Blood Flow to Hindquarters of Steers Measured by Transit Time Ultrasound and Indicator Dilution

J. H. EISEMANN, G. B. HUNTINGTON, and C. L. FERRELL
US Department of Agriculture
Agricultural Research Service
Roman L. Hruska US Meat Animal Research Center
Clay Center, NE 68933
and
Beltsville Agricultural Research Center
Beltsville, MD 20705

ABSTRACT
The objective was to compare blood flow to the hindquarters of steers measured by transit time ultrasound with blood flow determined by indicator (p-aminohippurate) dilution. Five Hereford steers had ultrasonic flow probes on the abdominal aorta and catheters in the abdominal aorta and inferior vena cava inserted through both sets of circumflex iliac vessels. Indicator was infused continuously into the abdominal aorta through both arterial catheters simultaneously, then through each of the arterial catheters in succession. Samples of blood from the inferior vena cava and jugular vein were taken during infusion for measurement of p-aminohippurate. Blood flow determined by the ultrasonic flow probe was averaged over each blood sampling interval. Compared with the ultrasonic measurement, there was no difference in mean blood flow measured by p-aminohippurate, regardless of method of infusion. Correlation of individual values between ultrasound and p-aminohippurate was .87 when p-aminohippurate was infused into both arterial catheters, .44 when p-aminohippurate was infused into the left arterial catheter, and .78 when p-aminohippurate was infused into the right arterial catheter. The respective ranges for ultrasonic measurements and p-aminohippurate were 3.62 to 10.99 L/min and 2.25 to 30.43 L/min. Although means by the two methods do not differ, there is a greater range and incidence of occasional high values with p-aminohippurate dilution.

INTRODUCTION
Blood flow is an important component of tissue metabolism and a key factor in determination of net and unidirectional uptake or release of nutrients across a tissue bed. It is therefore of interest to evaluate critically and compare the advantages and disadvantages of methods used to measure blood flow.

Several methods have been used to measure blood flow to the hind limbs of cattle or sheep including dye (2) or indicator (7, 11) dilution, tritiated water dilution equilibrium (10), and radioactive microspheres (10). Only a single method has been used in all studies except that of Oddy et al. (10), who showed no difference between flow values obtained with tritiated water and microspheres.

Dilution methods require adequate mixing between site of infusion and sampling, no loss of indicator from the blood stream between injection and sampling site, and determination of the concentration of infused marker in blood samples obtained during infusion. Average blood flow is therefore measured and pulsatile flow or change in blood flow over intervals of seconds or minutes is not detectable. Dilution methods have the advantage of determining flow in several vessels from a single infusion site (7); they are not dependent on the in vivo
stability of an implanted flow probe; and measurements can be repeated over time in the same animal. By infusion of radioactive microspheres, instantaneous flow to specific tissues is determined following tissue dissection and quantification of radioactivity. Although microspheres containing different isotopes can be used for successive measurements in a single animal, their expense and relatively short half-life [for example, $^{51}$Cr = 28 d, $^{141}$Ce = 33 d, $^{46}$Sc = 84 d, $^{85}$Sr = 65 d; reference (12)] limit this capability for long-term studies.

In addition, the number of successive injections is limited by the ability to separate energy peaks due to the principal photon of each isotope during quantitation.

Electronic cuff-type flow meters have the advantage of providing a direct estimate of flow, requiring no chemical analysis. They measure acute flow changes and can be used for chronic preparations as well. Electromagnetic (3, 5, 13, 15) and Doppler (13, 15)-type probes have been used extensively in various tissues or organ systems. These methods measure velocity and change in velocity well, but both require measurement of vessel diameter for determination of volume flow. Vessel diameter is taken from the size of the probe tightly fitted to each vessel. Recently, an ultrasonic flow probe has been designed that measures volume flow independent of vessel diameter (4). Flow measurements by ultrasonic probes have been validated against flow by microspheres when implanted on the cranial mesenteric and splenic arteries in the ewe (1) and against flow by electromagnetic probe when implanted on the external pudic artery in dairy cows (5) and the ascending aorta in dogs (6). Because of the stable zero offset, relatively loose vessel fit, and measurement of blood flow independent of vessel diameter, the ultrasonic flow probe is attractive for long-term studies of regional blood flow. Implants of 3 mo have been reported (6).

The objective of this study was to compare blood flow to the hindquarters of steers obtained by the ultrasonic flow probe with blood flow obtained by indicator dilution [p-aminohippurate (PAH)]. In addition, we were interested in the method of PAH infusion that would give most consistent agreement with values obtained using the ultrasonic flow probe.

**MATERIALS AND METHODS**

Five Hereford steers (263 ± 5 kg body weight) were used. They were adapted to confinement in individual stalls for 4 wk prior to surgery. Feed was withheld for 48 h and water for 24 h before surgical insertion of indwelling catheters and placement of an ultrasonic blood flow probe. The steers were initially sedated with an intrajugular injection of sodium thiomylal in sterile $0.15 M$ NaCl (1.26 g/100 kg BW) and then maintained under respiration anesthesia with 1 to 4% halothane. With the steer in right lateral recumbency, a 16-mm diameter ultrasonic flow probe (Transonic Systems, Inc., Ithaca, NY) was inserted around the abdominal aorta distal to the renal vessels through a side incision on the left flank perpendicular to the spine. The incision was extended to the patella to access the caudal branch of the circumflex iliac vessels. Teflon catheters (i.d., 1.27 mm; o.d., 2.29 mm) were inserted 25 to 40 cm into the circumflex iliac artery and vein until the catheter tips were located by palpation of the vessels distal (1 to 4 cm) to the probe body in the aorta and in a similar position in the inferior vena cava (IVC), respectively. The incision on the left side was then closed and the steer was moved to left lateral recumbency.

A similar incision was made on the right side for insertion of a second set of catheters into the right circumflex iliac vessels and palpation of catheter tips in the abdominal aorta and vena cava. The probe cable and catheters were exteriorized on the lumbar shelf and stored in a superficial gauze patch. A partially inflated inner tube was placed under the shoulder area of the steer during these procedures to minimize pressure trauma to the radial nerve. Following surgery and between sampling days, catheters were filled with a $0.15 M$ NaCl solution containing 200 U heparin/ml, 1% (vol/vol) benzyl alcohol, and 1% (vol/vol) procaine penicillin G. Catheter placement was again verified at necropsy. Following surgery and between sampling days, catheters were filled with a $0.15 M$ NaCl solution containing 200 U heparin/ml, 1% (vol/vol) benzyl alcohol, and 1% (vol/vol) procaine penicillin G. Catheters were flushed between samplings and filled with $0.15 M$ NaCl containing 25 U heparin/ml (8). Catheter placement was again verified at necropsy.

Before and after surgery, the steers were fed a high concentrate diet containing 18.4% crude protein and 2.96 Mcal calculated metabolizable energy/kg DM. The steers were fed in two equal aliquots at 12-h intervals with average

intake of 4.26 kg (SEM .07 kg) DM daily during measurements. Measurements were made 6 wk after surgery, following completion of another experimental protocol.

On the morning of infusion, a temporary catheter was inserted into the jugular vein (JV) to obtain blood samples for background concentration of PAH. Because PAH is not excreted or metabolized in the head, JV and arterial PAH concentrations are identical (7). A 5% (wt/vol) solution of PAH was prepared by combining 50 g PAH with 23.4 g NaOH, adjusting the pH to 7.4 with 1 N HCl, and diluting to volume with deionized water. A priming dose (15 ml) was infused into each arterial catheter followed by a continuous infusion at 1.2 ml/min using a syringe pump (Model 915, Harvard Apparatus, South Natick, MA). A filter (.22 μM) was placed between the infusion syringe and tubing to the catheter. Three methods were used to infuse 1.2 ml/min PAH. Following the priming dose, .6 ml/min was infused into each of the arterial catheters (method 1). After 25 min of continuous infusion, a set of six simultaneous blood samples were taken at 5-min intervals from the jugular vein and one, or both (where patency allowed), catheter(s) in the inferior vena cava. The infusion was then switched to the right artery only, (method 2) and after a 10-min lag period a second set of six simultaneous blood samples were taken at 5-min intervals. Finally, the infusion was switched to the left artery only (method 3) to take a third set of blood samples in like fashion. Data in Table 1 illustrate the stability of background PAH concentration.

Steers were sampled on 2 d within a weekly interval with the exception of one steer (steer 097). Its venous catheters were no longer patent after four samples on its first sampling day.

All blood samples were collected in heparinized syringes and immediately placed on ice. Packed cell volume (PCV) was determined and the samples centrifuged at 1500 x g for 20 min at 4°C to obtain plasma. Concentration of PAH in plasma was determined by automated procedures (Technicon Industrial Method No. 216-727, Technicon Industrial Systems, Tarrytown, NY). Standards were dilutions of the PAH infused. The average intraassay CV for determination of PAH was less than 1%. The

<table>
<thead>
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<th>Steer</th>
<th>Infusion d 1</th>
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<tr>
<td></td>
<td>X</td>
<td>SE</td>
</tr>
<tr>
<td>2358</td>
<td>11.3</td>
<td>.29</td>
</tr>
<tr>
<td>089</td>
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<tr>
<td>115</td>
<td>10.5</td>
<td>.07</td>
</tr>
</tbody>
</table>

Each mean is the average of 18 samples.

PCV was used to convert plasma PAH concentration to whole blood PAH concentration, assuming there was no PAH in cells (unpublished observations), as follows:

\[
\text{Blood } [\text{PAH}] = \text{Plasma } [\text{PAH}] \times (1-\text{PCV})
\]

Blood flow from PAH dilution was calculated by using the following equation:

\[
\text{Blood flow (L/min)} = \frac{\text{PAH infusion rate, mg/min}}{[\text{PAH}]_{\text{IVC}} - [\text{PAH}]_{\text{JV}}}
\]

where \([\text{PAH}]_{\text{IVC}} - [\text{PAH}]_{\text{JV}}\) is the concentration difference for PAH (mg/L) between the inferior vena cava and jugular vein.

The transit time ultrasonic blood flow meter (Model T101, Transonic Systems, Inc., Ithaca, NY) was used to measure blood flow over the interval (about 1 min) of each blood sampling during PAH infusion. Integrated average blood flow rates monitored at 3-s intervals were averaged to obtain a single blood flow rate, determined by ultrasound, for each blood sampling interval. Baseline output from all ultrasonic probes had been stable for several weeks.

A paired t statistic was calculated to compare the concentration of PAH in blood samples obtained from the two abdominal venous catheters and to compare blood flow rate determined ultrasonically with that determined by PAH dilution both within and across steers. Comparisons were made by method of infusion. Correlation coefficients comparing the individual values for ultrasonic flow probe with


TABLE 2. Whole blood p-aminohippurate (mg/L) from each of two catheters in the vena cava.  

<table>
<thead>
<tr>
<th>Steer</th>
<th>Infuse both</th>
<th>Infuse left</th>
<th>Infuse right</th>
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<tbody>
<tr>
<td></td>
<td>Left vein²</td>
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</tr>
<tr>
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<tr>
<td>Mean</td>
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<td>23.8</td>
<td>1.78</td>
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</table>

¹ Means differ: aP<.01, bP<.10. Each mean is for six samples.
² p-Aminohippurate (PAH) was infused into both arterial catheters in the abdominal aorta.
³ The PAH was infused into the abdominal aortic catheter inserted through the left circumflex iliac artery.
⁴ The PAH was infused into the abdominal aortic catheter inserted through the right circumflex iliac artery.
⁵ Left vein (LV) = Catheter in the inferior vena cava inserted through the left circumflex iliac vein.
⁶ Right vein (RV) = Catheter in the inferior vena cava inserted through the right circumflex iliac vein.
⁷ SEₐ = Standard error of the difference.

Indicator dilution were calculated for each method. Correlation, rather than regression, was used because all sample values were included and the slope of a regression line would be heavily biased by a few outlying values.

RESULTS

Two steers had two patent catheters in the inferior vena cava (Table 2). Overall, similar values were obtained for PAH concentration in blood sampled from the two catheters (P>.10). Concentration tended to differ between the two catheters in the vena cava of one steer (steer 089). There were no observations of catheter placement at necropsy to explain this discrepancy. This difference was most pronounced when the infusion included the right artery. During the infusions into the right artery only, PAH concentration was lower (P<.10) in blood sampled from the venous catheter inserted on the ipsilateral side.

Overall, there was no difference (P>.10) in blood flow estimated by the two methods regardless of the vessel(s) used for PAH infusion (Table 3). There was also no trend for one method to give higher mean values than the other. The two values obtained for steer 089 were different (P<.05) when PAH was infused on the right side only as a result of the low

TABLE 3. Blood flow comparison (L/min) between p-aminohippurate (PAH) dilution and transit time ultrasonic probe by method of PAH infusion. ¹

<table>
<thead>
<tr>
<th>Steer</th>
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<th>Infuse left</th>
<th>Infuse right</th>
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<td>PAH Probe</td>
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<td>.34</td>
<td>5.46</td>
</tr>
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</table>

¹ Means differ: ᵃP<.05. Each mean is for six samples.
² The PAH was infused into both arterial catheters in the abdominal aorta.
³ The PAH was infused into the abdominal aortic catheter inserted through the left circumflex iliac artery.
⁴ The PAH was infused into the abdominal aortic catheter inserted through the right circumflex iliac artery.

BLOOD FLOW WITH ULTRASOUND AND DILUTION

I2

Figure 1. Comparison of blood flow obtained by the ultrasonic flow probe and p-aminohippurate (PAH) dilution in individual samples. Values from individual steers are coded as follows: 089, •; 125, ○; 115, ▲; 2358, △; and 097, ■. Lines of unity between the two methods are included. See text for correlation between the two methods. A. p-Aminohippurate was infused into both arterial catheters; B. PAH was infused into the abdominal aortic catheter inserted through the left circumflex iliac artery; C. PAH was infused into the abdominal aortic catheter inserted through the right circumflex iliac artery. A single point (probe flow 8.9, PAH flow 30.4) was omitted from Figure 1C for clarity.

The correlation between individual pairs of values for blood flow estimates with PAH dilution and transit time ultrasound was .87 (n = 52, P<.001) when PAH was infused into both arteries, .44 (n = 48, P<.002) when PAH was infused into the left artery only, and .78 (n = 48, P<.001) when PAH was infused into the right artery only (Figure 1). For all sample values across the three infusion methods, the mean, median, mode, and range (L/min) for ultrasonic measurements were 5.99, 5.44, 4.33, and 3.62 to 10.99 and for PAH dilution were 6.15, 5.37, 5.14, and 2.25 to 30.43, respectively.

DISCUSSION

Data obtained from sampling two venous catheters suggest inadequate mixing of PAH with blood caused variation in measurement of blood flow to the hindquarters of at least one animal. Mixing problems in the use of indicators to measure regional blood flow have been discussed by Ushioda et al. (14), who recommended inclusion of the heart between the site of infusion and sampling when possible and indicated a venous infusion and sampling site will not give consistent mixing because of the laminar, rather than turbulent, nature of venous flow. They did not address arterial infusion with venous sampling, which should aid mixing by inclusion of the capillary bed. However, in studying blood flow to the hindquarters, indicator infused into the arterial system must be equally divided between the circulation of each hindquarter and thoroughly mixed at the confluence of the external and internal iliac veins to insure a completely mixed sample. The occasional high values, as indicated by the range in blood flow of PAH (2.25 to 30.43) compared with the ultrasonic probe (3.62 to 10.99), also indicate occasional mixing problems with the dilution method. Check of catheter placement at necropsy did not give consistent explanations for this observation.

The agreement between average flow (mean of six samples) obtained using PAH and the ultrasonic flow probe indicates the validity of either method when average flow is of interest. Dilution methods have been used to measure average hind limb (2) or hindquarters (7, 11) blood flow in previous studies, although values
obtained have not been compared with another method.

However, in evaluation of individual samples (Figure 1) it is evident that there is occasional discrepancy between the two methods, most evident when infusion was through a single arterial catheter. Thus, where acute changes in blood flow are of interest, the ultrasonic flow probe provides greater accuracy and capability than the dilution method. The need for several blood samples to determine an average blood flow for a given time was previously suggested for portal blood flow measured by dye dilution (9).

Overall, the method used to measure blood flow to the hindquarters depends on specific experimental objectives. Indicator dilution (PAH) is appropriate for determination of average flow rates. Inconsistencies in mixing and analytical errors in PAH determination contribute to variation in flow measurement by this method. In particular, flow will be overestimated due to streaming of marker if the sampling catheter is outside of the stream and underestimated if the sampling catheter is in the stream. However, repeated measures over time within animal are possible as long as catheters remain patent. If one does use dilution for measuring blood flow to the hindquarters, we recommend infusion into two arterial catheters for optimum mixing of PAH as well as sampling from two venous catheters to obtain diagnostic information on potential mixing problems. The agreement shown between the two methods (Figure 1A compared with Figure 1B,C) supports this concept. These observations also have application to isotopic infusions to a regional circulation for study of metabolism. Alternatively, the ultrasonic flow probe provides the capability to measure average as well as acute changes in blood flow. Bias will tend to underestimate flow, especially in cases where the ultrasonic signal is dampened by inclusion of adipose tissue in the signal path. The stable zero offset and nonconstrictive vessel fit also provide the capability for longitudinal studies with growing animals.

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