CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

ISCHEMIC INTERVALS DURING WARM BLOOD CARDIOPLEGIA IN THE CANINE HEART EVALUATED BY PHOSPHORUS 31-MAGNETIC RESONANCE SPECTROSCOPY

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Objective: Warm blood cardioplegia requires interruption by ischemic intervals to aid visualization. We evaluated the safety of repeated interruption of warm blood cardioplegia by normothermic ischemic periods of varying durations. **Methods:** In three groups of isolated cross-perfused canine hearts, left ventricular function was measured before and for 2 hours of recovery after arrest, which comprised four 15-minute periods of cardioplegia alternating with three ischemic intervals of 15, 20, or 30 minutes (I_{15} , I_{20} , and I_{30}). Metabolism was continuously measured by phosphorus 31-magnetic resonance spectroscopy. **Results:** Adenosine triphosphate levels fell progressively as ischemia was prolonged; after recovery, adenosine triphosphate was $99\% \pm 6\%$, $90\% \pm 1\%$ ($p = 0.0004$ vs control), and $68\% \pm 3\%$ ($p = 0.0002$) of control levels in I_{15} , I_{20} , and I_{30} , respectively. Intracellular acidosis with ischemia was most marked in I_{30} . After recovery, left ventricular maximal systolic elastance at constant heart rate and coronary perfusion pressure was maintained in I_{15} but fell to $85\% \pm 3\%$ in I_{20} ($p = 0.003$) and to $65\% \pm 6\%$ ($p = 0.003$) of control values in I_{30} , while relaxation (τ) was prolonged only in I_{30} ($p = 0.007$). **Conclusions:** Hearts recover fully after three 15-minute periods of ischemia during warm blood cardioplegia, but deterioration, significant with 20-minute periods, is profound when the ischemic periods are lengthened to 30 minutes. This suggests that in the clinical setting warm cardioplegia can be safely interrupted for short intervals, but longer interruptions require caution. (J Thorac Cardiovasc Surg 1997;114:1070-80)

Warm blood cardioplegia is accepted as a safe and efficacious method of myocardial protection,¹⁻³ used by 10% of practicing cardiac surgeons

in the United States.⁴ Since the first report of warm blood cardioplegia by Lichtenstein and coworkers,⁵ many clinical⁶⁻⁸ and experimental⁹⁻¹² studies have described its advantages and disadvantages and have suggested refinements to the technique.

Since continuous delivery of warm blood cardioplegia may limit visualization during surgery, intermittent ischemic periods as long as 15 minutes have been used.^{6, 13} The percentage of crossclamp time without cardioplegic infusion has been reported to be as high as 48.2% without apparent harm, provided no single ischemic interval exceeds 13 minutes.¹⁴ Only a few experimental studies have addressed the effects of ischemic intervals during warm blood cardioplegia.¹⁵⁻¹⁸

To evaluate the safety of repeated interruption of warm blood cardioplegia by normothermic ischemic periods of varying durations, we studied isolated

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This study was supported in part by grant HL12777 from the National Institutes of Health and by generous gifts from Mr. Anthony A. Borgatti, Jr., Mr. and Mrs. Milton J. Silverman, and the Leon S. Newton Foundation.

Received for publication Feb. 5, 1997; revisions requested May 15, 1997; revisions received June 23, 1997; accepted for publication June 25, 1997.

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0022-5223/97 \$5.00 + 0 12/1/84445

cross-perfused isovolumic canine hearts undergoing four 15-minute periods of cardioplegia alternating with three ischemic intervals of 15, 20, or 30 minutes. The preparation allows measurement of left ventricular (LV) function while its main hemodynamic determinants—coronary perfusion pressure (CPP), LV volume, and heart rate—are controlled. Phosphorus 31-magnetic resonance spectroscopy (MRS) enabled repeated nondestructive measurements of myocardial metabolism.

Methods

Animal preparation. The preparation has been described in detail previously.¹⁰ Dogs were tranquilized with acepromazine (0.4 mg/kg intramuscularly), anesthetized with intravenous chloralose (75 to 150 mg/kg) and urethane (0.75 to 1.5 gm/kg), intubated, and ventilated with oxygen-enriched air. A perfusion circuit was primed with 500 ml of 6% hetastarch (Hespan, DuPont Pharma, Wilmington, Del.), 1000 ml of 0.9% saline solution, 50 mEq of sodium bicarbonate, and 10,000 units of heparin. The heart of the donor dog (body weight 20.5 ± 0.7 kg) was isolated without interrupting coronary perfusion and was cross-perfused at a constant CPP by a larger support dog, with a third animal used as the blood donor. After the chordae tendineae were transected, a large compliant balloon primed with saline solution was placed in the LV cavity of the isolated heart. The sinus node was crushed and the heart atrially paced at a constant rate of 150 beats/min. Copper electrodes were positioned in contact with the right ventricle and the posterolateral aspect of the LV to allow defibrillation, if required.

Coronary blood flow (CBF), CPP, support dog aortic pressure, and myocardial temperature measurement have been described.¹⁰ LV pressure was measured by a high-fidelity micromanometer-tipped catheter (SPC 350 MR, Millar Instruments, Inc., Houston, Tex.) within the balloon and referenced to pressure measured through a fluid-filled catheter.¹⁰ LV pressure, CPP, and aortic pressure were monitored and recorded on a strip chart and the LV pressure on magnetic tape.

When its instrumentation was complete, the heart (maintained at 37° C) was supported by a pericardial sling within a water-jacketed acrylic cylinder (Plastic Design, Inc., Lexington, Mass.). The anterior LV surface was uppermost and positioned to maintain contact, without restricting cardiac pulsation, with an MRS surface coil mounted within the cylinder. The cylinder was placed in the center of a wide-bore horizontal magnet.¹⁰

Heparin (6000 units) was administered to the support dog for anticoagulation followed by 1000 units/hr. Indomethacin 50 mg (INN: indometacin), methylprednisolone 1 gm, chlorpheniramine maleate 10 mg (INN: chlorphenamine), and cimetidine (300 mg) intravenously were followed by a continuous infusion of 110 mg indomethacin, 1 gm methylprednisolone, 600 mg cimetidine, and 20 mg chlorpheniramine maleate in 500 ml normal saline solution at 50 ml/hr to maintain hemodynamic stability of the

support dog. Hetastarch was infused if needed. Chloralose (2 mg/kg per minute) and urethane (20 mg/kg per minute), given intravenously, maintained anesthesia. Ventilation was adjusted and sodium bicarbonate added as necessary to maintain arterial oxygen tension above 200 mm Hg and carbon dioxide tension and pH in the physiologic range.

LV function. LV function curves at constant CPP and heart rate were obtained by stepwise increases in balloon volume to an LV end-diastolic pressure not exceeding 15 mm Hg. At each LV volume, LV peak and end-diastolic pressures were recorded when heart rhythm and pressures were stable. LV systolic function was assessed from maximal systolic elastance, the slope of a line fit by least-squares regression to the peak LV pressure-volume relation.¹⁹ LV end-diastolic pressure, as a measure of LV chamber stiffness, and the LV relaxation constant, τ ²⁰ (averaged over 10 heart beats), were measured during stability at constant LV volume and CPP.¹⁰

Chemical measurements. Measurement of blood gases, serum Na^+ and K^+ , and plasma ionized Ca^{2+} has been described.¹⁰

MRS. Phosphorus 31 spectra were acquired in a 4.7-tesla GE-Omega MRS system (General Electric Co., Waterford, N.Y.) using a surface coil, as detailed previously.¹⁰ Before data acquisition, the field was shimmed to approximately 40 Hz (0.2 ppm) with the use of the 1H water signal. The radiofrequency pulse width was 40 msec. During the experiment, spectra were obtained continuously in approximately 1-minute blocks of 52 acquisitions using a pulse delay of 1 second and corrected to fully relaxed values with the use of data obtained at the beginning of each experiment with a pulse delay of 20 seconds.¹⁰

Data during cardioplegia or ischemia were summed over 1 minute and during control or reperfusion, over 10 minutes. Inorganic phosphate peaks, evaluated by means of intensity, and other peaks, evaluated by means of area, were scaled to an intensity standard¹⁰ and expressed as a percentage of their control values. Intracellular pH was calculated as previously described.¹⁰ B1-corrected areas were used to calculate the ratio of creatine phosphate (PCR) to adenosine triphosphate (ATP). When inorganic phosphate peaks were indistinguishable from noise—generally the case during cardioplegia after the third minute and during the first minute or two of ischemia—inorganic phosphate was taken to be zero and intracellular pH could not be measured. When PCR was indistinguishable from noise as in late ischemia, PCR was taken to be zero and intracellular pH was obtained from the chemical shift of the inorganic phosphate with respect to the standard.

Experimental groups. All hearts received four 15-minute periods of warm blood cardioplegia (Cp1, Cp2, Cp3, and Cp4) alternating with three periods of normothermic ischemia (Isc1, Isc2, and Isc3) and were randomly assigned to one of three groups (Fig. 1): group 1, three 15-minute periods of normothermic ischemia (I_{15}); group 2, three 20-minute periods of normothermic ischemia (I_{20}); and group 3, three 30-minute periods of normothermic ischemia (I_{30}).

Fig. 1. Experimental groups (I₁₅, I₂₀ and I₃₀). After a control period (CTL), each group underwent arrest, comprising four 15-minute periods of warm blood cardioplegia alternating with three normothermic ischemic periods of 15, 20, or 30 minutes' duration, followed by 2 hours of recovery (RCV). Control and recovery periods are not to scale. Dark bars = 15 minutes of warm blood cardioplegia; open bars = ischemic periods, with duration in minutes indicated inside each bar.

Protocol

Control period. CPP was maintained at 86 to 94 mm Hg and LV volume adjusted until LV end-diastolic pressure was 8 to 10 mm Hg. When LV function was stable, control MRS and hemodynamic data were acquired, and CBF was measured. Then LV function curves were obtained.

Cardioplegia. LV volume was decreased until LV end-diastolic pressure declined to 0 to 5 mm Hg, and pacing was discontinued. Then blood from the arterial reservoir and a modified Fremes cardioplegic solution,⁷ described later, were continuously mixed (4 parts blood to 1 part solution) and delivered to the heart via the coronary perfusion line. Arrest was induced by infusing high-potassium blood cardioplegic solution at approximately 100 ml/min for 5 minutes followed immediately by low-potassium blood cardioplegic solution. During Cp1, flow rate was adjusted if necessary to maintain CPP less than 125 mm Hg. In the following cardioplegic periods, flow was adjusted to maintain CPP at 86 to 94 mm Hg, allowing reactive hyperemia. A hemofiltrator was used to minimize hemodilution caused by cardioplegia.

Ischemia. After Cp1, Cp2, and Cp3, global ischemia was instituted for a duration appropriate to the experimental group.

Recovery. After Cp4, addition of cardioplegic solution to coronary arterial blood was discontinued and recovery began. CPP was returned to 86 to 94 mm Hg. Electrical defibrillation was performed if necessary. Temporary atrioventricular block occurred in all hearts, necessitating temporary ventricular pacing. Atrial pacing was resumed as soon as possible. After 30 minutes of recovery, the LV balloon was gradually inflated to the control LV volume. MRS and hemodynamic data were collected as during the control period at 60, 90, and 120 minutes of recovery. Finally, the LV was weighed.

Cardioplegic solutions. Before admixture with blood, the solution contained the following components (in

millimoles per liter) K⁺, 100 (high-potassium solution) or 30 (low-potassium solution); Mg²⁺, 9; Cl⁻, 75; SO₄²⁻, 9; glucose, 278; citrate, 1.8; and citric acid, 0.3. After admixture with blood, K⁺ was 22.8 ± 1 mEq/L in the high-potassium solution; in the low-potassium solution, K⁺ was 12.8 ± 0.3 mEq/L, ionized Ca²⁺ 0.8 ± 0.01 mmol/L, Na⁺ 104 ± 1.1 mEq/L, hematocrit 25.9% ± 0.7%, pH 7.36 ± 0.01, carbon dioxide tension 34.6 ± 0.7 mm Hg, and oxygen tension 329 ± 19 mm Hg.

Calculations. Warm blood cardioplegia volume (ml/100 gm LV) = crystalloid cardioplegia volume × 500/LV weight.

Statistical analysis. Data underwent univariate repeated-measures analysis of variance. Control data were contrasted with subsequent measures by paired *t* test if the *F* statistic for time or the interaction between group and time was significant. The incidence of ventricular fibrillation among the groups was compared by Fisher's exact test. Continuous variables measured only once were compared by Student's *t* test. Values of *p* < 0.05 were taken to indicate statistical significance. Data are expressed as mean ± standard error of the mean.

These studies were performed in accordance with institutional guidelines and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Results

Seven hearts were studied in each group. No functional or metabolic data during recovery were included for one heart because of inability to resume atrial pacing during recovery (I₁₅); for a second heart these data were excluded because of the

Table I. Hemodynamic variables

	Group	Control	60 min recovery	90 min recovery	120 min recovery
AoP (mm Hg)	I ₁₅	139 ± 3	133 ± 4	131 ± 4	134 ± 4
	I ₂₀	133 ± 5	122 ± 6	124 ± 6	121 ± 6
	I ₃₀	137 ± 5	128 ± 3	127 ± 5	127 ± 5
E _{max} (mm Hg/ml)	I ₁₅	4.1 ± 0.5	4.4 ± 0.6	4.1 ± 0.6*	4.4 ± 0.6
	I ₂₀	4.4 ± 0.4	3.7 ± 0.4*	3.6 ± 0.5†	3.7 ± 0.4*
	I ₃₀	5.0 ± 0.3	3.0 ± 0.3†	3.0 ± 0.3†	3.3 ± 0.4†
LVEDP (mm Hg)	I ₁₅	6.6 ± 0.4	9.9 ± 0.5†	7.6 ± 0.4*	7.3 ± 0.5*
	I ₂₀	6.7 ± 0.3	9.3 ± 1.0*	7.9 ± 1.0	7.6 ± 0.8
	I ₃₀	7.1 ± 0.4	12.5 ± 0.3†	10.3 ± 0.8†	9.7 ± 0.9*
Tau (msec)	I ₁₅	47 ± 1	48 ± 2	46 ± 2	48 ± 3
	I ₂₀	47 ± 2	51 ± 3	47 ± 2	48 ± 2
	I ₃₀	43 ± 2	72 ± 7†	70 ± 9*	68 ± 8*
CBF (ml/min/100 gm LV)	I ₁₅	47 ± 5	58 ± 4†	52 ± 3†	55 ± 6†
	I ₂₀	53 ± 9	68 ± 17	60 ± 13	56 ± 10
	I ₃₀	45 ± 4	58 ± 9	52 ± 8	49 ± 7

Values are mean ± standard error of the mean. AoP, Support dog aortic pressure; CBF, coronary blood flow; E_{max}, left ventricular maximal systolic elastance; LVEDP, left ventricular end-diastolic pressure; tau, left ventricular relaxation constant.

*p = 0.0051 to 0.0500 versus control.

†p = 0.0000 to 0.0050 versus control.

Table II. High-energy phosphates and intracellular pH

	Group	Control	Cp1	Isc1	Cp2	Isc2	Cp3	Isc3	Cp4	2 hr Rcv
% PCR	I ₁₅	100	108 ± 3*	20 ± 2†	103 ± 3	12 ± 2†	100 ± 2	11 ± 2†	97 ± 3	100 ± 3
	I ₂₀	100	105 ± 3	8 ± 2†	100 ± 2	6 ± 1†	98 ± 2	3 ± 1†	98 ± 3	109 ± 2†
	I ₃₀	100	109 ± 1†	1 ± 1†	107 ± 1†	1 ± 0.4†	112 ± 2†	2 ± 1†	112 ± 3*	115 ± 4*
% ATP	I ₁₅	100	96 ± 3	97 ± 4	96 ± 3	89 ± 4*	94 ± 2*	91 ± 1†	90 ± 4*	99 ± 6
	I ₂₀	100	94 ± 5	97 ± 6	88 ± 3*	86 ± 2†	81 ± 4†	79 ± 2†	78 ± 3†	90 ± 1†
	I ₃₀	100	102 ± 5	87 ± 5	75 ± 1†	71 ± 4†	62 ± 4†	58 ± 5†	61 ± 5†	68 ± 3†
PCR/ATP	I ₁₅	2.0 ± 0.1	2.8 ± 0.1*	0.41 ± 0.04†	2.2 ± 0.1	0.27 ± 0.04†	2.1 ± 0.1	0.25 ± 0.03†	2.2 ± 0.1*	2.1 ± 0.2
	I ₂₀	2.0 ± 0.2	2.2 ± 0.2	0.18 ± 0.07†	2.3 ± 0.2*	0.13 ± 0.03†	2.4 ± 0.2*	0.08 ± 0.03†	2.5 ± 0.2†	2.4 ± 0.2†
	I ₃₀	1.9 ± 0.1	2.1 ± 0.2	0.03 ± 0.01†	2.8 ± 0.2†	0.03 ± 0.01†	3.7 ± 0.4†	0.06 ± 0.03†	3.8 ± 0.5*	3.5 ± 0.3†
pH _i	I ₁₅	7.04 ± 0.05	—	6.87 ± 0.02*	—	6.82 ± 0.02*	—	6.80 ± 0.03†	—	7.02 ± 0.06
	I ₂₀	7.10 ± 0.07	—	6.77 ± 0.01†	—	6.74 ± 0.01†	—	6.68 ± 0.03†	—	7.00 ± 0.06*
	I ₃₀	7.05 ± 0.10	—	6.46 ± 0.07*	—	6.53 ± 0.06*	—	6.53 ± 0.03*	—	7.05 ± 0.07

Values are mean ± standard error of the mean. Cp1, Cp2, Cp3, and Cp4 indicate the ends of each cardioplegic period; Isc1, Isc2, and Isc3 indicate the ends of each ischemic period; pH_i, intracellular pH; Rcv, the end of 2 hours of recovery; %PCR, percentage of control creatine phosphate; %ATP, percentage of control ATP; PCR/ATP, ratio of PCR to ATP.

*p = 0.0051 to 0.0500 versus control.

†p = 0.0000 to 0.0050 versus control.

support dog's death (I₂₀); data were obtained through only 1 hour of recovery in a third heart because of failure of a mechanical pump (I₃₀). LV weight was 105 ± 5 gm and LV volume (corrected for balloon displacement volume) at which function was evaluated was 22.4 ± 1.3 ml (no significant differences among groups). There was no significant difference between groups in hemodynamic or metabolic variables before arrest (Tables I and II). At the control measurement, coronary arterial hematocrit value was 35.1% ± 0.7%, and plasma ionized calcium 1.18 ± 0.01 mmol/L (no significant differ-

ences among groups); neither changed significantly from these values during recovery.

Cardioplegic arrest. Volume of high-potassium blood cardioplegic solution for induction was 477 ± 38 ml/100 gm LV (no significant differences among groups). Volumes of low-potassium blood cardioplegic solution were 4130 ± 500, 3970 ± 590, and 6200 ± 1020 ml/100 gm LV in I₁₅, I₂₀, and I₃₀, respectively (no significant differences). Greater re-active hyperemia caused the higher volumes in I₃₀.

High-energy phosphate metabolism. ATP and PCR during arrest are represented in Fig. 2; values

Fig. 2. The effect of periods of normothermic ischemia in the isolated cross-perfused canine heart during arrest by warm blood cardioplegia on PCR and ATP. *Cp1*, *Cp2*, *Cp3*, and *Cp4* indicate successive cardioplegic periods; *Isc1*, *Isc2*, and *Isc3* indicate successive ischemic periods. ATP is shown by filled circles and PCR by open circles. Control levels of ATP and PCR (100%) are indicated by horizontal dotted lines. In the top panel, hearts were subjected to four 15-minute cardioplegic periods alternating with three 15-minute ischemic periods (I_{15}). In the middle panel, ischemic periods were extended to 20 minutes (I_{20}) and in the lower panel, to 30 minutes (I_{30}). Data are means $\pm$ standard error of the mean. For clarity, standard errors of the mean are shown for only the first two data points in each period.

and statistics for ATP, PCR and PCR/ATP are in Table II. The PCR/ATP ratio before arrest did not differ among groups. During *Cp1*, PCR increased slightly—significant in I_{15} and I_{30} . In all groups,

PCR decreased immediately during ischemia and rapidly fell to low levels before recovering completely during the cardioplegic periods (Fig. 2). In the first ischemic period, PCR fell to significantly

lower levels as the duration of ischemia was prolonged—to 20% of control in I_{15} , 8% in I_{20} , and 1% in I_{30} (Table II). There were further declines with repeated ischemic periods; at the end of Isc3, PCR was 11% in I_{15} , significantly above the PCR of 3% in I_{20} and 2% in I_{30} . During Cp2, Cp3, and Cp4, PCR returned to the control level in I_{15} and I_{20} but rose most rapidly and to a significantly higher level in I_{30} . PCR during recovery was at control levels in I_{15} (100%) but significantly elevated in I_{20} (109%) and I_{30} (115%).

ATP declined during arrest in all groups, but more severely as the duration of the ischemic periods increased (Fig. 2). During Isc1, ATP was at 97% of control in I_{15} and I_{20} but had fallen to 87% in I_{30} (no significant differences vs control). By the end of CP2, ATP was significantly less than its control value in I_{20} and I_{30} but not in I_{15} . By the end of Isc2, ATP was significantly below control in all groups and remained so through the end of arrest, when ATP was 90% of control in I_{15} , 78% in I_{20} , and 61% in I_{30} (Table II).

In I_{30} during Isc1 and Isc2, ATP was maintained until PCR fell to a very low level, approximately 5% of its control level, and then declined (Fig. 2). The declines continued into the following cardioplegic periods with ATP falling while PCR was increasing at least to its control level. Little or no decline was observed in ATP during Isc3 although PCR fell to 2%. During recovery, ATP recovered to control levels in I_{15} (99%), was slightly depressed in I_{20} (90%), and was substantially depressed in I_{30} (68%) (Table II).

Inorganic phosphate and intracellular pH. Intracellular pH during arrest is depicted in Fig. 3; statistics for intracellular pH are presented in Table II. Control inorganic phosphate peaks were small, and in most hearts the increase of PCR during cardioplegia was accompanied by a decline in inorganic phosphate until it was indistinguishable from background noise. Intracellular pH increased by approximately 0.1 unit during the first 3 to 5 minutes of the ischemic periods and then decreased progressively (Fig. 3). Intracellular pH was lower at the end of all ischemic periods in I_{20} than in I_{15} , and in I_{30} than in I_{20} . During recovery, intracellular pH recovered to control values in I_{15} and I_{30} but was slightly below control in I_{20} . During ischemia, inorganic phosphate increased ninefold to twelvefold as PCR declined. During recovery, inorganic phosphate returned to slightly above control values ($141\% \pm$

15% , $131\% \pm 19\%$, and $163\% \pm 25\%$ in I_{15} , I_{20} , and I_{30} , respectively).

Recovery. Ventricular fibrillation occurred in five of seven hearts in I_{30} , but in none of seven in I_{20} or I_{15} ($p = 0.02$, I_{20} or I_{15} vs I_{30}). Transient atrioventricular block occurred in all hearts. Aortic pressure during recovery was slightly but not significantly below control values (Table I).

Ventricular performance (Table I and Fig. 4). Maximal systolic elastance was essentially stable throughout recovery in all groups. In I_{15} , there was no evidence of deterioration of LV systolic performance from control levels. At 2 hours of recovery, percent maximal systolic elastance was $85\% \pm 2.8\%$ of control in I_{20} and $65\% \pm 6.3\%$ in I_{30} . Throughout recovery, tau remained at control levels in I_{15} and I_{20} , but was considerably prolonged in I_{30} . LV end-diastolic pressure was increased substantially at 1 hour of recovery in all groups, but most in I_{30} (Table I). By 2 hours there was some improvement, with LV end-diastolic pressure slightly above control in I_{15} , insignificantly increased in I_{20} , but still markedly raised in I_{30} . Increases in LV end-diastolic pressure did not parallel increases in CBF.

Discussion

Electromechanical arrest of the working heart reduces myocardial oxygen consumption by 90%.²¹ However, oxygen delivery adequate for the reduced requirements during warm blood cardioplegia remains critical to prevent ischemic damage. The fact that warm blood cardioplegia is often interrupted during coronary artery surgery threatens the physiologic premise of the method, which is said to be aerobic myocardial protection.^{6,7}

The use of intermittent periods of normothermic ischemia as long as 15 minutes during warm blood cardioplegia was reported by Lichtenstein and co-workers⁶ in the original description of the technique. Recent clinical trials^{1,3,8,14} report interruption of cardioplegia flow with good results. Our experimental data showed that four 15-minute periods of warm blood cardioplegia alternating with three 15-minute periods of normothermic ischemia do not cause measurable deterioration in systolic function and metabolic recovery in the uncompromised canine heart. However, a slight but significant increase in LV stiffness was observed (Table I). An increase in the duration of the ischemic periods to 20 minutes caused deterioration of function (Fig. 3) and a decrease in ATP that persisted to the end of recovery (Table II). With 30-minute periods of

Fig. 3. The effect of normothermic periods of ischemia during warm blood cardioplegia in intracellular pH (pH_i) in groups I_{15} , I_{20} , and I_{30} . Intracellular pH was not measurable for most of the cardioplegic periods when the inorganic phosphate peak was indistinguishable from noise (see *Methods* for details). Data points (means $\pm$ standard error of the mean) are included in the figure if n equals 5 or more. Note that intracellular pH increases during the first 3 to 5 minutes of ischemia and then progressively decreases, reaching the lowest levels in I_{30} .

ischemia, function and ATP were profoundly depressed.

Other experimental studies of normothermic ischemic periods during warm blood cardioplegia¹⁵⁻¹⁸ have come to contradictory conclusions. Much of the observed variation in the protective effect can be attributed to one of three variables: (1) length of the ischemic interval, (2) duration of cardioplegic reinfusion between ischemic intervals, and (3) adequacy of cardioplegic distribution. For example, Ko and associates¹⁵ reported that intermittent warm blood cardioplegia, administered antegradely every 10 minutes during 30 minutes of normothermic arrest

in a canine model, produced greater tissue acidosis and functional deterioration than cardioplegia given every 5 minutes. This implies a significantly shorter "grace period" than in our study. Of note, the volume of cardioplegic solution delivered during each infusion was 350 ml. On the basis of the reactive hyperemia observed in our study, this infusion would take only 2 to 3 minutes to infuse, meaning that 70% to 80% of the crossclamp time was ischemic in the 10-minute group, well beyond the percentage used clinically. In Ko's study, therefore, the cardioplegic reinfusion was probably too brief to be of optimal effect.

In contrast, Landymore, Marble, and Fris¹⁶ found satisfactory myocardial preservation with antegrade warm blood cardioplegia administered every 15 minutes during 90 minutes of normothermic arrest in the dog, apparently a more severe protocol than Ko's. The volume of cardioplegic solution delivered with each infusion in this study was only 200 to 250 ml, which again would have meant about 80% of the crossclamp time was ischemic. In both of these studies,^{15,16} normothermic ischemia was interrupted by brief infusions of warm blood cardioplegic solution while nearly all of the period of arrest was ischemic. Conceptually, this is quite different from the clinical use of warm blood cardioplegia, in which continuous infusion of cardioplegic solution is interrupted by brief intervals of ischemia. In our study, all hearts received four 15-minute periods of warm blood cardioplegia, with total ischemic times during normothermic arrest of 43%, 50%, and 60% of the total crossclamp time in I_{15} , I_{20} and I_{30} , respectively.

Tian and coworkers¹⁸ subjected isovolumic Langendorff-perfused swine hearts (evaluated by MRS) to 90 minutes' arrest with antegrade warm blood cardioplegia interrupted by six 10-minute ischemic periods. The total ischemic time was 67% of the arrest period. ATP declined slightly during arrest but recovered fully. With each ischemic episode, intracellular pH fell by an average of 0.12 unit without evidence of the initial alkalinization that we observed (Fig. 3), and PCR fell to approximately 50% of baseline. After a similar ischemic time we found that intracellular pH fell by 0.07 ± 0.04 unit and PCR to $36\% \pm 2\%$ of baseline (in the first ischemic period of all groups from the scan at an average of 10.1 minutes of ischemia). In Tian's study,¹⁸ PCR and intracellular pH changes were not cumulative with repeated ischemic intervals. During the 30-minute recovery period, function evaluated by rate of pressure rise and rate-pressure product was equal to that after continuous warm blood cardioplegia.

Even very brief ischemic periods may be damaging with a background of preexisting ischemia or with inadequate cardioplegic distribution. Matsuura and colleagues¹⁷ evaluated warm blood cardioplegia in a swine model of acute surgical revascularization to relieve regional ischemia imposed 90 minutes before cardioplegic arrest. Retrograde warm blood cardioplegia during 45 minutes of arrest, interrupted by three 7-minute periods of normothermic ischemia, increased tissue acidosis at the end of arrest and decreased echocardiographic wall motion

Fig. 4. The effect of normothermic periods of ischemia during warm blood cardioplegia on LV function at 2 hours of recovery. Statistics are in Table I. E_{max} , Maximal LV systolic elastance; τ , LV relaxation constant; these variables are expressed as a percentage of their respective control values. $LVEDP$, LV end-diastolic pressure; $\Delta LVEDP = (\text{LV end-diastolic pressure at 2 hours of recovery}) - (\text{control LV end-diastolic pressure})$. Bars indicate standard error of the mean.

scores during recovery when compared with intermittent antegrade/retrograde cold blood cardioplegia or continuous retrograde warm blood cardioplegia. In another study of the swine heart evaluated by MRS, Hoffenberg and colleagues²² showed that retrograde continuous warm blood cardioplegia resulted in metabolic changes characteristic of ischemia, which was attributed to the inhomogeneous distribution of retrograde cardioplegia.²³ In Matsuura's study,¹⁷ inadequate protection afforded by warm retrograde cardioplegia compounded by preexisting regional ischemia may account for the damaging effect of interrupting cardioplegic flow by

ischemic periods as short as 7 minutes. There was no MRS evidence of unintentional ischemia during antegrade delivery of cardioplegic solution in our model or in Tian's.¹⁸

We observed an initial increase of intracellular pH during ischemia to which the creatine kinase reaction may contribute²⁴:

Ischemia prevents the aerobic production of ATP, but initially ATP is replenished by the creatine kinase reaction as fast as it is consumed. PCR hydrolysis takes up hydrogen ions (H^+) whereas ATP hydrolysis produces them. Glycolytic formation and subsequent hydrolysis of ATP together are a net source of H^+ production.²⁴ In our study, the balance of H^+ production and consumption resulted in an overall decrease in $[\text{H}^+]$ in the first few minutes of ischemia while ATP remained more or less steady (Figs. 2 and 3). Comparable initial transient alkalinization has been observed by others.²⁵ In our study, as ischemia progressed, PCR reached very low levels, and ATP hydrolysis predominated with progressive acidosis. Other reactions that release or take up H^+ may have contributed to the H^+ balance.²⁴ Anaerobic glycolysis as a source of ATP during ischemia is inadequate and may be transient²⁴; it is inhibited by acidosis and the lack of oxidized nicotinamide-adenine dinucleotide (NAD^+).²⁶

In I_{30} in the first two ischemic periods, ATP stores began to fall sharply only when PCR declined to 5% to 10% of its control level at about 20 minutes of ischemia (Fig. 2). The declines in ATP in I_{30} continued through the initial minutes of cardioplegic reperfusion, as reported by others,²⁷ whereas PCR recovered (Fig. 2). In the third ischemic period, any additional fall in ATP was minimal. During the cardioplegic periods after ischemia, PCR recovered to control levels in I_{15} and I_{20} but exceeded control in I_{30} . The postischemic PCR overshoot,²⁸ and the continued ATP decline during cardioplegic reperfusion,²⁷ may be markers of severe injury. A low intracellular pH during arrest here and a low tissue pH in a prior study²⁹ both predicted poor recovery.

The isolated heart preparation has limitations, discussed previously.¹⁰ Coronary perfusion was uninterrupted during the surgical preparation to avoid preconditioning. We maintained support dog aortic pressure within physiologic limits to avoid effects on contractility of the isolated heart resulting from humoral influences arising in the support dog. Ionized calcium, also an influence on contractility, was

at the control level during recovery. Hemodilution, which increases CBF, was avoided. Increases in LV end-diastolic pressure during recovery did not parallel and cannot be attributed to the modest increases in CBF (Table I). In the absence of a collateral circulation, decreased washout of cardioplegic solution could be protective, but decreased washout of metabolites during ischemia could be deleterious. In the clinical setting, the presence of coronary artery disease may cause unintentional ischemia through maldistribution during antegrade delivery. In our model, antegrade delivery was used in an optimal manner with uninjured myocardium, which could be a factor in the level of recovery we found.

In conclusion, in this study of the uncompromised canine heart, the safe interval of normothermic ischemia during antegrade warm blood cardioplegia appears to be in the 15-minute range, but these results should not be extrapolated to the clinical setting, where injured myocardium or maldistribution of cardioplegic solution may make the margin finer. Our results and those of others¹⁸ suggest that warm blood cardioplegia can be safely interrupted for short periods of time. Longer periods of normothermic ischemia require caution.

We thank Linda Dell'Olio and Anne Manning for manuscript preparation and Jennifer Akins for technical assistance.

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Commentary

Dr. Carias de Oliveira and colleagues performed an elegant evaluation of intermittent warm blood cardioplegia and found that 15 minutes of cardioplegic interruption with 15 minutes of reperfusion was well tolerated by the normal canine heart. They also found that 30 minutes of cardioplegic interruption with 15 minutes of reperfusion resulted in substantial metabolic and functional deterioration that persisted at least 2 hours after cardioplegic arrest. They recommend that cardiac surgeons should limit warm cardioplegic interruptions, particularly in patients with poor preoperative left ventricular function, preoperative ischemia, or left ventricular hypertrophy.

This is an excellent study, which deserves careful consideration by cardiac surgeons interested in warm cardioplegia. The authors used magnetic resonance spectroscopy (MRS) to assess myocardial pH and high-energy phosphates during intermittent warm cardioplegia and reperfusion. MRS is a revolutionary assessment tool that allows noninvasive quantification of tissue metabolites *in vivo*.¹ However, a number of cautions must be exercised in the interpretation of the results of this study. The authors used blood-perfused isolated canine hearts. The tolerance of normal canine myocardium to interrupted cardioplegia may differ, as the authors suggest, from that of a heart with coronary disease and preoperative ischemia. In this study, an intraventricular balloon was used to assess ventricular function. The authors found a significant increase in left ventricular end-diastolic pressure and a decrease in diastolic function after cardioplegic interruptions of 20 or 30 minutes. This alteration in diastolic compliance renders the assessment of systolic function difficult. They found a decrease in end-systolic elastance after 30 minutes of cardioplegic interruption. Maximal elastance was not measured. A better measure of cardiac function would have been preload recruitable stroke work, which could have been calculated from pressure-volume loops. Unfortunately, the authors did not assess this measure of cardiac function.

Their evaluation of cardiac high-energy phosphates and myocardial acidosis provides clear evidence of progressive ischemia during cardioplegic interruptions. These results suggest cardiac surgeons should limit warm cardioplegic interruptions to less than 30 minutes. Altering the method of delivering cardioplegic solutions may decrease cardioplegic interruption times. In our experience, optimal visualization of distal anastomoses required interruption of warm antegrade cardioplegia for 10 to 20 minutes in 90% of patients, corresponding to 42% of the crossclamp

period.² The use of warm retrograde blood cardioplegia, in contrast, decreased cardioplegic interruptions to only 27% of the duration of crossclamping. This does not necessarily mean, however, that retrograde cardioplegia results in less ischemia. Retrograde cardioplegia may decrease cardioplegic interruption time simply by decreasing tissue perfusion, which in turn leads to decreased effluent from coronary arteriotomies and improved visualization of the anastomosis. Indeed, retrograde cardioplegia results in increased levels of inorganic phosphate and decreased levels of creatine phosphate compared with antegrade cardioplegia.³ Using a combination of antegrade and retrograde cardioplegia (i.e., simultaneous cardioplegia administration retrograde through the coronary sinus and antegrade through saphenous vein grafts) may be the optimal method of reducing the duration of cardioplegic interruption without significantly impairing visibility.⁴ In addition, lowering the temperature of the cardioplegic solution from 37° C to 29° C ("tepid" cardioplegia) may reduce myocardial oxygen requirements and improve the preservation of myocardial metabolism and ventricular function.⁴ These strategies may minimize the deleterious effects of cardioplegic interruption during cardiac surgery.

We have used arterial and coronary sinus serum samples to evaluate myocardial metabolism during cardioplegic delivery. We measured lactate production after resumption of antegrade or retrograde cardioplegia after interruptions of up to 15 minutes (unpublished data). Interruption of antegrade warm blood cardioplegia results in moderate lactate washout with resumption of cardioplegia; the magnitude of this lactate production is not related to the duration of cardioplegic interruption. In contrast, interruption of retrograde warm blood cardioplegia results in greater lactate production at all times, compared with antegrade cardioplegia, and lactate production and oxygen consumption increase significantly after only 7 minutes (implying washout of lactate accumulated during anaerobic metabolism, and repayment of an oxygen debt).

In addition to metabolic measurements, we have also assessed clinical outcomes during warm blood cardioplegia. A randomized clinical trial comparing normothermic and hypothermic blood cardioplegia was performed in 1732 patients at the University of Toronto.⁵ It demonstrated a significant reduction in the incidence of low cardiac output syndrome and perioperative enzymatic myocardial infarction in patients randomized to warm blood cardioplegia. The effects of cardioplegic interruption was subsequently assessed post hoc in 720 patients derived from the warm treatment arm.⁶ Intermittency was analyzed as cumulative ischemic time as a percentage of total crossclamp time, or as the single longest ischemic interval. Longest ischemic time (highest quartile > 13 minutes) was an important predictor of adverse cardiac outcomes (mortality, myocardial infarction, or low output syndrome, $p = 0.053$), but no consistent relationship was identified for cumulative ischemic time. We concluded that repeated interruptions of warm blood cardioplegia followed by sufficient cardioplegic reinfusion is a safe method of myocardial protection provided single interruptions are 13 minutes or less. Dr. Carias de Oliveira and colleagues used a model of ischemia with 15 minutes of reperfusion between each ischemic episode. Although the length of interruptions is compatible with clinical practice

(average longest ischemic interval 11.4 ± 4.0 minutes for 732 patients from the Warm Heart Trial),⁶ 15 minutes of reperfusion may represent an ideal rather than reflect routine practice. Additional studies testing shorter periods of reperfusion would provide valuable information that may more closely mimic typical surgeon behavior. It would appear from closer inspection of the first panel of Fig. 2, in conjunction with other animal data,⁷ that shorter periods of reperfusion would also be acceptable.

In summary, this is a well-designed study assessing the effects of warm intermittent blood cardioplegia. The results suggest that 15 minutes of repeated warm ischemia is safe, but that cardioplegic interruptions of 30 minutes are probably harmful. This length of time is clinically feasible in the majority of coronary bypass operations, particularly if combinations of antegrade and retrograde cardioplegia are used. Our myocardial metabolic and clinical data would also suggest that this length of warm ischemic time is well tolerated in coronary bypass surgery.

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*Dr. Carias de Oliveira and colleagues should cite our reference 6 in their manuscript, rather than the abstract that was cited (reference 14 in their manuscript).