Reply to the Editor:

I would like to respond to the issues addressed to me by Dr. Chanda concerning extent of glutaraldehyde fixation and calcification of bioprosthetic tissue. Our hydrothermal isometric temperature or denaturation temperature data indicate that dynamic fixation stabilized porcine aortic heart valve tissue within 3 hours 15 minutes and 24 hours when the tissue was fixed at room temperature in 0.5% and 0.05% glutaraldehyde, respectively. Previous authors have suggested that at concentrations from 0.625% down to 0.025%, glutaraldehyde cross-linking is rapid and the degree of cross-linking reaches a plateau within 24 hours, consistent with our study.¹⁻⁴ In contrast, Chachques and associates⁵ suggested that adequate fixation of pericardium could be achieved with a brief 10-minute immersion in standard glutaraldehyde solution (0.625%). Complete saturation remains elusive. Schoen, Tsao, and Levy² have shown that only 70% of the reactive sites of collagen react. This is due to limitations in the accessibility of the reactive sites and also resistance to diffusion of the glutaraldehyde reagent, a problem likely caused by glutaraldehyde polymer film formation (e.g., steric hindrance factors).^{6,7} We believe some of these limitations may be overcome by means of our dynamic fixation technique. The gradual depletion of glutaraldehyde in the preservative solution during long periods (exceeding 4 to 8 weeks) is less likely caused by completion of saturation than by rapid incorporation of glutaraldehyde monomers recruited during polymer formation. Polymer growth (i.e., alpha- or beta-unsaturated aldehyde polymers) can be catalyzed by amino acids (e.g., lysine, hydroxylysine), the rate of growth increasing rapidly with time and exponentially with temperature and pH.^{3,8} Thus the observation, by Chanda and associates, that shorter periods of time are required to achieve complete saturation at higher temperatures (i.e., more than 8 weeks at 4° or 10° C and less than 4 weeks at 37° C) is consistent with the hypothesis of glutaraldehyde consumption by polymer formation. The fact that glutaraldehyde incorporation finally reaches a limit in their study (i.e., after more than 8 weeks at 0.25%) may be associated with simple limitations in the extent of glutaraldehyde polymer growth.

With respect to glutaraldehyde-mediated calcification, although the mechanism is yet unclear, ample evidence indicates a direct relationship between the extent of tissue calcification and the amounts of glutaraldehyde used or incorporated during tissue fixation,^{1,2,9,10} along with the duration of tissue exposure to the glutaraldehyde solution.¹¹

Thus the use of a technique (e.g., dynamic fixation) that can both reduce glutaraldehyde levels and concomitantly achieve similar or more effective cross-linking can only be beneficial in potentially limiting the adverse side effects of glutaraldehyde used, such as calcification or cytotoxicity (not discussed here). We recognize, however, that further calcification studies are required to fully confirm this hypothesis.

We do not exclude the possible advantage of combining such a technique (i.e., dynamic fixation) with anticalcifi-

cation treatments to optimally reduce the risks of long-term calcification of bioprosthetic tissue.

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12/8/83228

Use of aprotinin after cardiac operations

To the Editor:

The article by Çiçek and colleagues¹ on the postoperative use of aprotinin (Trasylol) to reduce blood loss after

cardiac operations, a novel concept first introduced by Angelini and colleagues² and carefully evaluated by Kallis and associates,³ is of unusual interest.

The authors' comments in the discussion, "the satisfactory reduction in postoperative blood loss in the aprotinin group prompted us to analyze the results of blood loss in a smaller subgroup of patients undergoing coronary artery bypass who continued to take aspirin until 1 week before the operation," are disconcerting and require an explanation.

Çiçek and colleagues⁴ have already demonstrated such a benefit in the recent past in a similar group of patients. Is there an overlap between the patients in these two studies?

In the latter publication,⁴ the final sentence states that "higher risk patients need to be investigated." In this article,⁴ patients receiving aspirin within 7 days were excluded. It is this very group of 14 patients in the aprotinin group which received aspirin that is the crux of this more recent article,¹ but we are not informed as to whether they were truly prospectively randomized on the basis of this preoperative characteristic. If not, then although the reported differences in blood loss are striking, statistically speaking, the possibility of type II error rises to beyond 20%.^{5,6} Hence the strict scientific merit is lessened.

A new prospective randomized trial addressing patients expected to be at significant risk for bleeding after cardiac operations is required to answer whether postoperative aprotinin is effective in reducing blood product use after cardiac surgery.

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12/8/83068

Reply to the Editor:

We thank Dr. Alvarez for his interest in our articles. However, We are surprised by his implications regarding our patient population. There is no overlap between the

patients in these two studies. In regard to the randomization process, We would like to stress that patients in the more recent article are truly prospectively randomized with strict statistical criteria.

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Hypothermia during preconditioned ischemia-reperfusion attenuates the myocardial protection of preconditioning

To the Editor:

Perrault and colleagues¹ are to be congratulated on their elegant and sophisticated study in exploring ischemic preconditioning in cardiac surgery. However, the authors conclude that "preconditioning does not enhance cardioplegic protection and might even be deleterious." This potentially important conclusion was thrown into doubt by a number of significant limitations by Dr. Steven F. Bolling, in his discussion of the article: (1) Is the model actually a model of ischemic preconditioning? (2) Did preconditioning occur? (3) Why were right atrial biopsy specimens used? (4) Why were a few unimportant indices used, instead of the sensitive indices such as protein kinase C isoenzymes, in explaining the occurrence of preconditioning?

We are also part of a group that studies preconditioning both in animal experiments and in clinical trials in cardiac surgery.^{2,3} Many factors can interfere with the induction of preconditioning. Recently, we conducted research on the effects of hypothermia during preconditioned ischemia-reperfusion on the myocardial protection of preconditioning in isolated perfused rat hearts (Table I). Male Sprague-Dawley rat hearts were used in the model of modified Langendorff perfusion. Preconditioning was elicited by a single 5-minute period of ischemia and a 10-minute period of reperfusion. After preconditioning, all hearts were arrested by infusion of 4° C St. Thomas' Hospital cardioplegic solution, 5 ml per dose, every 30 minutes for 3 hours and then reperfused by 37° C Krebs-Henseleit buffer solution for 45 minutes. The results indicated that hypothermic preconditioning attenuates the myocardial protection of preconditioning, as shown in Table I. We therefore doubt that the hearts in the experimental group in Perrault's study were really preconditioned. For the purpose of exploring the effectiveness of preconditioning protocol in cardiac operations, ST-segment depression was used as a predictor of preconditioned ischemia in our studies (unpublished data). In addition, many details throughout the operation can disturb the results of preconditioning from an opposite view in cardiac surgery. These factors mainly include unstable anesthesia, cardiopulmonary bypass, body temperature,