A TECHNIQUE FOR MONITORING NUTRIENT ABSORPTION IN THE CONSCIOUS, UNRESTRAINED PIG


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Summary

A surgical preparation allowing for the determination of apparent absorption in the conscious, unrestrained pig is described. The technique allows the collection of blood from the hepatic portal vein and vena cava, as well as the simultaneous determination of rate of flow through the portal vessel. A chronic electromagnetic blood flow probe is placed around, and a mechanical, hydraulically activated sampling needle attached to, the hepatic portal vein. An indwelling catheter is placed within the vena cava. By simultaneously determining nutrient concentration of hepatic portal and vena cava blood and then multiplying their difference by the rate of blood flow through the hepatic portal vein, one can make a point-in-time estimate of apparent absorption from the gastrointestinal tract.

(Key Words: Absorption, Hepatic Portal Vein, Pig.)

Introduction

An understanding of nutrient absorption patterns, as affected by manner of feeding and content of the diet, is of both academic and practical interest. A small alteration in total amount absorbed and (or) relative time of maximum absorption of a particular nutrient could significantly affect diet utilization. In the pig absorption from the gastrointestinal tract (GIT) has been estimated by monitoring the disappearance of a given nutrient from the lumen of the GIT by serial slaughter or gut cannulation techniques. Serial slaughter is the simpler of the two and allows for simultaneous assessment throughout the GIT. However, a large number of animals is required, and there is also the potential confounding effect of the stresses associated with death (Low, 1976, 1977). The surgical introduction of GIT cannulas has been used extensively (Low, 1976, 1977, 1978; Low and Zebrowska, 1977). Although this approach has a number of advantages over serial slaughter, as with serial slaughter, the ubiquitous intestinal microbes (Savage and Blumershine, 1974), as well as varying levels of endogenous protein (as reviewed by Kidder and Manners, 1978), may have a confounding effect on the estimation of nutrient absorption. The effects of the intestinal mucosa are also not reflected. The solution to the problem with these techniques, then, involves correlating the apparent disappearance of a given nutrient from the lumen of the GIT with its actual absorption or availability to the animal.

Following is a description of surgical and postoperative procedures allowing the collection of blood from the hepatic portal vein and vena cava. The procedure also allows simultaneous measurement of portal blood flow.

Experimental Procedure

Since the lumen of the digestive tract is actually external to the animal's systemic environment, the entry of a substance into the systemic environment from the intestinal lumen would seem a more critical measurement of absorption than would disappearance per se from the GIT environment. A natural site for monitoring the initial appearance of a substance from the GIT is the hepatic portal vein, through which all blood from the intestinal capillaries is...
funneled on its way to the liver (Romer, 1970). By comparing concentrations of a substance in blood before and after its entry into the intestinal circulation, one can estimate the net uptake from or loss to the GIT. By simultaneously measuring the rate of portal blood flow and multiplying this value by the above-mentioned before and after concentration differences, one can obtain an estimate of net nutrient flux or apparent absorption (AA). Rérat (1977) and Rérat et al. (1976, 1977, 1979) have used a similar technique for estimating various aspects of protein and carbohydrate absorption.

Since these samples and measurements are obtained in the conscious pig, free of either physical or chemical restraint, resultant estimates of nutrient absorption should closely reflect that of a "normal" situation.

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6 Carolina Medical Electronics, King, NC.
7 Tygon Microbore Tubing, Formulation S-54-HL, Norton Plastics and Synthetics Division, Akron, OH.

The surgery was performed on crossbred female pigs weighing between 12 and 15 kilograms. Females were used because they offer a surgically preferable anatomical profile. In each pig a sine wave electromagnetic chronic blood flow probe (BFP)6 (figure 1) was placed around, and a mechanical, hydraulically activated sample needle (HSN) (figure 2) (McGilliard, 1971) was attached to, the hepatic portal vein. Samples of systemic blood were obtained via an indwelling catheter (JC) consisting of a 45-cm length of medical grade PVC tubing7 (ID = 1.27 mm, OD = 2.29 mm) placed in the vena cava.

The BFP and JC were sterilized by soaking in an aqueous solution of benzalkonium chloride (750:1). The HSN was sterilized in ethylene oxide. All other surgical equipment and materials were autoclaved.

Fourteen hours before surgery, a group of previously isolated pigs was deprived of food and water. On the basis of general health and disposition, one animal from this group for surgery was selected. In preparation for the operation, the pig was injected with atropine

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Figure 1. Chronic blood flow probe (BFP). Note the plug end and attached skin "button" (upper center), the cable and probe collar (right center).
sulfate and anesthetized with halothane in oxygen administered via face mask. When sufficiently relaxed, the animal was intubated and anesthesia was maintained with halothane (2 to 3%) in an oxygen-nitrous oxide (2:1) mixture (Dziuk et al., 1964). Oxygen flow rate averaged 1 liter/min.

Hair was clipped and the associated area shaved clean from appropriate areas along the mid-ventral line and the right side of the animal. These and adjacent areas were thoroughly scrubbed with a germicidal surgical detergent, rinsed and dried. Shaved areas were then bathed with an aqueous solution of benzalkonium chloride (750:1) before the animal was draped. The pig was placed in left lateral recumbency in preparation for externalization of the receptacle end of the BFP and the tubes of the HSN. A 4-cm incision was made through the skin 3 to 4 cm posterior to the last rib and slightly ventral to the median of this side. Skin was separated from the underlying muscle over a radius of 3 centimeters. A curved intestinal clamp was used to separate skin and its underlying muscle from the point of the incision to a point on the mid-ventral line approximately 4 to 5 cm posterior to the sternum.

To externalize the tubes of the HSN, a 2-cm incision was made in the area of the eighth rib, slightly dorsal to the lateral median. The curved intestinal clamp was then used to open a subcutaneous route to the mid-ventral line.

The incisions were covered with sterile gauze and toweling, and the pig was placed on its back, secured and draped. An incision was made from the base of the sternum to the navel. The peritoneal cavity was exposed and the BFP “threaded” through the previously prepared subcutaneous channel. Sterile cotton towels, moistened with warm (37 C) saline solution, were used to “pack back” the intestine, stomach and liver from the hepatoduodenal ligament. Two orientation reference points, (1) the gastroduodenal vein that enters from the left side of the portal vein and (2) a ganglion of lymph nodes lying posterior to the gastroduodenal vein and on top of the portal vein, were located (figure 3).

In the young pig (12 to 15 kg) it was neces-
nary to place the HSN anterior to, and the BFP collar posterior to, the junction of the gastro-duodenal and portal veins. Although there was variation from pig to pig, the general area occupied by the lymph ganglion was usually the point at which the BFP collar was attached. Depending upon their exact orientation, these nodes were either partially stripped away and laid back or removed. Occasionally, a portion of the pancreas also had to be teased back.

Because the BFP and HSN were both attached directly to the wall of the vessel, the connective tissue of the hepato-duodenal ligament was stripped back. So that the collar of the BFP could be attached, the circumference of the portal vein along a 1.2 to 2.0 cm band was freed of connective tissue. The portal vein was then squeezed into the lumen of the BFP collar and the collar snapped shut. The associated cable was routed cranially along the hepato-duodenal ligament and up between the lobes of the liver to the point where it exited the peritoneal cavity. Two sutures along this route were usually sufficient to secure the cable. For the HSN, a section 1.5 cm in diameter on the ventral side of the vein was cleared of connective tissue. The base of the HSN, to which a patch of dacron cloth had previously been cemented, was attached to the wall of the vessel by tissue adhesive\(^8\). The associated tubes that were to be exteriorized were threaded through the previously prepared subcutaneous route. The towel packing was then removed, a final examination made and the incision immediately sutured shut. The animal was again placed in left lateral recumbency, and the BFP plug and tubing of the HSN was sutured to the skin at their respective site of externalization.

For insertion of the vena cava catheter, a 2-cm incision was made mid-dorsal anterior to the right scapula and a second incision was made mid-lateral along the right side of the neck. The curved intestinal clamps were forced subcutaneously between the two incisions and used to pull the tubing through the opening. The pig was placed in dorsal recumbency, and a

\(^8\) Histoacryl blue, obtained through Tri Hawk International, Quebec, Canada.
5- to 6-cm incision was made 3 to 4 cm to the right of and parallel to the mid-ventral line. The intestinal clamps were forced dorsally from the last incision toward the mid-lateral incision. By gripping the end of the catheter between the jaws of the clamp and pulling back, we brought the catheter into the mid-ventral incision. The end of the catheter was cut to form a beveled tip. The catheter was flushed and then filled with heparin-saline solution (200 IU heparin/ml). By blunt dissection, the external jugular was located and a 5-cm length was stripped free of associated connective tissue. Two 15-cm long pieces of nonabsorbable suture (Vetafil size 0) were looped under the vessel, approximately 3 cm apart, and used to lift the vessel upward. Thus distended, the vessel was punctured between the two supporting loops and the beveled end of the catheter threaded caudally into the lumen of the vessel. An appropriate amount of tubing was passed through the vein to allow the tip to rest within the vena cava. The two loops of suture around the jugular were tied snugly and the incisions sutured. At its point of externalization, the catheter was sutured to the skin. Alpha cyanoacrylate contact cement was used to glue nylon pouches (figure 4) over the sites of catheter externalization. These pouches were designed to contain the exteriorized tubes, to facilitate monitoring of wounds and to protect the point of externalization. The animal was immediately removed to a recovery room.

The pig was placed in a 1.2 x 0.76 m solid partition crate with a slotted floor in an isolation room of 23 C ambient temperature. A heat lamp provided supplemental heat. Usually, within 8 to 12 hr the pigs had regained coordinated movement. Water was first offered 12 hr and feed 24 hr after surgery. Water was provided ad libitum, and a fortified 18% crude

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Figure 4. Pouches used to protect the exteriorized portions of the HSN and the vena cava catheter. The pouch is shown in open (left) and closed (right) position.
protein, corn-soybean meal diet was available for 2 hr daily from 0800 to 0900 hr and from 2000 to 2100 hours. Wounds were frequently monitored and a .2% nitrofurazone powder was applied topically. The vena cava catheter was periodically flushed with a sterile solution of heparin in saline (200 IU heparin/ml). The HSN was not activated until the first sampling. The animal was handled daily starting on day 3 to condition it for the sampling procedure. Extreme care was exercised to prevent damage to and irritation of the healing wounds.

Although measurements usually could have been made within 5 days after surgery, we preferred to allow a 10-day period to ensure complete recovery.

Discussion

The main problem encountered in the first surgeries was post-operative intestinal strangulation due to entanglement of the intestines and the BFP cable. Suturing the cable to the hepatoduodenal ligament and running it between the lobes of the liver to the wall of the peritoneal cavity eliminated further intestinal strangulations. The first 72 hr postsurgery were critical. Sepsis was controlled through an emphasis on cleanliness and sanitation and close attention to the areas of equipment externalization. Maintenance of this environment minimized the irritation that could have resulted in considerable rubbing behavior. The deflection piece around the inside perimeter of the cage prevented the pig from rubbing the wall because it did not allow a firm footing for such activity (figure 5). The retaining pouches (figure 4) were a great help in preventing sepsis, not only by protecting the wound but by making the area accessible for observation and treatment, as well.

Initial experience indicated that daily postoperative handling of the animals markedly affected the ease and accuracy of the various measurements. Daily sham collections accustomed the pigs to handling. Thus, when actual

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10 Furacin, Eaton Veterinary Laboratories, Norwich, NY.
samplings were begun, the behavior pattern of the unrestrained pig had been established.

We noted in necropsy of certain pigs that within 5 days after surgery, adhesions had usually engulfed and, in the process, firmly attached the HSN, BFP and tubes within the peritoneal cavity. The body of the HSN sometimes shifted from its position perpendicular to the portal vein, and, when activated, the needle tip would pass completely through the vessel and end up outside the lumen. We were able to overcome this problem by pulling a vacuum on the sampling port while the needle was slowly being moved. Orientation of the needle tip within the lumen was indicated by the appearance of blood within the sampling tube. If repeated samplings were to be made within a short time, the needle tip was kept extended. After withdrawal of blood, the sampling port was flushed with the heparin-saline solution. Barring occasional mechanical failure of either the BFP or HSN, animal growth and associated increases in diameter of the portal vein became the time-limit factor because of the fixed orifice size of the BFP. In time, the BFP began to restrict the flow of blood through the portal vein. This, of course, would result in an inverse relationship between time and our estimates of portal blood flow and, thus, apparent absorption. Duration of usefulness, then, depended on the correctness of initial fit of the BFP and rate of growth of the portal vein. Injectable atropine was used immediately before surgery to counter the decreases in cardiac output and blood pressure associated with the use of halothane (Parker and Adams, 1978). This aided the proper matching of BFP orifice and portal vein diameter. There would seemingly be less of a time-limit factor if larger pigs were used, because the larger the initial portal vein diameter is, the slower its increase in circumference in relation to increases in cross-sectional area.

The technique described allows for the simultaneous measurement of (1) nutrient concentrations in the blood of the hepatic portal vein and vena cava and (2) rate of blood flow in the hepatic portal vein in the conscious, unrestrained pig. The values thus obtained can be used for estimating apparent absorption from the intestine at a given point in time.

**Literature Cited**


