

# Evaluation of High-energy Phosphate Metabolism During Cardioplegic Arrest and Reperfusion: A Phosphorus-31 Nuclear Magnetic Resonance Study

ANNE-CATHERINE PERNOT, M.D., JOANNE S. INGWALL, Ph.D., PHILLIPPE MENASCHE, M.D.,  
CHRISTIAN GROUSSET, Ph.D., MICHEL BERCOT, M.D., ARMAND PIWNICA, M.D.,  
AND ERIC T. FOSSEL, Ph.D.

**SUMMARY** Hypothermic potassium cardioplegia is now commonly used to protect the myocardium during surgically induced ischemia. Because the potassium-related membrane depolarization has been shown to increase calcium influx, we undertook this study to define the effects of varying the calcium content in hyperkalemic perfusates and the effects of using magnesium instead of or in addition to potassium as the arresting agent on the ability of hearts to recover normal function after ischemic arrest. We subjected isolated perfused working rat hearts to 60 minutes of cardioplegic arrest followed by 30 minutes of reperfusion, and measured high-energy phosphate levels every 2½ minutes by phosphorus-31 nuclear magnetic resonance spectroscopy. These data were correlated with postischemic recovery of function. Our results show that potassium cardioplegia may be harmful when the calcium concentration is greater than 1 mM. The kalemic injury is significantly reduced when the calcium content is lowered to 0.25 mM and the greatest extent of preservation is provided by a calcium-poor perfusate (0.25 mM) containing 13 mM magnesium. The beneficial effects of magnesium are not enhanced by subsequent addition of potassium. Close correlations were found between all observed metabolic changes during arrest and the degree of recovery of contractile performance after reperfusion. We conclude that the ability of the myocardium to maintain or resynthesize high-energy phosphate after cardioplegic arrest may be an important determinant of postischemic mechanical performance. These results show that phosphorus-31 nuclear magnetic resonance spectroscopy is a valuable method for evaluating interventions to reduce the severity of ischemic damage.

PRESERVATION of the myocardium during surgically induced ischemic arrest is commonly achieved through a combination of hypothermia and administration of cardioplegic solutions. The requirement for immediate arrest has been emphasized.<sup>1</sup> Studies have documented that significant utilization of high-energy phosphates occurs during the brief period of electromechanical activity between the onset of ischemia and onset of asystole.<sup>2</sup> The most widely used cardioplegic agent is potassium chloride. Although at the concentrations generally used (30 mM), authentic contracture is unlikely to occur,<sup>3</sup> hyperkalemically mediated calcium entry into the cell could result in increased myocardial wall tension and, consequently, increased ATP breakdown during asystole.<sup>4</sup> We undertook the present study to assess the effects of calcium in hyperkalemic perfusates and the effects of magnesium and potassium, alone and in combination, on high-energy phosphate metabolism during hypothermic cardioplegic arrest.

Generally, such a problem requires extensive analysis of freeze-clamped myocardial tissue, from which metabolites are extracted for analysis. This is a destructive and tedious process that offers ample opportunity for the introduction of artifacts.

Phosphorus-31 nuclear magnetic resonance (P-31 NMR) spectroscopy has been used as a "noninvasive" method of providing serial measurements of high-energy phosphate content of isolated, intact, beating, perfused hearts and, at the same time, of obtaining measurements of mechanical performance.<sup>5</sup> We used this method to study an isolated, perfused, working rat heart model subjected to 60 minutes of hypothermic ischemic arrest followed by 30 minutes of normothermic reperfusion.

## Methods

### Heart Perfusion

The preparation used for these studies was a modification of the working rat heart model of Neely et al.<sup>5,6</sup> The oxygenated perfusion fluid entered the cannulated left atrium at a pressure of 14 mm Hg. The aortic and coronary flow rates were recorded at timed intervals. Aortic pressure was monitored by a pressure transducer connected to a Hewlett-Packard four-channel recorder. The cardioplegic solutions were infused through a side arm on the aortic cannula at a pressure of 60 mm Hg.

The circulating fluid was Krebs-Henseleit bicarbonate buffer (pH 7.40 at 37°C) supplemented with 11 mM glucose and gassed with 95% carbon dioxide. The perfusate  $P_{O_2}$  was thus maintained over 60 cm Hg. Before use, the perfusion was filtered through a 0.45-μ Millipore filter.

### P-31 Nuclear Magnetic Resonance Spectroscopy

NMR experiments were performed on a Nicolet NT-360 wide-bore spectrometer operating at a phosphorus frequency of 145.75 MHz. Spectra (fig. 1) were ob-

From the Departments of Medicine and Physiology and Biophysics, Harvard Medical School, and Brigham and Womens Hospital, Boston, Massachusetts; and the Service de Chirurgie Cardio-Vasculaire and INSERM U-127, Hôpital Lariboisière, Paris, France.

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Address for correspondence: Eric T. Fossel, Ph.D., Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115.

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tained using 2K data points and 30° radiofrequency pulses every 0.8 second. Use of a 30° pulse instead allows more rapid repetition of the pulse without introduction of signal saturation. Two hundred to 400 free induction decays (FIDs) were signal averaged, taking 2.5 or 5 minutes, and the resulting accumulated FID was Fourier transformed to produce spectra as shown in figure 1. These data represent time averages of the phosphate content of the heart during these time periods. The sequential spectra taken during a protocol were all acquired automatically and stored on the computer storage disc. After the experiment, the data were processed and analyzed. Concentrations of phosphorus-containing metabolites were determined by integration of the P-31 resonance areas. Under the conditions that spectra were acquired (using 30° pulses), saturation of all resonances was avoided; therefore, equal resonance areas represent equal concentrations of metabolites.

In this study, NMR determinations focused on the concentrations of inorganic phosphates (Pi), creatine phosphate and adenosine triphosphate (ATP). The concentration of ATP was determined from the resonance of the beta phosphate, which is thought to be uncontaminated by resonances from any other phosphate. Resonance assignments were made from comparison with authentic samples and agreed with literature data.<sup>7</sup>

### Experimental Design

Male Sprague Dawley rats that weighed 400–600 g were anesthetized with intraperitoneally administered Nembutal (10 mg/100 g of body weight). Immediately after excision, the heart was connected to the aortic cannula and retrograde perfusion (Langendorff) was initiated for a 5-minute washout and equilibration period. During this interval, cannulation of the left atrium was completed. The preparation was converted to a working heart by initiating left atrial perfusion, and the heart, contained in an NMR tube (outside diameter 25 mm), was then placed into the magnet. After a 15-

minute control period, total ischemia was induced by clamping both cannulas. The heart was subjected to a 2-minute coronary infusion of the cardioplegic solution (at 4°C) under study. Ischemia was then maintained for 60 minutes. Although the hearts were perfused at 37°C during the control and recovery periods, the use of dual temperature circuits permitted perfusion with the cardioplegic solution at 4°C. Cardiac hypothermia was induced throughout ischemia by blowing 15°C dry air through the probe and over the NMR sample tube containing the heart. Rewarming of the heart chamber was begun 2.5 minutes before reperfusion and was completed by the time reflow was reinitiated. Reperfusion was initially instituted in the Langendorff mode for 1 minute to allow the residual cardioplegic solution to be washed out. Left atrial perfusion was then resumed for a 30-minute recovery period. During the entire experimental protocol, coronary effluent was continuously removed through a vacuum line in the NMR tube. Thus, any NMR signal originating from a phosphate-containing medium surrounding the heart was avoided.<sup>8</sup>

Hemodynamic studies were performed throughout the control (prearrest) period and at 3, 5, 10, 15, 20 and 30 minutes of reperfusion. NMR spectra were recorded at 2.5-minute intervals during the control period, the first 15 minutes of ischemia and the first 10 minutes of reperfusion. Spectra were collected at 5-minute intervals during the remaining experimental time course.

### Experimental Groups

The hearts from 30 rats were divided into five groups of six hearts each. In group 1 (control series), myocardial protection was achieved by hypothermia alone, without cardioplegia. In the four other groups, cardioplegic protection was used in addition to hypothermia. The composition of the cardioplegic solutions (table 1) can be divided into two categories: two hyperkalemic (30 mM) perfusates (groups 2 and 3), which differ in calcium content (the calcium-rich solution [1 mM calcium concentration] and the calcium-poor solution [0.25 mM calcium concentration]), and three calcium-poor perfusates, which contain different electrolytes (potassium in group 3, magnesium in group 4, and both potassium and magnesium in group 5). In group 5, a two-stage perfusion was used: the magnesium-containing solution was first infused for 30 seconds, immediately followed by addition of potassium (30 mM) to the perfusate. The sodium content was the same in all solutions (100 mM). Osmolarity was adjusted by addition of 37 mosmol of mannitol. All the

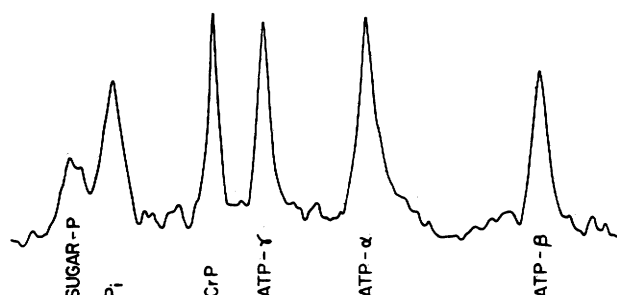


FIGURE 1. Phosphorus-31 nuclear magnetic resonance spectrum at 145.75 MHz in an isolated, perfused, working rat heart during the preischemic control period. This spectrum resulted from signal averaging 400 free induction decays resulting from 30° pulses repeated every 0.8 second. The signal-to-noise ratio was improved by multiplication of the accumulated free induction decay by a sensitivity-enhancing function (a decaying exponential), resulting in a line broadening of 25 Hz.  $P_i$  = inorganic phosphates; CrP = creatine phosphate; P = phosphates.

TABLE 1. Composition of the Cardioplegic Solutions

Group	Sodium (mM)	Potassium (mM)	Magnesium (mM)	Calcium (mM)	Mannitol (g/l)	Histidine (g/l)
2	100	30	0	1	12	10
3	100	30	0	0.25	12	10
4	100	4	13	0.25	12	10
5	100	30	13	0.25	12	10

solutions were buffered with histidine to pH 7.40 at 20°C. L-histidine has good buffering capacity at pH 6.0–8.0 at 20°C and remains stable over a wide range of temperatures.

Concentrations of high-energy phosphate compounds are expressed as a percentage of values found during preischemic or control periods. Similarly, values for the various measures of cardiac function were compared and expressed as a percentage of the control values. This method eliminates the effect of variability between individual hearts and allows the recovery of each variable to be related to the composition of the cardioplegic perfusate.

All results are expressed as the mean  $\pm$  SEM. Significance was determined by *t* test using  $p = 0.05$  as the limit of significance. Each heart served as its own control, obviating the requirement for analysis of variance.

## Results

### Changes in ATP Content

As expected, the ATP content in hearts of all groups declined throughout the ischemic period (figs. 2 and 3). Sequential NMR determinations showed that the rate of decline was more pronounced during the first 30 minutes of ischemia. The rate of ATP depletion was slow compared with hearts subjected to normothermic total ischemia, probably due to the hypothermic conditions of all experiments.

In comparing the effect of different interventions at the end of 1 hour of ischemia (table 2), it was found that the rates of ATP depletion were similar in groups 1 and 2. Thus, hyperkalemia, in the presence of 1 mM calcium, did not provide better preservation than hypothermia alone (fig. 2). Lowering the calcium content of

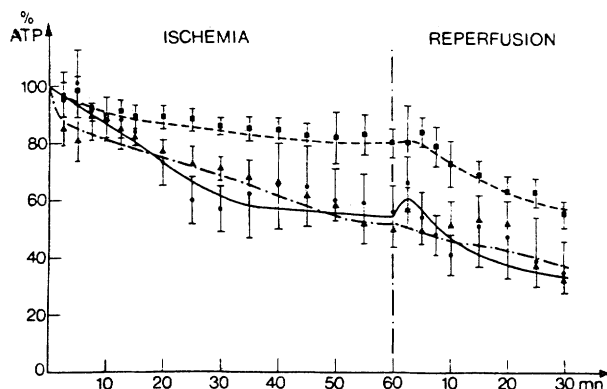


FIGURE 2. Influence of the calcium content of hyperkalemic perfusates on ATP levels. The concentration of ATP was determined from sequential spectra taken throughout the protocol. Initial ATP concentrations were  $25 \pm 2 \mu\text{mol/g}$  dry weight. Data are displayed as percentage of control. The protocol consisted of a 10-minute control period followed by 60 minutes of hypothermic (15°C) ischemia and 30 minutes of normothermic (37°C) reperfusion. ● = control group (hypothermia with no cardioplegia); ▲ = group 2 (potassium, calcium-rich); ■ = group 3 (potassium, calcium-poor). Each point is the mean of six hearts.

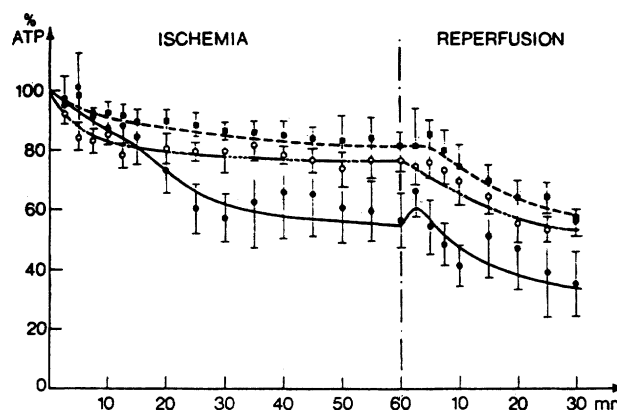


FIGURE 3. Comparative influence of potassium and magnesium in calcium-poor perfusates on ATP levels. The concentration of ATP was determined from sequential phosphorus-31 nuclear magnetic resonance spectra taken throughout a protocol consisting of a 10-minute control period followed by a 60-minute period of hypothermic (15°C) ischemia and a 30-minute period of normothermic (37°C) reperfusion. ● = control group (hypothermia with no cardioplegia); ■ = group 3 (potassium, calcium-poor); ○ = group 4 (magnesium, calcium-poor). Each point represents the mean of six hearts.

the perfusate from 1 mM to 0.25 mM, however, led to significantly higher ATP content after 60 minutes of ischemia ( $80 \pm 13\%$  of control in group 1 vs  $50 \pm 15\%$  in group 2,  $p < 0.01$ ). The rates of ATP depletion in hearts arrested with calcium-poor perfusate with potassium or magnesium are shown in figure 3. Replacing potassium with magnesium did not change the ATP content in hearts at the end of ischemia ( $80 \pm 13\%$  in group 3 and  $76 \pm 10\%$  in group 4). Similarly, the combination of potassium and magnesium failed to show any additive protection to that conferred by potassium or magnesium alone.

Upon reperfusion, ATP continued to decline in all groups. Compared with values after ischemia, the ATP content in hearts of all groups was 20–30% lower after 30 minutes of reflow. The largest decrease was in group 5 ( $75 \pm 9\%$  at the end of ischemia vs  $42 \pm 14\%$  at the end of 30 minutes of reperfusion,  $p < 0.001$ ).

### Changes in Creatine Phosphate Content

The creatine phosphate content also decreased during the ischemic period in all groups and differences between groups closely paralleled the differences observed for ATP (table 2). As observed for ATP, no significant difference was observed between groups 1 and 2.

Compared with all other groups, the creatine phosphate content at the end of ischemic arrest was highest for magnesium-containing, calcium-poor solution. This difference was statistically significant when compared with the “standard” potassium cardioplegia (group 4 vs group 2,  $p < 0.02$ ). At low calcium concentrations, there were no significant differences between creatine phosphate levels in hearts arrested with potassium alone, magnesium alone or a combination of both ( $28 \pm 9\%$ ,  $42 \pm 13\%$  and  $31 \pm 11\%$ , respec-



TABLE 2. High-energy Phosphate Content and Total Phosphate Resonance Areas (TPR) at End of Ischemic Arrest, After a 30-minute Reperfusion, and Recovery of Cardiac Output and Aortic Flow After a 30-minute Reperfusion

Group	End of ischemia (% of control)			End of reperfusion (% of control)			Cardiac output
	ATP	CrP	TPR	ATP	TPR	Aortic flow	
1 (Hypothermia)	55 ± 23	14 ± 1	88 ± 10	34 ± 23	66 ± 13	85 ± 5	92 ± 4
2 (K, Ca-rich)	50 ± 15	23 ± 7	89 ± 11	33 ± 12	67 ± 16	83 ± 8	89 ± 8
3 (K, Ca-poor)	80 ± 13	28 ± 9	101 ± 14	56 ± 13	76 ± 8	88 ± 3	93 ± 3
4 (Mg, Ca-poor)	76 ± 18	42 ± 13	90 ± 11	56 ± 14	80 ± 11	92 ± 5	96 ± 3
5 (Mg, Ca-poor, K)	75 ± 9	31 ± 11	98 ± 10	42 ± 14	75 ± 12	91 ± 3	94 ± 3

Abbreviations: CrP = creatine phosphate; TPR = total phosphate resonance area.

tively; NS). However, in contrast to the observation made for ATP, lowering the calcium content in the hyperkalemic perfusate did not improve creatine phosphate preservation after 60 minutes of ischemia ( $22 \pm 8\%$  in group 2 vs  $28 \pm 9\%$  in group 3). Perfusing with calcium-poor buffer protected against loss of creatine phosphate content compared with hypothermia alone (group 3 vs group 1,  $p < 0.01$ ), whereas the calcium-rich solution (group 2 vs group 1,  $p < 0.05$ ) did not.

Furthermore, in the three hypocalemic groups (3, 4 and 5), NMR spectra recorded during the early minutes of ischemia showed an initial rise of creatine phosphate up to 120% of the control values and a subsequent decline after approximately 5 minutes of ischemia. This pattern was not observed with the control hearts or hearts perfused with hyperkalemic calcium-rich perfusate. The calcium-poor perfusate might have caused rapid electromechanical inactivity and the resulting decreased energy expenditure might have resulted in a transient period of increased energy expenditure relative to demand. Although this hypothesis is speculative, a similar "overshoot" in creatine phosphate has been reported in the same experimental model after the rapid induction of cardiac arrest by sudden acidification of the perfusion solution.<sup>9</sup>

Upon reperfusion, creatine phosphate content immediately increased in all groups; its highest value was recorded on the first NMR spectrum obtained 2.5 minutes after resumption of flow. The greatest increases were found in groups 3 and 4 ( $97 \pm 16\%$  and  $92 \pm 10\%$  of control values, respectively). Recovery of creatine phosphate remained lower in the three other groups ( $63 \pm 12\%$  in group 1,  $70 \pm 12\%$  in group 2, and  $63 \pm 5\%$  in group 5).

#### Inorganic Phosphate Content

In all groups, Pi rose markedly throughout the ischemic period and abruptly fell upon reperfusion (fig. 4). The shape of the Pi curve closely followed the mirror image of the creatine phosphate curve. The peak value of Pi occurred during acquisition of the last NMR spectrum recorded immediately before resumption of flow. The magnitude of increase in Pi was not significantly different in groups 1, 2, and 3 ( $207 \pm 34\%$ ,  $199 \pm 33\%$ , and  $194 \pm 49\%$ , respectively). Pi accumulation was significantly lower with the magnesium-containing perfusate ( $153 \pm 31\%$ ,  $p < 0.05$  vs groups 1 and 2).

#### Total Phosphate Content

The quality of protection afforded by the magnesium-containing, calcium-poor perfusate was further documented by studying the total phosphate content of the heart tissue measured by summing the intensities of all phosphorus resonance areas (total phosphate resonance area, TPR). In all groups, TPR was lower at the end of the experimental protocol, suggesting a washout of phosphate from the heart. The rate of phosphate washout was minimal during ischemia (values of TPR recorded at the end of the ischemic period were not significantly different from control in any group), but significant phosphate washout occurred during reflow. As shown in table 2, the major loss of Pi was found in the hypothermic group, with TPR values averaging  $66 \pm 13\%$  of control after 30 minutes of reperfusion vs  $88 \pm 10\%$  after 60 minutes of ischemia ( $p < 0.05$ ); in the two hyperkalemic series (group 2:  $67 \pm 16\%$  vs  $89 \pm 11\%$ ,  $p < 0.05$ ; group 3:  $76 \pm 8\%$  vs  $101 \pm 14\%$ ,  $p < 0.05$ ).

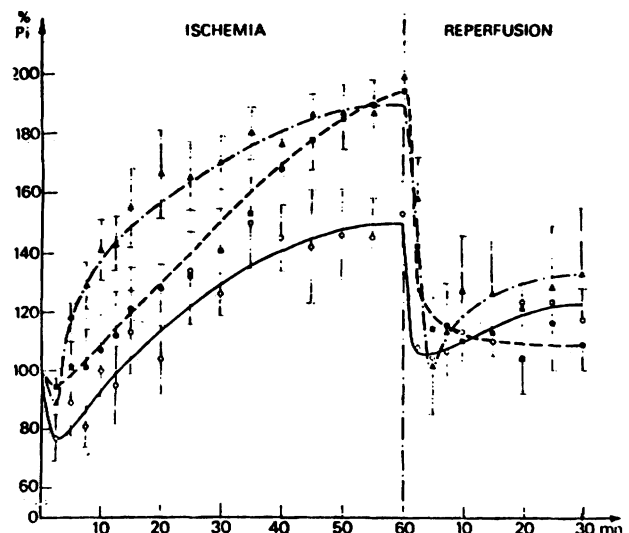


FIGURE 4. Comparative influence of potassium, calcium-poor perfusates on inorganic phosphate (Pi) levels. The concentration of Pi was determined from sequential phosphorus-31 nuclear magnetic resonance spectra taken throughout the same protocol described in figure 3.  $\Delta$  = group 2 (potassium, calcium-rich);  $\blacksquare$  = group 3 (potassium, calcium-poor);  $\circ$  = group 4 (magnesium, calcium-poor). Each point represents the mean of six hearts.

0.01); and in the potassium-magnesium group (group 5:  $75 \pm 12\%$  vs  $98 \pm 10\%$ ,  $p < 0.01$ ). In contrast, the magnesium-containing perfusate (group 4) resulted in the lowest rate of Pi washout, for values of TPR recorded at the end of reperfusion ( $80 \pm 11\%$ ) were not significantly different from those attained at the end of ischemia ( $90 \pm 11\%$ ).

### Hemodynamic Results

Hemodynamic characteristics of the isolated hearts are summarized in figures 5 and 6. The preischemic values of heart rate (HR), coronary flow rate (CFR), aortic flow rate (AFR), cardiac output (CO) and peak aortic systolic pressure (AP) were similar in all groups.

Upon reperfusion, all hearts defibrillated spontaneously within a few seconds. Throughout the recovery period, HR and CFR remained basically unchanged from control values in all series; however, postischemic values of AFR, CO and AP differed, depending on the experimental condition.

Comparison of all mechanical variables in groups 1 and 2 revealed that after 30 minutes of reperfusion there was no significant difference between them and that during the initial 15 minutes of reperfusion, the percentages of recovery of CO, AFR and AP were more depressed with the hyperkalemic calcium-rich solution than with hypothermia alone ( $p < 0.01$  for all three variables at 3, 5, 10 and 15 minutes). Lowering the calcium content of hyperkalemic perfusates produced a significant improvement in the initial recovery of mechanical function, since AFR remained statistically higher in group 3 than in group 2 up to 15 minutes after the onset of reperfusion ( $p < 0.05$  at 3 and 15

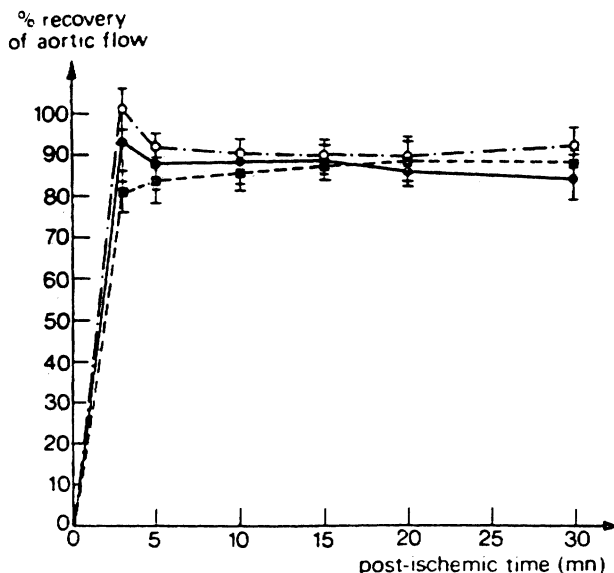


FIGURE 5. Influence of the calcium content in hyperkalemic perfusates on the recovery of the aortic flow after a 60-minute period of hypothermic ( $15^{\circ}\text{C}$ ) ischemia. ● = control group (hypothermia alone); ▲ = group 2 (potassium, calcium-rich); ■ = group 3 (potassium, calcium-poor). Each point represents the mean of six hearts.

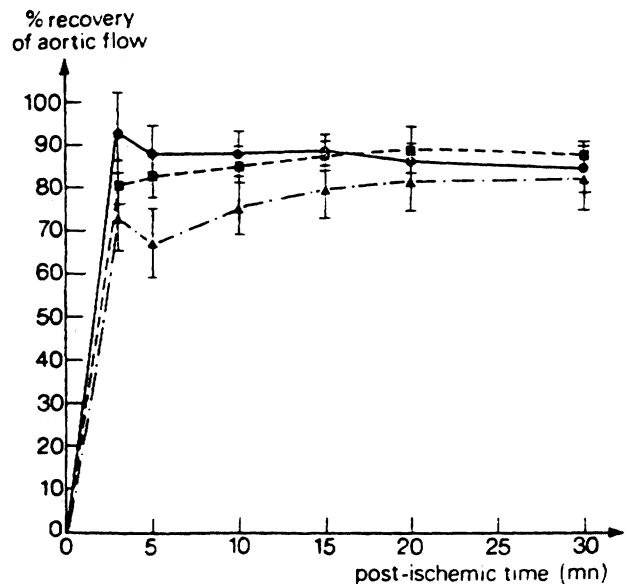


FIGURE 6. Comparative influence of potassium and magnesium in calcium-poor perfusates on the recovery of aortic flow after a 60-minute period of hypothermic ( $15^{\circ}\text{C}$ ) ischemia. ● = control group (hypothermia alone); ■ = group 3 (potassium, calcium-poor); ○ = group 4 (magnesium, calcium-poor). Each point represents the mean of six hearts.

minutes,  $p < 0.01$  at 5 and 10 minutes) and CO remained statistically higher in group 3 than in group 2 up to 20 minutes of reperfusion ( $p < 0.01$  up to 20 minutes and  $p < 0.05$  at 20 minutes). The magnesium-rich, calcium-poor cardioplegic solution provided the highest postischemic recovery of aortic flow ( $92 \pm 5\%$ ) and CO ( $96 \pm 3\%$ ) at 30 minutes (table 2).

The efficacy of the magnesium-induced protection was further evidenced by the recovery profile: peak values of CO, AFR and AP were recorded during the first minutes after reperfusion and subsequently remained stable throughout the reperfusion phase. Differences in these variables with the hyperkalemic calcium-rich perfusate (group 2) were highly significant at all intervals during the postischemic course ( $0.05 > p > 0.01$ ). In close correlation with NMR results, potassium addition failed to potentiate the magnesium-induced protective effects. In the setting of calcium-poor cardioplegic perfusates, comparison of potassium and magnesium groups revealed that recovery of CO, AFR and AP were similar at 30 minutes and that during the initial 5 minutes of reperfusion, cardiac function resumed more efficiently in the magnesium group, as documented by significantly higher CO and AFR recovery (group 4 vs group 3,  $p < 0.01$  at 3 minutes,  $p < 0.05$  at 5 minutes for both variables). Since the reperfusion conditions were similar in both groups, this finding suggests that there is minimal initial reperfusion injury after magnesium cardioplegia.

Considering data from all groups, strong correlations were found between NMR determinations of ATP levels at the end of ischemia and the percentage of

recovery of CO at 30 minutes of reperfusion ( $r = 0.93$ ) and AFR ( $r = 0.89$ ).

## Discussion

### P-31 Nuclear Magnetic Resonance Spectroscopy

Traditional techniques used to correlate biochemical variables with function in metabolically active tissues have serious limitations. For the perfused heart, such techniques include measurement of surface NADH fluorescence, comparison of coronary arteriovenous composition, and biochemical analysis of intracellular metabolites from biopsy specimens.<sup>10</sup> Although the latter approach has yielded critical information, it is limited because local injury prevents accurate measurement and interpretation of mechanical function.

Recently, P-31 NMR spectroscopy has been introduced as a new method for measurement of the content of phosphate metabolites in intact tissues.<sup>5, 11</sup> P-31 NMR has several favorable attributes for the study of cardiac metabolism: phosphate occurs in high concentration in only a few compounds; these compounds play important roles in the tissue's viability; and all naturally occurring phosphorus exists as P-31, allowing rapid acquisition of spectra with the use of modern Fourier transform signal-averaging techniques. The P-31 NMR data reported here represent spectra accumulation times of 2.5 or 5 minutes. NMR is a nondestructive method that allows kinetic analysis of high-energy phosphate changes associated with ischemia and reperfusion.

The causal relationship between high-energy phosphate compounds and mechanical function has been debated. Contractile failure after the onset of myocardial ischemia reportedly occurs when ATP content is only slightly reduced.<sup>12, 13</sup> These findings do not preclude the critical importance of maintaining high-energy phosphates at the highest level possible during ischemic arrest.<sup>14, 15</sup> By assaying ATP with either conventional quick freeze-clamping techniques or P-31 NMR, other investigators<sup>14, 15</sup> reported an almost linear relationship between ATP levels at the end of arrest and postischemic mechanical function. We also found a significant correlation between ATP concentrations after 60 minutes of hypothermic ischemia and the percentage of recovery of aortic flow rate after 30 minutes of reflow. In addition, we found a decrease in ATP content (and more generally, in total phosphorus resonance areas) upon reperfusion.<sup>16-18</sup> These observations provide indirect support for studies demonstrating that the ATP<sup>19</sup> or the total adenine nucleotide<sup>18</sup> content at the end of ischemia is an accurate predictor of the potential ATP resynthesis during reperfusion. This observation is particularly relevant because postischemic ATP concentration is one of the factors that correlated with the recovery of left ventricular function.<sup>17</sup>

### Protective Effects of Different Concentrations of Calcium in Cardioplegic Solutions

Since one of the most important considerations in

the design of a cardioplegic solution is its ability to cause rapid and complete cardiac arrest, potassium cardioplegia is now commonly used during clinical open heart surgery. However, the membrane depolarization induced by an elevated extracellular potassium concentration also increases calcium permeability and, hence, calcium influx,<sup>4, 20</sup> leading to increased phasic tension.<sup>21</sup> Results from Rich *et al.*<sup>4</sup> suggest that the large rise in internal calcium associated with prolonged kalemic depolarization greatly stimulates the energy-dependent sarcoplasmic reticulum (SR) calcium pump. Furthermore, given the functional limitation (or overloading) of the SR sequestering capacity in hypoxia,<sup>22, 23</sup> it is likely that a mitochondrial calcium accumulation also occurs,<sup>24</sup> for this energy-linked process is mainly determined by a rise in cytosolic calcium.<sup>9</sup> It is therefore probable that these energy-dependent calcium uptake mechanisms account for the high-energy phosphate breakdown and the subsequent depression in postischemic functional recovery observed in our group 2 (hyperkalemic, calcium-rich perfusate). Consequently, this cardioplegic approach failed to show any superiority over hypothermia alone, a finding consistent with experimental data from Engleman *et al.*<sup>25</sup> and recent clinical reports.<sup>26, 27</sup>

Lowering the calcium content (from 1 mM to 0.25 mM) in the hyperkalemic perfusate (group 3) significantly improved ATP levels at the end of ischemia. Upon reperfusion, mechanical properties of hearts protected with the potassium and calcium-poor solution recovered at a greater rate and to a greater extent than those supplied with the potassium and calcium-rich perfusate. Concomitantly, ATP levels were more depressed in group 2 than in group 3. This latter finding might be ascribed to a mitochondrial accumulation of calcium;<sup>28, 29</sup> or to a reduced amount of available substrate for ATP resynthesis, since the rate of Pi leakage was higher in group 2 (33%) than in group 3 (24%). Our results are thus consistent with Nierdergerke's<sup>3</sup> experiments showing that calcium enhances energy consumption at all levels of depolarization. They also closely agree with more recent results from Shine *et al.*<sup>30</sup> demonstrating a higher postanoxic recovery of developed tension in isolated septa exposed to low-calcium perfusates.

The critical role of a low calcium content in cardioplegic perfusates is also related to the maintenance of high external levels of sodium, for any rise in the extracellular potassium/sodium ratio increases the calcium influx<sup>4, 31</sup> and its subsequent mitochondrial uptake.<sup>32, 33</sup> Hence, maximal tension can be obtained even with little depolarization when the calcium/sodium ratio is increased.<sup>34</sup> Using creatine kinase release as an index of myocardial ischemic injury, Jynge<sup>35</sup> demonstrated that the protective effects of potassium or magnesium were optimally enhanced when the sodium concentration in the perfusate was 90–120 mM. All of our cardioplegic solutions contained 100 mM sodium.

Although our results have demonstrated the beneficial effects of hypocalcemic cardioplegic perfusates,



we did not evaluate a calcium-free solution to avoid the potential problems related to the "calcium paradox."<sup>36</sup>

### Comparison of Magnesium and Potassium

In an attempt to further reduce the calcium-related high-energy phosphate breakdown, we evaluated a calcium-poor solution containing magnesium (group 4). Extracellular magnesium rapidly enters the cell in rat myocardium.<sup>38</sup> Thus, the rationale for using magnesium instead of potassium includes the reduction of the passive magnesium loss which has been reported during myocardial ischemia<sup>37</sup> and, subsequently, the conservation of magnesium as an essential cofactor (as MgATP);<sup>14</sup> the calcium antagonist effects of magnesium, including reduction of the transsarcolemmal calcium influx,<sup>14</sup> decreases in mitochondrial calcium uptake, protection of mitochondrial phosphorylating mechanisms during active uptake of calcium, and inhibition of the external calcium-triggered calcium release from the SR, thus enhancing rapid asystole;<sup>32</sup> and a direct relaxing effect on myofibrils, which diminishes the demand for ATP.

Although the high-energy phosphate stores were preserved to the same extent after 60 minutes of ischemia in the potassium and in the magnesium groups, the superiority of magnesium cardioplegia was evidenced by an almost immediate functional recovery within the first minutes of reflow, whereas the potassium-treated hearts still remained significantly depressed; a lower peak value of Pi at the end of ischemia ( $153 \pm 31\%$  in the magnesium group vs  $193 \pm 49\%$  in the potassium group), which may reduce the detrimental trapping of calcium in the SR and mitochondria as calcium-phosphate precipitates;<sup>13</sup> and the greatest preservation of phosphate-containing moieties, since group 4 was the only experimental series in which TPR at the end of reperfusion was not significantly different from control.

Thus, our data support previous experimental<sup>14</sup> and clinical reports emphasizing the value of magnesium-induced preservation during cardiac ischemic arrest. Although differences between experimental groups 3 and 4 were statistically significant for only some of the variables measured, the results support use of magnesium instead of potassium to protect the ischemic myocardium.

### Additive Protective Effects of Potassium and Magnesium

A potential problem with the use of magnesium as a cardioplegic agent is that one of its basic calcium antagonist effects is a low level of depolarization compared with potassium. It was thus conceivable that cells might further depolarize during the ischemic period with a subsequent delayed calcium influx. We therefore investigated a two-stage cardioplegic perfusion: the calcium-poor solution containing magnesium was first injected into the coronary arteries to arrest the heart. We assumed that a significant amount of the sarcolemmal-bound calcium would be washed out by perfusate. Potassium was then added to the cardioplegic solution to complete the membrane depolarization.

However, because of the previously induced decrease in calcium availability, we expected that this depolarization would be predominantly related to the abolition of the transmembrane potassium gradient with only a minimal calcium influx. In fact, both biochemical and hemodynamic comparisons between groups 4 and 5 failed to demonstrate any advantage of adding potassium to magnesium perfusate. Magnesium-treated hearts remained completely motionless during the entire ischemic period. Our results thus differ from those of Hearse et al.<sup>14</sup> by showing additive protective effects of potassium and magnesium. The explanation of this discrepancy may be that their experiments were performed at normothermia, whereas the strict hypothermia in our protocol would account for the maintenance of the magnesium-induced depolarization throughout ischemia and the subsequent lack of further improvement after potassium addition.

Continuous monitoring of high-energy phosphate contents of the hearts during ischemic arrest and reperfusion by P-31 NMR shows that the tissue concentrations of ATP at the end of ischemic arrest is a correlate of postischemic recovery of function. This correlation, however, is over a relatively small range of ATP concentrations and functional variables. We have also demonstrated the value of lowering the concentration of calcium in the cardioplegic solution from 1 mM to 0.25 mM and of replacing potassium with magnesium as the arresting agent.

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A C Pernot, J S Ingwall, P Menasche, C Grousset, M Bercot, A Piwnica and E T Fossel

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