Nucleotide Degradation and Functional Impairment During Cardioplegia: Amelioration by Inosine

DONALD F. DEWITT, PH.D., KENNETH E. JOCHIM, PH.D., AND DOUGLAS M. BEHERENDT, M.D.

SUMMARY The degradation of adenine nucleotide levels and impairment of functional recovery associated with exposure to hypothermic (20°C) cardioplegia was studied in 84 isolated working rat hearts. After a 1-hour control period, hearts were exposed to 1 hour of cardioplegia that consisted of increasingly longer periods of cardiopulmonary solution (CPS) infusion (30 seconds and 10, 30 and 60 minutes), followed by increasingly shorter periods of global ischemia (59½ minutes and 50, 30 and 0 minutes). Hearts were then reperfused for 1 hour with control perfusate, during which recovery of cardiac output was monitored. Additional hearts were freeze-clamped at various points in the protocols to determine adenine nucleotide levels (ATP, ADP, AMP and their sum TAN). Exposure to increasingly longer periods of CPS perfusion resulted in proportionally greater degradation of nucleotides and poorer recovery of cardiac output. Inclusion of inosine in the CPS reduced the degradation of ATP and TAN and improved functional recovery. Addition of inosine to the recovery perfusate as well as the CPS further improved nucleotide levels and recovery of cardiac output. These results suggest that washout of nucleotide degradation products in the CPS or reperfusion prevents their salvage for nucleotide resynthesis and impairs functional recovery from cardioplegia.

PROTECTION of the myocardium during cardiopulmonary bypass and ischemic arrest is a major problem of cardiac surgery, and no ideal method has been found. Cold cardioplegic solutions (CPS) are widely used for this purpose, but the characterization of the ideal composition and method of administration is incomplete.

A major objective of myocardial protection is conservation of the adenine nucleotide pool during the ischemic period. At any moment, myocardial ATP content is a function of its rates of synthesis and degradation. Although hypothermic cardioplegia greatly reduces energy use during ischemia, ATP degradation still outstrips production and nucleotide degradation products such as adenosine, inosine and hypoxanthine accumulate in the myocardium. These constitute a purine base pool available for resynthesis of ATP during the postischemic recovery period. Failure to recover normal cardiac function after cardiopulmonary bypass correlates well with the inability to resynthesize normal levels of ATP. However, this may be a consequence of the loss of ATP precursors rather than

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a defect in the mechanisms of energy production (fig. 1).

The purpose of the present study was to evaluate the possibility that (1) inadequate resynthesis of ATP during reperfusion of isolated working rat hearts after perfusion with anoxic, hypothermic and substrate-free CPS is a consequence of the reduction of the total adenine nucleotide (TAN = ATP + ADP + AMP) pool; (2) reduction of ATP and TAN as a consequence of purine base loss during cardioplegia is associated with reduced functional recovery; and (3) supplementation of the CPS and recovery perfusate with inosine improves recovery of cardiac output by reducing ATP and TAN loss during cardioplegia and the recovery periods.

Methods

Experimental Preparation

Hearts from 84 Sprague-Dawley rats (200–300 g) were studied in an isolated working heart preparation similar to that described in detail by Neely and Rotto and Hearse and co-workers. The rats were anesthetized with 15 mg of sodium pentobarbital. The hearts were excised rapidly, dropped into ice-cold saline, and mounted on the perfusion cannula such that retrograde aortic perfusion (Langendorf perfusion) with oxygenated physiologic salt solution (PSS) (table 1) was initiated within 30 seconds. After a 15-minute period of Langendorf perfusion (used for left atrial cannulation and washout of blood elements), left atrial perfusion was initiated to begin a 60-minute control period during which the hearts ejected PSS through a 100-cm column (afterload) into a collection chamber

FIGURE 1. Myocardial adenine nucleotide degradation pathways. ATP, ADP, AMP = adenosine tri-, di- and monophosphate; IMP = inosine monophosphate.

<table>
<thead>
<tr>
<th>Component</th>
<th>PSS</th>
<th>CPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>117.0</td>
<td>91.6</td>
</tr>
<tr>
<td>KCl</td>
<td>4.7</td>
<td>14.8</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1.2</td>
<td>15.0</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>3.0</td>
<td>1.7</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>11.5</td>
<td>0</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>23.7</td>
<td>25.0</td>
</tr>
<tr>
<td>Inosine</td>
<td>±200.0</td>
<td>±200.0</td>
</tr>
<tr>
<td>Equilibrated with</td>
<td>95% O₂/5% CO₂</td>
<td>98% O₂/2% CO₂</td>
</tr>
<tr>
<td>pH</td>
<td>7.40</td>
<td>7.85</td>
</tr>
<tr>
<td>Temperature</td>
<td>37°C</td>
<td>20°C</td>
</tr>
</tbody>
</table>

*All values are in mM except inosine, which is in µM. Abbreviations: PSS = physiologic saline solution; CPS = cardioplegic solution; EDTA = ethylenediamine-tetraacetic acid.

in which aortic flow was periodically measured. The height of the left atrial perfusion chamber was adjusted so that the actual left atrial pressure (preload) recorded at the tip of the left atrial cannula was held constant at 8 cm H₂O at all subsequent levels of cardiac output. Coronary sinus flow was periodically measured as it dripped from the pulmonary artery and continuously discarded. Fresh PSS was infused into the preparation at a rate equal to the discarded coronary flow. Heart rate was counted with an electronic counter from the arterial pressure trace. Cardiac output was determined by adding the aortic and coronary flows. During the control period, aortic and coronary flow, pulsatile and mean aortic pressure, and heart rate were measured every 15 minutes.

In eight hearts, the control conditions were continued for 3 hours (i.e., the expected duration of the subsequent experiments) to demonstrate stability of the preparation. With other hearts after the control period, left atrial inflow was stopped and Langendorf perfusion established for 3 minutes. Then the hearts were exposed to a 60-minute period of cardioplegia at 20°C. The initial segment of this period consisted of CPS administration (30 seconds and 10, 30 or 60 minutes), followed by aortic clamping and hypothermic total ischemia during the remainder of the cardioplegia period. The CPS was administered using Langendorf perfusion at 75 mm Hg, which resulted in coronary flows that decreased with time and that were always less than that of control hearts (table 2). The CPS was a hypothermic (20°C) bicarbonate buffer (table 1). The magnesium concentration (15 mM) was elevated in the CPS in addition to the potassium concentration (15 mM) because of the protection it afforded in a previous study by Hearse et al. The CPS was anoxic (Po₂ < 100 mm Hg) and substrate-free because similar solutions are often used clinically and we wanted to evaluate a worst-case situation, in that the hearts would need to rely solely on endogenous energy stores by anaerobic metabolism. The pH was elevated (7.85), as suggested by Buckberg, to counter the developing acidosis dur-
TABLE 2. Cumulative Volume of Cardioplegic or Physiologic Solution Administered During Cardioplegia or Control Perfusion

<table>
<thead>
<tr>
<th>Perfusion duration (min)</th>
<th>Cumulative volume (ml)</th>
<th>3-hr control</th>
<th>CPS</th>
<th>% control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>7 ± 0.5</td>
<td>7 ± 0.4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>30 ± 1.0</td>
<td>24 ± 0.9*</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>75 ± 1.0</td>
<td>53 ± 1.3*</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>150 ± 3.0</td>
<td>91 ± 2.2*</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>225 ± 3.2</td>
<td>125 ± 2.9*</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>300 ± 3.5</td>
<td>155 ± 3.1*</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>450 ± 3.9</td>
<td>206 ± 3.2*</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>900 ± 5.0</td>
<td>356 ± 4.5*</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 15.
*Paired t test: significantly different from control at p < 0.05.

The cumulative volume of coronary sinus drainage during constant pressure (75 mm Hg) retrograde aortic perfusion from 3-hour control hearts during the second hour in the working mode and from hearts receiving cardioplegic solution for 1 hour. These data show that arrested hearts were not overperfused compared to control hearts and that coronary resistance steadily increased during administration of cardioplegic solution.

Abbreviation: CPS = cardioplegic solution.

ing ischemia that might inhibit anaerobic metabolism. Ethylenediamine-tetraacetic acid (EDTA) was added to both solutions to chelate trace quantities of heavy metals present in reagents.

At the end of cardioplegia, the hearts were reperfused with 37°C PSS for 3 minutes with Langendorff perfusion. Then, the left atrial infusion was resumed. The recovery of cardiac output, expressed as a percentage of the 1-hour control values, was recorded every 5 minutes during the first 15 minutes and every 15 minutes thereafter for 45 minutes. Some hearts did not immediately generate an aortic flow upon initiation of the recovery period. If not, Langendorff perfusion was maintained until they overcame the afterload. Then, the working mode was reestablished.

In a second series of experiments, CPS that contained 200 μM inosine was administered for 30 minutes, followed by 30 minutes of ischemia; inosine was added to the reperfusion PSS after 30 minutes of CPS administration and 30 minutes of ischemia; or inosine was added to both the CPS and reperfusion PSS.

Finally, hearts exposed to 1 hour of control perfusion plus 1 hour of perfusion with PSS containing 200 μM inosine were compared with hearts exposed to control PSS for 2 hours.

Biochemical Assessment
In a parallel series of studies, hearts were freeze-clamped between aluminum blocks cooled in liquid nitrogen for determination of ATP, ADP, AMP content at the end of the control period; after 30 seconds, 10 minutes, 20 minutes and 60 minutes of CPS administration; after 30 seconds of CPS administration plus 59.5 minutes of ischemia, after 10 minutes of CPS plus 50 minutes of ischemia, after 30 minutes of CPS plus 30 minutes of ischemia; and at the end of the recovery period, after all of the experiments described above.

Two pieces of frozen ventricle, approximately 0.3 g each, were obtained by breaking the heart into pieces on a block of dry ice. One piece was broken into 10-mg fragments. Four fragments were placed in each of three centrifuge tubes containing 0.6 ml of frozen 3 M perchloric acid. The tubes were thawed to −10°C and then refrozen on dry ice four times. The solution was then thawed and neutralized to pH 6.5 by addition of 2 M KHCO3. Each solution was then centrifuged (60 minutes at 10,000 g) and the supernatant was collected and stored at −70°C until analyzed for nucleotides by the fluorometric assays of Lowry and Passoneau. The other 0.3-g piece of tissue was then thawed, weighed to determine the wet weight, dried for 24 hours at 40°C and reweighed for the dry weight. In all instances the nucleotide tissue content was expressed as nmol/mg dry weight.

Statistical Analysis
The statistical significance of the changes in cardiac output or biochemical status produced by cardioplegia was determined by paired t test or analysis of variance (ANOVA). In addition, simultaneous multiple comparisons were made using Dunnett’s method for multiple comparisons between treatments and controls and Bonferroni’s method for multiple comparisons between all treatments. A p value less than 0.05 was considered significantly different from controls or other treatments.

Results
The stability of the isolated working rat heart preparation used in this study is demonstrated by the constant cardiac output of hearts perfused for 3 hours, and by the maintenance of the adenine nucleotide levels (table 3) of hearts perfused for 1, 2 and 3 hours.

The values of cardiac output at the beginning and the end of the recovery period of hearts exposed to 1 hour of cardioplegia (consisting of increasing durations of CPS administration followed by total ischemia) are shown in figure 2. The longer the initial exposure to CPS, the lower the cardiac output both at the beginning and at the end of the recovery period. In addition, only hearts perfused with CPS 10 minutes or less showed significantly improved performance during the recovery period and only those exposed to 30 seconds of CPS recovered completely.

Table 3 shows the changes in tissue adenine nucleotide levels during and after cardioplegia. Hearts exposed to 30 or 60 minutes of CPS administration lost almost all of their ATP. In contrast, the hearts given CPS for shorter intervals lost ATP at a slower rate and had significantly higher levels of ATP at the end of cardioplegia. During the recovery period, the return of ATP content toward control levels was inversely related to the duration of the CPS administration; i.e., the less CPS given, the greater the recovery. There was also a significant drop in the ADP content during cardioplegia of all hearts exposed to CPS for longer than 30 seconds. The ADP content returned to control levels during the recovery period only in hearts exposed to CPS 10 minutes or less. In contrast to the loss of ATP
TABLE 3. Adenine Nucleotide Levels of Control Hearts and Hearts Exposed to Cardioplegia

<table>
<thead>
<tr>
<th>Freeze clamping time</th>
<th>ATP (nmol/mg dry wt, n = 4)</th>
<th>ADP (nmol/mg dry wt, n = 4)</th>
<th>AMP (nmol/mg dry wt, n = 4)</th>
<th>TAN (nmol/mg dry wt, n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-hr control</td>
<td>18.1 ± 0.6</td>
<td>6.3 ± 0.4</td>
<td>3.4 ± 0.6</td>
<td>27.9 ± 1.1</td>
</tr>
<tr>
<td>2-hr control</td>
<td>18.6 ± 1.2</td>
<td>6.0 ± 0.4</td>
<td>2.7 ± 0.2</td>
<td>27.2 ± 1.0</td>
</tr>
<tr>
<td>3-hr control</td>
<td>18.3 ± 1.6</td>
<td>6.1 ± 0.5</td>
<td>2.1 ± 0.4</td>
<td>27.1 ± 1.4</td>
</tr>
<tr>
<td>30°CPS</td>
<td>12.5 ± 0.2†</td>
<td>7.8 ± 0.6</td>
<td>3.2 ± 0.4</td>
<td>23.5 ± 0.5*</td>
</tr>
<tr>
<td>30°CPS + 60'Isch</td>
<td>7.2 ± 0.6†</td>
<td>7.8 ± 0.3</td>
<td>8.6 ± 0.6†</td>
<td>23.8 ± 0.8*</td>
</tr>
<tr>
<td>30°CPS + Isch + 60'Rec</td>
<td>15.7 ± 0.2*</td>
<td>5.8 ± 0.4</td>
<td>2.3 ± 0.5</td>
<td>23.8 ± 0.6*</td>
</tr>
<tr>
<td>10°CPS</td>
<td>9.4 ± 0.6†</td>
<td>5.9 ± 0.3</td>
<td>7.6 ± 0.4*</td>
<td>22.9 ± 0.9*</td>
</tr>
<tr>
<td>10°CPS + 50'Isch</td>
<td>3.2 ± 0.7†</td>
<td>4.8 ± 0.2</td>
<td>13.8 ± 0.4†</td>
<td>21.8 ± 0.5*</td>
</tr>
<tr>
<td>10°CPS + Isch + 60'Rec</td>
<td>10.9 ± 1.0†</td>
<td>5.4 ± 0.6</td>
<td>2.0 ± 0.5</td>
<td>17.3 ± 0.9†</td>
</tr>
<tr>
<td>30°CPS</td>
<td>1.0 ± 0.3†</td>
<td>4.8 ± 0.9</td>
<td>14.7 ± 0.8†</td>
<td>20.4 ± 1.1†</td>
</tr>
<tr>
<td>30°CPS + 30'Isch</td>
<td>&lt;0.1 ± 0.0†</td>
<td>2.7 ± 0.3†</td>
<td>17.1 ± 1.4†</td>
<td>19.8 ± 1.3†</td>
</tr>
<tr>
<td>30°CPS + Isch + 60'Rec</td>
<td>7.5 ± 1.1†</td>
<td>3.1 ± 0.1†</td>
<td>2.0 ± 0.2</td>
<td>13.6 ± 1.2†</td>
</tr>
<tr>
<td>60°CPS</td>
<td>&lt;0.1 ± 0.0†</td>
<td>4.3 ± 0.5*</td>
<td>10.9 ± 1.0†</td>
<td>15.2 ± 1.1†</td>
</tr>
<tr>
<td>60°CPS + 60'Rec</td>
<td>3.1 ± 0.5†</td>
<td>3.3 ± 0.2*</td>
<td>2.1 ± 0.1</td>
<td>8.5 ± 1.5†</td>
</tr>
<tr>
<td>30°CPS + 30'Isch + 60'Rec(Ino)</td>
<td>7.0 ± 1.1†</td>
<td>3.4 ± 0.4*</td>
<td>2.5 ± 0.8</td>
<td>12.8 ± 1.4†</td>
</tr>
<tr>
<td>30°CPS(Ino)</td>
<td>10.0 ± 1.0†</td>
<td>5.8 ± 0.7</td>
<td>12.1 ± 0.9†</td>
<td>28.2 ± 2.3</td>
</tr>
<tr>
<td>30°CPS(Ino) + 30'Isch</td>
<td>6.6 ± 0.3†</td>
<td>2.9 ± 0.2†</td>
<td>16.5 ± 0.4†</td>
<td>25.4 ± 0.6*</td>
</tr>
<tr>
<td>30°CPS(Ino) + Isch + 60'Rec</td>
<td>10.2 ± 1.1†</td>
<td>4.4 ± 0.2*</td>
<td>2.0 ± 0.6</td>
<td>16.6 ± 1.4†</td>
</tr>
<tr>
<td>30°CPS(Ino) + Isch + Rec(Ino)</td>
<td>13.8 ± 1.6*</td>
<td>5.7 ± 0.6</td>
<td>2.0 ± 0.6</td>
<td>21.3 ± 2.6†</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Analysis of variance and Dunnett's simultaneous multiple comparison tests between 1-hour control values and all other values.

* p < 0.05.
† p < 0.01.

Hearts were freeze-clamped at the indicated times during the protocol for tissue analysis of adenine nucleotide levels; e.g., 30°CPS + 30'Isch + 60'Rec(Ino) hearts were freeze-clamped at the end of the recovery period after (1) a 60-minute period of cardioplegia consisting of 30 minutes of CPS perfusion followed by 30 minutes of total ischemia, and (2) a recovery period during which the hearts were perfused with PSS containing 200 μM inosine.

Abbreviation: TAN = sum of ATP, ADP and AMP levels; CPS = cardioplegic solution; Isch = hypothermic (20°C) total ischemia; (Ino) = supplementation with inosine in CPS or PSS; Rec = recovery.

and ADP during cardioplegia, there was a continual accumulation of AMP during CPS administration and ischemia in hearts exposed to CPS for up to 30 minutes. In hearts perfused with CPS for an additional 30 minutes, however, AMP content decreased from the elevated levels found at 30 minutes. All AMP levels returned to normal by the end of the recovery period. Finally, there was significant loss of TAN during CPS administration due to a greater loss of ATP and ADP than the accumulation of AMP. In contrast, there was no loss of TAN during the ischemic periods, when there was no perfusion of the myocardium. When perfusion was resumed in all hearts except those exposed to 30 seconds of CPS administration, however, there was continued loss of TAN during the recovery period because of the greater loss of AMP and ADP than the regeneration of ATP.

In the last series of experiments, the effects of reducing the concentration gradient causing the release of inosine during 30 minutes of continuous CPS administration was studied by increasing the extracellular concentration, i.e., CPS concentration, of inosine. A 30-minute period of CPS administration was chosen because in previous experiments (table 3, fig. 2) it consistently resulted in severe loss of ATP and failure of cardiac output to improve during the recovery period. Inosine was chosen because it is released from...
hypoxic rodent, canine and human myocardium in greater quantity than adenosine or hypoxanthine. 5, 17-20

The values of cardiac output at the beginning and end of the recovery period of hearts exposed to 30 minutes of perfusion with CPS containing 200 μM inosine followed by 30 minutes of ischemia are shown in figure 3. Inclusion of inosine in the CPS significantly improved functional recovery from cardioplegia compared with that seen in hearts given CPS without inosine. Because the cardiac output still did not improve to control levels, inosine was added to the recovery PSS (200 μM) as well as the CPS. This combination was even more effective in restoring functional performance (fig. 3). When inosine was added to the recovery PSS only, there was no improvement in the recovery of function over that when inosine was not added.

Jones and co-workers19, 20 have shown that inosine has a positive inotropic effect when administered to in situ dog hearts. Therefore, hearts that were working for 2 hours (i.e., nonarrested) were compared with hearts that were exposed to PSS containing 200 μM inosine during the second hour (table 4). Inosine is not a positive inotrope in the rat; there was no significant difference between the cardiac output of control hearts and hearts exposed to inosine.

The changes in adenine nucleotide content during 30-minute CPS administration plus 30-minute ischemia and recovery perfusion with and without inosine are shown in table 3. Inclusion of inosine in the CPS slowed the degradation rate of ATP and prevented the loss of TAN during cardioplegia. The improved ATP and TAN content at the end of the cardioplegia period correlated well with the improved functional recovery after administration of CPS containing inosine. However, there was continued loss of TAN during the recovery period. Inclusion of inosine in the CPS and the recovery PSS resulted in improved recovery of ATP content and reduced loss of TAN. Finally, inclusion of inosine in the recovery PSS only did not improve recovery of ATP or reduce TAN loss.

**Discussion**

The progressive degradation of the adenine nucleotide pool during myocardial ischemia or hypoxia with release of adenosine, inosine and hypoxanthine has been documented extensively under normothermic conditions,5-7, 17-23 The effects of cardioplegia on this process, however, have not been appreciated. The combination of hypothermia and cardioplegia greatly reduces myocardial oxygen consumption4 and allows for better functional recovery after ischemia. However, the present study clearly demonstrates that administration of CPS is not without undesirable consequences; i.e., the metabolic and functional recoveries are inversely related to the amount of CPS given at the beginning of the ischemic period. We hypothesize that this is a consequence of the reduction in the total adenine nucleotide pool because of washout of degradation products during CPS perfusion (fig. 1).

To maintain cellular viability, an adequate supply of ATP is necessary. The development of lethal cellular injury in ischemic myocardium is closely related to the degree of high-energy phosphate depletion.23 The ability to recover from cardioplegia also is related to ATP

**TABLE 4. Functional Performance of Nonarrested Hearts Exposed to 200 μM Inosine**

<table>
<thead>
<tr>
<th>Time during second hour (min)</th>
<th>Cardiac output*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-hr control</td>
</tr>
<tr>
<td>0</td>
<td>100†</td>
</tr>
<tr>
<td>15</td>
<td>100 ± 1.1</td>
</tr>
<tr>
<td>30</td>
<td>99 ± 2.0</td>
</tr>
<tr>
<td>45</td>
<td>99 ± 2.1</td>
</tr>
<tr>
<td>60</td>
<td>99 ± 2.3</td>
</tr>
</tbody>
</table>

*Values are mean percentage ± SEM. Mean percentage of the 1-hour control cardiac output (†) which was 60 ± 3.0 ml/min at a heart rate of 290(14) beats/min.

Hearts were exposed to either 2 hours of control perfusion with physiologic saline solution (PSS) in the working mode or 1 hour with control PSS and 1 hour with PSS containing 200 μM inosine. Analysis of variance was used to show that there was no significant difference with respect to time or treatments between the mean cardiac outputs during the second hour of perfusion in the working mode.

**FIGURE 3. Recovery of cardiac output after 1 hour of cardioplegia with exposure to 200 μM inosine (INO) during perfusion with cardioplegic solution (CPS) or during recovery perfusion. Recovery was expressed as the mean percentage of the 60-minute control cardiac output (n = 4 for all groups except the hearts that were not exposed to inosine [NOINO], where n = 8). The cardioplegia period consisted of CPS perfusion for 30 minutes followed by 30 minutes of total ischemia. Exposure to inosine in the CPS improved recovery but inclusion of inosine in the recovery physiologic saline solution (PSS) as well was the most effective. Inclusion of inosine in the recovery PSS (REC PSS) only did not improve recovery of cardiac output over that seen with NOINO hearts. Comparisons were made between mean recovery values to detect significant differences; the asterisk, compared with 60-minute recovery values of NOINO hearts; the circle, compared with 5-minute recovery values of NOINO hearts; the dot, compared with the recovery at 5 minutes.
content at the end of the arrest period and the ability to regenerate ATP during reperfusion (fig. 4). If the degradation products of ATP are allowed to accumulate under conditions in which purine salvage is possible, then they may be used to regenerate ATP and thus slow the net degradation of ATP. This was shown in the experiments in which administration of inosine in the CPS not only maintained the TAN pool, but also reduced the degradation of ATP (table 3). This protection of the ATP pool by administration of inosine was also reported by Ward and co-workers. Williams et al. reported the protection of rat hearts exposed to 1 hour of anoxia by treatment with a nucleoside transport inhibitor (concanavalin A); there was complete recovery of tissue ATP levels and 84% recovery of functional performance.

There are many opportunities for washout of these degradation products: by prolonged anoxic CPS administration, by noncoronary collateral blood flow or in the initial effluent during reperfusion. The most important period of washout appears to be during cardioplegia. This was shown by the degradation of ATP and the nucleotide pool during cardioplegia and by the lack of improved ATP content or functional recovery when inosine was supplied only during the reperfusion period. Additional support for this is found in other studies in which inosine was not effective in increasing ATP levels after periods of asphyxia in rabbits.

The reperfusion period can contribute to the inability to recover control ATP levels. This was seen in this study by the results that provision of inosine during reperfusion after its inclusion in the CPS, improved ATP and functional recovery over that when inosine was supplied in the CPS only. Thus, even if the myocardial cells are capable of nucleotide synthesis when reperfused, the purine substrate may not be available for incorporation into the adenine nucleotide pool and, indeed, there may be continued loss before these purines, which have accumulated during ischemia, can be utilized. Therefore, reduced recovery of ATP and reduced performance after periods of low-flow ischemia during cardioplegia may be a consequence of lost purines rather than the lack of damage of some other critical cellular component.

These data do not reveal exactly how provision of inosine during CPS administration protects the adenine nucleotide pool. Because the deamination of adenosine to inosine is essentially irreversible in the myocardium, inosine may be acting by inhibiting adenosine deaminase via product inhibition. This reduction of adenosine breakdown might thereby make more adenosine available for rephosphorylation to AMP (fig. 5). This process is not likely, however, because inhibition of adenosine deaminase by EHNA (erythro-9-(2-hydroxy-3-nonyl) adenine) does not increase nucleotide levels in ischemic dog hearts unless the release of adenosine is reduced by establishment of a high plasma level of adenosine. Incomplete recovery of nucleotides occurred in this case also, which may be due to deamination of AMP to IMP, then dephosphorylation to inosine and loss from the myocardium (fig. 1 and 5). The most likely salvage pathway is the degradation of inosine to hypoxanthine and then its combination with PP-ribose-P (1-pyrophosphate-5 phosphoribosyl) (fig. 5) to form IMP which is then reaminated to AMP. The importance of this pathway has recently been demonstrated by Pasque et al., who reported that provision of the PP-ribose-P precursor, ribose, to rat hearts before and after total ischemia improved
Finally, after workers found that when during administration. They even found that by Duvall-Arnold et al. They found that 4 mM inosine improved ATP levels in isolated working rat hearts exposed to 2 hours of ischemic cardioplegia when administered every 30 minutes. In addition, recovery of ATP was slightly improved after 30 minutes of reperfusion. The ATP regeneration and functional recovery might have been improved further if inosine had been included in the reperfusate as well. Finally, after our preliminary report, Ward and coworkers found that blockage of adenosine deamination by EHNA and infusion of inosine (500 μg/min/kg) during normothermic global ischemia and cardiopulmonary bypass in dogs resulted in better preservation of ATP concentrations at the end of ischemia and recovery than when EHNA or inosine were provided alone.

These data suggest that the amount of cardioplegic solution administered to patients should be reduced as much as possible both in amount and in frequency of administration. They also suggest that the collateral coronary blood flow that occurs in variable amounts during cardiopulmonary bypass in humans may have several detrimental effects. It may act to augment the washout of degradation products already occurring during CPS administration. Furthermore, by restoring electromechanical activity to the myocardium as it washes out the CPS, the collateral blood flow causes the surgeon to administer CPS more often than he might otherwise wish, additionally washing out more degradation product. In addition, the restoration of electromechanical activity brought about by the loss of CPS and wrinkling of the myocardium in the presence of low oxygen availability exacerbates the situation, causing a greater degradation of ATP and the TAN pool. Finally, these data and those of others confirm Buckberg’s concern for the damage that may be caused by the conditions during reperfusion, in that there is continued washout of nucleotide degradation products even as ATP is regenerated.

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Radionuclide Angiographic Evaluation of Right and Left Ventricular Function During Exercise After Repair of Transposition of the Great Arteries

Comparison with Normal Subjects and Patients with Congenitally Corrected Transposition

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SUMMARY We assessed the incidence, clinical significance and etiology of ventricular dysfunction after intraatrial repair of d-transposition of the great arteries in 11 patients, mean age 9 ± 3 years, who had had Mustard operations. We compared the results to 15 patients who were considered to have normal ventricular function, two patients who had Rastelli operations and five patients with congenitally corrected transposition. Gated equilibrium radionuclide angiography with supine exercise stress testing was used to assess these children. We found no significant difference between our patient groups in exercise capacity, heart rate, or blood pressure response to exercise. However, we found a high incidence of right ventricular dysfunction in the patient groups, manifested by an abnormal right ventricular ejection fraction response to exercise in six of 11 patients with a Mustard repair, both patients with a Rastelli repair and all five with congenitally corrected transposition. In addition, the left ventricular response to exercise was abnormal in 10 of 11 patients who had undergone a Mustard repair, both patients with a Rastelli repair, and two of five patients with congenitally corrected transposition. We conclude that biventricular dysfunction is frequently present after intraatrial repair of d-transposition of the great arteries. Despite this dysfunction, no significant decrease in exercise tolerance is found in childhood.

RIGHT VENTRICULAR dysfunction has been reported in many patients after repair of d-transposition of the great arteries.1-6 Although left ventricular dysfunction has also been reported in these patients,7 it is not a uniform finding.8 The incidence, clinical significance and etiology of these functional disturbances is unknown.

We assessed the incidence and severity of right and left ventricular dysfunction after operation in our patients with d-transposition of the great arteries, using radionuclide angiography and exercise stress testing. These results were contrasted to findings in a small group of patients with congenitally corrected transposition of the great arteries and a third group of patients without significant cardiac defects. We also assessed various surgical factors and hemodynamic residua as contributors to functional disturbances.

Patients and Methods

We performed radionuclide ventriculograms on 13 children with d-transposition of the great arteries, all of whom had undergone intracardiac repair. All patients older than age 6 years who had undergone repair of transposition of the great arteries or who had congenitally corrected transposition and who were evaluated in our Cardiology Clinic between June 1980 and January 1982 were referred for radionuclide ventriculography. Studies were performed 3 months to 14 years after repair. Eleven patients, mean age 9.1 ± 3 years, received a Mustard-type repair9 and two patients, mean age 9.9 ± 4.9 years, required a Rastelli repair.10 The two patients who had a Rastelli repair were significantly older than the group of patients with a Mustard repair at the time of complete repair (9.6 ± 4.9 vs 1.5 ± 1.2 years). Eight of the 11 patients with a Mustard repair had significant hemodynamic residua postoperatively: Five had mildly obstructed systemic venous
Nucleotide degradation and functional impairment during cardioplegia: amelioration by inosine.

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