Preservation of myocardial high-energy phosphates correlates with the heart's ability to resume normal function following aortic crossclamping (AXC). The ability of the canine myocardium to synthesize and maintain ATP during 180 minutes of AXC was evaluated in 12 hearts subjected to either blood or crystalloid cardioplegic arrest. Group 1 hearts were arrested by infusion of 750 ml of blood potassium cardioplegia (BKC) solution into the aortic root initially and every 30 minutes, as were group 2 (six) hearts but with a crystalloid cardioplegia (CC) solution. Transmural left ventricular biopsy specimens were obtained for ATP analysis prior to AXC (control), before and after cardioplegia injections 2, 4, and 6, prior to unclamping (180 minutes of AXC), and 30 minutes following reperfusion. ATP levels increased significantly above control (p<0.005) during the 180 minutes of AXC immediately following infusion of BKC. At the end of 180 minutes of AXC and following 30 minutes of reperfusion, ATP was noted to be normal in this group (p = NS). In contrast, ATP levels fell significantly (p<0.005) during the period of aortic crossclamping in the crystalloid cardioplegia group and did not return to normal even after 30 minutes of reperfusion (p<0.005). We concluded that BKC, by presenting the arrested myocyte with adequate oxygen and substrate, allows for synthesis and preservation of myocardial ATP during periods of AXC as long as three hours. In this respect, it should be regarded as superior to CC, which permits a statistically significant depletion of ATP (p<0.005) uncorrected, even after 30 minutes of reperfusion in the beating, nonworking state.

The importance of insuring maximum metabolic protection during elective cardiac arrest, especially with respect to energy metabolism, and of using only the method of arrest that causes minimum metabolic derangement, cannot be overemphasized. Numerous studies have illustrated that the method of cardioplegia used will affect the survival and subsequent recovery of the heart. In a large percentage of those reports, both clinical and experimental, myocardial protection was shown to be closely related to preservation of tissue ATP, and, in general, the higher the total of this high-energy phosphate, the greater the rate and extent of hemodynamic recovery.

Furthermore, it has been well established experimentally that hearts that are chronically ischemic or hypertrophied have lower control high-energy phosphate levels and develop ischemic contracture sooner than normal hearts. Clinically, in hearts of this nature, it follows that even moderate depletion of available ATP stores during the aortic crossclamp interval could produce disastrous results. It thus behooves the surgeon to select that method of myocardial protection and arrest which will permit full recovery by virtue of its preservation of normal cellular metabolism and maintenance of energy stores. With this in mind, we undertook a study to determine which of the two most widely used methods of elective cardiac arrest and myocardial protection could best fulfill this requirement. The ability of the canine myocardium to maintain a normal ATP level, through decreased utilization and/or continued synthesis, was evaluated in dogs subjected to 180 minutes of continuous blood or crystalloid cardioplegic arrest.

### Materials and Methods

**Experimental Preparation**

Twelve dogs weighing 25 to 30 kg were anesthetized with morphine sulfate (2.5 mg/kg) and chloralose (100 mg/kg). After intubation, respiration was controlled with room air positive-pressure ventilation. A median sternotomy was performed, the heart was suspended in a pericardial cradle, and sodium heparin (3 mg/kg) was administered. Total cardiopulmonary bypass was instituted with a...
bubble oxygenator (Bentley model BOX-10) primed equally with fresh homologous blood and balanced electrolyte solution. The superior and inferior cavae were cannulated, the azygous vein ligated, and venous return was delivered to the oxygenator by gravity drainage. Coronary sinus and the thebesian venous return were measured by placing a third cannula in the right ventricle and snaring the main pulmonary artery. Two 14-gauge plastic catheters were placed into the aortic root. The first was connected to a previously described cardioplegia delivery system for aortic root perfusion following crossclamping, and the second was connected to a transducer for measurement of aortic root pressure (coronary perfusion pressure).

A left ventricular vent was inserted through the left atrial appendage and left atrial pressure monitored continuously through a polyethylene catheter. Continuous measurement of myocardial temperature was obtained by insertion of a thermistor probe into the middle of the interventricular septum and maintained between 10 and 15°C in both groups. This temperature range has previously been determined to provide optimal preservation of high-energy substrate, myocardial ultrastructure, and performance. Arterial blood gases were measured every 30 minutes, and systemic pH maintained at 7.4 throughout the procedure. Continuous ECG monitoring was obtained utilizing standard limb leads and a V5 precordial lead. The hematocrit was maintained at 30 percent, and systemic arterial pressure maintained at 80 mm Hg.

Experimental Protocol

Following a 30-minute period of stabilization on bypass, with hearts in normal sinus rhythm and a nonworking state, a control transmural myocardial biopsy of the left ventricle, weighing from 8 to 10 mg, was obtained using a Travenol biopsy needle (Fig 1). These samples were immediately frozen in liquid nitrogen for subsequent ATP analysis. Myocardial tissue was analyzed for ATP by a modification of the bioluminescent method. Firefly luciferase catalyzes the oxidation of luciferin in the presence of ATP and magnesium as shown in the chemical reactions outlined in Figure 2. The final reaction results in the production of light. This is the basis on which the amount of light produced is directly proportional to the amount of ATP present.

Experimental Groups

Group 1 (blood cardioplegia): In six dogs following control biopsies, systemic cooling to 25°C was carried out. The aorta was crossclamped, and hearts were arrested by infusion of cold blood cardioplegia solution into the aortic root. The arrest solution consisted of blood from the pump oxygenator system altered by the addition of potassium to a concentration of 30 mEq/L and buffered to a pH of 8.0 by the addition of THAM. Initial arrest was achieved by instillation of 750 ml of the cold injectate (12.5 ± 0.03°C) at an aortic root pressure of 80 mm Hg. Reinfusion of 750 ml was routinely repeated every 30 minutes, additional 500 ml of topical iced lavage with normal saline at 4°C was used initially to lower myocardial temperature and again during the period of each reinjection. Transmural left ventricular biopsy specimens were obtained before and immediately after cardioplegia injections at 30, 90, and 150 minutes of arrest, and at 180 minutes of arrest immediately prior to aortic unclamping. Following 180 minutes of continuous arrest, during which no heart exhibited signs of electric or mechanical activity, the aorta was unclamped, and systemic rewarming to 37°C was carried out. Myocardial tissue specimens were obtained by insertion of a thermistor probe into the middle of the interventricular septum and maintained between 10 and 15°C in both groups. This temperature range has previously been determined to provide optimal preservation of high-energy substrate, myocardial ultrastructure, and performance.

Group 2 (crystalloid cardioplegia): Six dogs underwent control biopsies as in group 1. The aorta was crossclamped, and hearts were arrested in this group by infusion of hypothermic crystalloid cardioplegic solution into the aortic root. The arrest solution consisted of 30 mEq of potassium chloride, 1 g of methyprednisolone, and 16 ml of D-50W in 1 L of Plasmalyte, buffered as in group 1 to a pH of 8.0. Initial arrest was achieved in this group by instillation of 750 ml of the cold injectate (12.7 ± 0.04°C) at an aortic root pressure of 80 mm Hg. Reinfusion of 750 ml was routinely repeated every 30 minutes and 500 ml of topical iced lavage with normal saline at 4°C was additionally added to lower myocardial temperature during the period of each injection. Transmural left ventricular biopsies for ATP analysis were carried out during time intervals identical to group 1. Unless otherwise indicated, all data are reported as mean ± SEM, and statistical significance was determined using Student's t test.

RESULTS

Figure 3 illustrates the sequential changes in myocardial ATP levels throughout the experimental period.
in both groups of animals. Hearts in the crystalloid cardioplegia group (group 2) underwent a steady decline in tissue ATP levels, beginning as early as 30 minutes following aortic crossclamping. This change was not reversed by subsequent infusions of the cardioplegia solution, nor had the ATP returned to control values when measured as long as 30 minutes following aortic unclamping (p<0.005). In marked contrast, myocardial ATP in the blood cardioplegia group (group 1) showed no significant change from control values (p = NS) both at 180 minutes of aortic crossclamping and over the subsequent 30 minutes of reperfusion. These changes are even more dramatically shown in Figure 4, which depicts the percent change from control in myocardial ATP level for both groups of animals following 180 minutes of aortic crossclamping and 30 minutes of reperfusion. The blood cardioplegia group was essentially unchanged from control values (p = NS), while the crystalloid cardioplegia group exhibited a highly significant (p<0.005) decline in ATP values of 18 ± 4 percent.

If Figure 3 is examined more closely, it can be noted that in both groups, myocardial ATP levels consistently declined in the interval between each successive cardioplegia injection. Nevertheless, as stated previously, hearts in the blood group showed no significant change from control values at the end of the experimental period. This phenomenon can be easily understood by examining Figures 5 and 6, which represent the change in myocardial ATP levels immediately following injection of the crystalloid and blood cardioplegia solutions, respectively. Figure 5 shows that injection of up to 750 ml of crystalloid cardioplegia solution in the arrested heart was unable to compensate for the steady decline in myocardial ATP that occurred during the interval between injections. The mean percent change in ATP level following crystalloid injection actually showed a slight decrease (3 ± 0.6 percent, p = NS). In marked contradistinction to this, as illustrated in Figure 6, myocardial ATP levels routinely rose an average of 15 ± 0.9 percent following perfusion of the heart with the blood cardioplegia solution. Statistically, this change was noted to be highly significant (p<0.005).

The above changes in myocardial ATP levels for both groups of animals are outlined in greater detail in Table 1.

**DISCUSSION**

Numerous investigators have demonstrated a high correlation between myocardial high-energy phosphate levels at the end of arrest and subsequent myocardial performance. Lowe et al have reported that cell death first occurs in the canine heart when depletion of ATP exceeds 65 percent. They found that alterations in cell volume regulation, ion distribution, and ultrastructure were reversible if more than 35 percent of ATP was preserved. In a further study by Schaper and colleagues, a standardization of criteria for the severity of reversible ischemic injury was established during ultrastructural, biochemical, and functional parameters. It was concluded that a very narrow range of ATP values might determine whether irreversible ischemic injury occurred, and that a close correlation existed between the rates of structural, metabolic, and functional deterioration at the end of an ischemic interval.

In previous clinical and experimental studies from this institution, there also appeared to be a parallel...
between the maintenance of normal myocardial ultrastructure, ventricular function, and the preservation of high energy phosphate. Changes in ATP, therefore, probably are sensitive indicators of myocardial protection and help to define the facility with which the myocyte can resume normal function.

Several groups of investigators, utilizing highly sophisticated techniques such as nuclear magnetic resonance, have attempted to determine whether additional high-energy intermediates (most notably creatinine phosphate) could be used as a more precise measure of the adequacy of myocardial preservation. Many important observations regarding the earliest irreversible metabolic changes resulting from myocardial ischemia have arisen from these studies. Ultimately, however, cell integrity and function were always found to depend on adequate ATP levels, whereas changes in the content of other energy intermediates were indiscriminatory of the grade of ischemic damage. Nevertheless, it should be mentioned that in several published reports comparing the efficacy of sanguineous vs asanguineous cardioplegia techniques, all high-energy phosphates were significantly better preserved with blood cardioplegia.

The presence of normal ATP levels after blood cardioplegia in this experiment presumably reflects the fact that the myocardium was quickly arrested in a well oxygenated state with normal energy stores. Maintenance of these stores was made possible thereafter by adequate presentation of oxygen and substrate to the arrested myocardial cell to satisfy its ongoing demands.

Hearse et al have determined that the oxygen demands of the arrested myocardium at 15°C is 0.4 ml/100 g/min. Based on this finding, we calculated the myocardial oxygen demand of a 250-g canine heart during a five-minute cardioplegia injection to be 5 ml. As utilized in this experiment, 750 ml of unoxygenated, hypothermic, crystalloid cardioplegia was capable of carrying 3.23 ml of oxygen during each five-minute cardioplegia injection, while a similar volume of cold blood cardioplegia (hematocrit 30 percent, Po2 400 mm Hg, 89 percent oxyhemoglobin) carried 12.38 ml of free oxygen. Assuming that as much as 80 percent of the available free oxygen can be extracted by the myocardiun, unoxygenated hypothermic crystalloid cardioplegia would be capable of delivering only 2.58 ml of oxygen per five-minute cardioplegia injection, while blood cardioplegia could deliver 9.9 ml of free oxygen over the same period. The calculation of myocardial oxygen supply and demand ratio shows that crystalloid cardioplegia, therefore, delivers only 51.6 percent or approximately one half of the myocardial oxygen demand during the cardioplegia delivery period. In contrast, blood cardioplegia delivered 98 percent or nearly twice the myocardial oxygen demand during the same period. This finding readily explains the previously demonstrated differences in ATP production during cardioplegia injection by a shift to aerobic metabolism during this period in the blood group.

From these data we have concluded that asanguineous cardioplegic solutions, although providing a minimal degree of myocardial protection during aortic crossclamping, would permit a steady and significant depletion of myocardial high-energy phosphate stores due to uninterrupted anaerobic metabolism and, therefore, lack of ATP replenishment during cardioplegic injection. This ATP depletion begins almost immediately following aortic crossclamping, and will continue throughout its duration. In marked contrast, blood cardioplegia has been shown to provide superior preservation of myocardial ATP. This is accomplished by supplying the arrested myocyte adequate oxygen and substrate, and, hence, a shift to aerobic metabolism during the period of injection, allowing for synthesis of myocardial ATP. Furthermore, this synthesis has been shown to completely replenish the ATP depleted during the ischemic period between cardioplegic injections, and thus preserve myocardial 

<table>
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<tr>
<th>Table 1—Comparison of Myocardial ATP Levels</th>
<th>Minutes of Aortic Crossclamp</th>
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<tr>
<td>Control</td>
<td>30</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>(Blood)</td>
<td></td>
</tr>
<tr>
<td>ATP μ mole/100 mg PTN</td>
<td>4.20 ± 1.0</td>
</tr>
<tr>
<td>% Change</td>
<td>↓</td>
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<tr>
<td>p*</td>
<td>NS</td>
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<tr>
<td>Group 2</td>
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<tr>
<td>(Crystalloid)</td>
<td></td>
</tr>
<tr>
<td>ATP μ mole/100 mg PTN</td>
<td>4.10 ± 1.0</td>
</tr>
<tr>
<td>% Change</td>
<td>↓</td>
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<td>p*</td>
<td>NS</td>
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*p vs control.

*p<.005 blood vs crystalloid.
ATP during periods of aortic crossclamping for as long as three hours. In this respect, blood cardioplegia should be regarded as superior to crystalloid cardioplegia. In light of these findings, further investigations to determine the efficacy of blood cardioplegia during periods of prolonged aortic crossclamping in both hypertrophied myocardium and in the presence of diffuse coronary artery disease are now indicated.

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