The Effect of Mannitol Following Permanent Coronary Occlusion

HEINZ O. HIRZEL, M.D., AND EDWARD S. KIRK, PH.D.

With the technical assistance of Ross Raymond and Robert Schouler

SUMMARY Quantitative comparisons of infaracts 24 hours after ligation of the left anterior descending coronary artery (LAD) via thoracotomy were made in 13 control and 13 dogs treated with i.v. 25% mannitol, 2 ml/min for 4 hours following occlusion. Mannitol increased serum osmolality by 44 ± 4 mOsm/L (mean ± 1 SE) with hemodynamic effects limited to a small increase in left ventricular dP/dt. Nonperfusable tissue measured by planimetry at 24 hours was similar in both groups (46 ± 4% of area defined with dye injected into the distal LAD for control versus 48 ± 5% for mannitol treated dogs, P = NS). Creatine phosphokinase activity in infarcted tissues was also similar in both groups. Myocardial blood flows measured with radioactive microspheres were also similar in both groups. Collateral conductance calculated from retrograde flow and aortic pressure increased within the 24 hour period by 146 ± 23% in the control dogs; in the mannitol treated dogs, collateral increased only 38 ± 14% (P < 0.001). Thus mannitol had no effect on ultimate infarct size. Moreover, mannitol appeared to hinder the development of collateral vessels.

THE OBSERVATION of Sheehan and Davies1 of failing renal reflow after 3 hours of total ischemia evoked a series of extensive studies directed to elucidate the role of early vascular changes in tissue rendered ischemic. In experimental animal models it has been shown that endothelial cells of the small vessels begin to swell within 20 to 120 minutes following the onset of ischemia in the brain,2 heart,3 and kidney.4, 5 This intracellular edema may, by narrowing of the vascular lumen, hinder the passage of red blood cells, thus causing a further reduction of blood supply to the ischemic tissue.6, 7 It has been proposed that increasing serum osmolality by infusion of hyperosmotic agents such as mannitol may prevent these circulatory defects.8 Hypertonic mannitol given to increase serum osmolality by approximately 40 mOsm has been shown to increase flow to the heart following the release of a prolonged coronary occlusion in anesthetized dogs on right heart bypass9 and increases in blood flow following ischemia were also found in kidneys from rats10 and dogs11, 12 as well as in brains from rabbits13 and baboons14 when treated with mannitol. Changes in regional coronary blood flow in the ischemic as well as in the normal myocardium after treatment with mannitol were also observed in the conscious dogs during acute and chronic myocardial ischemia.15–18 In addition, hyperosmolality was found to improve myocardial performance both in isolated cardiac muscle19–21 and in the intact heart with normal coronary perfusion and after regional ischemia.22–24

These results led to the speculation that treatment with hypertonic mannitol might be of value in salvaging ischemic
myocardium. However, quantitative effects of mannitol on infarct size have not been reported in a preparation with permanent coronary occlusion. Accordingly, the present study was undertaken to evaluate the effects of a four hour treatment with hypertonic mannitol on the extent of myocardial damage 24 hours after acute coronary occlusion in dogs.

Methods

Experimental Preparation and Procedure

Thirty-one mongrel dogs weighing between 12 and 20 kg (average weight 15.1 kg) were anesthetized with 27.5 mg/kg sodium pentobarbital. Additional amounts were administered as needed to maintain prolonged anesthesia. The dogs were intubated with an endotracheal tube and ventilated with 100% O₂ via a Harvard respirator. Through a left-sided thoracotomy performed under clean conditions the heart was exposed and the left anterior descending coronary artery (LAD) isolated below its first diagonal branch. Polyvinyl catheters were inserted through the left atrial appendage for injecting radioactive microspheres and into the left ventricle to measure left ventricular pressure and its first derivative with respect to time, LV dp/dt. In addition, a third catheter was introduced into the thoracic aorta via the right femoral artery to measure systemic blood pressure. All pressures measured by means of Statham P 23 Db pressure transducers (Statham Instruments, Inc.) were recorded continuously on a Beckman Type S II multichannel oscillograph (Beckman Instrument Co.).

The LAD was then ligated and a cannula inserted distal to the occlusion. Perfusion was subsequently continued with arterial blood from the left carotid artery derived through a large bore cannula and a polyvinyl tubing system. Through a first side-arm in this bypass-system near the inserted cannula either the perfusion or the peripheral coronary pressure could be measured (fig. 1). A second side-arm allowed measurements of retrograde flow during back-bleeding against atmospheric pressure using a graduated cylinder and a stop watch. This side-arm was placed below the level of the heart. This was based on preliminary experiments which showed that retrograde flow was maximal and constant as the level of the outflow tubing was lowered below the heart. The procedure of inserting the cannula into the LAD ordinarily caused an interruption in perfusion of no longer than 2 to 3 minutes. After restoring flow in the distal LAD, the area was perfused through the bypass system for a recovery period of approximately 20 minutes. At the time of distal LAD-bypass, the animals were anticoagulated with 5,000 units of heparin.

The LAD was occluded by clamping the bypass system near the second side-arm, thus keeping the additional vascular dead space minimal. Ten minutes after occlusion, a set of radioactive microspheres was injected to measure myocardial blood flow in the normal and in the ischemic region. Thereafter, retrograde flow was measured and blood samples were taken to determine the hematocrit in all 31 dogs, the serum osmolality in the 18 dogs which subsequently received the treatment and the serum electrolytes Na⁺, K⁺, Cl⁻ and Ca²⁺ in seven of the treated dogs.

Fifteen minutes after occlusion the treatment with mannitol was instituted in 18 of the 31 dogs. Mannitol was infused intravenously as a 25% solution at a rate of 2 ml/min. This infusion was continued over a period of four hours. Forty-five minutes after occlusion and 30 min after the beginning of the treatment a third set of microspheres was injected, the hemodynamic measurements repeated, and blood samples taken for the determination of the hematocrit and the serum osmolality to determine the initial response to the treatment. Three hours after the beginning of the treatment, at a time when serum osmolality was presumably at an equilibrium level, serum osmolality and hematocrit were again determined.

After the third set of microspheres was injected, the catheters inserted through the left atrial appendage and the LAD cannula were removed. Fifty mg of protamine sulfate were administered and the pericardium and chest were closed. Air and fluid were evacuated from the chest cavity by a tube that remained under suction for about four hours. Premature ventricular beats and tachyarrhythmias which occurred frequently within the first 20 min after LAD occlusion were treated in five animals receiving mannitol as well as in five animals that served as controls with bolus injections of 50 to 100 mg of a 2% solution of lidocaine hydrochloride. The five mannitol-treated dogs received an average of 120 mg, the five control dogs an average of 80 mg of the antiarrhythmic drug. Ventricular fibrillation occurred in one dog from each group within 14 min after occlusion and was successfully treated with electric countershock. Both dogs survived the 24 hour study period.

Mannitol caused an extraordinary fluid loss due to osmotic diuresis which was monitored during the first 5 hours after occlusion by collecting the urine through a catheter in-
roduced into the urinary bladder. The fluid loss was continuously compensated for by infusion of Ringer-lactate solution and a positive net fluid balance of 200 ml was attempted. The 13 control dogs were each infused with 200 ml of saline.

In addition a single dose of sodium ampicillin of 500 mg was administered intramuscularly and morphine sulfate at a dose of 15 mg was given subcutaneously to each dog to insure the comfort of the animal when it was awake. Additional morphine was given if needed.

The following day the dogs were reanesthetized with about one half of the initial dose of sodium pentobarbital (irreversible cardiac arrests were observed in an earlier series of animals undergoing the same experimental procedure when the full dose of pentobarbital was employed). The animals were then prepared as before, and after administration of 5,000 units of heparin, the LAD was recannulated at the site of ligation. Twenty-four hours after occlusion another set of radioactive microspheres was injected followed by the hemodynamic and retrograde flow measurements. In addition, a final blood sample was taken for the determinations of the hematocrit, the serum osmolarity and the serum electrolytes. At the end of the experiment a 5 to 8 ml bolus of a 2% solution of Evans blue dye was hand-injected slowly through the inserted cannula to stain the region originally supplied by the occluded distal portion of the LAD (fig. 2). Simultaneously ventricular fibrillation was induced by intravenous injection of 10 to 15 ml of a saturated potassium solution. The heart was then removed and frozen quickly in a bath of dry ice and alcohol.

Delineation of the Tissue at Risk

The myocardium jeopardized by acute occlusion of a normal coronary artery is the tissue normally supplied by the occluded vessel. Since the amount of tissue supplied by an artery occluded at a specific site varies from heart to heart methods were devised to identify this tissue. In this way the various indices of infarct size could be normalized relative to the amount of tissue at risk. Although the dye injected at the end of each experiment provided a rough guide of the size of the tissue normally perfused by the occluded LAD and allowed identification and dissection with only gross examinations, several factors may limit its usefulness. The injection of the dye at pressures differing from the systemic blood pressure may cause a spread of the dye into normal tissue or incomplete staining of the region at risk. Since the dye has to be concentrated to effectively stain the tissue, small inclusions of tissue may go undetected. Finally the highly convoluted border makes the exact separation between normal and stained tissue extremely difficult, if not impossible.

To delineate this tissue portion with accuracy the normal myocardium, i.e., the myocardium perfused by vessels other than the distal portion of the LAD (which subsequently was occluded), was labelled with radioactive microspheres prior to occlusion. The device used for this purpose is shown in figure 1. Two additional side-arms in the bypass-system led to a 50 ml reservoir containing a balloon and arranged so that filling of the balloon resulted in a simultaneous emptying of the reservoir. The balloon was large enough to remain flaccid even when it occupied the entire reservoir volume. Prior to assembly of the reservoir the balloon was tested for leaks by distending it with saline. After assembly the saline remaining in the balloon was withdrawn through its connecting side-arm so that no air remained trapped inside the balloon. The myocardium supplied by the distal LAD could thus be perfused with reservoir blood at the pressure transmitted to the outflow by distention of the flaccid rubber balloon (see below). Initially clamps A and B were closed and clamp C open, thus permitting normal perfusion of the artery.

Immediately prior to the first microsphere injection, the stopcock at the top of the reservoir was opened and the reservoir was filled with arterial blood by opening clamp B. The stopcock then was closed. Clamp A then was opened and clamp C closed, causing the empty balloon in the closed chamber to fill and thus displace reservoir blood into the distal LAD. Under these conditions, the pressure at the level of the LAD cannula was identical to the pressure prior to initiating perfusion from the reservoir and corresponded closely to aortic pressures. Mean perfusion pressure was within 2 mm Hg of aortic root pressure while phasic pressures transmitted through the carotid artery and the bypass system were similar to the distal aortic pressures. During diastole the aortic root pressure exceeded perfusion pressure by only 5–10 mm Hg, and then only during a portion of diastole. Perfusion from the reservoir could be continued without reduction in pressures for at least 2½ min. Thus, all coronary arteries were perfused at similar pressures and blood flow in interarterial anastomoses should be minimal. At this time the first set of microspheres was injected into the left atrium and ones destined for the LAD were collected in the balloon. Microspheres were deposited in the heart except in the area supplied by the distal LAD. Any myocardial samples subsequently obtained from the LAD area which were found to contain these microspheres must have contained some tissue that was normally perfused by blood delivered through adjacent arteries. Perfusion in this tissue presumably continues after LAD occlusion and this tissue, therefore, cannot be properly considered as ischemic tissue. In our view, only the tissue supplied by the LAD is made ischemic by LAD occlusion. Thus, the presence of microspheres from this first injection in samples from the LAD area identifies adequately perfused tissue which “contaminates” tissue made ischemic by LAD occlusion. If blood flow in this contaminating tissue is equal to that in more distal samples from the myocardium perfused by the circumflex artery the amount of normal tissue contaminating samples from the LAD region can be estimated from this first set of microspheres.

Guided by the blue staining tissue supplied by the distal portion of the LAD was separated from the unstained normal myocardium by careful dissection along the blue border. And as was expected, samples from the border of this region contained significant numbers of microspheres from this first injection, indicating that tissue supplied by the LAD interdigitates to various degrees with adjacent normally perfused tissue and cannot be easily separated.

Since perfusion of the normal tissue contaminating these border samples remains following LAD occlusion and may even become hyperemic, flows calculated for these samples represent an average of flow in ischemic and normal tissue and may not represent the degree of ischemia for any par-
Tissue Separation and Analysis

First, prior to freezing of the hearts, a representative transverse slice of 0.5 to 1 mm in thickness was cut out of the heart. The slice extended from the apparent center of the stained region to normal tissue on either side. This small piece of myocardium was fixed in a 10% buffered formaldehyde solution for further histologic examination. The hearts then were frozen as described above and subsequently cut into slices of 4 mm in thickness from the apex to the base using an electric slicing machine (fig. 2). The region supplied by the distal LAD was clearly delineated from the normal myocardium supplied by unoccluded vessels by the Evans blue dye injected into the distal LAD. The blue dye, however, failed to stain the subendocardial region to a variable extent which appeared more or less homogeneously white and was often surrounded by a hemorrhagic border. This part of the tissue was therefore considered to be a region of nonperfusion and was, as a detailed histologic examination revealed, grossly necrotic. In contrast, the blue stained tissue appeared to be normal and was therefore called ischemic tissue. The white and the blue stained tissue together comprised the total of the involved tissue as defined by this staining technique. Pictures were taken from each sliced heart to assess the different areas by planimetry. The areas of grossly necrotic tissue could then be expressed in percent of the total of the involved tissue and in percent of the left ventricular area.

A transmural slightly sector-shaped piece of myocardium was cut out of the center of the involved area in each slice and divided into five equal layers from the endo- to the epicardium. Corresponding layers from adjacent slices were pooled and the five samples obtained in this way used for the determination of creatine phosphokinase (CPK) activity and microspheres. Control samples were taken out of the normal myocardium of the posterior wall of the left ventricle. The weight of these tissue samples from the ischemic and infarcted zone averaged 0.71 ± 0.02 g and from the normal myocardium averaged 0.97 ± 0.03 g. In addition, all of the remaining blue stained and nonperfusable tissue was cut out of the heart. Special attention was given to include all the blue stained tissue, to the extent that small amounts of unstained tissue had to be included. These samples were processed in the same way as the five individual samples to obtain the amount of CPK activity left in the entirely involved tissue.

Tissue Creatine Phosphokinase Activity

CPK was analyzed in all samples using the method described earlier by Kjekshus and Sobel. The samples obtained from the center of the involved area as well as the remaining blue stained and nonperfusable tissue were placed in 20 ml of iced ethylenediamine tetroacetic acid-2-mercaptopropanol-sucrose-buffer and then disrupted in a Virtis homogenizer (Virtis Company) in two bursts of 15 sec duration, speed no. 4. The homogenate was then centrifuged at 17,300 × g for 20 min which allowed complete recovery of the microspheres in the pellet for determination of the myocardial flows, while the corresponding CPK levels could be measured in the supernatant. The CPK activity was expressed in international units (I.U.) per g wet tissue weight. Specific activity (CPK activity per mg of supernatant protein), which is useful in identifying the purity of an enzyme, was not used since in our experience the protein determination only adds variance to the results. Furthermore, the protein determination by the Biuret method was influenced by the Evans blue dye in the samples from the ischemic region since the dye absorbs light of the same wave length.
used in the protein assay. This was not a problem for the CPK determination. However, in the control samples CPK specific activity was quite similar to values reported recently by others, averaging 45.1 ± 2.0 I.U./mg protein.

**Administration of Microspheres and Determination of Tissue Flows**

Myocardial blood flow and cardiac output were measured using standard carbonized microspheres of 15 ± 5 μ in diameter labelled with the nuclides 141Ce, 85Sr, 51Cr and 103Nb (3M Company) as described by Rudolph and Heymann. The microspheres were suspended in a 63% sucrose solution. Prior to injection they were dispersed by mechanical agitation and sonication in an ultrasonic bath for 5 min. Ten ml of the solution containing 109 beads were injected over a period of 20–25 sec through the left atrial cannula, which was subsequently flushed with 10 ml of saline. Usually no changes in the hemodynamic parameters recorded could be observed following the injections, but occasionally a decrease in aortic pressure of 10–20 mm Hg lasting for about 10–15 sec was noted. An arterial blood sample was withdrawn starting just prior to the administration of the microspheres and continuing for 30 sec beyond the end of the injection using a Harvard pump at a withdrawal rate of 11.6 ml/min (Harvard Apparatus Co.) to permit calculation of absolute tissue flows and the cardiac output.

All tissue and the corresponding blood samples were subjected to gamma-ray spectrometry using a Searle Analytic Model 1185 3-channel gamma-ray counter with a two inch crystal (Searle Analytic). The number of microbeads present per g of tissue averaged 495 ± 25 in the control samples; it was subsequently less in the samples from the ischemic or infarcted tissue, where it averaged 85 ± 12 beads/g. The data were corrected for background and cross-over counts on a PDP-11 computer (Digital Equipment Corp.) and the tissue flows and cardiac outputs expressed in absolute units.

**Histologic Preparations**

The myocardial tissue fixed with formaldehyde solution was processed in a routine fashion. The histologic sections were stained with hematoxylin-eosin and subjected to qualitative light microscopic examination. The examiner had no knowledge which sample was from a treated dog and which one from a control dog.

**Statistics**

All results were expressed as means of ± 1 se. Paired and unpaired comparisons with Student's t-test were used to evaluate the statistical significance of differences in the data within and between the groups. The differences in mortality rate between treated and untreated animals were analyzed with the χ²-test using Yates' correction since the sample numbers were small.

**Results**

Thirteen of the 18 dogs treated with mannitol recovered well from the first surgical intervention and could be restudied the next day. Five treated dogs survived coronary artery occlusion for only 4, 5, 8, 12 and 23 hours respectively and could not be restudied at 24 hours. Although the exact cause of death is not known, the animals showed unusually rapid increases in serum osmolarity and three cases were characterized by progressive cardiovascular collapse. In general, the treated animals appeared significantly more debilitated on the day of the restudy than untreated ones. None of the 13 control dogs died. Although the difference in mortality is not significant, a larger series of animals would be needed to rule out a detrimental effect of mannitol. It is of interest, however, to note that in the only other study to report mortality figures, the death rate was also higher in animals treated with mannitol (71%) than in untreated animals (44%). It must be emphasized that significant differences between the present study and this other study limit direct comparisons of the mortality figures.

In the remaining 13 mannitol treated dogs, serum osmolarity increased by 13.0 ± 3.2 mOsm/L from a control value of 309.2 ± 2.6 mOsm/L within the first 30 min of the treatment (P < 0.01) and by 43.7 ± 3.6 mOsm/L three hours after the beginning of the mannitol infusion (P < 0.001). This value is similar to the ones reported by others. Twenty-four hours after occlusion, serum osmolarity had again returned to normal levels.

The hematocrit decreased significantly during the 24 hour period within each group without statistical difference between the groups (from 42 ± 1% to 36 ± 1% [P < 0.001] in the mannitol treated and from 40 ± 1% to 34 ± 1% [P < 0.01] in the control dogs). The decline is explained as the result of blood loss and mobilization of extravascular fluid. During the time of treatment the hematocrit decreased, as expected, as serum osmolarity increased. The treatment with hypertonic mannitol led to an extraordinary osmotic diuresis resulting in an average fluid loss of 1770 ± 180 ml (< 10% of body weight) during the time of treatment, whereas the control dogs lost virtually no volume. The fluid loss in the treated animals was compensated for by a continuous infusion of Ringer lactate solution of 1970 ± 190 ml which resulted in a net positive fluid balance of approximately + 200 ml, the amount of saline which was routinely administered to the control dogs.

Because of the excessive fluid loss caused by mannitol which was observed in a first group of treated dogs, serum electrolytes were measured in a second group of the treated animals. Sodium and chloride did not change during the 24 hour period. Although potassium and calcium decreased from 4.05 ± 0.16 mEq/L to 3.42 ± 0.18 mEq/L (P < 0.05) and from 11.0 ± 0.7 mg% to 9.1 ± 0.3 mg% (P < 0.05), respectively, they remained within normal limits.

**Hemodynamic Findings**

The hemodynamics in both groups were similar (table 1). The heart rate did not change appreciably during the entire period of observation. Mean aortic pressure decreased significantly in both groups, from 131 ± 4 mm Hg to 94 ± 5 mm Hg (P < 0.001) in the treated and from 120 ± 5 mm Hg to 87 ± 3 mm Hg (P < 0.001) in the control group, but without statistical difference between the groups. Mean left atrial pressure increased slightly over the 24 hour period but
again was similar in both groups. Mean peripheral coronary pressure tended to increase in both groups but the differences were not statistically significant. A small increase in left ventricular dP/dt from 2930 ± 220 mm Hg/sec to 3230 ± 200 mm Hg/sec (P < 0.05) was noted in the mannitol treated animals during the time of treatment. The cardiac output decreased in each group averaging 2310 ± 110 ml/min initially and 1970 ± 120 ml/min (P < 0.001) 24 hours after occlusion in the mannitol treated animals and 2330 ± 270 ml/min initially and 1840 ± 140 ml/min (P < 0.001) in the control animals respectively. Again no difference was found between the two groups. Retrograde flow did not change in the treated animals but increased substantially in the control animals (3.5 ± 0.5 ml/min at the beginning and 3.2 ± 0.5 ml/min at 24 hours [P = NS] in the mannitol groups and 2.8 ± 0.6 ml/min at the beginning and 4.4 ± 0.6 ml/min [P < 0.001] in the control group.). The difference between the two groups, however, was statistically not significant.

Gross and Histologic Examination of the Hearts

The volume of myocardium supplied by the distal LAD was well delineated by the Evans blue dye injected at the end of the experiment. In each heart, however, the dye failed to penetrate the subendocardial layers and, to a variety extent, the midwall, and sometimes even parts of the epicardium, which then appeared white throughout, occasionally slushy brown-grey. These regions were often surrounded by a narrow hemorrhagic rim (< 1 mm) which was not always sharp and generally paralleled the curvature of the endocardium. Since the absence of patent vessels did not allow the blue dye to penetrate, and thus stain these regions and since the fiber-like fine structure was absent, the tissue within the hemorrhagic rim was considered to be grossly necrotic. This nonperfusable tissue occupied approximately the same thickness of wall up to the lateral borders of the lesion and was separated from clearly normal myocardium by only a very small blue rim of tissue. In contrast to this nonperfusable tissue the remaining blue-stained myocardium appeared normal except for an occasional isolated focus. This tissue, although ischemic to some extent, could still be perfused and thus might be capable of survival. This pattern of perfusable and nonperfusable tissue was observed with regularity in the control as well as in the mannitol-treated dogs. Since we were unable to identify the degree of damage within the blue-stained tissue it was considered ischemic but surviving. This classification is of course an oversimplification.

Qualitative histologic routine examination of representative tissue samples from all hearts by light microscopy confirmed the gross differentiation. The areas designated as grossly necrotic revealed a wide variety of tissue damage with absent or disturbed cross striation of the fibers, pyknotic nuclei, hypereosinophilic muscle fragments and massive waviness of the fibers, extravasation of red blood cells and infiltration of polymorphonuclear leucocytes. The blue-stained tissue, even though damaged to a much lesser degree, also showed some mild interstitial edema, infiltration of leucocytes, mainly at the epicardial surface, and areas of focal necrosis or wavy fibers. All these changes were

<table>
<thead>
<tr>
<th>Heart Rate (beats/min)</th>
<th>Control (n=9)</th>
<th>Mannitol (n=8)</th>
<th>Initial</th>
<th>Control (n=9)</th>
<th>Mannitol (n=8)</th>
<th>24 hr</th>
<th>Initial</th>
<th>Control (n=9)</th>
<th>Mannitol (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>161 ± 5</td>
<td>168 ± 5</td>
<td>163 ± 4</td>
<td>165 ± 7</td>
<td>164 ± 4</td>
<td>162 ± 3</td>
<td>161 ± 8</td>
<td>165 ± 4</td>
<td>164 ± 3</td>
<td>162 ± 3</td>
</tr>
<tr>
<td>121 ± 4</td>
<td>124 ± 3</td>
<td>120 ± 2</td>
<td>114 ± 5</td>
<td>111 ± 3</td>
<td>110 ± 2</td>
<td>121 ± 3</td>
<td>122 ± 4</td>
<td>120 ± 2</td>
<td>118 ± 2</td>
</tr>
<tr>
<td>2310 ± 110</td>
<td>2330 ± 120</td>
<td>2330 ± 120</td>
<td>2330 ± 270</td>
<td>2330 ± 270</td>
<td>2330 ± 270</td>
<td>2330 ± 270</td>
<td>2330 ± 270</td>
<td>2330 ± 270</td>
<td>2330 ± 270</td>
</tr>
<tr>
<td>5.6 ± 0.5</td>
<td>5.7 ± 0.5</td>
<td>5.6 ± 0.5</td>
<td>5.7 ± 0.5</td>
<td>5.6 ± 0.5</td>
<td>5.7 ± 0.5</td>
<td>5.6 ± 0.5</td>
<td>5.7 ± 0.5</td>
<td>5.6 ± 0.5</td>
<td>5.7 ± 0.5</td>
</tr>
<tr>
<td>0.025 ± 0.004</td>
<td>0.025 ± 0.004</td>
<td>0.025 ± 0.004</td>
<td>0.025 ± 0.004</td>
<td>0.025 ± 0.004</td>
<td>0.025 ± 0.004</td>
<td>0.025 ± 0.004</td>
<td>0.025 ± 0.004</td>
<td>0.025 ± 0.004</td>
<td>0.025 ± 0.004</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SE. *P < 0.05; **P < 0.01. Each value of one parameter was compared with the first one measured within each group and with the corresponding value of the second group. After MANNITOL treatment.
present in the tissue of both control as well as mannitol treated dogs. The extent of necrosis was not quantified by morphometric measurements nor was examination by electronmicroscopy to identify the degree of edema performed.

Estimation of the Nonperfusible Tissue by Planimetry

The LAD perfused 41.4 ± 2.4% of the total left ventricular mass in the control group and 44.5 ± 3.4% in the group treated with mannitol (P = NS). The size of the nonperfusable tissue estimated by planimetry averaged 47.9 ± 5.3% in the mannitol treated dogs, and 45.5 ± 4.3% of the LAD perfused tissue in the control dogs (P = NS) (table 2). Thus no difference in the size of the nonperfusable tissue mass could be detected with this technique 24 hours after occlusion.

Infarct Size by Determination of Tissue Creatine Phosphokinase Activity

The tissue creatine phosphokinase activity left in the center transmural sample averaged 549 ± 75 I.U./g or 29.9 ± 4.1% of control in the mannitol treated animals and did not differ significantly from the activity found in the center transmural sample of the control dogs which was 617 ± 105 I.U./g or 34.0 ± 5.8% of control (table 2).

The CPK activity per gram tissue of the entire stained and nonperfusable region did not differ in the two groups, averaging 1061 ± 91 I.U./g for the mannitol treated group and 1003 ± 83 I.U./g for the control group. However, the method used to define the tissue mass supplied by the distal portion of the occluded artery clearly indicated the presence of a substantial amount of tissue normally perfused by adjacent vessels within the entire stained region. Since flow continued to perfuse this “contaminating” tissue, the CPK activity of the included normal tissue was assumed to be the same as in the control sample. Thus, the CPK activity was corrected for the apparent admixture of various degrees of normal CPK activity. The CPK activity calculated as being representative for only the tissue which was supplied by the occluded portion of the LAD was 584 ± 681 I.U./g or 31.8 ± 3.7% of the CPK activity in normal tissue for the mannitol treated dogs and 643 ± 84 I.U./g or 35.7 ± 4.6% of the CPK activity in normal tissue for the control dogs. Thus no difference in the extent of necrosis could be found by further refining these data.

Regional Myocardial Flows and Collateral Vessel Functions

The tissue flow in the center transmural sample of the involved area and in the normal myocardium was examined to determine if the increase in osmolarity had resulted in at least a change of the flow patterns during the 24 hour period.

In the normal myocardium, the transmural flow increased significantly 10 minutes after occlusion in both groups, averaging 1.66 ± 0.05 ml/min-g⁻¹ at 10 min as opposed to 1.16 ± 0.02 ml/min-g⁻¹ initially (P < 0.001) in the treated dogs, and 1.52 ± 0.14 ml/min-g⁻¹ at 10 min and 1.22 ± 0.12 ml/min-g⁻¹ initially (P < 0.001) in the control dogs. There was no significant difference between the groups nor was there a significant increase in the flow in the normal myocardium during the time of treatment (table 1). Twenty-four hours after occlusion the transmural flow did not differ from the initially measured flow, being 1.11 ± 0.11 ml/min-g⁻¹ for the mannitol treated dogs and 1.18 ± 0.09 ml/min-g⁻¹ for the control (NS).

### Table 2. Infarct Size Measurements

<table>
<thead>
<tr>
<th>Method of estimation</th>
<th>Mannitol treated dogs (N = 13)</th>
<th>Control dogs (N = 12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planimetry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Ischemia</td>
<td>47.9 ± 5.3</td>
<td>45.5 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>% of LV</td>
<td>20.1 ± 2.4</td>
<td>19.5 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Tissue CPK activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmural sample (% of control)</td>
<td>29.9 ± 4.1</td>
<td>34.0 ± 5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total area at risk (% of control)</td>
<td>31.8 ± 3.7</td>
<td>35.7 ± 4.6*</td>
<td>NS</td>
</tr>
</tbody>
</table>

*12 determinations.
Values are expressed as mean ± se.
Statistical analysis was performed using unpaired t-tests.
CPK = creatine phosphokinase.

In the ischemic myocardium the transmural blood flow delivered by collateral vessels measured within 10 minutes of occlusion was somewhat greater for the mannitol group but did not differ significantly from the control group. After 24 hours, transmural flow increased significantly in both groups averaging 0.17 ± 0.03 ml/min-g⁻¹ for the mannitol group and 0.18 ± 0.04 ml/min-g⁻¹ for the control group. Although the absolute increase in flow for the mannitol group was only 60% of the increase for the control group, the difference did not achieve statistical significance. An increase in ischemic flow during mannitol administration was not observed. The flow patterns within the myocardial wall showed the characteristic redistribution as described earlier, with blood flow decreasing significantly in the subendocardial layers between 10 minutes and 24 hours after occlusion, while flow increased in the epicardial layers (fig. 3). This change in flow gradient across the wall is most likely conditioned by an increase in resistance to flow in endocardial layers. However, the same kind of flow redistribution occurred in the dogs treated with mannitol. Thus, mannitol did not prevent the increase in resistance to flow in the infarcting subendocardium.

Mannitol treatment appeared to hinder the development of collateral vessel function. Collateral conductance, calculated from retrograde flow and mean aortic pressure, increased by 146% in the control dogs from 0.24 ± 0.055 ml/min-mm Hg⁻¹ to 0.53 ± 0.009 ml/min-mm Hg⁻¹ (P < 0.001), while it increased only by 38% in the mannitol treated dogs from 0.26 ± 0.004 ml/min-mm Hg⁻¹ to 0.34 ± 0.005 ml/min-mm Hg⁻¹ (NS) (table 1, fig. 4). This difference accounts for the smaller increase in flow observed in the ischemic myocardium in the mannitol-treated animals.

### Discussion

In the present study, treatment with hypertonic mannitol did not reduce infarct size following coronary occlusion. The extent of tissue damage, determined 24 hours after occlusion by planimetry of the sectioned heart and by tissue creatine phosphokinase activity, was the same in control animals and in those treated with a 4 hour infusion of hypertonic mannitol instituted 15 minutes after coronary artery occlusion. Negative results were obtained despite significant elevations of serum osmolalities and profound alterations in fluid balance. Although previous studies have suggested that increases in serum osmolarity produced by mannitol may result in beneficial changes during myocardial ischemia, no measurements have thus far been reported showing the effect
of mannitol on infarct size in a preparation with permanent coronary occlusion.

In this study mannitol administration was given 15 minutes after coronary occlusion and was continued for 4 hrs of the 24 hr period prior to measurements of infarct size. This regime was selected based on available evidence which suggested that a beneficial effect would result. First, it has been postulated that the beneficial effect of mannitol in ischemia is due to its ability to increase serum osmolarity and thus prevent swelling of the damaged cells within the ischemic region, especially the endothelial cells of the microvasculature.8 Secondly, several authors7'8 have shown that obstruction of the vascular bed does occur within 90 to 120 minutes following occlusion. Thus treatment during this time appeared likely to improve the perfusion of the ischemic tissue. Moreover, it has been shown that infarct sizes can be altered by treatments limited to the first few hours following occlusion.32 Finally, extreme difficulties in the management of the fluid balance in the treated animals as a result of massive fluid losses due to the osmotic diuresis stimulated by the mannitol infusion forced us to limit the duration of the infusion to 4 hours. After 4 hours and 15 minutes following occlusion serum osmolarity was expected to fall gradually to normal levels. It cannot be ruled out,
however, that a prolonged maintenance of a hyperosmotic state would finally have had a beneficial effect on infarct size, even though the disturbances of the fluid balance and the intra- and extracellular electrolyte concentrations cause serious problems in the management of the treatment, and may ultimately even be related to the increased mortality rate in animals treated with mannitol.

The experimental model used in the present study which was developed by Maroko et al. is one of the most widely used animal models for the purpose of testing interventions for their ability to alter infarct size. At 24 hours the infarct is clearly visible in the sectioned heart and its size can thus be estimated even by gross techniques. The tissue shows clear histological changes and the extent of cellular damage can also be assessed by determination of the loss on intracellular enzymes such as creatine phosphokinase. Additional advantages of limiting the experiment to 24 hrs are that severe adhesions between the heart and the pericardium and the lungs do not develop in this short interval so that the ligated artery can easily be recannulated for measurements of the peripheral coronary pressure and the retrograde flow. Finally severe infections rarely occur in this short period of time.

An infarct induced by ligation of a coronary artery is certainly a single event. We therefore compared in the present study the size of infarcts in control and treated animals on the basis of each animal representing a single observation, thus being equal to one infarct. In contrast, many studies obtain measurements from multiple sites in each heart and treat each measurement as an independent observation. Thus, a few animals appear to provide many independent tests of the effect of an intervention. However, the independence of several measurements within a single heart has not been proven and the validity of the statistical comparison is questionable. It is unlikely that the singular action of occluding a coronary artery would create a situation where ischemia and infarction in one region would be independent of events in another. Even so called "border zones" are likely to depend on events in the center of the infarct. In fact, recent experiments in our laboratory showed that the blood flow in the ischemic tissue at the lateral border of an ischemic area following acute coronary occlusion was the same as in the center. Moreover, 24 hours later, the depletion of creatine phosphokinase enzyme was the same at the border and the center of the infarct.

This study introduces the concept of normalizing infarct size relative to the mass of tissue at risk. The myocardium at risk is identified with the tissue normally supplied by the occluded artery and was outlined visually by injecting Evans blue dye into the occluded artery, and by a technique developed in this laboratory in which myocardium perfused by adjacent vessels is marked prior to occlusion with radioactive microspheres. Infarct sizes normalized in this way should compensate for the differences between animals in the amount of tissue perfused by the occluded vessels. The results presented in table 2 show that in the canine model, infarction indicated by enzyme levels involves most of the myocardial mass at risk. Viewed in this way, control animals have large infarcts and effective treatments could salvage large amounts of jeopardized tissue.

In the present study, mannitol did not increase myocardial blood flows in either the normal or ischemic areas 30 minutes after institution of the treatment, in contrast to reports by Willerson, Hutton et al., The reason for this discrepancy is not entirely clear but several points should be noted. First, serum osmolarity had risen only 13 mOsm/L on the average in the present experiment at the time when myocardial blood flows were measured, compared to increases of 17–40 mOsm/L in previous experiments. Moreover, the regulation of coronary blood flow in isolated perfused hearts and in anesthetized dogs on right heart bypass may differ from the physiologically more normal preparation used in the present studies. In addition, the increases in myocardial blood flow observed in unanesthetized preparations following mannitol infusion were accompanied by marked increases in aortic pressure and cardiac output in contrast to the measurements reported here. Flows reported by one group studying mannitol in ischemic myocardium are significantly higher than those reported in the present study, which raises the possibility that the tissue labelled as "ischemic" in some studies may have included portions of tissue perfused by adjacent, unoccluded vessels. In this view, the increases in flow attributed to the ischemic area may simply reflect increases in blood flow in normal myocardium. This possibility emphasizes the need to separate and correctly identify the location of tissue samples. In the experiments reported here, the microspheres used to mark myocardium perfused by unoccluded vessels provide assurance that the samples are correctly identified.

Following coronary occlusion, changes in the ischemic tissue such as interstitial and intracellular edema have been associated with disturbances in the blood supply, especially to the deeper layers of the wall. Swelling of endothelial cells in the small vessels ultimately leads to complete vascular obstruction and an inability to reperfuse the ischemic tissue. This so-called no reflow phenomenon has been observed in brain and heart. Recently we have demonstrated that the increase in resistance to flow in the infarcting myocardium redistributes collateral blood flow to the surviving epicardial layers. Renal perfusion is improved if mannitol is added to the perfusate before and during periods of ischemia, presumably as the result of reduced endothelial cell swelling. Other investigators, however, observed no gross effect on the vascular patency in the heart but did observe a reduction of swelling in intracellular organelles. In the present study, mannitol did not alter the redistribution of collateral blood flow within the infarct. Myocardial blood flows were not altered by the treatment either 30 minutes after initiation of the treatment or 24 hours later. Since the supposed benefit of increasing serum osmolarity with mannitol is an increase in collateral blood flow, it is not surprising that the extent of necrosis was unaffected in the present study. Moreover, there appears to be no direct benefit of mannitol through its effect on fluid and electrolyte balance.

Since the work of Koch-Weser and Wildenthal and co-workers, it has been known that mild changes in the osmolarity of perfusion solutions are associated with increased cardiac contractile strength in vitro. In addition, Willerson et al. have published results showing increases in max dP/dt in open-chest dogs on right-heart bypass and with fixed heart rates when serum osmolarity was increased by as little as 17 mOsm/L above control levels during mannitol infusion.
Similar effects have been obtained by Atkins and co-workers\(^2\) in anesthetized and conscious dogs after increasing serum osmolality with mannitol. In this study, this phenomenon was also observed. The exact mechanism of this increase in LV dp/dt is not yet fully clarified. In isolated heart muscle, increased tonicity per se may exert a positive or a negative inotropic influence, depending on frequency of contraction, calcium levels in the bath, or temperature.\(^3, 4\)

Although mannitol is widely used to enhance urinary output in cases with impending renal failure, the drug requires cautious administration. In addition to the rare occurrence of mannitol intoxication,\(^5\) high doses of mannitol can increase central venous pressure due to shifts of fluid from intracellular into the interstitial and intravascular spaces.\(^6\) More serious changes in ionic shifts across the cell membranes may accompany cellular dehydration and would be difficult to detect and relatively unmanageable therapeutically. By itself the massive fluid loss due to osmotic diuresis creates serious problems in the management of fluid balance.

The finding that mannitol hinders the development of collateral vessels, however, adds additional doubts on the usefulness of this drug in the treatment of myocardial infarction. In the critical period following coronary occlusion, the enlargement of collateral vessels is essential in stabilizing the infarct. Inhibition of this vital process may prolong the jeopardy of the surviving myocardium.

Acknowledgment

We wish to thank Dr. Edmund H. Sonnenblick for the encouragement given throughout the study and the generous help he gave in the preparation of the manuscript. We are also grateful to Dr. Sandor Szabo for performing the histologic examinations, to Stephen Rothman for the CPR determinations, and to Rebecca Covino for her expert secretarial assistance.

References

23. Braunwald E, Moroko PR: The reduction of infarct size — An idea whose time (for testing) has come. Circulation 50: 206, 1974
30. Reimer KA, Kloner RA, Willerson JT, Jennings RB: Reduction of infarct size by mannitol during acute myocardial ischemia. Circulation 52 (suppl II): 119-249, 1975

MANNITOL AFTER CORONARY OCCLUSION/Hirzel, Kirk
The effect of mannitol following permanent coronary occlusion.

H O Hirzel and E S Kirk

*Circulation.* 1977;56:1006-1015
doi: 10.1161/01.CIR.56.6.1006

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1977 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/56/6/1006

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/