J. Membrane Biol. 52, 173–179 (1980)

The Journal of
Membrane Biology

Exchange of HCO_3^- for Monovalent Anions across the Human Erythrocyte Membrane

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Summary. A stopped-flow rapid reaction apparatus was used for measuring changes in extracellular pH (pH_o) of red cell suspensions under conditions where dpH_o/dt was determined by the rate of HCO_3^-/X^- exchange across the membrane ($X^- = Cl^-$, Br^-, F^-, I^-, NO_3^- or SCN^-). The rate of the exchange at 37 °C decreased for X^- in the order: $Cl^- > Br^- > F^- > I^- > NO_3^- > SCN^-$, with rate constants in the ratios 1:0.86:0.77:0.55:0.52:0.31. When HCO_3^- is exchanged for Cl^-, Br^-, F^-, NO_3^- or SCN^-, a change in the rate-limiting step of the process takes place at a transition temperature (T_T) between 16 and 26 °C. In I^- medium, however, no transition temperature is detected between 3 and 42 °C. Although T_T varies with X^-, the activation energies both above and below T_T are similar for Cl^-, Br^-, NO_3^- and F^-. The values of activation energy are considerably higher when $X^- = I^-$ or SCN^-. The apparent turnover numbers calculated for HCO_3^-/X^- exchange (except for $X^- = I^-$) at the corresponding T_T ranged from 140 to 460 ions/site ·sec for our experimental conditions. These findings suggest that: (i) HCO_3^-/X^- exchange for all X^- studied takes place via the rapid anion exchange pathway; (ii) the rate of HCO_3^-/X^- exchange is influenced by the specific anions involved in the 1:1 obligatory exchange; and (iii) the different transition temperatures in the Arrhenius diagrams of the HCO_3^-/X^- exchange do not seem to be directly related to a critical turnover number, but may be dependent upon the influence of X^- on protein-lipid interactions in the red blood cell membrane.

Recent investigations (Obaid & Crandall, 1979) have shown that HCO_3^-/Cl^- exchange across the human erythrocyte membrane takes place through the rapid anion transport pathway first described and extensively studied for halide self-exchange (Gunn, 1972; 1977; Gunn et al., 1973; Dalmark, 1976; Brahm, 1977). Cl^-/Cl^- and HCO_3^-/Cl^- exchange exhibit marked similarities in their dependence on pH and temperature, as well as in their response to specific inhibitors (Gunn et al., 1973; Gunn, Wieth & Tosteson, 1975; Brahm, 1977; Crandall, Obaid & Forster, 1978; Obaid & Crandall, 1979). In particular, both exchanges exhibit different activation energies above and below a transition temperature of about 16 °C and maximum and minimum fluxes occurring at about pH 7.6 and 5.0, respectively.

It has been known for some time that the presence of different monovalent anions can have a significant influence on the rate of membrane anion exchanges (Tosteson, 1959; Adrian, 1961; Hutter & Warner, 1967; Wieth, 1970). Furthermore, the characteristics of the self-exchange of anions other than chloride have been reported to differ from those of Cl^-/Cl^- exchange with respect to rates and temperature dependence (Tosteson, 1959; Wieth, 1970; Dalmark & Wieth, 1972; Brahm, 1977). Inhibition of Cl^-/Cl^- exchange by other small anions (Dalmark, 1976) and of HCO_3^-/Cl^- exchange by F^-, Br^- and I^- at low temperatures (Lambert & Lowe, 1978) have been reported.

In the present work, the temperature dependence of the exchange of HCO_3^- for several monovalent anions was studied in order to gain further insight into anionic hetero-exchanges across the human erythrocyte membrane. HCO_3^-/X^- exchange kinetics were determined between 3 and 42 °C by studying the rate of extracellular pH change in red cell suspensions due to flux through the Jacobs-Stewart cycle (Jacobs & Stewart, 1942). The experimental method for studying rapid HCO_3^- transport utilizing the Jacobs-Stewart cycle in the rapid reaction apparatus

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0022-2631/80/0052-0173 $01.40

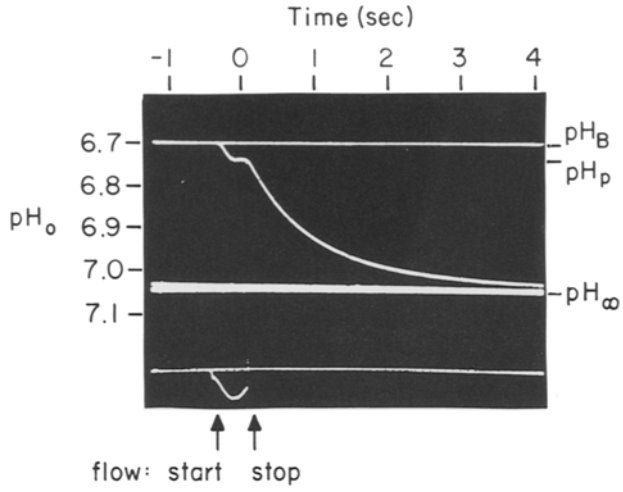

Fig. 1. Oscilloscope tracing of a typical experimental record at 37 °C. In this experiment, equal volumes of suspension A_{Br^-} (hematocrit = 16.3%; $pH_{A_{Br^-}}$ 7.7), containing 800 U/ml bovine carbonic anhydrase and 4.4 mM $NaHCO_3$, and solution B_{Br^-} (pH 6.7) were mixed in the stopped-flow rapid reaction apparatus. The lower trace indicates when the flow of reactants starts and stops. The upper trace represents the extracellular pH 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 discussion

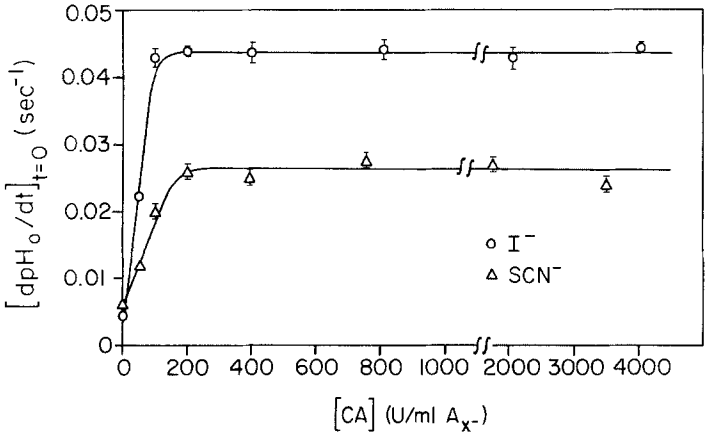

Fig. 2. Effect of extracellular bovine carbonic anhydrase concentration on the rate of pH equilibration via the Jacobs-Stewart cycle involving HCO_3^-/I^- or HCO_3^-/SCN^- exchange at 25 °C. The abscissa is bovine carbonic anhydrase concentration in suspension A_{X^-}. The ordinate is the initial rate of pH change (dpH_o/dt) obtained after mixing an equal volume of B_{X^-} with A_{X^-} containing a given concentration of bovine carbonic anhydrase

has been described in detail elsewhere (Chow, Crandall & Forster, 1976; Crandall et al., 1978; Obaid & Crandall, 1979; Obaid, Critz & Crandall, 1979).

Materials and Methods

Apparatus

The stopped-flow rapid reaction apparatus used in these experiments has been extensively described previously (Crandall, Klocke & Forster, 1971; Chow et al., 1976; Crandall et al., 1978). In the apparatus, equal volumes of a red cell suspension (A) and phosphate-buffered saline solution (B) are forced through a four-jet mixer (0.004 ml) into a 0.1-ml pH measuring chamber. A pH-sensitive glass electrode (Leeds and Northrup 117145) is used to follow the pH of the suspension ($A+B$) as a function of time after the instant of mixing.

Preparation of Solutions

Freshly drawn human blood from normal adults was used; coagulation was prevented with heparin (2 U/ml). The blood was centrifuged at $1,750 \times g$ for 10 min and the plasma and buffy coat were removed by aspiration. The remaining cells were washed 3 times at less than 5% hematocrit in 146.5 mM Na^+, 3.5 mM K^+, 150 mM X^- ($X^- = Cl^-$, Br^-, F^-, NO_3^-, I^- or SCN^-). Between centrifugations cells were allowed to stand for 30 min at room temperature with occasional agitation. The cells, washed and equilibrated with X^- as the only anion present in the extracellular medium, were then resuspended to 20% hematocrit in the same solution. NaOH was added to the suspension medium to reach a final pH of about 7.7. Bovine carbonic anhydrase (Sigma Chem. Co., # C-7500, St. Louis, Mo.) was added to the suspension to a concentration of 800 Wilbur-Anderson units/ml. Freshly prepared $NaHCO_3$ was then added to a final concentration of 4.4 mM, and the suspension (A_{X^-}) maintained in a closed tonometer thereafter.

The phosphate-buffered solution (B_{X^-}) was made up of 112.5 mM NaX, 15 mM KH_2PO_4, 15 mM Na_2HPO_4 (pH 6.7). In some experiments, suspension A_{X^-} (before adding the $NaHCO_3$) and solution B_{X^-} were degassed and equilibrated 3 times with 10% O_2, 90% N_2 gas mixture, but this procedure did not affect the results. All suspensions and solutions were prepared at room temperature.

Experimental Procedures

A calibration curve for the stopped-flow pH electrode apparatus was obtained before each experiment by flowing standard buffer

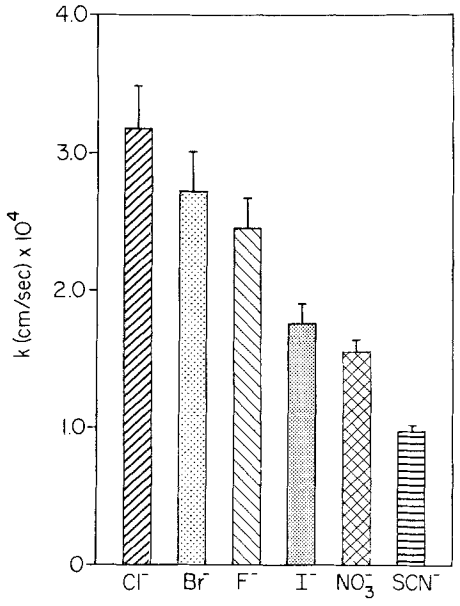

Fig. 3. Rate constants of HCO_3^-/X^- exchange at 37 °C. Each bar represents the average rate constant k (± SEM) from several experimental runs in each of two to six different populations of cells

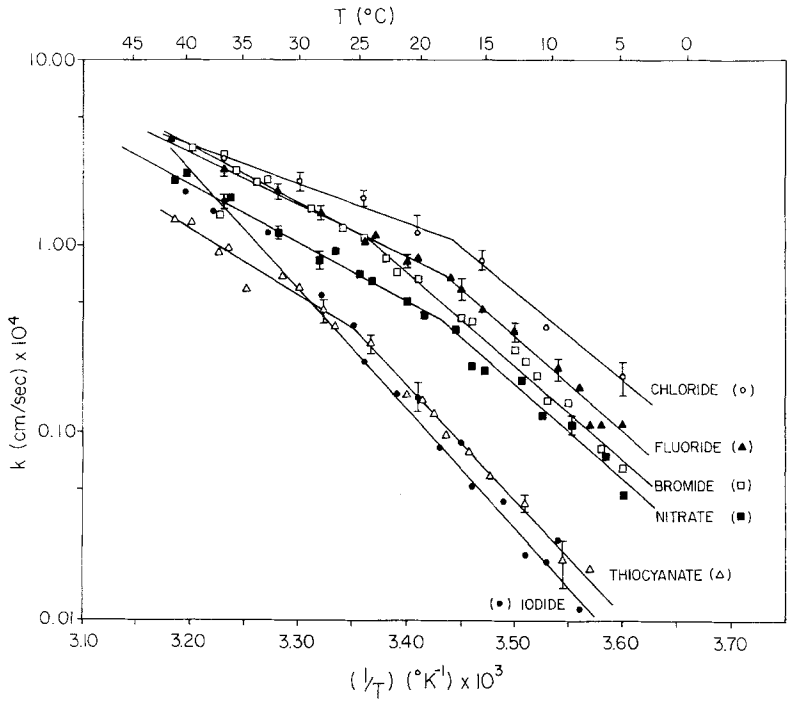

Fig. 4. Temperature dependence of HCO_3^-/X^- exchange. The logarithm of the rate constant k for HCO_3^-/X^- exchange is plotted against the reciprocal of the absolute temperature ($1/T$). Experiments were performed between 3 and 42 °C. Each point represents the average k from several experimental runs in each of two to ten different cell populations. The error bars indicate the standard error when three or more different cell populations were studied at the same temperature

solutions (50-B-X, Fisher Sci. Co., New Jersey) through the electrode chamber. Solution B_{X^-} was then passed through the electrode chamber, and the output stored on the oscilloscope screen as a reference value for each particular experimental run. Subsequently, equal volumes (2.5 ml) of A_{X^-} and B_{X^-} were driven through the mixing chamber at approximately constant velocity until flow was abruptly halted. pH equilibration in the mixture was recorded on the storage oscilloscope screen. When the reaction was complete, a photograph was taken of the experimental record. At the conclusion of each experiment, hematocrit of suspension A_{X^-} was measured in standard Wintrobe tubes after centrifugation at approximately 2,300 × g for 15 min. Intracellular pH (pH_{lys}) was measured on a lysate of cells from A_{X^-}, prepared by rapidly freezing and thawing packed cells twice in dry ice and acetone. pH_{lys}, as well as $pH_{A_{X^-}}$ and $pH_{B_{X^-}}$, were determined anaerobically (Radiometer BMS3 Mk2 blood gas machine). The supernatant from a mixture of equal volumes of A_{X^-} and B_{X^-} that had passed through the stopped-flow rapid reaction apparatus was titrated anaerobically (Radiometer ABU 13 autoburette with TTA 60 titration assembly) to obtain the buffer capacity of the extracellular fluid as a function of pH. Experimental runs were performed at temperatures between 3 and 42 °C.

Computations

The initial flux Φ of HCO_3^- in exchange for X^- was determined from the dpH/dt observed in the extracellular fluid of the mixture immediately after stopping flow in the rapid reaction apparatus (Obaid & Crandall, 1979). A rate constant k for HCO_3^-/X^- exchange was defined by

$$k = \Phi/(\{HCO_3^-\}_t - \{HCO_3^-\}_o)t = 0$$

where the denominator (initital HCO_3^- concentration difference) was calculated using equations described previously (Chow et al., 1976; Crandall et al., 1978). The values of Arrhenius activation energy (E_a) of HCO_3^-/X^- exchange between 3 and 42 °C were calculated by linear regression analysis of the relationship between the natural logarithm of k and the reciprocal of the absolute temperature. The turnover number (T_N) of HCO_3^-/X^- exchange at

Table 1. Transition temperature (T_T), Turnover number at T_T (T_N), and activation energies of HCO_3^-/X^- exchange in human erythrocyte suspensions between 3–42 °C

X^-	n[a]	T_T (°C)	T_N (ions/site·sec)	E_{a_I}[b] (kcal/mol)	$E_{a_{II}}$[b] (kcal/mol)
CL^-	10	16.2±0.4	3.8±0.3×10²	11.7±0.1	19.6±0.2
BR^-	4	23.6±0.1	4.6±0.1×10²	14.0±0.7	23.7±0.7
F^-	4	17.5±0.4	2.6±0.2×10²	12.8±0.5	23.5±0.6
NO_3^-	4	17.5±0.5	1.5±0.1×10²	14.1±0.1	23.0±1.2
SCN^-	4	25.5±0.6	1.4±0.2×10²	17.2±1.4	28.1±2.1
I^-	5	–	–	28.2±0.7[c]	28.2±0.7[c]

Errors represent standard errors of the mean.

[a] n indicates the number of different populations of cells studied.

[b] E_{a_I} is activation energy above T_T; $E_{a_{II}}$ is activation energy below T_T.

[c] Since no T_T was observed for I^-, E_{a_I} and $E_{a_{II}}$ are shown with the same value.

the transition temperature (T_T) at which a change in E_a takes place was calculated from the HCO_3^-/X^- flux at that temperature, assuming 10^6 transport sites/cell (Zaki et al., 1975; Lepke et al., 1976) and 6.023×10^{23} ions/mole.

Results

Figure 1 shows a typical experimental record obtained when solution B_{Br^-} (pH 6.70) was mixed with suspension A_{Br^-} (16.3% hematocrit; pH 7.70) at 37 °C. Similar records were obtained for all X^- studied. The upper tracing represents the pH of the fluid in the measuring chamber as a function of time. The lower trace indicates when flow of reactants starts and stops. Before flow starts, solution B_{Br^-} is in the measuring chamber (pH_B). During flow, the "plateau" pH (pH_p) is that of the extracellular fluid after rapid redistribution of HCO_3^- into the cells has taken place, but before significant HCO_3^-/X^- exchange has had time to occur. After flow stops, the extracellular pH (pH_o) of the mixture in the measuring chamber rises toward its final equilibrated value (pH_∞ 7.06) as the Jacobs-Stewart cycle effects the transfer of H^+-equivalents from outside to inside the erythrocytes.

In order to study HCO_3^-/X^- exchange kinetics using the Jacobs-Stewart cycle, it is necessary to ensure that the cycle is rate-limited by the exchange. This requires, among other factors, acceleration of the extracellular dehydration of H_2CO_3 by exogenous bovine carbonic anhydrase. Some monovalent anions, and especially I^- and SCN^-, have been reported to markedly inhibit carbonic anhydrase activity (Maren, Rayburn & Liddell, 1976; Wright & Diamond, 1977). We therefore studied the rate of the Jacobs-Stewart cycle as a function of added bovine carbonic anhydrase concentration, when $X^- = I^-$ or SCN^-. The results, shown in Fig. 2, indicate that at very low extracellular enzyme concentration, dpH_o/dt increases with increasing enzyme concentration. At carbonic anhydrase concentrations greater than 200 U/ml, however, dpH_o/dt remains constant. These findings show that the extracellular dehydration of H_2CO_3 is not rate-limiting at the exogenous carbonic anhydrase levels (800 U/ml) used in the experiments for the investigation of HCO_3^-/X^- exchange.

Figure 3 shows the rate of HCO_3^-/X^- exchange at 37 °C. The rate constant decreases in the order $Cl^- > Br^- > F^- > I^- > NO_3^- > SCN^-$. This sequence is the same as that reported for X^-/X^- exchange (Tosteson, 1959; Wieth, 1970) and for inhibition of Cl^-/Cl^- and $SO_4^=/SO_4^=$ exchange by X^- (Wieth, 1970; Dalmark, 1976).

The relationship between the rate constant for HCO_3^-/X^- exchange and the inverse of the absolute temperature between 3 and 42 °C is shown in Fig. 4. These Arrhenius relationships show a transition temperature (T_T) at approximately 17 °C for Cl^-, F^- and NO_3^-, and 25 °C for Br^- and SCN^-. However, for $X^- = I^-$, only one straight line is obtained over the whole range of temperatures studied. Similar behavior as a function of temperature has been observed for X^- self-exchange with $X^- = Cl^-$, Br^-, I^- and SCN^- (Dalmark & Wieth, 1972; Brahm, 1977). Table 1 summarizes the values of activation energies for HCO_3^-/X^- exchange obtained by linear regression analysis of the curves presented in Fig. 4, as well as the turnover number T_N for HCO_3^-/X^- exchange at T_T.

Discussion

The experiments reported above were performed in a stopped-flow pH electrode rapid reaction apparatus. This technique is useful for measuring the rate of HCO_3^-/X^- exchange, provided that it can be shown that HCO_3^- and X^- move through the Jacobs-Stewart cycle (Jacobs & Stewart, 1942) and that their exchange is the rate-limiting step (Chow et al., 1976). The different steps involved in this cycle have been extensively described for HCO_3^-/Cl^- exchange (Chow et al., 1976; Obaid & Crandall, 1979; Obaid et al., 1979). CO_2 diffusion across the membrane, while perhaps limited by unstirred layer effects (Gutknecht, Bisson & Tosteson, 1977), is very much faster (Gros & Moll, 1971) than the other steps in the cycle. The catalysis of CO_2 hydration-dehydration reactions by both bovine and human carbonic anhydrases, however, has been shown to be inhibited by some small anions, including Cl^-, Br^-, F^-, I^-, NO_3^- and SCN^- (Maren et al., 1976). The results shown in Fig. 2 indicate that the extracellular reactions in the presence of the added bovine enzyme were not rate-limiting under the conditions of our experiments. While we have no direct measurements of the intracellular carbonic anhydrase activity, it is normally (in Cl^- medium) so high (Silverman, Tu & Wynns, 1976; Itada & Forster, 1977) as to make it extremely unlikely that some further inhibition (e.g., by I^- or SCN^- instead of Cl^-) could slow the intracellular hydration reaction to the extent that it becomes rate-limiting in the Jacobs-Stewart cycle. Furthermore, since the activity of the added bovine carbonic anhydrase remained sufficient in the presence of I^- or SCN^- to accelerate the extracellular reactions to the point where they were not rate-limiting (Fig. 2), and since bovine carbonic anhydrase and human red cell carbonic anhydrase C are very similar enzymes (Maren et al., 1976), it would be surprising if the intracellular enzyme did not also retain sufficient ac-

tivity to prevent the intracellular reactions from becoming rate-limiting.

As shown in Fig. 3, the rate of HCO_3^-/X^- exchange at 37 °C decreases for X^- in the order: $Cl^- > Br^- > F^- > I^- > NO_3^- > SCN^-$. This sequence is the same as that reported for X^-/X^- exchange, and for Cl^-/Cl^- exchange at room temperature and $SO_4^=/SO_4^=$ exchange at 38 °C in the presence of X^- (Tosteson, 1959; Wieth, 1970). The different sequence of rates of X^- self-exchange at 0 °C found by Dalmark and Wieth (1972) is most likely due to the differences in temperature dependence that characterize the transport of these various anions across the red blood cell membrane (*see* Fig. 3 and below). It is interesting to note that the inhibition of X^- transport by other ions is not confined to red cells, since the outflow of $^{36}Cl^-$ from frog muscle is inhibited by NO_3^- (Adrian, 1961), Br^- and I^- (Hutter & Warner, 1967).

Our experiments differ somewhat from those previously reported in the literature, in that we are measuring net HCO_3^- movement when X^- is the major anion in both intra- and extracellular mediums. This establishes a situation in which HCO_3^- must compete with X^- on the internal side of the membrane. As a result, it is not surprising that the rates of HCO_3^-/X^- exchange decrease in the same sequence as the increasing inhibition by X^- of Cl^-/Cl^- and $SO_4^=/SO_4^=$ exchange. Furthermore, in our experiments the loading of the exchange site on the external side of the membrane depends almost entirely upon the affinity of X for the transport site (although perhaps with some competition from phosphate). Consequently, the similarity between the rates of HCO_3^-/X^- and X^-/X^- exchanges is also expected.

While the anion sequences discussed above are the same in our work as in previous reports, quantitative differences between HCO_3^-/X^- and X^-/X^- exchange rates have been observed. In particular, the ratio of Cl^-/Cl^- exchange rate to that of I^-/I^- at 0 °C is about 200 (Wieth, 1970), while the ratio of HCO_3^-/Cl^- exchange rate to that of HCO_3^-/I^- is calculated to be about 15 at the same temperature and only about 2 at 37 °C (Fig. 4). One possible explanation of these quantitative differences in the flux ratios is that, since the exchanges are saturable processes, the various experiments were performed at anion concentrations differing from the K_m or K_i of the process by variable amounts. This possibility makes comparisons of absolute fluxes of different anion exchanges somewhat risky. There are no data available on the K_m for the X^- self-exchanges studied here (other than Cl^- and Br^-) (Gunn et al., 1973; Brahm, 1977) or on the K_i for X^- inhibition of HCO_3^- transport under the conditions of our experiments.

Our studies of the temperature dependence of HCO_3^-/X^- exchange demonstrated that for $X^- = Cl^-$, Br^-, F^-, NO_3^- and SCN^-, the Arrhenius relationships exhibit two slopes, suggesting a change in the rate-limiting step of the transport process within the range of temperature studied. However, when $X^- = I^-$, the Arrhenius diagram exhibits only one straight line. Furthermore, the transition temperatures (T_T) for $X^- = Cl^-$, Br^-, F^-, NO_3^- and SCN^- are not all the same (Table 1).

There are several possible explanations for the differences in T_T observed for the various HCO_3^-/X^- exchanges. Chapman et al. (1977) have shown that the temperature at which a phase transition in a lipid bilayer occurs depends upon the salt with which it is in contact. The identity of both the anion and the cation may affect the phase transition. I^- and SCN^- had the greatest influence on the transition temperature compared to the other anions. Chapman et al. (1977) suggest that these anionic effects can be explained qualitatively by differing interactions of the various anions with the polar regions of the lipid bilayer, leading to an alteration in the packing of polar and nonpolar components. The effects of SCN^- were attributed in particular to its tendency to accumulate at the polar lipid-water interface (Chapman et al., 1977). These interactions between lipid bilayers and various anions lead to the reasonable expectation of some effects of the same ions on the structure of membrane lipids, especially when both sides of the cell membrane are exposed to them. It has often been suggested (Chapman, 1975; Warren et al., 1975; Ross & McConnell, 1978; Obaid & Crandall, 1979; Obaid et al., 1979) that such changes in lipid structure could also alter the lipo-protein assembly of the cell membrane. If so, the kinetics of the anion transport pathway, which has been shown to be related to the Band 3 protein in the erythrocyte membrane (Rothstein, Cabantchik & Knauf, 1976; Cabantchick, Knauf & Rothstein, 1978), might be altered. These considerations could help explain the variability in T_T for $X^- = Cl^-$, Br^-, F^-, NO_3^- and SCN^-, and the absence of T_T for $X^- = I^-$.

Although T_T varies with X^-, the slopes of the Arrhenius diagrams both above and below T_T are not significantly different for Cl^-, Br^-, NO_3^- and F^-, suggesting that the transport mechanism is the same for all these X^- both above and below their transition temperatures. The values of activation energies associated with these exchanges (Table 1) are in the range expected for a carrier-mediated model (Parsegian, 1969; Gunn et al., 1973). When $X^- = I^-$ or SCN^-, anions which interact more strongly with membrane components (Scatchard & Black, 1949; Chapman et al., 1977), the values of activation energies for the exchange are significantly higher. These

results are not surprising, considering the extremely high affinity of these anions for charged groups of the proteins (Scatchard & Black, 1949) and their disruptive action on the structure of lipid bilayers (Chapman et al., 1977).

Another possible explanation for the behavior of T_T is that the affinity of each X^- for the transport site varies with temperature on the inside of the membrane in a manner different from that of HCO_3^- and other X^-, or on the outside of the membrane in a manner different from that of phosphate and other X^-. Unfortunately, no data are available on these affinities except for the fact that the K_m for chloride self-exchange does not vary markedly over the temperature range studied (Brahm, 1977). However, if the affinity of X^- with increasing temperature increases relative to that of HCO_3^- inside the cell or decreases relative to that of phosphate outside the cell, it is possible that binding of HCO_3^- inside or X^- outside is rate-limiting above T_T while translocations are rate-limiting below T_T. Differences of affinity behavior among the various X^- would then lead to differences in T_T. However, our values for T_T for HCO_3^-/Cl^- and HCO_3^-/Br^- exchange are almost identical to those for Cl^-/Cl^- and Br^-/Br^- self-exchange (Brahm, 1977) even though no X^- or phosphate was present in the self-exchange experiments. Thus, although an explanation based on changes of affinity of X^- for the transport site with temperature cannot be ruled out, it is considerably less likely than membrane lipid alterations to be the underlying mechanism for the presence and variability of T_T.

Brahm (1977), studying Cl^- and Br^- self-exchanges as functions of temperature, observed transition temperatures in the Arrhenius relationships (very similar to ours) which appeared at 15 °C for Cl^- and 24 °C for Br^-. Based on his experimental evidence that the rate of turnover at which these transitions take place was practically the same for both anions ($3.5–3.7 \times 10^3$ ions/site·sec), Brahm suggested, as a working hypothesis, that the activation energy changes when a critical turnover number (T_N) is reached. This could occur if the overall transport rate is determined by two "reactions" with different activation energies, in such a way that one step with high activation energy is rate-limiting at low temperatures, whereas another step with a lower activation energy becomes rate-limiting at high temperatures. While our T_T for Br^- and Cl^- are very close to those of Brahm (1977), our overall results do not support this hypothesis. I^- does not exhibit a transition temperature at all, despite having an apparent turnover number at 33 °C that is greater than the T_N for all the other anions studied under our experimental conditions. Furthermore, the apparent T_N for $X^- = Cl^-$, Br^-, F^-, NO_3^- and SCN^- at their respective T_T varies between 140 and 460 ions/site·sec. Finally, although the T_N under the conditions of our experiments are all of the same order of magnitude, they are one order of magnitude less than those reported by Brahm (1977) for Cl^-/Cl^- and Br^-/Br^- exchange (probably due to our working at lower absolute concentrations) even though the T_T are almost identical. Although, as noted above, there is some uncertainty in comparing absolute flux rates, these findings make it unlikely that a critical T_N determines T_T for a given anion exchange.

In summary, we conclude that:

1) HCO_3^-/X^- exchange across the human erythrocyte membrane for all X^- studied takes place via the rapid anion transport pathway.

2) The rate of HCO_3^-/X^- exchange is a function of the two anions involved in the 1:1 obligatory transport, decreasing at 37 °C in the order $X^- = Cl^- > Br^- > F^- > I^- > NO_3^- > SCN^-$.

3) The different transition temperatures of the HCO_3^-/X^- exchange are unlikely to be directly related to a critical turnover number and may be dependent upon the influence of X^- on protein-lipid interactions in the red blood cell membrane, although we cannot rule out the possibility that different T_T result from differing behavior of the affinity of the various X^- for the transport site with temperature.

We thank Dr. R.E. Forster for his continuing support, and Dr. Akhil Bidani for his assistance with the computations. This work was supported in part by research grants AHA 75-992 and NHLBI 19737. E.D. Crandall is the recipient of Research Career Development Award HL00134.

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Received 11 June 1979; revised 24 October 1979