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Effects of Salicylate on HCO_3^-/Cl^- Exchange across the Human Erythrocyte Membrane

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Summary. Changes in extracellular pH (pH_o) in human red cell suspensions were monitored in a stopped-flow rapid reaction apparatus. A 20% suspension of washed human RBC in saline at pH 7 containing $NaHCO_3$ and extracellular carbonic anhydrase was mixed with an equal volume of buffered saline solution at pH 6.7. Sodium salicylate, when present, was added to both the erythrocyte suspension and the buffer solution. The effects of salicylate in the therapeutic to toxic concentration range on HCO_3^-/Cl^- exchange were studied at 37 °C. HCO_3^-/Cl^- exchange flux was estimated using the extracellular buffer capacity and the difference between dpH_o/dt using a control RBC suspension and that using a suspension of RBC whose anion exchange pathway was markedly inhibited. The results show that salicylate competitively decreases the rate of HCO_3^-/Cl^- exchange, with inhibition increasing as salicylate concentration increases. K_I is ~2.4 mM. At a salicylate concentration of 10 mM, HCO_3^-/Cl^- exchange under the conditions of our experiments was inhibited by more than 70%. These findings are consistent with the possibility that CO_2 transfer in capillary beds *in vivo* may be diminished in the presence of salicylate due to slowing of red cell HCO_3^-/Cl^- exchange.

Key words salicylate · erythrocyte · anion exchange · pH · DIDS · CO_2

Introduction

The importance of the kinetics of bicarbonate/chloride exchange across the erythrocyte membrane during CO_2 uptake in the tissues and elimination in the lungs is related to the possibility that it might limit the amount of CO_2 that can be transferred during capillary transit (Wieth & Brahm, 1978, 1980; Crandall & Bidani, 1981; Crandall et al., 1981). This observation is due to the fact that the half time for HCO_3^-/Cl^- exchange *in vivo* may be of the same order of magnitude as the transit time of red cells through capillaries (Obaid & Crandall, 1979; Crandall & Bidani, 1981). Inhibitors of the specialized red cell anion exchange pathway, therefore, may have a profound effect on the transfer of metabolically-produced CO_2 from cells to environment under certain conditions.

Salicylate has been shown to cause inhibition of phosphate and sulfate exchange across the red cell membrane (Passow & Schnell, 1969; Deuticke, 1970; Schnell, 1972), with a K_I of ~3 mM for sulfate exchange. It has also been demonstrated that salicylate is a potent inhibitor of Cl^-/Cl^- self-exchange across the red cell membrane (Wieth, 1970). Dalmark and Wieth (1972) found that at a concentration of 120 mM, salicylate reduced the rate of efflux of chloride ions from red cells by a factor of about 500. However, no information has been obtained on the effects of salicylate on the physiologically important HCO_3^-/Cl^- exchange, especially in the clinically relevant concentration range.

In the present work, HCO_3^-/Cl^- exchange in human red cell suspensions was studied in the stopped-flow rapid reaction apparatus using a modification of a technique described previously (Chow, Crandall & Forster, 1976; Obaid & Crandall, 1979). In the absence of salicylate, if a large quantity of H^+ is added to a red cell suspension which contains carbonic anhydrase in its extracellular fluid, some of the H^+ will rapidly combine with HCO_3^- in the extracellular fluid to form CO_2, which quickly enters the red cells where it is hydrated in the presence of intracellular carbonic anhydrase to form H^+ and HCO_3^- (Fig. 1). This "redistribution," which takes place in less than 20 msec (Chow et al., 1976), acts to quickly increase the intracellular concentration of HCO_3^-. The intracellular HCO_3^- then begins to move out of the cells in exchange for Cl^-. As HCO_3^- enters the extracellular fluid, it combines with H^+ there to form CO_2 and raise extracellular pH. The CO_2 re-enters the red cells and rehydrates to form HCO_3^- and H^+. The net result of a complete cycle of one bicarbonate leaving the red cells and CO_2 entering to reform HCO_3^- is the transfer of one H^+ and one Cl^- from outside to inside the cells (with water following passively). This cycle of CO_2

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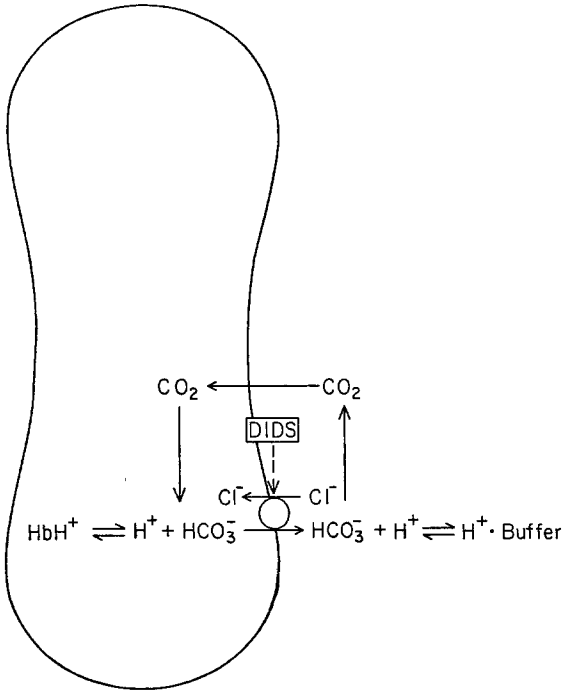

Fig. 1. Schematic diagram of the Jacobs-Stewart cycle. This cycle as drawn effects the transfer of one H^+ and one Cl^- from outside to inside the red cell. *See* text for further discussion

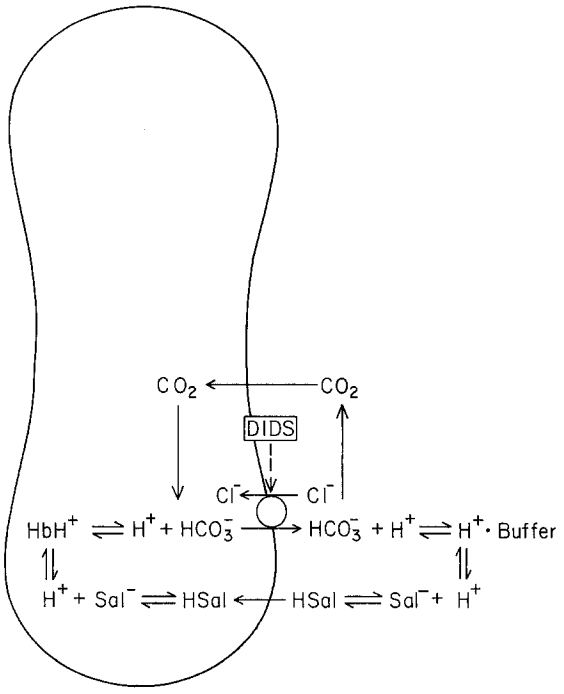

Fig. 2. Schematic diagram of the Jacobs-Stewart cycle in the presence of salicylate (Sal^-). When Sal^- is present in both the intra- and extracellular fluids, the Jacobs-Stewart cycle accounts for only part of the flux of H^+ into the red cells. The remainder of the H^+ flux is mediated by movement of undissociated H · Sal into the red cell. *See* text for further discussion

and HCO_3^- entering and exiting the cells has been called the Jacobs-Stewart cycle (Jacobs & Stewart, 1942). dpH/dt in the extracellular fluid after the initial rapid redistribution of intra- and extracellular HCO_3^- is rate-limited by the exchange of HCO_3^- for Cl^- across the red cell membrane. The rate of HCO_3^-/Cl^- exchange can therefore be measured by monitoring dpH_o/dt. HCO_3^-/Cl^- exchange is markedly inhibited by pretreatment of the red cells with DIDS (4-4′-diisothiocyano-2-2′-disulfonic stilbene) (Cabantchik & Rothstein, 1972).

When salicylate (Sal^-) is present in both intra- and extracellular fluids (Fig. 2), the Jacobs-Stewart cycle accounts for only part of dpH_o/dt measured experimentally after addition of H^+ to the extracellular fluid. The remainder of dpH_o/dt is due to the movement of undissociated H · Sal into the cells (Dalmark & Wieth, 1972). Because DIDS does not inhibit H · Sal flux (*see* below), the rate of HCO_3^-/Cl^- exchange can be determined from the difference between dpH_o/dt in suspensions of normal RBC and dpH_o/dt in suspensions of RBC pretreated with DIDS. A small correction is made for the fraction of HCO_3^- flux that continues to occur in the presence of DIDS.

We have studied HCO_3^-/Cl^- exchange at 37 °C in the presence of 0–10 mM sodium salicylate. The results indicate that salicylate is a potent inhibitor of the exchange with a $K_I \sim 2.4$ mM. At 10 mM salicylate, HCO_3^-/Cl^- exchange is inhibited $>70\%$ under the conditions of our experiments.

Materials and Methods

Apparatus

The stopped-flow rapid reaction apparatus used in these experiments has been described previously (Crandall, Klocke & Forster, 1971; Chow et al., 1976). In the apparatus, a hydraulic drive system forces equal volumes of a red cell suspension (*A*) and an acidic buffered saline solution (*B*) through a four-jet mixer (0.004 ml) into a 0.1 ml reaction chamber. In the chamber, a pH-sensitive glass electrode (Leeds and Northrup 117145) is used to follow pH_o as a function of time. The reference electrode liquid junction is a KCl-saturated cotton wick bridging a snug-fitting Teflon plug, and is pressure- and flow-insensitive (Crandall et al., 1971). The voltage across the electrodes is amplified (Transidyne General MPA-6) and monitored on a storage oscilloscope screen (Tektronix 5103N). The voltage signal from a magnet-in-coil device mounted on the drive block of the apparatus allows the flow velocity to be simultaneously monitored on the oscilloscope screen. The entire apparatus is water-jacketed, and the experiments reported here were performed at 37 °C. Using a ramp change in pH due to dehydration of carbonic acid, the response time of the electrode system has been estimated to be less than 5 msec (Crandall et al., 1971). At the flow rates used in these experiments (25–50 cm/sec), the elapsed time between mixing and reaching the glass electrode is less than 20 msec.

Preparation of Solutions

Outdated human blood was centrifuged at $1000 \times g$ for 10 min and the plasma and buffy coat removed by aspiration. The cells were then washed (resuspended to about 20% hematocrit and centrifuged for 10 min at $1000 \times g$) three times in a solution containing 146.5 mM NaCl, 3.5 mM KCl, and 0–10 mM sodium salicylate ($NaC_7H_5O_3$). After the third wash, the cells were incubated at 10% hematocrit and 37 °C for 10 min with constant agitation in 146.5 mM NaCl, 3.5 mM KCl, 0–10 mM sodium salicylate, 20 mM Tris buffer (pH 7.4), and 0.2% ethanol. The cells were then washed twice more as described above. After the final wash, the cells were resuspended in the wash solution to approximately 20% hematocrit. Bovine carbonic anhydrase (Sigma Chemical Co., #3-7500, St. Louis, Mo.) and sodium bicarbonate were added anaerobically to the suspensions to concentrations of 800 Wilbur-Anderson U/ml and 2.2–8.8 mM, respectively. The resulting cell suspensions (A) were then titrated to an equilibrium pH between 6.95 and 7.05.

Suspensions A' were prepared in exactly the same manner as were suspensions A, except that the red cells were treated with the anion exchange inhibitor, DIDS. After the third wash, the cells were incubated at 10% hematocrit and 37 °C for 10 min with constant agitation in 146.5 mM NaCl, 3.5 mM KCl, 0–10 mM sodium salicylate, 20 mM Tris buffer (pH 7.4), 0.2% ethanol, and 0.1 mM DIDS. The cells were then washed two times and suspended to 20% hematocrit (as described above). Eight hundred Wilbur-Anderson U/ml of bovine carbonic anhydrase and 2.2–8.8 mM $NaHCO_3$ were then added anaerobically to the suspensions. The resulting cell suspensions (A') were titrated to an equilibrium pH between 6.95 and 7.05.

The acidic buffered saline solutions B were composed of 117.5 mM NaCl, 15 mM KH_2PO_4, 15 mM Na_2HPO_4, and the same concentrations of sodium salicylate as in the respective red cell suspensions with which they were to be mixed. The pH of solutions B was always 6.7.

Suspensions C and C' were prepared in the same manner as were suspensions A and A' above, except that the wash solution consisted only of 150 mM sodium salicylate. The final pH was between 6.95 and 7.05. Solution B' was composed of 117.5 mM sodium salicylate, 15 mM Na_2HPO_4, and 15 mM KH_2PO_4. The pH of this solution was ~6.7.

Experimental Procedure

The stopped-flow apparatus was calibrated by passing standard buffer solutions through the electrode chamber. Solution B, at a given salicylate concentration, was then placed in the chamber and its output stored on the oscilloscope screen to serve as a reference value for a particular experimental run. Equal volumes of solution B and suspension A at the same salicylate concentration were then driven into the electrode chamber at approximately constant speed until flow was abruptly halted. The subsequent time course of pH_o of the mixture was monitored, stored on the oscilloscope screen, and photographed. The above experimental run was then repeated using the corresponding suspension A' and solution B. These experiments were performed over the entire range of salicylate concentrations. Several experiments were also performed using suspension C (or C') and solution B'. All experiments were performed at 37 °C.

At the conclusion of each experimental run, intracellular pH was measured in a lysate of cells from the suspension used, prepared by freezing and thawing packed cells. All pH determinations were performed anaerobically (Radiometer BMS3 MK2 blood gas machine). A sample of the effluent mixture from each experimental run was collected after having passed through the stopped-flow apparatus. The hematocrit of this sample was measured in standard Wintrobe tubes, after centrifugation at $1000 \times g$ for 10 min. The remainder of the effluent mixture was centrifuged and the supernatant titrated (Radiometer ABU13 autoburette, with TTA60 titration assembly). The buffer capacity of the extracellular fluid was determined as a function of pH.

Computations

The total initial flux of acid (H^+) into the red cells after "redistribution" (*see* above) per unit of membrane surface area is given by

$$\phi_T = \beta \cdot \left(\frac{dpH_o}{dt}\right) \cdot \frac{1-HCT}{(HCT)\,A/V} \tag{1}$$

where (dpH_o/dt) is the initial rate of change of extracellular pH after stopping flow, β is the buffer capacity of the extracellular fluid at the "plateau" pH (immediately after redistribution), V is volume/cell, and A is surface area/cell. HCT is the hematocrit of the suspension after mixing of equal volumes of the red cell suspension and the buffered saline solution in the stopped-flow rapid reaction apparatus. ϕ_T includes H^+ flux due to HCO_3^-/Cl^- exchange plus that due to H·Sal flux, and is obtained when suspensions A are mixed with solutions B.

The initial flux of HCO_3^- (ϕ) out of the red cells is given by the difference between the total flux (ϕ_T) and the flux of H^+ into the red cells obtained when DIDS-treated red cell suspensions A' are mixed with an equal volume of the acidic buffered saline solutions B:

$$\phi = \phi_T - \phi_{DIDS} \tag{2}$$

where

$$\phi_{DIDS} = \phi_{H\cdot Sal} + (\phi_{HCO_3^-/Cl^-})_{DIDS}. \tag{3}$$

In Eq. (3), $\phi_{H\cdot Sal}$ represents the flux of salicylic acid H·Sal, and $(\phi_{HCO_3^-/Cl^-})_{DIDS}$ represents the small but finite HCO_3^-/Cl^- flux in DIDS-inhibited red cell suspensions.

To obtain the true HCO_3^-/Cl^- flux at any salicylate concentration, we use a concentration factor "a" such that

$$\phi_{HCO_3^-/Cl^-} = \frac{\phi}{a} \tag{4}$$

where

$$a = \left[\frac{\phi_T - (\phi_{HCO_3^-/Cl^-})_{DIDS}}{\phi_T}\right]_{Sal^- = 0}. \tag{5}$$

Substituting Eqs. (1) and (4) into Eq. (2) yields the following overall expression:

$$\phi_{HCO_3^-/Cl^-} = \left[\beta_A \cdot \left(\frac{dpH_o}{dt}\right)_A \cdot \frac{1-(HCT)_A}{(HCT)_A \cdot A/V} - \beta_{A'} \cdot \left(\frac{dpH_o}{dt}\right)_{A'} \cdot \frac{1-(HCT)_{A'}}{(HCT)_{A'} \cdot A/V}\right] \Big/ a \tag{6}$$

where subscript A in Eq. (6) represents experiments with red cell suspensions A and solutions B, and subscript A' represents corresponding experiments with DIDS-treated red cell suspensions A' and solutions B. If HCT and β are constant, Eq. (6) could be further simplified. However, there were generally small differences in β_A and $\beta_{A'}$, and $(HCT)_A$ and $(HCT)_{A'}$, necessitating the use of the general form of Eq. (6) above.

In the absence of Cl^-, the flux of H·Sal can be determined using Eq. (1) directly (assuming Sal^-/HCO_3^- exchange to be negligible as discussed below). This equation was used to calculate initial fluxes in the experiments using suspensions C and C' with solution B'.

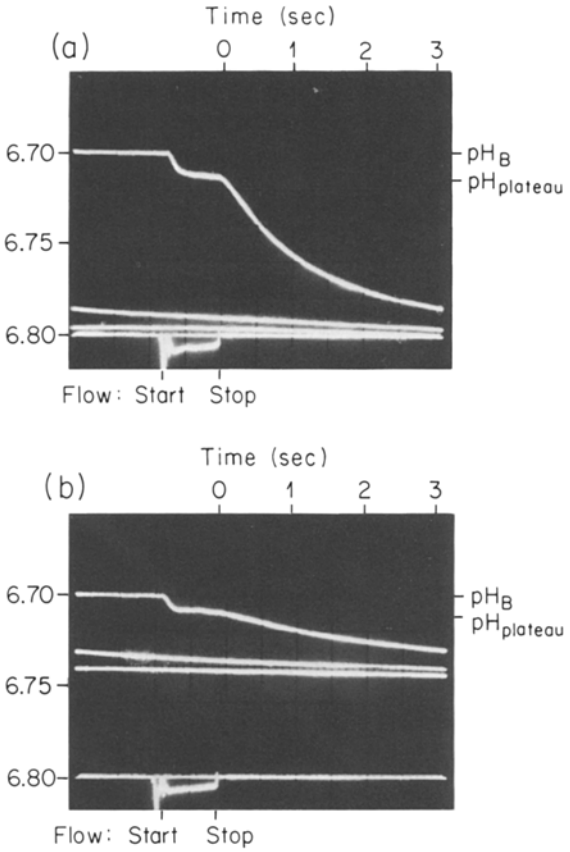

Fig. 3. Oscilloscope tracings obtained at 37 °C when equal volumes of solution *B* (pH 6.7, 5 mM salicylate) and suspension *A* or *A'* (pH 7.0, 8.8 mM $NaHCO_3$ and 5 mM salicylate) were mixed in the stopped-flow rapid reaction apparatus. The lower traces indicate times of starting and stopping flow. The upper traces represent the extracellular pH (pH_o) of the fluid in the measuring chamber as a function of time. Each trace was swept across the screen several times. *See* text for further details of the experimental records. (*a*): Solution *B* + suspension *A* (HCT = 19%). (*b*): Solution *B* + suspension *A'* (HCT = 21%)

Results

Figure 3*a* shows a typical experimental record obtained when equal volumes of solution *B* containing 5 mM salicylate (pH 6.7) and suspension *A* (pH 7.0, hematocrit 19%) containing 800 U/ml of carbonic anhydrase, 8.8 mM $NaHCO_3$ and 5 mM salicylate were mixed in the stopped-flow apparatus. The upper tracing shows the pH of the fluid in the chamber as a function of time. The lowest tracing represents flow velocity, where a downward deflection indicates an increase in flow speed. Prior to the initiation of flow, solution *B* is in the electrode chamber and its pH is indicated as the left uppermost part of the upper trace in Fig. 3*a*. When flow starts, indicated by an abrupt downward deflection on the lower trace, pH increases to the "plateau" pH (after redistribution) which persists until the flow is stopped. After flow stops, the pH of the mixture rises toward its equilibrium value due to both the Jacobs-Stewart cycle effecting the transfer of H^+ and Cl^- from outside to inside the cells, and the movement of undissociated H · Sal into the red cells (Fig. 2). The initial dpH_o/dt immediately after flow stops is used to compute ϕ_T.

Figure 3*b* shows the experimental record obtained on a subsequent experimental run when DIDS-treated RBC suspension *A'* containing 800 U/ml of carbonic anhydrase, 8.8 mM $NaHCO_3$, and 5 mM salicylate (pH 7.0, hematocrit 21%) is mixed with buffered saline solution (pH 6.7) containing 5 mM sodium salicylate in the stopped-flow apparatus. After flow stops, the pH_o of the mixture is again seen to increase (more slowly), with a seemingly smaller total pH_o change than that with suspension *A* (Fig. 3*a*) to a "quasi-equilibrated" value. In fact, a very slow

Table 1. Summary of experimental flux data[a]

$[HCO_3^-]$ (mM)	[Salicylate] (mM)	dpH_o/dt (control) (sec^{-1})	dpH_o/dt (DIDS) (sec^{-1})	$\phi_{HCO_3^-/Cl^-}$ (moles/cm^2-sec $\times 10^{-10}$)
2.2	0	0.0323 ± 0.0030	0.0006 ± 0.0001	1.422 ± 0.122
2.2	2	0.0265 ± 0.0036	0.0046 ± 0.0005	0.858 ± 0.092
2.2	5	0.0230 ± 0.0019	0.0096 ± 0.0007	0.580 ± 0.051
2.2	7.5	0.0229 ± 0.0014	0.0127 ± 0.0009	0.454 ± 0.064
4.4	0	0.0479 ± 0.0051	0.0007 ± 0.0001	2.127 ± 0.259
4.4	2	0.0366 ± 0.0031	0.0047 ± 0.0006	1.217 ± 0.126
4.4	5	0.0285 ± 0.0049	0.0080 ± 0.0014	0.916 ± 0.161
4.4	7.5	0.0335 ± 0.0039	0.0136 ± 0.0008	0.854 ± 0.130
4.4	10	0.0329 ± 0.0012	0.0206 ± 0.0010	0.590 ± 0.061
8.8	0	0.0643 ± 0.0036	0.0011 ± 0.0002	3.371 ± 0.274
8.8	2	0.0547 ± 0.0058	0.0043 ± 0.0002	2.483 ± 0.258
8.8	5	0.0421 ± 0.0028	0.0088 ± 0.0004	1.780 ± 0.170
8.8	7.5	0.0377 ± 0.0027	0.0131 ± 0.0010	1.231 ± 0.115

[a] Entries are mean ± SEM ($n \geqq 3$).

subsequent pH_o increase occurs in these mixtures such that the total ΔpH_o with suspensions A and A' are about the same (*see* below). Initial dpH_o/dt is used to compute ϕ_{DIDS}. Using these measurements of the initial rate of pH change (dpH_o/dt) from Figs. 3*a* and *b*, we calculate true HCO_3^-/Cl^- flux at the given salicylate concentration of 5 mM.

As outlined above, the flux of bicarbonate out of the red cells in exchange for Cl^- can be obtained from records of dpH_o/dt using normal red cell suspensions A and DIDS-treated red cell suspensions A' in the presence of different concentrations of salicylate. This data is summarized in Table 1. The mean initial values of dpH_o/dt in control and DIDS-treated red cell suspensions at salicylate concentrations of 0–10 mM and total bicarbonate concentrations of 2.2–8.8 are given. The mean initial dpH_o/dt ($n=2$) obtained on mixing cell suspension C with buffer solution B' in the stopped-flow apparatus was 0.038, compared with 0.041 when DIDS-pretreated cell suspension C' was mixed with buffer solution B'.

Figure 4 shows the percentage inhibition of the HCO_3^-/Cl^- flux in the presence of salicylate as a function of the log of the salicylate concentration (mM). It can be seen that salicylate is a potent inhibitor of human RBC HCO_3^-/Cl^- exchange, causing a decrease in flux of >35% when $[Sal^-]=2$ mM and >70% when $[Sal^-]=10$ mM.

In the Dixon plot ($1/\phi_{HCO_3^-/Cl^-}$ *vs.* $[Sal^-]$) shown in Fig. 5, the straight lines through the data points

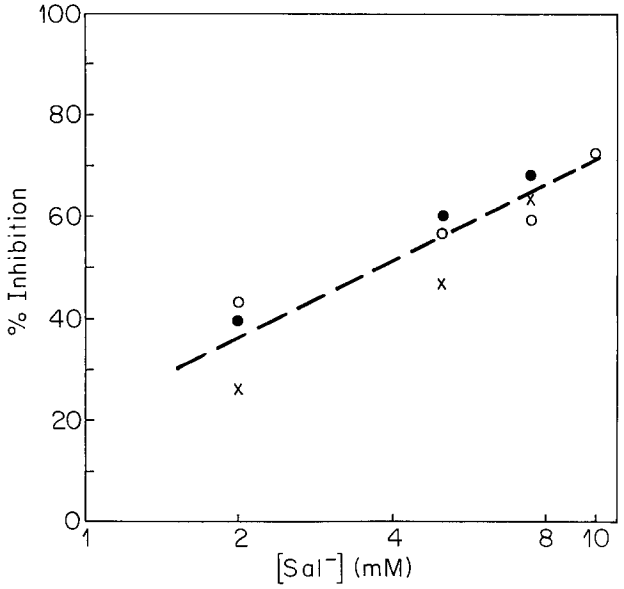

Fig. 4. Effects of salicylate on human red cell HCO_3^-/Cl^- exchange at 37 °C. o – 2.2 mM $NaHCO_3$ in red cell suspensions; • – 4.4 mM $NaHCO_3$ in red cell suspensions; × – 8.8 mM $NaHCO_3$ in red cell suspensions. The data is plotted as percentage inhibition of HCO_3^-/Cl^- exchange flux *vs.* log salicylate concentration

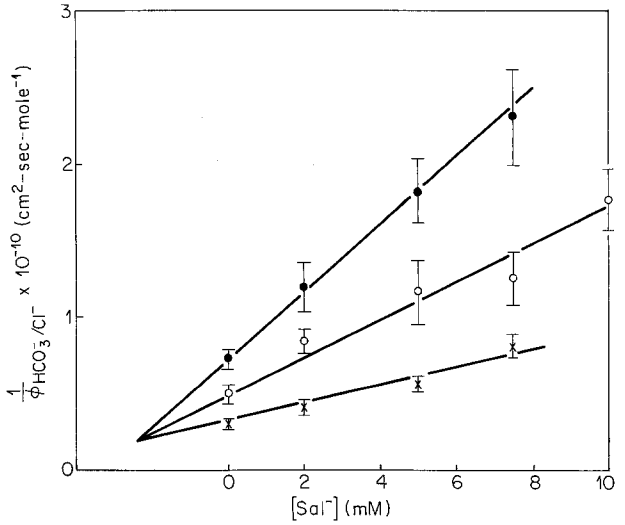

Fig. 5. Dixon plot ($1/\phi_{HCO_3^-/Cl^-}$ *vs.* $[Sal^-]$) for HCO_3^-/Cl^- exchange across the human erythrocyte membrane at 37 °C. o – 2.2 mM $NaHCO_3$ in red cell suspensions; • – 4.4 mM $NaHCO_3$ in red cell suspensions; × – 8.8 mM $NaHCO_3$ in red cell suspensions. $\phi_{HCO_3^-/Cl^-}$ – = HCO_3^-/Cl^- exchange flux; $[Sal^-]$ = salicylate concentration. From these data, K_I for salicylate inhibition of HCO_3^-/Cl^- exchange is 2.4 mM

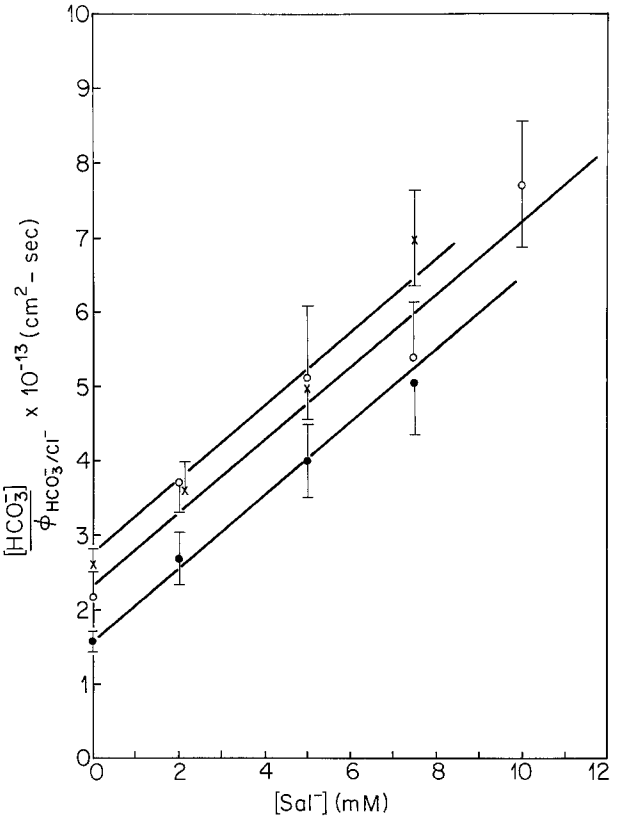

Fig. 6. Cornish-Bowden plot ($[HCO_3^-]/\phi_{HCO_3^-/Cl^-}$ *vs.* $[Sal^-]$) for HCO_3^-/Cl^- exchange across the human erythrocyte membrane at 37 °C. o – 2.2 mM $NaHCO_3$ in red cell suspensions; • – 4.4 mM $NaHCO_3$ in red cell suspensions; × – 8.8 mM $NaHCO_3$ in red cell suspensions. The approximately parallel lines show that salicylate inhibition of HCO_3^-/Cl^- exchange is strictly competitive

for each of the experimental bicarbonate (substrate) concentrations intersect at a point above the ascissa and to the left of the ordinate, compatible with competitive inhibition. The point of intersection of these lines corresponds to the K_I of 2.4 mM.

Figure 6 shows a Cornish-Bowden plot ($[HCO_3^-]/\phi_{HCO_3^-/Cl^-}$ *vs.* $[Sal^-]$) for HCO_3^-/Cl^- exchange across the red cell membrane at 37 °C. The approximately parallel lines for the different bicarbonate concentrations indicate that salicylate inhibition of RBC HCO_3^-/Cl^- exchange is strictly competitive.

Discussion

The experiments reported here were undertaken to quantitate the effects of salicylate on HCO_3^-/Cl^- exchange across the human red cell membrane at 37 °C. In the presence of a weak acid (such as salicylic acid), only a fraction of the initial dpH_o/dt measurable when a red cell suspension is mixed with more acidic buffered saline solution is effected via the Jacobs-Stewart cycle (in which HCO_3^-/Cl^- exchange kinetics are rate limiting). The other fraction of the initial dpH_o/dt is due to the movement of undissociated acid into the red cells. In these experiments, H · Sal flux occurs despite the fact that the pH at which these experiments were performed (~6.7) was much higher than the pK (2.97) of salicylic acid (*see* below and Dalmark & Wieth, 1972).

When red cell suspensions that have been pretreated with DIDS (a potent inhibitor of the anion exchange pathway) are mixed with acidic buffered saline solution in the presence of salicylate, HCO_3^-/Cl^- flux across the red cell membrane is markedly reduced, but the flux of undissociated H · Sal is essentially unaffected (*see* Table 1 and below). By subtracting the initial dpH_o/dt measured with DIDS-pretreated suspensions from that obtained with control suspensions, we were able to calculate the flux of HCO_3^-/Cl^- across the red cell membrane in the presence of varying concentrations of salicylate.

Fifty percent inhibition of the HCO_3^-/Cl^- exchange flux occurred at ~3.8 mM salicylate in our experiments (Fig. 4). This can be compared with ~5 mM salicylate reported by Deuticke (1970) and Schnell (1972) for 50% inhibition of phosphate and sulfate transfer across the red cell membrane, respectively. In a later review, Deuticke (1977) cites values of ~2.5 and ~1 mM for half-maximal inhibition of phosphate influx and sulfate efflux at 37 °C in human erythrocytes, respectively. The K_I of 2.4 mM (Fig. 5) that we obtained can be compared with a K_I of ~3 mM obtained by Schnell (1972) for sulfate transfer across the red cell membrane at 37 °C. The differences among these values are small considering the different anionic species and the variability in the systems used to study their exchange properties. Furthermore, our experiments were performed at pH ~6.7 compared to 7.2–7.3 in Schnell's (1972) work and ~7.35 in the study of phosphate transfer across the red cell membrane (Deuticke, 1970). It should also be pointed out that our data indicate a strictly competitive inhibition by salicylate of HCO_3^-/Cl^- exchange, whereas Schnell (1972) noted, with some surprise, that salicylate inhibition of sulfate exchange was noncompetitive.

On mixing DIDS-pretreated red cell suspensions containing salicylate with acid-buffered saline solution, not only is the rate of rise of extracellular pH of the mixture after stopping flow considerably slower than that in control suspensions, but the apparent extent of the rise is also smaller (Fig. 3). The apparent decrease in the total pH_o change after stopping flow in DIDS-treated red cell suspensions is due to the fact that H · Sal flux, predominant initially, rapidly declines due to the fall in extracellular $[Sal^-]$, resulting in the quasi-equilibrium pH_o seen in Fig. 3*b*. After the dissipation of the H · Sal flux at this point, H^+ transfer is subsequently effected almost solely via the Jacobs-Stewart cycle, rate limited by the markedly inhibited HCO_3^-/Cl^- exchange pathway. The continuing rise in extracellular pH beyond the quasi-equilibrated pH_o is therefore very slow and not seen in Fig. 3*b*. The fact that the eventual equilibrium pH_o using suspension A' was essentially the same as that using suspension A was verified in a number of experiments by observing pH_o over more than 10 min. Furthermore, the difference between plateau pH_o and quasi-equilibrated pH_o using suspensions A' increased as $[Sal^-]$ increased, indicating that the initial dpH_o/dt is in fact due to H · Sal flux.

In experiments where Cl^- was absent (i.e., red cell suspensions C containing 150 mM salicylate, and solution B'), the total flux ϕ_T was $\sim 1.7 \times 10^{-10}$ mole/cm^2-sec. When DIDS-pretreated red cell suspensions C', also containing no Cl^- and 150 mM salicylate, were mixed with acidic buffered saline solution B', the flux of H^+ ($\phi_{DIDS} \sim 2.1 \times 10^{-10}$ moles/cm^2-sec) was seen to be essentially the same. This indicates that DIDS has no observable effect on the movement of H · Sal across the red cell membrane, and suggests that the HCO_3^-/Sal^- exchange flux is negligible. It has previously been shown that the Cl^-/Sal^- exchange in a 150 mM sodium salicylate medium is virtually negligible at 0 °C (Burgin & Schatzmann, 1979).

Since the acidic buffered saline solutions B contained phosphate, it is possible that phosphate-anion exchange or phosphoric acid flux could be present

in our experiments. As noted above, the experiments with DIDS-pretreated red cell suspensions (containing salicylate but no Cl^-) showed no difference between ϕ_{DIDS} and ϕ_T. This suggests that $H_2PO_4^-/HCO_3^-$ exchange in our experiments was not significant. Furthermore, phosphate permeability of the red cell membrane is $\sim 1/10^4$ that for Cl^- (Deuticke, 1977). It is possible that some of the flux observed in our experiments interpreted as being due to H · Sal flux was due in part to the movement of phosphoric acid (H_3PO_4). Since the pK of phosphoric acid (~ 2) is much lower than the pK of salicylic acid (~ 3), it would be expected that, if these undissociated weak acids have about the same permeability through the RBC membrane, phosphoric acid flux would be only 1/10 that of salicylic acid. In experiments with DIDS-pretreated suspensions A' that contained no salicylate, ϕ_{DIDS} was noted to be extremely small (Table 1), suggesting that the movement of phosphoric acid across the red cell membrane in these experiments was not significant despite being present at the same concentration as in all the other experiments. Even if a fraction of the dpH_o/dt measured in our experiments were due to the movement of phosphoric acid, it would remain approximately constant under all conditions since the concentration of phosphate in all experiments was the same. This phosphoric acid flux would be included in dpH_o/dt in both terms of Eq. (6) and would thus not influence our calculated $\phi_{HCO_3^-/Cl^-}$ across the red cell membrane at various salicylate concentrations. Finally, no difference was noted in dpH_o/dt when phosphate was completely replaced by imidazole in solutions *B*.

Since salicylate is a primary metabolite of aspirin, a widely used drug, it is interesting to speculate on the possible *in vivo* implications of these results. The therapeutic range for salicylate (total) concentration is ~ 1.5–2.5 mM (Hart, 1976), with the toxic range > 3 mM. For these concentration ranges, the fraction of the free salicylate concentration *in vivo* is dependent not only on the total salicylate concentration itself but also on the serum albumin concentrations. In individuals with normal serum albumin levels (0.3–0.6 mM), the free (unbound) concentration of salicylate would be expected to be between 0.15–0.7 mM (Wosilait, 1976) when total salicylate is in the therapeutic range. In the toxic range, the concentration of free salicylate may be as high as 4–8 mM (Wosilait, 1976), where the inhibition of HCO_3^-/Cl^- exchange flux by salicylate might be physiologically significant (Fig. 4). Since it has been recently shown, both theoretically (Wieth & Brahm, 1978, 1980; Crandall & Bidani, 1981) and experimentally (Crandall et al., 1981), that inhibition of RBC HCO_3^-/Cl^- exchange might significantly affect CO_2 excretion, these data on the inhibition of HCO_3^-/Cl^- exchange flux by salicylate may be clinically relevant, especially in subjects with impaired cardiopulmonary function.

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