the vein and tying. Patency should be checked regularly during these procedures; if the catheter is not patent as anchored and retained, then sutures should be cut and the catheter repositioned. A balky catheter during surgery will almost always have limited patency after recovery. A third suture is attached 4 to 6 cm caudal to the primary anchor, using omentum as attachment and procedures described for anchor sutures on the hepatic catheter. The catheter is filled with heparinized saline and retained away from the surgical field as much as possible.

**Mesenteric Vein Catheterization.** Two mesenteric vein catheters are inserted to provide two opportunities for optimal mixing of paraaminohippurate (PAH) during blood flow determinations and as insurance in case of loss of patency. The omentum is gently pulled dorsally and cranially by grasping the ventral edge within the visceral cavity. The small intestine is then exteriorized and displayed on the surgical drape. Towels soaked with saline are useful for protecting the intestines and keeping them moist. Mesenteric vasculature (14) is more apparent on one side of the intestines than the other and the side with the clearest view should be used for catheter insertion. The primary arcade of mesenteric vein is located and two locations as caudal as possible from the porta hepatis are selected (Figure 1). Catheters are inserted into smaller branches between the major venous arcade and the small intestine. The vein is cleared of surrounding tissue by blunt dissection. Manual pressure is used to control bleeding when the vessel is snipped with iris scissors. Commercially available catheter inserters, vein spreaders, or the tip of a cutting suture needle help insertion. Speed and gentleness are key factors to minimize local trauma and collapse of the vessel before insertion is completed. Catheter tips are palpated within the major mesenteric venous arcade to verify that the catheter did not course into another distal branch. Once tip placement and patency are confirmed, the catheterized vessel is tied off distal to insertion and catheters are anchored by nonresorbable suture to the mesentery, taking care to avoid puncture of the intestine or other
blood vessels. Several individual sutures 5- to 7-cm apart are used to “lead” the catheter toward the portal vein and away from the small intestine. Catheters are passed through the omentum by blunt puncture as close to the pancreas as possible before exteriorization.

**Mesenteric Artery Catheterization.** Our current first choice for access to arterial blood is catheterization of a mesenteric artery. Procedures are similar to those described for mesenteric vein catheterization, except an arterial branch close to the anterior mesenteric artery as possible is selected in the area of the proximal duodenum. After insertion of about 50 to 70 cm (Appendix 2), the visceral tissue is palpated to confirm direction of the catheter in the mesenteric artery toward the caudal aorta rather than toward distal portions of the mesenteric arterial system. The catheter is passed through the omentum with the mesenteric vein catheters.

**Exteriorization of Catheters and Closure.** Intestines are rinsed with sterile saline, blood clots, and other detached tissue removed, and a final rinse of saline containing 3% (vol/vol) glycerol is administered before returning intestines to the visceral cavity and repositioning of the omentum. Hepatic, portal, and mesenteric catheters are exteriorized through the skin using a stylette. Exits should be caudal to the incision over the paralumbar shelf and spine. Catheters are exteriorized individually in a line parallel to the spine to aid postsurgical identification. Immediately before exteriorization catheters are swabbed with an antiseptic (Furacin) to minimize possibility of infection in the exit wound.

Viscera in the surgical field are rinsed with saline; blood clots and detached tissue are removed with special attention to the area between the diaphragm and liver; and a final rinse of glycerol-treated saline (3%, vol/vol) is administered before closing. Peritoneum, individual muscles, and skin are sutured separately with a continuous interlocking stitch. Catheters are filled with heparinized saline described previously, then sealed. Arterial catheters are pinched with hemostats after filling with saline and before sealing to avoid reentry of blood into the catheter. Catheters are covered with a pouch of gauze and adhesive tape that is constructed and attached to the hair around catheter exits with branding cement. Mesenteric catheters are coiled individually and stacked between layers of gauze soaked in antiseptic solution to facilitate their movement if they are drawn into the body after surgery.

**Alternative Surgical Procedures**

The peritoneal cavity can be accessed by resection of the 12th right rib (4). An incision lateral and parallel to the rib is used to clear the rib of surrounding tissue including periosteum. The rib is resected with surgical wire 10- to 15-cm ventral to the spine, dislocated at the articulation with the intercostal cartilage, removed, and discarded. The medial aspect of the periosteum is bisected carefully to avoid puncture of the duodenum or the diaphragm. Rib retractors are used to provide surgical field.

An arrowhead or round point of bone can be used for insertion of catheter into the portal vein (16). The point is handmade of metatarsal bone using a handheld electric craft tool with abrasive grinding stones. A 1 cm shaft fits the lumen of the catheter and a 1-cm head is made as sharp as possible. Procedures for catheter placement and anchoring are the same as described previously. The point is forced through the wall of the portal vein and the catheter inserted towards the liver. The point is expelled by injection of saline through the catheter or manually rubbing the vein against the point. The expelled point enters the liver and is encapsulated and resorbed with no apparent adverse effects on the animal.

A dual or double-catheter apparatus can be used to place one sampling tip caudal to the splenic vein and a second tip either in the splenic vein (18) or porta hepatitis. Both catheters are sheathed in one 20-cm section of silicone rubber tubing starting 14 cm from the tip of the shorter (mesenteric) catheter. The longer (splenic or portal) catheter is individually sheathed from the common sheath to 3 to 6 cm from the tip. Both catheters are individually sheathed from the common sheath to exterior end or tubing adapter. Both catheters are inserted through one incision into an anterior branch of the main mesenteric arcade. By palpation, tips are located caudal to the splenic (shorter catheter) and either in the splenic vein or porta hepatitis (longer catheter). The apparatus is anchored by suturing through the vein wall between the cuffs on the longer catheter and
back through the vein wall, with the knot outside the vessel. Ligation at insertion is to control blood loss and not to restrict postsurgical movement of the catheters. Catheters are passed through the omentum and exteriorized together.

The hepatic portal vein can be catheterized through the liver as described for hepatic vein catheterization, except an hepatic portal vein within the liver is selected in a left lateral location (R. N. Heitmann, personal communication). The catheter is inserted until it can be palpated in the portal vein, then withdrawn 4 to 7 cm before anchoring.

Chronic indwelling arterial catheters can be inserted through the saphenous or circumflex branches of the iliac system in the rear leg (4). Insertion of saphenous catheters is 10 cm dorsal to the tuber calcis process on the medial surface. The incision and exposure of the vessel is determined by visualization or palpation. About 40 cm of catheter is inserted to place the tip in the caudal aorta. The catheter can be exteriorized close to the incision or on the spine by serial passes of a trocar. We prefer catheterization of the circumflex branch of the iliac to the saphenous, because exteriorization with visceral catheters is more convenient, and tip placement can be confirmed by palpation of the caudal aorta (5). A 20-cm incision is made perpendicular to the spine beginning just ventral to the transverse vertebral processes in the paralumbar fossa. After locating the artery cranial and medial to the tensor fasciae muscle, the visceral cavity is opened to access the caudal aorta. After insertion of the catheter, the tip is palpated in the caudal aorta, then 5 to 15 cm is inserted to place the tip in the aorta cranial to the iliac bifurcation.

Arterial blood can be obtained by elevation of a carotid artery (15), then insertion of a temporary catheter on sampling days, thereby eliminating dependence on a chronic indwelling catheter. A 15-cm incision is made over the jugular vein, and the jugular is cleared of surrounding tissue. Muscles are dissected to locate the carotid artery, and the artery is separated from the vagus nerve and surrounding tissue. The jugular vein is depressed, the carotid artery elevated, and the sternoccephalicus muscle is sutured over the jugular and under the carotid. Skin is closed, taking care not to puncture the artery. On sampling days, a local anesthetic is used over the elevated artery, and palpation or real-time ultrasound is used to locate the vessel. Catheterization kits (I cath 14-gauge I. V. Placement Unit. Delmed, Inc., Canton, MA) are used to access arterial blood; then the catheter is removed after sampling.

RESULTS

Infusion and Sample Collection

Catheters are not disturbed for 1 wk after surgery. At that time patency is confirmed and PAH infused to check catheter placement. The PAH is infused into mesenteric catheters individually, then simultaneously, through separate lines into both mesenteric catheters. Other venous catheters are sampled for determination of blood PAH concentration. Arterial samples are not required; a jugular sample can be used for determination of peripheral PAH concentrations (9), or relative concentration of PAH in portal and hepatic blood can be used to evaluate differences in infusion site(s). Portal PAH concentration should be greater than hepatic PAH concentration.

Portal and hepatic blood flow are measured by downstream dilution of PAH (12). The PAH (Sigma Chemical #A1422, Sigma Chemical Co., St. Louis, MO 63178) is dissolved in H2O and NaOH (.47 g NaOH/g PAH) to about 3/4 desired volume, filtered through #42 Whatman paper (Whatman, Inc., Clifton, NJ), titrated with a solution of NaOH or HCl to pH 7.4, then brought to volume with water. Previously we and others have dissolved PAH in sterile (or nonsterile) saline but addition of substantial amounts of NaOH and concomitant hypertonicity of the solution preclude the need to use saline (5). After making to volume, the solution is filtered through a .22-μ filter into a sterile container and stored at 0 to 5°C until used.

Concentration of PAH varies with size of animals infused; those weighing 250 kg or less can be infused with 7 to 10% (wt/vol) PAH at 1 ml/min, but larger animals (lactating cows) require 10% PAH at >2 ml/min to provide adequate blood concentrations (Table 1). Our goal is to attain concentrations of about 20 mg/L in arterial blood; that concentration usually will provide reliable concentration differences for calculation of blood flow. Blood concentration
TABLE 1. Arterial para-aminohippurate (PAH) concentration, portal-arterial (P-A), and hepatic-arterial (H-A) concentration differences and calculated blood flow one sampling day.

<table>
<thead>
<tr>
<th>Item</th>
<th>Lactating Holstein cow</th>
<th>Beef heifer</th>
<th>Beef steer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight, kg</td>
<td>565</td>
<td>390</td>
<td>198</td>
</tr>
<tr>
<td>Number of sampling</td>
<td>12</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Sampling interval, min</td>
<td>60</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>PAH infusion rate, mg/h</td>
<td>14,400</td>
<td>6343</td>
<td>5760</td>
</tr>
<tr>
<td>Blood PAH, mg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>27.3 ± .3</td>
<td>20.1 ± .3</td>
<td>24.0 ± 1.3</td>
</tr>
<tr>
<td>P-A</td>
<td>11.3 ± 1.1</td>
<td>7.9 ± .5</td>
<td>9.0 ± 1.3</td>
</tr>
<tr>
<td>H-A</td>
<td>8.8 ± .1</td>
<td>6.4 ± .8</td>
<td>8.6 ± 1.2</td>
</tr>
<tr>
<td>Blood flow, L/h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal</td>
<td>1251 ± 132</td>
<td>814 ± 8</td>
<td>650 ± 86</td>
</tr>
<tr>
<td>Hepatic</td>
<td>1622 ± 253</td>
<td>1003 ± 73</td>
<td>683 ± 99</td>
</tr>
</tbody>
</table>

1Mean ± SD.

of PAH is measured colorimetrically by manual (10) or automated (5) procedures. Standards for the assay are dilutions of PAH used in that sampling session. Blood flow is calculated by the equations (11):

\[
\text{Portal flow (liter/hr)} = \frac{IR}{P - A}
\]

\[
\text{Hepatic flow (liter/hr)} = \frac{IR}{H - A}
\]

where IR is infusion rate of PAH (mg/h) and P, H, and A are PAH concentrations (mg/L) in portal, hepatic, and arterial blood, respectively.

At the beginning of sessions, a priming dose of PAH is infused into a mesenteric vein as a bolus 15 times the infusion rate per minute. The PAH is infused continuously at least 20 min before the first sampling, then throughout the session. Simultaneous blood samples are taken from catheters in the portal vein, hepatic vein and artery to form a sample set. A Harvard syringe pump model 941 (Harvard Apparatus Inc., South Natick, MA) and 50-ml plastic syringes are used for PAH infusion. The plastic syringes are calibrated beforehand; we have found insignificant variation among syringes of a given make or brand. Larger pumps (Harvard model 2260) and syringes can be used to reduce frequency of syringe transfers during a sampling session. Infusion lines to connect the syringe to the mesenteric catheter are heat-sensitive tubing (TM Thermoplastic Scientifics, Inc., Warren, NJ) with coils to allow movement of the animal. The lines are gas sterilized before each use. The lines have tubing adapters at both ends and are connected with male-to-male connectors. A 45-μ filter (Millipore Inc., Bedford, MA) is inserted into the infusion assembly at either end of the infusion line to minimize risk of infusion of toxic substances. This filter is changed periodically during a sampling session.

Highly concentrated (5250 IU/ml) heparin is used to treat syringes for blood samples. Syringes are treated by drawing the solution into the syringe, then expelling all but the portion that remains in the tip of the syringe. Blood can be collected in nonheparinized syringes and transferred to rotes containing anticoagulant. If protocol requires large (20 to 40 ml) sample volumes, we use heparinized collection syringes that serve as centrifuge tubes for plasma harvest (Sarstedt Inc., Princeton, NJ).

Procedures for Blocked or "Bloaky" Catheters

All catheters may not bleed freely after surgery. The order of procedures for patency evaluation is to remove seal and 1) attempt to withdraw blood; 2) infuse 1 ml of heparinized saline, then withdraw; 3) infuse 5 to 10 ml saline, then withdraw; and 4) in venous catheters only, rapidly infuse 1 to 5 ml air followed by 10 ml of saline, wait a few seconds, then withdraw. If the catheter still is not patent, a
wire guide like those used in surgery can be inserted to clear the catheter or reposition the tip. Small teflon tubing like that used in hepatic catheterization can be stabilized with an .46-mm wire guide and inserted to obtain blood. Catheter lengths must be known to allow correct placement of the tip of this temporary catheter. If the flow check previously described indicates suboptimum placement of the portal catheter, this technique may be used to extend the portal catheter beyond the tip of the chronic catheter, thereby providing an opportunity to use an otherwise nonfunctional preparation. Extension of the hepatic catheter by this technique must be monitored carefully to avoid sampling the vena cava. Alternatively, blood can be withdrawn around a .46-mm wire guide that is inserted at each sampling, then removed.

Mesenteric infusion catheters are not patent consistently, nor do they need to be. If they are not patent, then infusion of saline, removal of the syringe, and observation of inward movement of saline is indication of a valid catheter. If saline does not draw in, PAH infusion should be attempted and concentration in portal samples evaluated.

Actions to avoid include forceful withdrawal of blood; withdrawal of 5 ml/min is adequate for sampling, and more forceful withdrawal tends to draw the tip to a location that blocks flow. Forceful withdrawal on a blocked catheter is not helpful, but only exacerbates the problem. Infusion of air into an arterial catheter is painful to the animal and is not recommended. Infusion of air into a carotid catheter can kill the animal.

Permanent loss of patency of portal and hepatic catheters is indicated by back-pressure when saline is infused or appearance of saline around the exteriorization site. Blocked mesenteric venous catheters cause obvious discomfort to the animal when infusion of PAH is attempted.

Other Problems and Remedies

During sedation and anesthesia, the animal may regurgitate digesta. Quick and proper placement of the endotracheal tube and delayed removal until the animal can clearly swallow and chew is essential too avoid aspiration of digesta, which almost always is lethal.

Major branches of the portal vein can be severed during the operation, particularly the gastroduodenal vein. If a major branch is ruptured, application of gauze pads and pressure are used to control hemorrhage. Satin key forceps are clamped around the tear and the tear is sutured with a Parker-Kerr suture pattern. The clamp is removed and the suture tied.

If the diaphragm is torn or ruptured during surgery, the wound edges are brought into opposition with Ellis forceps. The wound is closed with a mattress suture of #1 Dexon®. Lungs are inflated before final closure of the tear by applying pressure to the bag on the respirator. The suture is reinforced with a second row of simple continuous sutures.

Anorexia, lack of defecation, and metabolic alkalosis are symptoms of a twisted or blocked intestine. The blockage usually is due to strangulation of the intestine by one of the mesenteric catheters. Mesenteric catheters should be checked to ensure free movement, or capacity of the exteriorized portion to be drawn into the animal. Rarely, the problem is rectified spontaneously; if this is not apparent by resumption of eating and defecation by 5 d after surgery, euthanasia is recommended. For several weeks following surgery, mesenteric catheters may be drawn into the animal by action of visceral organs without apparent distress to the animal. Catheters in danger of disappearing under the skin should be extended by 2-cm sections of stainless steel tubing (Appendix 2) forced into the ends of the catheter and extender tubing.

Infection around the exteriorization sites can be minimized by sheathing teflon catheters in silicone rubber or silastic as previously described, treatment with antiseptic before exteriorization and periodic topical application of antiseptic around exteriorization sites after surgery. Abcesses should be treated by approved veterinary procedures, taking care not to puncture the catheter. Iliac and saphenous catheters (particularly teflon catheters) occasionally result in blockage of major circulation and loss of function of a rear leg. We know of no remedy, and we recommend euthanasia. Infection resulting from catheterization of an elevated carotid artery can cause chronic swelling and rupture of the vessel. Euthanasia is recommended in this instance as well.

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Necropsy

Usual observations at necropsy include adhesion of the liver to the diaphragm and scar tissue around insertion and exteriorization sites of catheters. Occasionally adhesions are apparent in the small intestine. Infection around catheter exteriorization sites can be associated with abscesses in the abdominal visceral cavity around catheter insertion sites. In cases of severe infection, preparations 6-mo-old or older, or use of the double catheter assembly, partial or complete blockage of the portal vein has been observed, with establishment of collateral flow to the liver. Sections of catheters inside vessels may be completely encapsulated on the wall of the vessel, or expelled from the vessel or its remnant. In most cases, however, catheters are in patent vessels. Teflon catheters are not sheathed or encapsulated within the vessel, and mesenteric or arterial catheters have a small lump of fibrin at the tip, which may or may not have caused loss of patency.

CONCLUSIONS

Fifty-five surgeries were performed between January 1982 and October 1987. Four surgeries are excluded from the summary because the experiment was short by design and required killing the steers. Of the remaining 51 surgeries (on 11 cows, 22 steers, and 18 heifers) 5 animals died of surgical complications and 2 had incorrect placement of the portal catheter. Of the remaining 44 animals, 25 had hepatic catheters and 24 had elevated carotid arteries in lieu of a chronic arterial catheter. Measurements were precluded in 41 of 44 animals by failure of the portal catheter first, by killing animals with patent catheters, or by removal of patent catheters. Therefore, patency of the portal catheter or experimental design determined the longevity of the preparations. The range of portal catheter patency (including animals killed with patent catheters or removal of patent catheters) was 1 to 16 mo, with a mean and standard deviation of 6.8 ± 3.8 mo. Time from surgery in months and percent patent portal catheters were: 2, 98%; 4, 77%; 6, 50%; 8, 30%; 10, 20%; and 12, 18%.

In conclusion, we have described current approaches and techniques for establishing and maintaining catheters that provide valid measurements. We want to emphasize that there may be and likely are other techniques that are appropriate to attain valid measurements. We encourage and continually search for improvements that result in preparations that enhance longevity of preparations, precision and accuracy of measurements, and well-being of the animal subjects. Users of these or other techniques are encouraged to study anatomical depictions (8) and attend or perform necropsies to familiarize themselves with anatomy.

REFERENCES

1 Barcroft, J., R. A. McAnally, and A. T. Phillipson. 1944. Absorption of volatile acids from the alimentary tract of the sheep and other animals. J. Exp. Biol. 20:120.
APPENDIX 1

Figure A1. Instruments for surgical packs and description of suture.

I. Number and description of surgical instruments:

<table>
<thead>
<tr>
<th>Instrument Type</th>
<th>Quantity</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Oschner ribbon retractors</td>
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<td></td>
</tr>
<tr>
<td>Towel clamps</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Rochester-Pean forceps</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Satinskey forceps</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Allis tissue forceps</td>
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<td>Sponge forcep</td>
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<td></td>
</tr>
<tr>
<td>Thumb forcep</td>
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<td></td>
</tr>
<tr>
<td>Meeker hemostatic forceps</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Halsted or Kelly straight forceps</td>
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</tr>
<tr>
<td>Halsted or Kelly curved forceps</td>
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<td></td>
</tr>
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<td>30-in obstetrical chain</td>
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<td></td>
</tr>
<tr>
<td>3.5-in obstetrical hook, blunt point</td>
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<td></td>
</tr>
<tr>
<td>#3 Knife handle</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>#4 Knife handle</td>
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<td></td>
</tr>
<tr>
<td>12-in needle holders</td>
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<td></td>
</tr>
<tr>
<td>6-in needle holder</td>
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<td></td>
</tr>
<tr>
<td>Scissors</td>
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<td></td>
</tr>
<tr>
<td>Iris scissors</td>
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</tr>
</tbody>
</table>

II. Suture source, size, and description:

- Ethicon, Inc. Somerville, NJ
  #0 Silk braided with tapered needle, No. K835
  #1 Silk braided with tapered needle, No. K845
  #2 Silk braided with tapered needle, No. K846

- Davis and Geck, Inc. Manati, PA
  #1 Dexon braided with tapered needle, No. 7312-71
  #2 Dexon braided with tapered needle, No. 7322-81

- S. Jackson, Inc. Alexandria, VA
  #3 Supramid extra with 1/2 circle reverse cutting needle, No. JJ03
APPENDIX 2

Figure A2. Description of catheters and sources of materials.

I. Catheter dimensions

A. Portal vein catheter:
   Teflon white spaghetti tubing, 1.27 mm i.d., 2.29 mm o.d.
   Silicone (.062 mm i.d., .125 mm o.d.) to 3.81 or 7.62 cm from burnished tip,
   1-m long with 17-gauge, dulled tubing adapter

B. Hepatic vein catheter:
   Same as portal except 1.3-m long and silicone to the following distance from
   tip;
   - Adult dairy cow, 16 cm
   - Adult beef cattle, 12 cm
   - Cattle <300 kg, 8 cm

C. Hepatic guide:
   Teflon, .86-mm i.d., 1.17 mm o.d., 1.57 m long with 20-gauge, dulled tubing
   adapter

D. Mesenteric vein catheter:
   Tygon®, 1.0 mm i.d., 1.78 mm o.d., TDMAC-coated, two cuffs 20 cm from
   tip and one cuff 30 cm from tip with 19-gauge, dulled tubing adapter
   - Dairy cows, 2.44 m long
   - Others, 1.52 m long

E. Mesenteric artery catheter:
   Tygon®, 1.27 mm i.d., 2.29 mm o.d., TDMAC-coated, two cuffs 50 to 70 cm
   from tip and one cuff 60 to 80 cm from tip with 17-gauge, dulled tubing
   adapter. For animals 200 to 400 kg live weight, 50 cm from tip to cuff; 70
   cm for larger animals
   - Dairy cows, 2.44 m long
   - Others, 1.52 m long

II. Sources of materials

A. Tubing adapters and stopper caps:
   - Becton-Dickinson #8207 (17 gauge)
   - Becton-Dickinson #8209 (19 gauge)
   - Becton-Dickinson #8210 (20 gauge)
   - Becton-Dickinson #8330 Sterile Male Stopper Caps
     From:
     Becton-Dickinson
     Rutherford, NJ

B. 2% Heparin TDMAC, #3921
   From:
   Polysciences, Inc.
   Warrington, PA

C. Teflon tubing for catheters:
   Teflon, #FCT2160, Chemflour Standard Wall Spaghetti Tubing, AWG16 (.050 in
   i.d., .090 o.d.) or AWG18 (.040 in i.d., .070 o.d.), White TFE
   Made by:
   Chemplast
   150 Day Rd.
   Wayne, NJ 07470
Figure A2. (continued) Description of catheters and sources of materials.

Available from:
Geophysical Supplies
Houston, Texas
Teflon, #SST-20, 20 gauge tubing, light weight, .034 in i.d., .006 wall
From:
Small Parts, Inc.
6901 NE 3rd Ave.
Miami, FL 33138

D. Silicon rubber tubing for teflon catheter sheaths:
#T5715-8, Silicone medical grade silastic tubing, .062 in i.d., .125 o.d.
From:
American Scientific Products
1900 NW 97th Ave.
PO Box 520276
Miami, FL 33152

E. Tygon® For mesenteric catheters:
Tygon® Microbore Tubing, #14-170-15D, .040 in i.d., .070 in o.d.
From:
Fisher Scientific
711 Forbes Ave.
Pittsburgh, PA 15219

F. Wire guides:
#TSF-18-145-BH, .018 in, fixed core
#TSF-32-145-BH, .032 in, fixed core
#TSM-32-145-BH, .032 in, movable core
#TSF-38-145-BH, .038 in, fixed core
#TSM-38-145-BH, .038 in, movable core
From:
Cook, Inc.
PO Box 489
Bloomington, IN 47402

G. Stainless steel tubing:
HTX 17 hypodermic tubing
HTX 19 hypodermic tubing
From:
Small Parts, Inc.
6901 NE 3rd Ave.
Miami, FL 33138