

Reply to the Remarks of C. S. Johnson, Jr.

Dear Sir:

The objections raised by Johnson to the article of LaGattuta et al. (1) begin with his first-paragraph assertion that their work is "greatly at variance with previous findings." It seems, however, that there are no previous published findings that deal with the question of the dependence of the hemoglobin (Hb) mutual diffusion coefficient (D) upon hemoglobin concentration (CHb), when either $\text{pH} > \text{pI}$ or $\text{pH} < \text{pI}$. As for the region $\text{pH} \approx \text{pI}$, the data reported in reference 1, for oxy-HbA at $\text{pH} = 6.9 \approx \text{pI}$, compares reasonably well, we think, with that referred to by Johnson, i.e., to his published works (2, 3).

Indeed, as Johnson points out, for $\text{pH} \approx \text{pI}$ our D vs. CHb data (Fig. 2 of reference 1) suggest a linear fit almost as readily as a nonlinear. It was only after considering the D vs. CHb data taken at pH values away from the pI (Figs. 1 and 3 of reference 1) that we were led to propose the more general nonlinear form. Based upon this data, the need for such a proposal seems compelling. The nonlinear form and pronounced pH dependence have been reported for the bovine-serum-albumin system (4).

After all, the issues raised by Johnson revolve about the question of accuracy. At the very end of his Letter, Johnson suggests that a variation of 3% or less is necessary "for such studies to have validity." We disagree, since trends in the shapes of the D vs. CHb curves are evident although data precisions are only $\sim 15\%$. We believe these trends to be significant. If they were the result of membrane fragment inclusions or dust contamination, then they should not have been reproducible. We emphasize that the data reported in reference 1 were compiled in two entirely separate experimental trials, for each of the pH values considered. By this we mean that all experimental procedures were performed twice, beginning with the drawing of blood.

As for the additional comments of Johnson concerning, (a) sample heating and (b) heterodyne mixing of scattered light from the walls of the sample cell, we can say the following. We have calculated from the equation for heat conduction that the temperature rise at the midpoint of the 0.3-mm Diam sample cell due to absorption of the 5-mW He-Ne laser radiation within the oxy-HbA sample was $< 0.5^\circ\text{C}$. An estimate of the effect of convective motions upon the homodyne measurement showed this to be negligible. Effects of heterodyning were searched for by placing submicron-sized latex spheres in our sample cell and determining values of D and the variance in the usual way. Results obtained suggested that heterodyning was not a factor, since the D value was as expected from the Stokes-Einstein formula and the variance was very small. For this step we took the obvious precaution of using an extremely dilute solution of spheres.

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REFERENCES

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