# Improvement in Myocardial Function and Coronary Blood Flow in Ischemic Myocardium after Mannitol

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ABSTRACT The purpose of this study was to evaluate the effect of hyperosmolality on the performance of, and the collateral blood flow to, ischemic myocardium. The myocardial response to mannitol, a hyperosmolar agent which remains extracellular, was evaluated in anesthetized dogs. Mannitol was infused into the aortic roots of 31 isovolumic hearts and of 15 dogs on right heart bypass, before and during ischemia. Myocardial ischemia was produced by temporary ligation of either the proximal or mid-left anterior descending coronary artery.

Mannitol significantly improved the depressed ventricular function curves which occurred with left anterior descending coronary artery occlusion. Mannitol also significantly lessened the S-T segment elevation (epicardial electrocardiogram) occurring during myocardial ischemia in the isovolumic hearts and this reduction was associated with significant increases in total coronary blood flow ($P < 0.005$) and with increased collateral coronary blood flow to the ischemia area ($P < 0.005$).

Thus, increases in serum osmolality produced by mannitol result in the following beneficial changes during myocardial ischemia: (*a*) improved myocardial function, (*b*) reduced S-T segment elevation, (*c*) increased total coronary blood flow, and (*d*) increased collateral coronary blood flow.

## INTRODUCTION

It has been demonstrated that myocardial ischemia is accompanied by a reduction of organ perfusion when continuity of blood flow is restored (1). Similar findings have been noted in the brain (2) and the kidney (3). Furthermore, in these latter two organs, hyperosmolar agents which remain largely extracellular, through reduction of ischemic cell swelling, have been demonstrated to improve the reflow of blood after arterial occlusion (4, 5). In the kidney the resulting improvement in circulation has recently been noted to be associated with improved renal function (5). Because of the possibility that hyperosmolar agents might protect and improve the function of myocardium exposed to hypoxia, studies were undertaken to evaluate the influence of these agents on the performance of the ischemic left ventricle and, in particular, on collataral blood flow to ischemic myocardium.

The purpose of this communication is to present data evaluating the hemodynamic effect of hypertonic mannitol on the function of ischemic myocardium, on total coronary and collateral coronary blood flow, and on the degree of S-T segment elevation noted in the epicardial electrocardiogram which has been shown to be a reliable indicator of myocardial injury during ischemia (6).

## METHODS

Adult mongrel dogs of either sex weighing between 25 and 35 kg were anesthetized with intravenous chloralose (60 mg/kg) and urethan (600 mg/kg). The preparations used in this study included the right heart bypass and the isovolumic left ventricle. The sinus node was crushed and heart rate kept constant in both preparations by atrial pacing utilizing a Grass stimulator (Grass Instrument Co., Quincy, Mass.) (model SD-5).

*Preparations.* In the right heart bypass experiments, an endotracheal tube was inserted and ventilation provided by a Harvard respirator (Harvard Apparatus Co., Millis, Mass.) with 95% $O_2$ and 5% $CO_2$. The chest was opened through a median sternotomy and after the intravenous administration of heparin 3 mg/kg, the superior and inferior vena cavae were cannulated and the azygous vein divided. The caval return was directed to a reservoir, through a bubble oxygenator and heat exchanger (37°±0.5°C), and then returned through a variable speed calibrated roller pump to

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the main pulmonary artery. The rate of pumping into the pulmonary artery thereby determined cardiac input. The use of the bubble oxygenator allowed the option of switching to total cardiopulmonary bypass by altering the route of perfusion from the pulmonary artery to the left subclavian artery when it was necessary to prevent irreversible distention of the left ventricle. A ligature placed around the proximal pulmonary artery helped to isolate the right heart which only received coronary venous drainage. The coronary venous blood draining into the right ventricle was led by siphon drainage from the cannulated right atrium and ventricle to the venous reservoir. Timed volumetric collections provided a quantitation of total coronary blood flow. Aortic pressure was maintained constant by the use of a vertical cylinder (designed by Dr. J. H. Mitchell). The height of the column of blood could be varied by releasing a clamp from one sidearm of the column and reapplying the clamp to another sidearm; systemic pressure varied directly with the height of the column of blood. Blood was drained freely from the large cylinder by gravity and returned to the blood reservoir. Left ventricular pressures and left ventricular diastolic pressures were measured through a short, wide-bore, Y-shaped metal cannula inserted through the apex of the left ventricle. Proximal aortic pressures were measured through a short wide-bore polyethylene catheter inserted into the aortic arch through the left carotid artery. A snare was placed around the proximal left anterior descending coronary artery (LAD)[1] adjacent to its origin to permit reversible ligation of the LAD. Function curves were then constructed (7) in both the normal and ischemic canine left ventricle before and after treatment with mannitol. The ventricular function curves were obtained between 2 and 7–8 min after LAD occlusion. In two animals autonomic blockade was effected by the beta-receptor blocking agent propranolol (Inderal 0.5 mg/kg) and by ganglionic blockade with mecamylamine hydrochlorade (Inversine, 10 mg/kg). Autonomic blockade was verified at the completion of the experiment by the absence of a chronotropic or inotropic response to 5 $\mu$g of isoproterenol injected into the pulmonary artery and to the clamping of both common carotid arteries.

The isovolumetric preparation was utilized to measure total coronary blood flow, to measure collateral coronary blood flow, and to measure S-T segment elevation with epicardial electrocardiography before and during ischemia, both with and without the infusion of hypertonic mannitol. This preparation was chosen for these studies in order to make the above measurements in an environment relatively uninfluenced by exogenous factors. The heart was removed from a donor animal and retrograde perfusion of the aortic root from the femoral artery of the support dog provided. To maintain constant pressure perfusion of the coronary arteries, a Sarns roller pump (model No. 1800, Sarns, Inc., Ann Arbor Mich.) was interposed in the coronary perfusion line to assure a constant rate of pumping from the support dog independent of systemic blood pressure. The roller pump was adjusted to maintain a continuous overflow of blood from the top of a vertical column of tubing. The height of this column determined coronary perfusion pressure. Subtraction of the overflow from the amount of blood pumped allowed the determination of total coronary blood flow. A thin-walled distensible balloon was attached to a button on the end of a short, wide-bore cannula connected to a pressure transducer and inserted into the left ventricle through the mitral annulus. A purse string suture around the mitral annulus assured that the balloon remained within the left ventricular cavity. A small sidearm of the button obviated herniation into the left ventricular outflow tract. A drain was placed into the left ventricular apex to allow drainage of the small amount of coronary flow returning to the left ventricle. Right ventricular and right atrial drainage, which represented an approximation of total coronary flow, were returned to the support dog which was ventilated with 95% $O_2$ and 5% $CO_2$ by means of a Harvard respirator. As in the right heart bypass studies, a snare was placed around the LAD. For the epicardial mapping and total coronary blood flow experiments the area of LAD occlusion was at the midlevel of the LAD. The epicardial electrocardiogram was monitored before and at 5 and 14 min after ligation of the LAD. Epicardial mapping was accomplished utilizing an electrode with a smooth, rounded 2 mm tip. The tip of the electrode was gently applied to an area of the epicardium and the unipolar epicardial electrocardiogram obtained. Either a drawing or a Polaroid picture of the area of LAD occlusion and the 15 points to be mapped was made. This allowed one to repeat the epicardial mapping as often as necessary using virtually identical sites. For any given time interval S-T segment elevations were summed from 15 points within and adjacent to the territory supplied by the LAD.

*Collateral coronary blood flow.* An estimation of collateral coronary blood flow was obtained using the krypton washout technique in the isolated isovolumetric preparation described above. A polyethylene loop was inserted into the proximal portion of the LAD, usually at a location just distal to the origin of the first anterior interventricular branch. Occlusion of the proximal portion of this loop provided ischemia for that portion of the left ventricle supplied by the LAD. 2 min after the onset of ischemia, 1.5 cc of $^{85}Kr$ (32 mCi/cc) were injected just distal to the occlusion into the ischemic area. This volume insured that the $^{85}Kr$ was injected into that portion of the left ventricle now deprived of its major source of blood supply, i.e., the LAD. The rate of $^{85}Kr$ washout from the ischemic area of the left ventricle was then dependent on collateral coronary blood flow (8, 9). The $^{85}Kr$ washout was assessed by withdrawing blood from the venous drainage of the right ventricle through a counting chamber at 5 cc/min. Counting was continued for 7 min after the $^{85}Kr$ was injected and the slope of disappearance of the $^{85}Kr$ from within the ischemic area plotted on semilogarithmic paper. Collateral coronary blood flow was then calculated from the slope of the $^{85}Kr$ washout (8, 9) in the same canine heart for the: (*a*) control period (no ischemia), (*b*) period after LAD occlusion, (*c*) period after LAD occlusion and pretreatment with mannitol, (*d*) period after LAD occlusion, and (*e*) a final control (no ischemia) period.

*Mannitol administration.* Mannitol (12.5 g/50 cc) was infused at 3.82 cc/min with a Harvard infusion pump (model 600–000) for periods varying from 13–60 min into the aortic roots of the dogs before the onset of myocardial ischemia. In every experiment, the infusion was continued during the period of ischemia. Coronary venous and systemic arterial osmolalities were determined before, during, and after mannitol infusions. In all dogs the osmolality determination was repeated at the end of the ischemia period, before stopping the mannitol infusion, and was documented to have remained elevated. In the majority of the right heart bypass experiments, five separate ventricular function curves were obtained as demonstrated in Fig. 1. The first and last ones (marked 1 and 5 in Fig. 1) were without LAD occlusion to

[1] *Abbreviations used in this paper:* LAD, left anterior descending coronary artery; LVEDP, left ventricular end diastolic pressure.

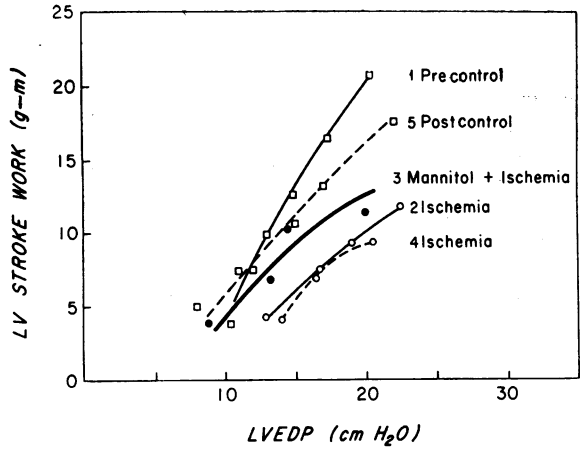


FIGURE 1 Ventricular function curves. The results from a representative right heart bypass experiment showing the effect of ischemia and the effect of mannitol and ischemia on ventricular function. The first and fifth curves were obtained without myocardial ischemia. The second and fourth ones were obtained during myocardial ischemia. The third function curve was also obtained during myocardial ischemia but after pretreatment with hyperosmotic mannitol. LVEDP: left ventricular end diastolic pressure.

be certain that the preparation had neither spontaneously deteriorated nor improved during the course of the experiments. The second and fourth function curves were obtained after LAD occlusion. The third function curve was obtained after LAD occlusion in a heart pretreated with mannitol. 30–45 min of rest on total cardiac bypass preceded each of the ventricular function curve runs.

In the isovolumetric studies, five separate measurements were also made of S-T segment elevation and total coronary blood flow or collateral coronary blood flow. The first and fifth were without LAD occlusion and served as controls. The second and fourth were with LAD occlusion and the third with LAD occlusion immediately after treatment with hyperosmolar mannitol. A recovery period of 30–45 min followed each of the initial four isovolumetric studies. Usually the 30–45 min of recovery was sufficient for the serum osmolality to return substantially toward control levels between the third and fourth runs, but on occasion it was still moderately elevated at the onset of the fourth run.

In the majority of the above experiments pre- and post-intervention control data were obtained in the same preparation. In the event that either spontaneous deterioration or improvement was found that experiment was not included in the data subsequently analyzed. For the above reasons four right heart bypass experiments, four isovolumetric studies, and two $^{85}Kr$ washout experiments could not be used in the statistical analyses.

*Pressure recordings.* Pressures were measured with Statham P23db pressure transducers (Statham Instruments, Inc., Oxnard, Calif.); the frequency response of the pressure measurement system was linear up to 30 cps. The rate of rise of left ventricular pressure LV $dP/dt$ was obtained utilizing an RC electronic differentiation of the full left ventricular pressure. Calibration of the LV $dP/dt$ differentiator was accomplished by supplying a wave form of known slope to the differentiating circuit which had a time contant of 0.0001 sec and a cut off at 160 cps.

All measured pressures were recorded on a multichannel Sanborn direct writing oscillograph (Hewlett-Packard Co., Waltham, Mass.).

*Statistics.* The data obtained in these studies were analyzed utilizing Student's $t$ test and mean values, standard errors, and standard deviations were obtained.

## RESULTS

*Ventricular function.* Mannitol was infused into the aortic root of 15 dogs on right heart bypass. This elevated the mean serum osmolality in the coronary circulation 17 (±3 SEM) mOsm above the control level during the mannitol infusion at the time of onset of the ischemia. Fig. 2 demonstrates that mannitol failed to improve the ventricular function curve in the normotensive animals without myocardial ischemia (four animals with a mean Δ stroke work of 0.0, 0.3, and 0.2 g-m at left ventricular end diastolic pressures (LVEDP) of 4, 6, 8, and 10 cm $H_2O$, respectively, NS). While the ventricular function curves were not changed in the normotensive animals without myocardial ischemia by the infusion of mannitol, left ventricular $dP/dt$ at an LVEDP of 10 cm $H_2O$ was slightly increased ($+413 \pm 114$ mm Hg/sec, $P < 0.05$) in four hearts in which this measurement was made.

Myocardial ischemia produced by reversible ligation of the LAD markedly depressed the ventricular function curves in nine dogs. Mannitol pretreatment significantly improved these depressed ischemic ventricular function curves. Fig. 1 illustrates the data from one animal and Tables I A–I D summarize the data from nine animals. Stroke work was significantly increased at three levels of

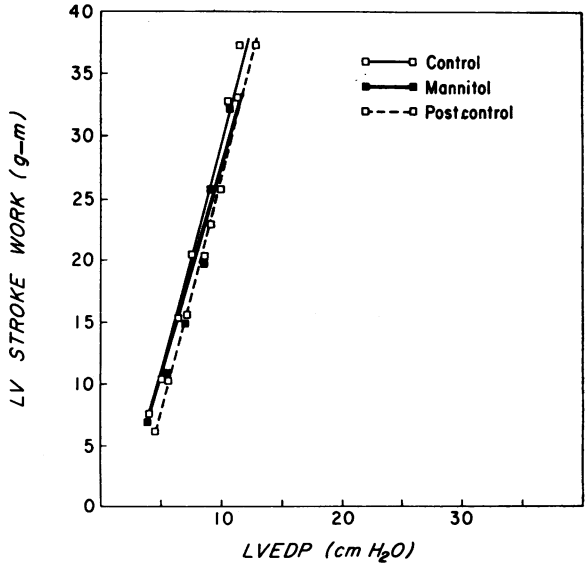


FIGURE 2 Nonischemic ventricular function curves. The lack of effect of mannitol on the ventricular function curve of the animal without myocardial ischemia is demonstrated.

TABLE I A

*Effect of Mannitol on Myocardial Stroke Work during Myocardial Ischemia*

| No. of exps. | LVEDP | Δ Stroke work | Mean Δ Stroke work |
|---|---|---|---|
| | *cm $H_2O$* | *g-m* | *% control* |
| 8 | 10 | +3.1±1* ($P < 0.025$) | +37±7*% |
| 9 | 13 | +3.9±1 ($P < 0.01$) | +46±8% |
| 8 | 15 | +4.1±1.2 ($P < 0.025$) | +47±9% |

* Standard error of the mean.

LVEDP in the mannitol pretreated dogs (Table I A and Fig. 3). Similarly, left ventricular end diastolic pressures were lower during ischemia in the mannitol-treated animals at seven comparable levels of stroke work (Table I B). Table I C and I D present the absolute data in each animal in the sequence in which the study was carried out. Directionally similar results were noted in the two animals with autonomic blockade. In these two animals, increments in stroke work of the ischemic left ventricle produced by mannitol were + 3.5 and + 1.0 g-m at an LVEDP of 15 cm $H_2O$, + 1.5 and + 3.0 g-m at 20 cm $H_2O$ and + 2.0 and + 5.5 g-m at 25 cm $H_2O$. Decreases in LVEDP at a stroke work of 7, 9, and 11 g-m were − 2 and − 2 cm $H_2O$, − 2 and − 7 cm $H_2O$ and − 4 and − 11 cm $H_2O$, respectively. In the right heart bypass preparation LV *dP/dt* measurements in five dogs made at a comparable LVEDP were also increased during myocardial ischemia after the administration of mannitol. The Δ*dP/dt* at an LVEDP of 13 cm $H_2O$ was + 280±122 mm Hg/sec ($P < 0.05$).

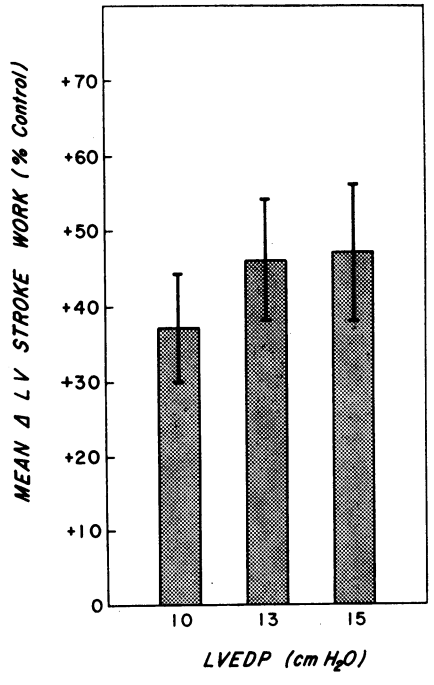


FIGURE 3 Summary of the ischemia ventricular function curve data. The mean change in left ventricular stroke work is along the vertical axis, expressed as the per cent of control. The three levels of left ventricular stroke work at which these data were obtained are along the horizontal axis. Note the per cent increase in stroke work at three levels of end diastolic pressure achieved by elevation of the serum osmolality during ischemia. The vertical bars represent the standard errors of the mean.

In 11 animals elevation of the serum osmolality by 18±2 mOsm from a pre-control level of 334±5 mOsm failed to change significantly (+ 0.2 ±0.5%) the hematocrit from the control level of 36±1%. During the post-control coronary ligation experiments in these animals the osmolality fell significantly ($P = 0.01$) but remained 12±4 mOsm above the precontrol level ($P = 0.01$). This intermediate osmolality corresponded to a level of ventricular function which was intermediate between the pre-control ischemic and the mannitol-ischemia values in a substantial number of experiments (Tables I C and I D).

*S-T segment elevation.* Mannitol infusions into the aortic roots of the isovolumetric left ventricles elevated mean serum osmolality 47±5 (SEM) mOsm in the coronary circulation. Preliminary experiments demonstrated that the administration of mannitol had no demonstrable effect on S-T segments in the nonischemic canine heart. In three animals, the reproducibility of S-T segment elevation during repeated 12-min periods of ischemia was documented. Fig. 4 depicts a representative experiment. The maximum S-T segment elevation during repeated ischemia was 43.5, 43, and 42 mm in the second dog and 31 and 33 mm in the third dog.

Myocardial ischemia produced marked elevation of the S-T segments in the six hearts evaluated. Fig. 5 depicts two representative examples. Pretreatment with mannitol markedly reduced the S-T segment elevation that occurred after 5 and 14 min of ischemia (Table II).

TABLE I B

*Effect of Mannitol on LVEDP during Myocardial Ischemia*

| No. of exps. | Stroke work | Δ LVEDP | Mean Δ LVEDP |
|---|---|---|---|
| | *g-m* | *cm $H_2O$* | *% control* |
| 9 | 4 | −2.3±0.5* ($P < 0.005$) | −32±9*% |
| 9 | 6 | −3.3±0.7 ($P < 0.025$) | −28±6% |
| 6 | 8 | −3.2±0.8 ($P < 0.05$) | −29±4% |
| 5 | 10 | −5.9±1.7 ($P < 0.025$) | −37±5% |
| 4 | 12 | −3.4±0.1 ($P < 0.05$) | −29±9% |
| 3 | 14 | −4.7 | −38% |
| 2 | 16 | −7.5 | −45% |

* Standard error of the mean.

The range of the reduction in ischemic S-T segments after mannitol was 0.5 mm to − 30 mm (mean − 8.2 mm) after 14 min of ischemia.

TABLE I C

*Effect of Mannitol on Myocardial Stroke Work (g-m) during Myocardial Ischemia at Three Levels of LVEDP*

| Dog No. | | LVEDP levels | | |
|---|---|---|---|---|
| | | 10 | 13 | 15 |
| | | *cm $H_2O$* | | |
| 1 | C* | 15.2 | 20 | — |
| | I | 5.8 | 8.0 | 9.0 |
| | I+M | 10.5 | 13.3 | 14.4 |
| | I | 7.3 | 9.3 | 9.7 |
| | C | — | — | — |
| 2 | C | 29.5 | — | — |
| | I | 13.2 | 14.2 | 15 |
| | I+M | 21 | 23 | 24.7 |
| | I | 12.3 | 12.9 | 13 |
| | C | — | — | — |
| 3 | C | 14.8 | 19.6 | 23 |
| | I | 9.8 | 14 | 16.8 |
| | I+M | 16.5 | 22.5 | 25.5 |
| | I | 13.5 | 18.6 | 22 |
| | C | — | — | — |
| 4 | C | — | 10.2 | 12.8 |
| | I | — | 4.2 | 4.8 |
| | I+M | — | 7.2 | 8.8 |
| | I | — | 3 | 5.4 |
| | C | — | 8.6 | 10.4 |
| 5 | C | 10.4 | 13.0 | — |
| | I | 6.3 | 7.9 | — |
| | I+M | 7.5 | 11.0 | — |
| | I | 8.6 | 11.0 | — |
| | C | 11.0 | 13.5 | — |
| 6 | C | 12.2 | — | — |
| | I | 4.0 | 5.7 | 6 |
| | I+M | 4.8 | 6.2 | 7.0 |
| | I | — | — | — |
| | C | 11.8 | 15.1 | 17.5 |
| 7 | C | 6.9 | 8.0 | 8.8 |
| | I | 5.2 | 5.0 | 5.9 |
| | I+M | 6.8 | 8.0 | 8.6 |
| | I | 4.3 | 5.0 | 5.6 |
| | C | 6.0 | 7.0 | 8.8 |
| 8 | C | 23.5 | 25.5 | 26.5 |
| | I | 5.9 | 7.5 | 8 |
| | I+M | 6.3‡ | 7.5‡ | 8‡ |
| | I | 6.3 | — | — |
| | C | 12.0 | 13 | 13.8 |
| 9 | C | 11.2 | 14.3 | 15.7 |
| | I | 4.0 | 5.2 | 6.3 |
| | I+M | 6.5 | 8.0 | 8.8 |
| | I | 5.9 | 7.2 | 7.7 |
| | C | 10.5 | 15.5 | 18.7 |

* C, Control; I, Ischemia: I+M, Ischemia and mannitol.
‡ The arterial osmolality only increased 5 mOsm/liter.

TABLE I D

*Effect of Mannitol on LVEDP (cm $H_2O$) during Myocardial Ischemia at Seven Levels of Stroke Work*

| Dog No. | | Stroke work levels | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
| | | | | | *g-m* | | | |
| 1 | C* | 5 | 6.8 | 7.0 | 7.5 | 8 | 9.5 | 10.5 |
| | I | 8.5 | 10.5 | 13 | 17 | >22 | >22 | — |
| | I+M | 5.6 | 6.5 | 7 | 9.5 | 11.5 | 13.5 | 18 |
| | I | 7.0 | 8.8 | 11 | 17 | >30 | >30 | — |
| | C | — | — | — | — | — | — | — |
| 2 | C | — | 0.2 | 0.8 | 1.5 | 2.5 | 3.3 | 4.2 |
| | I | — | 5 | 6 | 7.3 | 8.5 | 12.3 | 18 |
| | I+M | — | 2 | 2.8 | 4 | 5.3 | 6.5 | 7.6 |
| | I | — | — | 6.2 | 7.6 | 9 | — | — |
| | C | — | — | — | — | — | — | — |
| 3 | C | 4.0 | 5.1 | 6.2 | 7.3 | 8.3 | 9.5 | 10.8 |
| | I | 7.0 | 7.7 | 9.0 | 10.2 | 11.5 | 13.0 | 14.5 |
| | I+M | 4.0 | 5.1 | 6.1 | 7.0 | 8.0 | 9.0 | 9.8 |
| | I | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.1 | 10.7 |
| | C | 4.0 | 4.5 | 5.5 | 6.5 | 7.2 | — | — |
| 4 | C | 10.0 | 11.0 | 12.0 | 13.0 | 14.3 | 15.8 | 17 |
| | I | 13 | 15.5 | 18 | 20.5 | 23 | — | — |
| | I+M | 10 | 12 | 14 | 16.3 | 19.5 | — | — |
| | I | 14 | 15.5 | 18 | >22 | — | — | — |
| | C | 8 | 10.3 | 12.5 | 14.5 | 16.5 | 18.5 | 20.5 |
| 5 | C | 3 | 5.3 | 7.5 | 9.5 | 12 | | |
| | I | 7.5 | 9.5 | 13.8 | 12.5 | | | |
| | I+M | 6.5 | 8.5 | 10.5 | 11.5 | | | |
| | I | 5 | 7.5 | 10 | 12 | | | |
| | C | 5.5 | 7 | 8 | 9.5 | 10.5 | | |
| 6 | C | 2 | 3.2 | 5 | 6.5 | | | |
| | I | 10 | 16 | — | | | | |
| | I+M | 9 | 12.5 | 19 | | | | |
| | I | — | — | — | | | | |
| | C | 3.3 | 5 | 6.8 | 8.5 | | | |
| 7 | C | 5.5 | 8.5 | 13 | | | | |
| | I | 7.5 | 16 | — | | | | |
| | I+M | 6.5 | 9.2 | 13 | | | | |
| | I | 9 | 16.5 | — | | | | |
| | C | 7.5 | 10.5 | 15.5 | | | | |
| 8 | C | 1 | 1.5 | 1.7 | 2.2 | 3 | | |
| | I | 6.5 | 10.5 | 19 | — | — | | |
| | I+M | 5.5‡ | 9.5‡ | 19‡ | — | — | | |
| | I | 5.5 | 9.5 | — | — | — | | |
| | C | 2.5 | 4 | 6 | 7.8 | 10 | | |
| 9 | C | 3.7 | 5.5 | 7.3 | 9 | | | |
| | I | 10 | 14.5 | — | | | | |
| | I+M | 5 | 9 | 13.3 | | | | |
| | I | 6 | 10 | 16 | | | | |
| | C | 3 | 5.3 | 7.5 | 9.5 | | | |

* Abbreviations as in Table I C.
‡ The arterial osmolality only increased 5 mOsm/liter.

Directionally similar results were noted in three additional hearts into which control isotonic saline infusions at 3.82 cc/min were administered during the pre- and post-control ischemic periods, thereby indicating that a lowering of the temperature of ischemic myocardium by the infusion of a room temperature solution could not account for the changes noted in each of these three hearts. In each of the three hearts the sum of S-T segment elevation after 15 min of precontrol ischemia, ischemia with mannitol and post-control ischemia was 52 mm, 39 mm, and 47 mm; 23 mm, 15 mm, and 20 mm;

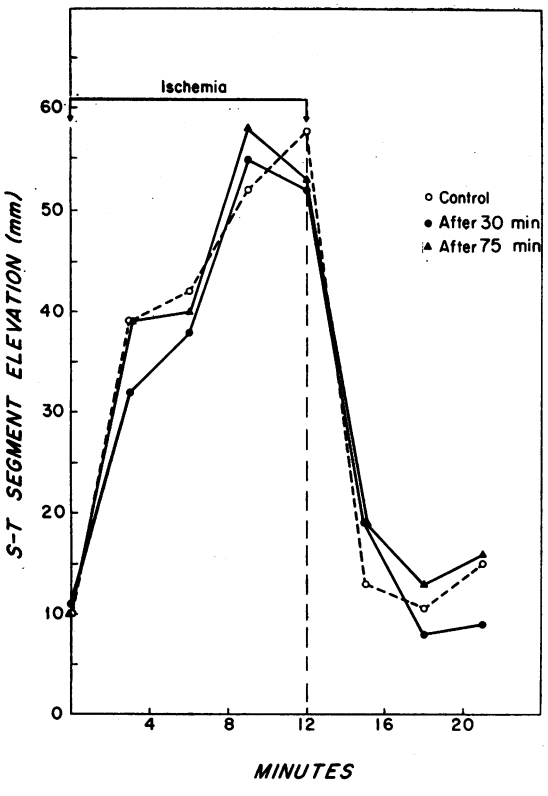


FIGURE 4 Reproducibility of S-T segment changes obtained with epicardial mapping by the technique described in the Methods section. Note that 1¼ hr after epicardial S-T segment mapping with the first reversible ligation of the LAD, the degree of S-T segment elevation associated with a subsequent ligation of the LAD is similar.

and 32 mm, 25 mm, and 32.5 mm. In each of these experimental runs measurement of the temperature with a Fisher Scientific Thermometer (Fisher Scientific Co., Pittsburg, Pa.) (− 10° + 260°C with 1° calibrations) placed in the LV cavity between the balloon and the endocardium and with another similar thermometer held against the center of the epicardial ischemic region failed to reveal a discernible change in temperature. The epicardial temperature in each of the three animals was 28, 25, and 27°C. The endocardial temperature in each of the three animals was 30, 27, and 30°C.

*Total coronary blood flow.* The administration of mannitol significantly increased total coronary blood flow in the isovolumic hearts studied both immediately before and during ischemia as demonstrated in Fig. 6 and Table II. The actual increases in total coronary blood flow by mannitol over the control ischemic flows ranged from + 30 cc/min per 100 g LV to + 70 cc/min per 100 g LV (mean + 44.0 cc) in the control period before ligation; from + 22 cc/min per 100 g LV to + 60 cc/min per 100 g LV (mean + 38.9 cc) for the 5 min ischemic period; and from + 25 cc/min per 100 g LV to + 40 cc/min per 100 g LV (mean + 31.6 cc) for the 14 min ischemic period. This increase in total coronary blood flow was not explainable by changes in wall tension as reflected in left ventricular peak systolic and end diastolic pressures. There were no statistically significant differences in these pressures in the isovolumic hearts before and after mannitol. Similarly the changes in coronary blood flow did not appear secondary to changes in hematocrit induced by the hyperosmolality. In 8 hearts elevation of the serum osmolality by 36±5 mOsm from a control of 316±3 mOsm was associated with a mean decrease in hematocrit of only 3±1% from a control level of 40±1%. In the postcontrol coronary ligation experiments the osmolality fell significantly ($P = 0.01$) but remained 12±3 mOsm above the pre-control level ($P = 0.01$). These intermediate osmolalities in the postcontrol ischemia experiments were associated with intermediate elevations of S-T segments which were 8.5±4.3 mm below the pre-control ischemic level ($P = 0.07$) but were significantly higher ($P < 0.05$) than the mannitol-ischemia level at 14 min of ischemia.

Figs. 5 and 6 demonstrate the reciprocal relationship between changes in coronary blood flow and changes in S-T segments in these experiments. The S-T segment data depicted in the left panel of Fig. 5 are from the same experiment as the coronary blood flow data in the center panel of Fig. 6. Similarly, the S-T segment data on the right of Fig. 5 correspond to those in the right panel of Fig. 6. For a given experiment the greatest S-T segment elevation was associated with the lowest total coronary blood flow and vice versa.

*Collateral coronary blood flow.* Collateral coronary blood flow as estimated by the $^{85}Kr$ injections into the ischemic region of the left ventricle was significantly increased relative to control ischemic runs in each of seven isovolumic hearts studied after pretreatment with mannitol (Table III and Fig. 7). The range of increase in collateral coronary blood flow during ischemia after mannitol as compared to ischemia alone was + 1.9 to + 16.4 cc/min per 100 g LV (mean + 9.5 cc). Postcontrol ischemic and subsequent postcontrol nonischemic collateral coronary blood flow determinations showed a return toward but not to the precontrol levels in all of the animals in which these determinations were performed (Table III). For these experiments the elevation of the serum osmolality of + 64±7 mOsm, from a control level of 327±9 mOsm during the mannitol-ischemia experiments fell significantly ($P = 0.001$) in the postcontrol coronary ligation experiments to + 17±4 mOsm above the precontrol levels ($P < 0.05$). These intermediate osmolalities in the postcontrol runs corresponded to collateral blood flow determinations which were intermediate between the precontrol ischemic and the ischemia

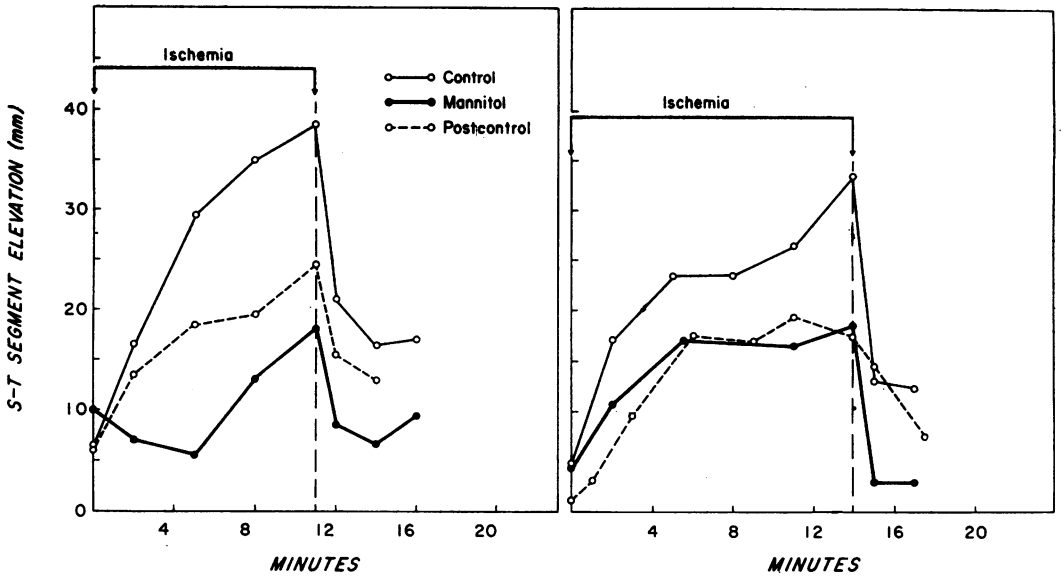


FIGURE 5 S-T segment changes. Two examples of the changes in S-T segments during myocardial ischemia with and without pretreatment with mannitol. Note in both experiments the decrease in S-T segment elevation during ischemia associated with mannitol pretreatment. In the panel on the right the post-control S-T segments appear similar to those noted with pretreatment with mannitol most likely because of persistent elevation of the serum osmolality after mannitol. In the panel on the left the ischemic period was shorter than usual, lasting only 11 min.

and mannitol values (Table III). In an additional heart which did not receive mannitol $^{85}Kr$ blood flow determinations were 72 cc/min in the precontrol period, 20.4 cc/min during the first ischemic period, 20.7 cc/min during the second ischemic period, and 73.0 cc/min during the postcontrol, nonischemic period.

In five additional hearts direct measurement of the backflow with a graduated cylinder showed directionally similar results to the $^{85}Kr$ data. The mean increase in backflow during the ischemia experiments associated with the administration of mannitol and an elevation of osmolality of 33±6 mOsm was 28±3% over the backflow during the control ischemic experiments.

## DISCUSSION

The data obtained from these studies demonstrate that hypertonic mannitol improves ventricular contractility during myocardial ischemia as evidenced both by a shift to the left of the ventricular function curve and by an increase in left ventricular *dP/dt*. The ability of mannitol to increase total coronary blood flow during ischemia and, even more importantly, to increase collateral coronary blood flow into the ischemic region of the left ventricle are demonstrated. It seems likely that the increase in coronary blood flow after mannitol importantly contributes to the improved ventricular function that occurs during myocardial ischemia. The reduction in ischemic

TABLE II

*Effect of Mannitol on S-T Segment Elevation and Total Coronary Blood Flow during Myocardial Ischemia*

| No. of Exps. | Time after onset of myocardial ischemia | Δ S-T segments* | Mean Δ S-T segments | Δ Total coronary blood flow | Mean Δ total coronary blood flow |
|---|---|---|---|---|---|
| | *min* | *mm* | *% control* | *cc/min per 100 g LV* | *% control* |
| 6 | 5 | −10.0±4.4‡ ($P < .05$) | 33±14‡ % | +38.9±5.0‡ ($P < .001$) | 147±49‡ % |
| 4 | 14 | −8.2±2.9 ($P < .025$) | 22±7% | +31.6±3.7 ($P < .005$) | 246±185% |

* Δ Sum of S-T segments from 15 points mapped epicardially.

‡ Standard error of the mean.

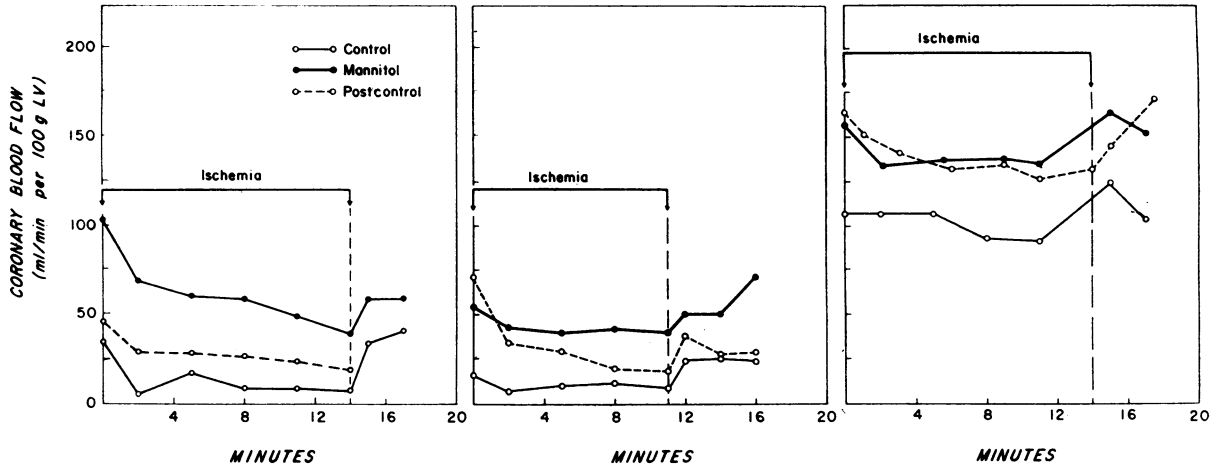


FIGURE 6 Coronary blood flow changes. The changes in total coronary blood flow during myocardial ischemia with and without pretreatment with mannitol are demonstrated. Note in all three experiments the increased coronary blood flow during ischemia associated with mannitol pretreatment. The data depicted in the center panel are from the same experiment as the S-T segment data in the left panel of Fig. 5. Similarly, the data in the right panel correspond to those on the right of Fig. 5.

S-T segment elevation is probably also the result of this increase in coronary blood flow.

This combination of beneficial factors, i.e. improved ventricular function, increased total and the fast compounds of collateral coronary blood flow, and reduced S-T segment elevation is to date unique for mannitol. Agents which previously have been noted to reduce S-T segments depress myocardial contractility (6). Other routinely used inotropic agents that increase myocardial contractility such as isoproterenol and digitalis also further elevate S-T segments during ischemia (6). It is likely that these latter agents act directly on the ischemic myocardium to increase contractility and myocardial oxygen demands. This increase in metabolism in the presence of a limited blood supply may result in further ischemia which would then explain a further increase of epicardial S-T segment elevation.

In the present experiments, the increase in contractility was not associated with further S-T segment elevation but was actually accompanied by a lowering of the S-T segments. This reduction of S-T segment elevation in the face of increasing contractility suggests that extracellular hyperosmolality does not augment tissue injury in association with increasing performance of the ischemic myocardium. There is, however, an increase in collateral blood flow to the ischemic area. Thus, it seems

TABLE III

*Collateral Coronary Blood Flow* as Measured by $^{85}Kr$ Washout*

| Exp. | No ischemia | Ischemia | Ischemia-mannitol | Δ Increase in collateral flow after mannitol | Ischemia | No ischemia |
|---|---|---|---|---|---|---|
| 1 | 34.7 | 9.9 | 23.1 | +13.2 | ‡ | 46.4 |
| 2 | 36.6 | 9 | 10.9 | +1.9 | 9.1 | 20.0 |
| 3 | 41.4 | 18.3 | 34.7 | +16.4 | 23.1 | 53.4 |
| 4 | 45.6 | 17.6 | 29 | +11.4 | 24 | 32.6 |
| 5 | 29.5 | 2.6 | 7.6 | +5.0 | ‡ | ‡ |
| 6 | ‡ | 10.9 | 20.4 | +9.5 | 19.7 | ‡ |
| 7 | ‡ | 2.2 | 11.4 | +9.2 | 7.7 | ‡ |
| Mean | 37.6 (±2.8) | 10.1 (±2.4) | 19.6 (±3.8) | +9.5 (±1.8) ($P < .005$) | | |

* Expressed as cc/min per 100 g left ventricle.

‡ Not measured.

likely that extracellular hyperosmolality improves the function of ischemic myocardium by directly increasing the blood supply to the ischemic area. This salutory effect may be a property of other hyperosmolar agents as well.

It is probable that the reduced S-T segment elevation accompanying the administration of mannitol is directly correlated with reduced myocardial cellular injury. Recent work by Maroko et al. (6) demonstrates a close correlation between the magnitude of early S-T segment elevation in epicardial recordings after acute coronary artery occlusion and the later development of cellular damage as evidence by depression of myocardial creatine phosphokinase activity. This inverse relationship between S-T segment elevation and myocardial creatine phosphokinase depression has also been demonstrated to be present during pharmacologic interventions (administration of isoproterenol and propranolol) which alter ischemic S-T segment elevation (6). Other investigators have also emphasized the correlation between S-T segment elevation and myocardial cellular injury (10, 11).

A number of questions remain to be answered regarding the mechanism(s) by which these beneficial changes occur. First, it remains to be determined whether the increase in coronary blood flow reflects reduced myocardial and endothelial cellular edema with subsequent increased patency of myocardial capillaries. It has been demonstrated in the heart (1, 12), the brain (2), and the kidney (5) that during ischemia cellular edema occurs as a consequence of impaired metabolism with the insufficient energy available to maintain active extrusion of sodium from cells. Recently, Leaf has emphasized that the regulation of intracellular volume may be important in ischemia and has speculated that the prevention of cell swelling during ischemia might have a beneficial effect on organ function by increasing blood flow and reducing tissue injury (13). Evidence for this has been accumulated in the brain (4) and the kidney (5). In these previous studies, elevation of the extracellular osmolality has been shown to reduce the area which is underperfused when the blood supply is re-established after ischemia. Presumably, this beneficial effect of hypertonic mannitol is the result of its remaining for the most part extracellularly and thus acting osmotically to reduce the intracellular edema that occurs during ischemia. Alternatively, the possibility exists that the increase in total coronary and collateral coronary blood flow observed in the present experiments may occur as the result of a direct reduction in coronary arteriolar resistance. Whatever the mechanism, improvement of myocardial blood flow by hyperosmotic mannitol during ischemia, can alone explain the improved ventricular function and reduced S-T segments demonstrated in this study.

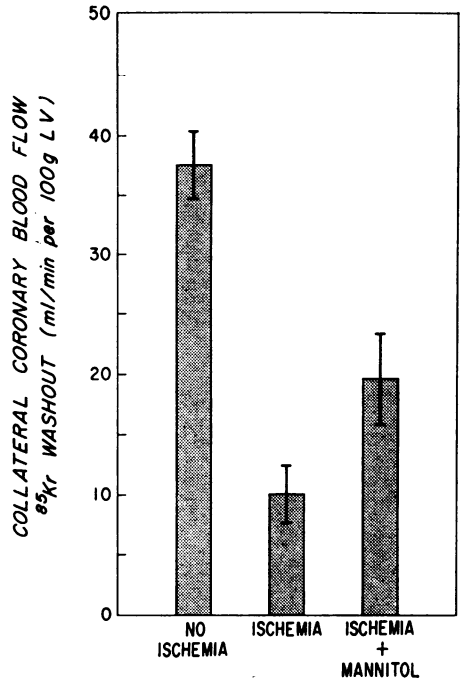


FIGURE 7 Summary of the $^{85}$Kr collateral blood flow data. This illustration shows the increase in collateral blood flow as measured by the $^{85}$Kr washout technique during ischemia which is associated with an elevation of the serum osmolality.

In addition to a consideration of coronary blood flow, other questions remain to be resolved. Specifically, is some of the positive inotropic effect of mannitol on ischemic myocardium independent of its influence on blood flow? Previous investigators have demonstrated that certain hyperosmolar agents, including mannitol, increase *dP/dt* in the whole heart and developed tension and its first derivative in the papillary muscle in well-oxygenated systems within a cetrain osmolality range (14–16). The present data which include the results of experiments done with autonomic blockade, and the recent study by Atkins, Wildenthal, and Horowitz (17) suggest that this increase in contractility is due to a direct effect on the myocardium rather than a neurohumoral response to stimulation of a peripheral receptor (18).

From this, it would appear that mannitol has inotropic properties independent of its effect on blood flow but whether increases in contractility occur in a hypoxic environment is as yet unknown. Similarly, it is not known whether mannitol is capable of improving the contractility of myocardium which is depressed by interventions other than ischemia. Also remaining to be determined is whether this inotropic property, if it is present in hypoxic situations, is mediated solely by maintenance of a more favorable intracellular volume or by another as

yet undetermined mechanism. It is of interest that Ferrans, Buja, Levitsky, and Roberts have recently demonstrated the importance of increasing the osmolality in tissue preservation specifically as regards the prevention of swelling of sarcoplasmic reticulum, mitochondrial damage, and interstitial edema (19).

The potential clinical application of the information obtained from these studies is, as yet, unknown. The exact method and circumstances involved in the administration of mannitol to patients with myocardial ischemia will have to be determined in the course of the clinical evaluation of the efficacy of hyperosmolar agents in myocardial ischemia.

It seems clear that hyperosmolar mannitol exerts a positive inotropic influence on ischemic myocardium while improving total coronary and the faster components of collateral coronary blood flow and reducing the area of ischemic damage. This particular combination of beneficial changes occurring during myocardial ischemia is thus far unique for mannitol and invites further investigation both in experimental and clinical settings.

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