Age-Dependent and Hypoxia-Related Differences in Myocardial Protection During Pediatric Open Heart Surgery

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Background—Current cardioplegic protection techniques used in pediatric cardiac surgery do not take into consideration age and cyanotic differences. The aim of the present work was to address this question by monitoring clinical outcome, myocardial metabolism, and reperfusion injury in pediatric patients protected by cold-crystalloid cardioplegia.

Methods and Results—Fifty-eight patients (31 children and 27 infants) with or without hypoxic stress (cyanosis) undergoing open heart surgery with cold-crystalloid cardioplegia were included in the study. Clinical outcome measures assessed included inotropic and ventilatory support, intensive care, and hospital stay. Ischemia-induced changes in metabolism (adenine nucleotides, purines, lactate, and amino acids) were determined in ventricular biopsies collected at the beginning and end of ischemic time (cross-clamp time). Reperfusion injury was assessed by measuring postoperative serial release of troponin I. Evidence was observed of ischemic stress during cardioplegic arrest in children and infants as shown by significant changes in cellular metabolites. Compared with infants, children had significantly less reperfusion injury and better clinical outcome, and these factors were related to duration of ischemic time. Cyanosis did not influence outcome in infants, but cyanotic children showed worse reperfusion injury and clinical outcome than acyanotic children.

Conclusions—Extent of myocardial protection with cold-crystalloid cardioplegia in pediatric open heart surgery is dependent on age and degree of cyanosis. (Circulation. 2001;103:1551-1556.)

Key Words: pediatrics ■ surgery, pediatric ■ cardioplegia ■ ischemia ■ metabolism

Since the introduction of cardioplegic arrest, major advances have been made in preservation of myocardial function during open heart surgery. However, despite variation in composition of cardioplegia, myocardial protection has been based primarily on use of high-potassium cold-cardioplegic solutions. Although these solutions originally were designed to protect adult hearts, they have been adopted for pediatric cardiac surgery.1 Available laboratory data has been inconclusive to explain vulnerability of immature myocardium to cardiac surgery. Most reports suggest that developing mammalian hearts are more resistant to damaging effects of cardiac insults than adult hearts.1–4 This improved tolerance has been attributed to differences in vascular resistance, calcium mobilization, and metabolism.5–8 However, others9,10 have reported that immature myocardium is more vulnerable to injury than adult heart. Experimental laboratory research that has investigated cardioprotective action of cardioplegic solutions also has been inconclusive, in part because of species-dependent and age-related differences.11–13

Current methods of myocardial protection during adult open heart surgery include a variety of cardioplegic techniques, several of which have been shown to provide good myocardial preservation.14,15 In contrast, myocardial protection during pediatric open heart surgery remains poor and associated with relatively more morbidity and mortality.16,17 This is largely because myocardial protection techniques used in adult hearts are uncritically extended to pediatric hearts. Furthermore, end points used in myocardial protection studies in pediatric surgery tend to focus on single aspects (eg, function or metabolism).16,18-20 No comprehensive studies have dealt with metabolism, myocardial reperfusion damage, and clinical outcome or have addressed the question of age and effect of chronic cyanosis. The aim of the present work was to investigate whether myocardial protection during pediatric open heart surgery depends on age and degree of cyanosis. To achieve this aim, we monitored myocardial metabolic changes during ischemia, postoperative troponin I release as a measure of reperfusion injury, and a variety of parameters of clinical outcome in children and infants with or
without evidence of cyanosis who were undergoing open heart surgery with St. Thomas’ Hospital cardioplegic solution 1.

Methods
Fifty-eight patients (27 infants <12 months and 31 children ≥12 months of age) who underwent elective open heart surgery for congenital heart disease between March 1998 and November 1999 at the Bristol Royal Hospital for Sick Children were recruited prospectively into the present study. The 2 groups were divided into cyanotic and acyanotic patients according to arterial blood oxygen saturation (oxygen saturation acyanotic, 90% to 100%; cyanotic, <90%). Preoperative characteristics are summarized in Table 1. All cyanotic patients were stable and none was acutely hypoxic. No emergency operations had occurred, and no patients were on preoperative respiratory or inotropic support. Ethical approval from the local authority and informed consent were obtained for all patients.

Operative Procedure
All operations were performed by use of cardiopulmonary bypass with ascending aortic and bicaval venous cannulation. Anesthetic technique was standardized for all patients. Slow induction with sevoflurane and 50% air-50% O2 followed by fentanyl 25 to 50 mg/Kg was used. Morphine 0.5 mg/Kg was infused during cardiopulmonary bypass, and neuromuscular blockade was achieved by 0.1 to 0.15 mg/Kg pancuronium bromide. Heparin 3 mg/Kg body wt was initially added and supplemented as required to maintain active clotting time of ≥480 seconds. Alpha-stat acid-base management was adopted. Cold-crystallloid St. Thomas’ Hospital cardioplegic solution 1 (4°C to 6°C) was used for myocardial protection (Martindale Pharmaceuticals). Cardioplegic arrest was achieved by an antegrade infusion of 25 mL·kg⁻¹·min⁻¹ for 4 minutes. No additional cardioplegia was administered. Topical cooling with ice slush was used to maintain myocardial cooling.

Assessment of Clinical Outcome
Intraoperative and postoperative clinical parameters were used to determine level of clinical outcome. These parameters included intraoperative requirement of inotropes for weaning patients from cardiopulmonary bypass and postoperative inotropic support. The latter was considered to be either minimal (<6 μg·kg⁻¹·min⁻¹ of dopamine) or significant (6 to 10 μg·kg⁻¹·min⁻¹ of dopamine with or without other inotropic agents such as adrenaline or noradrenaline). Other clinical parameters included length of inotropic and ventilatory support, intensive care, and hospital stay.

Collection of Ventricular Biopsies and Extraction of Metabolites
Myocardial biopsies (5.5±0.5 mg) were collected from right ventricle (free wall of trabecular portion) through tricuspid valve by direct resection with surgical scissors. Biopsies were collected from only 42 patients. First biopsy was taken immediately after cross-clamping the aorta (control biopsy), whereas second biopsy was taken before releasing the aortic cross-clamp (ischemic biopsy). Each specimen was immediately frozen in liquid nitrogen until processing for analysis of cellular metabolites.

Determination of Adenine Nucleotides, Purines, Lactate, and Amino Acids in Biopsy Specimens
Adenine nucleotides, purines, lactate, and free amino acids were measured in all biopsies collected. Adenine nucleotides and purines in neutralized extract were separated and quantified by use of a high-performance liquid chromatography method based on previous reports. Lactate was determined by use of a diagnostic kit from Sigma Chemical Co. Amino acids were determined according to the Pico-Tag method of Water as reported earlier.

Measurements of Myocardial Troponin I and Characteristics of Exclusion
Serum concentration of myocardial troponin I was determined before surgery and at 4, 12, 24, and 48 hours postoperatively with ACCESS system (Beckman, Inc). Corrective congenital heart surgery can involve significant ventricular incision or myocardial resection. In such patients, postoperative release of troponin I is likely to be due to reperfusion injury as well as damage from incision and resection. We found that postoperative release of troponin I in patients who had significant right ventricular incision was markedly higher than patients who had minimum or no incision or resection of ventricular muscle (data not shown). Eight patients with significant incision and resection therefore were excluded from troponin I analysis.

Data Collection and Analysis
Clinical outcome data were collected prospectively and analyzed for all 58 patients recruited. Ventricular biopsies were collected from 42 patients, all of which were used in the analysis. Postoperative release of troponin I was measured in all 58 patients, but results from only 50 were used for analysis (see exclusion criteria above). The 50 patients for whom troponin I analysis was completed included 26 children (6 with cyanosis) and 24 infants (10 with cyanosis). Clinical and biochemical data were expressed as mean±SEM. Statistical intragroup analysis was performed by use of paired t test or repeated measures ANOVA with Bonferroni post hoc test as appropriate. Intergroup analysis was performed by use of unpaired t test. Correlation coefficient was calculated and significance determined by use of Fisher’s r to z. Statistical analysis was performed with a StatView personal computing package (SAS Institute Inc).

Results
Clinical Outcome
No in hospital deaths occurred, and no differences were found between children and infants with regard to cardiopulmonary bypass and aortic cross-clamp time (Table 1). Significant

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**TABLE 1. Patient Characteristics and Clinical Outcome**

<table>
<thead>
<tr>
<th>Description</th>
<th>Infants (n=27)</th>
<th>Children (n=31)</th>
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| Age, mo                 | 4.7±0.6        | 46.8±5.9*
|                         | (1–11)         | (12–120)        |
| Body wt, kg             | 5.2±0.4        | 15.9±1.4*
| Sex, M/F                | 20/7           | 21/10           |
| Pathology, n            | 3               | 5               |
| Ventricular septal defect | 9              | 6               |
| Tetralogy of Fallot     | 4              | 7               |
| Atrioventricular septal defect | 4           | 0               |
| Pulmonary atresia or stenosis | 2         | 4               |
| Total anomalous pulmonary vein drainage | 3           | 0               |
| Atrial septal defect/partial defect | 0       | 5               |
| Mitral or aortic valve  | 0              | 4               |
| Other                   | 5              | 5               |
| Cardiopulmonary bypass time, min | 61.0±3.9 | 54.7±4.8 |
| Aortic cross-clamp time, min | 28.2±2.3 | 25.3±1.7 |
| Intotopic support duration, h | 61±9       | 28±7*           |
| Ventilatory support duration, h | 47±7       | 16±3*           |
| Intensive Care Unit stay, d | 4.9±0.7   | 2.9±0.5*        |
| Hospital stay, d        | 13.7±1.7       | 9.6±0.9*        |

*P<0.05 vs infants.
postoperative inotropic support was required for 67% of infants and 39% of children, and amount of dopamine needed for weaning patients from cardiopulmonary bypass was significantly higher in infants than children (6.9 ± 0.7 versus 4.6 ± 0.6 g·kg⁻¹·min⁻¹, respectively; P < 0.05). All clinical outcome parameters were significantly better for children (Table 1) and were dependent on ischemic cross-clamp time (Figure 1).

Clinical outcome in infants was not dependent on presence of cyanosis. However, despite similar cross-clamp times, cyanotic children had worse outcome compared with acyanotic children (Table 2).

Given the small number of patients in each pathology (Table 1), firm conclusions on the effect of different pathologies on clinical outcome are difficult to make from the present study. However, 2 pathologies were examined that included relatively more patients (Table 1); children with tetralogy of Fallot (n = 7) and infants with ventricular septal defect (n = 9). As a result of narrow range of cross-clamp time in children with tetralogy of Fallot (25 to 35 minutes), no correlation existed between cross-clamp time and clinical outcome. However, significant (P < 0.05) negative correlation was seen between increased degree of hypoxia and clinical parameters. As for infants with ventricular septal defect pathology, positive correlation was seen between cross-clamp time and inotropic duration (P < 0.05).

### Changes in Cellular Metabolites During Ischemia

During ischemia, a significant fall in myocardial concentration of ATP was seen in both groups: from 45.2 ± 3.5 to

### Table 2. Patient Characteristics and Clinical Outcome in Children and Infants With or Without Evidence of Cyanosis

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<tr>
<th></th>
<th>Infants (n=12)</th>
<th>Children (n=11)</th>
<th>Infants (n=27)</th>
<th>Children (n=20)</th>
</tr>
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</table>
| Cardiopulmonary bypass time, min | 65.4 ± 6.7 | 57.5 ± 4.7 | 75.6 ± 9.0 | 43.2 ± 3.7*
| Aortic cross-clamp time, min | 29 ± 4 | 28 ± 3 | 29 ± 2 | 24 ± 2
| Hemoglobin, g/L | 14.0 ± 0.6 | 11.3 ± 0.3 | 14.9 ± 0.4 | 12.1 ± 0.3*
| Blood O₂ saturation, % | 84.2 ± 1.0 | 95.3 ± 0.7* | 83.5 ± 1.5 | 97.9 ± 0.5*
| Postoperative inotropic support, n | 5 | 4 | 2 | 17
| Minimum | 7 | 11 | 9 | 3
| Significant | 7 | 11 | 9 | 3
| Dopamine, μg·kg⁻¹·min⁻¹ | 7.7 ± 0.8 | 6.2 ± 0.9 | 6.6 ± 1.2 | 3.3 ± 0.6†
| Inotropic duration, h | 61 ± 12 | 61 ± 14 | 56 ± 17 | 14 ± 3††
| Intubation time, h | 43 ± 10 | 50 ± 12 | 29 ± 6 | 9 ± 2‡‡
| ICU stay, d | 3.9 ± 0.7 | 5.6 ± 1.3 | 5.2 ± 1.2 | 1.7 ± 0.2‡‡
| Hospital stay, d | 10.3 ± 1.1 | 16.4 ± 2.7 | 11.1 ± 1.0 | 8.8 ± 1.3††

*P < 0.05 vs cyanotic patients in the same group; †P < 0.05 vs acyanotic infants; ‡P < 0.05 vs cyanotic infants.
Scattergrams show correlation between ischemic stress (% fall in ATP) and cross-clamp time (A) and between reperfusion injury (peak troponin I release) and cross-clamp time (B). Strong positive correlation existed (coefficients were 0.73 for both children (peak troponin I release) and cross-clamp time (B). Strong positive correlation existed (coefficients were 0.73 for both children and infants in A and 0.68 for children and 0.69 for infants in B; P < 0.0001).

Figure 2. Effect of cross-clamp time on ischemic stress and reperfusion injury in children (○, n = 20) and infants (●, n = 20). Scattergrams show correlation between ischemic stress (% fall in ATP) and cross-clamp time (A) and between reperfusion injury (peak troponin I release) and cross-clamp time (B). Strong positive correlation existed (coefficients were 0.73 for both children and infants in A and 0.68 for children and 0.69 for infants in B; P < 0.0001).

21.9 ± 2.2 nmol/mg protein for infants and from 42.2 ± 2.1 to 24.6 ± 3.3 nmol/mg protein for children (P < 0.05). Changes in ATP were strongly dependent on duration of ischemia (Figure 2A). Furthermore, fall in ATP was significantly greater in patients who needed a higher amount of inotropic support versus patients who had minimum postoperative inotropic requirement (P < 0.05). A significant rise in lactate during ischemia was seen only in children (from 164 ± 32 to 308 ± 71 nmol/mg protein for children, P < 0.05, versus 259 ± 55 to 263 ± 45 nmol/mg protein for infants). Although resting levels of lactate in children tended to be lower than in infants, this did not reach statistical significance.

Table 3 shows concentration of all metabolites before and after ischemia in children and infants, with each group further divided into cyanotic and acyanotic patients. A significant ischemia-induced fall in ATP was evident in cyanotic and acyanotic hearts in both children and infants. A significant fall in ADP occurred after ischemia only in cyanotic hearts of infants and children. The concentrations of inosine, adenosine, and hypoxanthine in control biopsies were similar in acyanotic and cyanotic children and infants and increased in a similar fashion at end of ischemia (Table 3). A significant fall in concentration of glutamate during ischemia was seen in all groups, although this was more marked in cyanotic compared with acyanotic patients. Furthermore, fall in concentration of aspartate was significant only in cyanotic patients. No difference in concentration of lactate was observed in biopsies collected at the beginning of ischemia in cyanotic or acyanotic infants. However, acyanotic hearts in children had significantly lower concentration of lactate compared with cyanotic children or infants. No significant change occurred in lactate concentration at end of ischemia in infant hearts (cyanotic or acyanotic) or cyanotic hearts of children. A significant increase in myocardial lactate during ischemia was seen only in acyanotic children.

Reperfusion Injury (Postoperative Troponin I Release)
Troponin I values 4 and 12 hours after operation were significantly higher for infants compared with children

### Table 3. Myocardial Concentration of Metabolites Before and After Ischemia in Cyanotic and Acyanotic Children and Infants

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Infants (n = 22)</th>
<th>Children (n = 20)</th>
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<tbody>
<tr>
<td></td>
<td>Cyanotic (n = 9)</td>
<td>Acyanotic (n = 13)</td>
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<tr>
<td></td>
<td>Cyanotic (n = 10)</td>
<td>Acyanotic (n = 10)</td>
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<thead>
<tr>
<th>Metabolite</th>
<th>Infants</th>
<th>Children</th>
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<tbody>
<tr>
<td>ATP</td>
<td>Control</td>
<td>Ischemia</td>
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<tr>
<td>ADP</td>
<td>Control</td>
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<td>AMP</td>
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<td>Glutamate</td>
<td>Control</td>
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<td>Lactate</td>
<td>Control</td>
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Values are nmol/mg of protein.

*P < 0.05 vs corresponding control biopsy; †P < 0.05 vs control biopsy in cyanotic infants; ‡P < 0.05 vs control biopsies in cyanotic children and infants; and §P < 0.05 vs corresponding ischemic biopsy in cyanotic infants for glutamate and vs acyanotic children for aspartate.
The present work shows for the first time that myocardial protection with cold-crystalloid cardioplegia in pediatric open heart surgery is age dependent and cyanosis dependent postoperative troponin I release in acyanotic children ($\bullet$, $n=20$) and acyanotic infants ($\circ$, $n=14$). B, Time-dependent postoperative troponin I release in cyanotic children ($\bullet$, $n=6$) and cyanotic infants ($\circ$, $n=10$). *$P<0.05$ vs troponin I levels at corresponding cross-clamp time in acyanotic children.

($5.5\pm0.6$ versus $3.2\pm0.3$ ng/mL at 4 hours and $4.0\pm0.4$ versus $2.5\pm0.3$ ng/mL at 12 hours after operation; $P<0.05$). Peak troponin I release correlated positively with cross-clamp time in both infants and children (Figure 2B). Total and peak troponin I release in infants but not in children correlated positively with intensive care unit stay, hospital stay, and duration of inotropic support (all $P<0.01$; data not shown).

Time-dependent postoperative release of troponin I was significantly lower in acyanotic children compared with acyanotic infants (Figure 3A) but was similar for both cyanotic infants and children (Figure 3B).

**Discussion**

The present work shows for the first time that myocardial protection with cold-crystalloid cardioplegia in pediatric open heart surgery is dependent on age and cyanosis. This conclusion was reached by use of markers of myocardial ischemic stress, reperfusion injury, and clinical outcome.

**Myocardial Protection in Pediatric Surgery is Age and Cyanosis Dependent**

In the present study, we found that clinical outcome after pediatric open heart surgery is age dependent; children showed more resistance to reperfusion injury than infants. However, cyanotic children had worse outcome and more reperfusion injury compared with acyanotic children. Cyanosis did not influence outcome and injury in infants. The finding that cyanosis predisposes heart to more damage during open heart surgery has been confirmed indirectly in 2 recent studies, although neither addressed the question of age. However, these observations are in contrast to work on chronically hypoxic immature rabbit hearts, which were found to be more tolerant to ischemia compared with normoxic hearts. This difference occurred because hearts from animal models are different from well-compensated hypoxic pediatric human hearts. Underlying mechanisms responsible for hearts of infants and cyanotic children being more susceptible to ischemia and reperfusion injury than hearts of acyanotic children are not readily apparent. These mechanisms may include differences in myocardial calcium handling and in metabolic properties. Calcium handling properties have been used to explain differences between adult and immature animal hearts. Whether these properties also change during development of human heart is presently unknown.

Low myocardial lactate levels and their significant increase during ischemia seem to be the only metabolic change that distinguishes acyanotic children from both infants and cyanotic children. The observation that hearts of infants and cyanotic children do not accumulate lactate during ischemia is not consistent with known changes during myocardial anaerobic metabolism and suggests that anaerobic metabolism in these hearts is different from that of hearts of acyanotic children. Hypothermia has been shown to reduce significantly buildup of lactate during ischemia in newborn pig heart. However, given that all hearts were equally hypothermic in the present study, the difference is due to temperature. One can argue that the increase in lactate concentration in hearts of acyanotic children during ischemia can protect myocardium by lowering intracellular pH. In fact, mild acidification of cardioplegia was found to improve myocardial protection of immature rabbit heart. Intracellular acidosis early after reperfusion can protect myocardium by influencing several pathways implicated in myocardial protection (eg, inhibition of mitochondrial pore). One can also argue that, in contrast to hearts of infants and cyanotic children, hearts of acyanotic children develop the ability to use lactate as a substrate for energy production, particularly given that concentration of ATP at the end of ischemia tended to be higher in acyanotic children. Another possibility as to why lactate may protect the heart could be due to an increase in $K_{ATP}$ channel activity.

In our assessment of children and infants, we avoided the question of pathologies. Pathologies can be different for different age groups, and study would require a large sample size. However, in 2 pathologies with relatively more patients, we found correlation between clinical outcome and cross-clamp time in ventricular septal defect and between clinical outcome and degree of cyanosis in tetralogy of Fallot.

**Clinical Implications for Use of Cold-Crystalloid Cardioplegia**

Current cardioplegic techniques have contributed to decrease in mortality in pediatric patients during open heart surgery. However, as shown in this work and by others use of cold St. Thomas’ Hospital solutions 1 and 2 is still associated with significant reperfusion injury. Experimental studies have
shown that St. Thomas’ Hospital solution 2 provides better protection than solution 1. However, to the best of our knowledge, no clinical evidence has demonstrated superiority of St Thomas’ solution 2 over solution 1. In recent years, many surgeons, particularly in North America, have begun to use cold-blood cardioplegia in pediatric surgery, although little evidence suggests that cold-blood cardioplegia is superior to crystalloid cardioplegia. Limitations of the present study include absence of neonatal patients and patients with acute high degree of cyanosis. The former was due to technical difficulties of obtaining biopsies and the latter because ethical approval was granted to study only patients undergoing elective surgery. Finally, range of cross-clamp time was relatively narrow and in most patients did not exceed 70 minutes.

Summary
In the present study, we present novel data that show that cold-crystalloid cardioplegia in pediatric cardiac surgery is associated with significant ischemic stress and myocardial injury. Reperfusion injury and clinical outcome were dependent on age and cyanosis.

Acknowledgments
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