Cerebral autoregulation maintains blood flow relatively constant over a wide range of arterial pressure. Changes in pial artery pressure are smaller than associated changes in systemic pressure, thus attenuating large fluctuations in cerebral blood flow (rCBF). As systemic pressure is reduced, however, maximal autoregulatory vasodilatation is reached, and rCBF decreases passively during further reduction of systemic pressure and pial artery pressure. Pial artery pressure also correlates with rCBF after middle cerebral artery (MCA) occlusion in cats. Although previous studies have characterized autoregulation in normal cerebrum, they have not compared the pressure-flow relation in normal cerebrum and in cerebrum dependent on collateral flow.

In previous studies, we have characterized two regions that are present within the morphologically defined area at risk after occlusion of an MCA branch: a central zone with a profound reduction in rCBF, which is collateral dependent, and a peripheral zone of overlap flow with intermediate flow. Earlier studies of collateral-dependent cerebral blood flow have been limited by an inability to resolve the admixture of collateral and overlap flow.

The purpose of this investigation was to test the hypotheses that the pressure–flow relation is different in collateral-dependent and normal cerebrum and that collateral flow can be predicted from microvascular pressure measurements during profound hypotension. We compared the relations among pial artery pressure, large- and small-vessel resistance, and rCBF in normal and collateral-dependent cerebrum. Two novel approaches were used to study cerebral autoregulation. First, we employed a new approach to define collateral-dependent flow after occlusion of the MCA. Thus, flow was measured to normal and true collateral-dependent cerebrum. Second, we measured pressure in branches of the MCA, in addition to systemic arterial pressure, to calculate cerebral vascular resistance in both regions. In normal cerebrum, microvascular pressure is lower than systemic pressure. After occlusion of the MCA, microvascular pressure distal to the occlusion decreases further, despite little change in systemic pressure. Hence, accurate calculation of vascular resistance within the collateral-dependent zone requires measurement of pial artery pressure. Using these measurements, we have characterized the pressure–flow relation.
in normal and collateral-dependent brain during MCA/carotid occlusion and progressive hypotension.

Materials and Methods
Ten adult mongrel dogs weighing 18–25 kg were anesthetized with thiopental sodium (25 mg/kg i.v.) and intubated. Anesthesia was maintained with a 1% halothane and 45% O₂/55% N₂O mixture (Harvard pump respirator, Harvard Apparatus Company, Inc., Millis, Mass.), and expired CO₂ concentration was monitored.

The common carotid arteries were isolated through a midline incision. Catheters were inserted in both femoral veins for administration of fluid and drugs and measurement of core body temperature. A catheter was placed in a brachial artery to allow continuous measurement of mean arterial pressure (MAP) and to provide determination of core body temperature. A catheter was placed in a brachial artery to allow continuous measurement of mean arterial pressure (MAP) and to provide determination of core body temperature. A catheter was placed in a brachial artery to allow continuous measurement of mean arterial pressure (MAP) and to provide determination of core body temperature.

A 7F pigtail catheter was inserted into a femoral artery and advanced into the left ventricle for injection of radiolabeled microspheres. Brachial and femoral arterial catheters were placed for simultaneous withdrawal of reference arterial blood samples after microsphere injection. Arterial CO₂ tension (Paco₂) was 40±1 mm Hg and Pao₂ was 205±17 mm Hg. Arterial pH was 7.28±0.04 and did not change significantly until MAP was reduced to 25 mm Hg (pH decreased to 7.19±0.05; p<0.05).

A left frontotemporal craniectomy was performed, and the dura over the sylvian fissure was incised and tacked away from the brain. Temperature-regulated artificial cerebrospinal fluid was superfused over the exposed cortex. A large branch (700–1,000 μm) of the MCA was identified and measured with a video microscope (Colorado Video, Boulder, Colo.). Perfusion pressure in the selected artery and an adjacent vessel was determined with a glass micropipette (tip diameter, 2–4 μm) (Figure 1), using the servo-null micropressure recording system (model 5, Instruments for Physiology and Medicine, San Diego, Calif.).

FIGURE 1. Determination of shadow flow: A micropipette was used to measure intraluminal pressure (point A) in the cannulated vessel that perfused the 'area at risk' before occlusion and in a neighbor branch of the middle cerebral artery (MCA) (point B). Pressure was measured at point A during shadow flow. Pressure at point B is MCA branch stem pressure. After cannulation of the MCA branch, rate of infusion of microsphere-free blood was adjusted until pressure at point A was equal to pressure measured at point B.

The pressure in the distal MCA branch was then monitored continuously.

Before cannulation of the MCA branch, baseline rCBF was measured. Radiolabeled microspheres were selected in random fashion from a stock pool of six isotopes (¹⁴³Gd, ¹⁴¹Ce, ¹¹²Sn, ⁹⁵Nb, ⁸⁸Sr, and ⁸⁶Sc). 1×10⁶ microspheres 15 μm in diameter were injected into the left ventricle and flushed with 5 ml saline over 5 seconds. Reference arterial blood samples were withdrawn from the brachial and femoral arteries at 1.31 ml/min for a total of 2.5 minutes starting 30 seconds before microsphere injection.

After arterial cannulation, a second measurement of flow was obtained during shadow flow: the cannulated MCA branch was perfused from a reservoir with heparinized, autologous microsphere-free blood at a pressure equal to that measured by a micropipette in an adjacent MCA branch of similar diameter (Figure 1). This perfusion thus minimizes collateral flow (flow to a region that occurs only when a primary artery is occluded) to the area at risk (that region determined morphologically to be supplied by the cannulated artery). Because the area at risk was perfused with microsphere-free blood during shadow flow, blood flow through collateral channels was minimal. Thus, values for rCBF, measured with microspheres, to the region supplied by the MCA branch during the shadow flow reflect solely the contribution of overlap flow (flow to the area at risk that is not through collateral vessels and is present during both normal and demand conditions) from adjacent vascular territories.

Perfusion of the MCA branch was stopped, and, 30 minutes later, a third measurement of rCBF was obtained. During this measurement, because antegrade perfusion of the MCA branch was stopped, perfusion of the area at risk was entirely by collateral and overlap flow.

The common carotid arteries were clamped to reduce perfusion pressure, and, after 30 minutes of occlusion, a fourth measurement of rCBF was performed. Blood was removed to decrease MAP to 50 mm Hg, and a fifth determination of rCBF was obtained 30 minutes later. More blood was removed until a MAP of 25 mm Hg was established for 30 minutes, followed by a final measurement of rCBF.

Immediately before each measurement of rCBF, pressure was measured in both the cannulated MCA
branch and an adjacent branch of the MCA using the micropipette. In the cannulated vessel, pressure was measured from arterial back pressure and from micropipette pressure at a point approximately 2 cm distal to the cannulation site (Figure 1, point A). In all 10 dogs, both pressures were virtually identical (±1 mm Hg); values from micropipette measurements are presented in Table 1.

After reinjection of blood and restoration of systemic pressure to baseline, the cannulated MCA branch was again perfused through the closed system with heparinized autologous microsphere-free blood at stem pressure. Fifty milliliters of a 4% neutral red dye solution (Fisher Scientific Co., Fair Lawn, N. J.) was administered intravenously and allowed to circulate for 1–2 minutes. A negative “map” of the area at risk was thus obtained (Figure 2A). The animal was killed with pentobarbital (150 mg/kg i.v.), and the brain was removed.

The brain was sliced into 3-mm-thick coronal sections. The negatively stained area at risk was dissected from the surrounding tissue and divided into tissue samples of 0.4–0.5 g. Reference blood samples and samples from the area at risk, normally perfused surrounding tissue, and paired areas of contralateral cerebral tissue were counted in a 3-inch, well-type gamma scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.), and rCBF was calculated.

Neutral red dye was useful for the gross identification of the area at risk, but it was not sufficiently sensitive to differentiate overlap flow from collateral flow (Figure 2). Collateral-dependent tissues were defined as those with rCBF ≤10 ml/100 g per minute during shadow flow. This value was chosen because previous authors have suggested that when rCBF is sustained below this value, tissue infarction results.⁷–¹⁰ By subtracting the value for flow to the collateral-dependent tissues obtained during shadow flow from the value for flow obtained 30 minutes after cessation of MCA perfusion, an estimation of collateral flow was obtained.¹¹ Significant levels of overlap flow (rCBF>10 ml/100 g per minute during shadow flow) were present in the remaining tissues that were defined morphologically (by absence of neutral red staining) as within the risk area, however, and these tissues could have been mistaken as collateral dependent. Therefore the shadow technique was essential in identifying those tissues that were truly collateral dependent.

Resistance of large pial arteries was calculated from the following equation: large-artery resistance=(systemic pressure–pial artery pressure)/blood flow. Small-vessel resistance, downstream to the large pial vessels, was calculated for normal and collateral-dependent brain by dividing pressure in the vessel supplying each area by rCBF to this region. Venous pressure was assumed to be zero. Pressure for the collateral-dependent zone was measured in the cannulated MCA branch; in normal brain, pressure was measured in an adjacent MCA branch.

Analysis of variance and pairwise comparisons with Duncan’s test were used to compare flows in the collateral-dependent zone and ipsilateral normal hemisphere. The t test was used for paired data to compare the values for resistance. A value of p<0.05 was considered statistically significant.

### Results

Control blood flow was similar in the normal and collateral-dependent zone (Table 1). After MCA and carotid occlusion, there was no significant change in flow despite a significant decrease in MCA pressure.
During hypotension, flow to the collateral-dependent zone decreased to 6.1 ± 1 at 25 mm Hg, concurrent with a profound decrease in MCA intraluminal pressure. The relation between pressure in the cannulated branch of the MCA and rCBF in the collateral-dependent region is presented in Figure 3. In normal cerebrum, blood flow did not change significantly during hypotension to a MAP of 50 mm Hg despite a significant decrease in MCA pressure. With further hypotension, flow decreased significantly, to 39 ± 5 at 25 mm Hg.

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Large-artery resistance increased after carotid occlusion, but it returned and remained near baseline values during hypotension (Table 1). Small-vessel resistance (calculated from pressure in the cannulated MCA branch) in the collateral-dependent zone decreased significantly after carotid occlusion. During hypotension to 50 and 25 mm Hg, resistance in the collateral-dependent zone decreased further, suggesting that autoregulatory dilator capacity of small branches of the MCA was reached. In normal tissue outside of the area at risk, vascular resistance decreased after carotid occlusion, decreased further at 50 mm Hg, and tended to decrease further at 25 mm Hg. Thus, autoregulation of cerebral vessels outside of the collateral-dependent zone prevented a decrease in blood flow until MCA pressure was reduced to at least 29 mm Hg (Figure 3).

Discussion

There are several major findings in this study. After occlusion of an MCA branch and the carotid artery, small-vessel resistance is minimal within the collateral-dependent region and does not decrease further during progressive hypotension. In contrast, small-vessel resistance in normal brain (outside the distribution of the occluded MCA branch) continues to decrease as systemic pressure is reduced to 25 mm Hg. Although maximal autoregulatory dilatation in small vessels occurred in the collateral-dependent zone before it occurred in the surrounding normal tissue, the pressure-flow relation was virtually identical in collateral-dependent and normal cerebrum (Figure 3). There is profound hypoperfusion within the collateral-dependent tissue at 25 mm Hg after carotid and MCA occlusion, with maintenance of perfusion outside of the area at risk. Thus, autoregulatory dilatation is maximal in collateral-dependent vessels after MCA and carotid occlusion, so that hypotension produces profound focal hypoperfusion. During carotid occlusion and hypotension, flow correlated directly with pial artery pressure.

The lower limit of autoregulation has been described as the blood pressure below which cerebral blood flow begins to decrease. However, during progressive hypo-
tension, the level of pressure at which cerebral blood flow decreases is not associated with maximal vasodilatation. Thus, a decrease in resistance (autoregulatory vasodilatation) continues below the "lower limit" of autoregulation. Previous studies in rabbits have shown that autoregulatory vasodilatation is lost and cerebral vessels do not dilate in response to hypercapnia when systemic pressure is <35 mm Hg. When perfusion pressure is reduced from 65 to 35 mm Hg, however, autoregulation continues, and there is a persistent, albeit diminished, response to hypercapnia. This finding implies that there is a level of perfusion pressure below which resistance will not change because vessels are dilated maximally. Our data indicate that in normal brain, vascular resistance continues to decrease during hypotension after carotid occlusion. In the collateral-dependent zone, however, resistance fails to decrease during hypotension after MCA branch and carotid occlusion. The minimal resistance that was achieved in both areas was virtually identical (approximately 0.33 mm Hg/ml/100 g per minute). This does not exclude the possibility, however, that active changes continue in a portion of the vascular bed. It is possible that there is continuing relaxation in some vessels, but this is balanced by vascular collapse due to the decline in intravascular pressure in other vessels.

In the present study, when MCA pressure was approximately 30 mm Hg, blood flow was maintained at virtually normal levels in normal brain and in the collateral-dependent zone (Table 1). Below this pressure, rCBF decreased concurrently with a decrease in pial artery pressure. In light of its association with maintenance of blood flow in both normal and collateral-dependent areas of the brain, this pressure appears to define the lower limit of autoregulation under the conditions of this experiment. This resulted despite different methods of induction of hypotension—carotid and MCA occlusion in the collateral-dependent tissues, and carotid occlusion and hemorrhage to systemic pressure of 50 mm Hg in normal brain. The implication of this finding is that cerebral blood flow can be predicted from intraluminal pressure only when intraluminal pressure is <30 mm Hg. At this pressure, collateral and other vessels are probably maximally dilated. In all 10 dogs, the ratio of flow to pressure was approximately 3:1 (ml/100 g per minute : mm Hg) when MCA intraluminal pressure was <30 mm Hg. Profound regional hypoperfusion (rCBF <10 ml/100 g per minute) occurred when MCA pressure was 3–4 mm Hg (Figure 3).

In this study, cerebral blood flow and vascular resistance could not be predicted accurately from systemic pressure. After carotid occlusion, systemic pressure increased slightly, but rCBF was unchanged. Progressive hypotension did not affect blood flow significantly in normal brain until a systemic pressure of 25 mm Hg. In contrast, flow to the collateral-dependent zone was reduced during systemic pressure of 50 mm Hg. Vascular resistance in the collateral-dependent zone, as calculated from the pial artery pressure, decreased as expected after carotid occlusion. If systemic pressure is used to calculate resistance, however, the trends of the resultant values differ from that of those calculated using pial artery pressure (Table 1). Thus, cerebral vascular resistance can only be calculated accurately from the pial vessel pressure-flow relation. Two reasons for the differences between flow and microvascular pressure compared to systemic pressure include the autoregulatory capacity of the brain, which is greater than that of most other organs; and the pressure decrease from the aorta to the cerebral microvasculature. Pressure in the largest intracranial vessels is approximately 80% of aortic pressure, and pressure in small pial arteries (approximately 200 μm in diameter) is only 50–60% of systemic pressure. This segmental pressure drop would not be accounted for if resistance were calculated from systemic pressure.

Small-vessel resistance was calculated using pressure in large pial vessels (500–900 μm). The resultant value is a reflection of resistance of all vessels downstream from these large pial vessels, including small pial and intracerebral arterioles. Venous pressure was not measured in this study, but we have measured it in previous studies and it is low (0–9 mm Hg) under normal conditions (M.G. Muohon and D.D. Heistad, unpublished observations). We assumed that venous pressure was zero. One would need to measure venous pressure extremely precisely (perhaps to 0.1 mm Hg) to calculate accurately perfusion pressure and resistance during extreme hypotension because MCA branch pressure is very low (Table 1) and probably nearly equal to venous pressure. The small-vessel segment is heterogeneous (capillaries, venules, veins), and we assume that the vessels are passive and do not contribute to active changes in cerebral vascular resistance during hypotension. Each of these assumptions probably leads to a slight reduction in precision of our calculation of small-vessel resistance.

Large-artery resistance increased after carotid occlusion (Table 1), presumably primarily as a direct result of arterial occlusion. In addition, carotid occlusion increases sympathetic discharge, and sympathetic stimulation increases resistance of large cerebral arteries.

The extensive collateral circulation through pial anastomoses, as well as effective autoregulation, protect against focal cerebral hypoperfusion after arterial oc-
clusion. Both mechanisms contribute to preservation of blood flow within the territory of an occluded cerebral artery that is usually sufficient to prevent infarction. Compensatory mechanisms are sufficient to maintain cerebral blood flow in dogs above the threshold of tissue ischemia despite bilateral carotid and vertebral artery occlusion. When a branch of the MCA is occluded, the greatest contribution to the maintenance of blood flow comes from other branches of the MCA, which provide both overlap and collateral flow. In the present study, this collateral flow was impaired progressively to induce focal, profound hypoperfusion.

The cerebral collateral circulation has been studied previously in several animal models. It is difficult to differentiate overlap from collateral flow and thus to study truly collateral-dependent tissues. We have characterized a method that allows separation of overlap flow from true collateral flow and therefore allows identification of regions of cerebral tissue that are solely dependent on collateral flow. A similar method has been used extensively in studies of coronary collateral flow, but the application of this concept to the cerebral circulation is new. Other studies would have mistakenly identified the entire area at risk as collateral dependent by failing to exclude the portion of the area at risk that is perfused by overlap flow.

In cerebrovascular studies using arterial occlusion, there are a priori reasons to differentiate collateral from overlap flow. First, there are important differences in regulation of collateral and overlap flow in the cerebrum. For example, during seizures and hypercarbia, resistance of collateral vessels increases while resistance of normal vessels (including overlap) decreases (M.G. Muhonen, C.M. Loftus, and D.D. Heistad, unpublished observations). Second, we have found recently that collateral and overlap flow differ in response to inhibition of nitric oxide synthesis. Blood flow to normal brain decreased after infusion of L-Nω-nitro-arginine, and collateral flow increased (M.G. Muhonen, C.M. Loftus, and D.D. Heistad, unpublished observations). Third, separation of collateral from overlap flow in the coronary circulation has proven critical in the myocardium. Sharp margins surround an area of ischemia, with a minimal lateral border zone which has led to a concept of “overlapping peninsulas” in the myocardium. In the brain, there is more proximal admixture of blood flow between adjacent vascular territories. Nevertheless, if overlap flow is not differentiated from collateral flow, values for true collateral blood flow value are not accurate in cerebrum as well as in myocardium.

In this study, post hoc analysis indicated that the pressure–flow relation (i.e., autoregulation) is similar in normal regions and collateral vessels. This conclusion would not be valid without accurate separation of collateral and overlap flow.

In summary, there are several new major contributions from this study. The pressure–flow relation in collateral-dependent and normal brain is virtually identical, with a decrease in small-vessel resistance accounting for the maintenance of flow after carotid occlusion and during hypertension. The lower limit of autoregulatory vasodilatation was manifested by the lowest level of resistance occurring in the face of decreasing blood flow. In addition, we have developed a model that allows prediction of profound cerebral hypoperfusion from micropipette measurement of MCA pressure. This experimental approach may be useful as a model for reproducible focal cerebral hypoperfusion and for studies of regulation of collateral flow to severely hypoperfused cerebrum.

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