MYOCARDIAL PROTECTION IN NORMAL AND HYPOXICALLY STRESSED NEONATAL HEARTS: THE SUPERIORITY OF HYPOCALCEMIC VERSUS NORMOCALCEMIC BLOOD CARDIOPLEGIA

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Objectives: The ideal cardioplegic calcium (Ca^{+2}) concentration in newborns continues to be debated. Most studies examining cardioplegia calcium concentrations have been done with a nonclinical model (i.e., isolated heart preparation), the results of which may not be clinically applicable, and they have not examined the effect of calcium concentration in a clinically relevant stressed (hypoxic) heart. **Methods:** Twenty neonatal piglets 5 to 18 days old were placed on cardiopulmonary bypass, and their aortas were crossclamped for 70 minutes with hypocalcemic or normocalcemic multidose blood cardioplegic infusions. Group 1 ($n = 5$; low Ca^{+2} , 0.2 to 0.4 mmol/L) and group 2 ($n = 5$; normal Ca^{+2} , 1.0 to 1.3 mmol/L) were nonhypoxic (uninjured) hearts. Ten other piglets were first ventilated at an FiO_2 of 8% to 10% (O_2 saturation 65% to 70%) for 60 minutes (i.e., causing hypoxia) and then reoxygenated at an FiO_2 of 100% with cardiopulmonary bypass, which produces a clinically relevant stress injury. They then underwent cardioplegic arrest (as described above) with a hypocalcemic ($n = 5$, group 3) or normocalcemic ($n = 5$, group 4) blood cardioplegic solution. Myocardial function was assessed with pressure volume loops and expressed as a percentage of control values. Coronary vascular resistance was measured during each cardioplegic infusion. All values were reported as the mean $\pm$ standard error. **Results:** In nonhypoxic hearts (groups 1 and 2), good myocardial protection was achieved at either concentration of cardioplegia calcium, as demonstrated by preservation of postbypass systolic function (104% vs 99% end-systolic elastance), minimally increased diastolic stiffness (152% vs 162%), no difference in myocardial water (78.9% vs 78.9%), and no change in adenosine triphosphate levels or coronary vascular resistance. Low-calcium blood cardioplegia solution repaired the hypoxic reoxygenation injury in stressed hearts (group 3), resulting in no statistical difference in myocardial function, coronary vascular resistance, or adenosine triphosphate levels compared with nonhypoxic hearts (groups 1 and 2). Conversely, when a normocalcemic cardioplegia solution was used in hypoxic hearts (group 4), there was marked reduction in postbypass systolic function ($49\% \pm 4\%$ end-systolic elastance; $p < 0.05$), increased diastolic stiffness ($276\% \pm 9\%$; $p < 0.05$), increased myocardial water ($80.1\% \pm 0.2\%$; $p < 0.05$), rise in coronary vascular resistance ($p < 0.05$), and lower adenosine triphosphate levels compared with groups 1, 2, and 3. **Conclusions:** This study demonstrates that, in the clinically relevant, intact

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animal model, good myocardial protection is independent of cardioplegia calcium concentration in nonhypoxic (noninjured) hearts; hypoxic (stressed) hearts are extremely sensitive to the cardioplegic calcium concentration; and normocalcemic cardioplegia is detrimental to neonatal myocardium subjected to a preoperative hypoxic stress. (J Thorac Cardiovasc Surg 1996;112:1193-201)

Neonatal myocardial protection remains suboptimal, resulting in an increased operative mortality compared with the results for older children and adults.¹⁻³ In view of the structural, functional, and metabolic differences, extrapolation of adult cardioprotective strategies to the neonate is fundamentally imprudent and potentially harmful.¹⁻⁶ Myocardial protective strategies and cardioplegia solutions must be studied in the infant heart if this problem is to be overcome and the results are to be clinically applicable.

An important consideration regarding myocardial protection is calcium concentration, because high levels have been implicated as a major component in cellular injury during ischemia and reperfusion.^{2,7,8} Although hypocalcemic cardioplegic solutions are superior for protecting adult hearts, the ideal cardioplegia calcium concentration in newborns continue to be debated.^{2,3,8} Most studies examining cardioplegic calcium concentrations in pediatric hearts were performed using an isolated heart preparation, which does not mimic clinical conditions. In addition, these studies examined only normal neonatal hearts, although many newborn hearts are altered by preoperative stresses such as hypoxia. We therefore investigated the appropriate cardioplegic calcium concentration using a model that mimics the clinical conditions of nonhypoxic (noninjured) and hypoxic (stressed) neonatal hearts.

Materials and methods

Twenty neonatal, 5- to 18-day-old piglets (3.5 to 5 kg) were premedicated with 40 mg/kg ketamine hydrochloride intramuscularly and anesthetized with 30 mg/kg pentobarbital intraperitoneally, followed by 5 mg/kg intravenously each hour. The lungs were ventilated through a tracheotomy with a volume ventilator (Servo 900B, Siemens/Elema, Solna, Sweden). All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication no. 80023, revised 1978).

The femoral artery and vein were cannulated to monitor arterial pressure, for blood gas determinations, and for

intravenous infusions. After left thorocotomy, the coronary sinus was cannulated for blood sampling and pressure monitoring of the ligated hemizygous vein. The heart was exposed by means of median sternotomy, and transducer-tip catheters (Millar Instruments Inc., Houston, Tex.) were placed into the left ventricle, thoracic aorta (through the internal mammary), left atrium, and pulmonary artery. The signals were routed to a recorder (model 4586C, Hewlett-Packard, Palo Alto, Calif.) by means of signal conditioners (model 8805C, Hewlett-Packard). An eight-electrode-equipped conductance catheter (Webster Laboratories, Baldwin Park, Calif.) was inserted through the left ventricular apex, and the signals were routed to a Sigma-5 dual-field signal conditioner processor (Leycon, Cardiodynamics, Leiden, Netherlands). After systemic heparin administration (3 mg/kg), a thin-walled venous cannula (18F) and an aortic cannula (8F) were inserted into the right atrial appendage and left subclavian artery, respectively. An 18-gauge cardioplegia needle (DLP, Inc., Grand Rapids, Mich.) was inserted into the aorta for delivery of cardioplegic solutions. Arterial blood gases, electrolytes, and hemoglobin (Blood Gas System 288, Ciba Corning, Medfield, Mass.) were measured every 15 minutes to ensure optimal levels. A heating blanket was placed below the piglet, and a heating pad was placed on the abdomen to maintain a continuously monitored rectal temperature of 37° C to 38° C. All external heat sources were discontinued during systemic cooling.

The cardiopulmonary bypass (CPB) circuit was prepared with heparin, primed with packed red blood cells from donor pigs, and made normocalcemic with CaCl₂. The hematocrit value was adjusted to 25% to 35% with 0.9% normal saline (Baxter Healthcare, Deerfield, Ill.). A Shiley Plexus membrane oxygenator (Shiley Corporation, Irvine, Calif.), was used, and the aortic pressure was maintained between 30 and 50 mm Hg by adjustment of the systemic flow to approximately 100 ml/kg/min.

Cardioplegia protocol. Cardioplegia solutions (CAPS Service, Research Medical Inc., Salt Lake City, Utah) are shown in Tables I and II. The aorta was crossclamped for 70 minutes and cardioplegia delivered by using a protocol of 5 minutes of warm (37° C) followed by 5 minutes of cold (4° C) blood cardioplegic induction, a 2-minute cold (4° C) cardioplegic infusion every 20 minutes, and a 4-minute warm (37° C) cardioplegic reperfusate ("hot shot") before aortic unclamping. Cardioplegia was infused at a continuously measured aortic pressure of 40 to 50 mm Hg. Immediately after crossclamping the aorta, all piglets were cooled to a systemic temperature of 25° C, and rewarming to 37° C was begun 16 minutes before unclamping. CPB was discontinued 30 minutes after aortic unclamping, and final functional and biochemical mea-

Table I. Low-calcium, warm blood cardioplegic solution

Cardioplegic additive	Volume added (ml)	Component modified	Concentration delivered*
KCl (2 meq/ml)	10	K ⁺	8–10 mEq/L
Tham (0.3 mol/L)	225	pH	pH 7.5–7.6
CPD	225	Ca ⁺³	0.2–0.3 mmol/L
Aspartate/glutamate	250	Substrate	13 mmol/L each
D ₅₀ W	40	Glucose	>400 mg/dl
D ₅ W	200	Osmolarity	380–400 mOsm

Tham, Tromethamine; CPD, citrate-phosphate-dextrose; D₅₀W, 50% dextrose in water.

*When mixed in 4:1 ratio with blood. For normocalcemic cardioplegia, CaCl₂ was added to make the ionized Ca⁺², 1.1 to 1.3 μmol/L when mixed in a 4:1 ratio with blood.

surements were made 30 minutes later, after arterial blood gases and the levels of Ca⁺² and K⁺ were normalized.

Physiological measurements. Coronary vascular resistance was determined during each cardioplegic infusion by measuring coronary sinus pressure and cardioplegic flow after a constant infusion rate with an aortic pressure between 40 and 50 mm Hg was achieved. Coronary vascular resistance was calculated with the following formula:

$$\text{CVR (dynes} \cdot \text{sec}^{-1} \cdot \text{cm}^{-5}) = \frac{\text{CIP} - \text{CSP}}{\text{Cardioplegic flow}} \times 80$$

In the equation CIP is cardioplegia infusion pressure, CSP is coronary sinus pressure, and CVR is coronary vascular resistance.

Myocardial performance. Left ventricular (LV) pressure and conductance catheter signals were amplified and digitized to inscribe LV pressure volume loops after first correcting for parallel conductance (myocardial tissue and blood viscosity) with hypertonic saline solution according to the method of Baan.⁹ A series of pressure volume loops was generated under various conditions by transient occlusion of the inferior vena cava during an 8-second apnea. Measurements were made before hypoxia or bypass (i.e., control) and 30 minutes after CPB was discontinued.

The end-systolic pressure-volume relationship, end-diastolic pressure-volume relationship, and the preload recruitable stroke relationship were analyzed with the use of a computer graphics program (Spectrum, Bowman-Gray School of Medicine, N.C.), on a 33 mHz 486 Dell personal computer. LV systolic performance was determined from the descending slope of the end-systolic pressure-volume relationship by linear regression analysis and designated as the end-systolic elastance. End diastolic compliance was determined from the exponential regression of the end-diastolic pressure-volume relationship. Global myocardial performance was assessed by preload recruitable stroke work, which was calculated as the integral of left ventricular transmural pressure and cavity volume over each cardiac cycle. Functional measurements are expressed as percent recovery of baseline values, with each piglet acting as its own control. After final hemodynamic measurements were made, all piglets were placed

Table II. Low-calcium, cold blood cardioplegic solution

Cardioplegic additive	Volume added (ml)	Component modified	Concentration delivered*
KCl (2 mEq/ml)	10	K ⁺	8–10 mEq/L
Tham (0.3 mmol/L)	200	pH	pH 7.6–7.8
CPD	50	Ca ⁺²	0.5–0.6 mmol/L
D ₅ W 1/4 NS	550	Osmolarity	340–360 mOsm

Tham, Tromethamine; CPD, citrate-phosphate-dextrose; D₅₀W, 50% dextrose in water; NS, normal saline.

*When mixed in 4:1 ratio with blood. For normocalcemic cardioplegia, CaCl₂ was added to make the ionized Ca⁺², 1.1 to 1.3 μmol/L when mixed in a 4:1 ratio with blood.

back on bypass and cooled to 25° C. Hearts were then arrested with cold (4° C) blood cardioplegia, and transmural LV biopsy specimens were obtained. Endocardial and epicardial portions were separated, frozen quickly in liquid nitrogen, and stored for biochemical analysis. A separate sample was obtained for myocardial water.

Biochemical analysis. Samples were crushed in a liquid nitrogen-cooled mortar and pestle and lyophilized (Savant Speed Vac Systems, Farmingdale, N.Y.). The adenosine pool was determined according to the method of Sarin and colleagues.¹⁰ Ten to 15 mg of dry tissue was extracted with 0.6 mol/L perchloric acid, the protein-free supernatant neutralized by a mixture of KHCO₃ in 0.5 mol/L Tris buffer (pH 7.5), and a neutralized solution allowed to stand at 4° C to precipitate KClO₄. Aliquots of the neutralized extract were chromatographed with high-performance liquid chromatograph (Waters Inc., Milford, Mass.), equipped with a Waters Nova-Pak column. The adenosine pool was isocratically eluted with a mobile phase consisting of 0.1 mol/L ammonium phosphate (5.0 nmol/L) (tetrabutylammonium hydroxide pH 5.0) and detected at 233 nm. ATP levels were expressed as micrograms per gram of dry tissue.

Myocardial water. Ventricular samples were placed in a preweighed vial and dried to a constant weight at a temperature of 85° C. Myocardial water was calculated with the following formula:

$$\% \text{ Myocardial water} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

Experimental groups

Nonhypoxemic (uninjured) studies. Five minutes after initiating CPB, 10 piglets underwent 70 minutes of cardioplegic arrest by the cardioplegia protocol outlined above. Group 1 (*n* = 5, low calcium) used the hypocalcemic cardioplegic solutions shown in Tables I and II, and in group 2 (*n* = 5, normal calcium) CaCl₂ was added to these solutions to make the cardioplegic ionized calcium concentration normal (1.1 to 1.3 μmol/L) when mixed with blood in a 4:1 ratio.

Hypoxemic (stress) studies. Ten other piglets underwent 60 minutes of ventilator hypoxia by lowering the fraction of inspired oxygen (FiO₂) to 8% to 10%, producing an arterial Po₂ of 25 to 35 mm Hg and an oxygen saturation of 65% to 70%. Before hypoxemia was initiated, piglets

Fig. 1. Left ventricular systolic function as measured by end-systolic elastance and expressed as a percentage of control. There is complete preservation of systolic function independent of cardioplegic calcium concentration in noninjured (nonhypoxic) hearts. In hypoxic hearts low-calcium blood cardioplegia solution allows cellular repair of the hypoxic reoxygenation injury, with complete return of systolic function. In contrast, there is diminished systolic function in hypoxic hearts protected with a normocalcemic (NL) cardioplegia solution. * $p < 0.05$.

were transfused as necessary to increase their hematocrit levels to greater than 35%. This simulates the chronic adaptive change of erythrocytosis that occurs in the cyanotic infant and increases oxygen-carrying capacity, thereby allowing ischemia to be avoided during hypoxia. All piglets remained hemodynamically stable during the entire 60 minutes of ventilator hypoxia. At the end of 60 minutes, all piglets were placed on CPB at an FiO_2 of 100% for 5 minutes to produce a reoxygenation injury. The aorta was cross-clamped, and the heart underwent 70 minutes of cardioplegic arrest with the cardioplegic protocol previously outlined, with a hypocalcemic (group 3, $n = 5$) or normocalcemic (group 4, $n = 5$) cardioplegic solution.

Statistics. Data were analyzed with use of JMP V2.0 (SAS Institute, Inc., Cary, N.C.) on a Macintosh IIVX computer (Apple Inc, Cupertino, Calif.). A paired Student's t test was used for comparison of variables among experimental groups at a probability level of $p < 0.05$. Group data are expressed as the mean $\pm$ standard error of the mean.

Results

There was no difference between groups for control values of LV contractility, diastolic compliance, or global myocardial function. Hypoxia resulted in an increase in heart rate from 130 to 150 to a rate of 190 to 220 beats/min, decreased systemic vascular resistance, increased pulmonary vascular resistance, and did not significantly affect cardiac output. All piglets tolerated 60 minutes of hypoxia with stable hemodynamics.

Hemodynamic and physiologic measurements. Results are depicted in Figs. 1 through 4. There was

Fig. 2. Left ventricular diastolic compliance as measured by the end-diastolic pressure-volume relationship and expressed as a percentage of stiffness compared with control determinations. There is only a minimal increase in diastolic stiffness in noninjured hearts independent of cardioplegia calcium concentration. In hypoxic hearts hypocalcemic blood cardioplegia solution allows cellular repair of the hypoxic reoxygenation injury, resulting in only a mild increase in diastolic stiffness, which is not statistically different from noninjured hearts. Conversely, there was a marked increase in diastolic stiffness in hypoxic hearts protected with a normocalcemic (NL) cardioplegic solution. * $p < 0.05$.

no change or difference in the x intercept (V_0) for end-systolic elastance or preload recruitable stroke work between before (control) and after bypass values for any experimental group. Therefore, end-systolic elastance, preload recruitable stroke work, and diastolic compliance are expressed as changes in slope compared with control measurements. The nonhypoxic hearts (group 1 and 2) had complete preservation of myocardial function with either concentration of cardioplegic calcium (see Figs. 1 through 3). Hypoxia followed by reoxygenation produces an injury that results in myocardial depression.^{11,12} A hypocalcemic cardioplegic solution facilitated cellular repair of the hypoxic reoxygenation injury (group 3), resulting in complete preservation of normal postbypass myocardial function (see Figs. 1 through 3). Conversely, when a normocalcemic cardioplegic solution was used in hypoxic hearts (group 4), there was marked reduction in systolic, diastolic, and global myocardial function (see Figs. 1 through 3), implying cellular damage. Similarly, there were no significant differences in CVR among groups 1, 2, and 3 (see Fig. 4) during cardioplegic infusions. In contrast, there was a marked increase in CVR with each cardioplegic infusion in hypoxic hearts protected with a normocalcemic cardioplegic solution (group 4). Because these hearts underwent

the same hypoxic stress as group 3, this finding implies a vascular injury.

Tissue studies. Results are summarized in Table III. Although the levels were slightly lower than laboratory control values, there was no difference in endocardial ATP levels among groups 1, 2, and 3. Endocardial ATP levels were the lowest in hypoxic hearts protected with a normocalcemic solution (group 4). The ATP/ADP ratio, which reflects the ability of mitochondria to phosphorylate ADP to ATP, was markedly reduced in hypoxic hearts protected with normocalcemic cardioplegia (group 4), suggesting mitochondrial damage. Group 4 also had the highest percentage of myocardial water, indicating the greatest degree of cellular injury of any group.

Discussion

This study demonstrates that, in a clinically relevant intact animal model simulating conditions in the operating room, good myocardial protection is independent of the cardioplegic calcium concentration in nonhypoxic hearts; hypoxic neonatal hearts are more sensitive to cardioplegic calcium concentration; normocalcemic cardioplegia solutions are detrimental to hearts subjected to preischemic hypoxia; and the optimal method of myocardial protection of hypoxic hearts is achieved with a hypocalcemic cardioplegia solution.

Intracellular accumulation of calcium during ischemia and reperfusion has been associated with cellular injury.^{2, 7, 8, 13, 14} Several mechanisms of injury have been proposed, which include increased ATP use through activation of calcium-dependent ATPases; impaired ATP synthesis because of mitochondrial calcium uptake; activation of numerous calcium-dependent degradative enzymes such as proteases, lipases, and phospholipases; and enhancement by calcium of various free radical mediated reactions.^{13, 14} The neonatal heart may be more susceptible to a calcium-mediated cellular injury because the sarcoplasmic reticulum of the immature myocardium possesses a diminished capacity to sequester calcium and because of different characteristics of the calcium transport system.^{2, 14}

Although many studies have demonstrated the beneficial effects of hypocalcemic cardioplegia solutions in protecting the adult myocardium, the optimal cardioplegic calcium concentration in neonatal hearts continues to be debated.^{2, 3, 8} The groups of Caspi¹⁵ and Kofsky¹⁶ demonstrated better recovery of postischemic myocardial function with hypocalce-

Fig. 3. Overall left ventricular myocardial function as measured by preload recruitable stroke work and expressed as a percentage of control. There is complete preservation of global myocardial function independent of cardioplegia calcium concentration in noninjured (nonhypoxic) hearts. In hypoxic hearts hypocalcemic cardioplegia solution allowed cellular repair of the hypoxic reoxygenation injury, with complete preservation of global myocardial function. In contrast, global myocardial function is depressed in hypoxic hearts protected with a normocalcemic (NL) cardioplegia solution, indicating cellular damage. * $p < 0.05$.

mic cardioplegic solutions, but those of Pearl¹⁷ and Corno¹⁸ found better recovery with normocalcemic solutions. These seemingly conflicting results may reflect several factors. Most neonatal studies have been conducted with an isolated heart model. Although this allows precise experimental control, it does not mimic the clinical conditions in the operating room. For instance, bronchial blood return and noncoronary collateral flow are absent in the isolated heart preparation, but they may have a profound effect in the *in vivo* model.⁷ Cardioplegic magnesium concentrations may also be responsible for differences, because magnesium competes with calcium at entry points on the cell membrane.^{2, 8} The optimal calcium concentration determined for a solution containing magnesium may be different if magnesium is omitted.² Despite the prevalence in the pediatric population of preoperative stresses such as hypoxia, almost no previous studies have included stressed hearts in their investigations. However, hypoxia may profoundly alter the neonatal myocardium, influencing the effects of cardioplegic solutions.^{2, 3, 11, 12} We therefore examined different cardioplegic calcium concentrations in neonatal hearts using a model that mimics conditions of the operating room, without added magnesium, and in noninjured and hypoxic hearts.

Fig. 4. Coronary vascular resistance (CVR) measured during each cardioplegic infusion. Cardioplegia infusions 1 and 4 were during warm (37°C) induction and reperfusion, respectively, and 2 and 3 were during cold (4°C) multidose infusions. There is no difference in CVR in noninjured (nonhypoxic) hearts independent of cardioplegia calcium concentration, indicating that both solutions preserve vascular endothelial cell function. In hypoxic hearts, a hypocalcemic blood cardioplegia solution resulted in CVRs that were similar to nonhypoxic hearts, indicating avoidance of a vascular injury. Conversely, there was a marked rise in CVR when a normocalcemic (NL) cardioplegic solution was infused in hypoxic hearts, indicating that high calcium caused a vascular injury. $*p < 0.05$.

Table III. Endocardial tissue results

Group	ATP ($\mu\text{g/gm tissue}$)	ATP/ADP ratio	Myocardial water (%)
Laboratory control	19.1 ± 0.6	4.5 ± 0.2	77.3 ± 0.3
NL heart/low Ca^{+2} (group 1)	15.7 ± 0.4	4.3 ± 0.3	78.9 ± 0.4
NL heart/NL Ca^{+2} (group 2)	15.4 ± 1.9	4.2 ± 0.5	78.9 ± 0.1
Hypoxic heart/low Ca^{+2} (group 3)	15.2 ± 1.1	4.1 ± 0.2	79.6 ± 0.6
Hypoxic heart/NL Ca^{+2} (group 4)	$12.2 \pm 0.9^*$	$2.3 \pm 0.2^{\dagger}$	$80.1 \pm 0.2^{\ddagger}$

NL heart, Normal (noninjured) heart; NL Ca^{+2} , normocalcemic cardioplegia.

* $p < 0.05$, group 4 compared with group 1.

$^{\dagger}p < 0.05$, group 4 compared with group 1, 2, or 3.

$^{\ddagger}p < 0.05$ group 4 compared with group 1 or 2.

In nonhypoxic hearts, there was complete preservation of myocardial function with either cardioplegic calcium concentration (see Figs. 1 through 3). There was also no change in CVR (see Fig. 4), myocardial edema, or ATP levels, indicating that both levels of cardioplegic calcium provide similar protection. However, the ischemic (crossclamp) time was relatively short in this study, and the neonatal myocardium is more tolerant of ischemia than that of the adult.^{2,3} Because the reperfusion injury depends on the duration of ischemia, these factors may have accounted for the lack of difference between the two groups. Moreover, congenital lesions seen in clinical practice usually result in hypoxia or a pressure-volume overload, and there-

fore "normal" hearts are probably uncommon in the neonatal population.³

Increasing numbers of infants with cyanotic congenital heart disease are undergoing primary repair. Although this may be preferable to palliation, it subjects the immature hypoxic heart to CPB and high levels of oxygen, which can cause an unintended reoxygenation injury.^{11, 12, 19, 20} This injury is mediated by oxygen free radicals, results in myocardial depression, and may explain why impairment of ventricular function is common after apparently satisfactory surgical correction of cyanotic congenital defects.^{1, 3, 21, 22} Despite the prevalence of this condition, no studies have investigated the effects of cardioplegic calcium concentrations in this clinically

relevant hypoxic model. Including stressed hearts in any investigation of cardioplegia solutions is important because the results may be dramatically altered. This is why adult studies imposed a preischemic stress when examining cardioplegia solutions.⁷ Because pediatric hearts rarely undergo preoperative ischemia, the injury must be changed to reflect a clinically relevant stress, such as hypoxia.

Subjecting the neonatal heart to hypoxia profoundly altered the effect different cardioplegia calcium concentrations had on the myocardium. Hypocalcemic cardioplegic solutions protected the heart from further damage, and they facilitated repair of the injury caused by hypoxia and reoxygenation; resulting in complete preservation of myocardial and vascular endothelial cell function (see Figs. 1 through 4). Conversely, hypoxia followed by reoxygenation altered the myocardium, resulting in an increased cellular injury when normocalcemic solutions were used. This injury was manifested by depression in postbypass myocardial and endothelial cell function. These findings are not surprising, because a similar phenomenon occurred when adult hearts were subjected to ischemic stress.⁷ Although our model of acute hypoxia does not allow for the chronic adaptive changes that may occur in cyanotic newborns, several studies have documented a similar oxygen-mediated injury with reoxygenation of the chronically hypoxic infant.^{3, 19, 20} In addition, this sensitivity to cardioplegic solutions after acute hypoxia parallels the findings in cyanotic infants and chronically hypoxic animals,^{1, 2, 23-25} leading us to believe these experimental findings are clinically relevant.

Vascular endothelial cell function was determined by measuring CVR during each cardioplegic infusion when the heart was arrested and the flow and infusion pressure were constant. Although the myocardial metabolic demands and CVR may change between warm and cold cardioplegia, they should be identical for each heart at any given temperature. Because our experimental model of hypoxia does not result in ischemia, restoration of normoxic perfusion (reoxygenation) should also result in similar CVR in both groups of hypoxic hearts. In contrast, there was a marked increase in CVR during each cardioplegic infusion in hypoxic hearts protected with a normocalcemic solution (group 4). The only other reason for this increased CVR would be vascular compression from increased myocardial water. However, because edema was the same in both hypoxic groups, the increased CVR during

cardioplegic infusions appears to reflect a derangement in vascular function secondary to the higher cardioplegic calcium concentration. This increased CVR may have resulted in a less homogeneous cardioplegia distribution, compromising myocardial protection during the 70 minutes of myocardial arrest. We did not measure vascular function after discontinuing the bypass. However, because myocardial function was still depressed after bypass in hypoxic hearts protected with a normocalcemic solution, it is likely that vascular function was similarly affected.

Calcium homeostasis is maintained by several ATP-dependent mechanisms. Unlike ischemia, our model of acute experimental hypoxia with or without reoxygenation does not significantly deplete ATP levels (unpublished data). The increased susceptibility of hypoxic hearts to high cardioplegic calcium therefore must depend on a mechanism other than depleted ATP. After cardioplegic arrest, the endocardial ATP levels were lowest in hypoxic hearts receiving normocalcemic cardioplegic solution (group 4). However, the ATP levels were not statistically different from hypoxic hearts receiving low-calcium cardioplegic solution, despite decreased ventricular function in the normocalcemic group. Conversely, when the ATP/ADP ratio was examined, it was found to be markedly reduced in group 4. This seems to indicate that there is some calcium-mediated mitochondrial damage resulting in a decreased ability to convert ADP to ATP. However, this cannot be proved with our data, because we did not directly examine mitochondrial function.

During ischemia, an elevated calcium level accelerates the breakdown of ATP by activation of calcium-dependent ATPases. This energy depletion may further impair ATP-dependent mechanisms that maintain calcium homeostasis. Our experimental model of hypoxia does not cause significant depletion of nucleotide stores, but chronically cyanotic hearts are predisposed to accelerated depletion of these high-energy compounds during the periods of increased myocardial oxygenation demand associated with exercise or ischemia induced during operative repair.^{3, 26, 27} Therefore the effects of a normocalcemic cardioplegia solution may be even more pronounced in the cyanotic infant.

This study, conducted with a clinically relevant model, demonstrates that high and low cardioplegic calcium concentrations provide good myocardial protection in nonhypoxic hearts subjected to short

ischemic intervals. Conversely, when the heart is subjected to a preoperative hypoxic stress, cardioplegic calcium concentration takes on increased importance, with hypocalcemic solutions providing markedly superior myocardial protection. This study suggests that, because cyanotic hearts are often depressed after apparently successful surgical repair, hypocalcemic cardioplegic solutions should always be used in cyanotic infants.

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Discussion

Dr. John H. Kennedy (*Cambridge, England*). I enjoyed your presentation very much. It is a sophisticated study and well worked out.

The leaching of calcium from the presumably normo-

calcemic heart is biphasic. The first rapid clearance is from the extracellular space, and the slower phase is the cytosol and later the mitochondrial portions. Can you tell us what the pH was, because that phenomenon is pH dependent and theoretically could explain the difference in the two groups, unless they were both the same pH.

Dr. Bolling. Because the cardioplegia in both groups had the same pH, it could not have accounted for the differences. In addition, the calcium movements you have described occur with active myocardial contraction, but hearts in this study were exposed to different calcium concentrations only during cardioplegic arrest.

Dr. Jakob Vinten-Johansen (*Winston-Salem, N.C.*). What is the mechanism of postischemic dysfunction in the hypoxic hearts? Did you take any other measurements of injury—creatine kinase activity, for example? You concluded that the increase in postischemic vascular resistance in the hypoxic normocalcemic cardioplegia group resulted from endothelial dysfunction. Did

you measure endothelial function in vivo or in vitro to confirm this?

Dr. Bolling. Calcium is detrimental to ischemic cells, because it activates proteases and increases ATP use. We believe that high-calcium cardioplegia damages the myocardium by both of these mechanisms. Several investigators have also demonstrated that hypoxic hearts are more sensitive to ischemic injury and to ATP depletion, making them more vulnerable to calcium. Besides the physiologic measurements, we did examine tissue ATP levels and myocardial edema, and both findings support the presence of increased cellular injury in the high-calcium cardioplegia group. With regard to the changes in vascular resistance, we did not examine the coronary artery in vitro. Therefore, we can only say that there was a vascular injury but cannot be sure of the site of this injury. Although we believe that the injury involves the vascular endothelial cell, this cannot be proved with this study's data.