CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

ASPARTATE/GLUTAMATE-ENRICHED BLOOD DOES NOT IMPROVE MYOCARDIAL ENERGY METABOLISM DURING ISCHEMIA-REPERFUSION: A ^{31}P MAGNETIC RESONANCE SPECTROSCOPIC STUDY IN ISOLATED PIG HEARTS

Hooman R. Ghomeshi, BSc^a
Ganghong Tian, MD, PhD^a
Jian Ye, MD^a
Jiankang Sun, MSc^a
Edward F. Hoffenberg, MSc^a
Tomás A. Salerno, MD, MSc^b
Roxanne Deslauriers, PhD^a

Objective: Our objective was to test the effects of exogenous L-aspartate and L-glutamate on myocardial energy metabolism during ischemia-reperfusion. **Methods:** Phosphorus 31-magnetic resonance spectroscopy was used to observe cellular energetics and intracellular pH in isolated pig hearts perfused with blood (group A, $n = 8$) or blood enriched with 13 mmol/L each of L-aspartate and L-glutamate (group B, $n = 6$). The hearts were subjected to 30 minutes of total normothermic ischemia and then reperfused for 40 minutes. Two hearts from each group were inotropically stimulated by titration with calcium after normokalemic reperfusion. Left ventricular function was measured with the use of a compliant balloon and oxygen consumption was calculated. **Results:** Magnetic resonance spectroscopy showed no decrease in the rate of energy decline during ischemia for group B versus group A. No significant differences were observed between the two groups in terms of myocardial function, oxygen consumption, or the rate or extent of high-energy phosphate recovery after normokalemic reperfusion or inotropic stimulation. Inotropic stimulation of postischemic hearts, however, led to dramatic improvement in myocardial function in both groups ($p < 0.05$ for all parameters) and significant improvement in oxygen consumption ($p = 0.01$). **Conclusions:** In a normal, isolated, blood-perfused pig heart subjected to 30 minutes of total normothermic ischemia, (1) enrichment of the perfusate with aspartate/glutamate before and after ischemia affects neither myocardial energy metabolism during ischemia-reperfusion nor postischemic recovery of myocardial function or oxygen consumption and (2) inotropic stimulation can recruit significant postischemic function and sufficient aerobic respiration to support it, irrespective of aspartate/glutamate enrichment. (J Thorac Cardiovasc Surg 1997;113:1068-80)

Since the introduction of substrate enhancement by Lazar and coworkers^{1,2} in 1980, the benefits of L-aspartate and L-glutamate in cardioplegic solutions have been controversial. Nevertheless, aspar-

tate/glutamate-enriched blood cardioplegic solutions are used during elective and emergency cardiac operations,³⁻⁵ with possible potential for use during cardiac harvesting⁶ and neonatal cardiac surgery.⁷

We have used phosphorus 31 (^{31}P) magnetic

From the Institute for Biodiagnostics, National Research Council of Canada, Winnipeg, Manitoba, Canada,^a and the Department of Cardiothoracic Surgery, State University of New York at Buffalo, Buffalo, N.Y.^b

Supported by grants from the Medical Research Council of Canada. Partial support for Hooman R. Ghomeshi was provided by the Natural Sciences and Engineering Research Council of Canada.

Read at the Seventy-sixth Annual Meeting of The American Association for Thoracic Surgery, San Diego, Calif., April 28-May 1, 1996.

Received for publication May 6, 1996; revisions requested June 18, 1996; revisions received Jan. 2, 1997; accepted for publication Feb. 13, 1997.

Address for reprints: Dr. Roxanne Deslauriers, Institute for Biodiagnostics, National Research Council of Canada, 435 Ellice Ave., Winnipeg, Manitoba, Canada R3B 1Y6.

Copyright © 1997 by Mosby-Year Book, Inc.

0022-5223/97 \$5.00 + 0 12/6/81120

resonance (MR) spectroscopy to investigate the effects of exogenous aspartate/glutamate on myocardial adenosine triphosphate (ATP), phosphocreatine (PCr), inorganic phosphate (Pi), and intracellular pH in isolated pig hearts subjected to 30 minutes of total normothermic ischemia and 40 minutes of reperfusion.

Materials and methods

This study was performed in accordance with Canadian Council on Animal Care guidelines and approved by the Animal Care Committee at the Institute for Biodiagnostics.

Solutions. St. Thomas' Hospital II solution (used to arrest hearts during isolation) contained (in millimoles per liter) NaCl 100, NaHCO₃ 25, KCl 26, MgCl₂ 16, and CaCl₂ 1.2; the pH was adjusted to 7.4 by HCl. Modified Krebs-Henseleit solution (for topical hypothermia and hemodilution) for group A (the control group) contained (in millimoles per liter) NaCl 118, MgSO₄ 1.2, NaH₂PO₄ 1.2, Na₂ ethylenediaminetetraacetic acid 0.5, D-glucose 11, CaCl₂ 1.75, NaHCO₃ 25, and bovine serum albumin 5 gm/L. For group B (aspartate/glutamate) the Krebs-Henseleit NaCl content was lowered to 25 mmol/L.

Heart isolation. The isolated, blood-perfused pig heart preparation has been described in detail previously.⁸ Domestic pigs (40 to 55 kg) were acclimatized for 2 weeks, fasted overnight, and premedicated with an intramuscular injection of ketamine hydrochloride (25 mg/kg), midazolam (400 µg/kg), and atropine sulfate (0.05 mg/kg). Surgical anesthesia was induced and maintained with isoflurane (1.5% to 2.0%) in 50% oxygen (in air). After a median thoracotomy, the hearts were isolated and arrested with cold St. Thomas' Hospital II solution. Cold Krebs-Henseleit solution was used for topical hypothermia in the chest. After excision, the hearts were fitted with a compliant latex balloon (unstressed volume >50 ml) in the left ventricle (LV) and a 1.0 ml round-bottom flask containing phenyl phosphonic acid, a ³¹P-MR intensity reference, in the right ventricle. A catheter was then loosely placed inside the coronary sinus (for collection of venous blood) and secured to the wall of the right atrium.

Blood perfusate preparation. After heart excision, blood collecting in the chest was removed by suction and Krebs-Henseleit solution was used to adjust the hematocrit value to 20% to 25% (normal pig blood hematocrit value is about 30%). In both groups, KCl was omitted from the Krebs-Henseleit to compensate for any rise in [K⁺] from residual mixing of cardioplegic solution with the blood during cardioplegic infusion. In group B, low-NaCl Krebs-Henseleit solution was used to lower the total [Na⁺] below 120 mmol/L to allow for addition of 13 mmol/L each of monosodium aspartate and monosodium glutamate (from 1.0 molar stock solution). The final [Na⁺] was adjusted to 140 ± 5 mmol/L. The final [K⁺] was adjusted to 4.5 ± 0.5 mmol/L. The osmolarity of the prepared perfusate was similar in all experiments and comparable with that of the pig's own blood (270 to 280 mOsm/L).

Perfusion system. The perfusion system was designed to minimize prime volume (<400 ml). Blood was pumped from the reservoir with the aid of a Cobe Precision Blood Pump (Cobe Laboratories, Inc., Lakewood, Colo.) through a hollow-fiber oxygenator (Capiiox 308, Terumo Corp., Tokyo, Japan) followed by a 20 µm arterial blood filter (D735, Dideco, Mirandola, Italy). The arterial line entered the Faraday cage housing the MR magnet through a special radiofrequency filter and connected to the brachiocephalic artery cannula. Venous blood was collected at the bottom of the MR probe container and returned to the reservoir by suction. The system allowed blood to recirculate independently of the arterial line, to prevent excessive settling or clotting during periods of ischemia. Anticoagulation was maintained by an additional bolus of heparin (5000 IU) every hour. Arterial blood temperature was maintained at 37° C and blood gas partial pressures as follows: oxygen tension greater than 200 mm Hg and carbon dioxide tension between 35 and 45 mm Hg.

Heart perfusion. Hearts were perfused through the brachiocephalic artery cannula, with the subclavian artery cannula initially being used to remove air from the filling aorta. The subclavian artery cannula and the LV balloon were then connected to pressure transducers (Cobe), which were connected to a multichannel polygraph recorder (Gould, Valley View, Ohio). The arterial flow was increased gradually over a 5-minute period and adjusted to maintain the perfusion pressure at 60 mm Hg until initiation of data acquisition, after which constant flow rate was maintained (about 1.0 to 1.5 ml/gm heart tissue). During initial warming, the hearts were defibrillated as required. Once stable, the heart preparation was placed inside the MR probe and inserted into the bore of the MR magnet. The LV balloon was gradually filled with filtered water to achieve a stable diastolic pressure of 0 to 5 mm Hg, after which the balloon volume remained constant.

Experimental protocol. Control hearts (group A, *n* = 8) were perfused with blood without added substrate. Aspartate/glutamate hearts (group B, *n* = 6) were perfused with blood enriched with 13 mmol/L each of L-aspartate and L-glutamate (dose commonly suggested to be beneficial experimentally^{9,10} and clinically^{3,5}). All hearts were perfused for 20 minutes (control perfusion), subjected to 30 minutes of total normothermic ischemia (heart temperature decreased during ischemia to 32° to 35° C), and then reperfused for 40 minutes with the respective perfusate used for each group (stage I, Fig. 1).

To assess possible causes for the observed contractile dysfunction, we subjected two hearts from each group to inotropic stimulation with calcium after stage I reperfusion. The perfusate [Ca⁺⁺] was gradually increased (over 20 minutes) until myocardial function reached a plateau. Heart function was allowed to stabilize for 10 minutes and data were collected (stage II) for an additional 10 minutes (total 40 minutes).

Data acquisition. Once the intraventricular balloon had been filled, MR magnet shim values were adjusted where necessary, after which continuous acquisition of ³¹P-MR spectra was initiated. Functional data from the LV balloon were collected continuously throughout the protocols. Arterial and venous (from coronary sinus)

Fig. 1. Outline of experimental protocols. *Asp*, Aspartate; *glu*, glutamate.

blood samples were taken every 10 minutes to measure myocardial oxygen consumption (MVO_2). At the end of each protocol, LV biopsy samples were taken for quantification of energy metabolites by high performance liquid chromatography. A piece of LV tissue was taken to determine the dry/wet weight ratio.

MR spectroscopy methods. MR spectroscopy was performed in a 40 cm horizontal bore magnet at 7.0-tesla with the use of a Bruker Biospec spectrometer (Bruker, Karlsruhe, Germany). The heart was suspended inside a home-built MR probe consisting of a radiofrequency coil surrounding a glass container. After the coil had been tuned and the ^1H signal used to optimize the magnetic field homogeneity, the coil was switched to a frequency of 121.47 MHz for acquisition of ^{31}P spectra. Free induction decay signals were obtained using 2 K data points and a sweep width of 12 KHz. Single radiofrequency pulses were used with a pulse length and repetition time of 80 μsec and 2 seconds, respectively. Sixty free induction decay signals were accumulated for each spectrum (2-minute time resolution).

Data analysis. Accumulated free induction decay signals were transferred to in-house software (Xprep), Fourier transformed, and phase and baseline corrected. The spectra were subjected to 20 Hz line broadening by exponential multiplication of the time function and transferred to another in-house analysis software package (X-Allfit), where they were fitted with Lorentzian curves and quantified using the integrals of the curve. To compensate for changes in spectral intensity during experiments, peak integral values for the β -ATP, PCr, and Pi peaks were corrected to the reference (phenyl phosphonic acid) peak. The β peak was used to quantify ATP. Intracellular pH was determined from the relative positions of the Pi and PCr peaks.¹¹ All data are presented as means $\pm$ standard errors of the means. Statistical analyses were performed with the use of Microsoft Excel 5.0 and Statistica 5.0 (Statsoft, Tulsa, Okla.) software. Student's *t* test and the Mann-Whitney U test were used to compare data between groups. The paired Student's *t* test was used to compare data across time within groups.

Results

Stage I

Myocardial energy phosphates

MR SPECTROSCOPY. Fig. 2 shows representative ^{31}P -MR spectra from a control heart (group A). The spectra show that PCr levels decrease immediately

on initiation of ischemia, Pi levels increase, and the three ATP peaks decrease slightly. In addition, the Pi peak shifts upfield (i.e., toward the PCr peak), indicating decreased intracellular pH. By the end of 30 minutes of normothermic ischemia, the PCr peak has all but vanished, the Pi peak is very large and shifted significantly owing to acidification of the medium, and the ATP peaks are significantly reduced in size. These parameters appear to return to near normal levels during the first few minutes of reperfusion, with the exception of the ATP peaks, which show only modest recovery.

The rate and extent of energetic changes throughout ischemia and reperfusion are similar in the two groups (Fig. 3). The only apparent difference between the two groups is in levels of Pi during control perfusion, which was most likely due to difficulties in fitting of the Pi peak created by its overlap with peaks arising from phosphomonoesters and phosphodiesteres (see Fig. 2). Comparison of ATP, PCr, and Pi levels in the two groups indicates that aspartate/glutamate enrichment of the perfusate did not decrease the rate of energetic decline during ischemia or improve energy production upon reperfusion (Table I).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. The ATP and PCr content in LV biopsies, normalized to total tissue creatine, are similar in the two groups (Table II). Using ATP concentrations measured by high performance liquid chromatography and the initial and final ATP levels from MR spectroscopy, the preischemic ATP concentration is estimated at $28 \pm 3 \mu\text{mol/gm}$ and $26 \pm 1 \mu\text{mol/gm}$ dry weight for groups A and B, respectively. These values are in good agreement with those obtained for pig hearts in other studies.¹²

Intracellular pH. Myocardial intracellular pH during control perfusion averaged 7.22 ± 0.15 and 7.30 ± 0.07 for groups A and B, respectively. The changes in intracellular pH during the protocols are shown in Fig. 4 and summarized in Table I.

Fig. 2. Representative ^{31}P -MR spectra obtained from a blood-perfused pig heart during control perfusion, normo-thermic ischemia, and reperfusion. The main resonances are from phenyl phosphonic acid (PPA, spectrum intensity reference), phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiester (PDE), phosphocreatine (PCr), and the three phosphate groups (α , β , and γ) of adenosine triphosphate (ATP). The chemical shift is expressed as parts per million (PPM) relative to the PCr peak.

Fig. 3. Changes in myocardial energy phosphates during ischemia-reperfusion. *Asp/Glu*, Aspartate/glutamate; *ATP*, adenosine triphosphate; *PCr*, phosphocreatine; *Pi*, inorganic phosphate. *Time points* represent average metabolites over a 2-minute period. *Data points* represent means $\pm$ standard errors of the means.

Myocardial function, oxygen consumption, and edema. In all hearts, postischemic recovery of LV rate-pressure product (RPP), rate of systolic increase in ventricular pressure, and rate of diastolic decrease in ventricular pressure were poor relative to preischemic values (Table III, Fig. 5). As seen in

Table I. Changes in myocardial energy phosphates and intracellular pH during ischemia-reperfusion

	Control perfusion	End of ischemia	Initial reperfusion	End of reperfusion
Group A (control) (<i>n</i> = 8)				
ATP	100 ± 3	47 ± 7	65 ± 4	66 ± 5
PCr	260 ± 22	75 ± 21	298 ± 23*	318 ± 24*
Pi	171 ± 21	463 ± 72	182 ± 21	194 ± 22
pHi	7.22 ± 0.15	6.33 ± 0.16	7.18 ± 0.16	7.20 ± 0.07
Group B (asp/glu) (<i>n</i> = 6)				
ATP	100 ± 1	49 ± 4	63 ± 3	65 ± 4
PCr	233 ± 13	46 ± 14	310 ± 29*	334 ± 29*
Pi	116 ± 7	396 ± 29	155 ± 8	170 ± 9
pHi	7.30 ± 0.07	6.44 ± 0.19	7.28 ± 0.11	7.35 ± 0.09

ATP, Adenosine triphosphate; PCr, phosphocreatine; Pi, inorganic phosphate; pHi, intracellular pH; asp/glu, aspartate/glutamate. ATP, PCr, and Pi are expressed as a percentage of initial ATP levels. The values shown are averages over a 10-minute period (6 minutes for pHi) at the indicated stage of the protocol. There were no significant differences between groups ($p > 0.05$).

* $p < 0.01$ with respect to control perfusion

Table II. High-energy metabolites measured by HPLC at the end of stage I reperfusion

	ATP ($\mu\text{mol/gm dry wt}$)	ATP (% total Cr)	PCr ($\mu\text{mol/gm dry wt}$)	PCr (% total Cr)
Group A (control)	17.0 ± 1.7	10.1 ± 0.5	106 ± 6	62.7 ± 4.5
Group B (asp/glu)	15.1 ± 0.6	9.4 ± 0.6	104 ± 7	63.8 ± 2.9

HPLC, High performance liquid chromatography; ATP, adenosine triphosphate; PCr, phosphocreatine; Cr, creatine. Total creatine is equal to the sum of PCr and Cr. All data are presented as mean ± standard error of the mean. There were no significant differences between groups ($p > 0.05$).

Table III. Preischemic myocardial function and oxygen consumption

	RPP (mm Hg/min)	+dP/dt (mm Hg/sec)	-dP/dt (mm Hg/sec)	MVO ₂ (ml O ₂ /100 gm/min)	RPP/MVO ₂ (mm Hg/ml)
Group A (control)	10900 ± 1300	1220 ± 90	1040 ± 100	5.5 ± 1.4	1100 ± 200
Group B (asp/glu)	12100 ± 800	1200 ± 90	1090 ± 80	6.3 ± 1.1	950 ± 90

RPP, Rate-pressure product; +dP/dt, rate of contraction; -dP/dt, rate of relaxation; MVO₂, myocardial oxygen consumption; asp/glu, aspartate/glutamate. All data are presented as mean ± standard error of the mean. There were no significant differences between groups ($p > 0.05$).

Table III, aspartate/glutamate did not affect preischemic MVO₂ or the efficiency of conversion of chemical energy to mechanical function (RPP/MVO₂). Postischemic recovery of these parameters (Fig. 6) was similar in the two groups.

The total water content at the end of reperfusion was similar for the two groups ($p = 0.33$), with a dry weight/wet weight of 16.1% ± 0.4% and 17.6% ± 1.3% for groups A and B, respectively.

Stage II. Tracings of cardiac LV pressure before and after titration with Ca⁺⁺ show that LV developed pressure and the rate of contraction and relaxation increased dramatically in stage II (Figs. 7 and 8). These improvements in myocardial function remained relatively stable for the duration of heart perfusion.

Stimulation of the hearts resulted in considerable improvements in MVO₂ and in the efficiency of conversion of chemical energy to mechanical function (RPP/MVO₂) (Fig. 9). Although the improvements in MVO₂ and RPP/MVO₂ were significant ($p = 0.03$ for both) only for the control group, the lack of significance in the aspartate/glutamate group is due to the small number of hearts. When the data for the control and aspartate/glutamate inotropically tested hearts are combined ($n = 4$), MVO₂ (as percent of control) is 52 ± 7 and 86 ± 7 ($p = 0.01$), whereas RPP/MVO₂ is 59 ± 7 and 102 ± 10 ($p = 0.01$) for stage I and stage II, respectively. The MR spectroscopy spectra showed no change in ATP (66 ± 3 vs 66 ± 2) or Pi (144 ± 5 vs 150 ± 5) after titration with Ca⁺⁺ (Fig. 10). Although PCr levels

Fig. 4. Myocardial intracellular pH during ischemia-reperfusion as measured from ^{31}P -MR spectra. Each *data point* represents average pH values over a 6-minute period. *Data points* represent means $\pm$ standard errors of the means. No statistically significant differences were observed between the groups ($p > 0.05$) at any time points. *Asp/Glu*, Aspartate/glutamate.

decreased from 307 ± 9 to 273 ± 8 ($p = 0.008$), PCr levels in stage II were still higher than the preischemic levels in Table I. These observations are consistent with high performance liquid chromatography data from stage I ($n = 10$) and stage II ($n = 4$), showing no change in ATP/total creatine (9.6 ± 0.4 vs 10.7 ± 0.4 , $p = 0.14$), but a decrease in PCr/total creatine (63.4 ± 1.1 vs 54.8 ± 2.7 , $p = 0.04$). Intracellular pH in stage II was maintained at 7.23 ± 0.02 (see Table I for control values), negating the presence of significant anaerobic metabolism during stage II.

Discussion

Interpretation of results. Several mechanisms have been suggested by which aspartate/glutamate may exert beneficial effects on myocardial energetics.¹³ During ischemia, aspartate and glutamate may protect myocytes by contributing to anaerobic energy production through substrate-level phosphorylation in the citric acid cycle. Through transfer of reducing equivalents across the mitochondrial membrane, aspartate/glutamate may also enhance glycolysis during ischemia by maintaining a supply of oxidized nicotinamide adenine dinucleotide in the

Fig. 5. Postischemic recovery of LV functional parameters. *Asp/Glu*, Aspartate/glutamate; RPP, rate-pressure product; $+dP/dt$, rate of systolic increase in ventricular pressure; $-dP/dt$, rate of diastolic decrease in ventricular pressure. *Data points* represent means $\pm$ standard errors of the means. No statistically significant differences were observed between the groups ($p > 0.05$).

cytoplasm. Stimulation of glycolysis may also have an “oxygen sparing” effect by diverting substrate selection away from fatty acids. In addition, during ischemia-reperfusion, aspartate/glutamate could

Fig. 6. Postischemic recovery of myocardial oxygen consumption (MVO_2) and the efficiency of conversion of chemical energy to mechanical work (RPP/MVO_2). Data points represent means $\pm$ standard errors of the means. No statistically significant differences were observed between the groups ($p > 0.05$). *Asp/Glu*, Aspartate/glutamate.

stimulate anaplerosis and improve the potential for aerobic respiration on reperfusion.

Our hypothesis was that the mechanisms listed herein should improve the energy profile of the myocardium during ischemia or reperfusion and that continuous observation of high-energy phosphate levels should detect any beneficial effects of aspartate/glutamate. Hearts in this study were subjected to 30 minutes of total normothermic ischemia, resulting in extensive depletion of high-energy phosphates and poor recovery of MVO_2 and contractile function. The severity of ischemia is confirmed by the significant decline in intracellular pH. The choice of ischemic insult was such that improvements in these parameters with aspartate/glutamate should have been detected.

Both groups of hearts showed minimal recovery of ATP after ischemia but rapid recovery of PCr (see Table I),¹⁴ indicating good mitochondrial function. In addition, significant recovery of myocardial function, MVO_2 , and RPP/MVO_2 was observed in response to inotropic stimulation, suggesting that poor contractile function and MVO_2 during stage I reperfusion were caused by inability of the contractile machinery to use the available chemical energy, possibly because of a decrease in calcium sensitivity of the myofibrils.¹² These results show that low postischemic MVO_2 is not necessarily indicative of lack of potential for efficient aerobic respiration, and decreased myocardial function is not necessarily due to energy depletion. Therefore the use of agents (such as aspartate/glutamate) intended to improve aerobic respiration during reperfusion may be misguided in instances in which poor contractile function or MVO_2 does not represent an inability to produce energy.

The aspartate/glutamate controversy. Numerous studies have been conducted on aspartate/glutamate enrichment and related topics. Some studies using aspartate- and/or glutamate-enriched cardioplegic solutions have reported extraordinary benefits,^{3, 4, 9, 15, 16} whereas other studies have failed to show any benefit whatsoever.¹⁷⁻¹⁹ Yet others observed only short-lived benefits^{20, 21} or attributed any benefits to coincident changes in perfusate ionic composition.²² Similar disagreements are found among studies using aspartate/glutamate-enriched perfusates without cardioplegia.^{1, 23-25} Confusion is also prevalent on specific issues relating to aspartate/glutamate. For example, Wiesner and associates²⁶ showed that flow deprivation in dog hearts causes reductions in tissue glutamate levels but has no effect on aspartate. On the other hand, a study by Pisarenko and coworkers²⁷ correlated postischemic recovery of function and energy status in rat hearts with maintenance of tissue aspartate, not glutamate, an observation that contradicts other studies, including a previous clinical study headed by Pisarenko and colleagues.²⁸

The variability in experimental results relating to aspartate/glutamate enrichment may stem largely from variations in experimental design. These variations include choice of experimental species and model, extent and type of ischemic insult, composition of the perfusate and reperfusate, concentration of amino acid used, temperature, experimental parameters, diagnostic tools, and methods of data analysis. Two such factors have contributed significantly to the conflicting results in the literature. First

Fig. 7. Representative polygraph tracing showing myocardial function in postischemic hearts before (stage I) and after (stage II) inotropic stimulation. The hearts showed dramatic recovery of functional parameters, which was sustained for the duration of high Ca^{++} perfusion. LV, Left ventricular.

Fig. 8. Improvements in postischemic recovery of myocardial function after inotropic stimulation (stage II). Error bars represent standard errors of the means. * $p < 0.05$ with respect to stage I. Asp/Glu, Aspartate/glutamate.

Fig. 9. Improvements in myocardial oxygen consumption (MVO_2) and the efficiency of conversion of chemical energy to mechanical work (RPP/MVO_2) with inotropic stimulation (stage II). Error bars represent standard errors of the means. * $p = 0.01$ with respect to stage I. Asp/Glu, Aspartate/glutamate.

is the lack of adequate controls in many studies. For example, Galiñanes and coworkers²² have shown the importance of correcting for coincidental changes in ionic composition of cardioplegic solutions when using salts of amino acids, a factor overlooked in many studies.^{2, 9, 10, 29} Second is the choice of diagnostic tools and parameters. The failure to provide a solution to the aspartate/glutamate controversy may be largely due to a lack of adequate tools for direct observation of metabolic events throughout an episode of ischemia-reperfusion.

The MR approach. MR spectroscopy allows direct, continuous observation of metabolic events

noninvasively and nondestructively. In this study, we used ^{31}P -MR spectroscopy to observe myocardial energy metabolism and found that a 13 mmol/L concentration of aspartate and glutamate in the perfusate did not affect the energetic status of pig hearts during ischemia or reperfusion. However, we did not gain any insight into the metabolic reactions involving aspartate/glutamate. Such information could have been provided by carbon 13 (^{13}C) MR techniques.

Fig. 10. Changes in myocardial energy phosphates after 30 minutes of inotropic stimulation (titration with calcium). *ATP*, Adenosine triphosphate; *PCr*, phosphocreatine; *Pi*, inorganic phosphate. Error bars represent standard errors of the means.

^{13}C -MR spectroscopy is well-suited to the study of myocardial metabolism, without the need for assuming metabolic or isotopic steady state. Jessen and coworkers²³ recently used ^{13}C isotopomer analysis to study the effects of a 5 mmol/L concentration of aspartate/glutamate on substrate selection and anaplerosis in rat hearts subjected to 25 minutes of total normothermic ischemia. The results showed no effects of aspartate/glutamate on functional recovery, substrate selection, or anaplerosis. In fact, these investigators did not detect any metabolism whatsoever of ^{13}C -labeled aspartate or glutamate. These observations are consistent with the results of the current study and suggest that under certain conditions, exogenous aspartate/glutamate may not be able to enter the cell or be incorporated into biochemical pathways.

Limitations of the study. The pig heart was chosen for this study because, with the exception of primates, it most closely resembles the human heart. Nevertheless, application of the findings to the

clinical situation requires caution: First, the hearts used for this study were initially normal. Diseased hearts may have altered metabolism of aspartate/glutamate³⁰ and thus could respond differently to aspartate/glutamate enrichment. Second, an MR-compatible modified Langendorff heart was used. It is possible that energetic requirements of a working model heart would be higher, and thus its response to substrate enrichment would be different. Third, the MR probe used for this study acquired spectra from the whole heart. It is conceivable that aspartate/glutamate could have had some effects on specific small regions of the heart, but that the effect was not large enough to be detected in the whole-heart spectra. Fourth, ^{31}P -MR spectroscopy cannot distinguish between cytosolic or mitochondrial ATP. Aspartate/glutamate may stimulate production of ATP by way of substrate-level phosphorylation in the mitochondria and protect the mitochondria without significant effects on overall cellular energetics.²⁴ Finally, the extent of functional recovery of the hearts may have been limited by the length of the reperfusion period (40 minutes).

The efforts of the following persons have been critical to the success of the study. Shelley Gernscheid, AHT, and Lori Gregorash, AHT, BSc, provided technical assistance, and Lori Shoemaker, BSc, carried out biochemical analyses. All statistical analyses were performed by, or in consultation with, Randy Summers, MSc. Our most sincere appreciation to all of them.

REFERENCES

1. Lazar HL, Buckberg GD, Manganaro AJ, Becker H, Maloney JV Jr. Reversal of ischemic damage with amino acid substrate enhancement during reperfusion. *Surgery* 1980;88:702-9.
2. Lazar HL, Buckberg GD, Manganaro AM, Becker H. Myocardial energy replenishment and reversal of ischemic damage by substrate enhancement of secondary blood cardioplegia with amino acids during reperfusion. *J Thorac Cardiovasc Surg* 1980;80:350-9.
3. Allen BS, Buckberg GD, Kirsh MM, Popoff G, Beyersdorf F, Fabiani JN, et al. Superiority of controlled surgical reperfusion versus percutaneous transluminal coronary angioplasty in acute coronary occlusion. *J Thorac Cardiovasc Surg* 1993;105:864-84.
4. Beyersdorf F, Kirsh M, Buckberg GD, Allen BS. Warm glutamate/aspartate-enriched blood cardioplegic solution for perioperative sudden death. *J Thorac Cardiovasc Surg* 1992;104:1141-7.
5. Buckberg GD, Beyersdorf F, Allen BS, Robertson JM. Integrated myocardial management: background and initial application. *J Card Surg* 1995;10:68-89.
6. Tixier D, Matheis G, Buckberg GD, Young HH. Donor hearts with impaired hemodynamics: benefit of warm substrate-enriched blood cardioplegic solution for induction of

- cardioplegia during cardiac harvesting. *J Thorac Cardiovasc Surg* 1991;102:207-14.
7. Kofsky E, Julia P, Buckberg GD, Young H, Tixier D. Studies of myocardial protection in the immature heart. V. Safety of prolonged aortic clamping with hypocalcemic glutamate/aspartate blood cardioplegia. *J Thorac Cardiovasc Surg* 1991;101:33-43.
 8. Tian G, Xiang B, Butler K, Camazzola D, Calafiore A, Mezzetti A, et al. Effects of intermittent warm blood cardioplegia on myocardial high energy phosphates, intracellular pH, and contractile function: a ^{31}P NMR study in isolated rat and pig hearts. *J Thorac Cardiovasc Surg* 1995;109:1155-63.
 9. Allen BS, Okamoto F, Buckberg GD, Bugyi H, Young H, Leaf J, et al. Immediate functional recovery after six hours of regional ischemia by careful control of conditions of reperfusion and composition of reperfusate. *J Thorac Cardiovasc Surg* 1986;92:621-35.
 10. Rosenkranz ER, Okamoto F, Buckberg GD, Robertson JM, Vinten-Johansen J, Bugyi HI. Safety of prolonged aortic clamping with blood cardioplegia. III. Aspartate enrichment of glutamate blood cardioplegia in energy-depleted hearts after ischemia in reperfusion injury. *J Thorac Cardiovasc Surg* 1986;91:428-35.
 11. Garlick PB, Radda GK, Seeley PJ. Studies of acidosis in the ischaemic heart by phosphorus nuclear magnetic resonance. *Biochem J* 1979;184:547-54.
 12. Kupriyanov V, St. Jean M, Xiang B, Butler KW, Deslauriers R. Contractile dysfunction caused by normothermic ischemia and KCl arrest in the isolated pig heart: a ^{31}P NMR study. *J Mol Cell Cardiol* 1995;27:1715-30.
 13. Svedjeholm R, Hakanson E, Vanhanen I. Rationale for metabolic support with amino acids and glucose-insulin-potassium (GIK) in cardiac surgery. *Ann Thorac Surg* 1995; 59(2 Suppl):S15-22.
 14. Kaplan LJ, Bellows CF, Carter S, Blum H, Whitman GJR. The phosphocreatine overshoot occurs independent of myocardial work. *Biochimie* 1995;77:245-8.
 15. Stein DG, Permut LC, Drinkwater DC Jr, Bhuta S, Chang P, Wu A, et al. Complete functional recovery after 24-hour heart preservation with University of Wisconsin solution and modified reperfusion. *Circulation* 1991;84(5 Suppl):III316-23.
 16. Weldner PW, Myers JL, Miller CA, Arenas JD, Waldhausen JA. Improved recovery of immature myocardium with L-glutamate blood cardioplegia. *Ann Thorac Surg* 1993;55: 102-5.
 17. Crooke GA, Harris LJ, Grossi EA, Baumann FG, Esposito R, Spencer FC, et al. Role of amino acids and enhancement cardioplegia in routine myocardial protection: experimental results. *J Thorac Cardiovasc Surg* 1993;106:497-501.
 18. Frierson JH, Penn MS, Lafont AM, Kultursay H, Marwick TH, Kottke-Marchant K, et al. Effect of Buckberg cardioplegia and peripheral cardiopulmonary bypass on infarct size in the closed chest dog. *J Am Coll Cardiol* 1992;20:1642-9.
 19. Axelrod HI, Galloway AC, Murphy MS, Laschinger JC, Grossi EA, Baumann FG, et al. A comparison of methods for limiting myocardial infarct expansion during acute reperfusion—primary role of unloading. *Circulation* 1987;76(5 Pt 2):V28-32.
 20. Asai T, Grossi EA, Leboutillier ML, Parish MA, Baumann FG, Spencer FC, et al. Resuscitative retrograde blood cardioplegia: Are amino acids or continuous warm techniques necessary? *J Thorac Cardiovasc Surg* 1995;109:242-8.
 21. Bonchek LI, Burlingame MW, Vazales BE, Ferdinand NJ. Coronary bypass with substrate-enhanced cardioplegia versus non-cardioplegic technique for early revascularization in acute infarction. *Eur J Cardiothorac Surg* 1990;4:124-9.
 22. Galiñanes M, Chambers DJ, Hearse DJ. Effect of sodium aspartate on the recovery of the rat heart from long-term hypothermic storage. *J Thorac Cardiovasc Surg* 1992;103: 521-31.
 23. Jessen ME, Kovarik TE, Jeffrey FM, Sherry AD, Storey CJ, Chao RY, et al. Effects of amino acids on substrate selection, anaplerosis, and left ventricular function in the ischemic reperfused rat heart. *J Clin Invest* 1993;92:831-9.
 24. Bittl JA, Shine KI. Protection of ischemic rabbit myocardium by glutamic acid. *Am J Physiol* 1983;245:H406-12.
 25. Bush LR, Warren S, Mesh CL, Lucchesi BR. Comparative effects of aspartate and glutamate during myocardial ischemia. *Pharmacology* 1981;23:297-304.
 26. Wiesner RJ, Deussen A, Borst M, Schrader J, Grieshaber MK. Glutamate degradation in the ischemic dog heart: contribution to anaerobic energy production. *J Mol Cell Cardiol* 1989;21:49-59.
 27. Pisarenko OI, Rosenfeldt FL, Langley L, Conyers RA, Richards SM. Differing protection with aspartate and glutamate cardioplegia in the isolated rat heart. *Ann Thorac Surg* 1995;59:1541-8.
 28. Pisarenko OI, Portnoy VF, Studneva IM, Arapov AD, Korostylev AN. Glutamate-blood cardioplegia improves ATP preservation in human myocardium. *Biomed Biochim Acta* 1987;46:499-504.
 29. Haan CK, Lazar HL, Rivers S, Coady C, Shemin RJ. Improved myocardial preservation during cold storage using substrate enhancement. *Ann Thorac Surg* 1990;50:80-5.
 30. Mudge GH Jr, Mills RM Jr, Taegtmeyer H, Gorlin R, Lesch M. Alterations of myocardial amino acid metabolism in chronic ischemic heart disease. *J Clin Invest* 1976;58:1185-92.

Discussion

Dr. Bradley S. Allen (*Chicago, Ill.*). You have used a very elegant model to examine the effects of aspartate and glutamate, but I have several problems with your conclusions. First, most investigators have administered aspartate and glutamate either as a continuous infusion or as an additive to cardioplegic solutions. In addition, no magic bullet exists; in other words, if aspartate or glutamate is simply added to unmodified blood containing high calcium and low osmolarity, the beneficial effects of amino acid supplementation may be negated. Therefore my first question is this: Why didn't you use a cardioplegic solution, inasmuch as this is where most of the beneficial effects of aspartate and glutamate have been demonstrated, and do you think this may have changed your results?

Mr. Ghameshi. Yes, we are aware that many studies on aspartate/glutamate enrichment have used cardioplegic solutions. The literature in this area is not only extensive, but also very inconclusive, because of significant inconsistencies from one study to the next. These inconsistencies concern the overall effects of aspartate/glutamate, as well as the specifics. In fact, there is disagreement over prac-

tically everything pertaining to these amino acids, the details of which I will not discuss here.

In this study, we were interested in looking at the independent effects of aspartate/glutamate on myocardial energy metabolism during ischemia-reperfusion. Thus, to avoid the multiplicity of factors that may have caused some of the confusion in the literature, we decided to use aspartate and glutamate alone, without cardioplegia. Our study is not unique in testing the effects of aspartate/glutamate without cardioplegia. Several previous studies, including the original aspartate/glutamate article by Dr. Lazar in 1980, have used aspartate/glutamate without cardioplegia and have reported benefits. We therefore expected that, using this model, we would be able to see any beneficial effects from aspartate/glutamate, but we were unable to see any effects.

I do, however, agree that using cardioplegic ischemia might have produced different results. The rate of energy decline during cardioplegic ischemia is significantly lower than that which was observed with noncardioplegic ischemia in this study. It is therefore possible that aspartate and glutamate could have had very slight effects on myocardial energetics that might have been better detected had we used a cardioplegic solution.

Dr. Allen. In all of our previous studies, we always gave aspartate/glutamate continuously to maintain a constant level. However, you used an isolated heart preparation and administered these amino acids only once. Therefore the aspartate/glutamate you gave could have been metabolized, resulting in declining levels throughout the study. Did you measure levels to determine whether the aspartate/glutamate concentration remained adequate during your measurements? If these levels indeed declined, could this not have been partly responsible for your results?

Mr. Ghomeshi. I agree that it is possible that the levels of aspartate/glutamate in the perfusate declined during our protocol. However, the volume of perfusate used (2.5 to 3.0 L) was large compared with the size of the hearts (approximately 200 gm), which would make a rapid decline in aspartate/glutamate concentration unlikely. In addition, most studies on aspartate/glutamate agree that they have no effects on the myocardium during periods of normal perfusion. Therefore it is safe to say that the aspartate/glutamate levels in the perfusate should not have been affected during the initial perfusion period, eliminating this as an explanation for lack of beneficial effects during ischemia. It is, however, theoretically possible that aspartate/glutamate levels declined during the reperfusion period, thus preventing us from seeing beneficial effects that might otherwise have been observed.

Dr. Allen. An isolated heart preparation is not the same as the clinical setting or an intact experimental model. For instance, an isolated heart preparation has no noncoronary collateral flow. This can yield results different from those obtained in the clinical setting. For instance, high glucose solutions have been shown to be detrimental when given in an isolated heart preparation, whereas in clinical cardioplegic solutions they have been shown to be beneficial. Could the use of your model have explained your findings?

Mr. Ghomeshi. It absolutely could. This study was in an isolated heart model, and it was in the pig. Obviously,

applicability of our conclusions to the clinical setting, or to an in vivo model, needs further study and could be very limited.

Dr. Allen. I noticed that you added calcium to obtain a steady state in stage II of your experiment. Did the different groups require different amounts of calcium? In other words, were certain hearts more or less sensitive to calcium and were the final ionized calcium levels the same? If hearts in different groups had different responses or needed different levels of calcium to achieve the same response, they may have indeed been different.

Mr. Ghomeshi. Unfortunately, I cannot answer that question, because we tested only two hearts per group with calcium. Had we expected the results obtained in the study, we would have used inotropic testing in all hearts, but we did not. I thus cannot comment on what the results might have been had we used a full group of inotropic stimulation with or without aspartate/glutamate.

Dr. Allen. Last, the majority of investigations have demonstrated increased oxygen uptake during administration of aspartate/glutamate cardioplegic solutions, with 100% recovery of myocardial function, even after an ischemic insult. This increased oxygen uptake usually lasts for only the first several minutes of cardioplegic administration and then returns to basal requirements. Not only did we see this experimentally, but in a recent clinical study reported in the *Annals of Surgery* we saw an increased oxygen uptake with aspartate/glutamate cardioplegia even in hemodynamically stable patients undergoing routine coronary surgery. Once again, the increased oxygen uptake was back to baseline after several minutes. Conversely, you examined your oxygen uptakes after a much longer time interval. Did you measure oxygen uptake early during the administration of aspartate/glutamate? If so, did oxygen uptake differ between groups? Did you measure the oxygen uptake across the myocardium during the first few minutes of reperfusion, or did you only look at oxygen uptake at 15 and 25 minutes after reperfusion?

Mr. Ghomeshi. MVO₂ and functional recovery were presented here after 5 minutes, 15 minutes, 25 minutes, and 35 minutes of reperfusion. Therefore postischemic MVO₂ was measured relatively early, but not before 5 minutes of reperfusion.

Dr. Harold L. Lazar (Boston, Mass.). [Slide] This slide shows the oxygen uptake in dog hearts after 45 minutes of normothermic ischemic arrest in a study that was presented before this Association 16 years ago. After aortic unclamping, hearts were either kept in the beating state on bypass, the so-called unmodified group, or arrested for 5 minutes with either blood cardioplegia or a glutamate-enriched blood cardioplegic solution.

After aortic unclamping, the unmodified group showed the characteristic depression in oxygen uptake that was seen in this present study and is indicative of an injured postischemic heart. In contrast, both the cardioplegia groups showed a significant increase in oxygen uptake, the most significant being in the cardioplegia group that was supplemented with glutamate. Subsequently, both the cardioplegia and glutamate groups had more complete recovery of ATP and both systolic and diastolic function. The best recovery was seen in the glutamate group.

Since that study, numerous clinical studies have been conducted, as you have alluded to, which showed the benefits of glutamate in patients with angina, congestive heart failure, and as a cardioplegic additive to patients in cryogenic shock and after cardiac transplantation. In view of these results, what are the mechanisms that you think are responsible for the inability of glutamate and aspartate to improve both the metabolic and ventricular function in your study? Dr. Allen has alluded to the fact that you used an isolated preparation with a beating empty heart. The energy requirements of an intact working heart are much higher than in an isolated preparation. Do you think that substrate enhancement would be less likely to be effective in your model, which is essentially a nonworking reperfusion model?

Mr. Ghomeshi. The various mechanisms suggested in the literature for beneficial effects from aspartate/glutamate should all lead to the common effect of improving myocardial energy levels during either ischemia or reperfusion. These energy levels were measured continuously in this study, but no effects were observed. Nevertheless, this study by itself cannot give information regarding potential mechanisms, specific metabolic pathways, or involvement of aspartate and glutamate in these pathways.

[Slide] In 1993, however, Jessen and coworkers reported using ^{13}C -MR techniques to provide some insight into aspartate/glutamate metabolism during ischemia-reperfusion. ^{13}C -MR spectroscopy allows studying myocardial metabolic pathways without assumptions of metabolic or isotopic steady state. Using isolated rat hearts, 25 minutes of total normothermic ischemia, and a 5 mmol/L concentration of aspartate/glutamate before and after ischemia, these investigators were unable to detect any effects on myocardial function, on substrate selection for the Krebs cycle, or on anaplerosis (pathways that replenish Krebs cycle intermediates). In fact, using ^{13}C -labeled aspartate or glutamate, they were unable to detect any metabolism of these amino acids whatsoever. These results, together with those of the current study, suggest that at least under certain circumstances, which, as Dr. Lazar points out, could be those of using an isolated heart, exogenous aspartate and glutamate may not be able to enter the cells. Whether that in any way applies to some *in vivo* models or to certain clinical situations, I cannot say.

The second question was regarding the use of a working model. It is true that the working model would use more energy than an isolated Langendorff model. Therefore it is possible that if we had used a working model, the energy consumption would be higher and we might have been able to see benefits from aspartate and glutamate. Using MR involves technical limitations that make it difficult to use what might perhaps be the most relevant model. In this case, we used the best model we could find that was also relatively simple to do in combination with MR.

Dr. R. Svedjeholm (Linköping, Sweden). Inasmuch as the heart has a capacity to use a wide range of substrates, it implies that under many conditions the addition of extra substrates may have little influence on the metabolic and hemodynamic function. Therefore it would be surprising to find one substrate that in every experimental condition improved the metabolic and hemodynamic function of the heart. On the other hand, I am sure Mr. Ghomeshi would

agree that the heart needs substrates to survive and produce energy. In Mr. Ghomeshi's own study, for example, the control hearts were provided substantial amounts of amino acids and carbohydrate substrates from normal blood.

Mr. Ghomeshi's study has too many limitations to make any conclusions regarding the role of glutamate/aspartate for the ischemic and postischemic human heart. These include the choice of species, the use of an isolated heart preparation, the use of "healthy hearts" not subjected to chronic bouts of ischemia, and the study design, to name a few. Dr. Kimose from Denmark published a paper in 1993 demonstrating transient myocardial leakage of glutamate early after cardioplegic arrest in the isolated pig heart. This implies that part of Mr. Ghomeshi's study may have been conducted under conditions when uptake of glutamate was not to be expected.

As for species and adaptation to ischemia, several investigators, including Mudge, Thomassen, and Pisarenko, have shown that in human beings the single most characteristic metabolic feature of chronic ischemic heart disease is the increased uptake of glutamate and the increased release of alanine.

However, the most important limitation of Mr. Ghomeshi's study is the use of the isolated heart preparation. The impact of systemic metabolic and immunologic changes are not accounted for. As for systemic metabolic changes, available data from human beings suggest that the combined effects of systemic metabolic changes and metabolic changes in the heart resulting from ischemia make amino acids important for postischemic recovery of myocardial metabolism. The explanation for this is that Krebs cycle intermediates lost during ischemia are replenished primarily by carbohydrates and amino acids. As a result of the systemic metabolic changes, the uptake of glucose and other carbohydrates is impeded and, hence, the relative importance of amino acids increased. Moreover, high catecholamine levels in conjunction with elevated free fatty acid levels contribute to oxygen wasting and accumulation of toxic free fatty acid metabolites, thereby providing an unfavorable metabolic environment for the ischemic and postischemic heart. Available data from human beings undergoing coronary artery bypass grafting procedures demonstrate that glutamate uptake precedes recovery of myocardial metabolism. Furthermore, uptake of glutamate seems to be limited by substrate availability, that is, arterial levels of glutamate. Accordingly, Pisarenko demonstrated enhanced metabolic and hemodynamic recovery by intravenous glutamate infusion in patients with heart failure after cardiac operations. In a recent study from Linköping, we were able to emulate these results after routine coronary bypass procedures. Moreover, Thomassen demonstrated improved myocardial tolerance to ischemia in coronary patients during exercise testing and pacing by intravenous glutamate administration. Thus a number of independent studies in human beings suggest an important role for glutamate during and after ischemia.

To conclude, I have some questions for Mr. Ghomeshi. First, the hearts in the control group were perfused with blood that I assume contained substantial amounts of substrate, including glutamate. How much glutamate and what alternate substrates were used by the control hearts?

Second, Mr. Ghomeshi uses the term *preischemic level*, although the hearts have been subjected to excision during crystalloid cardioplegic arrest. We would consider this a postischemic condition; therefore, the degree of recovery reported may not be related to the true preischemic level. Would Mr. Ghomeshi like to comment on this? Third, the number of hearts studied was few; for instance, only two hearts in each group were subjected to intervention with calcium. How did Mr. Ghomeshi arrive at these sample sizes?

Mr. Ghomeshi. I would like to thank Dr. Svedjeholm for his interesting comments on this paper. Dr. Svedjeholm's first question is regarding any measurements of substrate use by control hearts. Unfortunately, the extraction of substrates from the perfusate by hearts was not measured in our study. The principal purpose of this study was to determine whether substrate enrichment of normal blood with aspartate and glutamate could provide any additional benefits to the ischemic-reperfused hearts. The parameters used were myocardial function and oxygen consumption but, more important, a direct measurement of myocardial high-energy phosphate levels (from MR spectroscopy) during the entire protocol. Therefore, in the absence of demonstrated beneficial effects of using such a direct measure of energy metabolism, measurements of substrate use by the hearts would be of little relevance to the study's purpose.

Dr. Svedjeholm next questions our use of the term *preischemic level* in presenting the data. I would like to

clarify that the terms *preischemic level* and *preischemic value* in this paper refer to measurements taken during the period of control perfusion at the start of the experimental protocol (see Fig. 1). These terms are used instead of the term *control* solely to prevent confusion with data from control hearts (group A). Despite the excision of hearts using cold crystalloid cardioplegia, as Dr. Svedjeholm points out, these "control" measurements may be different from what the true preischemic values may have been. Nevertheless, as all hearts were subjected to the same excision technique, it is unlikely that the comparative functional or metabolic recovery of hearts in any one group would change if reported relative to "true" preischemic levels. The interpretation of results is therefore unaffected by our use of terminology.

The last question concerns the sample sizes in the study. The intended sample size for each of groups A and B was eight, based on previous studies using similar models. Because of the significant expenses involved, experiments in group B were stopped at six, as it became clear that aspartate and glutamate had no effects whatsoever on any of the parameters studied. As for inotropically tested hearts, despite the small numbers, the changes induced with calcium are dramatic enough to allow the conservative conclusions made based on these data. Clearly, as I am sure Dr. Svedjeholm would agree, statistical analysis of any data set depends more on the quality and type of the data than on the mere number of experiments.

Bound volumes available to subscribers

Bound volumes of The Journal of Thoracic and Cardiovascular Surgery are available to subscribers (only) for the 1997 issues from the Publisher, at a cost of \$110.50 for domestic, \$139.64 for Canadian, and \$130.50 for international subscribers for Vol. 113 (January-June) and Vol. 114 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue of the volume. The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone 800-453-4351 or 314-453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular Journal subscription.