CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

CAN RETROGRADE PERFUSION MITIGATE CEREBRAL INJURY AFTER PARTICULATE EMBOLIZATION? A STUDY IN A CHRONIC PORCINE MODEL

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Objective: We assessed the impact on histologic and behavioral outcome of an interval of retrograde cerebral perfusion after arterial embolization, comparing retrograde cerebral perfusion with and without inferior vena caval occlusion with continued antegrade perfusion. **Methods:** Sixty Yorkshire pigs (27 to 30 kg) were randomly assigned to the following groups: antegrade cerebral perfusion control; antegrade cerebral perfusion after embolization; retrograde cerebral perfusion control; retrograde cerebral perfusion after embolization; retrograde cerebral perfusion with inferior vena cava occlusion, retrograde cerebral perfusion with inferior vena cava occlusion control, and retrograde cerebral perfusion with inferior vena cava occlusion after embolization. After cooling to 20° C, a bolus of 200 mg of polystyrene microspheres 250 to 750 (μ m diameter (or saline solution) was injected into the isolated aortic arch. After 5 minutes of antegrade cerebral perfusion, 25 minutes of antegrade cerebral perfusion, retrograde cerebral perfusion, or retrograde cerebral perfusion with inferior vena cava occlusion was instituted. After the operation, all animals underwent daily assessment of neurologic status until the time of death on day 7. **Results:** Aortic arch return, cerebral vascular resistance, and oxygen extraction data during retrograde cerebral perfusion showed differences, suggesting that more effective flow occurs during retrograde cerebral perfusion with inferior vena cava occlusion, which also resulted in more pronounced fluid sequestration. Microsphere recovery from the brain revealed significantly fewer emboli after retrograde cerebral perfusion with inferior vena cava occlusion. Behavioral scores showed full recovery in all but one control animal (after retrograde cerebral perfusion with inferior vena cava occlusion) by day 7 but were considerably lower after embolization, with no significant differences between groups. The extent of histopathologic injury was not significantly different among embolized groups. Although no histopathologic lesions were present in either the antegrade cerebral perfusion control group or the retrograde cerebral perfusion control group, mild significant ischemic damage occurred after retrograde cerebral perfusion with inferior vena cava occlusion even in control animals. **Conclusions:** Although effective washout of particulate emboli from the brain can

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be achieved with retrograde cerebral perfusion with inferior vena cava occlusion, no advantage of retrograde cerebral perfusion with inferior vena cava occlusion after embolization is seen from behavioral scores, electroencephalographic recovery, or histopathologic examination; retrograde cerebral perfusion with inferior vena cava occlusion results in greater fluid sequestration and mild histopathologic injury even in control animals. Retrograde cerebral perfusion with inferior vena cava occlusion shows clear promise in the management of embolization, but further refinements must be sought to address its still worrisome potential for harm. (*J Thorac Cardiovasc Surg* 1998;115:1142-59)

Although use of hypothermic circulatory arrest (HCA) in the management of complex cardiovascular surgical procedures continues to enjoy widespread clinical acceptance, there is persistent concern about its efficacy in protecting the brain when longer durations of HCA are required. Because highly complex problems requiring lengthy periods of HCA are now being addressed more frequently by operation, there is widespread interest in the study of methods that might provide improved cerebral safety under these circumstances. The use of retrograde cerebral perfusion (RCP) was proposed some years ago as a possible means of prolonging the safe duration of HCA and of dealing with the catastrophic effects of both air and particulate emboli.^{1,2}

Fortunately, the incidence of severe cerebral damage directly attributable to HCA remains relatively small, but recent studies have confirmed the suspicion that subclinical cerebral injury occurs in a significant proportion of neonates after prolonged HCA³⁻⁵ and that some degree of transient neurologic injury occurs in a substantial number of adult patients with long durations of HCA during aortic arch operations.⁶ Because the overall results of cardiac and aortic operations have steadily improved in recent years and because the adult population undergoing cardiac operations has gradually aged, the patients in whom morbidity and/or death are clearly a consequence of cerebral dysfunction have increasingly become a major focus of concern. Currently, there is a great deal of interest in strategies to avoid cerebral emboli in patients with extensive atheromatous disease who are undergoing either an aortic or coronary artery bypass operation.⁷ In this atmosphere of the heightened awareness of possible cerebral sequelae of cardiac and aortic operations, RCP is especially appealing because it may provide a way of improving neurologic outcome

both by reducing the incidence or mitigating the severity of embolic injury and by increasing cerebral protection during HCA.⁸⁻¹⁰

We have developed a chronic porcine model to study RCP. We have thus far been able to demonstrate that RCP results in a small amount of nutritive flow and provides a degree of cerebral protection during prolonged HCA that appears to be superior to HCA alone, even when the head is packed in ice.¹¹ We have also investigated the possibility that RCP might improve neurologic outcome after particulate embolization to the brain, but we were unable to demonstrate an unequivocal benefit from the use of RCP in this context, although we accumulated some encouraging data.¹²

The present study was undertaken to explore the hypothesis that occluding the inferior vena cava (IVC) during RCP (RCP-O) may provide more effective washout of emboli than RCP without IVC occlusion. The anticipated superiority of RCP-O was based on two observations: that much of the flow into the superior vena cava (SVC) during RCP can be seen to be shunting into the lower resistance IVC and that RCP in human cadavers takes place largely via spinal cord collaterals fed by the azygos and hemiazygos systems.¹³ We therefore expected that we might be able to show improved outcome after particulate embolization in our chronic porcine model using this new approach to pressurize the entire venous system during RCP.

In the current study, we have compared an interval of RCP-O, RCP without IVC occlusion, and continued antegrade cerebral perfusion (ACP) after an ascending aortic injection of polystyrene microspheres. Using saline solution-injected control animals, we have examined the impact of these interventions on the retention of microspheres in the brain, cerebrovascular physiology and metabolism,

Fig. 1. Mean ($\pm$ standard error of mean) esophageal, rectal, and epidural temperatures at various time points in 60 pigs.

electrophysiologic recovery, behavioral outcome, and cerebral histologic evidence.

Materials and methods

Sixty juvenile Yorkshire pigs (Thomas D. Morris, Inc, Reisterstown, Md.), 3 to 4 months of age, weighing 27 to 30 kg, were randomly assigned to groups to undergo 25 minutes of one of the following procedures at 20°C: ACP control (AC); ACP after embolization (AE); RCP control (RC); RCP after embolization (RE); RCP-O control (RCO), and RCP-O after embolization (REO).

Preoperative management. All animals received humane care in accordance 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 Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 85-23 revised 1985). The protocol for these experiments was approved by the Mount Sinai Institutional Animal Care and Use Committee.

Anesthesia and hemodynamic monitoring. Anesthesia was induced with ketamine hydrochloride (10 mg/kg intramuscularly), and muscular paralysis was maintained with pancuronium (0.1 mg/kg intravenously). After endotracheal intubation, the animals were maintained on positive-pressure ventilation with 100% oxygen and isoflurane anesthesia (1%). For monitoring, appropriate catheters were positioned in the left femoral artery and vein, in the pulmonary artery to measure cardiac output by thermodilution, in the esophagus and rectum for temperature, and in the bladder for urine output. The sagittal sinus was also cannulated as previously described for monitoring of pressure and blood withdrawal, and a fine-wire temperature probe (IT-18; Physiotemp Instruments Inc, Clifton, N.J.) was inserted into the epidural space.^{11, 12}

Electroencephalography (EEG) and evoked-potential monitoring. Cortical electrical activity was monitored from four stainless-steel screw electrodes (5 mm diame-

ter) implanted in the skull over the parietal and frontal areas of the cortex. All EEG recordings were referenced to a frontal screw electrode, which, with a ground screw electrode, was implanted over the frontal sinuses. Processing of the EEG, including amplification, filtration, digitization, storage, and analysis, was conducted on a Spectrum 32 data acquisition system (Cadwell Laboratories, Kennewick, Wash.). Continuous EEG activity was recorded for 3 minutes at the time of each measurement.

Somatosensory-evoked potentials (SSEPs). SSEPs were recorded from the cervical spine and bilateral skull sites in response to stimulation of the median nerve. Electrical stimulation (25 mA, 0.1 msec duration) of the median nerve was accomplished through a pair of stainless-steel needle electrodes that were inserted at the most distal joint on the posterior surface of each forelimb.

The cervical recording electrode was a long stainless-steel needle that was insulated except at the tip (impedance < 3000 Ω). The electrode was inserted at approximately the second cervical vertebra and lowered until the spine was contacted. The electrode and its wire lead were held in place by a suture. For all recordings, a nose reference was used. The most repeatable potential from the cervical site was a negative-positive wave; the latency of the negative wave was 6 to 8 msec, and the latency of the positive peak was 8 to 10 msec.

Stainless-steel screws to which wire leads were soldered served as the skull recording electrodes. The exact sites of these electrodes were determined empirically in pilot experiments by mapping the skull region overlying the cerebral cortex while stimulating the median nerve. With the use of a nose reference, a large negative-positive complex could be recorded in all animals. The peak latency of the negative potential was 17 to 19 msec. The largest potentials were recorded at a site approximately 50% of the distance between the frontal and occipital poles and approximately 10 mm lateral to the midline on the side contralateral to the stimulation. The exact coordinates were 10 mm anterior and 10 mm lateral to bregma.

Two sets of SSEPs were recorded for each median nerve at five of the six measurement time points described in the experimental protocol. Each waveform was obtained by averaging the responses to 500 stimuli. The latencies and amplitudes were measured at 7 to 9 msec for the positive cervical potential and at 19 to 21 msec for the negative cortical potential in all animals. The amplitudes of the responses at the first recording session were used as a baseline. All subsequent amplitude measurements were converted to a percent of the baseline.

Auditory brainstem-evoked responses (BSERs). BSERs were recorded from all animals by averaging the responses to 1000 presentations of an auditory click stimulus (95 db) that was presented to the right ear through an ear insert (Cadwell Laboratories). The sites of the recordings were determined in pilot experiments. Probably because of the mass and shape of the pig's head, recordings from surface electrodes placed near the ear produced unacceptable levels of variability in the shape of the responses. On the other hand, recordings from a cervical electrode (described in the previous section) and referenced to a

Table I. Vascular resistance during stable selective perfusion

Group	n	CPB flow (ml/min)	Vascular resistance (mm Hg/ml/min)				
			Pressure (mm Hg)		Upper body		Lower body
			SVC	IVC	SVC determined	SS determined	IVC determined
AC	10	188 ± 47	—	—	—	7.77 ± 1.91	—
AE	10	108 ± 32*	—	—	—	14.45 ± 3.95	—
RC	10	254 ± 158	67 ± 39	-1 ± 4	9.23 ± 11.04	3.70 ± 4.5	—
RE	10	249 ± 106	47 ± 23	2 ± 5	4.47 ± 2.0	2.42 ± 1.0	—
RCO	10	131 ± 52	36 ± 15	17 ± 5	2.30 ± 1.87	1.52 ± 0.69	0.38 ± 0.18
REO	10	177 ± 52	44 ± 28	22 ± 5	1.94 ± 1.03	1.34 ± 0.51	0.33 ± 0.13

AC, Saline control followed by antegrade cerebral perfusion; AE, microsphere embolization followed by antegrade cerebral perfusion; RC, saline injection followed by retrograde cerebral perfusion; RE, microsphere embolization followed by retrograde cerebral perfusion; RCO, saline injection followed by retrograde cerebral perfusion with occlusion of the inferior vena cava; REO, microsphere embolization followed by retrograde cerebral perfusion with occlusion of the inferior vena cava; CPB, cardiopulmonary bypass; IVC, inferior vena cava, SVC, superior vena cava, SS, sagittal sinus. Values are shown as mean ± standard deviation.

**p* = 0.0003 compared with controls.

cortical screw produced consistent waveforms in the pilot animals.

The first repeatable wave was a positive potential at a latency of 1.5 to 1.8 msec. This first wave was followed consistently by a series of waves with interpeak latencies of approximately 1.0 msec. In some animals, the second positive wave was not distinct but was masked by the third positive wave. In addition, the fifth positive wave in many animals was not distinct and was merged with the fourth wave. Although the first and fourth peaks in the BSERs were the most consistent and could have been used for analysis, we opted instead to measure the total spectral power in all waves of the BSERs from 1.25 to 6.25 msec after stimulus presentation. The total power of the BSER at the first measurement time point was used as a baseline for all subsequent measurements.

Cardiopulmonary bypass (CPB). Through a right thoracotomy in the fourth intercostal space, the azygos and hemiazygos veins were ligated (the porcine hemiazygos vein drains independently into the coronary sinus). The SVC and aortic arch were mobilized, and the descending aorta just distal to the left subclavian artery was exposed by a retrosophageal approach. Twenty-gauge catheters were positioned in the isolated aortic arch, the SVC, and the IVC.

After heparinization (300 IU/kg), the ascending aorta was cannulated with a 16F arterial cannula, and the right atrial appendage was cannulated with a single 24F atrial cannula; nonpulsatile CPB was initiated at 100 ml/kg per minute. A cannula was passed from the right superior pulmonary vein into the left ventricle to permit the decompression of the left ventricle during CPB. Core cooling was carried out with a heat exchanger, and surface cooling was accomplished with a cooling blanket. A membrane oxygenator (VPCML plus; Cobe Laboratories, Inc., Lakewood, Colo.) was primed with 1 liter 0.9% NaCl, 1 unit 5% albumin, furosemide (1 mg/kg), heparin (5000 IU), and KCl (1 mEq/kg). The pH was maintained, with alpha-stat principles, at 7.40 ± 0.05 with an arterial carbon dioxide tension of 35 to 40 mm Hg, uncorrected for temperature.

Perfusion cooling was carried out for 25 minutes to attain an esophageal temperature of 20° C. Cardiac arrest

was induced with potassium chloride (1 mEq/kg), and topical cardiac cooling was begun and maintained throughout the procedure. The ascending aorta was crossclamped just proximal to the aortic cannula, and the descending aorta was crossclamped distal to the left subclavian artery, thereby isolating the transverse aortic arch.

Experimental protocol. After isolation of the aortic arch, aortic arch pressure was slowly increased to 50 mm Hg. When this pressure had been maintained for 60 seconds, a flow rate designated as *preinjection flow* was recorded. Two hundred milligrams of polystyrene microspheres 250 to 750 μ m in diameter (coated with albumin to minimize clustering and suspended in 10 ml saline solution) were injected into the isolated aortic arch through the aortic perfusion cannula in the embolized groups; control groups were injected with saline solution.

If aortic arch pressure changed after the injection, CPB flow was increased or decreased to maintain a pressure of 50 mm Hg. The resulting antegrade flow was then stabilized (*postinjection flow*) and maintained for 5 minutes in all groups; it was followed by 5 minutes of HCA during which preparations for RCP were made as necessary. For RCP, a 14F cannula was inserted into the SVC and advanced as cranially as possible, snared in place, and connected to the arterial line with a Y connector. In the RCP-O groups the IVC was also snared, and a 10F cannula was inserted into the descending aorta just distal to the second crossclamp to permit collection of descending aortic return.

As dictated by the randomization protocol, a 25-minute interval of ACP, RCP, or RCP-O was then initiated. Retrograde flow was slowly increased and regulated to achieve a pressure of 25 mm Hg in the sagittal sinus during the first 10 minutes (*prestable period*). During the subsequent 15 minutes, the CPB flow was kept constant despite slight alterations in sagittal sinus pressure (*stable period*). In animals undergoing RCP-O, maintenance of adequate retrograde flow required infusion of 200 to 300 ml of blood obtained from donor pigs. In the ACP groups, a pressure of 50 mm Hg in the aortic arch was maintained throughout.

In the retrograde groups, perfusate returning from the upper body to the isolated aortic arch, right atrium, and

Table II. Blood return and sequestration during selective perfusion

Group	n	Return blood volume (ml)						Return blood flow (ml/min)		
		Prestable period			Stable period			Stable period		
		Ascending aorta	Descending aorta	Right atrium	Ascending aorta	Descending aorta	Right atrium	Ascending aorta	Descending aorta	Right atrium
AC	10	—	—	—	—	—	—	—	—	—
AE	10	—	—	—	—	—	—	—	—	—
RC	10	73 ± 81	—	871 ± 917	183 ± 142	—	2147 ± 1650	12 ± 9	—	143 ± 110
RE	10	84 ± 27	—	1466 ± 718	179 ± 90	—	2718 ± 1302	12 ± 6	—	181 ± 87
RCO	10	124 ± 86	224 ± 192	15 ± 34	297 ± 151*	802 ± 359	9 ± 18	20 ± 10*	53 ± 24	0.5 ± 1.2
REO	10	100 ± 59	203 ± 144	2 ± 4	295 ± 119*	1106 ± 395	2 ± 7	20 ± 8*	74 ± 26	0.15 ± 0.5

Values are shown as mean ± standard deviation. Abbreviations as in Table I.

*When these control and embolism data for each perfusion method are pooled, they differ significantly from one another, as discussed in the text.

descending aorta (only in RCP-O) was drained to collecting chambers; its volume was measured and returned to the pump. The amount of sequestered fluid was assessed in all groups. In the retrograde groups, the microspheres were recovered from the perfusate with a 40 μ m transfusion filter.

After 25 minutes of selective perfusion, all animals were switched to ACP and rewarming was initiated. Ice slush was removed from the chest; the blanket was turned to the heating position, and an external heating source (lamp) was used. Weaning from CPB occurred approximately 60 minutes after the beginning of rewarming, with cardiac support provided by dobutamine; a single dose of furosemide (60 mg) and mannitol (12.5 gm) was administered. Animals were extubated 1 hour after the last measurement.

During the experiments, measurements were recorded at six different times:

1. At baseline, 37° C (esophageal)
2. At the end of cooling, at 20° C, immediately before institution of the intervention
3. During stable perfusion, at 20° C, 15 minutes after the start of the intervention
4. During rewarming, at 30° C
5. Two hours after the start of rewarming
6. Four hours after the start of rewarming

Postoperative evaluation. Postoperatively, all animals were evaluated daily with a previously described quantitative behavioral score.^{11, 12} Each surviving animal was killed on day 7. After being weighed, the brain was divided in half, and the left side was immediately immersed in 4% formalin for histologic analysis; the right side was processed for microsphere recovery.

Microsphere recovery. After being sliced into small pieces to increase its exposed surface area, the entire right side of the brain was digested with 250 ml of a 10 mol/L potassium hydroxide solution for at least 24 hours to allow recovery of the microspheres. The solution was passed through a 45 μ m filter and flushed thoroughly; if necessary, digestion and filtering were repeated 24 hours later until no tissue was left. After being dried, the microspheres were collected and weighed.

Histopathologic analysis. After fixation, the left hemisphere of each brain was examined grossly. The cerebrum was sectioned in the coronal plane; the brainstem, cerebellum, and 1.5 inches of the spinal cord were sectioned in a horizontal plane. Sections were then processed into paraffin, and 5 μ m sections were stained with hematoxylin and eosin.

Alternate coronal sections of the entire brain of each animal were systematically examined by a single experienced senior neuropathologist (D.W.), blinded both to the experimental design and to the identity and fate of individual animals. Each section was carefully screened for the presence or absence of any infarctive or other damage.

Definition of the anatomic regions scored was as previously described. Morphometric analysis of the volume of ischemic injury of the cerebral cortex was classified in five groups: 0 = no microscopic ischemic damage identified in any sections; 1 = <5% of total neocortex infarcted; 2 = >5% but <30% of total neocortex infarcted; 3 = >30% but <70% of total neocortex infarcted; 4 = >70% of total neocortex infarcted. For all other anatomic regions, the classifications were: 0 = no microscopic ischemic damage identified; 1 = a single or multiple small infarctive lesions, and 2 = a large area of infarctive damage.

To allow quantitative comparisons, a total histologic score was calculated by adding all the regional scores, although we recognize that this is by no means a rigorous quantitative assessment. A score of more than 4 means that the animal had significant extracortical and cortical injury.

Other measurements. Systemic arterial, venous, and sagittal sinus blood samples were obtained to determine pH, oxygen tension, carbon dioxide tension, oxygen saturation, oxygen content, hematocrit, and hemoglobin (Ciba-Corning Diagnostic Corp., Medfield, Mass.). Glucose and lactate were analyzed with a YSI 2300 Stat (Yellow Springs Instrument Co., Yellow Springs, Ohio). Temperatures were also recorded at intervals throughout the study.

Statistical analysis. Summary statistics for continuous or ordinal variables were expressed as the mean ± standard deviation, mean ± standard error of mean, or as

<i>Blood sequestration (ml)</i>		
<i>Prestable period</i>	<i>Stable period</i>	<i>Total</i>
76 ± 121	-17 ± 58	59 ± 124
90 ± 122	25 ± 72	115 ± 145
541 ± 286	1245 ± 772	1786 ± 1869
570 ± 108	838 ± 366	1408 ± 406
1550 ± 491*	1130 ± 588	2680 ± 967*
1458 ± 464*	1722 ± 737	3263 ± 894*

the median. Statistical significance was determined by one-way analysis of variance between the treatment groups. Comparisons between each time point and baseline were done by a set of paired *t* tests or Wilcoxon signed-rank tests. Normality was tested first, and if it failed, the analysis was performed with the corresponding non-parametric tests (Wilcoxon or Kruskal-Wallis tests). If significant differences were found by the one-way analysis of variance, relevant pairwise comparisons were performed and the significance levels were reported. Categorical variables were compared by the use of χ^2 tests. Significance levels are reported for comparisons with $p < 0.05$. However, the levels of statistical significance should be interpreted with caution, given the large number of statistical tests performed. Analyses were performed with a standard statistical program (SigmaStat, Jandel Corporation, San Rafael, Calif.).

Results

Physiologic data

Comparability of experimental groups. The mean animal weight was 28.8 ± 2.0 kg (mean $\pm$ standard deviation), with no significant differences between groups. The CPB cooling time was 51 ± 7 minutes, and the warming time was 64 ± 10 minutes (mean $\pm$ standard deviation); these values also did not differ significantly between experimental groups. Temperature measurements also showed no significant differences between experimental groups; after being cooled to an esophageal temperature of 20°C , brain (epidural) temperature was best approximated by esophageal temperature, with rectal temperature lagging behind. During rewarming, however, epidural temperature more closely paralleled rectal temperature (Fig. 1).

Hemodynamic data showed that all animals were stable before, during, and after CPB, with only minor variations in blood pressures and cardiac output. No significant differences in these measurements between embolized and control groups or between groups undergoing different methods of perfusion were recorded during the interventions,

but mean arterial pressures, for example, were significantly different from baseline values at all subsequent time points, $p < 0.001$, dropping from a mean ($\pm$ standard deviation) of 86 ± 16 mm Hg at baseline to 52 ± 12 at the end of cooling, 57 ± 10 at 30°C , and gradually reaching 62 ± 9 by 4 hours after the start of rewarming. Cardiac output also did not differ significantly among the groups: 4.6 ± 0.8 L/min at baseline, 4.3 ± 1.0 at 2 hours, and 3.9 ± 0.01 at 4 hours. CPB flow, too, was comparable in all the groups, with a mean of 1.94 ± 0.4 L/min at the end of cooling and 2.4 ± 0.4 L/min during rewarming.

Blood gas and hematocrit measurements (data not shown) did not differ significantly between embolized and control animals or between groups undergoing different methods of perfusion except during the period of the different interventions. A significantly alkaline pH was seen in the RCP group (RC, 7.59 ± 0.11 ; RE 7.56 ± 0.08), probably reflecting the considerable volume of blood infused into the SVC which is shunted into the IVC during RCP if the IVC is not clamped, and is then recirculated without having perfused any tissues.

A transient but significant drop in hematocrit during RCP-O (RCO, 0.16 ± 0.05 ; REO, 0.18 ± 0.03 , in contrast to 0.21 ± 0.03 in the RCP group [RC and RE] and 0.23 ± 0.02 in the ACP group [AC and AE]) reflects the considerable quantity of saline solution that is required, in addition to donor blood, to maintain adequate perfusion pressures during RCP-O. Extracellular fluid sequestration during and immediately after RCP-O resulted in the correction of the hemodilution early during rewarming, with no significant differences among the groups at that point or thereafter.

Flow and vascular resistance before and after embolization. The mean perfusion flow rate in the isolated aortic arch during the 5 minutes of ACP before embolization (preinjection flow) was 296 ± 176 ml/min with the pressure maintained at 50 mm Hg; it did not differ among the experimental groups. The mean flow, with the aortic arch pressure still maintained at 50 mm Hg, decreased distinctly after microsphere injection but only slightly after saline-solution injection ($p < 0.0001$). A statistically significant difference in vascular resistance after injection was also seen between microsphere-injected and saline solution control groups, $p < 0.0001$.

During the subsequent 25 minutes of ACP, the mean flow rate was significantly higher in the AC group than in the AE group, $p = 0.001$ (Table I).

Table III. Cerebral metabolism

	<i>n</i>	Baseline (37° C)	End of CBP cooling (20° C)	Stable ACP/ RCP (20° C)	After start of rewarming		
					(30° C)	2 Hr	4 Hr
Sagittal sinus lactate (mmol/L)							
AC	10	1.1 ± 0.5	1.7 ± 0.8**	2.5 ± 0.7**	4.2 ± 1.3***	3.3 ± 1.5**	1.8 ± 1.0
AE	10	1.1 ± 0.4	1.7 ± 0.4**	2.6 ± 0.5***	5.2 ± 0.9***	3.9 ± 1.0***	2.3 ± 0.9**
RC	10	1.2 ± 0.4	1.8 ± 0.6**	2.9 ± 0.9**	5.1 ± 1.2**	4.0 ± 1.3***	2.3 ± 0.8**
RE	10	1.3 ± 0.4	2.1 ± 0.8**	3.5 ± 1.2***	6.0 ± 1.9***	4.3 ± 1.8***	2.6 ± 0.9**
RCO	10	1.5 ± 0.8	2.1 ± 0.5*	3.4 ± 0.7***	6.1 ± 1.4***	4.6 ± 2.6**	3.4 ± 2.7
REO	10	1.4 ± 0.7	2.1 ± 0.3**	3.6 ± 0.4***	5.8 ± 0.8***	4.3 ± 1.2***	3.3 ± 1.4**
Mean	10	1.3 ± 0.5	1.9 ± 0.6*	3.2 ± 0.9***	5.4 ± 1.4***	4.4 ± 1.8***	2.6 ± 1.5**
Cerebral glucose difference (mg/dl)							
AC	10	4.7 ± 2.5	3.7 ± 2.3	5.7 ± 7.0	11.9 ± 4.9**	4.9 ± 4.8	7.0 ± 11.5
AE	10	6.5 ± 2.3	5.1 ± 7.0	5.8 ± 4.6	9.0 ± 11.0	7.9 ± 2.8	8.6 ± 4.1
RC	10	6.1 ± 2.7	3.4 ± 2.3	28.0 ± 28.7	8.6 ± 5.8	11.3 ± 6.0	4.8 ± 3.2
RE	10	6.3 ± 4.2	3.6 ± 2.6	13.5 ± 7.8	10.3 ± 6.1	3.0 ± 5.4	7.8 ± 6.5
RCO	10	8.2 ± 3.4	4.3 ± 4.8	-6.8 ± 13.5**	9.9 ± 13.4	9.6 ± 3.2	7.5 ± 4.0
REO	10	5.7 ± 5.5	4.3 ± 3.0	-2.1 ± 26.1	12.0 ± 8.4	7.0 ± 6.5	8.0 ± 10.0
Cerebral oxygen difference (mg/dl)							
AC	10	3.0 ± 1.5	2.2 ± 0.7	3.4 ± 3.0	4.4 ± 1.1	4.2 ± 1.2	4.8 ± 1.9*
AE	10	3.4 ± 0.6	2.1 ± 1.1*	3.2 ± 1.3	3.3 ± 1.0	4.7 ± 0.9**	5.2 ± 1.7*
RC	10	3.2 ± 0.6	1.7 ± 0.7*	7.1 ± 1.4**	2.2 ± 0.3**	3.0 ± 0.7*	4.4 ± 1.8
RE	10	4.1 ± 1.5	1.3 ± 0.9**	7.7 ± 1.1**	2.6 ± 1.3	3.4 ± 1.5	4.3 ± 2.1
RCO	10	3.4 ± 1.1	2.0 ± 1.0**	4.8 ± 2.0	2.3 ± 1.3*	5.3 ± 0.9***	5.1 ± 1.1*
REO	10	3.5 ± 1.4	2.2 ± 1.2**	5.5 ± 1.5**	2.6 ± 1.1**	3.7 ± 2.0	4.1 ± 2.3

Values are shown as mean ± standard deviation. Abbreviations as in Table I.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.0001$ compared to baseline.

During the stable period of RCP, the flow rate was higher in the groups without IVC occlusion whether or not embolism had taken place: RCP, 251 ± 31 ml/min versus RCP-O, 154 ± 56 ml/min, $p = 0.004$. During the stable period, the mean SVC pressures were also higher in the RCP than in the RCP-O groups (RCP, 58 ± 33 mm Hg versus RCP-O, 40 ± 22 mm Hg, $p = 0.03$) although the sagittal sinus pressures, which determined flow, remained the same. These data are consistent with the observation that much of the retrograde flow into the SVC during RCP is shunted into the IVC if the IVC is not clamped.

Vascular resistances during the stable period were calculated separately based on the SVC and sagittal sinus pressures (Table I). Although no statistically significant differences were found between control and embolized groups (RC versus RE or RCO versus REO), a highly significant difference ($p < 0.0001$) was found in vascular resistance when data from embolized and saline solution groups were pooled (20 animals in each group) to compare RCP without and with IVC occlusion. Vascular resistance

during RCP was 6.9 ± 8.1 mm Hg/ml per minute versus 2.1 ± 1.5 mm Hg/ml per minute during RCP-O (with the use of SVC pressures). Sagittal sinus-determined vascular resistances also differed between the pooled groups: RCP, 3.1 ± 3.3 mm Hg/min versus RCP-O, 1.4 ± 0.6 mm Hg/ml per minute, $p = 0.03$. The higher vascular resistance during RCP when compared with RCP-O may be due in part to the higher pH of the perfusate in RCP; alkaline pH is known to cause cerebral vasoconstriction.

Thus embolization had a significant impact on flow and vascular resistance in the ACP group. In the various RCP groups, the differences in flow and vascular resistance as the result of embolization did not reach statistical significance. However, occlusion of the IVC during RCP did induce statistically significant differences in flow and vascular resistance in the combined saline solution and embolism groups. The animals with RCP-O had significantly lower absolute flow rates and consistently lower SVC pressures and cerebral vascular resistances compared with RCP without IVC occlusion.

Table IV. Oxygen extraction and consumption

Group	n	Blood flow (ml/min)		Oxygen extraction (ml/dl)		Oxygen consumption (ml/min)	
		Upper body	Lower body	Upper body	Lower body	Upper body	Lower body
AC	10	188 ± 48	—	3.5 ± 1.0	—	6.1 ± 1.5*	—
AE	10	108 ± 32	—	4.4 ± 1.2	—	4.5 ± 1.1*	—
RC	10	12 ± 9	143 ± 110	7.1 ± 1.4*	0.4 ± 0.3	0.8 ± 0.6	0.5 ± 0.5
RE	10	12 ± 6	181 ± 87	7.7 ± 1.1*	1.2 ± 3.5	0.9 ± 0.5	1.4 ± 4.1
RCO	10	20 ± 10	53 ± 24	4.8 ± 2.1	5.5 ± 2.2	1.3 ± 1.6	2.5 ± 08
REO	10	20 ± 8	74 ± 26	5.5 ± 1.5	5.8 ± 1.8	1.2 ± 07	4.5 ± 1.9

Values are shown as mean ± standard deviation. Abbreviations as in Table I.

*When these control and embolism data for each perfusion method are pooled, they differ significantly from one another, as discussed in the text.

Blood return/fluid sequestration. Little if any fluid sequestration, calculated as the difference between total inflow and venous return, was detected in the ACP groups during the 25 minutes after injection whether or not embolization had taken place (Table II); there was no difference between the embolized group and the control. Similarly, in the RCP groups, no significant differences were found in venous return or sequestration rates when comparing RC versus RE or RCO versus REO groups, but significant differences between the two methods of retrograde perfusion (RCP versus RCP-O) were found when data from the RC and RE groups were pooled and compared with pooled data from RCE and RCO groups. Although the volume of desaturated blood returned to the aortic arch was slightly smaller with RCP during the prestable period, this difference did not reach statistical significance. However, during the subsequent period of stable perfusion, there was significantly less blood returning to the aortic arch in the RCP group: RCP, 208 ± 136 ml versus RCP-O, 296 ± 132 ml, $p = 0.02$. In terms of flow rates, the difference was also significant (RCP, 12 ± 9 ml/min versus RCP-O, 20 ± 9 ml/min, $p = 0.02$), with higher flow rates when the IVC was occluded.

However, the total amount of fluid sequestered (Table II) was distinctly higher in the RCP-O groups (2956 ± 955 ml with RCP-O versus 1621 ± 776 ml with RCP, $p < 0.0001$). The major difference between the RCP groups occurred during the prestable period (RCP-O, 1504 ± 468 ml versus RCP, 587 ± 256 ml, $p < 0.0001$) whereas sequestration during the stable period had only marginal statistical significance (RCP-O, 1426 ± 716 ml versus RCP, 1035 ± 641 ml, $p = 0.08$).

Thus more effective cerebral perfusion during RCP-O was seen, judged both by a higher rate of aortic arch flow and a higher volume of blood

returned to the arch, but this more effective cerebral blood flow was accompanied by increased extracellular fluid sequestration, especially during the first few minutes of RCP.

Metabolic data. Sagittal sinus lactate and arteriovenous cerebral glucose differences at six measurement time points are depicted in Table III. As was the case with the hemodynamic measurements, significant differences were seen between embolized groups and their control groups only in the groups with ACP; but there were a number of measurements that differed among pooled embolism and control groups subjected to different methods of perfusion.

Sagittal sinus lactate increased progressively during cooling to 20° C and during the stable perfusion period, with the highest values being found during rewarming at 30° C. Significantly higher values ($p = 0.02$) were seen in all retrograde groups than were present during or after ACP, suggesting a greater degree of anaerobic metabolism during and immediately after RCP than during continued ACP.

Distinct differences were found between ACP and the two methods of RCP in glucose metabolism during the 25 minutes of differing perfusion (Table III). In the ACP groups, the mean cerebral glucose extraction was 5.7 ± 5.9 mmol/L, whereas in the RCP groups the equivalent extraction (between the SVC arterial line and the aortic arch return) was 20.3 ± 21.0 mmol/L. In the RCP-O groups, by contrast, it would seem that no glucose was extracted; the mean pooled value was -4.4 ± 20.3 mmol/L, ($p < 0.001$). The fact that glucose extraction did take place in the RCP group argues that some nutritive function is performed by retrograde flow; the greater glucose extraction during RCP than during ACP probably reflects compensation for the much lower cerebral blood flow that occurs during RCP. Although RCP-O provided greater

Fig. 2. Microsphere recovery from the right hemisphere of the brain after digestion with potassium hydroxide in animals treated with different methods of perfusion after embolization. All data points and median recovery in each group are depicted. A significantly lower number of residual emboli were found after RCP-O than in the other perfusion groups. Abbreviations as in Table I.

effective flow than RCP, flow was still not as high as with ACP; so the failure to note any glucose extraction during RCP-O most likely reflects some unexpected confounding factor, such as admixture of hepatic venous blood, which is high in glucose, during RCP-O.

This interpretation of the glucose extraction data was upheld when the oxygen extraction data was examined (Tables III and IV), which are inversely related to effective flow and therefore, as expected, show lower extraction with ACP than with either RCP ($p < 0.0001$) or RCP-O ($p = 0.01$) and significantly lower extraction with RCP-O than with RCP, $p < 0.001$ (the mean values in upper-body oxygen extraction were ACP, 3.3 ± 2.3 ml/dl; RCP, 7.4 ± 1.2 ml/dl; and RCP-O, 5.2 ml/dl).

The oxygen consumption data (Table IV) reflect the physiologic consequences of flow rates and oxygen extraction. Oxygen consumption was highest during ACP: ACP, 5.35 ± 1.5 ml/min; RCP, 0.86 ± 0.54 ml/min; RCP-O, 1.25 ± 1.16 ml/min; ACP versus all forms of RCP, $p < 0.001$. The oxygen consumption during RCP-O was not significantly higher than cerebral metabolic rate of oxygen during RCP, however.

Summary of physiologic data. When the physiologic data are examined overall, it is clear that ACP provides the most efficient flow, sustaining much higher levels of cerebral oxygen consumption than retrograde flow, although still falling short of ideal

perfusion as evidenced by the presence of lactate levels significantly higher than baseline. RCP-O allows better perfusion than RCP, with significantly lower vascular resistance, higher flow, and lower oxygen extraction, although not significantly higher oxygen consumption. However, RCP-O also results in significantly higher levels of fluid sequestration than RCP. Some of the enhanced flow seen with RCP-O compared with RCP may be due to vasoconstriction induced by the higher pH of the perfusate during RCP.

Microsphere recovery (Fig. 2). There was a significant difference in microsphere recovery among the three embolized groups; nearly twice the number of microspheres were recovered from the brains in the AE group and the RE group than from the REO group ($p = 0.03$). The differences in microsphere recovery indicate that RCP-O was quite effective in the desired goal of washing out microspheres from the brains of embolized animals, whereas RCP without IVC occlusion left as many microspheres lodged in the brain as continued ACP. The success in removal of microspheres from the brains of the animals that were subjected to RCP-O is in accord with the data on aortic arch return and oxygen extraction, which also indicate that effective cerebral perfusion takes place during RCP-O.

Morbidity and death. All animals were stable during the surgical procedures. Forty-one of the 60 animals survived for the full 7 days of the experiment, enabling their daily behavioral scores to be recorded throughout. Premature deaths occurred in 18 of 30 embolized animals and in 1 of 30 control animals ($p < 0.0001$).

The 7-day deaths in the various embolized groups did not differ significantly among the groups: AE = 5, RE = 7, and REO = 6. The often severely affected animals in the embolized groups were frequently unable to stand, had poor appetite and abnormal reflexes, and either died spontaneously or were killed early to avoid excessive suffering. The only control animal that failed to survive for the full 7 days was from the RCO group; this pig exhibited substantial neurologic impairment, and postmortem examination revealed a large subdural hematoma that covered the entire cortex and brainstem and extended along the spinal cord.

Behavioral outcome. As shown in Fig. 3, neurologic recovery after 7 days was essentially complete in the control animals, although some minor behavioral abnormalities were evident in the first few days in some control animals after RCP-O (Fig. 4). After

embolization, the behavioral outcomes of the animals were significantly poorer than in the control groups but similar to one another, with no significant differences between AE, RE, and REO groups.

Among the animals that survived for 7 days, the neurologic outcome was distinctly better in control animals ($p = 0.001$); all control animals had a score of more than 8 one week after intervention, whereas only four animals in the AE group and two animals in each of the RE and REO groups had scores of more than 8 after 1 week.

EEG and evoked potentials (QEEG; Fig. 5). No difference was found in total EEG power (0.5 to 20 Hz) between any of the groups at baseline or at the end of cooling; EEG was isoelectric at 20° C in all of the animals. At 30° C during rewarming, animals in the antegrade groups had earlier EEG return than those in the retrograde groups. The EEG return 4 hours after intervention reflects a much more rapid recovery with ACP than with either method of RCP. In addition, there is a strong effect of embolism on QEEG return; the AC and RC groups show significantly higher return of baseline QEEG 4 hours after the start of rewarming than the AE and RE groups, $p < 0.001$, although there is no significant difference between the RCO and RCE groups in the pattern of EEG recovery.

SSEP. In general, the recovery of cortical SSEPs followed a pattern similar to the recovery of cortical QEEG (data not shown). During rewarming and 2 and 4 hours thereafter, the AC group showed the greatest recovery of cortical amplitude. As with QEEG, by 4 hours after the start of rewarming, the amplitude of cortical response to somatosensory stimulation in the AC group was significantly greater than AE amplitude, and RC amplitude was significantly better than RE amplitude. Again, as with QEEG, however, both groups with RCP-O showed delayed recovery. At no time did the RCO group show greater recovery than the REO group. In contrast to cortical SSEP recovery, cervical SSEP recovery (data not shown) was quite prompt, with essentially full recovery by 2 hours after the start of rewarming and no significant differences between embolized groups and control groups or between ACP, RCP, and RCP-O groups.

BSERs. Analysis of BSER potentials (Fig. 6) was restricted to those animals (35 of 60) in which repeatable responses could be obtained at all measurement time points. Many animals were excluded because some of their tracings had poor signal-to-noise levels and was not due to any characteristics of

Fig. 3. Median daily behavioral scores after embolization, or after saline solution injection in control animals, followed by various methods of hypothermic perfusion. There was almost complete recovery by day 7 in most of the control animals, whereas persistent impairment was present in many of the embolized animals in all perfusion categories, with no significant differences among embolized groups or among control groups. The behavioral score reflects appetite, mental status, and motor function. A score of 9 indicates complete recovery, and 0 indicates coma or death. Abbreviations as in Table I.

the animals, as far as we are aware. Like the cervical responses to somatosensory stimuli, BSERs showed early recovery. At 30° C, approximately 40% recovery of BSERs was seen in all except the REO group. By 2 hours after the start of rewarming, however, the total power in the RCO group had dropped, and this trend continued at the 4-hour time point in the RCO group although the response in the other groups remained stable. Analysis of variance of BSER recovery at 4 hours revealed a significant effect of perfusion method ($p = 0.006$), which was due solely to the poorer recovery in the two retrograde occlusion groups.

Analysis of the individual BSERs in the RCO group revealed that four of the six animals in this group showed a pattern like the one in Fig. 7, a good recovery at 30° C followed by a complete loss of the response by 4 hours after the start of rewarming. This pattern suggests that the ultimate failure of complete return of function in this control group may not reflect intraoperative injury, as seen in the embolized animals, but rather a later insult. Such a dampening of brainstem auditory-evoked potentials late after the operation could be attributed to the development of cerebral edema.

Fig. 4. Daily behavioral score of each animal in each group after embolization or saline solution injection. Abbreviations as in Table I and scores as in Fig. 3.

Histopathologic results. Brains could be recovered for histopathologic studies in 54 animals. In 81% (21 of 26) of control animals, histopathologic scoring of the cortex was 0, indicating absence of any pathologic damage, but this was true in only 7% (2 of 27) of the embolized animals ($p < 0.0001$).

Among the control animals, 9 of 9 AC, 8 of 8 RC, but only 4 of 9 RCO animals had no cortical pathologic damage ($p = 0.003$), paralleling the pattern of behavioral recovery in which RCO animals fared more poorly than the other control animals. Although the median cortical histopatho-

logic score for the RCO group was 1, indicating only mild pathologic damage, two animals in this control group had a moderate number of cortical defects. No differences in the intrinsic characteristics of histopathologic lesions were evident when embolized animals and RCO groups were compared. It should be borne in mind that any direct evidence of cerebral edema, which may have initiated the ischemic damage seen in the control animals, would no longer be expected to be present 1 week after the operation.

By adding together the scores from each area of the brain examined, one can arrive at a total his-

Fig. 5. Mean ($\pm$ standard error of mean) total EEG power, as a percent of baseline recordings at 37° C, in each group during recovery from embolization or saline solution injection followed by different methods of perfusion. Recovery of EEG was monitored during early rewarming (at 30° C) and at 2 and 4 hours after the start of rewarming. Return of EEG was significantly more rapid after ACP than after either method of RCP. Four hours after the start of rewarming, EEG power was significantly greater in control animals than in embolized animals when either ACP or RCP without IVC occlusion were used, AC versus AE and RC versus RE, $p < 0.001$, but no difference in recovery of EEG was seen between embolized and saline solution-injected control animals subsequently subjected to RCP-O (RCO versus REO). Abbreviations as in Table I.

topathologic score for each animal; the individual scores and the median for each experimental group are shown in Fig. 8. After embolization, the highest score was in the AE group and the lowest score was in the RCP-O group, but the trend toward lower scores after embolization with increasingly effective retrograde perfusion is not statistically significant.

The median scores for histopathologic outcome just in the cerebral neocortex were AE = 1, RE = 3, and REO = 3, $p = 0.15$, suggesting that no improved histologic outcome after embolization resulted from either form of retrograde perfusion. These results are in accord with the evidence from behavioral scores, which also failed to show improvement in

Fig. 6. Mean ($\pm$ standard error of mean) total power of brainstem auditory-evoked potentials (as a percent of baseline BSERs) during recovery after embolization or saline solution injection followed by various methods of hypothermic cerebral perfusion, with groups and time points as in Figure 5. Results are from 35 of 60 animals because low signal-to-noise levels precluded a complete set of reliable recordings in the remaining animals. Early recovery to $>40\%$ of baseline values was present in all but the REO group, which (as also observed with cervical SSEP) never showed a good response after the operation. Whereas the rapid recovery of BSERs in most groups was sustained, in the RCO group there was a drop in BSER power at 2 hours and a further drop at 4 hours. At the 4-hour time point, the recovery of BSER in both groups in which RCP-O was used was significantly poorer than recovery after either ACP or RCP, $p = 0.002$. Abbreviations as in Table I.

outcome if retrograde perfusion was instituted after embolization.

If the distribution of lesions within the brain is examined in those groups in which histologic damage was observed, which includes all the embolized

groups and the RCO control (Fig. 9), it can be seen that some differences are present. The pure embolism pattern seen in the AE group shows lesions in the neocortex, deep cerebrum, and cerebellum; whereas with pure retrograde perfusion-induced

damage (seen in the RCO group), most of the lesions are in the neocortex. The findings in both the deep cerebrum ($p = 0.02$) and in the cerebellum ($p = 0.005$) show highly significant differences between the AE and RCO groups. The patterns in the RE and REO groups are very similar to one another, with more neocortical lesions than in either the AE or RCO groups, and suggest the possibility that embolic and retroperfusion-induced histopathology may be additive. There is a marginally significant difference between the AE and RE groups in the neocortex, $p = 0.06$. In the deep cerebrum, the pattern in the RE and REO groups is compatible with the possibility that retrograde perfusion may have mitigated embolic injury. The only statistically significant differences are between the RCO and all embolized groups.

Discussion

The data from this study confirm earlier work that suggested that RCP can provide effective retrograde flow to the brain. To our knowledge, ours is the first study that indicates that more efficient RCP can be achieved via the SVC if the IVC is occluded (RCP-O). The higher rate of return of blood to the isolated aortic arch, the lower cerebral-vascular resistance, and the reduced oxygen extraction all suggest that RCP-O is more effective than RCP without IVC occlusion. The rise in the pH of the perfusate when RCP is carried out without the IVC clamped may have contributed to the decreased flow and enhanced vascular resistance seen with this method; but although a higher pH would be expected to result in enhanced vasoconstriction and therefore diminished flow, studies by others^{14, 15} have indicated that intracellular pH falls quite rapidly during RCP. This intracellular acidosis would probably effectively counteract any vasoconstrictive impact of the small volume of alkaline retrograde flow.

In addition to the hemodynamic and metabolic evidence, the demonstrated washout of microspheres from the brain in the RCP-O group confirms the efficacy of this technique. The unequivocal microsphere data should finally lay to rest the contention that RCP cannot perfuse the brain and that retrograde flow is confined to the meninges.¹⁶ Our results are based on a comparison of microspheres recovered when the parenchyma of the brains of the animals were digested after embolization and subsequent ACP or RCP. Because fewer emboli remained in the REO group than the AE or

Fig. 7. The brainstem auditory-evoked potential response pattern in four of six saline solution-injected control animals subsequently perfused retrograde with the IVC occluded (RCO). An initial recovery of the BSER can be seen by comparing the response at 30°C with the baseline recording. At 2 hours after the start of rewarming, however, the BSER was flat (1.6 msec loss of wave 1), and there was still no response 4 hours after the start of rewarming. The loss of the BSER after initial recovery suggests that these animals had a time-dependent or temperature-dependent insult later during recovery than in the REO group, where a likely intraoperative insult prevented even early return of the BSER. At zero latency, the stimulus would be expected to reach the tympanic membrane.

RE group, one must conclude that the microspheres were indeed flushed from the brain during RCP-O.

The successful removal of microspheres by RCP-O after embolization is encouraging for those who see promise in this method for the prevention of strokes after cardiac or aortic operation. But the absence of improved neurologic or histopathologic outcome in the REO animals, despite the effective removal of emboli, suggests that the conditions under which RCP were being carried out in this study are still far from optimal and echo the results from our previous study, which suggested that some animals may be harmed by RCP after embolization.¹² The neurologic morbidity evident even in control animals after RCP-O suggests that there is inherent potential for cerebral injury with this form

Fig. 8. Total histopathologic score in all animals after embolization or saline solution injection followed by different methods of hypothermic perfusion, with medians in all groups. A higher score indicates a greater degree of cerebral histopathologic damage. The total was derived by adding the scores of the different regions of the brain examined and is weighted toward neocortical pathology damage. Although a trend toward lower median scores after embolization when followed by increasingly effective retrograde perfusion is evident, these differences did not reach statistical significance. It should be noted that the only histopathologic injury among the control groups occurred after RCP-O. Abbreviations as in Table I.

of RCP, at least under the circumstances that prevailed in this study, even in the absence of any additional insult such as embolization. If RCP is to be considered for clinical use with the hope of reducing embolic injury after cardiac and aortic operations, it is important to try to determine whether the possible benefit of RCP can be dissociated from its potential for harm.

If one looks carefully at all the data, the most striking finding is the large amount of sequestered fluid that accumulates during RCP, particularly if the IVC is occluded. It seems quite plausible that this sequestered fluid might manifest itself to some degree as cerebral edema, which is certainly a potential cause of neurologic morbidity. The fact that the mean weight of the brains in the 19 animals that were killed within the first 24 hours was 86 ± 7 g, significantly higher than the mean brain weight (78 ± 5 g) of the remaining 41 animals that lived until day 7 ($p < 0.001$) suggests that cerebral edema was present in the animals that died early. Cerebral edema has been shown by a number of investigators to occur after RCP. Its severity has been observed to be related both to perfusion pressure and to dura-

tion of RCP.^{17, 18} In our previous study, high SVC perfusion pressures appeared to be associated with a poorer outcome despite uniformity of sagittal sinus pressures. In this study, several technical modifications were introduced, and the relationship between high SVC pressure and poor outcome was no longer observed.¹² In the current study, ligation of the hemiazygos vein was routinely carried out; higher perfusion pressures, when necessary, were achieved more gradually, and the sagittal sinus pressures that regulated flow were lower. These modifications may have reduced the development of cerebral edema during RCP.

In careful scrutiny of electrophysiologic recovery, the initial rapid early recovery of brainstem auditory-evoked potentials in all control groups, including the RCO, and the selective diminution of this response selectively in the RCO group at the later time points is also striking. This pattern is in contrast to the recovery of EEG and cortical-evoked responses in embolized animals, in which a consistent pattern of delayed and diminished but steadily progressive recovery throughout the period of postoperative surveillance is seen. The disappearance of the auditory-evoked response after an initial good recovery in several animals in the RCO group (Fig. 7) is even more impressive than the mean response and is suggestive of a postoperative rather than an intraoperative insult. Such a postoperative insult could be a consequence of the development of cerebral edema after RCP-O.

In the animals with embolization followed by RCP-O, the pattern of recovery followed by diminution in the brainstem-evoked potentials was not seen, probably because there was also an intraoperative insult occasioned by the microspheres not removed by RCP, which prevented the early recovery seen in the control animals. The combination of even minor intraoperative injury and the later development of cerebral edema would explain our failure to see any statistically significant benefit in terms of death or improved histologic or behavioral recovery in the REO group despite the successful removal of a significant number of emboli. But if absolutely no benefit had been rendered by removal of emboli by RCP-O, one would expect that the REO group would have had a significantly worse outcome not only than the RCO group but also compared with the RE and the AE groups. This was not apparent; in fact, the histologic result in the REO group was somewhat better than in the other embolized groups.

The improvement in behavioral scores between day 1 and 7 after the operation in a number of survivors of the current protocol could reflect resolution of mild, transient cerebral edema in many of these animals. Cerebral edema was probably most severe in the groups with RCP-O, in whom the greatest degree of fluid sequestration was observed. We speculate that more aggressive attempts to prevent or treat cerebral edema might have allowed more rapid recovery in more animals both in the embolized and in the control groups and might even have improved survival in those that were killed early or died spontaneously, in which the presence of cerebral edema was suspected on the basis of higher brain weight. Because the animals in this experiment were killed 1 week after the operation, one would not anticipate being able to document the presence of cerebral edema on histopathologic examination but only its sequelae.

If it is true that the late development of cerebral edema can explain both the failure of complete recovery in the control animals after RCP-O and the failure to see an unequivocal benefit in terms of histologic and behavioral outcome from the removal of emboli in the REO group, then it is conceivable that, with some modifications, RCP could still prove useful as a treatment for particulate embolization. Perhaps the interval of RCP could be shortened, or the potential for cerebral edema could be combated with diuretics and other pharmacologic strategies more vigorously and for a much greater interval after the operation. In the current protocol, only minimal precautions were taken to forestall development of cerebral edema; steroids were not administered, and only one dose of furosemide and mannitol were given at the time of weaning from CPB. Yoshimura and his colleagues¹⁹ have shown that the cerebral edema that develops in dogs after RCP can be significantly reduced with mannitol and antivasospastic substance, especially if drugs are administered both during CPB and for several hours after the operation. It is also conceivable that adjustments in the rate of retrograde flow, which in this protocol was regulated to achieve a given sagittal sinus pressure, might be helpful in limiting the development of cerebral edema without diminishing the effectiveness of RCP.

In summary, our data clearly show that RCP-O provides effective cerebral perfusion, including measurably significant removal of particulate emboli. The potential benefit of this strategy, however, seems unfortunately to be accompanied by a poten-

Fig. 9. Histopathologic scores in different areas of the brain in the four experimental groups in which histopathologic injury was evident, with median values. The distribution of lesions in the AE group can be considered the pattern of damage after embolization and the distribution in the RCO group, in which no emboli were involved, the pattern of damage induced by RCP. In the deep cerebrum ($p = 0.02$) and in the cerebellum ($p = 0.005$), the differences between the AE and RCO groups are highly significant. The damage in the RE and REO groups exhibit some features of each pattern; the relatively high neocortical scores in both these groups look as though there might be an additive effect of embolic and perfusion-related injury. In fact, the difference between AE and RE groups in the neocortex is marginally statistically significant, $p = 0.06$. In the deep cerebrum, RCP may have mitigated injury, but only the differences between the RCO and all the embolism groups were statistically significant. Again in the cerebellum, RCO was significantly different ($p = 0.03$) from the REO and the AE group. Abbreviations as in Table I.

tial for cerebral injury, even with a relatively short interval of RCP, even in control animals. Until the mechanism of this injury is better understood, RCP should be used sparingly in the clinical setting, and precautions should be taken to minimize postoperative cerebral edema.

It should perhaps be noted that the central observation of this study, that RCP-O can remove particulate emboli, might have been interpreted quite differently had behavioral evaluation, neurophysiologic recovery, and cerebral histologic examination not been carried out. Given the enthusiasm with which RCP has been adopted for clinical use despite only fragmentary understanding of its physiology,⁸⁻¹⁰ it seems especially important to remember that laboratory studies that do not include some reliable measures of neurologic outcome must be interpreted with caution.

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Discussion

Dr. D. Craig Miller (*Stanford, Calif.*). The first thing I would like to do is remind the audience that we are now celebrating the twenty-second anniversary of the first meeting of the Samson Thoracic Surgical Society held in Santa Barbara where Dr. Griep initially presented his seminal work on the use of HCA for aortic arch surgery, something the Western Thoracic Surgical Association can certainly be proud of (*J Thorac Cardiovasc Surg* 1975;70:1051-63).

Dr. Juvonen is a cardiac surgeon from Finland who has been working in Dr. Griep's laboratory for 2½ years. Dr. Griep and Dr. Juvonen, in typical surgical fashion, have taken something we think works clinically back to the laboratory to figure out if it works, and if it does, why it works.

You are correct, Dr. Juvonen, in that there is growing clinical uncertainty about the purported benefits and the unknown risks of RCP.

I thought the most constructive thing I could do was try to distill for the audience the important central points that you have made:

1. As one might expect, blowing small pieces of plastic up into the brain is not good, even in pigs.
2. RCP blood flow does, indeed, get to the deep cerebral and cerebellar brain matter; your work is, to the best of my knowledge, the first time that this effect has been firmly established. Many have thought in the past that

RCP flow mostly perfuses the dural sinuses and the meninges.

3. RCP can actually flush out particulate emboli from the brain, something that has only been assumed before.
4. RCP done properly, meaning with the IVC occluded, is superior in terms of removing emboli compared with the usual fashion in which RCP is carried out but, as you have illustrated, does appear to have separate injurious and deleterious affects on the brain.

As you mentioned, this brain injury was even observed in the control animals without embolization; it is an important point that has not been widely appreciated before. Furthermore, the mode of injury and the time of injury are different compared with that caused by the embolic ischemic insult, which is also new information.

Finally, it is critically important not only to focus on removing emboli and cerebral hemodynamics and oxygen consumption but also to assess some indicator of functional neurologic recovery and the pathologic extent of cerebral injury. Otherwise, your results in this experiment could have been interpreted entirely differently. The bottom line is that we have to refine how to use RCP if we are going to continue to use it to maximal patient advantage.

I am concerned about the relevance of this porcine model to man, because much work has been done previously in subhuman primates and other species. Can you tell us if you and your colleagues expect that these observations will apply to man as well as they do to the pig, given the anatomic and physiologic differences between species?

Dr. Juvonen. First, I would like to thank Dr. Miller for his kind remarks regarding our paper. We acknowledge that there are several problems in using animal models overall. If we specifically look at cerebral perfusion, we have to have a model in which it is feasible carry out RCP. In this respect, the dog model, which is used frequently in cardiac surgery, failed because there exist competent jugular valves; it is more complicated to create RCP beyond jugular veins. Although there are many papers in the literature with a dog model, this was the reason we selected a porcine model for RCP studies. We have found that the rate of competent jugular valves in pigs is approximately 20%. In this subgroup of animals, there is a distinct gradient between SVC and sagittal sinus pressures at least at the beginning of the experiment. The human cadaver study by de Brux and colleagues¹³ indicated that there are competent jugular valves in 85% of human beings and that the azygos vein system is the major pathway to the central nervous system. This finding was also present in an RCP study performed on nonhuman primates, baboon monkeys by Boeckxstaens and Flameng,¹⁶ indicating that less than 1% of blood returned to the aortic arch during retrograde perfusion and more than 90% was circulating and shunting to the IVC. Our point is that there should be little doubt that, during RCP without IVC occlusion, the high-capacity and low-resistance descending venous bed will drain most of RCP volume regardless of whether the jugular veins are valvulated or not.

It is also important to notice that we monitored sagittal sinus pressure and maintained it at 25 mm Hg during the

protocol and assumed that the sagittal sinus pathway is at least a significant pathway for retrograde perfusion in the pig. In this respect, this material is homogeneous; all animals were subject to the same pressure during retrograde perfusion. And we found that effective blood flow return to the aortic arch was much lower in RCP when the IVC was opened supporting the presence of a shunt. Moreover, we would like to emphasize that the major interest in our current study was RCP-O, and that all our major findings and conclusions concerned the IVC occlusion method. We feel strongly that the existence of valves and differences between species is less important if we want to pressurize an entire venous system. Actually we have data showing how SVC, IVC, and sagittal sinus pressures equalize during RCP in animals after the vascular bed is filled.

Dr. Miller. Are you saying that the pig is the best model short of a human?

Dr. Juvonen. I believe that it is a relevant model and one of the best so far.

Dr. Miller. My second question is about these polystyrene microspheres. They are very small; they are very light, and they are certainly not atheromatous or thrombotic debris, which have more mass. How much do these differences limit the interpretation of your results when we go back home to the operating room?

Dr. Juvonen. We admit that albumin-coated microspheres 250 to 750 μ m in diameter are very different from atheroma debris, but the finding that we were able to generate effective RCP flow to wash out microspheres from the deep brain suggests that this could be a potent method to wash out larger particles from extracranial arteries as well. In this context, I would like to review our current clinical experience at Mount Sinai Hospital. Results of this kind from our experimental laboratory have modified our clinical RCP protocol in a way that we are using RCP when the IVC is occluded only in cases in which a loose clot or atheromatous debris is present during aortic surgery, and the central venous pressure is kept below 20 mm Hg. After adopting this RCP protocol for clinical use, the incidence of strokes has decreased, but the observation is that these patients wake up more slowly and are extubated later than those who have undergone HCA alone. This is in line with our experimental findings.

Dr. Miller. That leads to my wrap-up statement. We must remember that Dr. Griepp's group has shown clinically in a multivariate analysis that RCP is actually a predictor of stroke during aortic arch procedures in their experience, mainly because they only use it when there is a lot of debris in the arch, that is, in patients at relatively high risk of stroke. So, as I understand your conclusions, clinically you are now advocating RCP with low pressure and with IVC occlusion in selected patients. Are you using RCP for a brief period of time or throughout the entire circulatory arrest interval?

Dr. Juvonen. It is used either for a brief period of time or intermittently.

Dr. Miller. So you advise that we limit the time duration of RCP and keep the pressure low to try to minimize the cerebral edema?

Dr. Juvonen. Yes I recommend, if an entire venous vascular bed is pressurized.