To the Editor:

The article by Steigbigel et al. is the latest of a series of investigations from different laboratories describing functional alterations in vitro in granulocytes obtained by FL. Both modest and rather pronounced decrements in function have been noted, perhaps related in part to variations in the vigorousness of the elution procedure, the eluting solution used, donor premedication, and other collection factors. Significant morphologic abnormalities have also been described in a proportion of granulocytes collected by FL with some evidence indicating that different populations of cells may be present, perhaps related to the duration of time the cells have adhered to the fibers.

Further evidence of the heterogeneity of FL granulocytes is provided by the experiments described below. As part of a clinical “followup” to observations in vitro that incubation in plasma could improve the morphology of FL granulocytes, granulocytes obtained by 3-hr FL donations and concentrated by low speed centrifugation at 280 g for 10 min were suspended in ABO-identical heparinized plasma at 22°C (four experiments) or 37°C (four experiments) for 15 min. The granulocytes were then recentrifuged (280 g for 10 min) and resuspended in heparinized plasma prior to transfusion. Overall, the mean loss of granulocytes ([preincubation – postincubation]/preincubation] = 31% (range 16%, 65%). The “lost” cells could not be accounted for in the supernatant, suggesting that they were lysed by this relatively atraumatic handling. Because of the cell loss, we have abandoned this technique as a means of improving the quality of granulocytes obtained by FL, although the rate of transfusion reactions was somewhat decreased in the recipients of these preparations.

Prior to testing in vitro granulocytes obtained by FL, it is necessary to wash the granulocytes, resuspend them in physiologic media, and often perform RBC lysis procedures for purification purposes. Neither Steigbigel et al. nor any of the other investigators (including us in our previous report commented on granulocyte loss during these maneuvers, which as noted above can be considerable. It is likely that the more morphologically and functionally abnormal cells are lost during this preparation and therefore that the tests in vitro are carried out on a “selected” population of granulocytes, thereby considerably overestimating the functional integrity of the original yield of granulocytes collected. The fact that the material transfused contains damaged cells is in keeping with the much increased incidence of transfusion reactions following administration of FL granulocytes, the presence of “smudged” (presumably more fragile cells) seen on Wright-stained preparations, and the morphologic variability and may also perhaps contribute to the apparent decreased corrected postransfusion count increments noted by most investigators.

Because a variety of studies have shown the clinical efficacy of granulocytes obtained by FL in the treatment of infection in granulocytopenic patients, FL should not be abandoned as a therapeutic modality; studies aimed at improving and standardizing the FL product should be encouraged. All such functional studies in vitro, however, should take into account granulocyte loss during cell preparation and purification prior to extrapolating the results in vitro to the product actually being transfused.

REFERENCES
3. Klock IC, Bainton DF: Degranulation


Filtration Leukapheresis (FL) and Granulocytes: Reply

To the Editor:

Dr. Schiffer and Dr. Aisner raise an important point; certainly potential loss of cells during the preparation procedure should be looked for in future studies. It does not seem appropriate, however, to assume that the “loss” of cells noted by Schiffer and Aisner during their suspension of the cells in plasma and 280 g centrifugation would occur during the preparation of cells used for our studies in vitro. During the latter preparation, the maximum force exerted is 190 g. It would be of interest to know if some of the “loss” noted by Schiffer and Aisner might be accounted for by clumping of the cells during the two 280 g centrifugations or by adherence of the cells to the vessel. If their cells are counted with a Coulter counter, clumped cells would obviously yield falsely low cell numbers. Our counts in a hemocytometer showed little evidence of clumping. In addition, in excess of 98%, of our cells were viable by trypan blue dye exclusion and had relatively normal bactericidal activity. If the preparation caused lysis of a significant proportion of cells, it is reasonable to expect that another proportion would be injured without lysis; this type of population has not been identified in our studies.

Obviously, the question of cell loss can be properly answered only by including the examination for such loss as suggested by Schiffer and Aisner.

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Filtration leukapheresis (FL) and granulocytes [letter]

CA Schiffer and J Aisner