Coronary Perfusate Composition Influences Diastolic Properties, Myocardial Water Content, and Histologic Characteristics of the Rat Left Ventricle

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Background. **Recent studies found that edema, histology, and left ventricular diastolic compliance exhibit quantitative relationships in rats. Edema due to low osmolarity coronary perfusates increases myocardial water content and histologic edema score and decreases left ventricular filling. The present study examined effects of perfusate osmolarity and chemical composition on rat hearts.**

Methods. **Arrested American Cancer Institute (ACI) rat hearts (4°C) were perfused with different cardioplegia solutions, including Plegisol (289 mOsm/L), dilute Plegisol (172 mOsm/L), Stanford solution (409 mOsm/L), and University of Wisconsin solution (315 mOsm/L). Controls had blood perfusion (310 mOsm/L). Postmortem left ventricular pressure-volume curves and myocardial water content were measured. After glutaraldehyde or formalin fixation, dehydration, and paraffin embedding, edema was graded subjectively.**

Results. **Myocardial water content reflected perfusate osmolarity, being lowest in Stanford and University of Wisconsin solutions ($p < 0.05$ versus other groups) and highest in dilute Plegisol ($p < 0.05$). Left ventricular filling volumes were smallest in dilute Plegisol and Plegisol ($p < 0.05$). Osmolarity was not a major determinant of myocardial edema grade, which was highest with University of Wisconsin solution and dilute Plegisol ($p < 0.05$ versus other groups).**

Conclusions. **Perfusate osmolarity determined myocardial water content and left ventricular filling volume. However, perfusate chemical composition influenced the histologic appearance of edema. Pathologic grading of edema can be influenced by factors other than osmolarity alone.**

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Myocardial edema is well described in both experimental and clinical settings such as heart failure, cardiopulmonary bypass, and cardiac transplantation. Its relation to left ventricular (LV) function has also been described [1–3]. Recently, our laboratory studied coronary perfusion with crystalloid solutions in isolated hearts from both large (pig, monkey) and small (rat) animals [4–8]. These studies generally involved a single, brief period of coronary perfusion, similar to clinical cardioplegia for open heart operations or cardiac transplantation. Results have shown that crystalloid coronary perfusion induces myocardial edema and LV compliance decreases; the extent of these changes is accentuated by decreasing osmolarity and onconicity of the perfusates.

The interrelation of myocardial water content, LV volume at 15 mm Hg filling pressure, and histologic edema score were quantitated in rats by Carter and colleagues [4] in a study of the effect of myocardial edema on diastolic properties. They found that postperfusion myocardial water content and histologic edema score increased as perfusate osmolarity decreased. Osmolarity was also inversely related to postperfusion filling volume. The changes observed were predictable and directly reflected osmolarity of a single perfusate that was altered by dilution or evaporative concentration.

The study by Carter and colleagues suggested that the histologic characteristics of edema in myocardium from transmural or endomyocardial biopsies might be used to infer the extent of edema in the myocardium, and, in turn, related impairment of diastolic properties. To accomplish that, we must understand not only the effect of changes in perfusate osmolarity but also the effect of the chemical composition of common coronary perfusates. This knowledge is particularly important because for histologic studies myocardium might be immersed in solutions of diverse chemical composition, particularly when the parent organ has been involved in cardiac operation or preservation for transplantation.

Accordingly, in the present study, three crystalloid solutions commonly used for cardiac preservation (University of Wisconsin solution, 315 mOsm/L [UW_{315}]) or clinical cardioplegia (Stanford solution, 409 mOsm/L [S_{409}] and Plegisol, 289 mOsm/L [P_{289}]) were studied. The solutions differed from each other substantially not only

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in osmolarity but also in chemical composition. For the control group, the only perfusate was whole blood (310 mOsm/L [C_{310}]). A fifth group was studied, in which dilution of one of the crystalloids (dilute Plegisol, 172 mOsm/L [DP_{172}]) was used. Methods for coronary perfusion and analysis were standardized to allow comparison with prior studies. Effects of the perfusates on filling volume, myocardial water content, and histology were compared. The results showed that when the chemical composition of the perfusate varies, the histologic manifestations of edema in the myocardium are not predictable from osmolarity alone. The purpose of this study was to investigate the role of perfusate composition on diastolic properties, myocardial water content, and histology in the rat left ventricle. We hypothesized that lower perfusate osmolarity would lead to worsened diastolic properties, increased myocardial water content, and altered histologic characteristics in the rat left ventricle.

Material and Methods

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal Resources and the "Guide for the Care and Use of Laboratory Animal Resources" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication no. 86–23, revised 1985).

Thirty-five American Cancer Institute (ACI) rats (Harlan Sprague-Dawley Inc, Indianapolis, IN) were divided into the following five groups according to coronary perfusate: S_{409} (n = 7), UW_{315} (n = 6), P_{289} (n = 7), DP_{172} (n = 8), and C_{310} (n = 8).

Details of the preparation have been described previously [4]. Animals averaged 258 ± 3 g (mean ± standard error of the mean) (range, 217 to 299 g) before perfusion and were anesthetized with intraperitoneal ketamine (40 to 80 mg/kg) and xyalazine (5 to 10 mg/kg). A tracheostomy and mechanical ventilation (Harvard Apparatus, Cambridge, MA) were used. A transverse incision below the diaphragm and bilateral thoracotomy incisions were used. After heparinization (300 units/kg), the innominate artery was ligated.

In the four perfusion groups, the abdominal aorta, pulmonary artery, and venae cavae were transected, followed by cardiac arrest and perfusion. Hearts were protected from distension. Perfusate temperature was 4°C. The volume infused was approximately 5 mL at 60 mm Hg aortic root pressure over 2 minutes. Perfusate osmolarity was measured by microosmometer (Advanced Instruments Inc, Norwood, MA) before use. Excised hearts were immersed in their perfusate at 4°C until fixation. Control hearts, group C_{310}, were arrested with potassium chloride (4 mEq) injected into the aortic root and immersed in lactated Ringer's solution after excision.

After arrest and excision of the heart, the LV pressure-volume curve was measured. A 16-gauge angiocatheter connected to a three-way stopcock was advanced into the LV through the aortic valve. The aorta was tied around the catheter at the level of the coronary ostia. A clamp 1 mm on the atrial side of the mitral annulus sealed the LV. The right ventricle was incised to avoid fluid accumulation and pressure on the interventricular septum.

Volume was infused into the LV in 0.05-mL increments, with simultaneous recording of LV pressures with a 5-F micromanometer (Millar Instruments, Houston, TX) and an analog-to-digital converter (MacLab Inc, Milford, MA) until a LV pressure of 20 mm Hg was reached. Pressure-volume curves were recorded in duplicate. If less than 95% of infused volume was recovered, leakage was considered excessive, and data were discarded. Pressure-volume measurements were begun within 3 minutes of heart excision. Mean time from arrest to completion of the pressure-volume data collection was 11.1 ± 0.6 minutes. The heart was then transected perpendicular to the long axis, and half was fixed for histologic analysis and half was dried to determine myocardial water content.

Midheart cross-sections were fixed in phosphate-buffered 10% formalin or phosphate-buffered 2.5% glutaraldehyde overnight. Tissue was dehydrated, embedded, sectioned, and stained with hematoxylin-eosin. Pathologists graded each section for edema on scale from 0 to 3 (0 = no edema, 1 = mild edema, 2 = moderate edema, and 3 = severe edema). The LV free wall, interventricular septum, and right ventricular free wall were scored individually, and numeric scores were averaged to provide a mean grade for the whole heart.

The remaining heart was gently blotted dry, placed in a preweighed Petri dish, and weighed on an analytical balance (H16; Mettler Instruments Corp, Highston, NJ) to obtain the initial wet heart weight (WHW). The remaining heart was then dried to constant dry heart weight (DHW) in an oven maintained at 60°C for 48 hours. Myocardial water content (MWC) was calculated as

$$MWC = (WHW - DHW/WHW) \times 100\%.$$

Pressure-volume data were analyzed as described previously [5]. The mean curve was calculated for each animal by averaging ventricular pressures corresponding to the volume injected. To facilitate comparison of animals of different body weight (W), LV raw volumes (V) were normalized (Vn) to a body weight of 258 g (mean weight for the series) using the relation Vn = V (258/W). Normalized volumes were grouped into five pressure intervals: −2.5 to 2.4, 2.5 to 7.4, 7.5 to 12.4, 12.5 to 17.4, and 17.5 to 22.5 mm Hg. Pressure-volume data were analyzed using two-way repeated measures analysis of variance with mean volume within each of five pressure ranges as the repeated measure and experimental group as the grouping factor. If significance was found for the group effect or the group by pressure interaction, post hoc comparisons of volumes at each pressure range were calculated among the groups using Tukey's procedure with Bonferroni adjustment for the number of comparisons. Statistical significance was defined as p less than 0.05.

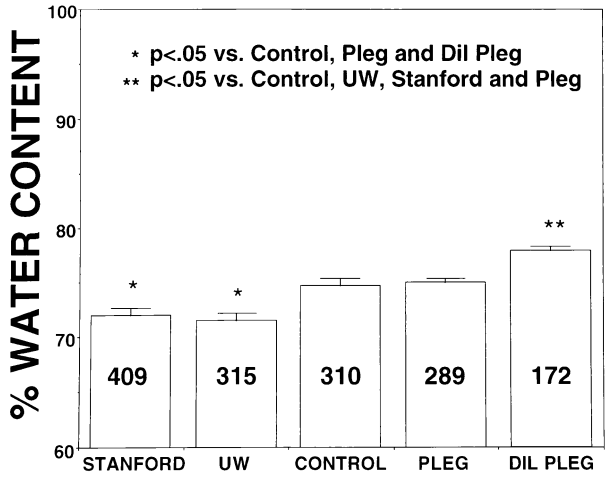

Fig 1. Relation between mean myocardial water content (% water content*) and perfusate group. Standard errors are indicated by whiskers. Mean myocardial water content for dilute Plegisol (*Dil Pleg*) is significantly higher than for all other groups; mean myocardial water content for both Stanford and University of Wisconsin solution (*UW*) is significantly lower than the other three groups ($p < 0.05$). (PLEG = Plegisol).*

Results

Average MWC increased as perfusate osmolarity decreased, as illustrated in Figure 1. Myocardial water content for DP_{172} was significantly higher ($p < 0.05$, analysis of variance) than that of the other groups. Conversely, MWC for S_{409} and UW_{315} was significantly lower than in the remaining groups. The relation between perfusate osmolarity and myocardial water content for samples from each LV is illustrated in Figure 2.

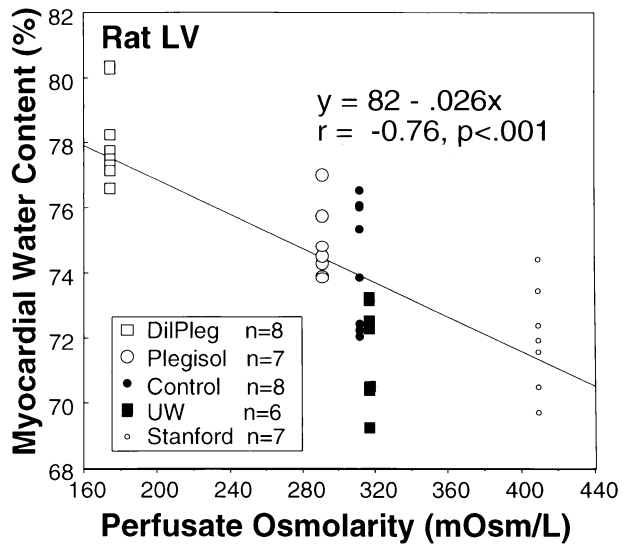

Fig 2. Relation between coronary perfusate osmolarity and myocardial water content. Individual data points are identified for each perfusate group. The fitted linear regression demonstrates a negative correlation; increasing perfusate osmolarity is associated with decreasing water content. Abbreviations as in Figure 1.

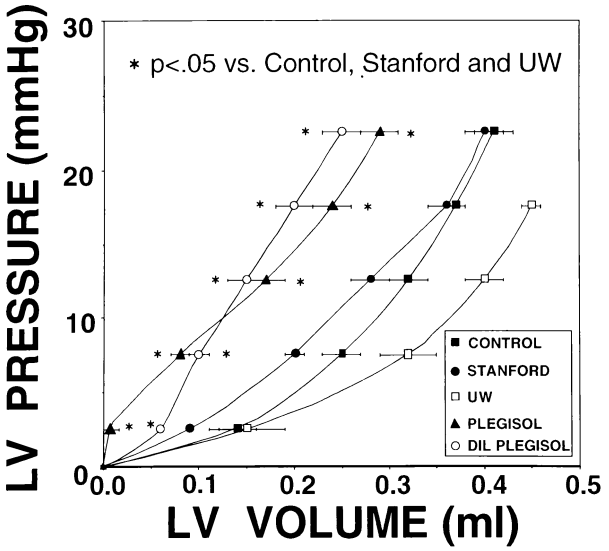

*Fig 3. Effect of perfusate osmolarity on the left ventricular (LV) diastolic pressure-volume relation. Mean LV pressure and LV volume are presented for pressure intervals averaging 2.5, 7.5, 12.5, 17.5, and 22.5 mm Hg. Standard errors are indicated by whiskers. The LV volumes for the dilute Plegisol (*Dil Pleg*) and Plegisol groups at all filing pressures are significantly smaller than for the other three groups. Abbreviations as in Figure 1.*

Average LV pressure-volume curves shifted toward decreased filling volume as perfusate osmolarity decreased (Fig 3). Volume was significantly less in all pressure intervals in the P_{289} and DP_{172} groups when compared with the remaining groups.

The relation between histologic edema grade and perfusate is illustrated in Figure 4. There was no significant difference in edema grade between the formalin- and glutaraldehyde-fixed tissues for any perfusate. With both formalin and glutaraldehyde fixation, edema grade was significantly higher ($p < 0.05$) with DP_{172} than with P_{289} or S_{409}. With glutaraldehyde fixation, edema grade for DP_{172} was also significantly higher than for C_{310}. With formalin fixation, edema grade was significantly higher for UW_{315} than for P_{289}. Edema grade did not correlate well with MWC or the LV pressure-volume curves. Histology for the individual regions did not differ qualitatively or quantitatively from the data averaged in Figure 4.

Representative photomicrographs of formalin-fixed tissues, arranged in order of osmolarity of the perfusate, are illustrated for the following groups: Figure 5 is UW_{315}, Figure 6 is C_{310} (no perfusion), Figure 7 is P_{289}, and Figure 8 is DP_{172}. The appearance of edema in Figure 5 is similar to that in Figure 8, and both samples appear more edematous than tissues in Figures 6 and 7. This finding is contrary to other effects of the perfusates, because mean MWC for the tissue in Figure 8 was higher and for Figure 5 was lower than that of the other tissues illustrated.

In Figure 9, linear regression relates MWC and edema grade for the present study. The data for UW_{315}, excluded from the analysis, are indicated by the open circle. Data

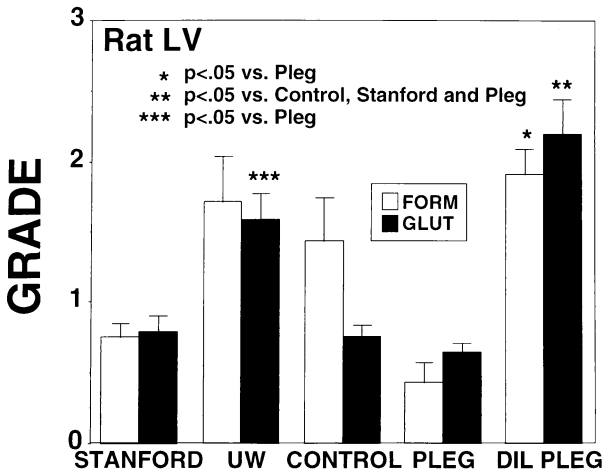

*Fig 4. Relation between mean histologic edema grade and perfusate group for samples taken from left ventricle (LV), right ventricle, and septum. Standard errors are indicated by whiskers. Samples fixed in glutaraldehyde (*GLUT*) in the dilute Plegisol (*Dil Pleg*) group were significantly different than GLUT fixed samples in control, Stanford, and Plegisol groups; UW samples fixed in GLUT were significantly different than Plegisol (*Pleg*) GLUT samples; dilute Plegisol samples fixed in formalin (*FORM*) were significantly different than Plegisol FORM samples. Abbreviations as in Figure 1.*

from a previous study by our laboratory [4] are also illustrated. These data suggest an atypical effect of impermeants in UW_{315} on histology.

Comment

The present study confirmed, consistent with our previous study [4], that lower osmolarity of coronary artery perfusates is associated with higher myocardial water content (Fig 1) and impaired diastolic filling (Fig 3). This study also demonstrated that histologic manifestations of edema appear in some situations to be influenced more by chemical composition than by osmolarity of the perfusate. This observation is important mechanistically because it implies that histologic sections might not reliably detect levels of edema associated with impairment of diastolic function. Further, it suggests that the history of myocardial samples—what fluids they are immersed in and what coronary perfusates have been used on the parent organ—are a critically important part of the histologic analysis of edema.

When the present results were compared with those of Carter and colleagues (Fig 9), we found that the histologic characteristics of tissue perfused with the UW solution are atypical of all the other data in this study as well as our previous study. Figure 5 also shows the abnormal histology, as there appear to be heterogeneous blebs of fluid within the tissue. These blebs contribute to the overall impression of edema but could indicate resistance of UW_{315}-perfused tissue to dehydration by standard

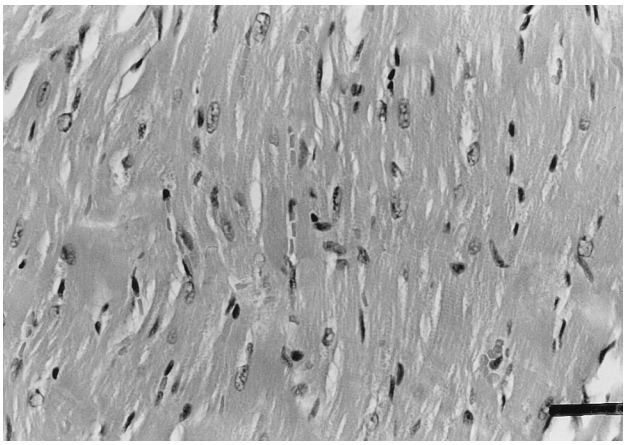

Fig 6. Representative photomicrograph of longitudinal section of left ventricular myocardium fixed with formalin from a blood (310 mOsm/L) perfused heart. Interstitial spaces are less prominent than in Figure 5. Length of mark is 10 μm.

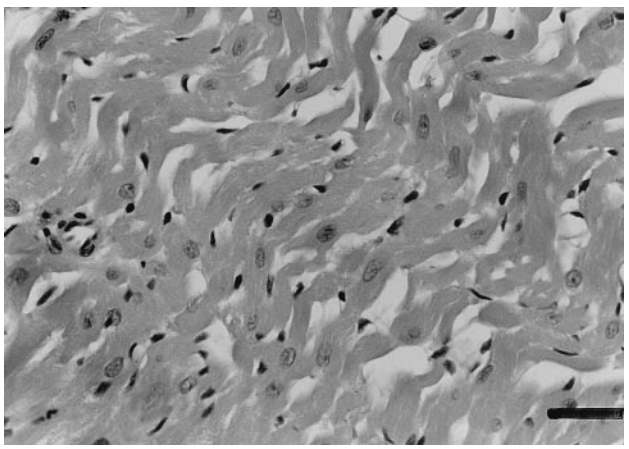

Fig 5. Representative photomicrograph of longitudinal section of left ventricular myocardium fixed with formalin after coronary perfusion with University of Wisconsin solution (315 mOsm/L). Interstitial spaces are readily apparent and there is a heterogeneous pattern of fluid accumulation suggesting loculation. Length of mark is 10 μm.

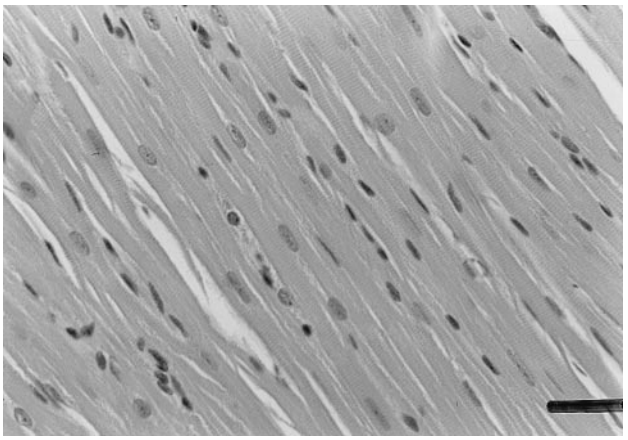

Fig 7. Representative photomicrograph of longitudinal section of left ventricular myocardium fixed with formalin after coronary perfusion with Plegisol (289 mOsm/L). Interstitial spaces are less prominent than Figure 5 and similar to Figure 6. Length of mark is 10 μm.

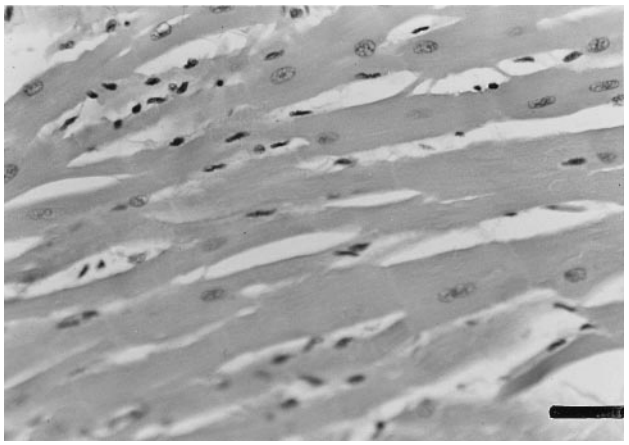

Fig 8. Representative photomicrograph of longitudinal section of left ventricular myocardium fixed with formalin after coronary perfusion with dilute Plegisol (172 mOsm/L). Interstitial spaces are readily apparent. The prominence of these spaces is similar to Figure 5, but fluid appears more uniformly distributed. Length of mark is 10 μm.

alcohol processing. Furthermore, the water content of tissue perfused with UW_{315} in this study might have been lower than that in the other groups (Fig 2). We believe that all the atypical effects of UW_{315} in this study reflect the action of impermeants incorporated into UW_{315} to minimize edema in organs stored for transplantation. Tissue perfusion with fluids containing impermeants might interfere with physiologically meaningful estimation of edema.

Continuous perfusion methods developed in the 1970s and early 1980s for kidney preservation proved unsuccessful for long-term pancreas preservation because of a marked tendency for edema to develop [9]. Wahlberg and associates [10] developed the cold storage University of Wisconsin solution by adding lactobionate to different versions of the kidney perfusion solutions that contained hydroxyethyl starch and raffinose. Those three components prevent cell swelling during cold ischemic storage. Sumimoto and Kamada [11] suggested that lactobionate (a lactose that has been oxidized and has a net negative charge) is the key component of the UW solution. Lactobionate has been shown to suppress hypothermically induced cell swelling in all tissues tested and is a relatively strong chelator of calcium, which might partially explain its efficacy in cold storage. Additionally, lactobionate chelates iron, a reaction that might reduce oxidative injury in cold-storage tissues [12]. Other agents unique to UW_{315} include glutathione and adenosine agents that could stimulate recovery of normal metabolism upon reperfusion by augmenting the antioxidant capacity of the organs (glutathione) or by stimulating high-energy phosphate generation (adenosine) [13].

Methodologic problems with the present study include heterogeneous distribution of edema within the heart. Thus, the percentage of MWC has been found to be lower at the base compared with the apex [Jia XC, Dean DA, Rabkin DG, Cabreriza SE, Weinberg AD, Spotnitz HM, unpublished data, 1996] but is similar in the right ventricle and LV when perfusion conditions and extent of injury are homogeneous [6]. As sample size decreases, technical issues become increasingly important, including effects of evaporation [14]. Inconsistency in sample blotting or artifactual dehydration might explain difficulties in reproducing absolute levels of water content in studies that should have comparable results.

Normalization of percent MWC can compensate for variation in heart size, based on reported correlations of ventricular weight and filling volume [15]. Stress-strain analysis, an alternate approach, is hampered by the ambiguity of unstressed volume and wall stress in the presence of edema [1]. Volume can be normalized to dry weight of the dehydrated heart but was impractical in the present study because specimens were bisected. Dry weight is increased by rejection in rats [1]. Normalization to body weight is favored when animals are genetically identical, healthy, and similar in age; these circumstances tighten the correlation of body weight and heart weight [1].

Quantitation of the predicted effect of percent MWC on diastolic filling volume might improve definition of the mechanism of alterations in diastolic properties. Discriminating between myocardial edema and other sources of diastolic dysfunction, including impaired calcium sequestration [16], could improve understanding of the mechanism of impaired diastolic filling during allograft rejection [17].

The present findings support the view expressed in previous studies that hypotonic crystalloid perfusion of rat hearts can cause myocardial edema and impair diastolic filling. Accordingly, the use of a crystalloid-

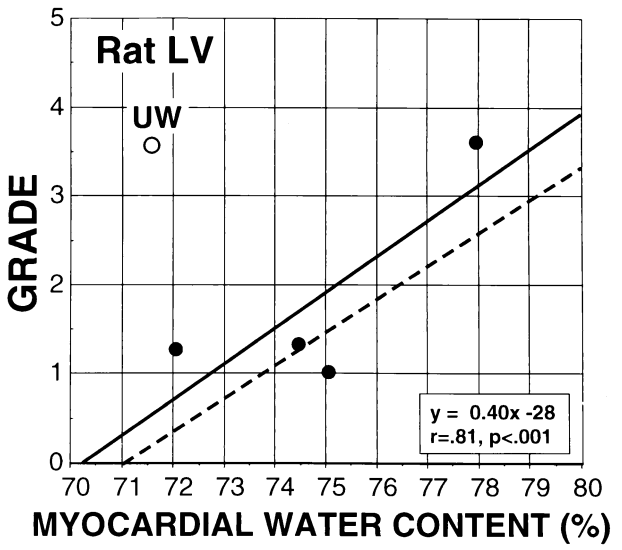

Fig 9. Linear regression relating myocardial water content and edema grade for the present study (solid line *and* solid circles*). The data for University of Wisconsin solution* (UW*), excluded from the analysis, are indicated by the* open circle. *Data from a previous study by Carter and colleagues [4] are also shown (*dashed line*). The pattern suggests an atypical effect of impermeants in University of Wisconsin Solution on histology.*

perfused Langendorff apparatus to develop cardioplegia solutions could fail to predict accurately contributions of such solutions to causing or preventing edema. The present results also suggest that, although histologic estimates of edema in myocardial biopsies of transplanted hearts can provide some insight into the extent of edema and related diastolic dysfunction, tissue processing can cause important artifacts in this process. Better understanding of present tissue-processing methods or alternate methods, including frozen sections which do not involve aqueous dehydration, could improve the utility of histology for assessing edema. Finally, the ability of University of Wisconsin solution to minimize edema in organs intended for transplantation may be a liability is assessing edema with standard methods of tissue processing.

We conclude that, although osmolarity of coronary perfusates is a predictable determinant of MWC and LV filling volume, chemical composition of perfusates is an important independent determinant of the histologic appearance of edema. Thus, pathologic grading of edema can be determined by factors other than those most relevant to the physiologic effects of edema.

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References

1. Amirhamzeh MMR, Jia CX, Starr JP, Hsu DT, Spotnitz HM. Diastolic function in the heterotopic rat heart transplant model. J Thorac Cardiovasc Surg 1994;108:928–37.
2. Spotnitz HM, Hsu DT. Myocardial edema—importance in the study of left ventricular function. Adv Cardiac Surg 1994; 5:1–25.
3. Dean DA, Amirhamzeh MMR, Jia CX, et al. Reversal of iatrogenic myocardial edema and related abnormalities of diastolic properties in the pig left ventricle. J Thorac Cardiovasc Surg 1998;115:1209–14.
4. Carter YM, Jia CX, Soto PF, et al. Diastolic properties, myocardial water content, and histologic condition of the rat left ventricle: effect of varied osmolarity of a coronary perfusate. J Heart Lung Transplant 1998;17:140–9.
5. Takoudes TG, Amirhamzeh MMR, Hsu DT, Wise BR, Odeh SO, Spotnitz HM. Time course of perfusion-induced myocardial edema resolution in rats. J Surg Res 1994;57:641–6.
6. Weng ZC, Nicolosi AC, Detwiler PW, et al. Effects of crystalloid, blood, and UW perfusates on weight, water content, and LV compliance in an edema-prone isolated porcine heart model. J Thorac Cardiovasc Surg 1992;103:504–13.
7. Hsu DT, Weng ZC, Nicolosi AC, Detwiler PW, Sciacca R, Spotnitz HM. Quantitative effects of myocardial edema on the LV pressure-volume relation. J Thorac Cardiovasc Surg 1993;106:651–7.
8. Amirhamzeh MMR, Hsu DT, Cabreriza SE, Jia CX, Spotnitz HM. Myocardial edema: comparison of effects on filling volume and stiffness of the left ventricle in pigs and rats. Ann Thorac Surg 1997;63:1293–7.
9. Collins GM, Bravo-Shugarman MB, Terasaki PI. Kidney preservation for transplantation: initial perfusion and 30 hour ice storage. Lancet 1969;2:1219–22.
10. Wahlberg JA, Southard JH, Belzer FO. Development of cold storage solution for pancreas preservation. Cryobiology 1986;23:477–82.
11. Sumimoto R, Kamada N. Lactobionate is the most important component in the UW solution for liver preservation. Transplant Proc 1990;22:2198–9.
12. Isaacson Y, Salem O, Shepherd RE, et al. Lactobionic acid as an iron chelator: a rationale for its effectiveness as an organ preservant. Life Sci 1989;45:2373–80.
13. Southard JH, Belzer FO. Organ preservation. Annu Rev Med 1995;46:235–47.
14. Haasler GB, Rodigas PC, Collins RH, et al. Two-dimensional echocardiography in dogs: variation of LV mass, geometry, volume, and ejection fraction on cardiopulmonary bypass. J Thorac Cardiovasc Surg 1985;90:430–40.
15. Spotnitz HM, Sonnenblick EH, Spiro D. Relation of ultrastructure to function in the intact heart: sarcomere structure relative to pressure volume curves of intact left ventricles of the dog and cat. Circ Res 1966;18:49–66.
16. Limas CJ, Olivari MT, Benditt DG, Almquist A. Altered calcium uptake by the sarcoplasmic reticulum following cardiac transplantation in humans. Can J Cardiol 1987;5: 215–9.
17. Soto P, Jia CX, Carter YM, Starr JP, Amirhamzeh MA, Spotnitz HM. Diastolic properties during rejection of the chronically transplanted heart. Surg Forum 1995;46:452–5.