Tissue Osmolality, Cell Swelling, and Reperfusion in Acute Regional Myocardial Ischemia in the Isolated Porcine Heart

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SUMMARY We devised a method to determine tissue osmolality in intact beating hearts. After occlusion of the left anterior descending coronary artery (LAD) of isolated porcine hearts, tissue osmolality in the ischemic myocardium increased within 50 minutes by about 40 mOsm/kg. This rise in osmolality could be accounted for by metabolic processes, notably the conversion of glycogen into lactate, and the hydrolysis of high energy phosphates. Concomitant with the rise in osmolality, the ischemic myocardium during the 1-hour period of LAD occlusion took up fluid and increased tissue water volume by an average of 16.5%. We demonstrated that the osmolality of fixatives used for morphological studies markedly influences ischemic cell morphology. Thus, normotonic fixation of the ischemic myocardium accentuates cell swelling, whereas nearly normal cell volumes result from hypertonic fixation, adjusted according to the rise in ischemic tissue osmolality. Normotonic reperfusion of the ischemic area after 1 hour of LAD occlusion resulted in the "no-reflow" phenomenon in the midmural and subendocardial regions. Epicardial and intramural DC-electrograms showed persistent ischemic changes, i.e., T-Q depression, S-T elevation, and monophasic potentials. Tissue resistivity, which during ischemia had risen twofold, remained high. Lactate levels remained high, creatine-phosphate (CP) and adenosine-triphosphate (ATP) levels remained low. Selective hypertonic reperfusion of the LAD, followed by a gradual return to normotonic perfusion, resulted in a normalization of DC extracellular electrograms, restoration of electrical resistivity to near normal, low levels of lactate, and higher levels of CP and ATP although control values were not reached. Cell morphology was correspondingly normalized following this procedure. We conclude that ischemic cells become hyper-osmotic and consequently take up additional fluid when exposed to normotonic blood. This increased cell swelling compresses capillaries, prevents reperfusion, and may be a major factor in causing reperfusion damage. This damage can be prevented to a large extent by selective hypertonic reperfusion.


SWELLING of cells and cell organelles is recognized as an important pathogenic factor in ischemic disease, be it in brain (Ames et al., 1968), kidney (Flores et al., 1972), or heart (Leaf, 1970). A direct damage due to membrane distension, loss of permeability characteristics, and, ultimately, membrane rupture may be accompanied by indirect damage due to compression of the microvasculature whereby residual flow and reperfusion, if any, is restrained.

The mechanisms underlying the swelling phenomena are, however, less well identified. A basic concept, applicable to problems of volume regulation in most soft mammalian tissues, is that departures from osmotic equilibrium between intra- and extracellular compartments will exist only as short transients because water is rapidly displaceable across cell membranes (Dick, 1966, 1970), and these cannot in general carry hydrostatic pressure differences of any significant magnitude. Thus, under physiologically relevant conditions, swelling of a cell or a membrane-bound organelle implies that the net sum of osmotic effectors, confined by the membrane—allowing for their osmotic coefficients—has increased relative to that of the surroundings. In ischemia, at least two mechanisms may contribute to this effect. First, depletion of energy stores and consequent failure of active membrane transport progressively leads to equilibrium of small molecules—notably Na+, K+, and Cl—across the cell membrane. Hereby a Gibbs-Donnan equilibrium, governed by the higher intracellular concentration of proteins, is approached. Consequently the cell undergoes (colloid osmotic) swelling (Leaf, 1959; Tofteson and Hoffman, 1960; Roivie and Gilies, 1979). A second mechanism of cell swelling, pertinent to ischemic tissue, is the intracellular production of new osmotic effectors by metabolic conversion of osmotically less active larger molecular species. The possible contribution of this latter mechanism to ischemic cell swelling has received comparatively less attention, although it has

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long been known that anaerobic metabolic processes may produce a rapid and substantial increase in tissue osmolality (Conway et al., 1955; Maffly and Leaf, 1959).

In the present study we report quantitative data on the increase in tissue osmolality in acute regional myocardial ischemia, accounted for by production of osmotically active particles through anaerobic metabolism. Furthermore, the importance of osmotic phenomena in histological evaluation of ischemic cell damage is shown. Finally, it is demonstrated that successful reperfusion of ischemic myocardium depends on the osmolality of the perfusate.

**Methods**

Pigs weighing 20–25 kg were premedicated with atropine (0.012 mg/kg, im) and Strepsil (R 1929 azaperone, 12 mg/kg im), then anesthetized with Hypnodil (R 8315, metamitadum 4 mg/kg, iv) and sodium pentobarbital (15 mg/kg, iv). A midsternal incision was made, and blood was collected via a catheter in the superior caval vein after iv administration of 5000 I.U. of heparin. Pig plasma osmolality was determined by the vapor pressure method (see below) on the first blood drawn from the catheter. During collection of blood, 1 liter of modified Tyrode's solution was infused via a femoral vein to keep the circulating volume constant. The composition of this modified Tyrode's solution was (mm): Na+, 156.5; K+, 4.7; Ca2+, 1.5; Mg2+, 0.7; H2PO4−, 0.5; Cl−, 137.0; HCO3−, 28; glucose, 20.0; heparin, 5250 I.U./liter; insulin, 10 U/liter; dextran (macrodex, MW 70,000) 60, g/liter. By this procedure of intracorporeal mixing, a total of 1500 ml blood-Tyrode's mixture was obtained, in which the hemoglobin content was around half that of the undiluted blood. (Average Hb of mixture was 3.3, average Hb of pig blood was 6.4 mmol/liter.) Ventricular fibrillation was induced by a small DC current to prevent myocardial air embolism during excision of the heart. The heart was rapidly connected to a Langendorff system and perfused with the blood-Tyrode's mixture thermostated at 37°C. In one experiment we calibrated each needle by submerging the needle in a large container holding a solution of known resistivity and by relating the measured voltage to the known impressed current.

**Biochemical Methods**

A hollow cylindrical drill (internal diameter 4 mm) was used to obtain transmural tissue biopsies. For the determination of tissue levels of adenosinetriphosphatase (ATP), creatine phosphate (CP), lactate, glycogen, and glucose-6-phosphate, biopsies were, within seconds after drilling, plunged into liquid nitrogen and, later, freeze dried. ATP, CP, and lactate were determined according to standard methods (Lamprecht et al., 1970) and expressed as umol/g dry weight. Tissue glycogen content was determined as total glucose after complete hydrolysis with α-amylase and amyloglucosidase. To this end, the freeze-dried biopsy was homogenized in 4% perchloric acid, brought to pH 4.8 with acetate buffer, and incubated for 2 hours at 30°C with α-amylase plus amyloglucosidase. After centrifugation, the supernatant was assayed for glucose with a commercial kit (Merck, Darmstadt, prod. nr. 3389) and corrected for glucose content present before hydrolysis.

For determination of the wet weight:dry weight ratios, and for tissue levels of sodium and potassium, drilled biopsies were rolled once on filter paper without applying pressure and immediately sealed in preweighed tubes. Wet weight was then measured and the biopsy was freeze dried. Sodium and potassium were determined by flame photometry. Statistical analysis was performed using Student's t-test.

**Measurement of the Tissue Osmolality of the Intact Left Ventricle**

To determine tissue osmolality, we developed a device which, for want of a better word, will be
called an "osmode." It consisted of a 3-cm-long cellulose acetate dialysis fiber (Biofiber-80, Bio-Rad Lab., o.d. 180 μm, i.d. 130 μm, nominal mol. wt. cutoff 30,000) which, at both ends, was glued into a polyethylene tube in parallel with a 200-μm nylon fiber which provided tensile strength (see Fig. 1, bottom). The "osmode" was stitched into the left ventricular wall by means of a needle inserted into one of the polyethylene tubes; care was taken that the dialysis fiber was fully embedded in the myocardium, so that only the two polyethylene tubes were exposed on the outside of the heart (see Fig. 2). The "osmode" was perfused with modified Tyrode's solution (see above) at a rate of 1 μL/min by means of a syringe pump (Analytical Instrument

**FIGURE 1** Lower panel: Schematic representation of an "osmode." A dialysis fiber is glued into two polyethylene tubes with Araldite glue. A thin nylon fiber is glued in parallel with the dialysis fiber to give the osmode sufficient tensile strength to be stitched into myocardium. Fluid is infused into the dialysis fiber and sampled at the other end. Upper panel: Osmolality of "osmode" sample (ordinate) obtained at different infusion rates when the "osmode" is placed in a test chamber containing stirred fluid of different osmolalities (abscissa). Middle panel: Osmolality of "osmode" samples obtained at an infusion rate of 1 μL/min when the "osmode" is placed in a test chamber containing agar gels of different osmolalities (abscissa). Each dot is the average of three measurements from one "osmode." Three different "osmodes" were tested.

**FIGURE 2** Upper panel: Cross-section of "osmode" track in normal myocardium. A zone of cellular destruction approximately 100 μm wide surrounds the osmode. Outside this zone, cells are undamaged and capillaries are open. Arrows point to impressions of dialysis fiber and the supporting nylon fiber. Scale bar indicates 100 μm. Lower panel: Schematic drawing of the way the "osmodes" were positioned into the ventricular wall. The polyethylene tubes (o.d. 800 μm), the dialysis fiber (o.d. 180 μm), and the nylon filament (diameter 200 μm) are drawn much larger than they are in reality.
Specialities) attached to one of the polyethylene tubes. A volume of 10 μl was infused over a period of 10 minutes. The fluid, having passed the dialysis fiber, accumulated in the other polyethylene tube. This fluid was aspirated into a Hamilton syringe with a needle fitting loosely into the polyethylene tube. The osmolality of this sample was determined in a Wescor vapor pressure osmometer (type 5100 B), calibrated with 0.1566 and 0.5514 molal NaCl as standards of 290 and 1000 mOsm/kg, respectively.

The accuracy of the method was assessed in two ways. First, "osmodes" were passed through a thermostated (37°C) chamber containing stirred solutions of different osmolalities, varying from 330 to 420 mOsm/kg. The osmolality was varied by addition of dil-sodium lactate to the modified Tyrode's solution. The "osmodes" were perfused with Tyrode's solution at different rates to determine the maximum perfusion rate which allowed osmotic equilibrium between perfusate and surrounding fluid to be established. This rate was in the order of 3 μl/min, when the osmolality of the surrounding fluid was between 330 and 360 mOsm/kg (see Fig. 1, upper graph). At higher osmolalities, a slower perfusion rate was required. To ensure a margin of safety, we chose a standard perfusion rate of 1 μl/min. We performed a second test by inserting the "osmodes" in 4% agar gels, prepared from solutions of different osmolalities, according to variations in lactate concentration. The gels were thermostated at 37°C during measurements. The osmolality of the gel fluid was measured on fluid collected in a well cast in the gel. As shown in Figure 1, lower graph, the osmolalities of the "osmode" samples deviate from those of the surrounding agar by 2 to 4%. (Each dot indicates the average of three measurements from each of three different osmodes tested at three different osmolalities.)

After collection of preocclusion baseline samples from the contracting left ventricle, regional ischemia was produced by clamping the left anterior descending coronary artery (LAD) for periods of 50 to 70 minutes. During occlusion, osmode samples were collected at varying times after occlusion (see Fig. 3). Each sampling period lasted 10 minutes. In three preliminary experiments, three osmodes were inserted in parallel, 5 mm apart, in the central ischemic region. One osmode was perfused intermittently with a 10-minute pause between sampling periods. Perfusion of the second and third osmode was initiated simultaneously with the second and third perfusion of the first osmode. In this way, it was found that the osmolality of samples drawn intermittently from one osmode did not differ systematically from samples drawn after the first perfusion of another osmode. Tissue damage produced by insertion of osmodes was evaluated from biopsies cut across the osmode tracks following fixation of the hearts. It was found that a zone of destroyed cells of about 100 μm width surrounded the osmode (see Fig. 2). In most experiments, two osmodes were positioned centrally in the ischemic area, and one osmode (control) was placed in the normally perfused posterolateral wall of the left ventricle.

The border between ischemic and normally perfused myocardium was seen to be sharply demarcated on the epicardial surface during occlusion. These border sites were marked by insertion of thin (150-μm) needles to facilitate and ensure the identification of the border at later steps in the experiment.

Technique of Reperfusion

In some experiments, the ischemic area was reperfused after a period of LAD occlusion of 60 minutes. Either the area was reperfused with the normal perfusion medium (blood-Tyrode's mixture) by removing the clamp on the LAD, or the LAD was cannulated and selectively reperfused with perfusion medium of high osmolality. To this end, 500 ml of blood-Tyrode's mixture was made hypertonic (up to 480 mOsm/kg) by the addition of mannitol. This fluid was kept stirred and oxygenated, passed through a 37°C heat exchanger, and infused selectively into the cannulated LAD at a controlled...
constant pressure equal to the perfusion pressure in the aorta.

During hypertonic LAD perfusion, the first 500 ml of perfusate emerging from the right atrium and ventricle were collected in order to prevent major hypertonic contamination of the normotonic perfusion fluid supplying the rest of the heart. The tonicity of the LAD perfusate was lowered gradually to normal levels by addition of normal perfusion medium to the stirred reservoir feeding the LAD. Generally, within 15-20 minutes of selective LAD perfusion, slight but well-defined color differences permitted tracing of the previous ischemic border. Likewise, color differences permitted identification of nonperfused tissue. Patches of unfixed (soft) tissue were found in the ischemic area of some hearts. This was the case particularly when the hearts were fixed following normotonic reperfusion.

In reperfusion experiments, the volume ratio of reperfused to total (original) ischemic area was estimated by drawing the outline of the originally ischemic tissue together with the outlines of nonreperfused tissue on transparent acetate foil (14 mg/cm²) for each of the slabs cut transversely through the ischemic region. After the foil was cut, the volume ratio was estimated as the weight ratio of foil covering reperfused tissue to the foil covering the whole of the ischemic area.

Small blocks (1-2 mm³) were cut from the slabs at two different sites in the fixed ischemic and in the nonischemic myocardium at three different depths at each site, viz., subepicardially, midmurally, and subendocardially. Blocks from ischemic tissue were cut well away from the ischemic border and close to an intramural recording electrode to ensure that the tissue was representative of ischemic myocardium. The blocks were transferred to Na-phosphate buffer (110 mmol/liter, pH 7.2) through a slow and continuous gradient procedure described elsewhere (Rostgaard and Tranum-Jensen, 1980). The blocks were postfixed in 2% OsO₄, in 110 mmol/liter phosphate buffer for 2 hours at room temperature and washed three times in the buffer, followed by dehydration and infiltration with Epon by a continuous procedure (Rostgaard and Tranum-Jensen, 1990). Sections 2 μm thick were cut on glass knives and stained with p-phenylenediamin or toluidin blue for light microscopy. Only tissue exhibiting uniformly open and empty capillaries, indicating that fixative had flushed the vascular bed, was accepted in the material. Ultrathin sections were prepared with a diamond knife and stained with uranyl acetate and lead citrate for electron microscopy.

To obtain a quantitative estimate of differences in myocardial cell volume in the different groups and between normal and ischemic myocardium in individual hearts, we measured cell diameters at the nuclear level in 2-μm sections of blocks cut 3-4 mm below the epicardial surface. Measurements were performed on one section of each of two blocks taken at different sites in the ischemic zone and similarly on two blocks taken from the normal myocardium. The sections were scanned systematically at a magnification of 625:1 and the smallest diameter across nuclei was measured using an ocular screw micrometer (Leitz) calibrated with a precision object micrometer (Leitz). Fifty cell diameters were measured in each section to give a total of 100 measurements on ischemic and 100 measurements on normal myocardium in each heart.

Results
Changes in Tissue Osmolality during Regional Ischemia

Figure 3 shows measurements of tissue osmolality in ischemic and normal myocardium at various times after LAD occlusion. The results of 23 experi-
ments are pooled. The position on the abscissa indicates the midpoint of the 10-minute interval during which a sample was collected from an osmometer. Tissue osmolalities are plotted relative to the osmolality of the perfusion medium at the beginning of the occlusion. The osmolality of plasma taken from 23 pigs was 303 ± 2 mOsm/kg. Numerical figures here and elsewhere in the text are given as the arithmetic mean ± 2x standard error of the mean (SEM). Average osmolality of the blood-Tyrode’s mixture used to perfuse the hearts was 313 ± 2.5 mOsm/kg at the start of perfusion. As can be seen, with time, the osmolality of the perfusion fluid increased, as did the osmolality of the normal myocardium. This increase is due mainly to evaporation from the perfusion system during the experiment. At the start of occlusion, the osmolality of the perfusion fluid was 319.2 ± 2.5 mOsm/kg, the tissue osmolality in the future ischemic myocardium was 325.7 ± 2.7, and that in the posterolateral wall of the left ventricle 324.6 ± 2.6 mOsm/kg. It is evident that tissue osmolality in the ischemic region quickly rose above normal to reach a level of 360.4 ± 3.1 mOsm/kg 45-60 minutes after coronary artery occlusion. At this time the tissue osmolality of the normal myocardium was 330.4 ± 3.4 mOsm/kg. Based on the testing procedure in agar-gels, we estimated that our method underestimated true tissue osmolalities in the higher range by 5-8 mOsm/kg. Thus, relative to the start of occlusion, osmolality in the ischemic area had risen by roughly 40 mOsm/kg (360.4 − 325.7 + (5 to 8) after 45-60 minutes of LAD occlusion. At this time a difference of roughly 35 mOsm/kg existed between ischemic and normal myocardium.

**Changes in Tissue Hydration and Na⁺, K⁺, ATP, CP, Lactate, and Glycogen Content**

In four experiments the wet weight:dry weight ratios and tissue content of Na⁺ and K⁺ were determined in each biopsy taken after 60 minutes of LAD occlusion from the central ischemic area and from the normal myocardium. In the central ischemic area, the mean wet weight:dry weight ratio was 4.74 ± 0.10 (24 biopsies), whereas in normal myocardium this ratio was 4.21 ± 0.10 (21 biopsies) (Fig. 4, lower panel), the difference being significant (P < 0.01). It follows that the volume of total tissue water per gram dry weight in normal myocardium is 3.21 ml. The increase of 0.53 ml in ischemic myocardium thus amounts to a 16.5% (0.53/3.21) increase in tissue water volume.

The mean Na⁺ contents of ischemic and normal myocardium were 263.9 ± 12.5 and 239.9 ± 10.5 μmol/g dry weight, respectively. This difference was significant (P < 0.01). The K⁺ content was 415.6 ± 6.9 in ischemic tissue and 410.7 ± 12.4 in normal myocardium (no significant difference). Thus, there was a gain in Na⁺ content in ischemic myocardium whereas K⁺ content remained essentially unchanged.

Lactate content of ischemic myocardium, after 1 hour of coronary artery occlusion was 228.3 ± 15.0 μmol/g dry weight, determined in 29 biopsies from five hearts. Lactate content of normal myocardium in 24 biopsies of the same hearts was 27.3 ± 6.2 μmol/g dry weight. CP and ATP both declined: CP from 54.0 ± 6.6 (13 biopsies) to values less than 0.2 μmol/g dry weight (11 biopsies) and ATP from 18.6 ± 0.5 (13 biopsies) to 3.0 ± 0.2 (11 biopsies) μmol/g dry weight. To appreciate the osmotic consequences of these changes, it is useful to express the measured values of ionic and metabolite content as mmols per liter total tissue water. From the measured wet weight:dry weight ratios of individual biopsies used for Na⁺ and K⁺ determinations, and using the values of ischemic tissue dry weight and 3.21 ml total tissue water per gram normal tissue dry weight for calculations on lactate, CP and ATP, the following figures result. In ischemic myocardium, K⁺ was 110.5 ± 3.1 Na⁺ 71.0 ± 2.3 (the sum of both being 181.5), lactate 61.0 ± 4.0, CP 0.1, ATP 0.8 ± 0.1 mmol/liter total tissue water. In normal myocardium, K⁺ was 127.9 ± 3.1, Na⁺ 74.9 ± 3.2 (the sum of both being 202.8), lactate 8.5 ± 1.9, CP 16.8 ± 2.1, ATP 5.8 ± 0.2 mmol/liter tissue water. The figures for Na⁺ and K⁺ are displayed graphically in Figure 4 (upper panel). In round numbers ischemic myocardium loses 21 mmol/liter total tissue water of cations, 17 mmol of CP, and 5 mmol of ATP, whereas it gains 53 mmol of lactate.
In two experiments, total glucose (glycogen + free glucose) was determined from biopsies taken immediately before occlusion of the LAD. Average values were 148.1 ± 8.7 (n = 8) μmol/g dry weight. After 1 hour of LAD occlusion, total residual glucose was 20.3 ± 5.4 (n = 11). In the same biopsies, the change in lactate was found to be from 19.7 ± 5.8 before the occlusion to 262.3 ± 21.8 μmol/g dry weight after the occlusion. Thus, the increase in lactate of 243 μmol/g dry weight corresponded well to the increase in lactate of 243 μmol/g dry weight after the occlusion. This, together with the K⁺ figures given above, indicates that little washout occurs in the central ischemic area during the 1-hour period of occlusion. It should be stated here that biopsies taken from normal myocardium at the end of the experiment had a glycogen content that was nearly twice as high as in biopsies from normal myocardium taken at the beginning of the experiment. This indicates that, during the perfusion with blood-Tyrode's mixture containing at least 10 mmol of glucose per liter, the normally perfused cells accumulated glycogen.

**Influence of Osmolality of the Fixative on Cell Morphology**

When ischemic myocardium is exposed to a fixative, which is isotonic with respect to normal myocardium, the ischemic cells may be expected to take up water from the relatively hypo-osmotic fixative. We have tried to assess such artificial swelling phenomena by fixing ischemic myocardium at different osmolalities. In these studies, only such tissue was considered that exhibited open, empty capillaries, indicating that fixative had flushed the capillary bed. This was intended to ensure uniform conditions of exposure to the fixative. Figure 5 shows photomicrographs of ischemic and normal myocardium fixed 1 hour after occlusion of the LAD with a fixative that was normotonic with respect to normal myocardium (osmolality of the fixative vehicle 312 mOsm/kg). Under these conditions, a marked intracellular edema was observed in the ischemic cells, as is evident from the clear empty spaces around the nuclei and the separation of myofibrils. Myocardial cell nuclei are extremely swollen and exhibit marginal condensation of chromatin. Figure 6 depicts the histological appearance of ischemic and normal myocardium from a heart treated identically to that of Figure 5 except that the fixative was hypertonic. This particular heart developed an osmolality in the ischemic area of 372 mOsm/kg, and the fixative was adjusted by the addition of mannitol to achieve an osmolality of the vehicle identical to the tissue osmolality. Under these conditions the intracellular edema was much less pronounced. Cell nuclei also are less swollen, but do exhibit a distinct margination of chromatin. As expected, the normal myocardium of the hypertonically fixed heart is shrunken, compared to the normotonically fixed normal myocardium.

To obtain a quantitative estimate of cell size, we measured cell diameters across cell nuclei in ischemic and normal myocardium in three pairs of hearts fixed at fixative vehicle tonicities of 312, 372, and 400 mOsm/kg, respectively. The results are summarized in Table 1. Cell diameters in ischemic myocardium decrease with increasing fixative toxicity. Ischemic cell diameters of any of the two hearts fixed at 312 mOsm/kg differ significantly (P < 0.01) from any heart in the other two pairs. In normal myocardium, no further shrinkage is detected between 372 and 400 mOsm/kg, indicating that the cells have approached a maximally shrunken state already at 372 mOsm/kg. At all tonicities, the ischemic cell diameters are significantly (P < 0.001) larger than the non-ischemic cell diameters in the same heart. At the high tonicities, the ischemic cells exhibit diameters found in normal myocardium by normotonic fixation.

In Figure 7, electron micrographs of normotonically and hypertonically fixed ischemic myocardium (1 hour after LAD occlusion, corresponding to Figs. 5 and 6) are shown. In both cases the mitochondria are swollen, with distinct cristae in a clear matrix, though most pronounced in the first case. Cell nuclei exhibit, in both cases, a distinct margination of chromatin. However, in the normotonically fixed heart, the degree of intracellular edema is distinctly greater than in the hypertonically fixed ischemic myocardium. As expected, in both cases glycogen stores are depleted.

**Normotonic and Hypertonic Reperfusion**

Reperfusion of an ischemic area with normotonic fluid can be expected to induce a rapid additional cell swelling, which in itself may injure the cells, and also, because of accompanying compression of capillaries, may impede reperfusion. We therefore compared the effects of normotonic and hypertonic reperfusion of the LAD area after a 60-minute period of ischemia.

Figure 8 shows epicardial and intramural DC-extracellular electrograms recorded during ischemia and after normotonic and hypertonic reperfusion, respectively. Normotonic reperfusion was accomplished by simply removing the clamp on the LAD after a 60-minute period of LAD occlusion. Selective hypertonic reperfusion of the LAD area was performed by cannulating the LAD and perfusing it, after 1 hour of ischemia, with a blood-Tyrode's mixture made hypertonic by the addition of mannitol to achieve 480 mOsm/kg. The LAD was perfused with this hypertonic perfusate for 8 minutes, and then the osmolality was gradually decreased over a period of about 20 minutes to normal levels. As can be seen, in both experiments, the epicardial and intramural DC electrograms were monophasic after 60 minutes of LAD occlusion, indicating
FIGURE 5  a, c, and e: Low- and high-power micrographs of myocardium fixed by vascular perfusion with normotonic fixative (fixative vehicle: 312 mOsm/kg) after a 1-hour period of LAD occlusion (ischemic tissue osmolality measured in this heart: 365 mOsm/kg). For comparison are shown micrographs of normally perfused myocardium taken from the same heart (b, d, and f). The normally perfused myocardium is well preserved; in particular there are no signs of intracellular edema. In contrast, the ischemic myocardial cells are markedly swollen as evidenced by the clear empty spaces between myofibrils and around nuclei (N). The latter are swollen and exhibit margination of chromatin. Mitochondria (m) are enlarged and appear as round bodies. Hypercontraction of myofibrils is seen (x), but not in all cells. Note that capillaries (c) are open and empty to indicate that fixative has flown. p-phenylene diamine staining. Scale bars indicate 20 μm.
Figure 6  a, c, and e: Low- and high-power micrographs of myocardium fixed with hypertonic fixative (fixative vehicle: 372 mOsm/kg) after a 1-hour period of LAD occlusion (ischemic tissue osmolality measured: 372 mOsm/kg). For comparison are shown micrographs of normally perfused myocardium from the same heart (b, d, and f). The cells of the normally perfused myocardium appear slightly shrunken. The ischemic myocardial cells exhibit only a slight edema compared to the normotonically fixed heart of Figure 5. Also their nuclei (n) are less swollen but do exhibit margination of chromatin as in Figure 5. Myofibrils generally are hypercontracted. c: Open empty capillaries. p-phenylene diamine staining. Scale bars indicate 20 μm.
Table 1: Mean ± 2 SEM of Smallest Cell Diameters (μm) Measured across Cell Nuclei in Ischemic and Normal Myocardium Fixed at Different Tonicities

<table>
<thead>
<tr>
<th>Osmolality of fixative vehicle</th>
<th>Experiment</th>
<th>In ischemic</th>
<th>Normal</th>
<th>Ischemic (pooled)</th>
<th>Normal (pooled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 mOsm/kg</td>
<td>A</td>
<td>12.13 ± 0.34</td>
<td>10.49 ± 0.28</td>
<td>A + B</td>
<td>12.10 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12.07 ± 0.34</td>
<td>10.42 ± 0.24</td>
<td>A + B</td>
<td>12.10 ± 0.24</td>
</tr>
<tr>
<td>372 mOsm/kg</td>
<td>C</td>
<td>13.58 ± 0.38</td>
<td>10.17 ± 0.29</td>
<td>C + D</td>
<td>13.12 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>12.62 ± 0.37</td>
<td>10.73 ± 0.34</td>
<td>C + D</td>
<td>13.12 ± 0.27</td>
</tr>
<tr>
<td>312 mOsm/kg</td>
<td>E</td>
<td>15.46 ± 0.50</td>
<td>13.17 ± 0.38</td>
<td>E + F</td>
<td>15.10 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>14.70 ± 0.42</td>
<td>12.98 ± 0.34</td>
<td>E + F</td>
<td>15.10 ± 0.33</td>
</tr>
</tbody>
</table>

Each value is derived from 100 measurements obtained by pooling two sets of 50 measurements from two different sites in ischemic or normal myocardium. In the two columns on the right, measurements on similarly treated hearts are pooled.

unresponsiveness of the ischemic myocardium (Kleber et al., 1978). After normotonic reperfusion, some electrical activity returns in the subepicardial layers, whereas deeper tissue remains unresponsive. In all signals, there is an appreciable amount of TQ-segment depression and ST-elevation, indicating loss of resting membrane potential and decrease in action potential amplitude, respectively, or—in the

![Figure 7](http://circres.ahajournals.org/lookup/fig/1766218)

**Figure 7** a and b: Electron micrographs of cross-sectioned ischemic cells subjected to normotonic and hypertonic fixation, in a and b, respectively (Same hearts as in Figure 5 and 6.) The marked intracellular edema in the normotonically fixed ischemic cells produce large empty spaces separating myofibrils (mf) and mitochondria (m), and around the nucleus (N). This edema is less pronounced in the hypertonically fixed ischemic cells. c: Capillaries. Scale bars indicate 1 μm.
which hypertonic reperfusion was instituted 1 hour after coronary occlusion. In two of these, recovery of extracellular potentials was as complete as in the experiment of Figure 8. In one experiment, despite a return of intrinsic deflections in previously monophasic complexes, residual ischemic changes remained present as a slight depression of the TQ segment and elevation of the ST segment. In all four experiments in which normotonic reperfusion followed the 1-hour ischemic period, no improvement in DC electrograms was observed.

In eight experiments, tissue resistivity was measured under control conditions; after 15, 30, and 60 minutes of coronary occlusion; and 15 to 40 minutes after reperfusion. In four experiments, normotonic reperfusion, and in four, hypertonic reperfusion was performed. The data are summarized in Table 2. At all levels in the ventricular wall (a total of 106), an increase in tissue resistivity was measured after coronary occlusion. As shown in Figure 9A, tissue resistivity increased only mildly during the first 15 minutes after occlusion. The most dramatic rise occurred between 30 and 60 minutes. In the hypertonic reperfusion period, measurements from all 52 terminals showed a decrease in resistivity. However, as is evident from Table 2, and in Figure 9B, control values were not reached in all instances. In contrast, following normotonic reperfusion, measurements from 41 of the 54 levels showed a further increase in tissue resistivity, in 11 resistivity remained the same as during occlusion, and in only two was a decrease in resistivity measured.

In Figure 10, tissue levels of ATP, CP, and lactate are shown for normal myocardium, and for previously ischemic myocardium (1 hour of LAD occlusion) subjected to normotonic and hypertonic reperfusion, respectively. After hypertonic reperfusion, lactate, CP, and ATP levels were 53.2 ± 17.0, 29.8 ± 11.3, and 6.6 ± 1.5 μmol/g dry weight, respectively. After normotonic reperfusion, these values were 171.4 ± 35.8, 4.6 ± 2.6, and 2.7 ± 0.9. All differences were statistically significant (P < 0.01). It is to be noted that after normotonic reperfusion, lactate levels can be as high as during coronary artery occlusion (Janse et al., 1979), indicating that parts of the LAD area are not reperfused at all, since no washout occurred. Also, it should be noted that, although reperfusion improves the metabolic case of monophasic potentials—no active membrane responses at a reduced level of resting membrane potential (Kleber et al., 1978). After hypertonic reperfusion, the TQ- and ST-segments of epicardial and intramural electrograms have become iso-electric, and large intrinsic deflections are present. This indicates that, throughout the wall, resting membrane potentials are restored to control levels, as are the action potential amplitudes. The only difference with respect to the control situation is a slight delay in activation.

Intramural recordings of DC extracellular electrograms were made in three other experiments in which hypertonic reperfusion was instituted 1 hour after coronary occlusion. In two of these, recovery of extracellular potentials was as complete as in the experiment of Figure 8. In one experiment, despite a return of intrinsic deflections in previously monophasic complexes, residual ischemic changes remained present as a slight depression of the TQ segment and elevation of the ST segment. In all four experiments in which normotonic reperfusion followed the 1-hour ischemic period, no improvement in DC electrograms was observed.

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conditions, control values are not reached. On macroscopic inspection of cross-sections of the fixed left ventricle, reperfused and non-reperfused areas could be distinguished easily. (see Methods). Normotonic reperfusion resulted in reperfusion of subendocardial patches and a rim of subepicardial tissue. In contrast, a major part of the previously ischemic area was reperfused when the LAD was initially perfused with hypertonic solution, and only small islands of tissue remained unperfused. Such islands were always located in deeper layers. The volume of reperfused tissue was estimated by planimetry and expressed as a percent of the previously ischemic area. After normotonic reperfusion this percentage was on the average 22 ± 4% (for the individual four hearts, the percentages were 24, 22, 17, and 26). After hypertonic reperfusion, on the average, 67 ± 7% of the ischemic tissue was reperfused (in six hearts the percentages were 74, 75, 54, 61, 77, and 63%).

The microscopic appearance of normotonically reperfused myocardium was studied in blocks cut across the border between reperfused and non-reperfused myocardium. In this border, the non-perfused myocardium still could be fixed (as indicated by open, empty capillaries) because fixative was introduced at a pressure that was 50-80 cm H2O higher than the perfusion pressure. Such tissue presents the same morphology as ischemic tissue that is normotonically fixed (see Fig. 5) without preceding reperfusion. Samples taken more superficially in the epicardial rim of successfully reperfused tissue did exhibit a nearly normal histological
structure. In contrast, the hypertonically reperfused myocardium shows a nearly completely normal histological appearance, whether samples were taken from subepicardial, midmural, and subendocardial layers of reperfused tissue, sporadic necrotic cells and scattered bands of hypercontracted myofibrils being the only stigma of the previous ischemia (Fig. 11).

In Table 3 are given measurements of cell diameters at the nuclear level in midmural myocardium of four hearts subjected to hypertonic reperfusion. In the first three hearts, the differences in cell diameter in the non-ischemic and previously ischemic myocardium were statistically not significantly different. In heart iv, a significant difference was found; cells in the previously ischemic area remained swollen, whereas cells in the normal area for unknown reasons were unusually small (compare with Table 1).

In Figure 12, an electron micrograph of subendocardial myocardium hypertonicity reperfused after 1 hour of ischemia is compared with an electron micrograph of normal myocardium from the same heart. The only differences detected are a slightly woolly appearance of mitochondria in the previously ischemic area, and a higher content of glycogen granules in the normal myocardium. In all other respects, the ultrastructure of the previously ischemic cell is normalized. It does appear that normotonic reperfusion of the LAD prevents the reperfusion of large parts of the LAD area. If, however, reperfusion is achieved as in the subepicardium, the cells regain their normal histological appearance in the same way as does the major part of the myocardium following hypertonic LAD reperfusion.

Discussion

The main results of this study can be summarized as follows. Following coronary artery occlusion, tissue osmolality rises so that after 50 minutes a difference between ischemic and normal myocardium of about 35–40 mOsM/kg is established. This increase in osmolality can be accounted for by metabolic processes, in particular the conversion of glycogen to lactate, and the hydrolysis of high energy phosphate compounds. Concomitantly, the ischemic tissue takes up fluid whereby tissue water volume increases by an average of 16.5%. Part of this extra fluid is located intracellularly so that ischemic cells swell.

Because of the increased osmolality, ischemic cells will undergo an additional swelling when brought into contact with normotonic fluids, be it fixative or normotonic perfusion fluid. This implies on the one hand that morphometric measurements on ischemic tissue can be misleading unless the tonicity of the fixative is adjusted according to the increased osmolality of the ischemic tissue. On the other hand, normotonic reperfusion may induce sufficient cell swelling to cause failure of capillary circulation, especially in subendocardial and midmural layers. Reperfusion, and thus recovery of ischemic cells, can be accomplished by initial reperfusion of an ischemic area with hypertonic fluids, followed by a gradual return to normal levels.

The Mechanisms of the Increase in Tissue Osmolality in Ischemia

Cell swelling is known to occur in myocardial ischemia, and attention has been drawn to "the vicious cycle of cell swelling, vascular obstruction, sustained ischemia, and eventual cell death" (Leaf, 1970). Cell swelling in ischemia has been attributed to an insufficiency of the Na⁺-K⁺ exchange pump (Leaf, 1970). The insufficiency of active membrane transport mechanisms to balance passive leaks will lead ultimately to equilibrium of small molecules across the cell membrane. Hereby a Gibbs-Donnan equilibrium, governed by the higher intracellular concentration of colloid anions, is approached. The cell membranes cannot resist a hydrostatic pressure difference of any significant magnitude, and consequently the cells will swell (Leaf, 1959; Tosteson and Hoffman, 1960; Rorive and Gilles, 1979). The time scale and extent to which this mechanism will operate in ischemic myocardium is difficult to assess due to incomplete knowledge about the passive ionic permeabilities in ischemic myocardial cells, as well as to insufficient characterization of intracellular proteins. Pine et al. (1977, 1978) found that 60 minutes of total metabolic blockade of papillary muscle did not result in cell swelling despite inhibition of Na⁺-K⁺ exchange pump activity. This in our opinion would indicate that the above-mentioned colloid osmotic phenomena, at least on a relatively short time scale, are insufficient to explain ischemic swelling of myocardial cells, and other mechanisms must contribute, such as the osmotic gradients likely to be created by anaerobic metabolites.

A rough estimate of the increase in osmolality in ischemic tissue due to hydrolysis of ATP and CP and the production of lactate can be made based on the measurements of the tissue content of those compounds. When tissue levels are expressed as mmol/liter total tissue water (see Results), it can be calculated that, in an ischemic period of 1 hour, 5 mmol/liter of ATP and 17 mmol/liter of CP are hydrolyzed, whereas 53 mmol/liter of lactate are produced. Complete hydrolysis of 5 mmol/liter of ATP will produce 15 mmol/liter of inorganic phosphate. However, the net increase in inorganic phosphate will be less, due mainly to increases in AMP and glucose-6-phosphate. We estimate that the hydrolysis of ATP will give rise to an increase in osmotically active inorganic phosphate of 10 mmol/liter total tissue water. The increase in lactate in ischemic myocardium is due mainly to glycogenolysis, but also partly to breakdown of glucose, present at the start of occlusion. Glycogen originally
present does not contribute significantly to total osmolality, but glucose does. Therefore, the increase in tissue osmolality due to lactate production should be corrected for disappearance of glucose during ischemia. Since the perfusion medium contained 11 mmol/liter of glucose, we estimate that, of the 53 mmol/liter of lactate produced, only 43 mmol/liter contribute to the increase in tissue os-
TABLE 3  Mean ± 2 sem of Smallest Cell Diameters (μm) Measured across Cell Nuclei in Previously Ischemic and in Normal Midmural Myocardium in four Hearts Subjected to Selective Hypertonic Reperfusion of the LAD Followed by a Gradual Return to Normal Tonicities and Subsequently Fixed with a Normotonic Fixative

<table>
<thead>
<tr>
<th>Hypertonic reperfusion</th>
<th>Previously ischemic myocardium</th>
<th>Non-ischemic myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>11.32 ± 0.28</td>
<td>11.16 ± 0.22</td>
</tr>
<tr>
<td>II</td>
<td>12.79 ± 0.57</td>
<td>13.25 ± 0.34</td>
</tr>
<tr>
<td>III</td>
<td>13.24 ± 0.30</td>
<td>13.81 ± 0.40</td>
</tr>
<tr>
<td>IV</td>
<td>15.07 ± 0.35</td>
<td>10.83 ± 0.32</td>
</tr>
</tbody>
</table>

Each value is derived from 100 measurements obtained by pooling of two sets of 50 measurements from two different sites in the previously ischemic tissue or in the normally perfused myocardium.

The measured increase in tissue osmolality after 1 hour of ischemia relative to the tissue osmolality just before occlusion was 360–325 = 35 mOsm/kg.

To explain the difference between calculated and measured increases in osmolality, we have to take into account different factors. First, due to water uptake there is a decrease in concentration of cations per liter total tissue water in the ischemic tissue. The sum of Na⁺ and K⁺ expressed as mmol/liter total tissue water is 21 mmol/liter less in ischemic myocardium than in normal myocardium. Second, our method underestimates true tissue osmolality by at least 5 mOsm/kg (see Fig. 1). Furthermore, intracellular solutes are known to have low osmotic coefficients (Dalmark, 1975), and the amount of bound tissue water is unknown. For these reasons, our calculations cannot be more than rough estimates.

In view of the considerations made above, we believe that the early rise in tissue osmolality observed after coronary artery occlusion can satisfactorily be explained by the increase in osmotically active particles produced by the hydrolysis of ATP and CP and by the breakdown of glycogen.

![Electron micrographs of cross-sectioned myocardial cells from (a) hypertonically reperfused ischemic tissue (same heart as in Fig 11), and (b) normally perfused, non-ischemic tissue from the same heart. The structure of the previously ischemic cell is normalized to a large extent, except that glycogen stores (g) are smaller, and mitochondrial dense granules are few or absent. T-tubules (T) are of normal caliber c: Capillaries. Scale bars indicate 1 μm.](http://circres.ahajournals.org/)

FIGURE 12  Electron micrographs of cross-sectioned myocardial cells from (a) hypertonically reperfused ischemic tissue (same heart as in Fig 11), and (b) normally perfused, non-ischemic tissue from the same heart. The structure of the previously ischemic cell is normalized to a large extent, except that glycogen stores (g) are smaller, and mitochondrial dense granules are few or absent. T-tubules (T) are of normal caliber c: Capillaries. Scale bars indicate 1 μm.
Cell Swelling and Increase in Tissue Water

In 1928, Eggleton et al. determined the coefficient of diffusion of lactate in skeletal muscle and found it to decrease in fatigue. They wrote "as the osmotic pressure inside the fibers rises in fatigue, the lymph (i.e., the extracellular fluid) is absorbed and the diffusion constant (of lactate) gradually diminishes to the value characteristic of the fibers closely packed." This would indicate that the sarcolemma is a significant barrier to lactate diffusion. In rat myocardium made partially ischemic, in that a residual flow of anoxic medium was maintained. Rovetto et al. (1973) calculated, based on measurements of sorbitol space and lactate content of the ischemic myocardium and effluent, that a gradient between intra- and extracellular lactate as high as 20 mmol/liter can exist in ischemic tissue.

The reason why, in ischemia, lactate would diffuse with difficulty through the cell membrane is far from clear. However, it has been found that, in superfused skeletal muscle, under conditions in which extracellular buffer capacity was low, lactate efflux rate is decreased further when the muscle fibers are depolarized with high K+ (Mainwood and Worsley-Brown, 1975). It is known that myocardial cells depolarize rapidly after coronary artery occlusion (Klöber et al., 1978) and that lactate content of ischemic myocardium in the isolated pig heart rises fourfold within 10 minutes after coronary artery occlusion (Janse et al., 1979). Moreover, within 4 minutes of ischemia, CP levels have decreased to practically zero and ATP has decreased as well (Janse et al., 1979). Thus, it is very likely that, in the very first minutes of regional myocardial ischemia, the majority of the breakdown products of the high energy compounds and glycogen remain intracellularly, thus creating an osmotic gradient across the cell membrane by which extracellular water is drawn into the cell, and osmotic equilibrium between intra- and extracellular compartments is restored. A volume of normotonic fluid subsequently entering the ischemic tissue will be distributed roughly according to the proportion of intra- to extracellular lactate as high as 20 mmol/liter can exist in ischemic tissue.

Effects of Ischemic Tissue Osmolality on Morphology

Although cell swelling is recognized widely to be associated with ischemia, an accurate determination of the degree of swelling is very difficult to obtain. Classical methods of determining extracellular space, such as inulin or sorbitol space determinations, cannot readily be applied in a truly ischemic situation because they imply equilibrium with an external fluid after ischemia is established. This inherently will interfere with the ischemia, and the method is further complicated by the osmotic phenomena. A potentially direct method for determining cell volume is morphometry. However, the results of morphometric measurements can be grossly misleading for several reasons. The first of these is the use of standard fixation, dehydration, and embedding procedures.

Thus, it has been shown that the use of discontinuous dehydration and embedding procedures may result in a reduction of cell volume by as much as 40% (Rostgaard and Tranum-Jensen, 1980). Such effects are particularly pronounced when swollen cells of little mechanical strength are processed. Second, it is known that the osmolality of fixatives can significantly alter cell volumes when not adjusted to the actual osmolality of the tissue to be fixed (Maunsbach, 1966). Our results on the effect of variations in fixative tonicity indicate that ischemic cell volume is influenced significantly by this parameter. Thus, since the osmolality in ischemic myocardium increases, the fixative used should be adjusted accordingly to prevent artefactual changes in cell volume. Ideally, since the osmolality increase varies from heart to heart, fixatives should be adjusted according to the measured increase in osmolality in each individual heart. The use of a fixative which is normotonic with respect to normal myocardium will lead to a significant overestimation of cell swelling in ischemic myocardium. It is a general, though not well understood experience, that the osmotic effect of a glutaraldehyde fixative is determined primarily by the tonicity of the fixative vehicle, the fixative itself contributing much less, at least in the range of concentrations normally used (Maunsbach, 1966). The vehicle of our normotonic fixative used after 1 hour of occlusion had an osmolality of 312 mOsm/kg. At this stage of the experiment, the osmolality of the nor-
nally perfused myocardium was measured to be around 330 mOsm/kg; this allows about 18 mOsm/kg for the effective toxicity of the 2% glutaraldehyde. The addition of colloids, e.g., dextran, to simulate the colloid osmotic properties of plasma proteins is important to avoid artificial widening of extracellular spaces during fixation by vascular perfusion (Bohman and Maunsbach, 1970).

It is our opinion that data on ischemic tissue micromorphology should be interpreted with great caution because the interaction of fixatives with ischemic tissue presents special problems, among which correction for tissue osmolality changes may be an important one. The morphological presentation we obtained by using a fixative vehicle of 372 mOsm/kg thus would come closer to the true morphology of the ischemic cells than that obtained with a normotonic fixative.

Reperfusion of Ischemic Myocardium

A practical consequence of our findings is that reperfusion of ischemic tissue with normotonic fluids will lead to abrupt cell swelling. This may be so severe as to obstruct capillaries and result in the well known “no-reflow phenomenon” (Jennings et al., 1969; Flores et al., 1972; Whalen et al., 1974). Flores et al. (1972) prevented the no-reflow phenomenon in ischemic renal tissue by infusing the animal during renal artery occlusion with hypertonic mannitol. Willerson et al. (1975) prevented the increase in coronary artery resistance, or reduced it in the subendocardium after 2 hours of ischemia by hypertonic mannitol. They stated that the mechanism was unclear, but that it could be due both to a reduction of cell swelling and to a direct reduction of vascular smooth muscle tone. Powell et al. (1976) showed that an increase in the osmolality at reperfusion of ischemic dog myocardium could reduce myocardial necrosis. Their findings showed that the changes of myocardial cells after 1 hour of ischemia were not reversible and that reduction of cell swelling may reduce necrosis.

The dog heart differs from the pig heart in that pre-existing collaterals are nearly absent in the pig, whereas they are abundant in the dog (Schaper, 1971). Coronary artery occlusion in the pig leads to a sharply demarcated transmural ischemia (Janse et al., 1979) whereas, in the dog, coronary occlusion does not lead to a total absence of flow, especially in the subepicardium (Willerson et al., 1975), and ischemic damage is not distributed equally over the wall (Reimer et al., 1977). Our reperfusion experiments confirm earlier findings (Willerson et al., 1975; Powell et al., 1976) and show that even in transmural ischemia, hypertonic reperfusion can to a great extent reverse ischemic changes after a 1-hour ischemic period. Thus, normotonic reperfusion resulted in reperfusion of on the average 22% of the ischemic area, whereas, after hypertonic reperfusion, an average of 67% of the ischemic myocardium was reperfused.

As shown in Figure 8, intramural and epicardial extracellular potentials return to control values, or show at least a substantial recovery in electrical activity following hypertonic reperfusion, whereas normotonic reperfusion after 1 hour of ischemia did not improve the electrical activity. The results of tissue resistivity measurements are more difficult to interpret. Since part of the applied subthreshold current flows through the extracellular space, and another part through the intracellular compartments, both extracellular and intracellular resistance are represented, but each to an unknown degree, in the measured tissue resistivity. The absolute value determined will be influenced also by factors such as fiber direction and the presence of large blood vessels in the neighborhood which produce varying degrees of anisotropy. The increase in tissue resistivity following coronary artery occlusion could be due therefore both to an increase in extracellular resistance caused by cell swelling and to an increase in intracellular resistance. That the resistance of the intracellular compartment can increase as a consequence of an increase in coupling resistance in ischemia is more than likely, since this has been shown to occur in hypoxia (Wojtczak, 1979). It is possible that the fact that tissue resistivity does not completely return to control values after hypertonic reperfusion (Table 2, Fig. 9) reflects a lasting increase in coupling resistance caused by ischemia that is not (entirely) reversible. This could account for the slight delay in activation found after hypertonic reperfusion. The fall in tissue resistivity upon hypertonic reperfusion, in contrast to the increase measured after normotonic reperfusion, could easily be explained by a reduction of extracellular resistance due to a decrease in cell swelling.

In our studies, as in others, mannitol was used to increase the osmolality of the blood–Tyrode mixture used to reperfuse the LAD. There are indications, however, that mannitol may enter the intracellular compartment. Thus, Grochowski et al. (1976) found the mannitol space in dog myocardium to be larger than the inulin space. Therefore, hypertonic reperfusion with extra mannitol followed by a fairly rapid return to normal levels of osmolality may induce a rebound effect when mannitol is indeed taken up by ischemic cells. Possibly the choice of a larger molecule than mannitol may lead to better results.

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