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pH Equilibration in Human Erythrocyte Suspensions

Anne McElroy Critz and Edward D. Crandall

Department of Medicine, University of California, Los Angeles, California 90024, and Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Summary. A stopped-flow rapid reaction apparatus was used to study the rate of pH equilibration in human red cell suspensions. Flux of OH^- or H^+ was determined over a wide range of extracellular pH (4–11) in CO_2-free erythrocyte suspensions. In these experiments, an erythrocyte suspension at pH 7.3 is rapidly mixed with an equal volume of NaCl solution at $3.0 > pH > 11.5$. The pH of the extracellular fluid of the mixture changes rapidly as OH^- or H^+ moves across the red cell membrane. Flux and velocity constants can be calculated from the initial dpH/dt using the known initial intra- and extracellular pH. It was found that the further the extracellular pH is from 7.3 (in either direction from 4–11), the greater the absolute value of total OH^- and/or H^+ flux. Pretreatment with SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid), a potent anion exchange inhibitor, greatly reduces flux over the entire pH range, while exposure to valinomycin, a potassium ionophore, has no measurable effect. These data suggest that (i) both H^+ and OH^- may be moving across the red cell membrane at all pH; (ii) the species dominating pH equilibration is probably dependent on the extracellular pH, which determines the magnitude of the driving gradient for each ion; and (iii) the rapid exchange pathway of the erythrocyte membrane may be utilized for both H^+ and OH^- transport during CO_2-free pH equilibration.

The erythrocyte membrane is known to exhibit a very high selective permeability to anions. Its permeability to both Cl^- and HCO_3^- is of particular physiological significance due to the function of these anions in CO_2 transport in lung and tissue capillaries. Recent studies based on the observations of saturation kinetics (Gunn et al., 1973; Brahm, 1977), competitive inhibition (Dalmark, 1976), high activation energy (Dalmark & Wieth, 1972; Brahm, 1977), and flux as a function of pH (Gunn, 1972, 1977), have suggested that a specialized transport system facilitates Cl^-/Cl^- self-exchange across the membrane (Gunn et al., 1973; Gunn, 1977). Similar findings have also been reported for HCO_3^-/Cl^- exchange across erythrocyte membranes (Chow, Crandall & Forster, 1976; Crandall, Obaid & Forster, 1978; Lambert & Lowe, 1978). The exchange of these anions is probably obligatory and electrically neutral, since membrane conductance is much lower than the calculated exchange permeabilities would predict (Lassen, 1972). These findings have led to speculation that both exchange and net translocation pathways for anions exist in the red cell membrane and that both pathways may share common sites (Gunn, 1977). These data have been supported by chemical probe analysis which indicates that specific sites in the red cell membrane (Band 3 protein) are associated with anion exchange processes (Rothstein, Cabantchik & Knauf, 1976; Cabantchik, Knauf & Rothstein, 1978). Gunn (1972, 1977) has proposed a model for anion exchanges that explains the experimental data for both divalent and monovalent anions and involves a series of titratable groups at the "carrier" site.

Under physiological conditions, about 25 mM HCO_3^- is present in human blood. Any pH disequilibrium across the red cell membrane *in vivo* would be rapidly dissipated by HCO_3^-/Cl^- exchange and the Jacobs-Stewart cycle (Bidani & Crandall, 1978). In the absence of HCO_3^-, however, pH equilibration must take place by H^+ or OH^- flux across the membrane, a much slower process due to the low concentrations of the ions involved. In this case, it remains likely that pH equilibration in erythrocyte suspensions takes place predominantly through the membrane transport pathway described for rapid Cl^-/Cl^- and HCO_3^-/Cl^- exchange. However, it is

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not experimentally possible to distinguish between H^+ and/or OH^- fluxes directly. Jacobs and Parpart (1932) concluded OH^- was the ion actually moving, based on an indirect method for measuring intracellular pH changes. Crandall, Klocke and Forster (1971) reported calculated permeability values for OH^- assuming OH^-/Cl^- exchange by simple diffusion down an electrochemical potential gradient. When these data were interpreted as H^+/Cl^- cotransport, a mirror image for H^+ permeability as a function of pH was obtained. Recently, however, Jennings (1976) presented data suggesting the H^+ may be cotransported with $SO_4^=$ in exchange for Cl^- via the anion exchange pathway. In view of this finding, Jennings (1978) went on to measure rates of pH equilibration in CO_2-free red cell suspensions over the pH range 5.8 to 7.5. The data obtained, not dissimilar to those reported earlier (Crandall et al., 1971), were interpreted in light of the recent elucidation of the characteristics of the electroneutral red cell ion exchange pathway. Based on the fact that a rate constant assuming H^+/Cl^- cotransport changed much less than that for OH^-/Cl^- countertransport over this pH range, Jennings (1978) concluded that H^+/Cl^- cotransport is the primary mechanism for pH equilibration in erythrocyte suspensions. However, this conclusion was based on data obtained almost entirely under conditions where the transmembrane $[H^+]$ gradient was considerably greater than that for $[OH^-]$.

In the present study, we used a stopped-flow rapid reaction apparatus to study the rate of pH equilibration in human red cell suspensions over a wide range of extracellular pH between 4 and 11. The data were interpreted assuming that either H^+/Cl^- cotransport or OH^-/Cl^- countertransport is exclusively responsible for pH equilibration. Based on the behavior of the fluxes and rate constants defined for each of these processes as a function of pH, we conclude that (i) both H^+/Cl^- cotransport and OH^-/Cl^- countertransport can participate in pH equilibration across the human erythrocyte membrane, (ii) both processes probably utilize the rapid anion exchange pathway, and (iii) the process primarily responsible for pH equilibration in CO_2-free suspensions is that with the larger concentration gradient at any given pH.

Materials and Methods

Apparatus

The experiments were performed using a temperature controlled stopped-flow rapid reaction apparatus (SFRRA) that has been described in detail by Crandall et al. (1971) and Chow et al. (1976). In this apparatus, equal volumes of red cell suspension B and an unbuffered solution A are injected through a four-jet mixer (0.004 ml) into a 0.1 ml electrode chamber where pH of the mixture is followed as a function of time, both before and after flow stops, with a pH-sensitive glass electrode (#117145, Leeds & Northrup Co., North Wales, Pa.). The reference electrode (#117147, Leeds & Northrup Co., North Wales, Pa.) has a KCl-saturated cotton wick liquid junction bridging a Teflon plug and is pressure and flow insensitive. The voltage across the electrodes is amplified (MPA-6 with MPS-15 power source, Transidyne General Corp., Ann Arbor, Mich.) and monitored on a storage oscilloscope (5103N, Tektronix Inc., Beaverton, Ore.). Voltage from a magnet-in-coil device mounted on the SFRRA drive block gives a measure of flow velocity as well as a flow stop indication (Chow et al., 1976) and is monitored simultaneously on a second oscilloscope channel. The response time of this electrode system has been estimated to be less than 5 msec, using a ramp change in pH resulting from carbonic acid dehydration as a test reaction (Crandall et al., 1971). Total lag time of the apparatus (elapsed time between mixing and reaching the glass electrode) is less than 20 msec at the approximately linear flow rates used in these experiments (25–50 ml/sec). Further details on the characteristics of the SFRRA are available in the literature (Crandall et al., 1971; Chow et al., 1976). All experiments were performed at 37 °C.

Solutions and Preparations

Fresh heparinized human blood was centrifuged for 10 min at $\simeq 3000 \times g$ and the plasma and buffy coat discarded. The red cells were resuspended in approximately 10 times their volume of 150 mM NaCl and centrifuged. This washing process was repeated two additional times. The resulting washed red cells were then resuspended in 150 mM NaCl to about 20% hematocrit to form suspension B. The suspension was evacuated and re-equilibrated with 100% N_2 at least five times. pH of this CO_2-free suspension was always about 7.3 and was not altered during the final three repetitions of the evacuation procedure. All evacuations and re-equilibrations were carried out at room temperature. Solution A was composed of 150 mM NaCl titrated with a small volume of either NaOH or HCl to a pH between 3.0 and 11.5 and evacuated and re-equilibrated with 100% N_2 four times as described above.

The control erythrocyte suspension B described above was altered in several experiments by pretreatment with SITS (4-acetamido-4′-isothiocyanostilbene-2-2′-disulfonic acid), a potent anion exchange inhibitor. Fresh red cells were washed three times in 150 mM NaCl as described above and then resuspended to a 10% hematocrit in a solution containing 146.5 mM NaCl, 2.9 mM Tris buffer (pH 7.4) and 0.11 mM SITS dissolved in ethanol (0.2% of total volume). This suspension was incubated for 10 min at 37 °C with constant shaking. After pretreatment with SITS the cells were rewashed three times with 150 mM NaCl, resuspended to 20% hematocrit in NaCl, and then evacuated and re-equilibrated with 100% N_2 as described above. In one experiment, the rate of pH equilibration was measured in the presence of valinomycin, a K^+ ionophore. Immediately prior to an experimental run, 0.1 ml of 9×10^{-5} M valinomycin in 1% ethanol was added anaerobically to 10 ml of suspension B and mixed well. The experiment was then performed as soon as possible (2–4 min) and again after 10 min of incubation with valinomycin. A control experiment was run subsequently to test the effects of 1% ethanol by repeating the procedure in the absence of valinomycin.

The effectiveness of the CO_2 removal process by gas evacuation and N_2 re-equilibration was investigated by repeating the cycle up to 16 times for suspension B, and up to 8 times for solution A, in several experiments. The P_{CO_2} of some of these

suspensions and solutions was measured as a function of the number of gas evacuation/N_2 re-equilibration cycles using a specially modified sampling chamber (Itada & Forster, 1977) in a mass spectrometer (Model 21-620A, Consolidated Electrodynamics Corp., Pasadena, Calif.). The influence of the presence of CO_2 in the suspension was studied by performing experiments with nondegassed solutions and suspensions, and when carbonic anhydrase (60 mg%) and/or $NaHCO_3$ (5–5,000 μM) was added to suspension B.

Hematocrit of suspension B was measured in standard Wintrobe tubes. pH was determined anaerobically at 37 °C in a blood gas machine (BMS3 MK2, Radiometer, Copenhagen). Supernatant hemoglobin concentration in suspension B was measured spectrophotometrically at 541 nm (Coleman #124, Perkin-Elmer Corp., Norwalk, Conn.). pH was determined to be about 7.1 in red cell lysates produced by freezing and thawing cells that had been separated by centrifugation. Buffer capacity of the extracellular fluid was determined directly by titration (ABU 13 Autoburette, with TTT 60 Titrator, Radiometer, Copenhagen) from the supernatant of the mixture taken directly from the SFRRA. Buffer capacity was about 0.3 mM/pH at pH 7.

Computations

Initial flux (ϕ) of OH^- or H^+ across the erythrocyte membrane per unit of membrane surface area was calculated from the initial dpH/dt in the mixture observed after stopping flow in the rapid reaction apparatus:

$$\phi = \beta(dpH/dt)_o\,(1-\mathrm{Hct})/(\mathrm{Hct}\times A/V) \quad (1)$$

where β = extracellular fluid buffer capacity determined by titration, Hct = fractional hematocrit in cell volume/total volume, V = volume/red cell (assumed to be 87 μm^3) and A = surface area/red cell (assumed to be 163 μm^2). A velocity constant was defined and computed from the initial flux assuming that pH equilibration results exclusively from either OH^- or H^+ flux:

$$k_{OH^-} = \phi/([OH^-]_i - [OH^-]_o) \quad (2)$$

$$k_{H^+} = \phi/([H^+]_o - [H^+]_i). \quad (3)$$

This form of driving gradient was chosen because it is the simplest and does not necessarily require obligate co- or counter-transport. It assumes the rate of pH equilibration is proportional to $\Delta[H^+]$ or $\Delta[OH^-]$, consistent with the fact that $[Cl^-] \gg K_m$ both inside and outside the red cells. It is probable that neither H^+ nor OH^- flux is near saturation, since flux increases greatly as concentration gradient increases and $[H^+]$ and $[OH^-]$ are far below the K_m reported for any ion using the anion exchange pathway of the red cell membrane. The initial OH^- or H^+ driving gradient across the erythrocyte membrane was calculated using the measured extracellular pH of the mixture immediately after mixing and the initial intracellular pH (equal to pH of the lysate of cells from suspension B).

Results

As measured in the mass spectrometer, the pCO_2 of nondegassed solutions and suspensions was found to be about 0.3 mm Hg, as expected for liquids in equilibrium with ambient air. However, no dissolved CO_2 was detected in solutions and suspensions which had been degassed by four or more gas evacuation/N_2 re-equilibration cycles. The minimum pCO_2 that our mass spectrometer can detect is estimated to be <0.001 mm Hg.

Two typical experimental records are shown in Fig. 1. In each record, the upper tracings represent pH of the fluid in the measuring chamber as a function of time, and the lower traces indicate when flow of reactants started and stopped. The cell suspension B was mixed with solution A at pH 3 in Fig. 1*a* and at pH 11 in Fig. 1*b*. In each case, before flow started, solution A was in the electrode chamber. The "plateau" which appears during flow represents the pH of the mixture about 20 msec after the

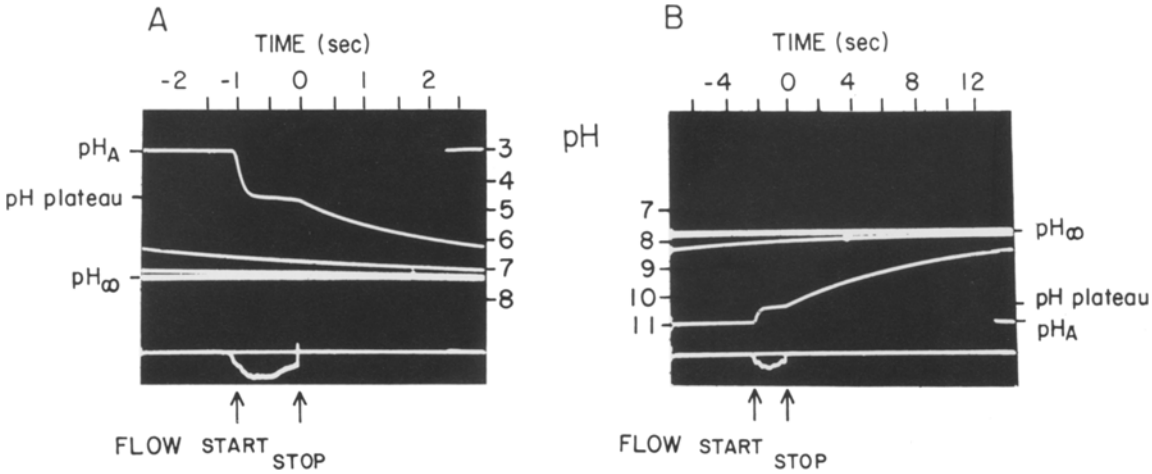

Fig. 1. Oscilloscope records from two experiments where a CO_2-free saline solution A was mixed with an equal volume of a CO_2-free 20% erythrocyte suspension B at 37 °C. In each case the upper trace represents pH of the extracellular fluid, and the lower trace indicates where flow starts and stops. Each trace was swept across the screen several times. Before flow starts, solution A is in the electrode chamber. The plateau pH is the pH of the mixture during flow. After flow stops, pH of the mixture either rises (*A*) or falls (*B*) toward an equilibrium value near 7.3. Initial flux was calculated from the initial rate of pH change after stopping flow

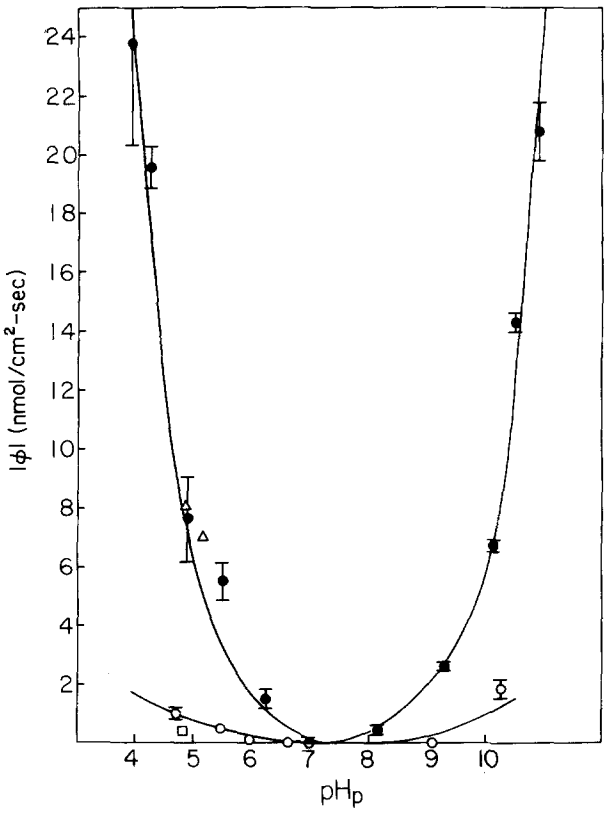

Fig. 2. Absolute value of flux of H^+ and/or OH^- *vs.* extracellular pH in CO_2-free erythrocyte suspensions. Points and bars represent mean ±SEM when $n>1$. The upper curve depicts control experiments (solid circles, $10 \leqq n < 25$) and the lower curve shows data from experiments where the erythrocytes were pretreated with SITS (open circles, $1 \leqq n \leqq 7$). Both curves were fit by eye. Also shown are data from experiments where the erythrocytes were exposed to valinomycin (open triangles, $n=1$) or pretreated with SITS and then exposed to valinomycin (open squares, $n=1$)

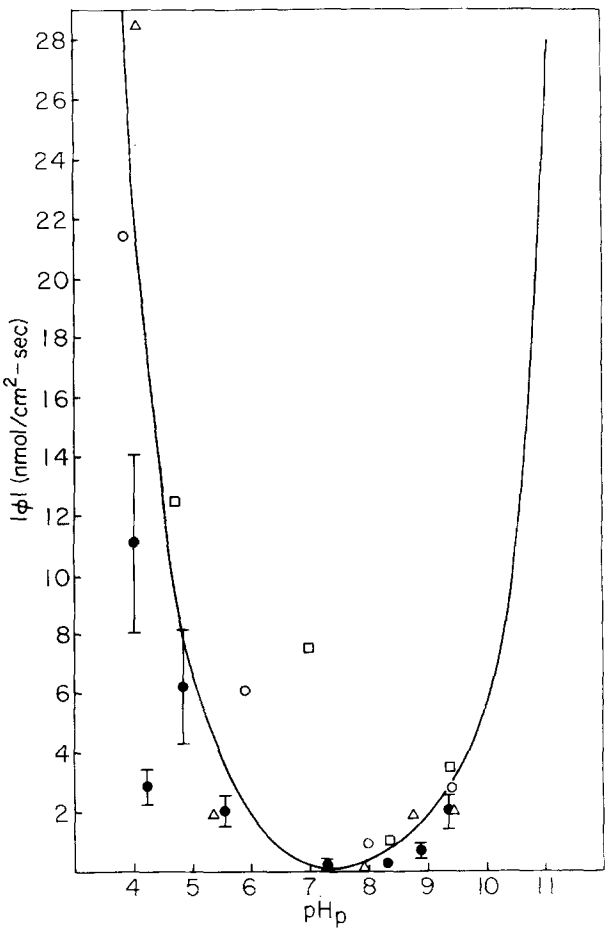

Fig. 3. Absolute value of flux of H^+ and/or OH^- *vs.* extracellular pH in erythrocyte suspensions. The data obtained from CO_2-free control experiments (solid line taken directly from Fig. 2) is shown along with data obtained in experiments using air-equilibrated solutions and suspensions (solid circles, $3 \leqq n \leqq 6$) or air-equilibrated suspensions supplemented ($1 \leqq n \leqq 2$) with carbonic anhydrase (60 mg%, open circles), $NaHCO_3$ (5–5,000 μM, open triangles), or both carbonic anhydrase (60 mg%) and $NaHCO_3$ (50–5,000 μM) simultaneously (open squares). Points and bars represent mean ±SEM when $n>1$

instant of mixing (Crandall et al., 1971) and is determined primarily by the pH of A and B and the buffer capacity of the extracellular fluid of the mixture. After flow stops, the pH of the mixture in the chamber rises (Fig. 1*a*) or falls (Fig. 1*b*) toward its final equilibrated value.

Figure 2 shows initial flux of OH^- or H^+ across the red cell membrane *vs.* extracellular pH in CO_2-free erythrocyte suspensions, calculated according to Eq. (1). In the control experiments (solid circles), the absolute value of flux increases almost symmetrically around pH 7.3 with either increased or decreased extracellular pH. Varying the number of gas evacuation/N_2 re-equilibration cycles from 4 to 8 for solution A and from 5 to 16 for suspension B resulted in no observable changes in the flux rates measured over the entire pH range studied. Pretreatment with SITS reduces flux by greater than 85%, shown by the lower line in Fig. 2 (open circles). The relationship between the absolute value of flux in SITS-pretreated cells and extracellular pH also appears to increase almost symmetrically as pH deviates from 7.3. Valinomycin, used to rapidly change the electrical potential across the erythrocyte membrane, had no measurable effect on flux when added to either control suspensions (open triangles) or SITS-pretreated suspensions (open squares).

Figure 3 compares data obtained from CO_2-free control experiments (solid line taken directly from Fig. 2) and data obtained under other conditions. The absolute value of flux in the experiments using nondegassed solutions and suspensions (solid circles) generally lies close to but below the curve for the CO_2-free control experiments, an observation for which we have no simple explanation. The addition of carbonic anhydrase to nondegassed suspensions also seems to

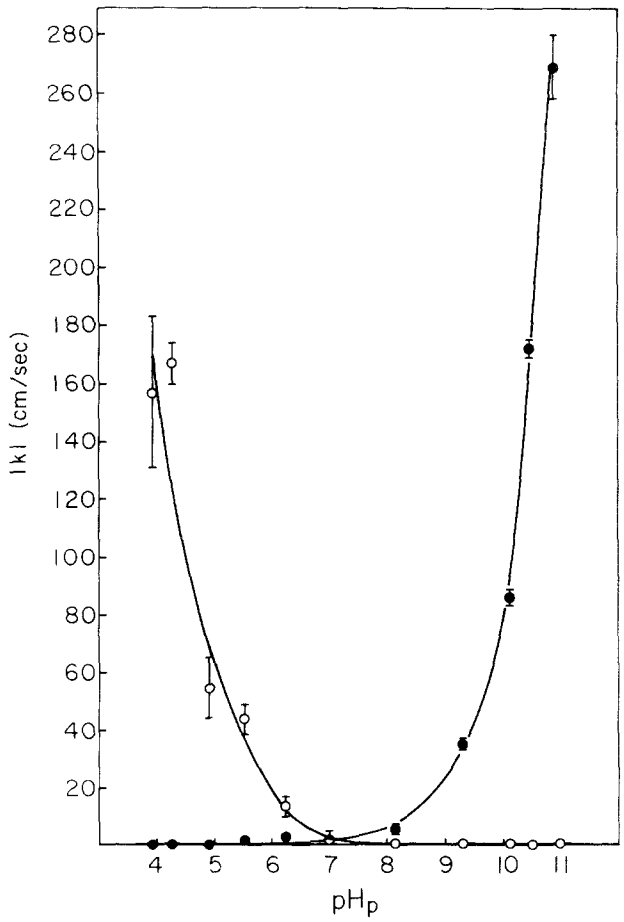

Fig. 4. Absolute values of the velocity constants k_{OH^-} (open circles) and k_{H^+} (solid circles) *vs.* extracellular pH. Points and bars represent mean ±SEM ($10 \leqq n \leqq 25$). The curves were fit by eye

have little effect on the rate of pH equilibration (open circles). $NaHCO_3$, when added to the nondegassed suspensions in amounts varying from 5–5,000 μM, leads to measured fluxes that are not significantly different from these measured CO_2-free controls. The average fluxes determined for all $NaHCO_3$ concentrations as a function of extracellular pH are shown in Fig. 3 (open triangles). The addition of carbonic anhydrase and $NaHCO_3$ (50–5,000 μM) simultaneously to nondegassed cell suspensions tends to increase the rate of pH equilibration somewhat, especially around neutral pH (open squares), although there was no good correlation between $[HCO_3^-]$ and rate.

Absolute values of the velocity constants, k_{OH^-} and k_{H^+}, are presented as a function of extracellular pH in Fig. 4. The curves are almost mirror images of each other. Below pH 7, the calculated k_{OH^-} increases markedly as the OH^- concentration gradient decreases, changing by a factor of about 120 between pH 4 and 7. However, k_{OH^-} asymptotically approaches a small value as the OH^- concentration gradient increases above 10^{-7} M, changing by a factor of about 20 between pH 7 and 10. The calculated k_{H^+} also increases with decreasing H^+ gradient and asymptotically approaches a small value as H^+ gradient rises above 10^{-7} M, changing by a factor of about 10 between pH 4 and 7, and about 30 between pH 7 and 10.

Discussion

The pCO_2 of solutions and suspensions equilibrated with air that had not been degassed was found to be about 0.3 mm Hg, as expected. In contrast, no detectable levels of dissolved CO_2 were found in solutions and suspensions which had undergone four or more evacuation/N_2 re-equilibration cycles. Increasing the number of cycles from 4 to 8 for solution A and from 5 to 16 for suspension B resulted in no observable change in the rate of pH equilibration over the entire pH range studied (4–11). In addition, use of air-equilibrated solutions and suspensions did not result in significant increases in measured H^+ and/or OH^- fluxes over the same pH range (Fig. 3). Even the addition of small amounts of $NaHCO_3$ (5–5,000 μM) or carbonic anhydrase (60 mg%) to nondegassed erythrocyte suspensions resulted in no consistent alteration in the measured fluxes, although addition of both $NaHCO_3$ (50–5,000 μM) and carbonic anhydrase (60 mg%) simultaneously to the cell suspensions tended to increase the rate of pH equilibration, particularly around neutral pH values.

The unexpected ineffectiveness of the presence of small amounts of CO_2 in accelerating pH equilibration at non-neutral pH has also been reported by Jennings (1978), while its accelerating influence at neutral pH has been reported by Jennings (1978) and Cousin, Motais and Sola (1975). One possible explanation for these findings is that at neutral pH, the driving force for H^+ or OH^- fluxes is extremely small and pH equilibration is effected by CO_2 movement via the Jacobs-Stewart cycle (Jacobs & Stewart, 1942), despite the fact that this cycle is slow due to its rate-limitation under ordinary circumstances by the uncatalyzed extracellular CO_2 hydration-dehydration reactions. On the other hand, away from neutral pH, the rate of the Jacobs-Stewart cycle remains approximately the same, while H^+ and/or OH^- fluxes are markedly increased due to the great increase in their driving gradients. The reason that addition of carbonic anhydrase alone to air-equilibrated suspensions has little effect may be that there is not enough total CO_2 present to have a significant influence on pH equilibration.

Figure 2 reveals an almost symmetrical and marked increase in the flux of H^+ or OH^- when extra-

cellular pH is increased or decreased from 7.3. These data, qualitatively similar between pH 5.8 and 7.5 to those obtained by Jennings (1978), suggest that both H^+ and OH^- fluxes may take place across the red cell membrane, but that H^+ flux dominates pH equilibration at acid pH while OH^- flux dominates at alkaline pH. When both ions can move across the membrane, this latter finding is not surprising, since the driving gradient for H^+ movement is very small ($\sim 10^{-7}$ M) at pH > 8 while that for OH^- movement is very small ($\sim 10^{-7}$ M) at pH < 6.5.

The dependence of the rate of pH equilibration on extracellular pH is markedly different from that reported for both Cl^-/Cl^- (Gunn et al., 1973) and HCO_3^-/Cl^- (Obaid & Crandall, 1979) exchange, which exhibit a maximum flux at about pH 7.6 and a minimum at about pH 5. However, a direct comparison between our pH equilibration behavior and the previous flux data is not possible, since in the Cl^-/Cl^- and HCO_3^-/Cl^- experiments, driving gradients were maintained approximately constant as pH varied, while in the present experiments the change in pH actually determined the driving gradients which changed over orders of magnitude. Velocity constants may be more directly comparable, but the overlap of the pH range in which we know reliably either k_{H^+} (4–6.5) or k_{OH^-} (8–11) and that in which Cl^-/Cl^- and HCO_3^-/Cl^- have been studied for intact red cells (5–9) is too small.

pH equilibration in SITS-treated red cell suspensions (SITS is a specific inhibitor of the Band 3 exchange pathway (Cabantchik et al., 1978)) was inhibited by at least 85% (Fig. 2) at the one concentration of SITS used to treat the cells. These data suggest that over the entire range of pH studied, equilibration probably occurs via the rapid anion exchange pathway of the erythrocyte membrane. Assuming that both H^+ and OH^- are moving as discussed above, this implies that both H^+/Cl^- cotransport and OH^-/Cl^- countertransport can be facilitated by the "anion" transport pathway. Flux of H^+ through this pathway is consistent with the earlier finding that H^+ can be cotransported with Cl^- in exchange for $SO_4^=$ (Jennings, 1976).

The erythrocyte membrane potential should have been greatly increased by the addition of valinomycin, a K^+-ionophore, but this had no observable effect on the rate of pH equilibration in either control suspensions or in suspensions where the erythrocytes had been pretreated with SITS. The absence of an effect on the rate of pH equilibration when the potential difference across the membrane has been changed indicates that this process probably occurs via an electroneutral pathway. These data are similar to those presented by Jennings (1978) for pH equilibration and are consistent with the fact that most of the flux of H^+ or OH^- leading to pH equilibration is electrically silent.

The velocity constant k_{H^+} calculated assuming only H^+ flux is responsible for pH equilibration increases dramatically for pH > 8, while k_{OH^-} calculated assuming only OH^- flux increases greatly for pH < 6.5 (Fig. 4). Using the argument that it is unlikely that such large changes in the velocity constants for these ions will take place over such a narrow pH range (Jennings, 1978), these data support the hypothesis that H^+ flux dominates pH equilibration at acid pH and OH^- flux dominates at alkaline pH. It should be noted, however, that the rate constants defined by Eqs. (2) and (3) are proportionality factors relating flux to an arbitrary driving gradient. If the mechanism of transport of OH^- or H^+ were known, a more specific gradient could be used. For example, if the ions were moving passively across the membrane down their electrochemical potential gradients, the Goldmann constant-field equations could be used. However, since it is now apparent that pH equilibration utilizes the SITS-sensitive ion exchange pathway in the red cell membrane, the choice of a driving gradient for defining a rate constant becomes somewhat arbitrary. In our calculations, we have assumed that the H^+ or OH^- concentration difference drives pH equilibration, since this is the simplest possible definition and takes into account the fact that Cl^- is present at concentrations much greater than K_m both inside and outside the membrane. Jennings (1978) utilized a gradient for H^+ of $(H_o^+Cl_o^- - H_i^+Cl_i^-)$ and for OH^- of $(OH_o^-Cl_i^- - OH_i^-Cl_o^-)$, similar to Jacobs and Parpart (1932). This gradient assumes obligate transport of H^+ or OH^- and Cl^-, and defines rate constants with unusual units. We have calculated rate constants from our data based on Jennings' (1978) definitions and find that the relationships between k_{H^+} or k_{OH^-} and pH is in general similar to those obtained using our Eqs. (2) and (3), the only difference being that the factors by which the rate constants change between pH 4 and 7 *vs.* pH 7 and 10 become similar. This difference points out that the conclusions about H^+ and OH^- movements as a function of pH based on arguments related to the rate constants are dependent in part upon the model used to define the constants.

Our conclusion that both H^+ and OH^- can move across the red cell membrane, and that the species dominating pH equilibration is determined by the magnitude of the driving gradients for each ion, has been based in part on kinetic arguments similar to those that have been utilized previously (Jacobs &

Parpart, 1932; Jennings, 1978). Jacobs and Parpart (1932) studied the rate of internal pH equilibration in very dilute suspensions of whole blood exposed to very acidic (pH 1–4) electrolyte and sucrose solutions. They concluded that their data was best explained by OH^-/Cl^- exchange because the deduced permeability to OH^- changed less than that for H^+. However, their method for determining transmembrane acid flux (time of color change due to denaturation of hemoglobin) quite possibly did not yield a precise measure of the rate of intracellular pH change. Jennings (1978) studied pH equilibration between pH 5.8 and 7.5, and concluded that H^+/Cl^- cotransport is responsible for pH equilibration in red cell suspensions, since k_{OH^-} would have to change about 500 times while k_{H^+} changes only by a factor of five. Although some uncertainty may have been present due to (i) measuring the rate of pH equilibration when the suspension was close to equilibrium, and (ii) calculating intracellular pH far from the initial condition, Jennings (1978) data are in qualitative agreement with our own. This earlier work (Jennings, 1978) was carried out over a relatively narrow range of pH where the H^+ concentration gradient was almost always larger than that for OH^-. Our extension of these studies to a wide range of pH has allowed us to investigate pH equilibration when the greater concentration gradient was present for either H^+ or OH^-, leading to our conclusion that both ions can utilize the rapid exchange pathway in the erythrocyte membrane. While the conclusion of Jennings (1978) agrees with ours that H^+/Cl^- cotransport dominates pH equilibration at low pH, it appears that OH^-/Cl^- exchange is probably responsible for pH equilibration at high pH.

The mechanism by which OH^-/Cl^- exchange takes place is probably the same as that for Cl^-/Cl^- self-exchange (Gunn, 1977), which has been suggested to be a ping-pong process (Gunn & Frohlich, 1979). The increasing OH^- flux with increased pH is expected, due to the increasing availability of OH^- to the transport site on the outside of the cell membrane as pH rises and the probability that $[OH^-]$ is much less than its K_m. The cotransport of H^+ with Cl^- via the same ping-pong pathway is less obviously explainable, but can be appreciated in view of the titratable character of the transport mechanism. When a large H^+ concentration gradient is present across the membrane at $pH < 7$, it is likely that more sites facing the low pH medium are titrated to a doubly-charged state than are those facing the higher pH medium (most of which would be singly-charged in this pH range). As a result, especially in the absence of divalent anions, those doubly-charged sites would tend to transport two Cl^- from low to high pH. The site then faces the high pH medium and one H^+ is released, returning the site to a singly-charged state and leading to the return of one Cl^- to the low pH medium. The result of one flip-flop cycle of the transport site is the net transfer of one H^+ and one Cl^- from low to high pH side. In both OH^-/Cl^- exchange and H^+/Cl^- cotransport under the conditions of our experiments, the rate should be proportional to the concentration difference of OH^- or H^+ across the membrane.

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