STUDIES OF HYPOXEMIC/ REOXYGENATION INJURY: WITHOUT AORTIC CLAMPING

IX. Importance of avoiding perioperative hyperoxemia in the setting of previous cyanosis

This study of an in vivo infantile piglet model of compensated hypoxemia tests the hypothesis that reoxygenation on hyperoxemic cardiopulmonary bypass produces oxygen-mediated myocardial injury that can be limited by normoxemic management of cardiopulmonary bypass and the interval after cardiopulmonary bypass. Twenty-five immature piglets (<3 weeks old) were placed on 120 minutes of cardiopulmonary bypass and five piglets served as a biochemical control group without cardiopulmonary bypass. Five piglets underwent cardiopulmonary bypass without hypoxemia (cardiopulmonary bypass control). Twenty others became hypoxemic on cardiopulmonary bypass for 60 minutes by lowering oxygen tension to about 25 mm Hg. The study was terminated in five piglets at the end of hypoxemia, whereas 15 others were reoxygenated at an oxygen tension about 400 mm Hg or about 100 mm Hg for 60 minutes. Oxygen delivery was maintained during hypoxemia by increasing cardiopulmonary bypass flow and hematocrit level to avoid metabolic acidosis and lactate production. Myocardial function after cardiopulmonary bypass was evaluated from end-systolic elastance (conductance catheter) and Starling curve analysis. Myocardial conjugated diene production and creatine kinase leakage were assessed as biochemical markers of injury, and antioxidant reserve capacity was determined by measuring malondialdehyde after cardiopulmonary bypass in myocardium incubated in the oxidant, *t*-butylhydroperoxide. Cardiopulmonary bypass without hypoxemia caused no oxidant or functional damage. Conversely, reoxygenation at an oxygen tension about 400 mm Hg raised myocardial conjugated diene level and creatine kinase production (CD: $3.5 \pm 0.7 A_{233}$ nm/min/100 g, creatine kinase: 8.5 ± 1.5 U/min/100 g, $p < 0.05$ vs cardiopulmonary bypass control), reduced antioxidant reserve capacity (malondialdehyde: 1115 ± 60 nmol/g protein at 4.0 mmol *t*-butylhydroperoxide, $p < 0.05$ vs control), and produced severe postbypass dysfunction (end-systolic elastance recovered only $39\% \pm 7\%$, $p < 0.05$ vs cardiopulmonary bypass control). Lowering oxygen tension to about 100 mm Hg during reoxygenation avoided conjugated diene production and creatine kinase release, retained normal antioxidant reserve, and improved functional recovery ($80\% \pm 11\%$, $p < 0.05$ vs oxygen tension about 400 mm Hg). These findings show that conventional hyperoxemic cardiopulmonary bypass causes unintended reoxygenation injury in hypoxemic immature hearts that may contribute to myocardial dysfunction after cardiopulmonary bypass and that normoxemic management may be used to surgical advantage. (J THORAC CARDIOVASC SURG 1995;110:1235-44)

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Chronic cyanosis results in metabolic adaptations that maintain normal aerobic metabolism despite severe hypoxemia.^{1,2} Simultaneously, the cya-

notic myocardium become depleted of endogenous antioxidants³ and thereby more vulnerable to oxidant stress when cardiopulmonary bypass (CPB) is

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initiated in cyanotic patients at hyperoxemic oxygen tension (Po_2) levels (i.e., about 400 mm Hg); we have speculated this causes oxygen-mediated myocardial injury, which intensifies intraoperative ischemic damage and accentuates post-CPB myocardial dysfunction.⁴ This hypothesis is supported by clinical reports showing cardiac lipid peroxidation in preischemic biopsy samples,⁵ inadequate cardioplegic protection in cyanotic patients,⁶ and myocardial dysfunction in infants placed on extracorporeal membrane oxygenation despite the absence of surgical ischemia.⁷

We have demonstrated previously in immature piglets that reoxygenation after acute hypoxemia induced by lowering inspired oxygen fraction (Fio_2) by ventilator causes myocardial damage, which is associated with lipid peroxidation.⁸ We have shown also that more gradual reoxygenation to hyperoxemic levels does not avoid this injury.⁹ Our prior studies have somewhat uncertain clinical relevance, because acute ventilator hypoxemia simulates acute asphyxia, which is not associated with the metabolic adaptations to chronic cyanosis (i.e., including polycythemia, augmented cardiac output, or both) and is usually characterized by hemodynamic deterioration and metabolic acidosis, which decreases oxygen delivery and superimposes ischemia. Experimental studies of chronic cyanosis confirm the increased susceptibility of cyanotic myocardium to ischemia/reperfusion injury^{10, 11} but are few in number because of high perioperative mortality and are limited by (1) an inability to produce a fixed Po_2 level and (2) the potential confounding hemodynamic consequences of cardiac pressure and volume overloading that makes changes caused by hypoxemia per se difficult to distinguish.¹²

This report describes an in vivo infantile piglet model of hypoxemia that is produced on CPB, whereby hematocrit level and perfusion rate are increased to ensure normal oxygen delivery and avoid anaerobic metabolism to simulate some of the metabolic adaptations occurring in cyanotic patients. This model of compensated hypoxemia was used to test the hypotheses that (1) sudden reoxygenation and hyperoxemic bypass produce oxygen-mediated injury after only a brief period of hypoxemia and (2) this oxygen-mediated damage can be limited by maintaining normoxemia during both CPB and after extracorporeal circulation is discontinued.

Material and methods

Experimental model. Twenty-five immature, 2- to 3-week-old Yorkshire-Duroc 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, published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Physiologic and biochemical determinations

Acid-base and blood gas analyses. Systemic arterial and venous blood gas, electrolyte, and hemoglobin measurements were made every 15 minutes during CPB (Blood Gas system 288, CIBA-Corning, Medfield, Mass.). Body oxygen delivery and uptake were calculated by the following equations:

Oxygen delivery (DO_2 , ml/min/kg) =

$$F \times \text{Cao}_2 / \text{body weight (kg)}$$

Oxygen utilization (VO_2 ml/min/kg) = $F \times (\text{Cao}_2$

$$- \text{Cvo}_2) / \text{body weight (kg)}$$

where F is pump flow (ml/min), Cao_2 is oxygen content in arterial blood, and Cvo_2 is oxygen content in systemic venous blood. Cardiac output was determined by duplicate injections of 1 ml of 4° C saline solution into a central venous catheter. Cardiac index (CI), systemic vascular resistance index (SVRI), and left ventricular (LV) stroke work index (LVSWI) were calculated with the following equations:

CI (ml/min/kg) = $\text{CO} / \text{body weight (kg)}$

SVRI ($\text{mm Hg} \cdot \text{min} \cdot \text{L}^{-1} \cdot \text{kg}$) = $(\text{MAP} - \text{CVP})$

$$/ (\text{mm Hg}) \cdot \text{CO}^{-1} (\text{L/min}) \cdot \text{BW (kg)}$$

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

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

where MAP is mean aortic pressure, LAP is mean left atrial pressure, CVP is central venous pressure, HR is heart rate (beats/min), and CO is cardiac output measured by thermodilution method or CPB pump flow rate in milliliters per minute.

Myocardial performance. LV pressure and conductance catheter signals were amplified and digitalized to inscribe LV pressure-volume loops. After the procedure for correction of parallel conductance, a series of pressure-volume loops under variable loading conditions was generated by rapid transient occlusion of the inferior vena cava during a 7-second period of apnea, at a control condition, and 30 minutes after CPB was discontinued.¹³

Fig. 1. Postbypass LV contractility. LV Ees after discontinuation of CPB, expressed as percent of individual prehypoxemic values. CPB: Piglets performed hyperoxemic bypass without hypoxemia; reoxygenation: piglets subjected to hypoxemia/reoxygenation on CPB at Po₂ about 400 mm Hg or Po₂ about 100 mm Hg. **p* < 0.05 vs CPB; †*p* < 0.05 vs Po₂ about 400 mm Hg.

The end-systolic pressure volume relationship (ESPVR) was analyzed by an interactive videographics data analysis computer program (Spectrum, Bowman Gray School of Medicine, Winston-Salem, N.C.) on an 386/33 MHz IBM computer (IBM, Armonk, N.Y.), and LV performance was described as the slope of linear regression (end-systolic elastance [Ees]), as described previously.¹⁴ Postbypass LV contractility was assessed by percent recovery of prebypass control value (percent Ees).

LV performance before and after CPB was also evaluated by infusing blood from the CPB circuit at 5 ml/min/kg over a 3-minute period while recording CO, MAP, and LAP to inscribe Starling function curves.

Myocardial oxidant injury

Myocardial conjugated diene and creatine kinase production. For blood biochemical assessments, the aorta was clamped temporarily (1 to 2 minutes) while the proximal aorta was perfused with blood via a calibrated pump to keep the aortic root pressure 50 to 60 mm Hg. Blood samples were withdrawn from the proximal aorta and the coronary effluent (coronary sinus blood) and analyzed for conjugated dienes and creatine kinase (CK) (see below). These analyses were made during control (normoxemia), hypoxemia, and 5, 30, and 60 minutes of reoxygenation period. Blood samples were centrifuged immediately for 5 minutes at 1000g, plasma was then stored in liquid nitrogen, and hydroxy conjugated diene levels were determined

as described by Lesnefsky and coworkers.¹⁵ The plasma concentration of CK was measured by the method of Oliver.¹⁶ Myocardial production of conjugated dienes and CK was calculated by the following equation: Myocardial production = (Ccs - Ca) × CBF/heart weight (100 gm) where Ccs and Ca are concentration in coronary sinus and arterial blood sample corrected by hematocrit (per milliliter of blood) and CBF (coronary blood flow, ml/min) is a flow rate via a calibrated pump into the proximal aorta during temporary aortic clamping.

Antioxidant reserve capacity. The myocardial endogenous antioxidant state was assessed by determining in vitro lipid peroxidation in LV subendocardial muscle after completion of each experiment by the method of Godin and colleagues¹⁷ as previously described.

Experimental groups

Nonhypoxemic studies. Five piglets underwent 2 hours of CPB at about 400 mm Hg Po₂ without hypoxemia. Perfusion flow rate averaged about 100 ml/min/kg at 50 to 60 mm Hg mean arterial pressure.

Hypoxemia on CPB studies. Twenty piglets were placed on CPB, and arterial Po₂ was reduced to approximately 25 mm Hg for 60 minutes by changing the blend of O₂ and N₂ in the oxygenator, as previously described.¹⁸

HYPOXEMIA WITHOUT REOXYGENATION. In five piglets the experiment was terminated after 60 minutes of hypox-

Fig. 2. LV performance by volume infusion before hypoxemia (control: open circles), after reoxygenation at normoxemic PO_2 (PO_2 about 100 mm Hg: open squares), and at hyperoxemic PO_2 (PO_2 about 400 mm Hg: closed circles). Function curves are different ($p < 0.05$) at all LAP > 15 mm Hg. LVSWI, Left ventricular stroke work index.

Table. Physiologic profile during hypoxemia versus normoxemia on CPB

	Normoxemia	Hypoxemia	<i>p</i> values*
pH	7.48 ± 0.02	7.41 ± 0.01	NS
PaO ₂ (mm Hg)	126.5 ± 14	24.0 ± 0.67	<0.01
Paco ₂ (mm Hg)	25.1 ± 2.0	29.3 ± 1.5	NS
BE (V-A)	1.55 ± 1.35	1.83 ± 0.45	NS
Hct (%)	31.5 ± 0.9	45.5 ± 1.4	<0.01
PFI (ml/min/kg)	109 ± 6	129.5 ± 5.9	0.0126
O ₂ delivery (cc/min/kg)	13.0 ± 0.34	13.4 ± 0.89	NS
VO ₂ (cc/min/kg)	7.4 ± 0.9	8.5 ± 0.5	NS
Lactate extraction	1271 ± 510	1223 ± 712	NS

PaO₂, Arterial PO₂; Paco₂, arterial PCO₂; BE, base excess; V, venous; A, arterial; Hct, hematocrit; PFI, pump flow index; VO₂, O₂ uptake.

*Paired *t* test.

emic perfusion to obtain cardiac biopsy specimens to quantify the effects of hypoxemia without subsequent reoxygenation.

HYPOXEMIA/REOXYGENATION. In fifteen other piglets this 60 minutes hypoxemic interval was followed by 60 minutes of reoxygenation on CPB.

1. Normoxemic reoxygenation (PO_2 about 100 mm Hg): in six piglets PO_2 is raised to normoxemic levels (about 100 mm Hg) and kept at this level during the 30-minute observation period after CPB.
2. Hyperoxemic reoxygenation (PO_2 about 400 mm Hg):

in nine piglets PO_2 was raised to hyperoxemic levels (about 400 mm Hg) during CPB, and post-CPB ventilation was with 100% oxygen to maintain hyperoxemia throughout the entire study interval.

Statistics. Data were analyzed with the use of the StatView V2.0 program (Abacus Concepts Inc., Berkeley, Calif.) on a Macintosh IIfx computer (Apple, Inc., Cupertino, Calif.). Analysis of variance was used for intergroup comparison, and the paired Student *t* test was used for comparison of variables within experimental groups. 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. Systemic (CPB) flow averaged 100 ml/min/kg at a mean arterial blood pressure of about 60 mm Hg in control studies. Systemic oxygen delivery (DO_2) was 13 ± 0.03 ml/min/kg, and pH remained about 7.4 without evidence of either cardiac or systemic lactate production. Comparable body oxygen delivery (DO_2) was also achieved during hypoxemia by increasing CPB flow 30% and raising hematocrit level to 45%; pH remained approximately 7.4, and no evidence of systemic or myocardial lactate production occurred (Table). Hypoxemia on CPB caused systemic vasodilation in all experiments as SVRI fell $62\% \pm 10\%$. SVRI re-

Fig. 3. Myocardial lactate production/extraction during hypoxemia-reoxygenation on CPB. CPB: Piglets performed hyperoxemic bypass without hypoxemia; reoxygenation: piglets performed reoxygenation on CPB at P_{O_2} about 400 mm Hg or about 100 mm Hg after hypoxemia. * $p < 0.05$ vs CPB; † $p < 0.05$ vs P_{O_2} about 400 mm Hg.

turned to control levels during reoxygenation and remained normal during the post-CPB observation interval.

LV contractility. In control studies, LV elastance (Ees) was unchanged after 2 hours of hyperoxemic cardiopulmonary bypass without superimposed hypoxemia (Fig. 1, $102\% \pm 9\%$ recovery). Conversely, hyperoxemic reoxygenation was associated with only $39\% \pm 7\%$ recovery of Ees ($p < 0.05$ vs control). Maintaining normoxemia (P_{O_2} about 100 mm Hg) throughout the reoxygenation interval restored LV contractility to $80\% \pm 11\%$ of pre-CPB levels ($p < 0.05$ vs hyperoxemia, Fig. 1). A parallel depression of myocardial performance occurred when functional reserve capacity was tested by volume infusion (Starling curve analysis) as piglets undergoing hyperoxemic reoxygenation recovered only $38\% \pm 9\%$ of LVSWI at LAP 12 mm Hg, whereas LVSWI recovered $64\% \pm 14\%$ at comparable LAP when P_{O_2} was kept normoxemic during reoxygenation on bypass and during the post-CPB observation period (Fig. 2).

Biochemistry results

Myocardial lactate metabolism. Lactate was consumed by the myocardium throughout the 120 minutes of hyperoxemic CPB in control studies when hypoxemia was not imposed. Comparable lactate

consumption occurred during hypoxemia (Table and Fig. 3), but lactate production was observed after 30 minutes of hyperoxemic reoxygenation. Conversely, the myocardium continued to consume lactate when reoxygenation was conducted at P_{O_2} 100 mm Hg.

Myocardial conjugated dienes. There was no conjugated diene production in piglets undergoing 120 minutes of CPB without hypoxemia (Fig. 4). Hyperoxemic reoxygenation raised conjugated diene production 210% above resting levels at 60 minutes of reoxygenation (3.45 ± 0.7 , A_{233} nm min/100 g, $p < 0.05$ vs CPB control), whereas negligible conjugated diene production occurred after normoxemic reoxygenation (Fig. 4).

CK release. No myocardial CK release occurred in piglets placed on CPB without hypoxemia (Fig. 5). Hyperoxemic reoxygenation caused progress of CK release and reached 330% above prehypoxemic values 60 minutes after reoxygenation (8.5 ± 1.5 U/min/100 g, $p < 0.05$ vs CPB control). In contrast, CK production was negligible and remained similar to control levels after normoxemic reoxygenation.

Antioxidant reserve capacity. Myocardial samples were analyzed for antioxidant reserve capacity in control piglets undergoing CPB without hypoxemia, at the end of the hypoxemic interval on CPB and

Fig. 4. Myocardial conjugated diene production during hypoxemia-reoxygenation on CPB. CPB: Piglets performed hyperoxemic bypass without hypoxemia; reoxygenation: piglets performed reoxygenation on CPB at P_{O_2} about 400 mm Hg or about 100 mm Hg after hypoxemia. * $p < 0.05$ vs CPB; † $p < 0.05$ vs P_{O_2} about 400 mm Hg.

after the 30-minute post-CPB observation period in piglets undergoing both hyperoxemic and normoxemic reoxygenation on CPB. Hypoxemia per se reduced antioxidant reserve capacity, as 25% more malondialdehyde was produced in myocardium exposed to hypoxemia without reoxygenation than in the control myocardium. Hyperoxemic reoxygenation reduced antioxidant reserve capacity further, whereas normal antioxidant reserve capacity was retained after normoxemic reoxygenation (Fig. 6).

Discussion

This study of immature piglets subjected to the model of "compensated" hypoxemia on CPB documents that (1) hyperoxemic CPB produces an oxygen-mediated myocardium injury characterized by lipid peroxidation, reduced endogenous antioxidant reserve capacity, membrane disruption (enzyme leak), and depressed myocardial performance, and (2) this iatrogenic damage is limited substantially by maintaining normoxemia during reoxygenation on CPB and during the subsequent observation period. These findings confirm the occurrence of an "unintended" surgical reoxygenation injury when cyanotic immature hearts are placed on conventional hyper-

oxemic CPB and emphasize the potentially important role of normoxemic management during surgical correction of defects causing cyanosis.

Chronic clinical cyanosis is associated with metabolic adaptations^{1,2} that allow normal aerobic metabolism to persist in the resting state; myocardial oxygen consumption, glucose and free fatty acid uptake, and lactate extraction remain comparable with that in noncyanotic patients,² and there is no shift toward anaerobic distribution of the lactate dehydrogenase isoenzyme.¹⁹ Studies of mitochondria from chronically cyanotic patients show a 30% to 40% greater oxidative capability (state 3 respiratory activity) than in the mitochondria of normoxemic myocardium. This enhanced respiratory capacity is sustained over several weeks of observation in the incubation medium.²⁰ This finding may in part explain the capacity to maintain aerobic metabolism under resting conditions in chronically cyanotic patients despite low oxygen tension. This compensatory mechanism is expended readily with stress, as atrial pacing causes myocardial lactate production and inorganic phosphate, indicating a shift toward anaerobic metabolism.²¹ This metabolic shift may occur also in cyanotic patients during the stresses of

Fig. 5. Myocardial CK production during hypoxemia-reoxygenation on CPB. CPB: Piglets performed hyperoxemic bypass without hypoxemia; reoxygenation: piglets performed reoxygenation on CPB at P_{O_2} about 400 mm Hg or about 100 mm Hg after hypoxemia. * $p < 0.05$ vs CPB; † $p < 0.05$ vs P_{O_2} about 400 mm Hg.

daily life, such as exercise, emotional upset, and tachycardia and become compounded during anoxic spells.

Our *in vivo* model of hypoxemia on bypass was an acute preparation so that the aforementioned mitochondrial adaptations were absent. The assurance of normal oxygen delivery by increasing perfusion flow rate and hematocrit level during hypoxemia avoided anaerobiosis and ensured a compensated state of *in vivo* hypoxemia that may be relevant to the chronically cyanotic condition.

Susceptibility of cyanotic myocardium to oxidant stress. Antioxidant reserve capacity was assessed by determining the extent of *in vitro* peroxidation of incubated myocytes exposed to the oxidant *t*-butylhydroperoxide. This measurement reflects the capacity of endogenous antioxidant enzymes (e.g., glutathione peroxidase) to limit $\cdot OH$ -initiated chain-reaction propagation of lipid peroxidation. The observed fall in antioxidant reserve capacity during hypoxemia is consistent with clinical reports of endogenous antioxidant depletion in cyanotic myocardium³ (i.e., superoxide dismutase, catalase, and glutathione peroxidase) that occurs despite metabolic adaptations. There is a strong correlation between P_{O_2} antioxidant enzyme levels, and suscep-

tibility to oxygen-mediated damage in myocytes cultured from cyanotic patients.²² These clinical and experimental findings suggest that endogenous antioxidant defense mechanisms are weakened by hypoxemia, therefore rendering the cyanotic myocardium more susceptible to subsequent intraoperative stress on reoxygenation and during and after temporary surgical ischemia.

These inferences are confirmed by a recent clinical report⁶ showing that cyanotic hearts are more vulnerable than noncyanotic hearts to ischemia/reperfusion damage despite comparable blood cardioplegic protection and shorter ischemic times. The clinical findings of accelerated adenosine triphosphate (ATP) depletion, lactate accumulation, and myocardial depression provide confirmation of experimental studies¹⁰⁻¹² showing that chronic cyanosis predisposes the myocardium to energy depletion¹⁰ and postischemic contractile dysfunction.^{11,12} These reports did not, however, consider the possibility that reoxygenation-induced damage before superimposed ischemia contributed to the final biochemical and functional findings. The present study intentionally excluded any ischemia to focus on the potential adverse effects of reinstitution of molecular oxygen to hypoxemic myocardium with

Fig. 6. Antioxidant reserve capacity: effects of P_{O_2} during reoxygenation. Data are expressed as malondialdehyde production in the myocardium incubated with 4.0 mmol *t*-butylhydroperoxide. Dotted bar shows range of mean values $\pm$ SE from control piglets without CPB. CPB: Piglets undergoing hyperoxemic bypass without hypoxemia; reoxygenation: piglets undergoing reoxygenation on CPB at P_{O_2} about 400 mm Hg or about 100 mm Hg after hypoxemia. * $p < 0.05$ vs CPB; † $p < 0.05$ vs P_{O_2} about 400 mm Hg.

depleted endogenous antioxidant defense mechanisms.

Surgical reoxygenation injury. This concept of surgical reoxygenation injury inferred by our experimental observations after hyperoxemic CPB is supported by clinical evidence of lipid peroxidation and reduced antioxidants in preischemic biopsy samples of the right ventricle from cyanotic patients placed on CPB,⁵ for correction of tetralogy of Fallot, and myocardial dysfunction after reoxygenation by extracorporeal membrane oxygenation⁷ in infants with pulmonary disorders causing cyanosis. Normal lactate metabolism persisted during "compensated hypoxemia" and normoxemic reoxygenation, but lactate production followed hyperoxemic reoxygenation. This metabolic shift toward anaerobic energy production may be related to mitochondrial dysfunction caused possibly by reoxygenation-induced mitochondrial calcium loading.²³ Our previous studies confirmed the inverse relationship between mitochondrial calcium and state 3 respiratory activity in the ischemic/reperfusion model,²⁴ and similar alterations may occur after reoxygenation despite the compensatory increase in mitochondrial activity during cyanosis.²⁰ The role of reactive oxygen spe-

cies in ischemic/reperfusion injury seems established, and our studies suggest that they may also play a major role in the pathogenesis of surgical reoxygenation injury. These data document an imbalance between antioxidant reserve capacity during hypoxemia and enhanced free radical generation after reoxygenation.

Our studies are purely observational and do not define the relative contributions of the multiple sources of oxygen free radicals including the xanthine oxidase system,²⁵ activated neutrophils,²⁵ leakage of electrons from electron transport system within the mitochondria,²⁶ and from the cyclooxygenase pathway of arachidonic acid metabolism²⁷ and catecholamine metabolism.²⁸ We suspect, however, that some potential sources of free radical generation operative during reperfusion may not necessarily play an important role in the pathogenesis of reoxygenation injury after compensated hypoxemia. In part the initial burst of free radicals after reperfusion results from the accumulation and availability of neutrophils for activation during stagnant flow and byproducts of ATP degradation (i.e., hypoxanthine) that can cause an immediate burst of oxygen radicals on reoxygenation with consequent lipid

peroxidation and alteration in electron carriers in mitochondria. The high flow condition during in vivo hypoxemia may limit the margination of neutrophils and buildup of byproducts of ATP degradation.

In contrast, under physiologic conditions, normal intact mitochondria and sarcoplasmic reticulum produce O_2^- and H_2O_2 . This production is enhanced by hyperoxemia²⁶ in a PO_2 -dependent way, and these oxygen free radicals are counteracted by endogenous antioxidants. Hyperoxemia sharply elevates the amount of oxygen dissolved in the membrane lipid matrix, thereby enhancing the possibility of O_2 interaction with reduced electron carriers and accentuating free radical production.^{26, 29-31} In addition, CPB per se causes complement-mediated activation of polymorphonuclear neutrophils (PMNs) leading to free radical production.³² This sequence may proceed in a self-propagating manner, because PMN-derived free radicals can trigger the secretion of chemotactic substances that further recruit and activate PMNs to produce more free radicals.³³ Consequently, initiation of hyperoxemic CPB may induce sufficient free radical generation to overwhelm reduced endogenous antioxidant defense system caused by hypoxemia and produce the signs of lipid peroxidation (conjugated dienes and subsequent cellular disruption) and CK enzyme release reported herein. We interpret the initial delay and subsequent progressive increase in CK and conjugated diene levels to reflect ongoing reoxygenation damage that may differ from the immediate oxidative reaction that follows reperfusion. Perhaps the systemic inflammatory response to CPB may play an important role in the pathogenesis of damage, because gradual increases in neutrophil³⁴ elastase and myeloperoxidase activity,³⁴ anaphylatoxins C3a and C5a,^{32, 34} and H_2O_2 production³² occur as the interval of CPB is prolonged.

Implications. Maintaining normoxemia rather than hyperoxemia substantially reduced oxidant damage (lipid peroxidation, enzyme leak, and depletion of antioxidant reserve capacity) while retaining normal lactate metabolism and decreasing the extent of myocardial stunning. These benefits coincide with the PO_2 -dependent nature of reoxygenation injury, because free radical production in vitro bioassay systems¹⁴ and myocardial injury after reoxygenation of isolated heart preparations^{35, 36} are proportionate to oxygen tension. In clinical practice hyperoxemic bypass is performed routinely but is likely never needed, because $PO_2 > 100$ to 150

mm Hg confers only negligible increase in O_2 content. The avoidance of hyperoxemia during reoxygenation in cyanotic infants to reduce injury may in a sense be comparable with restricting inspired F_{IO_2} in newborn infants to avoid retrolental hyperplasia.³⁷ Confirmation by others of the importance of normoxemic management may allow such treatment to be used to surgical advantage in the treatment of cyanotic infants.

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