CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

HIGHER HEMATOCRIT IMPROVES CEREBRAL OUTCOME AFTER DEEP HYPOTHERMIC CIRCULATORY ARREST

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Background: Various degrees of hemodilution are currently in clinical use during deep hypothermic circulatory arrest to counteract deleterious rheologic effects linked with brain injury by previous reports. *Material and methods:* Seventeen piglets were randomly assigned to three groups. Group I piglets ($n = 7$) received colloid and crystalloid prime (hematocrit < 10%), group II piglets ($n = 5$) received blood and crystalloid prime (hematocrit 20%), group III piglets ($n = 5$) received blood prime (hematocrit 30%). All groups underwent 60 minutes of deep hypothermic circulatory arrest at 15° C. with continuous magnetic resonance spectroscopy and near-infrared spectroscopy Neurologic recovery was evaluated for 4 days (neurologic deficit score 0, normal, to 500, brain death; overall performance category 1, normal, to 5, brain death). Neurohistologic score (0, normal, to 5+, necrosis) was assessed after the animals were euthanized on day 4. *Results:* Group I had significant loss of phosphocreatine and intracellular acidosis during early cooling (phosphocreatine in group I, 86.3% ± 26.8%; group II, 117.3% ± 8.6%; group III, 110.9% ± 2.68%; $p = 0.0008$; intracellular pH in group I, 6.95 ± 0.18; group II, 7.28 ± 0.04; group III, 7.49 ± 0.04; $p = 0.0048$). Final recovery was the same for all groups. Cytochrome aa_3 was more reduced in group I during deep hypothermic circulatory arrest than in either of the other groups (group I, −43.6 ± 2.6; group II, −16.0 ± 5.2; group III, 1.3 ± 3.1; $p < 0.0001$). Neurologic deficit score was best preserved in group III ($p < 0.05$ group II vs group III) on the first postoperative day, although this difference diminished with time and all animals were neurologically normal after 4 days. Histologic assessment was worst among group I in neocortex area (group I, 1.33 ± 0.3; group II, 0.22 ± 0.1; group III, 0.40 ± 0.2, $p < 0.05$, group I vs group II; $p = 0.0287$, group I vs group III). *Conclusion:* Extreme hemodilution during cardiopulmonary bypass may cause inadequate oxygen delivery during early cooling. The higher hematocrit with a blood prime is associated with improved cerebral recovery after deep hypothermic circulatory arrest. (J Thorac Cardiovasc Surg 1996;112:1610-21)

Hemodilution has been widely applied for hypo-
thermic cardiopulmonary bypass (CPB) for
many years. It was first introduced to decrease
homologous blood use,[1] thereby avoiding the "ho-
mologous blood syndrome."[2] Later, the effects of
hemodilution in counteracting the deleterious rheo-
logic consequences of deep hypothermia, such as
increased viscosity and red cell rigidity, came to be
considered important in minimizing brain injury
associated with deep hypothermic circulatory arrest
(DHCA).[3] However, hemodilution reduces the ox-
ygen carrying capacity of blood. In combination with
the leftward shift of the oxyhemoglobin dissociation
curve induced by hypothermia, which may be further
exacerbated by an alkaline pH strategy such as
alpha-stat, hemodilution may critically limit oxygen
delivery to neurons and other cells.[4]

Until recently, it was not possible to directly
examine the adequacy of oxygen delivery to neurons
during CPB. Both clinical and laboratory studies
have used surrogate end points, such as gross evi-
dence of neurologic injury or measurement of cere-
bral blood flow or cerebral metabolic rate. Recent
developments in magnetic resonance spectroscopy
(MRS) and near infra-red spectroscopy (NIRS)[5]
now allow more direct investigation of the adequacy
of oxygen delivery to cerebral cells and the ability of
cells to synthesize and maintain high-energy phos-
phates. We developed a piglet survival model of
DHCA that includes simultaneous MRS and NIRS,
followed by evaluation of neurologic status for 4
days and ending with the sacrifice and histologic
assessment of the animal. We used the model in this
study to evaluate the neurologic effects of perfusate
hematocrit during DHCA under conditions similar
to those used clinically.

Consistent with a lack of firm data from the past
on which to base a strategy for hematocrit manipu-
lation during DHCA is the observation that widely
disparate protocols have evolved at different centers
undertaking high volumes of neonatal and infant
cardiac operations.[6] At Loma Linda University, a
blood-free prime is routinely applied for neonatal
DHCA, resulting in a hematocrit less than 10%.[7]
Colloid osmotic pressure is elevated by means of
albumin. At Children's Hospital in Boston, a mix-
ture of whole blood (not packed cells) and crystal-
loid solution is used for the prime to achieve an
hematocrit of 20% during CPB.[8] No colloid other
than the plasma in the whole blood is added. At
Marie-Lannelongue in Paris, a blood-only prime is
used to achieve an hematocrit greater than 30%.[9]

Our study examines the adequacy of cerebral pro-
tection afforded by these three hemodilution proto-
cols.

Methods

Experimental preparation. Seventeen 5-week-old
Yorkshire piglets, weight 6.2 to 8.8 kg (mean 7.9 kg), were
anesthetized with intraperitoneal sodium methohexital
(45 mg/kg) and intubated with a 5 mm cuffed endotracheal
tube. Each animal was ventilated at a peak inspiratory
pressure of 20 cm H_2O, an inspired oxygen fraction of
0.21, and a rate of 12 breaths/min, by means of a pressure
control ventilator (Healthdyne model 105; Healthdyne
Technologies, Marietta, Ga.) to achieve a normal pH and
arterial carbon dioxide tension. After an intravenous
bolus of fentanyl (25 μg/kg) and pancuronium (0.5 mg/
kg), anesthesia was maintained by a continuous infusion
of fentanyl (25 μg $\cdot$ kg^{-1} $\cdot$ hr^{-1}), midazolam (0.2
mg $\cdot$ kg^{-1} $\cdot$ hr^{-1}), and pancuronium (0.2 mg/kg),
throughout the entire experiment except during the pe-
riod of circulatory arrest. Esophageal temperature was
recorded continuously until extubation. Before operation,
a 3.0 cm diameter surface coil for MRS was sutured on the
scalp overlying the cerebral hemispheres, and a pair of
fiberoptic optodes for NIRS were applied to the head over
the frontal lobes with an interoptode distance of 3 cm. The
superficial left femoral artery was cannulated for arterial
blood pressure monitoring and blood gas sampling. The
right femoral artery was exposed for the CPB arterial
cannula. A right anterolateral thoracotomy was per-
formed in the third intercostal space to expose the right
atrium for venous cannulation.

After systemic heparinization (300 IU/kg), an 8F Bio-
Medicus arterial cannula (Medtronic Bio-Medicus, Min-
neapolis, Minn.) and 24F venous cannula (Bard, Inc.,
USCI Division, Billerica, Mass.) were inserted into the
right femoral artery and right atrium, respectively. The
animal was then placed in a MRS horizontal-bore super-
conducting 4.7 magnet (Oxford Research System, Oxford,
England) and subjected to CPB and DHCA, as deter-
mined by the protocol (Fig. 1).

After 45 minutes of rewarming, the piglet was weaned
from CPB and decannulated outside the MRS bore.
Protamine (5 mg/kg) was administered intravenously.
Immediately after decannulation, the animal was reposi-
tioned in the bore for 3 hours for MRS and NIRS data
collection. After that, all incisions were closed in a sterile
fashion. The animal remained intubated during the first
12 postoperative hours.

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 Institute of Laboratory Animal Resources
and published by the National Institutes of Health (NIH
publication No. 86-23, revised in 1985).

Experimental groups. Piglets were randomly assigned
to three groups. Group I (n = 7, extreme hemodilution)
received colloid and crystalloid priming with a hematocrit
lower than 10%. The prime consisted of 800 ml Normosol
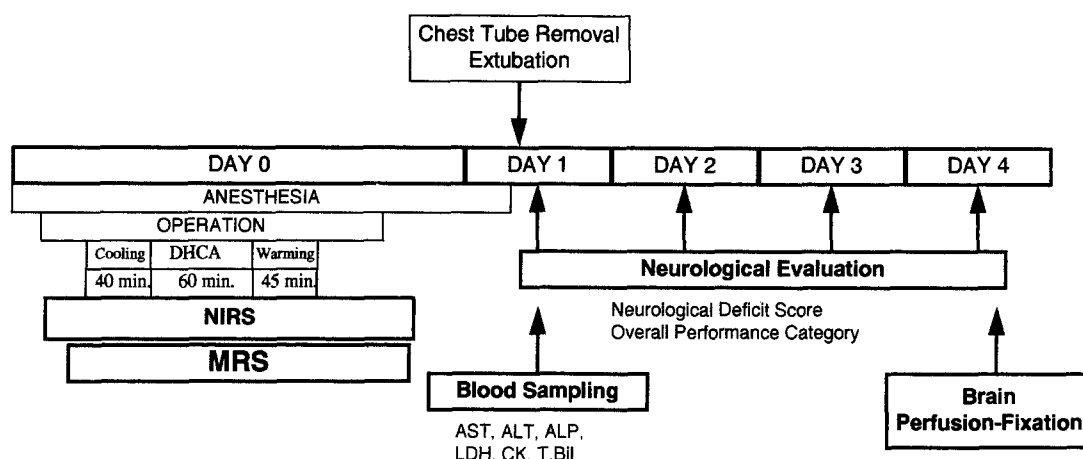R, pH 7.4 (Abbott Laboratories, North Chicago, Ill.), and

Fig. 1. Flow diagram depicting experimental protocol. *T.Bil,* Total bilirubin.

400 ml Hetastarch (Hespan; Du Pont Companies, Wilmington, Del.). Group II ($n = 5$) was moderately hemodiluted with 400 ml blood and 800 ml crystalloid solution (Normosol R, pH 7.4) with a hematocrit of 20%. Group III ($n = 5$, no hemodilution) was prepared with 1200 ml whole-blood prime and a hematocrit of 30%.

CPB technique. The circuit consisted of a roller pump (Cardiovascular Instrument Corp., Wakefield, Mass.), membrane oxygenator (VPCML plus; COBE Cardiovascular, Inc., Arvada, Colo.), and sterile tubing (Olson Medical Sales, Inc., Ashland, Mass.), with a 40 μm arterial filter (Pediatric extracorporeal blood filter; PALL Biomedical, Inc., East Hills, N.Y.). The pump prime was determined by the experimental protocols described previously. Cefazolin sodium (25 mg/kg), methylprednisolone sodium succinate (30 mg/kg), furosemide (0.25 mg/kg), and sodium bicarbonate (10 ml) were added to the prime. Full bypass flow was set at 100 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}. After cannulation and connection of the CPB circuit, animals were placed in the magnet bore for measurement of baseline MRS and NIRS data. CPB was then commenced and animals were immediately cooled to an esophageal temperature of 15° C during a 40-minute period by the pH-stat strategy. Phentolamine mesylate (0.2 mg/kg) was administered before cooling. Ventilation was stopped after the establishment of CPB.

On reperfusion, furosemide (0.25 mg/kg), mannitol (0.5 g/kg), phentolamine (0.2 mg/kg), and sodium bicarbonate (10 ml) were administered into the pump. The animal was warmed to a temperature of 35° C during a 45-minute period, maintaining a flow rate of 100 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}. The heart was defibrillated as necessary at 25° C. Fresh whole blood from a donor pig, drawn on the day of operation, was transfused into the pump as required to increase hematocrit to at least 25% in all groups during rewarming. In addition, ultrafiltration with a hemoconcentrator (Hemocor HPH 400; Minntech Corp., Minneapolis, Minn.) was performed during reperfusion in group I to achieve this hematocrit. Ventilation was restarted 10 minutes before weaning from CPB with an inspired oxygen fraction of 1.0. The animal was then weaned from

CPB and decannulated outside the bore. Intravenous protamine was administered when the animal was in hemodynamically stable condition. After decannulation, the animal was repositioned in the bore for a further 3 hours of MRS and NIRS data collection.

Postoperative management. All animals remained sedated, paralyzed, mechanically ventilated, and monitored continuously for 12 hours after operation, at which time chest tubes were removed, infusions were discontinued, and the animals were weaned from ventilation and extubated. Hemodynamic stability was observed in all animals, and none required postoperative inotropic or vasopressor support.

Data collection

Spectroscopy. Details of MRS[10] and NIRS[11] have been described previously elsewhere.

Biochemical analysis. Blood samples were taken on the day after the operation. Aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine kinase (CK), and total bilirubin were measured.

Other evaluations. Details of methods employed for neurologic and behavioral evaluations (neurologic deficit score [NDS] and overall performance category [OPC]) and for histologic evaluations have been described previously elsewhere.[12, 13]

Statistical analysis. All results were expressed as mean ($\pm$ standard error of the mean) and analyzed by a statistical analysis software package (Stat-View version 4.5, Abacus Concepts, Berkeley, Calif.). Analysis of variance and Bonferroni test were used to analyze the MRS data, NIRS data, enzyme deficits, and NDSs among and between groups. Kruskal-Wallis and Mann-Whitney tests were used for analysis of the OPC and histologic score. A p value less than 0.05 was considered statistically significant.

Results

Experimental conditions. The experimental conditions for each group are shown in Table I. Animals were similar in size, and there were no differences in

Table I. *Experimental conditions*

	Group I (mean ± SEM)	Group II (mean ± SEM)	Group III (mean ± SEM)	ANOVA p	Significant difference (p < 0.05)		
					I vs II	II vs III	III vs I
Body weight (kg)	8.03 ± 0.20	7.43 ± 0.34	8.13 ± 0.26	0.0800			
Hematocrit (%)							
Before operation	27.33 ± 1.43	27.63 ± 1.12	29.80 ± 1.39	0.3145			
Prime	0.00 ± 0.00	13.63 ± 0.53	28.80 ± 0.86	<0.0001	<0.0001	<0.0001	<0.0001
Before CA	8.44 ± 0.75	19.75 ± 0.46	31.00 ± 0.63	<0.0001	<0.0001	<0.0001	<0.0001
Before CPB weaning	27.44 ± 0.75	25.63 ± 0.75	30.20 ± 0.66	0.0004	<0.0001	<0.0001	<0.0001
POD1	29.00 ± 0.57	29.67 ± 1.15	30.20 ± 0.66	0.5855			
Esophagus temperature (°C)							
During cooling							
0 min	34.48 ± 0.82	33.85 ± 0.63	34.12 ± 0.42	0.7032			
40 min	14.12 ± 0.40	14.04 ± 0.40	14.63 ± 0.31	0.5251			
During rewarming							
0 min	15.81 ± 0.60	16.79 ± 0.62	15.53 ± 0.40	0.2259			
45 min	34.97 ± 0.47	35.43 ± 0.46	35.12 ± 0.24	0.6192			
Perfusion pressure (mm Hg)							
During cooling							
10 min	30.13 ± 3.77	47.88 ± 6.93	87.60 ± 3.96	<0.0001	0.0152	0.0001	<0.0001
20 min	27.00 ± 3.16	47.63 ± 5.89	85.20 ± 2.08	<0.0001	0.0025	<0.0001	<0.0001
30 min	26.38 ± 2.39	46.38 ± 5.53	89.60 ± 2.58	<0.0001	0.0021	<0.0001	<0.0001
40 min	25.63 ± 2.27	45.38 ± 5.27	81.20 ± 4.68	<0.0001	0.0016 ·	0.0002	0.0001
During rewarming							
10 min	33.75 ± 4.98	35.13 ± 4.07	41.00 ± 1.92	0.3340			
20 min	46.50 ± 8.24	40.13 ± 4.78	53.60 ± 4.27	0.0417		0.0413	
30 min	67.13 ± 11.18	66.25 ± 10.54	62.00 ± 6.22	0.9093			
40 min	77.13 ± 9.51	75.00 ± 8.21	78.20 ± 6.79	0.9467			
Ca (mmol/L)							
During cooling, 20 min	0.83 ± 0.05	1.04 ± 0.05	1.39 ± 0.05	<0.0001	0.0023	0.0003	0.0000
During rewarming, 20 min	1.08 ± 0.03	1.12 ± 0.05	1.45 ± 0.09	0.0001		0.0201	0.0152
Osmolarity (mOsm)							
Pump prime	270.71 ± 0.93	283.00 ± 2.87	280.60 ± 2.79	0.0008	0.0131		0.0221
After pump	280.33 ± 1.58	281.67 ± 2.27	277.20 ± 3.06	0.3976			

SEM, Standard error of the mean; *ANOVA,* analysis of variance; *CA,* circulatory arrest; *POD,* postoperative day; *Ca,* calcium.

hematocrit among groups before operation. During the cooling, arrest, and rewarming phases of CPB, there were no statistical differences in esophageal temperature among groups. During the cooling phase, hematocrit was set according to protocol, but there were no differences among groups after rewarming and on the first postoperative day. The mean perfusion pressure and calcium concentration changed with the hematocrit level during cooling and were therefore highest in group 3 and lowest in group 1 ($p < 0.0001$). The osmolarity of the pump prime was significantly lower in group I, but there were no significant differences among groups after rewarming and discontinuation of CPB.

Operative results. Two of seven animals in the extreme hemodilution group (group 1) developed severe hypotension and low-output state after weaning from CPB and could not be resuscitated. All data from these two animals were excluded from

subsequent analysis. All other animals were extubated within 24 hours of operation and survived until postoperative day 4, when they were sacrificed for histologic examination.

Cerebral high-energy phosphates and intracellular pH (pH$_i$). During the cooling phase a significant decrease in phosphocreatine (PCr) in group I was noted during the initial 10 and 20 minutes, whereas PCr increased in groups II and III (Fig. 2). An increase in pH$_i$ was noted in group III, whereas pH$_i$ significantly decreased during cooling in group I. During the circulatory arrest period, the adenosine triphosphate (ATP) signal was highest in group III and lowest in group I. PCr and pH$_i$ did not show any significant differences among groups during DHCA. During the rewarming phase, group III showed more rapid recovery of ATP, PCr, and pH$_i$ than in groups I and II. After discontinuation of CPB bypass, group III maintained higher levels of ATP,
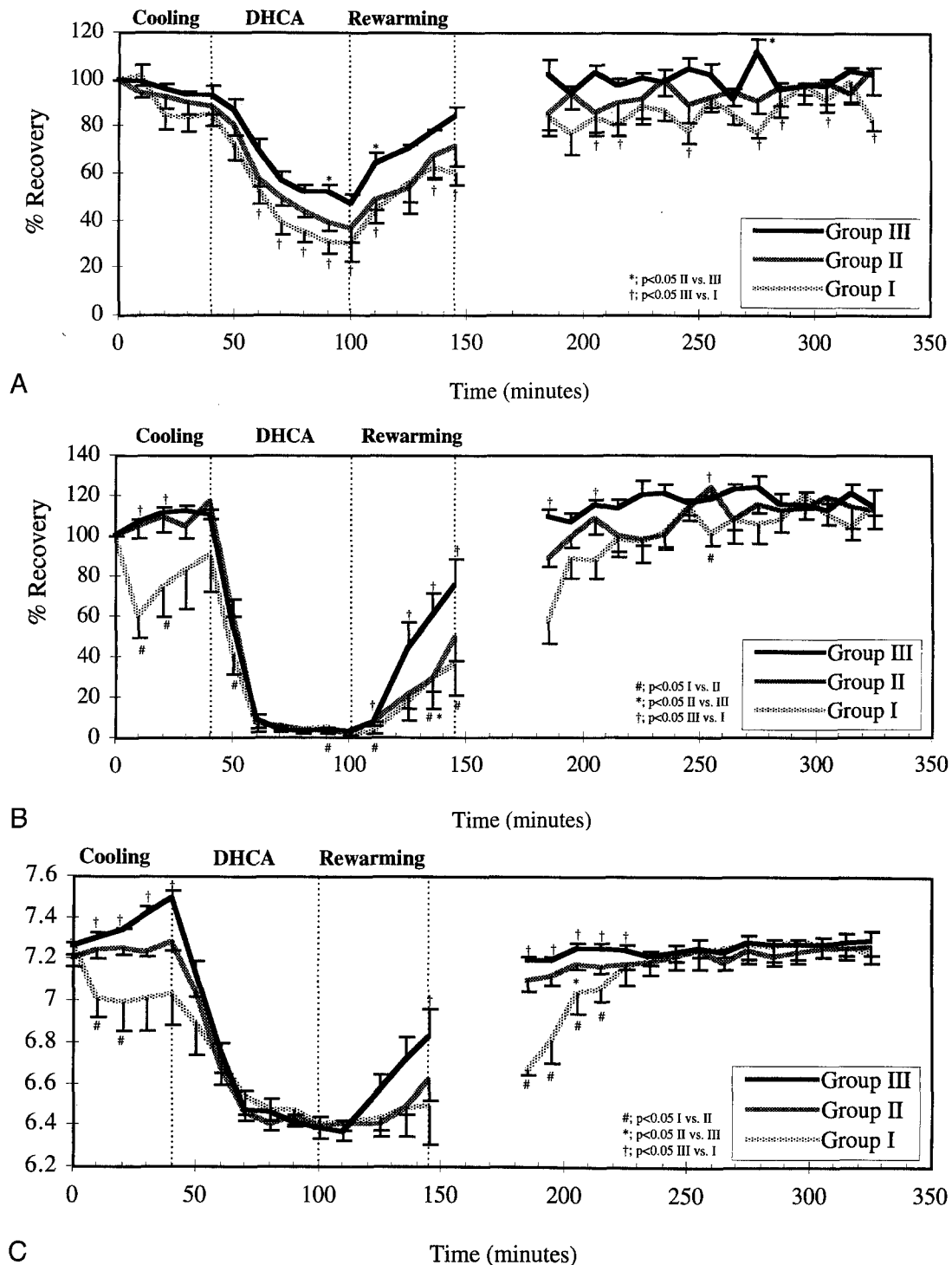
Fig. 2. Results of MRS. **A,** Cerebral ATP; **B,** cerebral PCr; **C,** cerebral pH_i.

PCr, and pH_i than did groups I and II. Three hours after CPB, however, there were no significant differences among groups with respect to PCr and pH_i, although group III showed a significantly higher ATP level than did group I.

NIRS. The oxyhemoglobin signal increased significantly during cooling in the whole-blood prime group (group III; Fig. 3). During cooling, the redox state of cytochrome aa_3 increased in group III but decreased significantly in groups I and II (Fig. 3).
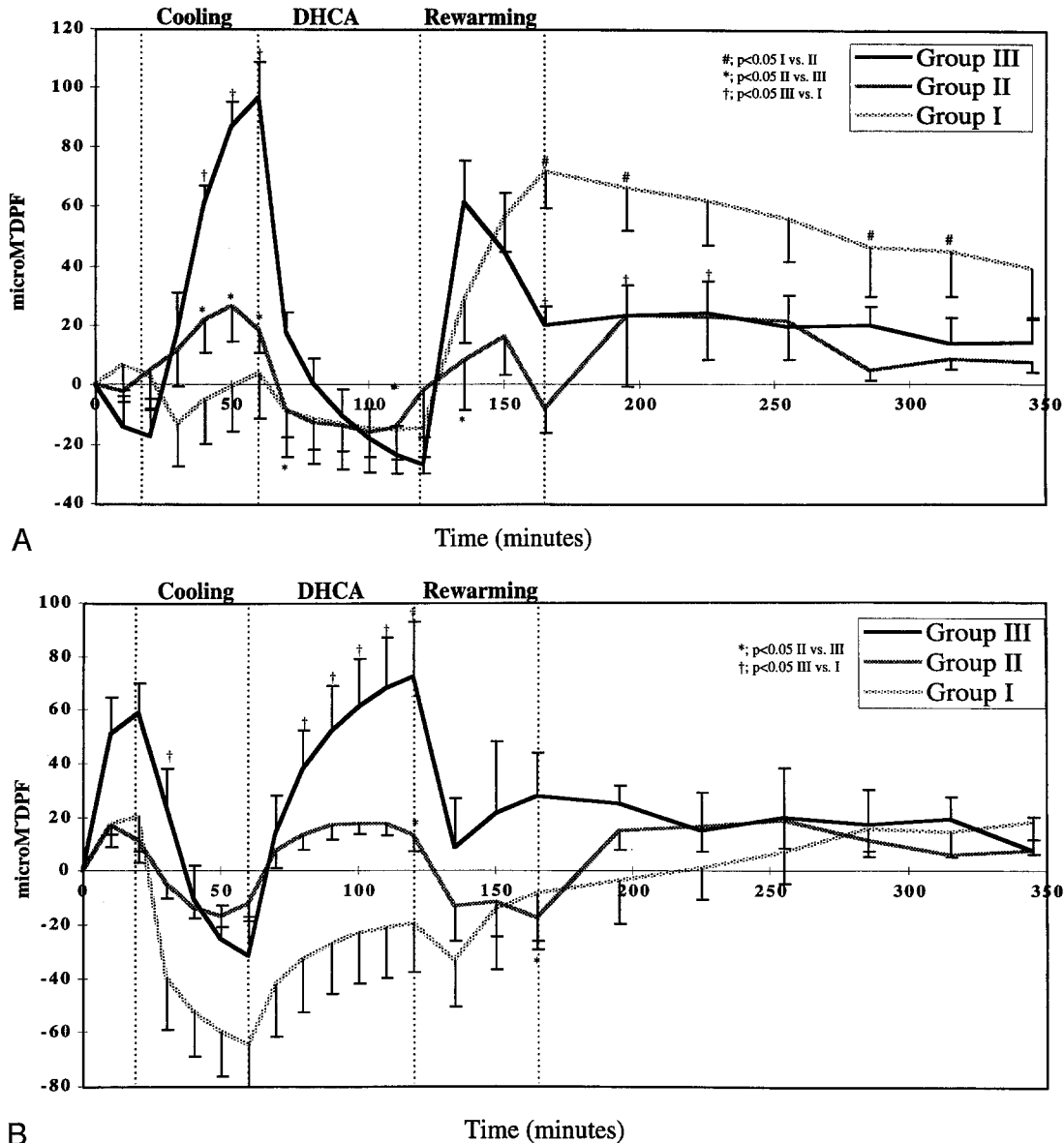
Fig. 3. Results of NIRS. **A,** Oxyhemoglobin; **B,** deoxyhemoglobin. *DPF,* Differential pathlength factor.

From the onset of DHCA, there was a decline in oxyhemoglobin and cytochrome aa_3 signals in all groups, and deoxyhemoglobin increased reciprocally. In group I and II, however, oxyhemoglobin and deoxyhemoglobin reached plateaus within approximately 30 minutes, whereas in group III there was no plateau in either signal.

Enzymes. There were no statistically significant differences among the three experimental groups (Table II). Levels of CK, AST, ALT, LDH, ALP, and total bilirubin tended to be lowest in group III.

Neurologic deficit score. The NDS and OPC demonstrated more rapid recovery in group III than

in groups I and II (Table II). On postoperative day 1, both the NDS and OPC in group III were significantly better than those in group II (p = 0.0965 for NDS, p = 0.0194 for OPC). On postoperative 2, the NDS in group III remained significantly better than that in group II (p = 0.0492). The NDS was generally worse in group I than group III, although this difference did not reach statistical significance. By postoperative days 3 and 4, most animals had recovered and showed normal performance, with no neurologic deficit.

Neuropathologic results. As in previous studies, neuropathologic damage was evaluated primarily by
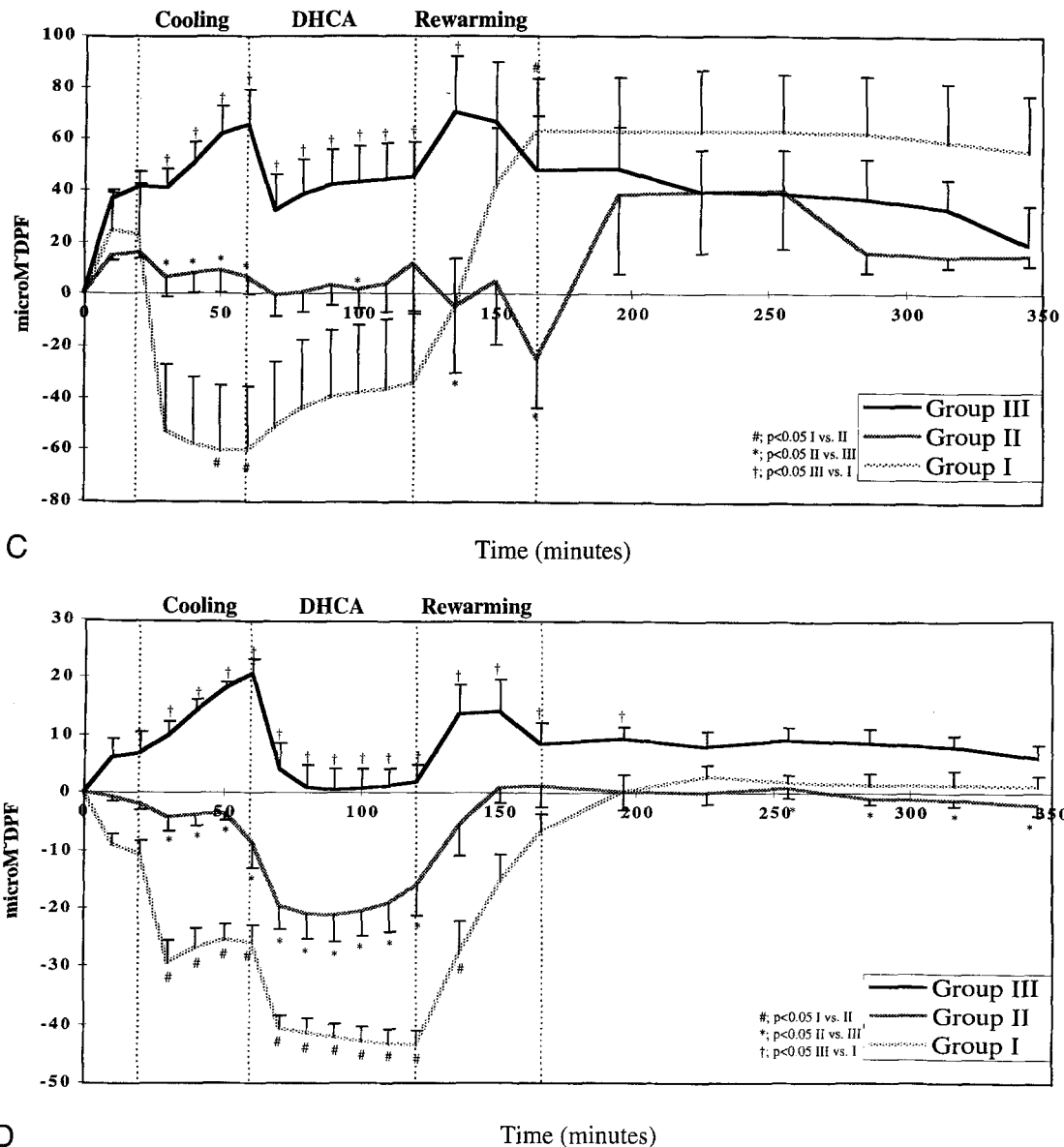
C   Time (minutes)

D   Time (minutes)

Fig. 3, Cont'd. C, Total hemoglobin; **D,** cytochrome *aa3. DPF,* Differential pathlength factor.

the presence of hypereosinophilic shrunken neurons with karyorrhectic nuclei, suggestive of recent hypoxic ischemic injury (Fig. 4). The patterns of injury followed those seen in the previous studies. Injury occurred primarily in the lateral frontal and parietal neocortex. A lesser degree of damage was present in the caudate nucleus; in one instance, a focal ischemic lesion of grade 2 was present in the hippocampal dentate gyrus. No damage that could be ascribed to hypoxic or ischemic injury was noted in the diencephalon, brain stem, or cerebellum. Rare, scat-

tered perivascular vacuolated lesions were noted, but these were not systematically recorded and their significance remains unclear at present. The hypoxic or ischemic injury was most pronounced in group I and was negligible in groups II and III (Fig. 5).

Discussion

In this study, a blood-free prime resulting in a hematocrit lower than 10% was associated with evidence of inadequate oxygen delivery to cerebral cells, as suggested by reduced cytochrome aa_3 by

Table II. *Results*

	Group I (mean ± SEM)	Group II (mean ± SEM	Group III (mean ± SEM)	ANOVA p K-W test	Significant difference (p < 0.05) I vs II	II vs III	III vs I
Neurologic scores							
NDS							
POD1	95.83 ± 18.73	112.50 ± 14.54	58.00 ± 7.52	0.0465		0.0077	
POD2	24.17 ± 12.68	39.17 ± 11.87	4.00 ± 4.00	0.0492		0.0213	
POD3	3.33 ± 3.65	3.33 ± 3.65	0.00 ± 0.00	0.6702			
POD4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	NA			
OPC							
POD1	2.67 ± 0.23	3.00 ± 0.00	2.00 ± 0.00	0.0194		0.0422	
POD2	1.33 ± 0.23	1.83 ± 0.34	1.00 ± 0.00	0.1588			
POD3	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	NA			
POD4	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	NA			
Enzymes							
AST	132.60 ± 18.95	119.40 ± 13.40	95.40 ± 11.41	0.1917			
ALT	31.00 ± 5.52	34.00 ± 3.61	28.80 ± 2.48	0.6190			
LDH	1125.60 ± 85.89	1228.50 ± 171.58	959.00 ± 114.75	0.2798			
ALP	157.80 ± 16.00	165.75 ± 17.31	202.20 ± 10.17	0.0700			
CK	4008.20 ± 483.76	3471.80 ± 607.08	2977.60 ± 831.51	0.5255			
TB	0.42 ± 0.04	0.43 ± 0.19	0.28 ± 0.07	0.4855			
Histology							
Neocortex	1.33 ± 0.33	0.22 ± 0.11	0.40 ± 0.18	0.0287	0.0054		0.0225
Hippocampus	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00				
Caudate	1.00 ± 0.71	0.17 ± 0.17	0.00 ± 0.00				

SEM, standard error of the mean; *ANOVA,* analysis of variance; *K-W,* Kruskal-Wallis; *POD,* postoperative day; *TB,* total bilirubin.

NIRS during the cooling phase on CPB before DHCA. Furthermore, cerebral PCr and pH_i fell significantly during cooling with severe hemodilution. Histologic assessment revealed neuronal necrosis after 4 days. In contrast, a hematocrit greater than 30 was associated with increased mitochondrial oxygen availability during cooling. In addition, oxyhemoglobin was markedly above baseline at the onset of DHCA, followed by a continuous decline during DHCA. This suggests that red blood cells continue to deliver oxygen throughout the arrest period; that is, hemoglobin may act as a reservoir of oxygen through 1 hour of DHCA. In contrast, oxyhemoglobin remained at baseline through cooling with a hematocrit lower than 10%. During DHCA, it appears that the oxygen reservoir is exhausted after approximately 30 minutes when the hematocrit is 20% or lower than 10%. The findings with oxyhemoglobin are reinforced by the reciprocal changes observed with deoxyhemoglobin, as measured by NIRS.

The least evidence of neurologic injury was observed in the animals with the highest hematocrits. The greatest histologic damage was seen in the animals with the lowest hematocrits.

The findings of this study are consistent with previous work from this laboratory, which suggested that a more alkaline pH strategy (alpha-stat) during cooling before DHCA with moderate hemodilution (hematocrit 20%) is associated with a greater degree of mitochondrial hypoxia, delayed recovery of cerebral high-energy phosphates, and worse neurodevelopmental outcome than is a more acidotic strategy (pH-stat).[11, 14] In the setting of deep hypothermia with severe or moderate hemodilution, this study suggests that hematocrit critically limits oxygen delivery. A more alkaline pH strategy further limits oxygen delivery through its effect on oxyhemoglobin dissociation.

It is interesting to speculate why the neurologic recovery of the moderate hemodilution group was delayed relative to the highest and lowest hematocrit groups. The moderate hemodilution group had no colloid added to the prime, whereas the severe hemodilution group had hetastarch added to model the addition of albumin, as is done clinically by the Loma Linda group. The colloid osmotic pressure was thus almost certainly higher in groups I and III than in group II. This may have been associated with more cerebral as well as generalized body edema in group II, which may have resulted in worse neurologic and general performance scores. We are cur-
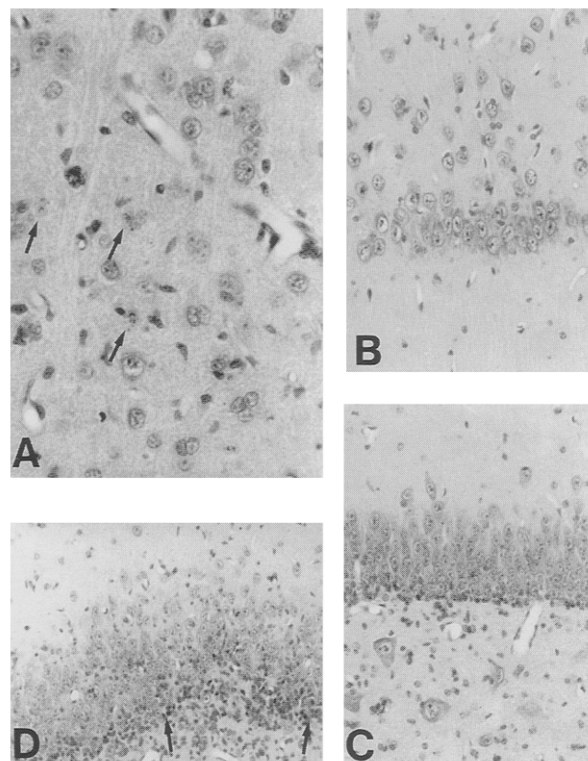
Fig. 4. A, Frontal neocortex (×40) from group I showing area of hypoxic-ischemic injury. This most severely injured animal in this group would be scored as having 2+ lesion. Note several neurons with hypereosinophic cytoplasm and karyorrhectic nuclei *(arrows)*. Blood vessel in upper right corner shows no pathologic change. **B,** Hippocampal pyramidal cell layers (from same animal as in **A**) showing no damage (×20). **C,** Fascia dentata (from same animal as **A**) showing no damage (×20). **D,** Fascia of hypoxic-ischemic damage between *arrows*. This animal had less injury to neocortex than did the other animal in this figure. No evidence of capillary damage or microemboli was present. (7 μm paraffin sections;

rently undertaking a study that will separate the effects of red blood cell and colloid hemodilution. This should help to define the relative importances of the inadequate oxygen delivery demonstrated by the intraoperative MRS and NIRS and of postoperative edema in determining postoperative outcome.

There has been a long-standing controversy regarding the optimal degree of hemodilution during hypothermic CPB.[15, 16] Kawashima, Yamamoto, and Manabe[17] and Michenfelder and Theye[18] found that oxygen delivery was limited by a hematocrit lower than 20%. A lower hematocrit resulted in acidosis.

In 1976, Koster and coworkers[19] demonstrated the beneficial effects of hemodilution on cerebral blood flow. In studies of three experimental groups with various degrees of hemodilution on CPB at hematocrits of 40%, 25%, and 15%, they concluded that hemodilution enhanced cerebral blood flow and maintained oxygen delivery to the brain in the dog. After 60 minutes of arrest, there was significant cerebral hyperemia during reperfusion, especially in the hemodilution group. This finding has been substantiated by many other investigators.[20-22] It can be argued, however, that there is a serious limitation to studies that derive inferences from cerebral blood flow and metabolic rate determined by oxygen consumption in any setting where oxyhemoglobin dissociation is shifted leftward, as is the case with hypothermic CPB, particularly profoundly hypothermic CPB. There are even more important limitations to the many studies that have examined the question of optimal hematocrit with non-CPB models.[21] In these models, hemodilution is always compensated for by a marked increase in cardiac output, usually also associated with an increase in cerebral blood flow. It is not clear whether the increases in cardiac output and cerebral blood flow are related to the decrease in viscosity caused by hemodilution or if there is an important component of hypoxic stress (flow-metabolism coupling in the case of the brain) that drives the increase in flow. In any event, cardiac output is fixed in the patient receiving CPB. Furthermore, the flow rate that is traditionally used at normothermia (2 to 2.5 L · min^{-1} · m^{-2}) would be considered a low cardiac output state in a normothermic person with a normal hematocrit. This could not be considered a flow rate that might compensate for the effects of hemodilution. Nevertheless, clinical experience suggests that hemodilution to a hematocrit between 20% and 30% in the adult undergoing continuous mildly or moderately hypothermic CPB is not associated with neurologic injury,[6] although comprehensive neuropsychometric testing has only recently been widely employed. One multiinstitutional study of adults found that lower hematocrit was associated with a postoperative decrease in cognitive score.[23]

The effects of deep hypothermia on microvascular circulation are complex and include red blood cell aggregation and arteriovenous shunting.[24] The role of hemodilution in reversing these effects was reviewed by Cooper and Elliott.[4] Edmunds and associates[25] suggested that the presence of microinfarction observed from sections of the brain taken from
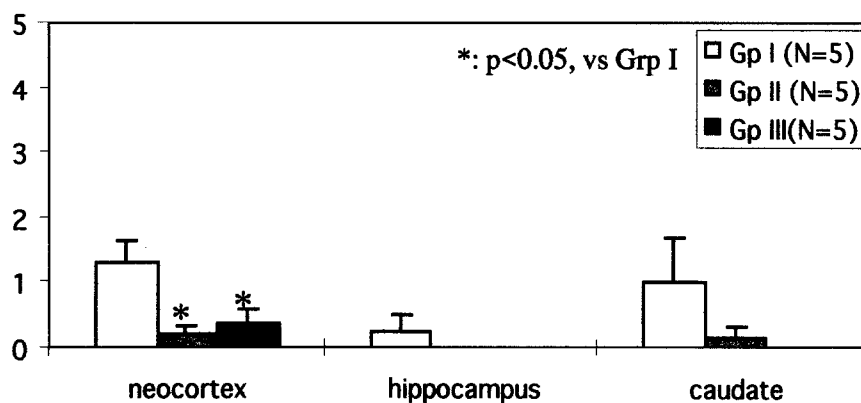
Fig. 5. Histologic score for neocortex, hippocampus, and caudate nucleus.

animals subjected to DHCA was related to the presence of blood within the brain during the period of DHCA. In a study in our laboratory with a blood-free solution (Hypothermosol; Cryomedical Sciences, Rockville, Md.) in the cerebral circulation during DHCA, however, outcome as determined by NIRS and by neurologic score was inferior to outcome with blood perfusate.[13] This study revealed no histologic evidence of microinfarcts. Most previous studies of microcirculatory disturbance in the setting of DHCA predate more recent interest in endothelial and white blood cell activation and its relationship to ischemia.[26] Future studies should focus on the role of upregulation of adhesion molecules in contributing to microcirculatory disturbance after DHCA. The role of red blood cell aggregation may have been overemphasized.

This is the first study from our laboratory that has combined MRS and NIRS with postoperative neurologic evaluation and histologic assessment in the same animals. We hoped to identify changes in intraoperative MRS and NIRS parameters that best predicted neurologic and histologic outcomes, and we therefore calculated Pearson coefficients. Although no definite predictors were identified, it is interesting to note the trends in r and p values. For example, for ATP at DHCA 60 minutes versus NDS on day 1, p was 0.98; for cytochrome aa_3 at DHCA 60 minutes versus NDS on day 1, p was 0.2; for pH at DHCA 60 minutes versus NDS on day 1, p was 0.12. The lack of statistically significant predictors is perhaps not surprising in light of the small numbers and the fact that DHCA time was limited to minimize mortality, rather than being extended to achieve severe neurologic injury as has been done in some previous studies from our laboratory.

In conclusion, extreme hemodilution results in evidence of inadequate oxygen delivery during the initial cooling phase and during DHCA. Whole-blood priming is associated with optimal preservation of mitochondrial redox state, optimal preservation of high-energy phosphates, and subsequently better early neurologic and histologic scores. The improved cerebral recovery with higher hematocrit after DHCA might in part be explained by greater oxygen availability and therefore preservation of high-energy phosphate metabolism. However, the effects of cerebral edema, low perfusion pressure, and low colloid oncotic pressure related to hemodilution could also be important. Further studies are underway to explore these variables.

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Discussion

Dr. Steven R. Gundry *(Loma Linda, Calif.)*. I congratulate you on designing a complex experimental model to once again look at the outcomes of various protocols for circulatory arrest. The fact that you were able to carry out this experiment successfully in a large number of piglets reflect your skills as surgeons and as managers of a bypass circuit.

You have attempted to compare three clinical protocols that are in use at Loma Linda, Boston, and Paris by means of three distinct methods of hemodilution or priming with whole blood. You have come to a remarkable conclusion in that you have concluded that your own protocol was not the winner, and you, the authors, are to be congratulated on admitting that. A review of the details of your perfusion mechanism, however, suggests that your conclusions are correct but your method of arriving at the conclusions may in fact be incorrect, and that you set yourself up to make the high hematocrit group win.

Specifically, I have these comments. You note in your article that the aortic cannula was placed in the femoral artery. This is far removed from the brain and completely opposite to the method used clinically, where the aortic cannula and thus the perfusate visit the brain first rather than last. This ensures that the brain is the last organ to be cooled in your model, rather than the first, as is done clinically.

Next, I noticed that your cooling occurs during a 40-minute period, and you specifically relate in your article that you used the entire 40 minutes to reach the cooling temperature. This implies that for most of the 40-minute cooling period the two groups with very low hemoglobin do in fact have very low oxygen-carrying capacity during periods of relative warmth of the brain. Your conclusions are therefore correct that during a prolonged, slow cooling period, the two groups that have the least oxygen carrying capacity and the slowest cooling of the brain would lose in this protocol, and that is exactly what you prove. Clinical protocols are exactly the opposite. The brain cannula is nearby and the brain is cooled rapidly, at Loma Linda specifically during a 10-minute period. Work by Schell and colleagues at Duke has shown fairly conclusively that rapid cooling of the brain results in much better brain metabolism after these short cooling periods compared with a long period for cooling of the brain.

Finally, I noticed that your group I was primed with hetastarch. Schell and colleagues at Duke have shown repeatedly that hetastarch undergoes viscosity changes at low temperatures. Specifically, hetastarch is not used in a priming solution at Loma Linda or at any other institution that I know of for circulatory arrest. I think that the fact that two animals out of the group I group died of hypotension suggests that this model was not tolerated systemically, either with the heart or any other organ, much less the brain; thus you set this model up to lose.

I have two questions. Do you believe that a more

clinically relevant cannulation and cooling period would have changed your results, and perhaps that your own method of cooling would then have prevailed?

Second, do you think that the fact that two animals in group I died of hypotension suggests that the hetastarch, which is not used in any clinical protocol, may have been the reason for some of your findings?

I again congratulate you on a difficult study, but I think examination of your protocol shows what you might expect—a lot of blood with warm temperatures is better than no blood at warm temperatures.

Dr. Shin'oka. Thank you, Dr. Gundry. In response to the first issue, regarding the site of arterial cannulation, we do not agree that femoral cannulation would bias the result against the low-hematocrit groups. In fact, we believe it possible that the bias would be in the opposite direction, for the following reason. The fundamental problem in the low-hematocrit groups is that there is inadequate oxygen carrying capacity in the early phase of cooling, as demonstrated by reduction in the cytochrome aa_3 signal and fall in PCr level. This is because of inadequate oxygen delivery from a low-hematocrit perfusate when the brain is still warm and the perfusate is cold. The more gradual cooling of the brain that results from femoral cannulation should, if anything, decrease this problem in the low-hematocrit groups.

Regarding the cooling duration of 40 minutes, we believe that the same issue applies here. In other words, a longer cooling duration allows any oxygen debt incurred in the early phase of cooling to be "paid off." We suspect that if a shorter cooling duration had been employed, the differences between the groups would have been exaggerated.

Finally, regarding the use of hetastarch, we have been interested in the work of Zikria and Oz at Columbia University regarding the biophysical properties of hetastarch when used during CPB and especially during DHCA. The group at Columbia uses hetastarch routinely in their CPB prime. They have demonstrated that not only is the use of hetastarch not deleterious during deep hypothermic circulatory arrest in the piglet model but in fact it is associated with a marked decrease in postoperative edema. Our inference from the deaths of two animals in the very low–hematocrit group, with no deaths in the other two groups, is therefore, that this is more likely related to inadequate oxygen delivery not only to the brain but also to other organs including the heart. We believe that the marked reduction of the cytochrome aa_3 signal in the low-hematocrit group as well as the fall in PCr is convincing evidence that extreme hemodilution results in inadequate oxygen availability during the early phases of cooling on CPB.