CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

BODY TEMPERATURE INFLUENCES REGIONAL TISSUE BLOOD FLOW DURING RETROGRADE CEREBRAL PERFUSION

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Objective: This study compared the cerebral microcirculation during retrograde cerebral perfusion with that during antegrade cardiopulmonary bypass under normothermic and hypothermic conditions. **Methods:** Brain tissue blood flow was measured with the hydrogen-clearance and colored microsphere (15 and 50 μ m) methods during antegrade cardiopulmonary bypass and retrograde cerebral perfusion. Measurements were performed during normothermia (37° C), moderate hypothermia (28° C) and deep hypothermia (20° C) in groups of mongrel dogs ($n = 8$). **Results:** During antegrade cardiopulmonary bypass, the microsphere method showed a significant decrease in cerebral blood flow as body temperature decreased (40.1 ± 20.8 ml/min/100 gm at 37° C, 16.2 ± 18.0 ml/min/100 gm at 20° C with 50 μ m microspheres). At 20° C, the cerebral blood flow measured with the 15 μ m microspheres was significantly lower than that assessed with the hydrogen-clearance method (11.3 ± 7.0 vs 24.8 ± 7.0 ml/min/100 gm). During retrograde cerebral perfusion, the microsphere method also showed a significant decrease in cerebral blood flow with cooling. At 37° C, the cerebral blood flow measured with the 15 μ m microspheres (0.8 ± 0.7 ml/min/100 gm) was significantly lower than that assessed with the hydrogen-clearance method (10.1 ± 3.5 ml/min/100 gm). At both 28° and 20° C, the hydrogen-clearance method showed significantly higher cerebral blood flow (10.1 ± 5.8 and 8.2 ± 3.7 ml/min/100 gm) than did the 50 μ m microspheres (1.8 ± 0.6 and 1.0 ± 0.8 ml/min/100 gm) and 15 μ m microspheres (0.23 ± 0.14 and 0.18 ± 0.15 ml/min/100 gm). **Conclusion:** (1) Cerebral blood flow that shunts to capillaries is increased during antegrade cardiopulmonary bypass under deep hypothermia. (2) During retrograde perfusion, the majority of the blood flow shunts away from brain capillaries, even under normothermic conditions, and blood flow through large venoarterial shunts increases as body temperature decreases. Although the cerebral microcirculation during retrograde perfusion is decreased, retrograde perfusion provides some degree of oxygenation to the body. (J Thorac Cardiovasc Surg 1997;114:440-7)

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Retrograde cerebral perfusion (RCP) is a new and simple technique used to protect the brain against interruption of the cerebral circulation during aortic arch surgery. As shown in previous studies, RCP can provide blood and oxygen to the brain, minimize the decrease in cerebral tissue adenosine triphosphate levels, and maintain brain cooling. RCP also can minimize ischemic damage and extend the duration of safe cerebral circulatory interruption.¹⁻⁴ The distribution of perfused blood has been measured with the colored microsphere method,

and it has been observed that RCP can perfuse the whole brain without lateralization of perfusion or significant areas of inadequate cerebral perfusion.⁵ Retrograde perfusion is not a physiologically usual condition, however, and the regional microcirculation during RCP remains to be clarified. In this study, we measured regional tissue blood flow (TBF) with small (15 μm) and large (50 μm) colored microspheres, as well as with the hydrogen-clearance method. Small microspheres are trapped only in capillaries, allowing evaluation of capillary blood flow. Large microspheres are trapped in vessels smaller than 50 μm in diameter, which include capillaries and arteriovenous shunts. The hydrogen-clearance method measures regional blood flow. Discrepancies among TBF measured by each method can yield information about the condition of the cerebral microcirculation. We therefore compared the cerebral microcirculation during RCP with that during antegrade cardiopulmonary bypass (CPB) by means of both the colored microsphere and hydrogen-clearance methods.

Materials and methods

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Animal preparation and RCP method. Sixteen mongrel dogs weighing 13.0 ± 1.1 kg (11.5 to 15.0 kg) were used in this study. Eight underwent RCP and the other eight underwent antegrade CPB as control animals. Anesthesia was induced with ketamine hydrochloride (10 mg/kg) given intramuscularly and thiopental sodium (5 mg/kg) given intravenously. After endotracheal intubation, each animal was mechanically ventilated with 100% oxygen. The ventilator rate and tidal volume were adjusted to maintain the arterial carbon dioxide tension at approximately 35 mm Hg by the alpha-stat method of pH control. Anesthesia was maintained with intravenous ketamine hydrochloride (2 mg/kg/hr) and pancuronium bromide (0.1 mg/kg). Catheters were placed in the internal carotid or femoral artery, the right external jugular vein, and the right femoral vein to monitor blood pressures. A perfusion cannula (8F) for RCP was placed in both internal maxillary veins. Temperatures were recorded by a thermometer placed in the nasopharyngeal cavity. Cerebral blood flow (CBF) was measured by both the colored microsphere method and the hydrogen-clearance method. Hydrogen-producing electrodes were inserted into the right parietal cortex through a burr hole placed in the skull. A right thoracotomy was made through the fourth

intercostal space. Heparin (300 U/kg) was given intravenously. The ascending aorta was cannulated with a 16F metal-tipped perfusion catheter. The right atrium was cannulated with two separate venous cannulas (28F), and vena caval tapes were applied before clamping of the azygous vein. CPB was established at a flow rate of 1000 mg/min under oxygenation with pure oxygen at 1.0 L/min. Mechanical ventilation was then discontinued and a cross-clamp was applied to the ascending aorta. The heart was arrested, and intermittent cold crystalloid cardioplegia was applied. RCP was then established by perfusing blood through bilateral internal maxillary vein cannulas by a small roller pump through a Y-shaped connector. The aortic cannula was opened, and blood was directed by gravity to the cardiotomy reservoir after bilateral clamping of the caval cannula. The pump circuit consisted of a membrane oxygenator (D705 MIDIFLO; Dideco, Mirandola, Italy) with a cardiotomy reservoir (3L CARDIF PLUS; Mallinckrodt Medical TPI, Inc., Irvine, Calif.) primed with electrolyte solution and 500 ml blood obtained from another dog to prevent hemodilution. No intervention to control blood pressure was made during each study.

Experimental protocol. In the RCP group, CPB was established at a flow rate of 1000 ml/min under normothermic conditions (37° C). Ten minutes after aortic cross-clamping, after stabilization of the vital signs, the perfusion was switched to RCP. A nasopharyngeal core temperature was maintained at approximately 37° C with intermittent core heating. After this, rapid core cooling was induced to reduce the nasopharyngeal core temperature to approximate 28° C and then to 20° C while remaining on RCP to avoid the release of any microspheres trapped in capillaries. During RCP, the external jugular venous pressure was maintained at approximate 25 mm Hg while the perfusion flow rate was varied. Nasopharyngeal temperatures of 37°, 28°, and 20° C were each maintained for 10 minutes. At the end of each 10-minute period, colored microspheres were injected through the cannula placed in each internal maxillary vein, and the blood pressure in each catheter was recorded. Blood samples were withdrawn from the inflow and outflow cannulas, and return blood flow rate was measured directly from the aorta. The hydrogen clearance was measured simultaneously.

In the control group, CPB was established at a flow rate of 1000 ml/min, and a nasopharyngeal temperature of 37° C was established by intermittent core heating. Core cooling was applied to reduce the nasopharyngeal temperature to 28° C and then to 20° C. The same measurements performed in the RCP group were obtained at 37°, 28°, and 20° C (nasopharyngeal temperatures) and compared with those obtained during RCP. The animals of both groups were killed immediately at the end of the study to avoid releasing any microspheres from the tissue to the circulation.

Analysis. Blood pressure was measured with a blood pressure monitor (HP7835; Hewlett Packard Co., Palo Alto, Calif.) with disposable transducers (SCK7178; Viggo Spectramed PTE Ltd., Singapore, Singapore). The zero-pressure level was set at the level of the operating table. Perfusion flow was calculated from the pump rotation

rate, which was calibrated for each pump circuit after each procedure. The blood samples were drawn into heparinized syringes and analyzed immediately at 37° C (the alpha-stat pH control) for pH, oxygen tension, carbon dioxide tension, oxygen saturation, oxygen content, total carbon dioxide, and hemoglobin (ABL-300; Radiometer A/S, Copenhagen, Denmark). Cerebral TBF was measured with the hydrogen-clearance method (RBF-2; Bio-medical Science, Inc., Kanazawa, Japan).^{6,7} Cerebral TBF was calculated by subtracting the baseline value measured during total circulatory arrest.

Colored microsphere method. TBF was also measured with the colored microsphere method, which allows calculation of TBF from the number of microspheres trapped in the capillaries.⁸ Nonradioactive colored microspheres (E-Z Trac, Los Angeles, Calif.) are made of a polystyrene-divinylbenzene bridging complex and labeled with chemically stable dyes.⁸ Two sizes (15 and 50 μ m) and three colors (red, blue, and green) were used in this study. Saline solution (10 ml) containing 200 thousand counts of 50 μ m microspheres and 4 million counts of 15 μ m microspheres of each color was injected at a constant rate over 1 minute through the inflow cannula at the end of each 10-minute study period. The animals were killed at the end of the study and their skulls were opened. Blood was perfused through each internal maxillary vein to verify that there was no interference by any venous valves. The whole brain and portions of the upper and lower spinal cord were dissected for analysis. The cerebrum was dissected bilaterally into frontal, parietal, and occipital lobes. Each part was dissected into white and gray matters. The brain stem was dissected into the thalamus, putamen, pons, and medulla oblongata. From each specimen a 2 to 3 gm sample was separated, weighed, and processed by enzymatic digestion and centrifugation. The number of microspheres in each sample was determined with a hemocytometer.

TBF in each sample was calculated according to the following formula: $TBF = A \times (Mo/Mi) \times (Q/W) \times (ml/min/100\text{ gm})$, where A is a constant (2381), Mo is the number of observed microspheres, Mi is the number of injected microspheres, Q is the perfused flow rate + 10 (in milliliters per minute), and W is the weight of the tissue sample (in grams). Total CBF (TCBF) was calculated as the sum total of blood flow in each specimen ($TBF \times W/100\text{ ml/min}$).

Calculation. Total vascular resistance (R) was calculated according to the following formula: $R = 79920 \times (Pi - Po)/Q$ (dynes $\cdot$ sec $\cdot$ cm⁻⁵), where during RCP, Pi is the external jugular venous pressure, Po is the internal carotid arterial pressure, and Q is the blood flow (in milliliters per minute) returned through the aortic cannula, and during CPB, Pi is the femoral arterial pressure, Po is the central venous pressure, and Q is the perfusion flow rate. Whole-body oxygen consumption (Vo_2) was calculated from the following equation: $Vo_2 = (Cao_2 - Cvo_2) \times Q/100 \times (ml/min)$, where Cao_2 is the oxygen content of the perfusate blood, Cvo_2 is the oxygen content of returned blood, and Q is either the blood flow (in milliliters per minute) returned through the aortic cannula during RCP or the perfusion flow rate during CPB.

Results are expressed as means plus or minus standard deviation. Statistical significance was determined with the paired t test after confirming normal distribution. The p values on multiple comparison were corrected by the Bonferroni method, and a p value less than 0.05 was determined to represent significance.

Results

Perfusion flow rates, vascular resistance and oxygen consumption. Antegrade CPB was performed at a perfusion flow rate of 1000 ml/min. Vascular resistance did not change significantly during antegrade CPB as the body temperature decreased (Fig. 1). However, Vo_2 decreased significantly as the body was cooled. Oxygen consumption was 54.5 ± 19.1 ml/min at 37° C, 37.5 ± 11.7 ml/min at 28° C, and 16.0 ± 10.5 ml/min at 20° C. The difference between oxygen consumption at 37° C and at the lower temperatures was significant (Fig. 2). Retrograde perfusion flow rates were 335 ± 64 ml/min at 37° C, 253 ± 25 ml/min at 28° C, and 277 ± 60 ml/min at 20° C. Retrograde perfusion flow rates appeared to change slightly at different temperatures; however, these changes were not significant. Vascular resistances increased when the nasopharyngeal temperature decreased to 20° C; a significant difference compared with the value at 37° C (4817 ± 1235 vs 3035 ± 1063 dynes $\cdot$ sec $\cdot$ cm⁻⁵; Fig. 1). Oxygen consumption during RCP also decreased as the body temperature decreased. However, there were no significant differences among these values. Oxygen consumptions during RCP were one sixth and one fourth those during CPB at 37° C and at 20° C, respectively (Fig. 2).

Distribution of TBF according to colored microsphere method. The average rates of brain blood flow in the white matter, gray matter, brain stem, cerebellum, and spinal cord measured with the 50 μ m microspheres during normothermic RCP were 7.5 ± 7.0 , 4.7 ± 4.5 , 12.0 ± 10.0 , 9.7 ± 8.3 , and 9.7 ± 5.9 ml/min/100 gm, respectively. These values were 18.7%, 13.3%, 24.5%, 22.7%, and 20.4%, respectively, of the values measured during normothermic CPB (Fig. 3). There was no lateralization in the distribution of blood flow and no significant differences between the frontal, parietal, and occipital lobes in the white and gray matters of cerebrum. The average brain TBFs assessed with the 15 μ m microspheres were extremely low, measuring 0.8 ± 0.7 , 0.9 ± 0.8 , 1.5 ± 1.0 , and 0.5 ± 0.4 ml/min/100 gm in the white matter, gray matter, brain stem, and cerebellum, respectively. These values were only 3.1%, 3.4%, 3.7%, and 1.6% of the respective blood

Fig. 1. Vascular resistance during antegrade CPB and retrograde perfusion under conditions of normothermia, moderate hypothermia, and deep hypothermia. Error bars represent standard deviation.

flows measured with the 15 μ m microspheres during normothermic CPB (Fig. 3).

Cerebral TBF measured by colored microsphere and hydrogen-clearance methods. During antegrade CPB, TBF in the cerebral cortex measured with both the 50 μ m and 15 μ m colored microsphere decreased significantly as body temperature decreased. There were significant differences between the CBF measured at 37° C and at 20° C with both types of microspheres. However, the hydrogen-clearance method did not show a significant change in cerebral TBF as body temperature decreased. At a body temperature of 37° C, the CBF measured with the 50 μ m microspheres was significantly higher than that measured with the hydrogen-clearance method. During deep hypothermia (20° C), the CBF measured by the 15 μ m microspheres was significantly less than the blood flow measured with the hydrogen-clearance method (Fig. 4).

During RCP, the cerebral TBF measured by the hydrogen-clearance method did not change significantly with change in body temperature. Cerebral TBF measured with both the 50 and 15 μ m microspheres, however, decreased significantly as the body was cooled. There was a marked difference in results between the methods during RCP at 28° and 20° C. At 37° C, the CBF measured with the

15 μ m microspheres was significantly less than the CBF measured with both the hydrogen-clearance method and the 50 μ m microspheres. At 28° and 20° C, CBF measured by the hydrogen-clearance method was significantly greater than the values obtained with either the 50 or 15 μ m microspheres (Fig. 4).

TCBF and whole-body blood flow. During antegrade CPB, the TCBF, calculated as the sum total of the blood flows measured with the 50 μ m microsphere, were 33.7 ± 16.4 ml/min at 37° C, 24.3 ± 10.4 ml/min at 28° C, and 14.5 ± 14.0 ml/min at 20° C. These values significantly decreased as body temperature decreased and were 3.4%, 2.4%, and 1.5% of perfused blood flow rate at 37° C, 28° C, and 20° C, respectively. TCBFs measured with the 15 μ m microspheres were 22.4 ± 9.9 ml/min at 37° C, 23.5 ± 11.0 ml/min at 28° C, and 10.4 ± 6.2 ml/min at 20° C, representing 2.2%, 2.4%, and 1.0%, respectively, of the perfused blood flow. The TCBF was significantly decreased at 20° C; however, there were no significant differences between the values measured with the 50 and 15 μ m microspheres at any temperature.

During RCP, the TCBFs measured with the 50 μ m microsphere were 6.1 ± 5.5 ml/min at 37° C, 2.3 ± 1.2 ml/min at 28° C, and 1.0 ± 0.7 ml/min at

Fig. 2. Oxygen consumption during antegrade CPB and retrograde perfusion under conditions of normothermia, moderate hypothermia, and deep hypothermia. Error bars represent standard deviation.

20° C, which were 1.8%, 0.9% and 0.34%, respectively, of the perfused blood flow rate. The TCBF and the percentage of TCBF decreased significantly as body temperature decreased. TCBFs measured with the 15 μ m microsphere were extremely low, only 0.71 ± 0.57 ml/min at 37° C, 0.24 ± 0.13 ml/min at 28° C, and 0.20 ± 0.16 ml/min at 20° C, or only 0.21%, 0.10%, and 0.07%, respectively, of the perfused blood flow.

Discussion

Retrograde perfusion through a superior vena caval cannula has been reported as a new technique for brain protection during circulatory arrest. We also have reported the results of several experiments with RCP. In a previous study, we found that RCP can provide blood and oxygen to the brain; however, it is insufficient to maintain full brain function.² Nonetheless, RCP can minimize the oxygen debt and ischemic damage to the brain and extend the duration for which cerebral circulation can be safely interrupted.³ Blood flow to the brain and brain oxygen consumption do not increase once venous pressure exceeds 25 mm Hg.⁴ In our most recent study, we measured the distribution of regional blood flow during RCP with the colored micro-

sphere method.⁵ We concluded that RCP can perfuse the whole brain without lateralization of perfusion, causing no significant areas of inadequate cerebral perfusion. We also found that regional blood flow measured by the colored microsphere method varied according to the body temperature and with the size of microspheres. In this study, our aim was to further define the variations in regional blood flow associated with changes in body temperature.

We used the microsphere method with two different sizes of microspheres (50 and 15 μ m) and also used the hydrogen-clearance method to measure regional blood flow during normothermia (37° C), moderate hypothermia (28° C,) and deep hypothermia (20° C). The colored microsphere method measures TBF by counting the microspheres trapped in capillaries.⁸ If microspheres circulating in the body are not be trapped by capillaries, the TBF may be underestimated. The small microspheres tend to distribute in a fashion similar to that of red blood cells but are subject to shunting, particularly when physiologic changes in vascular tone occur.⁹⁻¹¹ On the other hand, the larger microspheres are preferentially trapped in areas of high flow because of their axial distribution in the bloodstream.⁹⁻¹¹ Mi-

Fig. 3. Regional blood flow measured with 15 and 50 μ m microspheres during antegrade CPB and retrograde perfusion on normothermia. Error bars represent standard deviation.

crosspheres 50 μ m in size are easily trapped in capillary beds that the 15 μ m microspheres run through. The difference in the regional blood flows measured with 15 μ m and 50 μ m microspheres should therefore represent blood flow that is shunted away from capillaries. We also measured regional blood flow with the hydrogen-clearance method. This method measures regional blood flow of 100 mg wet volume by evaluating the clearance curve of tissue hydrogen concentrations.^{6,7} The tissue hydrogen content should be cleared by capillary blood flow; however, it may be cleared by blood flow that shunts away from capillaries. It is therefore possible that the hydrogen-clearance method overestimates regional blood flow. When discrepancies between the regional blood flows measured with 50 μ m microspheres and the hydrogen-clearance method occur, the differences represent shunt blood flow through vessels larger than 50 μ m in diameter between the arterial and venous systems. Comparison of the regional blood flows measured with these methods therefore can yield more accurate information regarding the regional microcirculation.

We summarize our results as follows: (1) During antegrade CPB, CBF decreases as body temperature

decreases, and the CBF shunted away from capillaries increases at deep hypothermia. (2) During retrograde perfusion, the majority of the CBF is shunted away from capillaries, even during normothermia. The amount of blood that runs through larger shunts increases as body temperature decreases. There have been several reports regarding the microcirculation during normal perfusion. It has been reported that blood flow that is shunted away from capillaries increases during deep hypothermia¹² as arteriovenous shunts open. Our results concur with these reports. Blood flow at 20°C measured with the 15 μ m microspheres was significantly lower than that measured by other methods. This indicates that arteriovenous shunts larger than 15 μ m are open at 20°C.

The study of the microcirculation during retrograde perfusion has not been well defined. Theoretically, blood perfused in a retrograde manner runs from the venous system to the arterial system through capillaries. There is, however, no evidence to support this theory. This study indicates that most blood perfused retrogradely may be shunted away from capillaries, running through larger venoarterial shunts in the brain tissue. This conjecture is based

Fig. 4. TBF in cerebral cortex measured by the hydrogen-clearance and 15 and 50 μ m microsphere method during antegrade CPB and retrograde perfusion during normothermia, moderate hypothermia, and deep hypothermia. Error bars represent standard deviation.

on the findings that the 50 μ m microspheres were trapped and the 15 μ m microspheres passed through during normothermic RCP. During hypothermic perfusion, even the larger microspheres (50 μ m) were not trapped. This indicates that large venoarterial shunts, through vessels greater than 50 μ m in diameter, may open during hypothermia. The phenomenon may be related to functional arteriovenous shunts with a diameter of 70 μ m in the pia mater and spinal cord described in studies of primates.¹³

Retrograde perfusion is entirely different from antegrade perfusion. There is a small possibility that microspheres will not be trapped even when the microspheres run through capillary beds. It is, however, more probable that the majority of the blood runs through venoarterial shunts. Boeckxstaens and Flameng¹⁴ reported that significant CBF could not be detected by microsphere methods and less than 1% of the RCP inflow returned to the aortic arch during hypothermic RCP in a baboon experimental

study. They concluded that RCP does not perfuse the brain because of venovenous shunting. They performed RCP through bilateral internal jugular veins with the inferior vena caval drainage. The inferior vena caval drainage during RCP is subject to venovenous shunting¹; in their study, however, microspheres were traced even in returned blood to the aortic arch. Arteriovenous shunts in the brain are therefore probably responsible for this shunting during RCP. RCP can provide some oxygen to the brain tissue and reduce the decrease in tissue adenosine triphosphate levels. It is not clear, however, whether retrograde perfusion provides sufficient brain protection because the microcirculation during RCP is totally different from the physiologically normal circulation.

The dog may not be a good model for RCP studies because it has a small internal jugular vein and also has many cervical venous valves.¹⁵ Human beings, in contrast, have large internal jugular veins and few jugular venous valves.¹⁵ Venous valves

interfere with retrograde perfusion. We observed both internal maxillary veins after each procedure to confirm that there was no interference caused by venous valves.

The cerebral microcirculation during retrograde perfusion is still not fully defined; however, the majority of blood perfused in a retrograde fashion tends to shunt away from capillaries of the brain. The efficacy of the blood shunted away from capillaries is also unclear; however, retrograde perfusion provides some degree of oxygenation to the body. It is still unclear whether retrograde perfusion provides sufficient brain protection because of extreme differences between the microcirculation during retrograde perfusion and that under physiologic conditions. Further experimental and clinical studies are necessary to clarify how long RCP can prolong the time during which circulatory arrest is safe.

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REFERENCES

1. Usui A, Hotta T, Hiroura M, Murase M, Maeda M, Koyama T, et al. Retrograde cerebral perfusion through a superior vena caval cannula protects the brain. *Ann Thorac Surg* 1992;53:47-53.
2. Usui A, Hotta T, Hiroura M, Murase M, Maeda M, Koyama T, et al. Cerebral metabolism and function during normothermic retrograde cerebral perfusion. *J Cardiovasc Surg* 1993;1:107-12.
3. Usui A, Oohara K, Tong-lin L, Murase M, Tanaka M, Takeuchi E, et al. Determination of optimum retrograde cerebral perfusion conditions. *J Thorac Cardiovasc Surg* 1994;107:300-8.
4. Usui A, Oohara K, Tong-lin L, Murase M, Tanaka M, Takeuchi E, et al. Comparative experimental study between retrograde cerebral perfusion and circulatory arrest. *J Thorac Cardiovasc Surg* 1994;107:1228-36.
5. Oohara K, Usui A, Murase M, Tanaka M, Abe T. Regional cerebral tissue blood flow measured by the colored microsphere method during retrograde cerebral perfusion. *J Thorac Cardiovasc Surg* 1995;109:772-9.
6. Doyle TF, Martins AN, Kobrine AI. Estimating total cerebral blood flow from the initial slope of hydrogen washout. *Stroke* 1975;6:149-52.
7. Pasztor E, Symon L, Dorsch NWC, Branston NM. The hydrogen clearance method in assessment of blood flow in cortex, white matter and deep nuclei of baboons. *Stroke* 1973;4:556-67.
8. Hale SL, Alker K J, Kloner RA. Evaluation of nonradioactive, colored microspheres for measurement of regional myocardial blood flow in dogs. *Circulation* 1988;78:428-34.
9. Phibbs RH, Dong L. Nonuniform distribution of microspheres through a medium-sized artery. *Can J Physiol Pharmacol* 1970;48:415-21.
10. Yipintsoi T, Dobbs WA Jr, Scanlon PD, Knopp TJ, Bassingthwaite JB. Regional distribution of diffusible tracers and carbonized microspheres in the left ventricle of isolated dog hearts. *Circ Res* 1973;33:573-87.
11. Marcus ML, Heistad DD, Ehrhardt JC, Abboud FM. Total and regional cerebral blood flow measurement with 7-, 10-, 15-, 25- and 50- μ m microspheres. *J Appl Physiol* 1976;40:501-7.
12. Ogura K, Takayasu M, Dacey RG Jr. Effects of hypothermia and hyperthermia on the reactivity of rat intracerebral arterioles in vitro. *J Neurosurg* 1991;75:433-9.
13. Ogata J, Feigin I. Arteriovenous communications in the human brain. *J Neuropathol Exp Neurol* 1972;31:519-25.
14. Boeckxstaens CJ, Flameng WJ. Retrograde cerebral perfusion does not perfuse the brain in nonhuman primates. *Ann Thorac Surg* 1995;60:319-28.
15. Miller MM. The venous system. In: Miller MM, editor. *Anatomy of the dog*. Philadelphia, London: WB Saunders; 1964. p. 389-435.