phorylation, resulting in the rephosphorylation of ADP to ATP in accordance with the instantaneous myocardial energy requirements. Finally, although pyruvate decreases the NADH/NAD^+ ratio in the cytosol, it increases this ratio in the mitochondria. This increase in the mitochondrial NADH/NAD^+ ratio would tend to increase the thermodynamic driving force for mitochondrial electron transport and thus stimulate myocardial oxygen uptake.

While the relative importance of the cytosolic and mitochondrial effects of pyruvate remain to be elucidated, the studies of Bünger et al.[13,14] suggest that the overall result appears to be an enhancement of the cytosolic phosphorylation potential. Because the sarcoplasmic reticulum calcium pump establishes the concentration gradient for free calcium across the sarcoplasmic reticulum in accordance with the cytosolic phosphorylation potential, pyruvate may ultimately improve calcium handling by the sarcoplasmic reticulum, leading to improved contractile function. If this is in fact the mechanism of action of pyruvate, then pyruvate exerts a positive inotropic effect secondary to improvements in the myocardial energy state, an effect that distinguishes it from conventional inotropic agents.

In support of these proposed biochemical mechanisms, Bünger et al.[14] recently reported that the improvement in postischemic function and myocardial energy state in the isolated perfused guinea pig heart was associated with a concentration-dependent increase in myocardial oxygen consumption, reflecting increased mitochondrial respiration and oxidative phosphorylation. We feel that these observations, combined with the findings of the present study indicating that intracoronary pyruvate improves regional myocardial function in the *in vivo* stunned heart, warrant further consideration of the clinical use of pyruvate in the treatment of postischemic ventricular dysfunction.

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Discussion

DR. ROBERT B. WALLACE (Washington, D.C.): Thank you, Dr. Polk. Dr. Jones, Fellows, and Guests: The studies of Dr. Flint, Mentzer, and their colleagues exemplifies a continuing search for means of altering the effects of ischemia on the myocardium. The authors have attempted to reduce the effects of ischemia by metabolic substrate enhancement using pyruvate infusion prior to and following a five-minute period of myocardial ischemia.

Braunwald introduced the concept of myocardial stunning, which might be described as reversible postischemic myocardial injury. In addition to the adverse effects of ischemia, Jennings, Buckberg, and others have stressed the significance of reperfusion as an additional insult to the myocardium.

Dr. Dan Miller, one of our surgical residents, working with Dr. Marc Visner in our cardiovascular research laboratory, has studied the effects of myocardial ischemia and how they might be altered by modifying reperfusion.

The model used by Dr. Miller is similar to that used by Dr. Mentzer and his colleagues. Regional function was assessed by determining regional ejectional shortening before and after a 15-minute period of occlusion of the left anterior descending artery. Following unmodified reperfusion, functional recovery was 60% of baseline at six hours. Animals in whom the first 15 minutes of reperfusion was with normothermic cardioplegic

solution into the left anterior descending coronary artery showed recovery of function to baseline within four hours.

(Slide) The pooled data of the individual experiments is shown on this slide. The open circles represent animals treated with unmodified blood reperfusion and the closed circles represent the animals treated with cardioplegic reperfusion and clearly shows the return to preischemic function within four hours in animals receiving the modified cardioplegic reperfusion, whereas animals receiving unmodified reperfusion showed only a 60% rate of recovery of function at the end of six hours of reperfusion. This suggests to us that initial reperfusion with cardioplegic solution offers some protection against ischemically induced myocardial stunning.

We have incorporated these findings into our practice and believe that it has enhanced our results, especially in patients who have experienced severe unprotected periods of ischemia.

I would like to ask the authors two questions. One, their study showed an enhancement of ventricular function by pyruvate infusion prior to ischemia; have you found any effect on animals treated after the ischemic period? Second, your period of ischemia was only five minutes; have you studied animals with longer periods of ischemia?

DR. JOHN HAMMON (Nashville, Tennessee): Dr. Polk, Dr. Jones; Dr. Mentzer was kind enough to allow me to review the manuscript prior to presentation, and I have a few comments to make. They have very nicely shown that pyruvate improves ventricular function in the myocardium both prior to and after a brief period of ischemia.

Basically, impaired ventricular function relates to the failure of oxygen delivery, as in an occluded coronary artery or the inability of the myocardium to efficiently use delivered oxygen, such as in the stunned myocardium following intervention.

My question to the authors relates to an issue that they raised in their own manuscript, and that is inotropic agents such as Dopamine and Isoprel have been shown to improve the function of a stunned segment in experimental preparation.

Does pyruvate then have an efficiency advantage over conventional inotropes in providing increased ventricular function but at a lower oxygen cost?

DR. FREDERICK L. GROVER (San Antonio, Texas): I, too, enjoyed this interesting paper by Drs. Flint and Mentzer. They have taken an important clinical problem into the laboratory in an effort to improve myocardial preservation. This difficult-to-manage group of patients with acute ischemic heart disease is seen with increasing frequency in this time of acute intervention.

I have several questions for the authors.

Do you believe that snaring the LAD for a period of five minutes in an animal with significant collateral circulation is an adequate model of myocardial stunning?

How did you select the dosage of pyruvate?

What were the reasons for using the systolic wall thickness as opposed to segmental wall shortening?

And if there is an inotropic effect of pyruvate, have you investigated whether it elevates myocardial oxygen demands less than do the traditional inotropic agents? Does it increase global contractility, *i.e.*, what are its advantages?

And finally, have you also considered investigating the effects of pyruvate on the globally ischemic heart and its efficacy in cardioplegia solutions, both before global arrest and during reperfusion?

DR. ROBERT ZEPPA (Miami, Florida): I would like to congratulate Dr. Flint and his colleagues for an elegant presentation.

Not being a card-carrying cardiac surgeon, my questions are biochemical in nature.

It wasn't clear to me in the presentation what was used to control the nontreated animals in terms of the tonicity of the infusions because you infused 155 millimolar pyruvate.

Do you think that something that was isosmotic with that material also might alter myocardial contractility? I don't know, but I didn't get it from the presentation.

Second, if you were really interested in energetics and in building ATP, pyruvate is the wrong way around because it doesn't give you that much coming down the Embden-Meyerhof-Coricycle.

Have you tried succinate to see whether providing a substrate for mitochondrial function improved myocardial contractility?

DR. ROBERT M. MENTZER, JR. (Closing discussion): President Polk, Secretary Jones: We want to thank the discussants for their questions and their comments. Dr. Wallace reviewed their work at Georgetown University that addresses the problem of postischemic reperfusion injury. In so doing, he has raised the question as to whether ventricular function might not be better assessed using pressure dimension loops rather than changes in wall thickening. The use of pressure volume relationships to assess contractility is particularly helpful when ventricular performance is affected by changes in preload and afterload, as is often the case following global ischemia. We used a preparation in which a limited region of "stunning" was induced to minimize the effects of preload and afterload and to facilitate comparison with nonischemic regions in the same animal. In this preparation, changes in wall thickening were extremely sensitive to changes in regional blood flow and served as an excellent index of regional contractility. The use of this form of contractility assessment has been verified by other investigators. Our preparation did not lend itself, however, to determining whether the stunning was produced by the ischemic event itself or whether it ocurred during reperfusion. Nevertheless, we are confident in our observation that pyruvate had a beneficial effect with respect to amelioration of the stunned state. Dr. Wallace also indicated that his laboratory is studying the feasibility of administering cardioplegic solutions during reperfusion to minimize reperfusion injury. This may turn out to be an important contribution. We would only suggest that the administration of a single agent such as pyruvate during reperfusion might be easier and safer than using a cardioplegic solution.

Dr. Hammon asked what are the inotropic advantages of using pyruvate over other conventional agents. Pyruvate appears to be different in that it can decrease the cytosolic NADH/NAD^+ ratio while it increases this ratio in the mitochondria. The net effect is such that this agent probably exerts its effect by improving calcium handling by the sarcoplasmic reticulum and increasing the driving force for the electron transport system. If improved contractile function occurs as a result of enhanced calcium transport and improved myocardial energy state, this would distinguish pyruvate from other inotropic agents that are known to accelerate high-energy phosphate consumption.

Dr. Grover asked a number of questions, including why we selected five minutes for the ischemia period, why we chose the dose of pyruvate that was administered, and whether pyruvate should be added to cardioplegic solutions.

We selected the five-minute period initially to induce localized regional stunning without inducing irreversible damage. After the initial observation that pyruvate improved function in the stunned region, the experiments were extended to ten minutes of LAD ischemia and 90 minutes of reperfusion. This ten-minute period induced reproducible regional stunning that was stable over the course of three hours. The dose of 1 ml per minute of 150 mM sodium pyruvate was selected on the basis of dose-response curves that were performed in animals undergoing the five-minute ischemia protocol. At this dose, a reproducible effect was observed without any adverse effects on systemic hemodynamics. Finally, although the addition of pyruvate to cardiplesia solutions may be beneficial by favorably altering the pyruvate/lactate ratio, we did not study its use in the setting of hypothermia and global ischemic arrest. Thus, whether pyruvate would be as effective when added to a cardioplegic solution as it is as inotropic agent remains to be elucidated.

Dr. Zeppa, both the vehicle and sodium pyruvate solutions were isotonic and buffered at pH 7.4. Although we have not studied the effects of other glycoloytic or Krebs-cycle intermediates on contractility, it is reasonable to assume that many of these substrates could have a beneficial effect on cellular function if acetyl-CoA production is increased. We are currently examining the release of some of these substrates into the interstitium by collecting the interstitial fluid using a microdialysis probe designed by Dr. Van Wylen. With this probe and the crystals we can simiultaneously measure coronary blood flow, the release of metabolites, and changes in wall thickening. In this manner we hope to gain new insights that will enable us to better manipulate the energetics of the cell. Ultimately it is this kind of information that will allow us to maximize myocardial protection and salvage ischemic tissues.