patient with prolonged P-R had a normal A-V conduction but prolonged internodal conduction. The surface ECG thus may not accurately represent the status of the A-V conduction system in patients with ECD, particularly in the postoperative state. The prognostic significance of the abnormalities uncovered by IE remains to be determined.

References

The Protective Effect of Hyperosmotic Mannitol in Myocardial Ischemia and Necrosis

Wm. John Powell, Jr., M.D., Donald R. DiBona, Ph.D., Jorge Flores, M.D., and Alexander Leaf, M.D.

SUMMARY Morphologic and hemodynamic changes that occur following coronary occlusion are examined. The effectiveness of hyperosmotic mannitol in lessening the extent of myocardial damage is assessed and mechanisms for its action discussed. Forty and 60 min of coronary vascular occlusion followed by 15 and 45 min of reflow were associated with a persistence of ischaemia following reflow of blood, as established by infusions of silastic into the aortic root.

Electron microscopic studies demonstrated myocardial and endothelial cell swelling at the end of the reflow period. The process of cell swelling appeared to be initiated during the period of arterial occlusion. This cell swelling was reduced by elevation of serum osmolality by 30-40 mOsm above control with the administration of mannitol during and following occlusion. There was an associated 40-50% reduction of vascular resistance following occlusion if mannitol was administered. In addition, the extent of necrosis, which was widespread in untreated hearts 12 hours after occlusion, was strikingly less in the hearts of dogs which received mannitol. Thus, in ischemic myocardium, elevation of osmolality by mannitol reduces myocardial necrosis, probably through its restoration of normal cell volume.

The Hemodynamic Efficacy of the use of hyperosmotic mannitol in experimentally induced myocardial ischemia has been documented. Elevation of extracellular osmolality during a period of constriction of a coronary artery produced the following acute effects: 1) a decrease in amount of ST-segment elevation over the ischemic muscle; 2) an increase in collateral blood flow to the area of ischemia; 3) an improvement in the function of the ischemic myocardium; and 4) a reduction in oxygen consumption of ischemic cardiac muscle. These favorable effects on function were all discernible in the acute experimental animal preparation. The present study was designed to answer the important clinical question of whether or not treatment with hyperosmotic mannitol protects ischemic myocardial cells from necrosis. Our results, based on studies carried out in the experimental canine model, indicate that it does. The study attempts to determine the mechanism of action of mannitol using histologic measures. A preliminary report of the data has been presented.

Methods

The experiments were performed in 80 adult mongrel dogs, anesthetized with intravenous sodium pentobarbital

From the Department of Medicine (Cardiac, Renal Biophysics, and Biochemical Pharmacology Units), Massachusetts General Hospital, and the Department of Medicine and Anatomy, Harvard Medical School, Boston, Massachusetts. Supported by grants N01 HV 71443, HL 17665, PHS AM17372, and HL 06664 (HEPP) and NIH contract N01 HV 53002. This work was presented at the Annual Meeting of the American Society for Clinical Investigation in Atlantic City, New Jersey, in May 1973. Address for reprints: Wm. John Powell, M.D., Cardiac Unit, Massachusetts General Hospital, Fruit Street, Boston, Massachusetts 02114. Received September 2, 1975; revision accepted May 10, 1976.

(30 mg/kg). Following endotracheal intubation, the animals were ventilated with a Harvard positive pressure respirator (Harvard Apparatus Co., Inc.) at a rate of 14 to 18 cycles/min with a mixture of 95% oxygen and 5% carbon dioxide. A left thoracotomy was then performed.

Figure 1 shows the experimental preparation. In those experiments in which circumflex artery blood flow was monitored, the sinoatrial node was crushed, and the atria were paced at a rate slightly above the intrinsic heart rate (usually 150 beats/min) (Medtronic Model 5837 A-V pulse generator, Medtronic, Inc.). Systemic blood pressure was monitored through a short, rigid wide-bore cannula in the right brachial artery and a Statham P23Db pressure transducer.

Reversible ligation of the proximal circumflex branch of the left coronary artery was performed in 56 animals using a snare (fig. 1, center panel). In these experiments the artery was occluded for periods of either 40 or 60 min — followed in some experiments by 15 or 45 min of reflow of blood, respectively. Data from these experiments were used in the assessment of the extent of cell swelling. In other animals a prolonged period of reflow of 12 hours was carried out and these data were used to assess the extent of myocardial cell necrosis.

In an additional 24 dogs coronary vascular resistance was evaluated during a one hour reflow period following either 40 or 60 min of proximal circumflex artery occlusion. In these experiments a polyethylene loop of tubing was interposed between the proximal and distal ends of the divided circumflex branch of the left coronary artery (fig. 1) and was clamped to produce occlusion. An extracorporeal flow probe (Statham Model MDQH 5020) connected to a flowmeter (Statham Model SP 2202) was placed around the polyethylene tubing, and mean and phasic tracings of blood flow were obtained. Mean and end-diastolic coronary vascular resistance were calculated before occlusion and during reflow as the ratio of perfusion pressure to blood flow. Changes in coronary vascular resistance were expressed as percent changes from control. In these experiments mean systemic blood pressure was maintained nearly constant by means of a blood overflow column and reservoir (fig. 1, lower right) connected to the femoral arteries and veins. To prime the column and reservoir, blood from one donor dog was used for each experiment. The level of the column was selected for individual animals to incorporate a shunting level. Thus a tendency toward a slight increase or a slight decrease in the dog's systemic blood pressure resulted in an increase or a decrease in shunting and a near constant systemic blood pressure. Because the pressure could not be increased if it fell below the shunt level, arterial pressure fell in the latter part of some experiments. A roller pump in the venous return line of the column maintained the level of blood in the reservoir constant during a given experiment.

All animals were pretreated with 50 mg/kg propranolol divided in three different intramuscular sites one-half hour prior to the occlusion. Approximately one-quarter of the dogs studied developed ventricular fibrillation — usually at the time of release of the coronary ligation and none of these dogs was used in subsequent data analysis. All experimental animals were heparinized initially (6,000 U, i.v.). In the prolonged reflow experiments 2,000 units of heparin were administered intravenously every four hours.

During each of the experiments a mannitol, saline, or control plasma infusion was administered intravenously during the last 15 min of the period of occlusion and during the first 15 min of the period of reflow. The mannitol and saline were infused at 15.3 cc/min for 10 min and then 7.64 cc/min for 20 min. The control plasma infusions were carried out at 38.2 cc/min for 10 min and then 15.3 cc/min for 20 min — a rate which was sufficient to lower the hematocrit by the same amount as the mannitol. Throughout 20 min of the control period, the period of occlusion, and the period of reflow, repeated observations were made of mean and phasic aortic pressures and determinations were done of arterial pH, pCO2, pO2, hematocrit, serum sodium and potassium concentrations, and osmolality as previously described.1-4

In the histologic studies, the papillary muscles were rapidly removed from the heart, and cross sections were obtained from the distal, middle, and proximal thirds of the papillary muscles. These cross sections were immediately layered with glutaraldehyde (2.5% glutaraldehyde in a phosphate buffer of 300 mOsm/L at a pH of 7.4). The sec-

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**Figure 1.** The experimental preparations employed in this study. SG = strain gauge; EE = stimulating electrodes; LCA = left coronary artery.
MANNITOL AND CELL SWELLING IN ISCHEMIA/Powell et al.

Table 1. Physiologic Data from Silicone Rubber Perfusion Experiments

<table>
<thead>
<tr>
<th>Time after Occlusion</th>
<th>MAP (mm Hg)</th>
<th>pH</th>
<th>pCO₂ (mm Hg)</th>
<th>pO₂ (mm Hg)</th>
<th>Hct (%)</th>
<th>Na⁺ (mEq/L)</th>
<th>K⁺ (mEq/L)</th>
<th>Osm (mOsm/L)</th>
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<tr>
<td>S</td>
<td>95 ± 5</td>
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<td>28 ± 3</td>
<td>355 ± 16</td>
<td>39 ± 4</td>
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<td>80 ± 13</td>
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<td>S</td>
<td>88 ± 18</td>
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<td>P</td>
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<tr>
<td>S</td>
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<td>40 ± 5</td>
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<td>31 ± 3</td>
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<tr>
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<td>4.4 ± .5</td>
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</table>

*Each value is ± the standard error of the mean.

+P < 0.05.

Abbreviations: S = saline infusion experiments; P = control plasma infusion experiments; M = mannitol infusion experiments; MAP = mean aortic pressure; Hct = hematocrit; Osm = osmolality.

Sections were obtained with a Reichert Om-U2 ultramicrotome. Thin sections (500-700Å) for electron microscopy were stained with uranyl acetate and lead citrate for examination in a Phillips EM-200 electron microscope. Thick sections (1 μ) for optical microscopy were stained with 0.5% Toluidine Blue.

In the long reflow experiments the extent of necrosis was evaluated by light microscopy. Approximately 2,000 cells (range 1799 to 2117 cells) from each papillary muscle were counted and the percent of necrotic cells determined. This was done from photographs which were coded and randomized by one of us (DB) who had no knowledge as to whether the heart from which a particular photograph was taken had been treated with mannitol.

Patency of the vasculature was evaluated by the infusion of a silicone rubber suspension4 (Microfil, Canton Biochemical Products). Following circumflex artery occlusion and reflow, snares were tightened around the superior and inferior venae cavae andazygos vein, both atrial appendages were excised, and the ascending aorta was ligated. The silicone rubber suspension was then infused into the aortic root through a wide bore metal cannula at 100 mm Hg pressure from a pressure bottle for a period of 5 min for each experiment. Following dehydration with ethanol and subsequent clearing with methylsalicylate,6 serial slices of the myocardium, each of approximately ½ cm thickness, were obtained from apex to base and examined with a dissecting microscope for evaluation of the distribution of silicone rubber. Photographs of the slices which included comparable levels of each papillary muscle were obtained.

Throughout the tables, figures, and text, mean values plus and minus the standard error of the mean are given. Statistical analysis was performed using Student's t-test.

Results

Confirmation of Ischemia

Hearts were perfused with silicone rubber to establish a reproducible experimental protocol for producing ischemia. Varying periods of occlusion (20–80 min) and reperfusion of blood (15–45 min) were screened. Forty or 60 min of arterial clamping with 15 or 45 min of reflow, respectively, resulted in a reproducible lesion in the posterior papillary muscles with decreased filling in the posterior papillary muscles as revealed by silicone rubber infusions (fig. 2, top panel). Table 1 indicates the associated osmolality, hemodynamic, blood gas, electrolyte, and pH data in the silicone-treated dogs.

Differences in the associated findings in experiments at 40 min and at 60 min (table 1) were consonant with the relative differences in the duration of ischemia but reflected the same processes. For this reason only the data at 40 min of ischemia and 15 min of reflow are given in the subsequent tables.

Variation in the overall degree of filling with this technique did not impair demonstration of the lesion in these control samples, but indicated that in the mannitol experiments the procedure was not precise. For example, in four out of six hearts which had had a 60 min occlusion and mannitol infusion, there was restoration of balanced filling between the anterior and posterior papillary muscles (fig. 2, bottom panel); in the remaining two, such restoration was not clearly shown. (This assessment was made by three of us without prior knowledge as to which hearts had received mannitol.)

tions from the proximal, middle, and distal thirds of the papillary muscles were then cut into approximately 1-2 mm cubes which were placed in the same mixture of glutaraldehyde within one min of excision of the heart.4

Blocks of myocardial tissue underwent further fixation in glutaraldehyde at 4°C (overnight). After postfixation in OsO₄, dehydration was accomplished in a graded series of ethanol solutions and the tissue embedded in an Epon-Araldite mixture.5

The histology of perfused fixed material was carefully evaluated in two additional dogs. In these dogs a 60 min ligation of the proximal circumflex coronary artery followed by 45 min of reflow of blood was performed as described in the text. At 45 min of reflow the root of the aorta was perfused for 20 min at 110 mm Hg with 2.5% glutaraldehyde with the ascending aorta cross clamped and both atrial appendages excised. After 20 min of perfusion (200-250 ml of glutaraldehyde fixative) the papillary muscles were excised and treated as described for immersion fixation. The results showed excellent preservation of cell morphology in the anterior papillary muscles.7 However, tissue from the posterior papillary muscles showed excessive damage characterized by fragmented cell membranes and contraction bands. At least some of the apparent damage must be attributed to poor tissue fixation probably due to impaired perfusion. As is evident in the Results section, tissue fixed by rapid immersion following the reflow of blood (without perfusion fixation) did not reveal this excessive cell damage. Therefore, rapid immersion fixation was the procedure of choice.
Coronary Vascular Resistance

Vascular resistance, when measured directly following either 40 or 60 min of occlusion, was substantially lower in the period of reflow of blood when mannitol was administered late during the occlusion and early during reflow (fig. 3). Table 2 shows that the associated hemodynamic and blood gas data were comparable whether or not mannitol was administered. The changes in mean vascular resistance and end-diastolic vascular resistance were directionally similar. It is noteworthy that in three of the six animals that did not receive mannitol, vascular resistance during the reflow period following a 60 min arterial occlusion rose above the control level. This elevated vascular resistance was substantially lowered by mannitol. Figure 3, right panel, illustrates the experimental data from one of these three animals.

Histologic Studies

Cell Swelling

Microscopic examination of the myocardium was done with samples obtained after short periods of reflow of blood following 40 or 60 min of arterial occlusion. In general ischemic myocardium following reflow of blood showed changes compatible with an increase in intracellular fluid. When mannitol was administered, these changes were not apparent.

Figure 4 compares anterior (nonischemic) (4a) and posterior (ischemic) (4b) papillary muscles of a dog which underwent 60 min of occlusion of the proximal circumflex artery followed by 45 min of reflow of blood. Normal myocardial morphology was characterized by close apposition of the plasma membrane (sarcolemma), close packing of the myofibrils and mitochondria, and a patent T-tubular system (labelled “t”). In the ischemic posterior papillary muscle the presence of increased cytoplasmic fluid was suggested primarily by increased space between the sarcolemma and the underlying myofibrils and by increased space between myofibrils illustrated more clearly at a higher magnification (fig. 4d). There also appeared to be decreased patency of the T-tubular system. The ischemic posterior papillary muscle of another dog in which mannitol was administered (fig. 4c) histologically resembles that of the control nonischemic papillary muscle in figure 4a.

Figure 2. Top panel) The distribution of silastic in a transverse section of a heart which following 40 min of occlusion of the proximal left circumflex artery had 15 min of reflow of blood. A control saline infusion was administered during the last 15 min of occlusion and the 15 min of reflow. Note that there is diminished distribution of the white silastic in the posterior papillary muscle shown at the top of the figure compared with the anterior papillary muscle to the right of the figure. Bottom panel) The distribution of silastic in a heart which received mannitol during the last 15 min of a 40 min period of circumflex artery occlusion and during the first 15 min of reflow of blood through this artery. Note that the distribution of silastic in the posterior and the anterior papillary muscles appears equal.
Morphologic findings from each of these samples were quantified. Cells with increased subsarcolemmal space (labelled "S" in fig. 5a) were classified as swollen. Another measure of cell swelling could be increased intermyofibrillar-sarcomplasmic space. However, increased space can be found in the region of the cell nucleus even without experimental manipulation.* For this reason we used sarcolemmal elevation as the index of cell swelling.

Capillaries with expansion of the endothelium in which the density of the cytoplasmic ground substance was reduced were classified as swollen (fig. 5a). In evaluating the total number of capillaries with swollen endothelia, only those capillaries were included in the statistical analysis in which the myofibrils and the capillaries were sectioned perpendicular to the long axis of both of these structures. The percentages of the capillaries affected were determined from coded and randomized micrographs at a magnification of 6000.

Figures 6 and 7 show the percentages of swollen myocardial cells and the percentages of capillaries with swollen endothelium. As can be seen in the upper panel of figure 6, 26.5 ± 1.0 (mean ± SEM) percent of myocardial cells showed evidence of swelling (sarcolemmal elevation) in posterior papillary muscles which were subjected to 40 min of blood flow interruption followed by 15 min of reflow of blood. The control anterior papillary muscles from the same hearts showed almost no evidence of swelling (0.8 ± 0.7%).

In the ischemic posterior papillary muscles which received mannitol, however, the percentage of swollen cells as determined by sarcolemmal elevation was significantly reduced (P < 0.01) to 7.4 ± 2.8% (figs. 5b and 6). As shown in figure 7 which illustrates 60 min of blood flow occlusion followed by 45 min of reflow, the percentage of swollen myocardial cells with sarcolemmal elevation in the posterior papillary muscles increased to 81.6 ± 6.5%, and this percentage was substantially reduced (P < 0.001) to 2.8 ± 1.7% with the administration of mannitol.

The percentages of capillaries with swollen endothelia following 40 min of occlusion and 15 min of reflow and following 60 min of occlusion and 45 min of reflow are shown in the lower panels of figures 6 and 7, respectively. In the 40 min occlusion experiments the percentage of swollen capillaries was reduced from 13.5 ± 4.7% to 1.2 ± 0.9% (P < 0.05) with the administration of mannitol. As shown in the lower panel of figure 7 with a period of ischemia of 60 min and reflow of 45 min, the percentage of swollen capillaries was 22.8 ± 4.6%, and this percentage was reduced with the administration of mannitol to 2.7 ± 1.4% (P < 0.005). The associated hemodynamic data for the shorter period of occlusion and reflow of blood are shown in table 3. Because in the initial 40 min occlusion experiments the hematocrit was lowered by the administration of mannitol, in the subsequent 60 min occlusion experiments control plasma infusions were administered at a rate sufficient to produce comparable decreases in hematocrit.

To determine whether cell swelling occurred during the ischemic phase or if a period of reflow was necessary, in four animals the hearts were excised at the end of a 60 min occlusion before any reflow of blood. Every myocardial and endothelial cell was swollen according to the criteria described in each of six randomly selected fields. For the purposes of this study, it was necessary to establish only whether swelling was initiated before or after reflow.

Necrosis

Hearts were examined for necrosis following a prolonged reflow period. In 20 dogs 12 hours of reflow of blood followed either a 40 (12 dogs) or 60 (8 dogs) min period of ligation; half the dogs in each group received mannitol. Figure 8 is illustrative of the results obtained by light microscopy and shows the basis of our evaluation of necrosis. Figure 8a is from the nonischemic control anterior papillary muscle of a dog which underwent a 60 min occlusion, had an infusion of plasma, and 12 hours of reflow. As can be seen, the cellular architecture by phase microscopy in this nonischemic muscle appears normal. Figure 8b illustrates substantial disruption of cellular architecture in the ischemic posterior papillary muscle of the same dog. On the right (fig.
8c) is shown an ischemic muscle of another animal which received mannitol. Scattered foci of necrosis are illustrated by the arrows; however, for the most part, the cellular architecture appears maintained in this animal. Verification that the pale cells were necrotic was provided by electron microscopy of each sample (fig. 9).

The percent of necrotic cells from each group (random selection) was evaluated for the extent of myocardial necrosis. In three randomly selected dogs from the group which underwent a 40 min occlusion and which received a control saline infusion, 32.7%, 27.7%, and 21.3% of the posterior papillary muscle cells were necrotic. The mean percentage of necrotic cells was 27.2 ± 3.3%. In three randomly selected animals from the group which underwent an identical period of occlusion and reflow but which received mannitol instead of saline, the number of necrotic cells in the posterior papillary muscles was substantially ($P < 0.002$) reduced (2.3%, 3.9%, and 1.2%) (mean 2.5% ± 0.8%) (fig. 10). In these animals the serum osmolality at the end of the infusion of mannitol (at 15 min of reflow) was 345 ± 7 mOsm/L compared to 304 ± 2 mOsm/L in the control hearts.

In three randomly selected animals from the group which underwent a 60 min occlusion followed by 12 hours of reflow

**Figure 4.** Panel a is an electron micrograph obtained from a control anterior papillary muscle of a heart which sustained a 60 min occlusion of its circumflex artery followed by 45 min of reflow of blood. Note that the sarcolemma is not elevated, the T-tubular system (labelled “T”) appears patent, and the capillaries are of normal thickness. ×4000. Panel b is an electron micrograph of the posterior papillary muscle of the heart, the anterior papillary muscle of which was illustrated in a. Note that 60 min of ischemia following reflow of blood in the posterior papillary muscle results in elevation of the sarcolemma and a tendency toward obliteration of the T-tubular system. Note also the loose arrangement of myofibrils with an apparent increase in the intermyofibrillar-sarcoplasmic space. This is appreciated more clearly at a higher magnification in panel d. ×4000. Panel c is an electron micrograph from the posterior papillary muscle of another heart which underwent 60 min of circumflex artery occlusion followed by 45 min of reflow but which received mannitol during the last 15 min of occlusion and the first 15 min of the period of reflow of blood. Note that the sarcolemma is not elevated, the T-tubular system is patent, and the capillary endothelia appear normal in thickness. ×4000. Panel d is an electron micrograph from the same ischemic posterior papillary muscle as in b. At this magnification the criteria for swelling are readily seen. Note that the granular sarcoplasm (SP) is readily seen between adjacent myofibrils and the individual mitochondria (M). It is particularly clear here that the intermyofibrillar-sarcoplasmic space is distended whether the myofibrils are viewed slightly longitudinally (MFL) or in near-perfect cross-section (MFX). The intercellular space (I) is bounded by sarcolemma which is, at most of the levels shown, lifted off the subjacent cellular organelles. ×14,000.
and a similar plasma infusion regimen, the percentages of necrotic cells in the posterior papillary muscles were 80.2%, 64.5%, and 71% (mean 71.6% ± 4.6%). In three other randomly selected animals which underwent the same experimental protocol but which received mannitol instead of plasma, the percentages of necrotic cells in the posterior papillary muscles were 12.6%, 8.7%, and 20.7% (mean 14.0% ± 3.5%). The decrease in number of necrotic cells at 60 min occlusion with mannitol was significant (P < 0.001). The serum osmolality in the former group was 304 ± 3 mOsm/L and 344 ± 7 mOsm/L in the latter group. Thus, the percent of necrotic cells increased substantially with a longer period of ischemia, and the percent reduction in the mannitol-treated dogs is similarly great, although slightly less (approximately 80% as compared with approximately 90% in the dogs with 40 min occlusions).

| Table 3. Physiologic Data on Histology (Cell Swelling) Experiments† |
|----------------------|----------------|------------|----------------|----------------|----------------|
|                      | MAP (mm Hg)   | pH         | pCO₂ (mm Hg)  | pO₂ (mm Hg)   | Osm (mOsm/L)  |
| **40 min Arterial Occlusion Plus Reflow (N = 6)**                  |               |            |                |                |                |
| Pre-occlusion        | S 92 ± 3      | 7.34 ± .04 | 40 ± 2        | 271 ± 85      | 305 ± 7        |
|                      | M 91 ± 4      | 7.38 ± .09 | 28 ± 5        | 273 ± 70      | 299 ± 1        |
| 15 min reflow        | S 90 ± 4      | —          | 43 ± 1        | 202 ± 38      | 303 ± 2        |
|                      | M 87 ± 2      | —          | 34 ± 2*       | 280 ± 40      | 339 ± 2*       |

*P < 0.05.
†The data for the 60 minute occlusion plus reflow experiments are on file. See legend to table 2. Abbreviations as in table 1.
The associated hemodynamic, blood gas, and electrolyte data were comparable whether or not mannitol was administered (table 4).

**Discussion**

This study demonstrates that a transient period of regional myocardial ischemia results in a reproducible pattern of morphologic changes which include myocardial and capillary endothelial cell swelling. The administration of hyperosmotic mannitol reduced the amount of ischemic cell swelling and substantially lessened the extent of eventual myocardial necrosis.

The results of the ischemia experiments in which mannitol was not administered are consonant with those of Jennings and co-workers.\(^7\) In the present study the amount of eventual necrosis was strikingly reduced by elevation of extracellular osmolality by mannitol. A reassessment of the time of total interruption of blood flow required to produce histologic necrosis appears necessary. It may be that the administration of mannitol considerably later in the period of reflow than was studied here would similarly preserve ischemic myocardial cells.

There are several possible mechanisms for the reduction in necrosis by hyperosmotic mannitol. Since the amount of both necrosis and swelling of myocardial and capillary endothelial cells correlates with the duration of the ischemic insult, failure of cell volume regulation could be postulated as a contributing factor to cell death. Indeed, the present data show a close correlation between the percentages of swollen cells (26.5% and 81.6% after 40 and 60 min ischemia, respectively) and of necrotic cells (27.2% and 71.6%, respectively). Redistribution of fluid leading to cell swelling appears to occur during the time of interruption of blood flow. Therefore, reflow of blood seems unessential for initiation of cell swelling.\(^6\) This redistribution of fluid is probably related to interference during ischemia with the continuous metabolically driven extrusion of sodium from cells. In its absence, sodium and chloride accumulate within cells, leading to an accumulation of water within the cell, and an increase in cell volume. Lea\(^9\) demonstrated that these events accompany impaired metabolism in the renal cortex.
phenomenon that swelling is associated with progressive cell damage seems to be a general one in biology. Our data suggest that if one can prevent or diminish cell swelling, eventual necrosis will be prevented or diminished.

The viability of individual cells may be enhanced through restoring normal cellular configuration. Many important metabolic processes depend upon membrane-bound enzymes. With swelling of cells and their organelles, the critical spatial arrangements of the enzymes may be so distorted that reactions vital to the cell are impaired. Shrinking the cell may preserve the spatial arrangements.

Experimental evidence in the brain suggests that cell

Table 4. Physiologic Data in Histology (Necrosis) Experiments

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<td>P 103 ± 5</td>
<td>7.30 ± 0.03</td>
<td>49 ± 7</td>
<td>270 ± 52</td>
<td>302 ± 2</td>
</tr>
<tr>
<td></td>
<td>M 105 ± 6</td>
<td>7.27 ± 0.04</td>
<td>51 ± 8</td>
<td>289 ± 51</td>
<td>303 ± 2</td>
</tr>
<tr>
<td>Release of occlusion</td>
<td>P 89 ± 2</td>
<td>7.31 ± 0.03</td>
<td>48 ± 5</td>
<td>309 ± 41</td>
<td>303 ± 2</td>
</tr>
<tr>
<td>at 40 min</td>
<td>M 94 ± 4</td>
<td>7.22 ± 0.03</td>
<td>52 ± 6</td>
<td>323 ± 51</td>
<td>340 ± 5*</td>
</tr>
<tr>
<td>2 hr reflow</td>
<td>P 85 ± 6</td>
<td>7.30 ± 0.03</td>
<td>48 ± 6</td>
<td>244 ± 43</td>
<td>307 ± 2</td>
</tr>
<tr>
<td></td>
<td>M 80 ± 6</td>
<td>7.25 ± 0.02</td>
<td>43 ± 5</td>
<td>257 ± 59</td>
<td>330 ± 2*</td>
</tr>
<tr>
<td>8 hr reflow</td>
<td>P 86 ± 6</td>
<td>7.31 ± 0.01</td>
<td>43 ± 4</td>
<td>275 ± 41</td>
<td>307 ± 7</td>
</tr>
<tr>
<td></td>
<td>M 72 ± 6</td>
<td>7.32 ± 0.02</td>
<td>35 ± 4</td>
<td>296 ± 50</td>
<td>331 ± 3*</td>
</tr>
<tr>
<td>12 hr reflow</td>
<td>P 65 ± 6</td>
<td>7.23 ± 0.03</td>
<td>33 ± 0</td>
<td>217 ± 8</td>
<td>297 ± 2</td>
</tr>
<tr>
<td></td>
<td>M 56 ± 6</td>
<td>7.23 ± 0.03</td>
<td>36 ± 1*</td>
<td>230 ± 50</td>
<td>334 ± 4*</td>
</tr>
</tbody>
</table>

*P < 0.05.
†The data for the 60 minute occlusion plus reflow experiments are on file. See legend to table 2.
Abbreviations as in previous tables except plasma infusions were administered in all control experiments.
swelling may lead to compression of the lumina of small vessels. In the kidney similar cell swelling in the subcortical zone results in decreased perfusion to that area. We feel that this is a possible mechanism in the heart and that a reduction of cell swelling may result in less resistance to blood flow to ischemic myocardium.
two major coronary arteries. In these areas reactive hyperemia, secondary to build-up of metabolic products, most likely outweighed interference with blood flow due to cell swelling. It is still possible, and even likely, that within the totally ischemic posterior papillary muscle resistance to blood flow rose above the control level following release of the occlusion.

The analysis of myocardial electrolyte and water content during reflow of blood following temporary coronary artery occlusion by Whalen et al.14 strongly suggests that a rapid increase in intracellular water occurs early during the reflow period. In the present study there was maximal or near maximal vasodilation at the initiation of reflow. At a time which would be expected to correspond to the rapid increase in intracellular edema, there was a rapid rise in coronary vascular resistance in the saline or plasma-treated animals. This increase in vascular resistance was consistently less rapid in the mannitol-treated animals. These two studies are consistent with the prevention by hyperosmotic mannitol of a progressive increase in vascular resistance in the setting of a progressive increase in cell volume of at least some of the cells in the ischemic region.

In addition to reducing cell swelling in areas of ischemic myocardium, as documented in the present study, hyperosmotic agents directly lower vascular resistance at least in several organs1-15,16 including the heart.1-17 Substantial lowering of the hematocrit through the administration of mannitol can also, through changes in blood viscosity, influence blood flow, but in our study in which hematocrit changes were similar in both the mannitol and plasma-treated animals, the changes in blood flow persisted in mannitol-treated animals. Direct effects of mannitol on cell function may also be acting.

Irrespective of the relative contributions of these other effects of mannitol, elevation of extracellular osmolality appears to be an effective mode of reducing cell death in ischemic myocardium.

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References

The Similarity of Changes in Segmental Contraction Patterns Induced by Postextrasystolic Potentiation and Nitroglycerin

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SUMMARY Despite a fundamental difference in their underlying mechanisms, both postextrasystolic potentiation (PESP) and administration of nitroglycerin (TNG) have been utilized to predict reversibility of abnormal segmental wall motion in patients with ischemic heart disease. To determine whether these interventions induce the same changes in segmental contraction pattern, we analyzed biplane ventriculograms of 14 patients who had an adequately visualized PESP beat on a basal ventriculogram as well as a post-TNG ventriculogram. Four segments in each plane were defined and the area ejection fraction of each segment was calculated for a basal sinus, PESP, and post-TNG beat. To correct for global differences in the response to PESP and TNG, we normalized each segmental ejection fraction (NSEF) by the ventricular ejection fraction for that beat and then compared the differences in NSEF from the basal value after PESP and TNG. Eleven patients demonstrated similar responses to both interventions. The three patients whose responses were discordant had elevated or unchanged left ventricular systolic and end-diastolic pressures at the time of the TNG ventriculogram. Our data suggest that, provided these pressures are lower than basal values at the time of the TNG ventriculogram, PESP and TNG will induce similar changes in segmental contraction patterns. Seven patients with similar responses had a PESP beat on their post-TNG ventriculogram. Changes in NSEF after PESP+TNG were identical to those after either intervention. This implies that the combination of interventions does not induce further changes in segmental contraction pattern beyond that produced by either intervention alone.

IN PATIENTS WITH ISCHEMIC HEART DISEASE, localized segments of the ventricle which display decreased systolic wall motion may be composed of compromised but viable muscle fibers rather than scar tissue. With the development of coronary artery bypass surgery, the concept of restoring contractile function by improving blood supply to an ischemic area has received increased consideration and has been supported by documentation of improvement in left ventricular function in some patients after such surgery. To preoperatively identify ischemic regions of the ventricle with the potential to improve their contractile function after adequate revascularization, several groups of investigators have evaluated the response of abnormally contracting segments to postextrasystolic potentiation (PESP), which enhances myocardial contractility, or to administration of nitroglycerin (TNG), which usually decreases the workload of the heart. Both of these interventions have been reported to improve the systolic motion of some hypokinetic and akinetic segments, and limited postoperative studies appear to confirm the predictive value of these induced changes.

The use of PESP requires a ventriculogram with an early extrasystole followed by an adequately visualized sinus beat, whereas the administration of TNG requires the performance of a second ventriculogram. In an individual patient, the choice of which intervention to use may be limited. Therefore, we performed this study to ascertain whether the comparative effects of PESP and TNG are the same for all segments of a given ventricle regardless of the basal function of any segment and despite the difference in the underlying mechanisms of the two interventions.

Methods In patients with chest pain suggestive of angina, we performed retrograde left heart catheterization with the patient in the postabsorptive state and following premedication with diazepam, 10 mg i.m. A #7F NIH catheter was passed from the right brachial artery or a #8F pigtail catheter was passed from the right femoral artery. Left ventricular pressures were monitored using a Statham P23Db
The protective effect of hyperosmotic mannitol in myocardial ischemia and necrosis.
W J Powell, Jr, D R DiBona, J Flores and A Leaf

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