STUDIES OF HYPOXEMIC/ REOXYGENATION INJURY: WITHOUT AORTIC CLAMPING

II. Evidence for reoxygenation damage

This study tested the hypothesis that the developing heart is susceptible to oxygen-mediated damage after reintroduction of molecular oxygen and that this "unintended" reoxygenation injury causes lipid peroxidation and functional depression that may contribute to perioperative cardiac dysfunction. Among 49 Duroc-Yorkshire piglets (2 to 3 weeks old, 3 to 5 kg) 15 control studies were done without hypoxemia to test the effects of the surgical preparation ($n = 10$) and 60 minutes of cardiopulmonary bypass ($n = 5$). Twenty-nine piglets underwent up to 2 hours of ventilator hypoxemia (with inspired oxygen fraction reduced to 6% to 7%) to lower arterial oxygen tension to approximately 25 mm Hg. Five piglets did not undergo reoxygenation to determine alterations caused by hypoxemia alone. Twenty-four others received reoxygenation by either raising ventilator inspired oxygen fraction to 1.0 ($n = 12$) or instituting cardiopulmonary bypass at oxygen tension 400 mm Hg ($n = 12$). Ventilator hypoxemia produced sufficient hemodynamic compromise and metabolic acidosis that 18 piglets required premature reoxygenation (78 ± 12 minutes). To avoid the influence of acidosis and hemodynamic deterioration during ventilator hypoxemia, five others underwent 30 minutes of hypoxemia during cardiopulmonary bypass (circuit primed with blood at oxygen tension 25 mm Hg) and 30 minutes of reoxygenation (oxygen tension 400 mm Hg) during cardiopulmonary bypass. Biochemical markers of oxidant damage included measurement of coronary sinus and myocardial conjugated dienes to determine lipid peroxidation and antioxidant reserve capacity assessed by incubating myocardial tissue in the oxidant *t*-butylhydroperoxide. Functional recovery was determined by inscribing pressure volume loops to determine end-systolic elastance and Starling curves by volume infusion. No biochemical or functional changes occurred in control piglets. Hypoxemia without reoxygenation did not change plasma levels of conjugated dienes, but lowered antioxidant reserve capacity 24%. Reoxygenation by ventilator caused refractory ventricular arrhythmias in two piglets (17% mortality), raised levels of conjugated dienes 45%, and reduced antioxidant reserve capacity 40% with recovery of 39% of mechanical function in the survivors. Comparable biochemical and functional changes occurred in piglets undergoing ventilator hypoxemia and/or cardiopulmonary bypass hypoxemia and reoxygenation on cardiopulmonary bypass. We conclude that hypoxemia increases vulnerability to reoxygenation damage by reducing antioxidant reserve capacity and that reoxygenation by either ventilator or cardiopulmonary bypass produces oxidant damage with resultant functional depression that is not a result of cardiopulmonary bypass. These findings suggest that initiation of cardiopulmonary bypass in cyanotic immature subjects causes an unintended reoxygenation injury, which may increase vulnerability to subsequent ischemia during surgical repair. (*J THORAC CARDIOVASC SURG* 1995;110:1171-81)

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Perioperative myocardial depression is the principal cause of morbidity and mortality after repair of defects causing cyanosis¹ despite the greater tolerance of the immature heart to ischemia.²⁻⁴ Biochemical and functional damage during repair of these defects is also more severe than that after correction of acquired defects (i.e., coronary artery bypass grafting) in patients with normoxic conditions in whom longer ischemic intervals are required and who are subjected to comparable conditions of cardiopulmonary bypass (CPB) and cardioprotective techniques.⁵ The reintroduction of molecular oxygen when CPB is started, before ischemia, is a feature that distinguishes operations in the aforementioned infants with cyanotic conditions from the normoxic adult operations.

Experimental and clinical data show that reoxygenation, per se, may produce biochemical and functional changes that are comparable to alterations that follow reperfusion after ischemia,⁶⁻⁹ so that the possibility exists that an unintended reoxygenation injury is produced when CPB is initiated in cyanotic immature subjects that may increase the vulnerability to subsequent ischemia required for surgical correction of congenital defects. This inference is supported by clinical observations that cardiac dysfunction after reoxygenation develops in hypoxemic infants with respiratory distress syndrome after they are placed on extracorporeal membrane oxygenation support, and myocardial depression occurs without superimposed surgical ischemia.^{10, 11}

This study was designed to test the hypothesis that (1) hypoxemia per se reduces myocardial antioxidant reserve capacity of the immature heart and thereby increases susceptibility to oxidant damage, (2) reintroduction of molecular oxygen further impairs antioxidant reserve capacity and triggers lipid peroxidation and subsequent myocardial depression, and (3) this unintended injury occurs when reoxygenation is by ventilator or by extracorporeal circulation and therefore is independent of the effects of CPB per se.

Material and methods

Experimental model. Forty-nine immature, 2- to 3-week-old Duroc-Yorkshire piglets (3 to 5 kg) were premedicated with 0.5 mg/kg diazepam intramuscularly, anesthetized with 30 mg/kg pentobarbital intraperitoneally followed by 5 mg/kg intravenously each hour, and the lungs ventilated on a volume-limited respirator (Servo 900D, Siemens-Elema, Solna, Sweden) via a tracheostomy. 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. 86-23, revised 1985).

The ductus arteriosus was occluded with a surgical clip via a left thoracotomy and the coronary sinus was cannulated for blood sampling via the ligated hemiazygos vein. The heart was exposed by median sternotomy, and transducer-tipped catheters (Millar Instruments, Inc., Houston, Tex.) were placed into the left ventricle (LV), thoracic aorta, left atrium, and right atrium and pulmonary artery. The signals were routed to a recorder (MT 9000, Astro-Med Inc., West Warwick, R.I.) via signal conditioners (Model 13-4615, Gould Inc., Ohio). A thermodilution probe (No. 4) was directed into the main pulmonary artery and connected to a cardiac output computer (Model 9520A, American Edwards Laboratory, Santa Ana, Calif.). An eight-electrode-equipped conductance catheter (Webster Laboratories, Baldwin Park, Calif.) was inserted through the LV apex and connected to a Sigma-5-DF signal conditioner processor (Leycom, Oegstgeest, The Netherlands).

After systemic heparinization (3 mg/kg), a thin-walled venous cannula (20F) and an aortic cannula (8F) were inserted into the right atrial appendage and the left subclavian artery. Arterial levels of blood gases, electrolytes, and hemoglobin (Blood Gas System 228, Ciba-Corning, Medfield, Mass.) were measured to ensure optimum extracorporeal circulation, and a heating pad was placed to keep rectal temperature at 38° C. The CPB circuit was heparinized, primed with packed red cells from donor pigs, and made normocalcemic with CaCl₂. Hematocrit value was adjusted to 25% to 30% with hetastarch (Hespan, Dupont, Wilmington, Del.) and plasma-electrolyte solution (Baxter Healthcare, Deerfield, Ill.). A Sarns 16310 membrane oxygenator (Sarns, Ann Arbor, Mich.) was used and aortic pressure kept at 50 to 60 mm Hg by adjustment of systemic flow to approximately 100 ml/min/kg. Piglets were observed for 30 minutes after CPB, while arterial blood gas and electrolyte levels were restored to normal. Final measurements of biochemical variables and contractile function were made 30 minutes after CPB. Hearts were then arrested with cold (4° C) blood cardioplegia (KCl 30 mEq/L) to stop metabolism and a LV biopsy sample (approximately 200 mg) obtained transmurally. The subendocardial portion was separated, frozen quickly in liquid nitrogen, and stored for biochemical analyses.

Physiologic and biochemical determinations. Hemodynamic measurements were made before hypoxia was started (control), every 15 minutes during hypoxia, and 15 and 30 minutes after CPB was discontinued. Cardiac output was determined by duplicate injections of 1 ml of 4° C cold saline solution into a central venous catheter. Cardiac index (CI), systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), and LV stroke work index (LVSWI) were calculated by the following equations:

CI (ml/min/kg) = CO/body weight (kg)

SVRI (mm Hg · min · L⁻¹ · kg)

$$= (\text{MAP} - \text{CVP})(\text{mm Hg}) \cdot \text{CO}^{-1} (\text{ml/min}) \cdot \text{BW} (\text{kg})$$

PVRI (mm Hg · min · L⁻¹ · kg)

$$= (\text{PAP} - \text{LAP})(\text{mm Hg}) \cdot \text{CO}^{-1} (\text{ml/min}) \cdot \text{BW} (\text{kg})$$

and

$$\text{LVSWI} (\text{g} \cdot \text{m/kg}) = (\text{MAP} - \text{LAP}) \times \text{CO} (\text{ml/min})$$

$$\times 0.0136/(\text{HR} \times \text{body weight} [\text{kg}])$$

where MAP is mean aortic pressure, PAP is mean pulmonary artery pressure, LAP is mean left atrial pressure, CVP is central venous pressure, CO is cardiac output in milliliters per minute, and HR is heart rate.

Myocardial performance. LV pressure and conductance catheter signals were amplified and digitalized to inscribe LV pressure volume loops after correction for tissue conductance.¹² A series of pressure volume loops was generated under varying loading conditions by transient occlusion of the inferior vena cava during a 7-second period of apnea. Measurements were made before hypoxia (control) and 30 minutes after CPB was discontinued.

The end-systolic pressure volume relationship was analyzed with use of a computer graphics program (Spectrum, Bowman Gray School of Medicine, Winston-Salem, N.C.) on a 386/33 mHz IBM PC (IBM, Armonk, N.Y.), and LV performance was determined from the descending slope using linear regression and designated end-systolic elastance as described previously.¹³ Postbypass LV contractility was expressed as percent recovery of prebypass control values. Myocardial function before and after CPB was also evaluated using Starling curves by infusion of CPB blood into the inferior vena cava at 5 ml/min/kg while cardiac output, MAP, and LAP were recorded.

Myocardial oxidant injury

Conjugated dienes. As a marker of oxidant-mediated lipid peroxidation, levels of conjugated dienes were determined in the myocardial tissue and coronary sinus plasma.

CORONARY SINUS PLASMA CONCENTRATION. An aliquot of coronary sinus blood was taken before hypoxia, at 5, 30, 35, and 60 minutes of CPB, and 30 minutes after CPB was discontinued. The samples were placed immediately into ice, centrifuged for 5 minutes at 1000 g, and stored in liquid nitrogen for later measurements. Conjugated diene levels were determined spectrophotometrically after chloroform methanol 2:1 vol/vol extraction as described by Lesnfsky and coworkers,¹⁴ and conjugated diene concentration was expressed as absorbance (A) at a wavelength of 233 nm per 0.5 ml plasma.

MYOCARDIAL TISSUE. LV endocardial biopsy specimens were immediately frozen and stored in liquid nitrogen. Tissue level of conjugated dienes was determined as previously described and expressed as absorbance at a wavelength of 233 nm per milligram of lipid. Total myocardial lipid was measured by the spectrophotometric method of Chiang, Gessert, and Lowry.¹⁵

Antioxidant reserve capacity. The myocardial antioxidant state was assessed by determining in vitro lipid peroxidation in cardiac tissue that was homogenized and incubated with *t*-butylhydroperoxide at concentrations varying from 0 to 5 mmol/L for 30 minutes at 37°C.¹⁶ Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances spectrophotometrically at 532 nm. A standard curve is run simultaneously and lipid peroxidation is expressed as nanometers malondialdehyde per gram of protein. Protein was measured by the method of Lowry and associates.¹⁷

Experimental groups

Control

INSTRUMENTATION ONLY. Ten piglets were instrumented as previously described herein and observed for 5 hours to determine the effects of the open-chest preparation without CPB.

CPB WITHOUT HYPOXEMIA. Five normoxic piglets underwent CPB (oxygen tension [Po₂] about 400 mm Hg) for 1 hour without preceding hypoxemia to distinguish the effects of CPB without reoxygenation.

Experimental

VENTILATOR HYPOXEMIA. In 29 piglets, hypoxemia was imposed by lowering the inspired oxygen fraction (Fio₂) in the ventilator to 6% to 7%, producing an arterial Po₂ of about 25 mm Hg. Hypoxemia was maintained for up to 120 minutes. Five piglets did not undergo reoxygenation (see following section). In 24 piglets, reoxygenation was by either increasing ventilator Fio₂ to 100% (1.0) or initiating CPB at Po₂ 400 mm Hg to maintain MAP at 50 mm Hg (flow rate averaging 117 ± 5 ml/min). Reoxygenation was initiated before 120 min if (1) mean arterial pressure fell below 30 mm Hg and could not be raised by volume infusion or (2) three bolus injections of NaHCO₃ did not restore pH to greater than 7.3.

HYPOXEMIA WITHOUT REOXYGENATION. Five piglets underwent up to 120 minutes of ventilator hypoxemia without subsequent reoxygenation to determine the effects of hypoxemia on the antioxidant reserve capacity.

HYPOXEMIA WITH REOXYGENATION

1. Ventilator hypoxemia and reoxygenation: Twelve piglets underwent a period of ventilator hypoxemia for up to 120 minutes, followed by reoxygenation by ventilator and a subsequent 60-minute observation period.

2. Ventilator hypoxemia and CPB reoxygenation: Twelve piglets that underwent ventilator hypoxemia underwent reoxygenation by CPB for 60 minutes.

3. CPB hypoxemia and reoxygenation: Five other piglets were placed on Po₂ 400 mm Hg CPB and made hypoxemic for 30 minutes by addition of N₂ to the gas mixture of the extracorporeal circuit to lower Po₂ to approximately 25 mm Hg. Reoxygenation was done by raising Po₂ to about 400 mm Hg for 30 minutes.

Function after CPB was assessed 30 minutes after extracorporeal circulation was discontinued in all piglets reoxygenated by CPB.

Statistics. Data were analyzed with use of the Stat View V2.0 program (Abacus Concepts Inc., Berkeley, Calif.) on a Macintosh IICI computer (Apple, Inc., Cupertino, Calif.). Analysis of variance was used for intergroup comparison and the paired Student's *t* test used for comparison of variables within experimental groups. Final

Fig. 1. LV performance evaluated by volume infusion before hypoxemia (control) and after hypoxia/reoxygenation (ReO_2) in piglets subjected to ventilator hypoxemia and reoxygenation by CPB (see text for description). *LVSWI*, Left ventricular stroke work index.

Fig. 2. LV pressure volume loops, inscribed by recording from conductance catheter before and after CPB in nonhypoxemic piglets subject to CPB without hypoxemia (see text for description). *m*, Slope of end-systolic pressure volume relation; *n*, number of loops; *r*, correlation of coefficient.

statistical analyses were applied similarly to all piglets undergoing ventilator hypoxemia and reoxygenation, because the incidence of reoxygenation before 120 minutes (caused by hemodynamic deterioration) was similar in piglets reoxygenated by ventilator or by CPB, as described in the hemodynamic results section. Differences were considered significant at the probability level of $p < 0.05$. Group data were expressed as mean plus or minus standard error of the mean.

Results

Hemodynamic results. Hypoxemia produced tachycardia (heart rate was increased from 170 ± 10 to 206 ± 15 beats/min), raised cardiac index $22\% \pm 4\%$, lowered systemic vascular resistance index $23\% \pm 5\%$, and caused pulmonary vasoconstriction (pulmonary vascular resistance index rose $70\% \pm 23\%$ during the first hour). All hypoxemic piglets subsequently had peripheral acidosis (requiring an average of 2.5 bolus injections of $NaHCO_3$). Hemodynamic instability developed after the first hour, and premature reoxygenation was required in 18 of 29 piglets after an average of 78 ± 12 minutes of ventilator hypoxemia. The duration of hypoxemia was similar in all experimental groups undergoing ventilator hypoxemia.

Fig. 3. Percent recovery of LV end-systolic elastance in piglets subjected to CPB without hypoxemia (*CPB control*) and those subjected to ventilator hypoxemia and reoxygenation by ventilator (*ventilator*), ventilator hypoxemia and CPB reoxygenation (*CPB*), or entire hypoxemia/reoxygenation process by extracorporeal circulation (*CPB Hypoxia*) (see text for description). *LV*, Left ventricular.

Two of 12 piglets reoxygenated by ventilator died (17% mortality) because hemodynamic deterioration progressed and ventricular tachyarrhythmias occurred despite raising F_{iO_2} to 1.0, thereby increasing arterial P_{O_2} to greater than 300 mm Hg. Conversely, all piglets reoxygenated on CPB survived; functional and biochemical results are shown in Figs. 1 through 7. Postreoxygenation cardiac output could always be raised to near normal levels by volume infusion but higher LAP was needed to reach prehypoxemic baseline levels compared with stroke work index levels in control piglets that did not undergo hypoxemia (Fig. 1). Furthermore, augmentation higher than the baseline stroke work index before CPB could not be achieved by volume infusion beyond a LAP of 10 to 15 mm Hg in reoxygenated piglets, whereas stroke work index rose more than 200% when LAP was increased to 10 mm Hg in control piglets.

LV contractility. In control studies, LV end-systolic elastance (Figs. 2 and 3) was unaffected either by instrumentation ($103\% \pm 3\%$ recovery) or CPB without preceding hypoxemia ($97\% \pm 4\%$ recov-

ery). Conversely, postreoxygenation end-systolic elastance was reduced markedly and comparably in all hypoxemic piglets, including those that underwent the entire hypoxemic/reoxygenation process by CPB. Cardiac dysfunction after CPB was similar (33% versus 39% recovery, $p < 0.05$ vs control) in piglets subjected to ventilator hypoxemia that required premature reoxygenation because of hemodynamic deterioration and in those that tolerated the 2 hours of hypoxemic stress.

Biochemistry results

Conjugated dienes

CORONARY SINUS. No conjugated diene efflux occurred in coronary sinus blood during the observation period in control piglets or during the hypoxemic interval in piglets that underwent CPB hypoxemia (Fig. 4). In contrast, reoxygenation by CPB raised coronary sinus conjugated diene levels 77% higher than end-hypoxemic levels ($p < 0.05$) and levels remained elevated 30 minutes after CPB was discontinued (Fig. 4).

MYOCARDIAL. Cardiac tissue levels of conjugated dienes at termination of the study were unchanged

Conjugated Dienes (Coronary Sinus Blood)

* = $p < 0.05$ vs. CPB Control

Fig. 4. Coronary sinus levels of conjugated dienes during control period, during hypoxemia, and during reoxygenation (ReO_2) by CPB (see text for description). A, Absorbance.

by instrumentation only, 1 hour of CPB without hypoxemia, or hypoxemia without reoxygenation and were normal in piglets that underwent 30 minutes of hypoxemia and reoxygenation during CPB. Conversely, postreoxygenation levels rose 45% and 56%, respectively ($p < 0.05$) in piglets that underwent more prolonged ventilator hypoxemia with subsequent reoxygenation by either ventilator or CPB (Fig. 5).

Antioxidant reserve capacity. Endogenous antioxidant reserve capacity was unchanged by either instrumentation or CPB without hypoxemia, but was reduced 24% by hypoxemia alone (Fig. 6). Reoxygenation by ventilator and by CPB lowered antioxidant reserve capacity 40% and 61%, respectively, in piglets subjected to ventilator hypoxemia and by 44% in piglets undergoing hypoxemia and reoxygenation during CPB (Fig. 7) ($p < 0.05$).

Critique of experimental models. The advantages of an intact animal model have been enumerated

previously (unpublished data). The 2- to 3-week-old piglet approximates the physical conditions of a human neonate,¹⁹ but differences between it and the newborn infant must be evaluated subsequently.²⁰ Its size allows institution of CPB as it occurs in infants with cyanotic conditions. An important shortcoming of the short-term model is the absence of the compensatory adaptive mechanisms to chronic cyanosis, yet the postreoxygenation changes parallel those reported in patients with cyanotic conditions who undergo reoxygenation during bypass.^{9, 21, 22}

The ventilator hypoxemia model unfortunately simulates acute asphyxia and causes hemodynamic deterioration often necessitating premature reoxygenation. Consequently, the possible confounding effect of a reperfusion injury is imposed, and a standard hypoxemic interval under comparable hemodynamic conditions does not occur when molecular oxygen is reintroduced. These limitations not-

Fig. 5. Cardiac tissue levels of conjugated dienes (CD) in LV subendocardial muscle, when samples were obtained at completion of experiment (see text for description). *A*, Absorbance.

withstanding, recovery was depressed similarly in piglets requiring premature reoxygenation and those tolerating the entire 2-hour hypoxemic interval.

CPB introduces the clinically relevant variables of complement activation,²³ neutrophil activation, leukostasis,²⁴ and generation of reactive oxygen intermediates,²³ and the effects of CPB on the immature heart are unknown. Although the brief interval of CPB in control studies did not impair functional recovery or cause oxidant damage, longer periods of CPB and observation after CPB may expose CPB-related changes. Extracorporeal circulation may have been beneficial because 2 of 12 piglets reoxygenated by ventilator died of progressive hemodynamic deterioration and reoxygenation arrhythmias, whereas circulatory support provided by CPB allowed 100% survival.

Concern over the inconsistency of the hypoxemic interval and hemodynamic status before reoxygenation led us to develop the model of hypoxemia and reoxygenation during CPB, but this model also has

no clinical counterpart. Despite this, the reoxygenation hemodynamic and biochemical impairment paralleled changes after ventilator hypoxemia. We interpret these data to corroborate the occurrence of reoxygenation damage, independent of the mode of producing hypoxemia and instituting reoxygenation.

Discussion

These in vivo studies show that reoxygenation after hypoxemia causes myocardial contractile dysfunction that is linked to biochemical evidence of lipid peroxidation and expenditure of endogenous antioxidant reserve capacity. This reoxygenation damage occurs after reintroduction of molecular oxygen either by raising Fio₂ or instituting CPB and develops also when the entire hypoxemia/reoxygenation process occurs during CPB.

Our interest in reoxygenation injury stems from our previous studies of reperfusion injury after ischemia in which (1) reintroduction of molecular oxygen is one aspect of damage,²⁵ (2) pharmacologic

Fig. 6. Antioxidant reserve capacity in LV subendocardial muscle, sampled before hypoxemia (control), at end of hypoxemia, and after hypoxemia and reoxygenation (ReO_2) during CPB (see text for description). MDA, Malondialdehyde.

blockade of endogenous antioxidants in blood cardioplegia offset the benefits of controlled reperfusion,²⁵ (3) experimental evidence of the same spectrum of changes that follow reperfusion occurs also after reoxygenation *without* ischemia,⁶⁻⁸ and (4) clinical reports are given of myocardial functional depression in infants with cyanotic disease who underwent reoxygenation by CPB (extracorporeal membrane oxygenation) without surgical ischemia.^{10, 11} These data suggest strongly that reintroduction of molecular oxygen is an etiologic factor. This implication is reinforced by studies that show reoxygenation damage is proportionate to the rate of reoxygenation.^{26, 27} The burst of oxygen free radicals after reintroduction of oxygen²⁸ may overwhelm the scavenging capacity of reduced cellular defense mechanisms quantified by the reduced antioxidant reserve capacity measured in *in vitro* studies.

The cytotoxic effects of reactive oxygen species such as $\cdot OH$, H_2O_2 , or O_2^- are recognized,²⁹ and their role in causing both biochemical and functional damage is validated indirectly by studies that show the beneficial effects of antioxidants.^{30, 31} We reported previously that signs of oxidant damage are reduced by providing intravenous metabolic support during hypoxemia with a solution containing antioxidants (*N*-(2-mercaptopropionyl)-glycine, coenzyme Q_{10} [CoQ₁₀]),³² but their precise salutary role

could not be assessed because other intravenous constituents (glutamate/aspartate, glucose insulin potassium) may have improved recovery via different mechanisms.

All tissues contain endogenous antioxidants, and oxidant injury occurs when these natural barriers to oxidant stress become depleted. Our data indicate reoxygenation damage was not caused by CPB, because 60 minutes of extracorporeal circulation in nonhypoxemic control animals either did not diminish endogenous antioxidant reserve capacity or increase levels of conjugated dienes (the selected marker of lipid peroxidation) in coronary sinus blood or in cardiac tissue. Although hypoxemia *per se* did not raise coronary sinus or myocardial conjugated diene levels in this and other studies,³³ it increased cardiac susceptibility to oxidant damage by reducing antioxidant reserve capacity, which was depleted further after reoxygenation (Fig. 7). These findings are consistent with reports of hypoxemia-related decrease of antioxidants and their further depletion after reoxygenation.^{7, 33} We interpret the lipid peroxidation and coincident cardiac depression to reflect oxygen free radical damage as a result of an overwhelmed and impaired myocardial antioxidant reserve capacity.

We assessed oxidant damage by measuring conjugated dienes eluted into coronary sinus blood and

Fig. 7. Antioxidant reserve capacity in LV subendocardial muscle in control studies, after CPB without hypoxemia, and after hypoxemia/reoxygenation (ReO_2) during CPB (see text for description). *MDA*, Malondialdehyde.

remaining in the myocardium after CPB was discontinued; the small size of piglet hearts did not allow for sequential biopsies. Coronary sinus levels of conjugated dienes rose during reoxygenation and remained elevated during the first 30-minute post-bypass observation period, suggesting ongoing oxidant injury (Fig. 4). Tissue levels of conjugated dienes increased almost twofold in piglets subjected to ventilator hypoxia, but the role of reoxygenation versus reperfusion damage could not be evaluated because hemodynamic deterioration developed in most piglets and many required premature reoxygenation before the intended 2-hour hypoxemic interval elapsed. Conversely, tissue levels of conjugated dienes were normal in piglets undergoing a brief (30 minutes) hypoxemic interval during CPB even though conjugated dienes were eluted in coronary sinus blood during reoxygenation, suggesting a more severe oxidant injury occurred after prolonged ventilator hypoxemia, despite comparable functional depression 60 minutes after reoxygenation. The differences in hypoxemic duration and resultant hemodynamic changes did not influence function after CPB, which recovered 39% in piglets

that withstood the 2-hour hypoxemic interval, 33% in those that required premature reoxygenation (78 ± 12 minutes), and 36% in piglets that underwent a fixed 30-minute hypoxemic insult during CPB to avoid hemodynamic deterioration. The recovery pattern may have been different if we followed function for several hours, rather than terminating the experiment at the time final measurements were made.

Impaired antioxidant reserve capacity after hypoxemia and subsequent reoxygenation occurred without ischemia in the model of hypoxemia/reoxygenation by CPB, and the resultant functional damage has a clinical counterpart in impaired contractility, sometimes progressing to myocardial stunning, in hypoxemic infants who undergo extracorporeal membrane oxygenation because of pulmonary disorders.^{10, 11} In these infants, myocardial depression occurs despite the reduction of cardiac work by diverting more than 50% of venous return to the extracorporeal circuit. We would expect the reduced antioxidant reserve capacity to increase vulnerability to ischemic damage, and we have demonstrated recently that the ischemic tolerance of the immature heart is nullified by preceding hypoxemia.³⁴ Similar

impairment of postischemic functional recovery occurs in long-term cyanotic models.^{35,36} In each instance, reoxygenation during bypass was introduced *before* imposition of surgical ischemia, so that the role of reoxygenation damage and the resultant functional depression could not be evaluated.

Clinical signs of lipid peroxidation immediately after reoxygenation during bypass and before aortic clamping was reported by Del Nido,⁹ and the Toronto group also reported (1) reduced myocardial endogenous antioxidants (glutathione peroxidase in preischemic biopsy samples)²¹ and (2) lowered endogenous antioxidant levels and increased susceptibility to oxidant stress in myocytes from patients with cyanosis that were incubated in a hypoxemic environment.²²

CPB could be discontinued in all studies, and no piglet required inotropic drugs to produce near-normal cardiac index so that the functional impairment could be quantified only by inscribing pressure volume loops or by transfusion to generate Starling curves. The measurement of end-systolic elastance by transient inferior vena caval occlusion is not done clinically, whereas the response to transfusion is standard practice. Figure 1 shows that a near-normal stroke work index could be achieved by volume infusion, but no further augmentation was possible by raising filling pressures higher than 15 mm Hg, compared with the marked augmentation that occurred in control hearts. Consequently, further elevation of cardiac index in response to increased tissue demands (fever, sepsis, or anemia) would probably necessitate inotropic support. Catecholamine infusions are sometimes started routinely after repair of congenital defects causing cyanosis, so that identification of the role of reoxygenation injury may be difficult after operation.

The absence of surgical ischemia allowed assessment of the effects of reoxygenation itself in this study. The increased vulnerability to oxidant stress implies that a subsequent standard ischemic insult (i.e., aortic clamping) would produce more functional depression as reported by Del Nido and associates⁵ in a comparison of similar blood cardioplegic techniques in children with cyanosis who underwent repair of tetralogy of Fallot and normoxemic adults who underwent coronary bypass grafting. Biochemical changes and functional impairment were greater in children with cyanosis despite shorter ischemic times (40 minutes versus 115 minutes).⁵ The different operations for correcting surgical causes of cyanosis add yet another variable that makes clinical assessment of reoxygenation damage

difficult, but surgical ischemia and remodeling of ventricular anatomy are absent in cyanotic infants with pulmonary disorders in whom myocardial depression develops after reoxygenation by bypass (extracorporeal membrane oxygenation).

These data, coupled with those from our previous studies of the effects of reoxygenation, lead us to conclude that the reintroduction of molecular oxygen to previously hypoxemic subjects produces myocardial damage that is linked to oxidant injury. These observations suggest that institution of CPB in the hypoxemic heart introduces an unintended reoxygenation injury and simultaneously makes the heart more susceptible to the subsequent ischemic interval needed for surgical repair. The similarity of reoxygenation damage to reperfusion injury leads us to the inevitable conclusion that, as with reperfusion injury, control of the method of reoxygenation may offset damage that otherwise would occur if CPB were instituted in a standard manner. If the foregoing is true, the possibility exists that modification of the conditions of reoxygenation (arterial P_{O_2}) and composition of the prime in the extracorporeal circuit (i.e., antioxidants and other additions) and incorporation of some of the interventions that successfully reduce reperfusion injury (i.e., blood cardioplegic protocols) may be used to surgical advantage in limiting reoxygenation injury.

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