which in parallel recruits external conformers (Bamberg and Passow, 1992). Nevertheless, it must be expected that DIDS or other inhibitors induce specific deformations of the protein. Consequently, the induced changes of the protein's volume in the two monolayers may vary for different inhibitors and from those induced by pH, leading to different stages of echinocytosis.

Of course, the CCBC model does not exclude other mechanisms, like changing protein interactions in the cytoskeleton or the redistribution of membrane compounds, from influencing the erythrocyte shape. However, the redistribution of the major species of lipids after a pH jump, e.g., from 7.4 to 5.8, is rather slow. Moreover, the transbilayer steady-state distribution of these lipids most probably does not correlate with the equilibrium erythrocyte shape according to the bilayer couple model (Libera et al., 1997). Furthermore, one must ask whether equilibrium investigations may disclose a general mechanism that can explain quasiequilibrium cell shapes, as well as very quick changes. Surprisingly, the CCBC model may play a role in the mechanisms behind both observations: very rapid shape changes due to synchronized recruitment of the binding sites and a subsequent conformational change, and the erythrocyte's equilibrium shape due to the average conformer distribution, such as that at alkaline pH.

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Does the Transmembrane Potential ($\Delta\psi$) or the Intracellular pH (pH_i) Control the Shape of Human Erythrocytes?

In a number of papers we indicated a correlation between transmembrane potential ($\Delta\psi$) and the shape of human erythrocytes. Recently Gedde et al. (1997a,b) discussed our findings, on the basis of their new experiments, stressing, however, that not $\Delta\psi$ but intracellular pH (pH_i) is the factor, which is responsible for echinocyte and stomatocyte shape transformations. Already in 1984, Bifano et al. had argued in a similar way, claiming that amphotericin, which we used in our first experiments (Glaser, 1979, 1982), was the real reason for the shape changes found. However, our reexaminations of this matter showed that our results were reproducible under various other conditions, even without antibiotics. On the other hand, we pointed out that the absence of echinocytic transformations in Bifano's work was caused by their use of albumin as a stomatogenic agent (Glaser et al., 1988, 1989, 1991).

Although many of our publications are mentioned in the papers of Gedde et al., some of our findings contradicting their conclusion, unfortunately, were not noticed. A crucial experiment, which was reproduced many times, is the following (Glaser et al., 1980; Glaser, 1993). In isotonic solutions of 30 mM NaCl + sucrose, at pH_e 5.1, washed erythrocytes are stomatocytes in control experiments ($\Delta\psi$ = +46 mV, pH_i 5.9) and remain stomatocytes, even if they are shifted into a full Donnan equilibrium ($\Delta\psi$ = +46 mV, pH_i 5.9, volume of the cells in relation to control = 62%). At pH_e 7.4, control cells remain stomatocytes ($\Delta\psi$ = +29 mV, pH_i 7.9), but in contrast to the response at pH 5.1, they fully transform into echinocytes if they are shifted into Donnan equilibrium ($\Delta\psi$ = −19.5 mV, pH_i 7.1, volume of the cells in relation to control = 67%). These data clearly demon-

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strate that neither shrinkage nor pH_i, but only the transmembrane potential correlates with shape.

Gedde et al. (1997b) point out, as Bifano et al. (1984) already did, that "ionophores have independent shape effect." This is true for large concentrations and was published by us for high concentrations of valinomycin (Glaser et al., 1991). But, as already mentioned above, the same shape effects were found without ionophores, when the cation exchange leading to the Donnan equilibrium was induced by electric breakdown of the membrane. Similar shape transformations under electric breakdown conditions were also observed by Chang and Reese (1990). Furthermore, we indicated that valinomycin in lower concentrations only induces stomatocytes if the cells have been preloaded with sodium, and a positive diffusion potential is induced in potassium-rich solutions (Glaser et al., 1991).

Another argument against the "pH_i hypothesis" are our experiments on ghosts: Why do ghosts not show a pH dependence of their shapes? Why do only resealed but not open ghosts exhibit a shape dependence on external Cl^- concentration that correlates to $\Delta\psi$ (Herrmann et al., 1985; Müller et al., 1986)?

The crucial argument of Gedde et al. (1997b) against our "$\Delta\psi$ hypothesis" are the results of experiments in unbuffered solutions of different external conditions, indicating nearly no shape effect in the range between -45 mV and $+45$ mV. However, changing $\Delta\psi$ while "holding other parameters constant" is impossible. Although Gedde et al. could stabilize pH_i, and to some degree the volume of the cells, they strongly modified the external Cl^- concentration and the internal 2,3-diphosphoglycerate content, to generate these different membrane potentials. As we indicated (Glaser and Donath, 1992), however, the critical membrane potential that corresponds to the shape transformation itself depends on the external ionic strength. In solutions of low ionic strength it can be larger than $+30$ mV! The membrane potential is only one factor, influencing the cell shape. Echinocytogenic and stomatocytogenic drugs, for example, may shift the critical $\Delta\psi$ value toward higher or lower values (Glaser et al., 1987).

In conclusion, the answer to the above question is as complex as the process itself. On the one hand, the erythrocyte shape is governed by different interacting processes, and on the other hand, the transmembrane potential controls a number of membrane properties and functions (Glaser, 1996). In general, one must differentiate between true electric field effects on membrane constituents, and gradients of permeable ions like chloride, or the local pH as the result of their Nernst distribution. Gimsa et al. (Gimsa, 1995; Gimsa and Ried, 1995) proposed a mechanism based on the conformation of the band 3 protein in the membrane. The membrane potential can also influence the asymmetrical distribution of drugs, or probably fatty acids or other charged compounds in the membrane. In any case, the transmembrane potential is a physical parameter controlling a number of membrane and membrane-near properties. This makes the correlation between $\Delta\psi$ and erythrocyte shape, found by us, understandable.

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