ABSTRACT

A method of continuous in vivo flow measurement of plasma metabolites through the liver in calves was described. Five 2-wk old male calves were fitted with chronic catheters in the hepatic and portal veins and in the hepatic artery and with electromagnetic blood flow probes in the portal vein and in the hepatic artery. The reliability of measurements was tested during a 3-wk period in which calves were fed milk diets that curdled or did not curdle (uncurdled) in the abomasum. In comparison with a conventional curdled milk diet, the intake of uncurdled milk diet did not modify mean portal vein (47 to 49 ml•mn⁻¹•kg live weight⁻¹) or hepatic arterial (5.6 to 5.7 ml•mn⁻¹•kg live weight⁻¹) blood flows but did influence nycthemeral variations in portal blood flow rates, especially during the second part of the night.

INTRODUCTION

In ruminant nutrition studies, estimates of blood metabolite flow in the intact animal had mainly focused on portal drained viscera to assess the magnitude of digestion and absorption metabolites. Metabolite balances involving combined measurements of venous-arterial concentration differences and blood flows into vessels draining the gastrointestinal tract have been measured on dairy cows (20), beef steers (28), preruminant calves (7), sheep (6, 21, 30), and goats (4).

Received June 25, 1987.
Accepted November 18, 1987.
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TABLE 1. Curdled (CM) and uncurdled (UCM) milk diet composition.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>CM</th>
<th>UCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Protein</td>
<td>22.8</td>
<td>23.2</td>
</tr>
<tr>
<td>Mineral and vitamin mixture</td>
<td>2.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Following week, they received a milk replacer that did not coagulate in the abomasum (UCM) and in which caseins were substituted for whey proteins. The DM intake increased for all animals from 6 to 11 g/kg body weight and growth rate was $600 \pm 115$ g/d.

**Surgery**

General anesthesia by oxygen-halothane 4.5% (ICI Pharma, 51064 Reims, France) was induced using a mask, and anesthesia was maintained with oxygen-halothane 2 to 2.5% using an endotracheal tube connected to a closed-circuit anesthesia apparatus. The calf was placed in left lateral recumbency and an incision about 30 cm long was made along a line that was 2 cm behind the last rib. The incision was continued through muscle tissue and the peritoneal cavity was opened.

**Portal Vein and Mesenteric Artery Cannulations.** A polyvinyl catheter in the portal vein (Bruneau, Boulogne Billancourt, France) (1.5 mm i.d. by 2.5 mm o.d.) was introduced via a collateral mesenteric vein and directed through the main mesenteric vein for about 20 cm, until it reached the porta hepatis, as previously described in the calf (29) and goat (4). An arterial catheter (.8 mm i.d. by 1.2 mm o.d.) was placed 15 cm into a mesenteric artery. The catheters were fastened into place at their point of entry into the vessel with a tress cuff system. They were tested for patency and filled with 1250 units of heparin in .9% NaCl solution.

**Hepatic Vein Cannulation.** The hepatic vein catheter (1.5 mm i.d. by 2.5 mm o.d.) was introduced via the vena cava as described in sheep and in calves (18) but without a wire stilette. This catheter was then inserted through the vena cava wall, nearest its diaphragmatic passage, and manipulated caudally with the appropriate inclination to penetrate into one of the two major hepatic veins (Figure 1). The
A catheter was introduced 6 to 8 cm into this hepatic vein in order to avoid any contamination from blood flowing from the vena cava. The catheter was secured by purse string silk suture at the vena cava wall and tested and filled with a heparin-saline solution. All catheters were prepared as shown in Figure 2.

**Blood Flow Probe Implantation.** The portal vein was exposed between the porta hepatic and the gastrosplenic vein junction. An electromagnetic blood flow probe (14 to 15 mm i.d., GOULD INC., Statham Instruments Division, Oxnard, CA) was placed around the portal vein. The left branch of the hepatic artery, which was most accessible, was freed from its surrounding tissues and nerve for a length so that it did not kink when the sensor was in position. The blood flow probe (3 mm i.d.) was put around the artery. The flow sensor and cable were fastened to the surrounding mesenteric tissue so that they were kept perpendicular to the vessel.

**Blood Flow Measurement.** Blood flow was calculated from the graphic-recorder, flow-probe regression line established before surgery and a conversion factor determined from variations in packed cell volume:

\[ Y = \frac{(a + bX)}{CF} \]

where

- \( Y \) = blood flow (ml/mn),
- \( X \) = graphic blood flow,
- \( a \) and \( b \) = coefficients of linear regression equation, and
- \( CF \) = conversion factor.

Values obtained from blood flow hepatic artery were multiplied by 2 to account for the two branches after verifying that blood flows in both branches of the artery were similar. Acute blood flow measurements during surgery and size estimation at necropsy were used to prove that blood flows in both branches of the artery were similar.

**Postoperative Management.** Bicillin (10^6 units, 1 g) was administered into the abdominal cavity during surgery and thereafter, intramuscularly, every day for the following 5 d. Catheters were flushed twice daily for the 1st wk after surgery and then once daily. After flushing with about 5 ml of .9% NaCl solution, catheters were filled with an anticoagulant solution (1250 units heparin/ml of .9% NaCl) containing thimerosal (Sigma Chemical Co., St. Louis, MO) (.02 mg/ml) to prevent infection. Sterile solutions and syringes were used for verifications and catheter needles; catheter tips and plugs were swabbed with 95% ethanol containing thimerosal 1% (wt/vol).

**RESULTS**

Postoperative recovery was rapid in the five calves fitted with catheters and electromagnetic probes. Eating habits were resumed in 4 d. In four calves, catheters were functional during the 2 wk of the experiment. Necropsy results indicated that the end of portal vein catheters was located at the porta hepatis or 1 to 2 cm inside the liver. Hepatic vein catheters were positioned in a central hepatic vein in 4 calves and, in one case, in a small collateral vein. Some catheters were partially covered with a fibrous sheath that did not reach the tip of the catheter, except in one case. Contacts between probe electrodes and portal and artery vessels were satisfactorily established 3 to 5 d after surgery. Portal and arterial blood flow decreased 10 to 20% 3 wk after flow probe implantation. This corresponded to a thickening of the walls in these vessels. Arterial blood flow variations were partially modified.

![Figure 2. Catheter for three hepatic vessels.](image-url)
by the animal position, which required that the baseline be verified frequently.

During the complete nycthemere, mean blood flow rates in the portal vein and in the hepatic artery were not significantly different between diets. They amounted to 47.0 ± 8.4 ml • mn⁻¹ • kg LW⁻¹ (LW = live weight) and 5.6 ± 0.9 ml • mn⁻¹ • kg LW⁻¹ for the diet UCM and 49.2 ± 6.5 ml • mn⁻¹ • kg LW⁻¹ and 5.7 ± 1.3 ml • mn⁻¹ • kg LW⁻¹ for diet CM, respectively. The relative contribution of these two vessels in the total blood flow throughout the liver accounted for 89.3 and 89.7% for the portal vein and for 10.7 and 10.3% for the hepatic artery with the UCM and CM diets, respectively.

Nycthemeral variations in portal blood flow rate (percent of daily mean flow rate) were significantly different between diets. With the CM diet, portal flow rate decreased from 1 to 4 h after the morning meal (-12%; P<.05) and after the second meal (-7%; P<.05), but it increased progressively (+15%; P<.05) from 4 to 8 h after the second meal and remained constant throughout the night (Figure 3). With the UCM diet, the portal blood flow rate increased (+9%, P<.05) 1 h after the meal. Then it decreased from 2 to 5 h (-6%; NS) after the morning meal but rose during the same period after the second meal (until +8 h) but decreased during the second part of the night (-12%; P<.01) (Figure 3).

Nycthemeral variations in hepatic arterial blood flow were similar for both diets (Figure 4). The hepatic arterial flow increased immediately after the morning (+22%; P<.05) and the second (+16%; P<.05) meals and then decreased progressively for 5 h after the morning (-40%; P<.05) and the second (-20%; P<.05) meals. During the first part of the night the hepatic arterial flow rate was unchanged and low (85% of daily flow rate) but sharply increased during the second part of the night (+36%; P<.05).

**DISCUSSION**

**Cannulation Aspects**

Formation of blood clots in the catheters was easily avoided by daily flushing without administering any anticoagulant drugs, as suggested elsewhere (8). Introducing 10 to 20 cm of the catheter into the portal vein and hepatic artery usually prevented the development of fibrous sheath at the catheter tip. The implantation of a hepatic catheter through the vena cava wall close to the diaphragm allowed a rapid and reliable cannulation of a main hepatic vein, and, therefore, the most represen-
tative samples of hepatic blood could be collected.

**Blood Flow Measurements**

Electromagnetic flowmetry was advantageous because it provided instantaneous and continuous determinations of blood flow. This technique proved to be well-adapted to simultaneous and reliable measurements of portal and hepatic arterial blood flows in dairy calves for 3 wk. Later on, the blood flow signal intensity decreased and was the consequence of a vessel wall thickening, as observed at necroscopy. This was not due to the development of collateral circulation as reported in pigs (25).

The mean portal blood flow rate (47 to 49 ml·mn⁻¹·kg LW⁻¹) determined in our study by electromagnetic flowmetry is in agreement with previous data determined in dairy calves by dye dilution [i.e., 37 (32), 45 (17), and 40 (11) ml·mn⁻¹·kg LW⁻¹] and was similar to data measured by dye dilution in cows [31 ml·mn⁻¹·kg LW⁻¹ (20)] and sheep [43 ml·mn⁻¹·kg LW⁻¹ (21)] or by electromagnetic flowmetry in pigs [40 ml·mn⁻¹·kg LW⁻¹ (24)] and goats [39 ml·mn⁻¹·kg LW⁻¹ (4)].

Direct measurements of mean hepatic arterial blood flow rate in dairy calves are scarce. In the day-old calf, it amounted to 7.8 ml·mn⁻¹·kg LW⁻¹ (11), which was higher than our values based on 3-wk-old calves (5.7 ml·mn⁻¹·kg LW⁻¹). In comparison with data determined by differences between total blood flow rates throughout the liver and portal blood flow, the arterial value in our study was similar or lower than that in several adult animals: 13.2 (21) and 7.4 ml·mn⁻¹·kg LW⁻¹ (6) in sheep, 7 to 9 ml·mn⁻¹·kg LW⁻¹ (2) in cows, and 6.6 ml·mn⁻¹·kg LW⁻¹ (21) in dogs. The contribution of hepatic arterial blood flow (amounted to 10%) of the total blood flow throughout the liver was slightly lower than the hepatic arterial contribution in these above mentioned species (15 to 27%) except for data obtained from direct assessments of arterial blood flow (5 to 10%) (3).

Feed intake usually leads to hyperemia for 3 to 6 h in humans (22), cats (14), dogs (15), sheep (10, 13), and cows (20). Hyperemia is closely related to portal blood flow increase (14). This was confirmed in our study with a milk diet that did not curdle in the abomasum. Because digestion was not delayed, portal flow was high for 5 to 6 h after intake and decreased during the night due to reduced intestinal nutrient absorption (12). Feed intake also induced a greater cardiac rhythm and pressure (5), which explained the rapid but temporary increase in hepatic arterial flow 1 h after feeding, as previously observed in lambs (13). The important rise in hepatic arterial flow at the end of the night would probably be due to the recovery of animal activity and was an anticipation phase to feed intake, as in dogs (15) where the central and sympathetic nervous systems led to 14% of the hyperemia noted.

The intake of the milk diet that curdled in the abomasum deeply influenced the nycthemeral variations in portal blood flow. The rapid increase in portal blood flow after the meal intake was probably caused by the bolus when it reached the abomasum, and by the intestinal absorption of small molecules such as glucose and free amino acids (27, 31). The clot formation in the abomasum led to a decreased portal flow for 4 h, probably because of the drop in mesenteric blood flow [which contributed to 65% of the portal blood flow in young calves (11)], following a lack of nutrient absorption during this period. The slow and progressive reduction of clot from 5 h after intakes and throughout the night restored absorption (27, 31) and therefore kept higher mesenteric and portal blood flows until the end of nycthemere.

In conclusion, this technique of continuous measurement of plasma constituent hepatic balance in conscious calves, tested with five animals, proved to be reliable over an extended period (3 wk). Suitable for animals that weigh 20 kg or more, the technique was used successfully in 3-wk-old dairy calves for studies on the role of liver in the metabolism of plasma lipids and lipoproteins, amino acids, cortisol, and digestive and growth hormones (9, 12, 16).

**REFERENCES**


