it before publication.1 We have recently become aware of a published thesis by Margot M. Bartelings6 at the University of Leiden that discusses cardiac development and uses the new terminology of Pexieder et al7 as well as the classic nomenclature.

Although we are in general satisfied with this suggested nomenclature, we believe it would benefit from clarification on two points. For our own study, we used embryos that fall into the category of late "early organogenesis" or early "advanced organogenesis." We think that the demarcation between the two periods is ill defined. This is made even more confusing because the term "heart loop stage" is equated with "early organogenesis" when there is an "absence of any specific internal features." We consider our hearts to be at the "heart loop stage" based on van Mierop's terminology; however, they have a wealth of internal features. It would be helpful if Pexieder et al7 clarified this distinction by avoiding the use of previously existing vocabulary and adopting the use of stages of development for the vertebrates most commonly encountered in studies of heart development to delineate the periods of organogenesis. This would leave no doubt regarding the transition.

Our second suggestion regarding the new nomenclature involves the outflow tract, an area of particular interest to us. We assume that the terms "ventriculoarterial portion" and "arterial portion" refer to the region we designated the conotruncus2; however, they have a wealth of internal features. It would be helpful if Pexieder et al7 clarified this distinction by avoiding the use of previously existing vocabulary and adopting the use of stages of development for the vertebrates most commonly encountered in studies of heart development to delineate the periods of organogenesis. This would leave no doubt regarding the transition.

With the exception of the outflow tract, we intend to adopt the terminology of Pexieder et al7 and urge other cardiac embryologists to do the same.

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Left Ventricular Unloading During Reperfusion

The article by Van Winkle et al4 raised several points that directly contradict the findings of numerous research groups. In their introduction, the authors misstated that "To date...clear evidence that left ventricular decompression during reperfusion without confounding variables..., can induce salvage..., is unavailable." Experimental series reported from our laboratory have shown significant myocardial salvage by left ventricular bypass alone during reperfusion. These experiments were in in vivo canine hearts that were not fibrillating, nor was cardioplegia used, and the myocardial salvage appeared to be solely due to mechanical unloading during the reperfusion period.2,3 After 2 hours of left anterior descending coronary artery occlusion followed by reperfusion, the infarct size was reduced from a 55.4% ratio of area of infarct to area of risk (AI/AR) to a 16.6% AI/AR by pulsatile left heart bypass. We demonstrated similar salvage in a different experimental series in which the heart was unloaded with percutaneous total heart bypass.4 Reduction of infarct size by unloading during ischemia has been reported by Pennock et al5 and Laks et al6 independently, while Allen et al7 also demonstrated increased myocardial function and salvage during reperfusion with the addition of left heart unloading. Thus, we are compelled to closely examine the data and methodology reported by Van Winkle et al.

The complex ex vivo, cross-circulation model used by Van Winkle and associates does not quantitate the "isovolumetric work," a "time-tension index," or the myocardial oxygen consumption (MVO2) in the different groups. To achieve effective "unloading" and subsequent tissue salvage, MVO2 should be reduced by approximately 50%. The authors do not document that myocardial oxygen consumption is significantly reduced in the "unloaded" group, although these data should have been available from the effluent coronary blood. Conversely, the authors' "tension" hearts had a left ventricular end-diastolic pressure of 7 mm Hg, much less than expected after an in vivo myocardial infarction, suggesting that the difference in loading conditions between the two groups may be small.

The TTC methodology is standard for acute infarct determination. However, as acknowledged by the authors, TTC data obtained only 2 hours after an intervention are quite questionable.8 The authors do not explain why their protocol was not extended an additional 2 hours so that the TTC results would be more accurate.

Finally, the power of their experimental model is limited. With SEMs being 10.5% (6/57; n=10 for each group), assuming normal distribution, and setting the power of the test to be 80% to find a significant difference at the 5% significance level, only a magnitude of 41% difference between groups would be expected to be detected in this experiment.9

The conditions that affect myocardial recovery during reperfusion after acute infarction are complex. To reduce the ultimate size of infarction, hearts must be significantly unloaded compared with controls or the assay must be extremely sensitive. Certainly there has been extensive experimental work documenting the

References
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effects of unloading when these conditions are met, as well as a large clinical experience demonstrating myocardial recovery with left heart assist devices. The research reported by Van Winkle et al appears to contradict previous data, and their experimental design and conclusions warrant close scrutiny.

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References

Reply
Although it is true that our study results conflict with those of Laschinger et al,1 Grossi and colleagues (Laschinger et al2 and Alexrod et al3), Alexrod et al4,5 Pennock et al,6 and Laks et al,6 several points must be clarified. Our study is not alone in observing that left ventricular decompression during reperfusion is not cardioprotective: Lazar and colleagues, using a porcine model of cardiopulmonary bypass, concluded that “during heterogenous cardioplegic arrest, left ventricular venting offers no additional myocardial protection...”; Moreover, contrary to the inference of Dr. Grossi, Allen et al did not find that left ventricular venting in the absence of cardioplegia was cardioprotective. In their study, working blood-reperfused hearts sustained a mean infarct size of 44±5% of area at risk (AAR), whereas vented blood-reperfused hearts had a similar infarct size of 48±6% AAR.5

Unfortunately, it is still true that clear evidence of “myocardial salvage...solely due to mechanical unloading during the reperfusion period” is lacking. Studies that have reported positive results have been seriously flawed. Although myocyte necrosis results from a multifactorial process, three variables have been shown to strongly affect ultimate infarct size—duration of occlusion, size of AAR, and amount of collateral flow to the ischemic zone.6 The early positive studies (Laks et al4 and Pennock et al6) only reported infarct size as infarct weight or percent of left ventricle; they did not measure risk zone size. These earlier studies also suffered from another deficit; regional flows were not measured, and thus the potential impact of collateral flow on infarct size was not assessed. The studies by Axelrod et al3,4 were similarly flawed. Although Laschinger et al did measure endocardial-to-epicardial flow ratios, these data were presented as a change from baseline values for only the ischemic zone.1,2 Moreover, although the methods section states that radioactive microspheres were injected at intervals throughout the experiment, endocardial-to-epicardial flow data were presented for only one time point (during ischemia1 or after 1 hour of reperfusion2). The important task of using collateral flow as a covariate in the analysis of infarct size was not performed. Including collateral flow in the analysis of infarct size is particularly important in studies that use dogs because dogs have large and variable collateral circulations.8,11 It is possible that the positive results reported by Laschinger et al and Axelrod et al are the result of an unfortunate sampling error in which dogs in the vented groups had large collateral flows.

Grossi and colleagues state that “to achieve effective ‘unloading’ and subsequent tissue salvage, MV2 should be reduced by approximately 50%.” However, this statement is unsubstantiated, except for their research, which, as discussed above, suffers from serious flaws. We agree that in studying the effect of decompression on infarct size, hearts should be significantly unloaded. We feel that the unloading in our rabbit preparation was quite complete because the hearts were vented via a 16-mm tube directly to the atmosphere. This was not the case, however, in the dog experiments using cardiopulmonary bypass. As can be seen from Figure 3 in Laschinger et al1, there was pressure being developed by the left ventricle during bypass, although the absolute value cannot be determined because there was no scale on the record. Similarly, Pennock et al6 reported that left ventricular systolic pressure during bypass was 23±4 mm Hg.

In the isolated heart, diastolic pressure does not increase when a region becomes akinetic because it is not pumping blood. We purposely limited left ventricular end-diastolic pressure to 15 mm Hg or less to avoid overdistension of the ventricle and to isolate the potential effects of systolic loading on infarct size. Systolic tension did differ significantly between vented and nonvented hearts (approximately 90 mm Hg and atmospheric, respectively). They imply that our ex vivo model is complex and may contain hidden variables. In fact, the cardiopulmonary bypass procedure is also complex and affects many organs, so protection was not proven to be a direct result of unloading of the ventricle. In our study, that variable was almost completely isolated.

In regard to the TTC staining method, the reference cited was for nonreperfused tissue in which enzyme washout is slow. Horn- effer et al12 validated the 2-hour reperfusion time. They reported that with 30- or 90-minute coronary occlusions, infarct size measured at 2 hours’ reperfusion with tetrozolium was not significantly different than infarct sizes assessed at 48 hours’ reperfusion. We also compared 2- to 24-hour reperfused infarcts in the in situ rabbit heart and found no difference, indicating that the ultimate infarct size had been reached after 2 hours of reperfusion (unpublished data).

The power of this experimental model is not as limited as Grossi et al suggest. In our study, the infarct size of group 4 hearts (13±5% AAR) was found to be significantly different than group 1 controls (41±6% AAR, p=0.05). This difference would not be expected to be significant if, as Grossi et al suggest, the minimum detectable difference was 41%. Indeed, we calculated the minimum significant difference for the four groups, setting the power
Left ventricular unloading during reperfusion.
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Circulation. 1990;82:1543-1545
doi: 10.1161/01.CIR.82.4.1543

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