SUMMARY We measured cerebral blood flow using both the radioactive microsphere technique and the cerebral venous outflow technique in dogs anesthetized with chloralose. The effect of sympathetic stimulation on cerebral blood flow was observed during both normocapnia and prolonged hypercapnia using both blood flow techniques. The increase in blood flow with hypercapnia was the same with both methods. During hypercapnia the venous outflow method showed a 38% decrease and microspheres an 18% decrease in cerebral blood flow with sympathetic stimulation. At normal CO2 stimulation caused a decrease in cerebral venous flow: no change was observed with the microsphere method. Analysis of the blood flow patterns to extracerebral tissues and evaluation of extracerebral arterial reference samples failed to prove the existence of axial streaming and subsequent skimming of microspheres within the cephalic circulation. It is concluded that direct electrical stimulation of the sympathetic innervation of the cerebral vessels is capable of reducing cerebral blood flow even during a profound hypercapnic vasodilation.


PREVIOUS studies from this laboratory have demonstrated an α receptor-mediated cerebral vasoconstriction which occurred independently of changes in cerebral spinal fluid pressure, as well as arterial PO2, PCO2, and pH (D'Alecy and Feigl, 1972; D'Alcey, 1972). The studies reported here were designed to: (1) demonstrate the global and regional sensitivity of the cerebral circulation to elevations in arterial carbon dioxide tension using, simultaneously, the radioactive microsphere technique and the venous outflow technique: (2) determine by simultaneous flow determinations (microspheres and venous outflow) if sympathetic vasoconstriction could compete with hypercapnia-induced increases in cerebral blood flow; and (3) evaluate the contradictory evidence (Alm and Bill, 1973; Alm, 1974; Meyer et al., 1977; Heistad et al., 1977) that there is little or no change in cerebral blood flow as determined by radioactive microsphere technique when cerebral sympathetic nerves are activated.

Methods

General Preparation

Adult male dogs weighing between 20.4 and 30.0 kg were anesthetized with α-chloralose (100-120 mg/kg body weight, iv), and maintained by continuous infusion (40 mg/kg per hour). The induction solution was 60 mg/ml α-chloralose in 24.8 mg/ml sodium tetraborate decahydrate. Each dog was ventilated mechanically (Harvard 607 respiration pump) via an intratracheal tube at 15 breaths per minute. The tidal volume was adjusted to give an end-expiratory carbon dioxide tension of approximately 5%, as monitored by an infrared analyzer (Beckman LB-2). Esophageal temperature, at the level of the heart, was monitored and used for control of body temperature with a heating pad and proportional controller at 39°C. Arterial pH was adjusted to approximately 7.4 by intravenous infusion of 1.5% sodium bicarbonate. Central arterial pressure was measured in the arch of the aorta via a 75-cm polyethylene (PE 260 Intramedic) cannula passed from the femoral artery. Mean arterial pressure and heart rate were determined electronically from the arterial pressure pulse. In one group of dogs, femoral arterial blood flow was measured with a noncanulating electromagnetic flow transducer (Micron Instruments RC1000) calibrated in vitro with saline over the full range of blood flow encountered in this study. Continuous recordings of cerebral blood flow, mean arterial pressure, respiratory carbon dioxide, femoral blood flow, heart rate, and pulsatile arterial pressure were made on a six-channel oscillograph (Gould-Brush 200).

Cerebral Blood Flow Preparation

The cerebral venous outflow preparation used in this study has been described in three previous studies of the cerebral circulation (D'Alcey and Feigl, 1972; D'Alcey, 1972; D'Alcey and Rose, 1977). Briefly, an uncontaminated measurement of cerebral venous outflow is obtained from the anterior cranial fossa of the dog by diverting the venous effluent out of the left temporal sinus by occlusion of the right and left sigmoid sinuses and occlusive...
cannulation of the right temporal sinus. In vivo calibration of flow and retrograde cerebral perfusion of acrylic are used to document the accuracy of the flow measurement and the validity of the venous outflow preparation. We currently use an ultrasonic flowmeter (American Ultrasonics Laboratories model 1012), rather than the electromagnetic flowmeter used in previous studies. Doppler transducer crystals are positioned over the left temporal sinus, secured by stainless steel suture wire to screws, and coated with quick-cure acrylic. At the end of each experiment, the left temporal sinus is cannulated via the left retroglenoid vein, allowing calibration of each preparation by collection of a volume of blood for timed intervals. Calibration curves were plotted to show the relationship between collected cerebral venous outflow (ml/min) and the averaged oscillograph record of flowmeter output (millivolts). Each calibration extended over the full range of blood flow encountered in the experiment. Least-square regression analysis for each series of points was used to compute the calibration factor as the slope of the regression line.

The retrograde infusion of acrylic was used for anatomic verification of the separation of the dorsal-ventral venous sinus and serves as a test for the presence of the anastomotic branch of the dorsal petrosal sinus. At the end of each experiment, the dog was heparinized (750 U/kg body weight)* for the calibration procedure and killed by iv infusion of 50 ml of saturated potassium chloride. A thin mix (monomer-to-polymer ratio 1:1 by volume) of acrylic Stratford Cookson Tramix was infused into the cerebral venous drainage system via the calibration cannula in the left temporal sinus at a constant pressure of 25 mm Hg. The acrylic mixture remained fluid for 15-20 minutes and was left to harden for 30-40 minutes. Vessels as small as 125 μm regularly were filled when dissected postmortem. The head was removed from the atlas and the occlusions of the right and left sigmoid sinuses as well as the temporal sinus were examined. The cranium was opened and the brain removed for examination of the uniformity of perfusion. The perfused brain was weighed for calculation of cerebral blood flow in ml/min per 100 g. In practice, the weight of the brain in the anterior cranial fossa (see Tissue Sections) is used for this calculation of blood flow in ml/min per 100 g. Inaccuracies in identifying the exact volume of the brain to be used will affect the precision of this calculation but not the overall accuracy of the flow measurement. The cavernous sinuses were opened to check for the presence of acrylic in the area of the sella turcica. Acrylic at this point would indicate the existence of the anastomotic branch of the dorsal petrosal sinus. In each dog, the dura and bone in the area of the sigmoid and temporal sinus were removed from the inside to verify the adequacy of the occlusions in each area. Venous outflow data from dogs with incomplete occlusions (0 of 42 dogs) or anastomotic veins (17 of 42) were excluded from this study, since in these cases cerebral blood flow may have been contaminated.

Microsphere Technique

Total and regional cerebral blood flow was calculated from measurements made with radioactive microspheres (3M Co.) using the arterial reference procedure. Strontium- and scandium-labeled microspheres (^Sr, ^Sc) were infused into the left atrium, and two simultaneous arterial reference samples were drawn from the brachial and femoral arteries. The radioactive microspheres were supplied as nominal 15-μm spheres suspended in 10 or 20% dextran with Tween 80 (0.05%). The stability of the bond between the carbonized spheres and the radioactive isotope was checked by suspension of an aliquot of spheres in water, saline, plasma, and blood. In no case were significant amounts of the isotope found in the supernate after centrifugation, either before or after repeated sonic disruption. All radioactivity counting was done in a large well (28-cm diameter, 7.6-cm height), through hole, two-channel, refrigerated (15°C) gamma spectrometer (Packard 5220 Gamma Scintillation Spectrometer). Channel 1 window was set to count activity from 460 keV to 580 keV, thus bracketing the ^Sr photo peak at 514 keV. Channel 2 was set on integral to include both photo peaks of ^Sc (889 keV and 1120 keV) by counting all activity higher than 600 keV. The overlapping ratio was calculated for each counting session and averaged 6.98 ± 0.05 SEM (n = 62) (approximately 14.4%) for scandium into the scintillation channel and 399.18 ± 38.08 SEM (n = 62) (approximately 0.25%) for the strontium into the scandium channel. The high voltage calibration was done using a cesium (137-CS) standard (photo peak, 662 keV). A constant efficiency zone was determined for each isotope by counting a point source of each isotope at 5-mm intervals from the top to the bottom of the through hole crystal. A 1.5-cm zone, 2 to 3.5 cm from the bottom, was found to give essentially equal counts for equal isotopic activity. This constant efficiency zone is essentially in agreement with the manufacturer's specifications.

Infusion of Microspheres

The 20-ml stock solution vial (10 mCi/g and 1 mCi/20 ml) was sonically disrupted (Bransonic 220) for a minimum of 20 minutes with intermittent vortex (Cole-Parmer Super-Mixer) mixing every 3-5 minutes. A 1.2-ml aliquot was drawn into a 10-ml syringe through a 26-gauge needle and diluted to approximately 10 ml with warm (ca. 39°C) saline. The final concentration of Tween 80 in this infusion mixture was 0.006%. The syringe containing the spheres was kept in constant motion until placed in the syringe pump (Harvard 975). The mixture then

*Heparin was graciously supplied by The Upjohn Company, Kalamazoo, Michigan.
was infused at 12.1 ml/min through a 30-cm section of polyethylene tubing (PE 190, Intramedic), the tip of which had been secured 4-6 cm within the apex of the left atrial appendage. The infusion line then immediately was flushed with 10 ml of warm saline at the same infusion rate. The number of spheres per infusion was calculated from the manufacturer's estimate of mean sphere diameter supplied with each vial of spheres and the specified density of 1.23 ± 0.05 g/ml. The number ranged from 2 to 4 × 10⁷ spheres per infusion. The two isotopes were alternated between the control and experimental manipulations.

**Arterial Reference Flows**

Arterial reference samples were drawn from both the brachial and femoral arteries at 7.00 ml/min through 100-cm sections of polyethylene tubing (PE 260 Intramedic) into two 50-ml plastic syringes by a two-channel syringe pump (Harvard 600-950). The tip of each catheter was flared with the heat of a match and secured in the vessel, just distal to a small muscular branch of the artery, with a ligature pulled snugly against the flared tip. The syringe and catheter were primed with heparinized saline (40 U/ml) and flushed prior to and after each reference sample. The withdrawal was started 10-15 seconds prior to infusion of spheres and continued for 2 minutes after the completion of the catheter flush. Any dog with a ratio of the brachial-to-femoral reference counts that exceeded ±10% was excluded from analysis. The absolute value of the average brachial femoral ratio for each isotope in each condition is given in Table 1. Each arterial reference sample (21-28 ml) was portioned immediately into a total of five vials per reference sample. The first three vials then were filled with 2N KOH in methyl alcohol, and all vials were placed in a drying oven overnight at 70-75°C. This reduced the height of each solution to less than 1.5 cm, thereby restricting all radioactivity to the constant efficiency zone. This procedure was found necessary because the microspheres would otherwise "float" in the buffy coat. This "floating" would result in a falsely low count for the reference sample and, therefore, a falsely high calculated cerebral blood flow. The shunting of spheres through the cerebral bed was checked by a similar sampling procedure of the cerebral venous blood, and the results agreed with published (Heistad et al., 1977) estimates of approximately 1.5%.

**Tissue Sections**

The brain was divided into 14 sections. The brainstem and cerebellum were cut from the rest of the brain along the plane of the tentorial membrane and separated for counting. The contents of the anterior cranial fossa were divided at the midline and dissected into six sections. [The anterior cranial fossa in the dog corresponds with the combined anterior and middle cranial fossa in primates. Further, it is the contents of the anterior cranial fossa that is represented in the venous outflow preparation and constitutes 80-85% of the brain by weight (D'Alecy and Feigl, 1972)]. The frontal section was obtained by cutting at a right angle to the base of the brain just anterior to the genu of the corpus callosum. The occipital section was obtained by cutting from medial to lateral through the splenial sulcus. The temporal sections were taken from the ventrolateral aspect of the remaining tissue. The section-labeled thalamus and hypothalamus included the mediodorsal section of the anterior cranial fossa obtained by sectioning from the corpus callosum in a dorsolateral plane. The remaining cortex was divided into an anterior and a posterior section. Each of the 14 sections was placed in preweighed vials and weighed. The tissue then was covered with 2N KOH in methyl alcohol and placed in the drying oven overnight at 70-75°C to reduce the height of all samples to less than 1.5 cm.

In one group of dogs, 14 extracerebral tissue sections were taken for determination of microsphere-calculated blood flow. Right and left samples of the temporal muscle, digastric muscle, tongue base, tongue tip, upper lip, nose, and eye were placed in preweighed vials and processed as described for brain tissues. For the muscle sections, the tissue was taken from the belly of the muscle, thus avoiding as much as possible the facia and fat in the area. The aqueous and vitreous humor, as well as the lens, were removed from the eye by sectioning the eye and expelling the freely displaced fluid. The average number of spheres per brain tissue sample is given in Table 1. Serial dilutions of an aliquot of stock sphere solution were counted

---

**Table 1** The Average Brachial-Femoral Ratio and Number of Microspheres per Tissue Sample for the Four Experimental Conditions Tested

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spheres/ sample</td>
<td>Spheres/ sample</td>
</tr>
<tr>
<td><strong>Normocapnia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.8</td>
<td>3.4</td>
</tr>
<tr>
<td>SEM</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>n = 15</td>
<td>n = 18</td>
<td>n = 18</td>
</tr>
<tr>
<td></td>
<td>n = 15</td>
<td></td>
</tr>
<tr>
<td><strong>Hypercapnia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.4</td>
<td>3.9</td>
</tr>
<tr>
<td>SEM</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>n = 14</td>
<td>n = 14</td>
<td>n = 6</td>
</tr>
</tbody>
</table>

The brachial-femoral ratio is presented as the absolute value of the number of counts in the simultaneously drawn arterial reference samples. The number of microspheres per tissue sample is the average of the number of microspheres in each of the 14 brain sections as determined by serial dilution and counting of known numbers of spheres (see text for detail).
and plotted against calculated number of spheres per dilution to give a measurement of counts per minute per sphere, which then was used to calculate the number of spheres per tissue sample. In each case the counts were corrected for background, overlapping counts ratio, and isotopic decay.

Flow Calculations Using Microspheres

Each sample was counted for a minimum of 10,000 counts regardless of the time required. The background and overlapping ratio were determined for each counting session. Time-normalized background in each channel was subtracted from normalized counts for each sample. The correction for channel overlap was done by solving the simultaneous equations defining the corrected counts in each channel. The flow for each sample was calculated separately for the brachial and the femoral arterial reference sample. Weighted averages for the right and left sides of the anterior cranial fossa and averages of the brachial and femoral determinations were computed, as well as the percent change for each sample flow. The flow for each tissue sample was calculated by multiplying the ratio of the arterial reference flow (ml/min) to the counts in that reference sample, by the corrected counts in each tissue sample. The weight of each sample then was used to compute blood flow in ml/min per 100 g.

Blood Gas Determinations

Arterial and cerebral venous blood samples were taken and analyzed at 39°C for oxygen tension, carbon dioxide tension, and pH before, during, and after hypercapnic cerebral vasodilation. Six arterial and six cerebral venous samples were taken alternately at approximately 3-minute intervals, during three periods starting 15 minutes prior to hypercapnia, 5 minutes after the start of hypercapnia, and 35 minutes after the end of the hypercapnic period. For each period (before, during, and after), the sequence alternated from arterial to venous samples. A total of 12 samples was analyzed for each dog.

To facilitate arterial sampling, a 3-cm section of heavy-walled silicone tubing was substituted for a section of the femoral artery. The cerebral venous sample was taken from the transverse sinus via the right temporal sinus by drawing blood through a 1.5-ml blood sample into a heparinized 2-ml syringe. Each sample was counted for a minimum of 10,000 counts to give a measurement of counts per minute per sphere, which then was used to calculate the number of spheres per tissue sample. In each case the counts were corrected for background, overlapping counts ratio, and isotopic decay.

Experimental Protocols

Four experimental protocols were used in this study: hypercapnia with blood gas measurements, hypercapnia with sympathetic stimulation and microspheres, normocapnia with sympathetic stimulation and microspheres, and turbulence cannula-tils with sympathetic stimulation and microspheres.

Hypercapnia with Blood Gases

This protocol was designed to demonstrate, using the venous outflow technique, the time course and relative sensitivity of the cerebral and hindlimb circulations to hypercapnia. Five dogs were paralyzed (1.5 mg/kg Flaxedil) and subjected to 20 minutes of hypercapnia by ventilating with a gas mixture containing 10% carbon dioxide, 21% oxygen, and balance nitrogen. Simultaneous measurements were made of cerebral venous outflow and femoral blood flow, as well as pulsatile arterial pressure, heart rate, respiratory carbon dioxide, and cerebral spinal fluid pressure in the cisterna magna. Arterial and cerebral venous blood samples were taken before, during, and after the hypercapnic period and analyzed for carbon dioxide tension, oxygen tension, and pH. The blood flow measurements would show the relative sensitivity of muscle, bone, and skin of the femoral bed in contrast to the highly sensitive cerebral vascular bed. The arterial blood gas measurements would document the hypercapnia and the changes in cerebral venous blood gases would reflect the cerebral vasodilation.

Hypercapnia with Sympathetic Stimulation and Microspheres

This protocol was designed to determine whether sympathetic stimulation could compete with the hypercapnia-induced cerebral vasodilation. The protocol involved two simultaneous measurements of cerebral blood flow, one with the venous outflow technique and one with the radioactive microsphere technique. The hypercapnic period was induced with the same gas mixture used in the first protocol, but the duration of the period was extended to 40 minutes to allow for the development of a steady state pressure-flow condition and to make possible a relatively long stimulation period of 8 minutes. A requirement that can rarely be met in the use of
Microspheres should document not only the hypercapnic cerebral outflow method uses a flow transducer that is linear except that the dog was ventilated with room air same as those used in the hypercapnia protocol and microsphere procedure were essentially the same. The venous outflow measurement of cerebral blood flow indicated that, during the first 5-10 minutes of hypercapnia, only the initial rapid cerebral vasodilation had occurred and steady state took at least 15 minutes to develop. Therefore, in nine dogs, a 15-minute period was allowed for cerebral blood flow to respond to the hypercapnia, at which time the sympathetic stimulation began. The left stellate ganglion was stimulated (Grass S9 and CCUIA stimulator) with bipolar platinum wire (20-ga, 1.5-cm) electrodes placed on the dorsal and ventral surfaces of the ganglion parallel to its long axis. The stimulus parameters were rectangular waves at 10 Hz, 3 msec, 20-30 mA for a total duration of 8 minutes. Four minutes after the start of the stimulation the first microsphere infusion began (19 minutes after the start of hypercapnia). The infusion and catheter flush took just under 2 minutes and the arterial reference continued for an additional 2 minutes after the end of the flush. Eight minutes after the end of the stimulation (35 minutes after the start of hypercapnia) the second infusion of spheres was done following the same infusion, flush, and arterial reference procedure. In a second group of five dogs, the 19- and 35-minute starting points for the microspheres infusions were retained, but the sympathetic stimulation was not begun until 4 minutes prior to the second spheres infusion. This reversal of the protocol served as a time control for possible non-steady state conditions in cerebral blood flow between the 19- and 35-minute time points. The simultaneous cerebral blood flow measurements, using two totally independent measurements, should document not only the hypercapnic cerebral vasodilation, but the competing sympathetic cerebral vasoconstriction.

Normocapnia with Sympathetic Stimulation and Microspheres

This protocol was designed to determine whether the radioactive microsphere technique would show a decrease in cerebral blood flow when venous outflow decreased in response to sympathetic stimulation. The time sequence, stimulation procedure, and microsphere procedure were essentially the same as those used in the hypercapnia protocol except that the dog was ventilated with room air throughout the measurement period. The venous outflow method uses a flow transducer that is linear over the entire range of blood flow encountered in this study (see Fig. 9 and D'Alecy and Rose, 1977). A similar linearity has never been documented for the use of microspheres in the cerebral circulation. We were therefore concerned in this protocol about the ability of the radioactive microsphere method to detect changes in cerebral blood flow that had been reduced by anesthesia and then to be further reduced by sympathetic stimulation. In a recent study using microspheres (Mueller et al., 1977), hypocapnia ($P_{CO2} = 25$ mm Hg) reduced brain blood flow to ca. 20-25 ml/min per 100 g and profound hypotension (arterial pressure ca. 35 mm Hg) reduced flow to ca. 37 ml/min per 100 g. These values of flow determined by the microsphere method under these severe conditions, are only as low as the control, normocapnic blood flow observed in the current study. It is conceivable therefore that in the anesthetized dog the microsphere technique will not correctly represent values of total cerebral blood flow below ca. 20-25 ml/min per mg.

Turbulence Cannulation with Extracerebral Flow Using Microspheres

This protocol was used to test the following hypothesis: nonuniform distribution of microspheres within the cephalic circulation of the dog could account for the inability of the microsphere technique to detect the decrease in cerebral venous outflow that occurs in response to sympathetic stimulation. This hypothesis is put forth on the basis of the results of the first three experimental protocols. Extracerebral tissue samples were taken in an attempt to determine if the microspheres were distributed nonuniformly. In one group of dogs, the protocol was identical to the normocapnic procedure. In a second group, the lingual and infraorbital arteries were cannulated bilaterally. The lingual catheter was inserted so that the tip was in the midcervical section of the common carotid. The infraorbital catheter was inserted so the tip passed the branch point of the orbital and cerebral anastomotic arteries and reached the internal maxillary artery as it passes anterior to the tympanic bulla. Neither of these cannulas occluded the common carotid or internal maxillary artery into which they were placed. The purpose of the cannulations was to introduce turbulence in the parent vessel, thus minimizing axial streaming of the microspheres. Axial streaming of spheres has been described previously, and the logical consequence of axial streaming is “skimming” of sphere-deficient blood into branching vessels such as the internal carotid or the anastomotic artery. Therefore, excessive numbers of spheres would be delivered to larger branches and terminal vessels of the carotid system. The cannulations should diminish this tendency toward nonuniform distribution of the spheres. If nonuniform distribution is occurring, the intact animal should show unusually high microsphere-calculated extra-
CEREBRAL BLOOD FLOW (ml/min/100g)

FEMORAL BLOOD FLOW (ml/min)

RESPIRATORY CO2 (percent)

ARTERIAL BLOOD PRESSURE (mmHg)

HEART RATE (beats/min)

Figure 1: An oscillograph record showing the response of the cerebral and femoral circulations to hypercapnia induced by respiring with 10% carbon dioxide, 21% oxygen, and balance nitrogen. The deflections on the cerebral record are the result of sampling cerebral venous blood from the right temporal sinus. The two deflections at the end of the femoral record are the result of a bolus intraarterial infusion of 0.1 ml of 25 μg/ml acetylcholine to demonstrate the capacity of the bed to dilate. The expected profound cerebral vasodilation is observed, using the cerebral venous outflow technique with the ultrasonic flow transducer.

cerebral flow patterns, and the cannulated animals should show normal or near normal flow for the particular tissue type. In a third group of dogs, arterial references were taken from either the lingual artery or the infraorbital artery at the same rate or twice the rate at which the brachial and femoral arterial reference samples were taken. By comparing the radioactivity collected from these extracerebral reference sites to the activity in the brachial and femoral samples, we should be able to detect any concentrating effect of the microspheres in the extracerebral tissues as a result of “skimming.”

Results

Hypercapnia with Blood Gas Measurements

A profound increase in cerebral blood flow was observed with the cerebral venous outflow technique. Brain blood flow increased rapidly during the first 5–10 minutes of hypercapnia and reached near maximal response (450% of control) at 15–20 minutes, (Figs. 1 and 2). Femoral blood flow showed only minor changes before, during, and after the hypercapnic period. The capacity of the femoral bed to dilate was demonstrated in each preparation by the bolus intraarterial infusion of acetylcholine (Fig. 1). Arterial blood gases showed the expected increase in carbon dioxide tension, decrease in pH, and a slight increase in oxygen tension (Fig. 3). Cerebral venous blood showed an increase in carbon dioxide tension and a decrease in pH. Cerebral venous oxygen tension increased from 32.3 to 70.87 mm Hg, indicating excess delivery of oxygen to the brain. Similar hypercapnic cerebral vasodilation was observed using either venous outflow or radioactive microspheres. The data from dogs in which venous outflow and microsphere measurements were made simultaneously (paired methods) are presented in Figures 4–6. The overall average (nonpaired methods) normocapnic flow was 37.49 ± 1.75 (SEM) ml/min per 100 g (n = 12) using the venous outflow, and 26.22 ± 1.55 ml/min per 100 g (n = 18) using the radioactive microspheres. Hypercapnia increased cerebral blood flow to 155.54 ± 16.98 ml/min per 100 g (n = 8) using the venous outflow, and 167.55 ± 16.87 ml/min per 100 g (n = 14) using the radioactive microspheres, (Table 2). The sample sizes for the nonpaired data are larger than for the paired data because they include flow determina-
FIGURE 2. A composite graph of the response of the cerebral and femoral circulations to a 20-minute period of ventilation with 10% carbon dioxide, 21% oxygen, and balance nitrogen. Each point is the average of five observations (one each in five dogs). Standard error of the mean bars not seen are within the dot. Cerebral blood flow increases markedly, whereas femoral blood flow shows little or no change.

FIGURE 3. A composite graph of the response of the cerebral circulation and blood gases during hypercapnia. In the three blood gas panels, the small dots represent arterial values and the triangles represent cerebral venous values. Each small dot or triangle is the average of five observations, one each in five dogs (n = 5) giving 4 degrees of freedom. Standard error bars not seen are within the symbol.
Figure 4: A bar graph comparing simultaneous determinations of cerebral blood flow using the cerebral venous outflow technique and radioactive microspheres. These data include only dogs in which each known criteria for validity was met for both methods. The venous outflow method shows a statistically significant decrease in cerebral blood flow during both normocapnia and hypercapnia. The radioactive microsphere method only shows this decrease during hypercapnia. The stippled bars are control and the clear bars are flow during sympathetic stimulation. Standard error of the mean is presented for each mean value.

Figure 5: The graph shows comparison of individual data from simultaneous determinations of cerebral blood flow using the cerebral venous outflow technique and radioactive microspheres. These data include only

Figure 6: This graph includes the average of the data in Figure 5 (normocapnia) as well as the individual data during hypercapnia. Sympathetic stimulation decreases cerebral blood flow as measured by both techniques. The dashed line is the linear least square regression analysis for the combined normocapnia and hypercapnia data.

Turbulence Cannulation with Extracerebral Flow Using Microspheres

The number of microspheres, and hence the calculated blood flow to 12 of the 14 extracerebral tissues sampled, decreased in response to the placement of a cannula in the parent vessels. In the intact preparation, the average extracerebral blood flow was 2-3 times higher than in the cannulated dogs in which each known criteria for validity was met for both methods. The venous outflow technique shows a consistent decrease in cerebral blood flow in response to sympathetic stimulation during normocapnia. The microsphere determinations are equally consistent in showing no change in calculated blood flow in these conditions.
preparation (Table 4). Individual values for muscle blood flow ranged as high as 48.5 ml/min per 100 g, or 10 times the expected value for resting, paralyzed, anesthetized blood flow to skeletal muscle. Cerebral blood flow determined by either method was not statistically different in the cannulated and uncannulated preparations. The normocapnic cerebral blood flow data thus includes both cannulated and intact preparations. Stimulation of the left sympa-

Table 2: Cerebral Blood Flow (ml/min per 100 g)

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Anesthetic</th>
<th>Flow measurement technique</th>
<th>Percent reactivity of ACBF</th>
<th>∆ CBFI ∆ CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wasserman (1961)</td>
<td>Man</td>
<td>None</td>
<td>Internal jugular flowmeter</td>
<td>4.1</td>
<td>—</td>
</tr>
<tr>
<td>Reivich (1964)</td>
<td>Baboon</td>
<td>Nembutal</td>
<td>Internal jugular transducer</td>
<td>4.2</td>
<td>—</td>
</tr>
<tr>
<td>Olesen (1971)</td>
<td>Man</td>
<td>Sodium</td>
<td>Venous outflow</td>
<td>6.0</td>
<td>—</td>
</tr>
<tr>
<td>Aoyagi (1976)</td>
<td>Dog</td>
<td>a-Chloralose</td>
<td>Radioactive microspheres</td>
<td>3.9</td>
<td>2.87</td>
</tr>
<tr>
<td>Tominaga (1976)</td>
<td>Dog</td>
<td>a-Chloralose</td>
<td>EM transducer</td>
<td>&lt;0.1</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Discussion

The conclusions to be drawn from these studies involve both physiological control mechanisms for the cerebral circulation and methodological problems in evaluating cerebral blood flow. Hypercapnic cerebral vasodilation is well documented and extensively studied (Norcross, 1938; Kety and Schmidt, 1946, 1948; Patterson et al., 1955; Reivich, 1964). Sympathetic cerebral vasodilation is less well accepted (Alm and Bill, 1973; Alm, 1974; Meyer et al., 1977; Heistad et al., 1977) but nevertheless documented in several species, (Edvinsson et al., 1973; Lubsen, 1941; Sercombe et al., 1975a, 1975b; Lluch et al., 1973, 1975; Meyer et al., 1967; Krog, 1964). The potential dominance of this neural control vs. hypercapnic vasodilation is just beginning to be evaluated. Because of the growing use of radioactive microspheres for the measurement of cerebral blood flow, careful attention must be directed to the validity of this method as applied to various animals and various flow conditions.

Table 3: The Response of the Cerebral and Femoral Circulation to Ventilation with 10% Carbon Dioxide, 21% Oxygen, and Balance Nitrogen is Shown for our Studies

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Anesthetic</th>
<th>Flow measurement technique</th>
<th>Percent reactivity of ACBF</th>
<th>∆ CBFI ∆ CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wasserman (1961)</td>
<td>Man</td>
<td>None</td>
<td>Internal jugular flowmeter</td>
<td>4.1</td>
<td>—</td>
</tr>
<tr>
<td>Reivich (1964)</td>
<td>Baboon</td>
<td>Nembutal</td>
<td>Internal jugular transducer</td>
<td>4.2</td>
<td>—</td>
</tr>
<tr>
<td>Olesen (1971)</td>
<td>Man</td>
<td>Sodium</td>
<td>Venous outflow</td>
<td>6.0</td>
<td>—</td>
</tr>
<tr>
<td>Aoyagi (1976)</td>
<td>Dog</td>
<td>a-Chloralose</td>
<td>Radioactive microspheres</td>
<td>3.9</td>
<td>2.87</td>
</tr>
<tr>
<td>Tominaga (1976)</td>
<td>Dog</td>
<td>a-Chloralose</td>
<td>EM transducer</td>
<td>&lt;0.1</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Carbon dioxide sensitivity (Δ CBFI/Δ CO2) and percent reactivity (Olesen, 1971) calculated on the basis of cerebral venous outflow, radioactive microsphere calculations, and femoral blood flow measurements are presented for comparison to published values. The cerebral response by both flow methods is similar and close to 100 times the femoral response in the same animal preparation. EECO2 is the end-expiratory carbon dioxide measured with an infrared analyzer.
Figure 7: An oscillograph record showing the response of the cerebral circulation to 40 minutes of hypercapnia. At 15 minutes, the left stellate ganglion was stimulated at 10 Hz, 3 msec and 25 mA for 8 minutes. The bars at the bottom of the figure indicate the time of infusion of scandium- and strontium-labeled microspheres into the left atrium.

Figure 8: The regional distribution of microspheres in normocapnia (bottom panel) and hypercapnia (top panel) during control (stippled bars) and during sympathetic stimulation (clear bars). Fourteen sections are shown: the brainstem, cerebellum, six right side sections, and six left side sections in each panel. The P value (paired t-test) is given between each set of bars along with an arrow indicating either an increase (left pointing) or a decrease (right pointing) in blood flow during stimulation. The standard error of the mean is given on top of each bar for n = 18 for normocapnia and n = 9 for hypercapnia.
Physiological Observations

The distinctive sensitivity of the cerebral circulation to hypercapnia has been quantified by several investigators. In Table 3, the sensitivity and percent reactivity (Olesen, 1971) of our preparation, using both the venous outflow technique and the radioactive microsphere technique to measure flow, are presented in comparison to the sensitivity levels observed by other investigators. Both techniques show carbon dioxide sensitivity that is 100 times that of the femoral bed in the same animal. This level of sensitivity clearly distinguishes the cerebral venous outflow as cerebral blood, rather than muscle, bone, or skin blood flow. The overall correlation between venous outflow and microspheres is shown in Figure 6. A slope of 1.15 and R2 of 0.82 suggests that at high blood flow these two methods tend to be in reasonable agreement. The time course of hypercapnic cerebral vasodilation (Figs. 1, 2, and 3) appears to have a rapid initial component that dominates the first 3–5 minutes of the response and a much slower component that requires 15–20 minutes to reach a steady state. Whether this pattern is peculiar to the chloralose-anesthetized dog, or indeed reflects two mechanisms responsible for hypercapnic cerebral vasodilation, is impossible to determine from these data. In either case, a steady level of cerebral blood flow was not obtained until ca. 15 minutes of hypercapnia had been maintained.

We have interpreted the data from both flow techniques to indicate that cerebral blood flow is reduced by sympathetic stimulation even during the marked increase in flow coincident with hypercapnia. The microsphere determinations during hypercapnia demonstrate a reduction in blood flow with sympathetic stimulation regardless of the order in which the control and experimental flow determinations are made. Although the percent decrease observed with the microsphere technique is less (18 vs. 39%) than that observed with the venous outflow technique, the absolute value of the decrease in flow is sizable by either method (31 and 60 ml/min per 100 g).

All 14 regions of the brain showed similar increase in blood flow in response to hypercapnia. The thalamus, hypothalamus, and cerebellum appear to have the highest hypercapnic blood flow. However, the variability in these regional determinations is sufficiently large as to make impossible statistically reliable comparisons between individual tissue sections. The trend toward higher thalamic and lower cortical flow can be identified in these data by noting the similarity of the response of the right side to the left side of the brain (Fig. 8). The cerebellum and brain stem appear to be less sensitive to sympathetic vasoconstriction. Again, the variability of the data leaves this point open to further documentation. Other studies (Heistad et al., 1978) have reported only ipsilateral changes in cerebral blood flow. This difference could be attributed in part to the different site of stimulation. The superior cervical ganglia tend to be distributed to the ipsilateral brain (Heistad et al., 1978) but the left stellate ganglion is distributed bilaterally to the brain and eyes. In this study, bilateral pupil dilation and brain blood flow changes were observed with stimulation of the left sympathetic stellate ganglion. It is possible however that the left side of the brain had a more pronounced vasoconstriction, but the variability in the data prevented this difference from becoming statistically significant. As will be discussed later, the variability in this regional data cannot be attributed to "counting" statistics or to the number of microspheres per tissue sample.

In the present study and in earlier studies (Kobayashi et al., 1969; James et al., 1969), sympathetic stimulation produced a decrease in cerebral blood flow during hypercapnic cerebral vasodilation. This observation suggests that sympathetic control mechanisms are capable of competing with carbon dioxide vasodilator mechanisms within the cerebral circulation. The results from studies using stimulation of the sympathetic innervation can only com-
ment on the capacity of the system and not the physiological significance of the control mechanisms. Some studies on the reflex activation of this system (Heistad and Marcus, 1976; Rapela et al., 1967) conclude that sympathetic vasoconstriction in the brain is of little or no physiological significance. Other studies (Eidelman, 1976; Ponte and Purves, 1974) attribute 40–60% of the hypercapnic vasodilatation to neural mechanisms. The afferent side of such reflexes is presumably the autonomic nervous system. Presently, it is not clear whether the carotid and aortic baroreceptors and chemoreceptors are the afferent side of such reflexes. If autonomic control of the cerebral circulation is to be documented, the afferent side of such reflexes must be identified.

During normocapnia, several laboratories (Alm and Bill, 1973; Meyer et al., 1977; Heistad et al., 1977) including our own have been unable to detect a decrease in cerebral blood flow during sympathetic stimulation. The factor common to this group of studies is the use of radioactive microspheres. Varying degrees of sympathetic cerebral vasoconstriction have been observed in the mouse (Edvinsson et al., 1973), rabbit (Lubsen, 1941), goat (Sercombe et al., 1975a, 1975b; Lluch et al., 1973, 1975) monkey (Meyer et al., 1967), and in our own laboratory in dogs (D’Alecy and Feigl, 1972; D’Alecy, 1972). In general, studies from laboratories other than our own (Edvinsson et al., 1973; Lubsen, 1941; Sercombe et al., 1975a, 1975b; Lluch et al., 1973, 1975; Meyer et al., 1967; Krog, 1964) report decreases in cerebral blood flow that are much smaller in magnitude and shorter in duration. Thus, different laboratories, using flow measurement techniques other than microspheres, have documented some degree of sympathetic cerebral vasoconstriction. Radioactive microspheres, in our study and in numerous other studies, have been consistent in showing no change in cerebral blood flow in response to sympathetic stimulation. We have no simple explanation for this discrepancy but currently suspect methodological problems with the radioactive microsphere technique when used at low blood flow in the anesthetized dog.

Methodological Considerations

The radioactive microsphere technique as used in this study meets or exceeds all published criteria for the reliable application of microspheres to the measurement of regional blood flow. The question of the statistical accuracy of a regional flow determination based on the number of spheres per tissue sample is answered in Table 1. Buckberg et al. (1971) have calculated that 400 spheres per sample will give an accuracy of ±10% for an individual tissue flow determination. The smallest average of the four groups of experiments is 1759 spheres per sample, suggesting that our accuracy should be better than ±5% per individual tissue flow determination.

The single most critical assumption in the use of radioactive microspheres is that the microspheres are distributed uniformly with the blood flow. The agreement of two simultaneously drawn arterial reference samples generally is used to document the validity of this assumption. In our study the agreement of the brachial and femoral counts averaged 3.37% in the four groups, in contrast to the more generally used value of ±10%. We did, however, exclude from analysis any dog in which the brachial-femoral “split” exceeded ±10%. By this brachial-femoral ratio data, we would have to assume that the microspheres are uniformly distributed with blood flow. However, our data on the distribution of microspheres to the muscle and skin structures of the head suggest that, within the cephalic circulation, the microspheres are not distributed uniformly. The placement of a small catheter in the common carotid and the internal maxillary arteries grossly altered the microsphere-calculated blood flow to the extracerebral tissues. In 12 of the 14 tissues, the number of microspheres in the tissue samples decreased markedly. In one section the number was unchanged, and in another there was an increase in the number of spheres in the sample. It could be argued that the catheters simply restricted flow, particularly to the tongue, nose, and upper lip. We do not believe this was the case: first, because not all the tissues showed a decrease in calculated flow; second, the magnitude of the decrease varied substantially from tissue to tissue; and, third, the cannulated blood flow determinations are in much closer agreement with published values for blood flow to these types of tissue than with the unusually high values obtained in the intact preparations. We are suggesting that sphere deficient blood could be “skimmed” off at earlier branch points, leaving sphere “rich” blood in the terminal branches. The cannulas, by inducing turbulence in the stream, decrease axial migration and axial streaming and thereby decrease the tendency for microsphere deficient blood to be “skimmed” off at early branch point. When the concentration of microspheres in the infraorbital and lingual arteries was measured, only a 2% concentrating effect was observed. Although qualitatively consistent with the proposed “skimming” effect, the magnitude of this concentrating effect is much too small to account for a major part of the discrepancy between the venous outflow and the microspheres technique. Because of the differences between the normal flow rate and the extracerebral sampling rate, as well as the use of steady flow vs. pulsatile flow in these sampling sequences, these procedures may be inadequate tests of branch point skimming. Therefore, neither the extracerebral flow determinations nor the extracerebral arterial reference sample procedure has proven definitive in supporting or refuting the proposed skimming effect.
If "skimming" effects exist, they are not as dominant during hypercapnia. One possible explanation for this difference is the elevated blood flow during hypercapnia. The greater than 400% increase in blood flow to the brain raises the velocity of blood in these vessels, perhaps high enough that the Reynolds number is reached and turbulence is thus present in the intact preparation without the cannulas. The turbulence would facilitate the uniform distribution of spheres with blood flow. A second explanation is that the microsphere technique is nonlinear at a level of cerebral blood flow in the dog below 20-25 ml/min per 100 g but can give a reliable indication of blood flow from 30 to 150 ml/min per 100 g. This never has been tested other than in the present study (by comparison with the venous outflow technique).

These arguments for the nonuniform distribution of spheres (during normocapnia in the anesthetized dog) within the cephalic circulation do not explain definitively why no change in microsphere-calculated blood flow was observed during sympathetic stimulation. For example, nonstimulated cerebral blood flow (by both methods) was not different in the cannulated and intact preparations. Whereas this indicates that the cannulations did not restrict blood flow to the cerebral circulation, they likewise did not alter the cerebral distribution of microspheres in a statistically significant way. Therefore this suggestion of a "skimming" effect in the internal maxillary and common carotid arteries does not explain why microsphere data are not in agreement with the venous outflow data. It does, however, open the question of reliability of the microsphere method under these low blood flow circumstances.

The unusually high muscle blood flow seen in this study has been observed in the dog by others (Marcus et al., 1977). In this study, temporal muscle blood flow was 17 ± 6 ml/min per 100 g in the control condition, and 34 ± 10 ml/min per 100 g after sympathetic denervation. These values are 5 to 10 times what generally would be accepted for resting muscle blood flow. Mechanical models (Bugliarello and Hsiao, 1964) and biological experiments (Phibbs and Dong, 1970) both confirm the existence of axial migration. The brachial-femoral sampling technique would not detect the effect of axial migration and "skimming" unless it were to occur in the major branch points of the systemic circulation. The nonuniform distribution we are proposing here is within the cephalic circulation.

The second method for the measurement of cerebral blood flow in this study was the cerebral venous outflow technique (D'Alecy and Feigl, 1972; D'Alecy, 1972; D'Alecy and Rose, 1977). This outflow method has not been widely accepted (Traystman and Rapela, 1975; Heistad and Marcus, 1978). These authors have suggested extracerebral contamination as an explanation for the decrease in blood flow observed during sympathetic stimulation. The presence, in the measured flow, of venous blood of noncerebral origin, which responds to sympathetic stimulation, thus would account for changes observed with the venous outflow technique. A second possible mechanism to consider in this regard is the shunting of cerebral venous blood out of the brain by some other channel during stimulation. Inasmuch as the venous outflow technique has not been used to supply direct evidence for either of these proposed criticisms, there is no specific evidence to be discussed relating to these proposals. The probability of either explanation will thus be dealt with in light of the experience in our own laboratory with this venous outflow technique.

The acrylic verification procedure used in each preparation is considered by others an inadequate test of extracerebral contamination. Even though the acrylic is perfused retrograde at a pressure of 25 mm Hg and regularly fills vessels of 125 μm, there is concern that the vessels may have changed upon the death of the animal, or that this postmortem (30-60 seconds after cardiac arrest) procedure does not describe accurately the condition of the vessels during sympathetic stimulation. As mentioned previously, the precision of the acrylic in defining the exact weight of the brain may be in error, but this would in no way affect the accuracy of the method and, in particular, it would not alter the percent changes indicated with the venous outflow technique.

In the present study, the reactivity of the cerebral venous outflow to 10% carbon dioxide inhalation is 100 times that of the muscle and bone of the femoral bed. This high level sensitivity to carbon dioxide could not be demonstrated if a significant fraction of the cerebral venous effluent were of noncerebral origin. Similar sensitivity is demonstrated by both the microsphere technique and the cerebral venous outflow technique (Table 3). It is, therefore, not likely that a meaningful fraction of the venous effluent is of extracerebral origin.

The possibility does exist that the decrease in measured venous outflow during sympathetic stimulation results from a shunting of cerebral blood out of the brain by some channel other than that measured in our preparation. We have been unable to identify (D'Alecy and Feigl, 1972), and know of no anatomical evidence for, such a channel. Furthermore, we know of no physiological studies that support the existence of a venous shunt that is opened by sympathetic stimulation and/or closed by the death of the animal. The presence of such a channel cannot be denied on the basis of our data and, therefore, this proposed channel remains a possible explanation for the observed decrease in cerebral venous outflow. Further data are required to support or refute the proposed existence of such channels.

In a recent study (Fan et al., 1979) the detection of 133Xe in the sagittal sinus of dogs after injection of the isotope into the nasal mucosa or deep muscle of the neck was interpreted as indicating "the drain-
age of blood from these two areas into the sagittal sinus.” However, these investigators did not discuss the possible significance to their study of the carotid rete countercurrent exchange system. These multiple arterial inflow tracts to the brain are surrounded by venous channels from the face and nose. This arrangement has shown to be effective in the thermal exchanges between the blood draining the face and that being delivered to the brain (Hayward and Baker, 1969). The possibility of movement of a freely diffusible gas (¹³³Xe) from these venousplexuses or cavernous sinuses into the internal carotid artery and thus to the sagittal sinus via the brain should be considered before it is concluded that direct vascular channels exist between the nasal mucosa and the sagittal sinus.

Other laboratories (Traystman and Rapela, 1975), using an extracorporeal venous outflow procedure, have been unable to detect changes in cerebral blood during sympathetic stimulation. The use of heparin and an extracorporeal flow circuit, the systemic acidosis (7.275 ± 0.011 pH), and the injection of heated bone wax to occlude cerebral venous channels may explain why there appears to be a lack of sympathetic effect on cerebral blood flow in their studies. The decrease in indicated flow observed during intentional contamination of their venous outflow and its disappearance after occluding the “lateral sinuses” has been used as an explanation for our observation of sympathetic cerebral vasoconstriction. This extrapolation is clearly unwarranted: first, because the preparations differ substantially in the areas of the brain drained; and second, our acrylic perfusion can easily resolve the level of contamination they propose (40%, 10 of 25 ml/min, of measured venous outflow). Since vessels as small as 125 μm are detected by the acrylic casts, it is difficult to conceive of 40% of the venous effluent leaving the brain by vessels smaller than 125 μm. In addition, a 40% contamination of the venous outflow measurement would produce a carbon dioxide sensitivity, determined with the venous outflow, which was substantially less than that determined with microspheres. This is because muscle, bone, and skin, as represented by the femoral blood measurement, have a carbon dioxide sensitivity ca. one one-hundredth that of brain (Table 3).

The cerebral venous outflow method clearly shows a sympathetically mediated decrease in blood flow during both normocapnia and hypercapnia. The classical reactivity of the cerebral circulation to hypercapnia is demonstrated equally well with microspheres and venous outflow, indicating that the measured venous outflow is cerebral in origin. The calibration data on the venous outflow transducer show excellent linearity (Fig. 9) over the entire range of blood flow measured. The acrylic verification procedure approximates the volume of brain from which blood flow is measured. We conclude that the continuous cerebral venous outflow method is a reliable, accurate measure of cerebral blood flow during normocapnia, hypercapnia, and sympathetic stimulation.

In summary, we have shown that simultaneous measurement of cerebral blood flow using radioactive microspheres and the cerebral venous outflow technique can demonstrate the selective sensitivity of the cerebral circulation to hypercapnia. Direct electrical stimulation of the sympathetic innervation can reduce cerebral blood flow even during profound hypercapnic cerebral vasodilation, and sympathetic vasoconstriction has a fairly uniform effect on the various regions of the brain. During normocapnia, the results obtained with cerebral venous outflow and microspheres are different. During hypercapnia, the two methods agree qualitatively but not quantitatively. Although several possible factors were explored, the explanation for the remaining differences in these two methods is still not clear. For example, these experiments do not rule out conclusively the possibility that cerebral venous blood is shunted from cerebral to extracerebral channels during some experimental conditions.

Acknowledgments

We would like to thank Betty Martin and Susan Koch for their help in preparing this manuscript. Stephen J. Fisher for his help in the early development of the microsphere technique and associated computer programs, John P. Manfredi for his assistance in the analysis of the extracerebral blood flow patterns, John G. Wood for developing and programming the simultaneous equations used for correcting for overlapping ratios.
and Drs. Faulkner, Lucochii, Sparrus, and Turcotte for the financial assistance in the purchase of the gamma counter.

References


Sympathetic modulation of hypercapnic cerebral vasodilation in dogs.
L G D'Alecy, C J Rose and S A Sellers

Circ Res. 1979;45:771-785
doi: 10.1161/01.RES.45.6.771

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/45/6/771

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/