LETTER TO THE EDITOR

## *Response to Pilwat and Zimmermann*

Dear Sir:

We would first like to thank Drs. Pilwat and Zimmermann for pointing out that it was a technical problem in one of our resistive particle sizing experiments that led to the experimental observation of "disappearing ghosts" (1). We also have found that a problem existed with this particular experiment. It is desirable that this information be put into the literature to correct the record—the true disappearance of the disappearing ghost phenomenon, so to speak. The letter by Pilwat and Zimmermann can accomplish this purpose and thereby eliminate our obligation to do so in some other fashion. (As explained in paragraph 3, below, the nature of the problem, and the correct explanation for the observation of the "disappearing ghosts" are somewhat different from those given by Pilwat and Zimmermann, but the net effect is the same.)

We are pleased to read at the end of their letter Pilwat's and Zimmermann's comment on ". . . the great value of [other aspects of our] work." In fact, the issue of "disappearing ghosts" was peripheral to the main thrust of our paper (to the extent that the subject did not figure in our Abstract). The main thrust was, and remains, our presentation of . . . " the first *explicit* (emphasis added) calculations of particle specific resistivity from post–dielectric-breakdown apparent size, using traditional electronic sizing techniques." We are naturally happy to see Pilwat and Zimmermann following this lead in presenting their own explicit calculation of this character, and we agree with them that this biophysical parameter is likely to prove of considerable practical interest in the future.

Some additional comments are still called for, first, in explanation of "disappearing ghosts" and secondly, on other aspects of Pilwat's and Zimmermann's remarks. On the first point, the problem with the experiment of Fig. 5 (1) was not, as stated by Pilwat and Zimmermann, that we " . . . simply overlooked one of the two peaks at high electric field strengths in (their) measurements." The technical problem was, rather, that the lower threshold was inadvertently set too high for the particular current and gain used for the final times of the kinetic phenomena under investigation. Thus, near the end of the process that included the ghost peak's becoming electrically smaller, a portion of the high-field ghost counts passed below threshold and were uncounted (in a quite reproducible manner).

Returning to the matter of high-field vs. low-field counting and sizing of mixed intact cell-ghost populations, we refer to Fig. 3 from an earlier publication (2). The 300 s curves of Figs. 3 *a* and 3 *b* appear quite similar to those of Pilwat and Zimmermann's Figs. 1 *a* and 1 *b* in their Letter to the Editor. Reference 2 was also listed as reference 2 of our 1983 *Biophysical Journal* paper (1); we believe it indicates the somewhat different explanation of "disappearing ghosts" we present above.

Another point already alluded to in regard to the Pilwat and Zimmermann letter merits closer consideration: their use of electrically produced ghosts, and benzyl alcohol-treated ghosts, to investigate the properties of osmotically produced ghosts in the absence of benzyl alcohol. The presence of two ghost peaks for their electrically produced ghost cells measured in the presence of benzyl alcohol shows that such ghosts are not the same as our osmotically produced ghosts. There are not two ghost peaks for osmotically produced ghosts: only single, smooth, and reproducible ghost peaks (plus separate intact-cell peaks) are obtained under our usual experimental sizing conditions, even for field strengths up to 6 or 7 kV/cm ([2], Fig. 3 *b*). (A "higher field strength" reported by Pilwat and Zimmermann in their letter is 3.7 kV/cm.) The Pilwat and Zimmermann double–ghost-peak phenomenon (produced under their different conditions) is perhaps something of interest in its own right, however.

An additional comment should be made, correcting the Pilwat and Zimmermann statement (p. 4) that ". . . The two populations, erythrocytes and ghost cells, have the same mean volume (130 $\mu$m), so they cannot be distinguished when the size distribution is determined by electronic sizing at low field strength (or currents." Though this is true for long-term mixtures of ghosts and intact cells, it is not true for early-time mixtures (i.e. 45–60 s post exposure to hemolytic medium) if normal (rather than slow) flow is employed. This is clearly shown in reference 2, and has been discussed in previous publications (3), and referenced in (1). (By appropriate manipulation of medium conditions, quite accurate steady state osmotic fragility percentages can be determined in this way, as well.)

A final comment is in order on the explanation offered by Pilwat and Zimmermann for the (real or apparent) lower value of the internal resistivity of resealed ghosts, as compared with that of the external medium, especially in the light of the results of MacGregor and Tobias, 1972 ([4]; also referenced in [1]). We agree with the observation itself. However, it is difficult to understand how an incomplete exchange of intracellular potassium could occur without an even more incomplete loss of hemoglobin (and the presence of hemoglobin generally has a larger, opposite effect on cytoplasmic resistivity).

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### REFERENCES

1. Akeson, S. P., and H. C. Mel. 1983. Erythrocyte and ghost cytoplasmic resistivity and voltage-dependent apparent size. *Biophys. J.* 44:397–403.
2. Akeson, S. P., and H. C. Mel. 1982. Osmotic hemolysis and fragility: a new model based on membrane disruption, and a potential clinical test. *Biochim. Biophys. Acta.* 718:201–211.
3. Yee, J. P., and H. C. Mel. 1978. Cell-membrane and rheological mechanisms: dynamic osmotic hemolysis of human erythrocytes and repair of ghosts, as studied by resistive pulse spectroscopy. *Biorheology.* 15:321–339.
4. MacGregor, R. D., and C. A. Tobias. 1972. Molecular seiving of red cell membranes during gradual osmotic hemolysis. *J. Membr. Biol.* 10:345–356.

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