J. Physiol. (1978), **275**, *pp.* 51–63
With 6 text-figures
Printed in Great Britain

CHLORIDE/BICARBONATE EXCHANGE IN HUMAN ERYTHROCYTES

BY ANN LAMBERT AND A. G. LOWE

From the Department of Biochemistry, University of Manchester, Manchester M13 9PL

(*Received 17 June 1977*)

SUMMARY

1. The exchange of chloride and bicarbonate across the human erythrocyte membrane has been followed by measuring the changes in extracellular pH which occur when chloride-rich erythrocytes are added to chloride-free media containing varying concentrations of bicarbonate and carbonic anhydrase.

2. The dependence of the rate of chloride/bicarbonate exchange on the extracellular concentration of bicarbonate was consistent with the existence of a saturable membrane anion transporter exhibiting Michaelis–Menten kinetics. In a medium containing sodium gluconate buffered to pH 7·0 with imidazole–malate the K_m for bicarbonate activation of transport was 0·39 ($\pm$ 0·03) mM and the V_{max} was 2033 ($\pm$ 80) m-mole anions exchanged/3×10^{13} cells. min, at 10 °C.

3. Chloride/bicarbonate exchange was temperature-dependent with an Arrhenius activation energy of 19·4 kcal/mole in the temperature range 2–10 °C.

4. Exchange of intracellular chloride for extracellular bicarbonate was inhibited by the presence of extracellular halides. Inhibition by chloride, bromide and fluoride was competitive and the affinity of the transport system decreased in the order $HCO_3^- > Cl^- > Br^- > F^-$. The kinetics of inhibition by iodide were complex, but inhibitory effects of low concentrations of iodide were less than those of chloride and bromide.

INTRODUCTION

The kinetics of the self-exchange of chloride and other halide anions across the human erythrocyte membrane have been studied extensively (Dalmark and Wieth (1972), Dalmark (1975, 1976), Gunn, Dalmark, Tosteson & Wieth (1973), Tosteson (1959), Cousin and Motais (1976)) but less detailed investigations have been made of the physiologically important transmembrane exchange of chloride for bicarbonate (Luckner (1939), Piiper (1964), Dalmark (1972) Dirken & Mook (1931)). Recently there has been renewed interest in the latter exchange process and Aubert & Motais (1975) have monitored chloride/bicarbonate exchange by following changes in cell volume spectrophotometrically and Chow, Crandall & Forster (1976) have utilized the changes in extracellular pH associated with the Jacobs/Stewart cycle to follow these anion movements. Surprisingly, however, the latter workers found no evidence of saturation of the anion carrier by bicarbonate. In this paper we describe the development of a method in which the operation of the Jacobs/Stewart cycle can be studied as a function of bicarbonate concentration in a simple medium free from

interfering halide anions. This has made it possible for us to demonstrate saturation of the anion carrier system by low concentrations of bicarbonate, and also to establish the relative affinities of the carrier for chloride, fluoride, bromide and iodide.

METHODS

Preparation of cells

Heparinized or acid-citrate-dextrose human blood was used within 7 days of collection. Erythrocytes were sedimented by centrifugation at 2000 *g* for 5 min then washed three times with 0·165 M-NaCl with removal of the buffy coat and plasma or saline by aspiration after each centrifugation. Cells (at a haematocrit of about 50%) were then adjusted to pH 7·3–7·4 at 20 °C by titration with 0·33 M-NaOH, before packing by centrifugation at 26,000 *g* for 20 min and removal of the supernatant and surface layer of cells by aspiration. This yielded a pellet of packed cells with 4% extracellular fluid (Funder & Wieth, 1967). The intracellular pH of these cells measured after suspension in 0·165 M-NaCl and lysis with 1% (v/v) Triton X-100 was found to be 7·0–7·2. The intracellular chloride concentration was measured by coulometric titration using a Radiometer chloride titrator after precipitation of protein with perchloric acid.

Media for chloride/bicarbonate exchange experiments

Experiments were carried out in media based on either 0·165 M-sodium gluconate or 0·330 M-sucrose. These media were buffered by dilution with isosmolar solutions of either *N*,*N*-bis (2-hydroxyethyl)-2-amino-ethane sulphonic acid (BES) adjusted to pH 7·0 (10 °C) with NaOH, or imidazole adjusted to pH 7·0 (10 °C) with monosodium malate to give final concentrations of 20 mM-BES or 20 mM-imidazole in the gluconate or sucrose. When other anions were used they were added as sodium salts and replaced Na gluconate or sucrose. Carbonic anhydrase (0·67 μM) was present in all media.

Efflux experiments

Experiments were carried out in a 40 ml glass vessel jacketed with water at the required temperature and solutions were stirred with a magnetic flea. 15 ml of the selected medium was adjusted to the required bicarbonate concentration by addition of freshly prepared 30 mM-$NaHCO_3$ and equilibrated with the appropriate mixture of CO_2 and air. About 0·5 ml of this medium was withdrawn into a 1 ml. syringe with an extension tube and, after introduction of an air bubble spacer, 0·25 ml packed red cells were also loaded into the syringe. These red cells and the previously withdrawn solution were rapidly injected into the vigorously stirred efflux medium. The pH of this medium was recorded continuously before and after addition of the cells. Before carrying out any series of experiments a check was made to establish that observed changes in extracellular pH were not due to a difference between the pH of the cell suspension and the pH of the medium. For this purpose cells were added to 15 ml of the medium which had been made CO_2-free by evacuating and bubbling with CO_2-free nitrogen, and which contained acetazolamide (Diamox, 0·067 mM) to inhibit hydration of any residual extracellular CO_2 by carbonic anhydrase from lysed cells. In order to achieve a situation in which no significant pH change occurred when cells were added to a medium at pH 7·0 (10 °C) it was found necessary, in practice, to use cells which had previously been adjusted to pH 7·3–7·4 at 20 °C, while at a haematocrit of 50%. The use of cells which had been adjusted to higher or lower pH led to significant changes in extracellular pH on addition to the CO_2-free extracellular medium at pH 7·0. Recordings were made using a Beckman-Research pH meter linked to a WW-recorder, a Russel SL pH electrode, a Beckman type RLB reference electrode, and a 3 M-KCl–agar bridge.

Experiments in which chloride efflux from erythrocytes was measured using a chloride electrode were carried out in the same way as those in which extracellular pH was monitored, except that a Ag/AgCl electrode replaced the pH electrode and the 0·1 mM-NaCl was added to extracellular medium before addition of the cells. The electrode was calibrated by measuring responses to various concentrations of NaCl in the medium used in the efflux experiments.

At the end of each flux measurement the amount of haemoglobin present in the erythrocyte suspension was measured after conversion to reduced pyridine haemochromogen, using the method of De Duve (1948). The results obtained were used to calculate the number of erythrocytes used in each experiment assuming a haemoglobin content of 29 pg/erythrocyte.

RESULTS

Fig. 1 illustrates the changes in extracellular pH which occurred after addition of packed erythrocytes to chloride-free media of various compositions. When bicarbonate and carbonic anhydrase were present there was a rapid acidification of the medium but when cells were added to a nominally bicarbonate-free medium containing Diamox, very little change in pH took place. These observations can be explained by operation of the Jacobs/Stewart cycle (below) in a way similar to that described by Chow *et al.* (1976) but with acidification and not alkalinization of the medium.

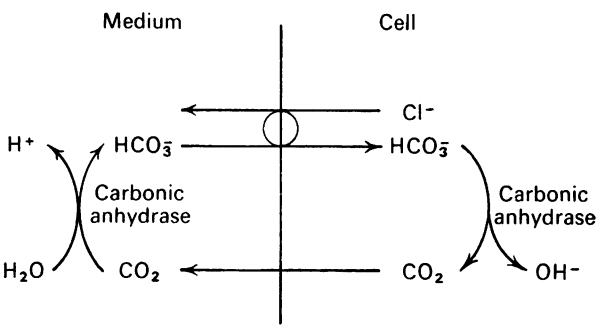


Fig. 1 also shows that addition of 0·067 mM-SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid) to a bicarbonate-containing medium prevented the pH change usually associated with addition of erythrocytes. Since SITS is known to inhibit the anion-exchange system of the erythrocyte membrane (Cabantchik & Rothstein, 1972) this is consistent with the hypothesis that changes in extracellular pH result from movements of chloride and bicarbonate through the anion transport system.

Several conditions must be satisfied before the rate of acidification of the medium can be considered to represent the rate of exchange of cellular chloride for bicarbonate.

1. The exchange must be the rate-limiting step in the Jacobs/Stewart cycle. Exchange of CO_2 across cell membranes is very fast (Gros & Moll, 1971), but uncatalysed hydration of CO_2 is slow relative to the observed acidification. However the high intracellular concentration of carbonic anhydrase makes hydration very fast inside the cell and carbonic anhydrase was added to the medium at a concentration (0·67 μM) sufficient to make extracellular CO_2-hydration at least five times faster than the observed rates of change of pH in the medium.

2. The observed rates of acidification of the medium must be closely related to the rates of chloride efflux from the cells and not substantially reduced by movements of protons or hydroxyl ions down the trans-membrane pH gradient. To investigate this the rate of chloride efflux, measured using a Ag/AgCl electrode, was compared to the rate of acidification of the medium following addition of 0·25 ml erythrocytes to 15 ml imidazole–malate buffered gluconate medium (pH 7·0) containing 0·5 mM bicarbonate. The rate of chloride efflux was $1·04 \pm 0·08$ μmole/sec. 0·25 ml cells (S.D., $n = 6$), while the rate of acidification of the medium was $1·01 \pm 0·06$ μmole acid/sec. 0·25 ml cells (S.D., $n = 6$). This gives a chloride to proton ratio of 1:0·97 and this stoicheiometry, together with the fact that the final extracellular pH after completion of exchange was stable for at least 10 sec under normal conditions, implies that the rates of permeation of protons and hydroxyl ions across the cell membrane are much slower than chloride/bicarbonate exchange.

3. The change in intracellular pH that occurs during chloride/bicarbonate exchange must not be great enough to inhibit the transport process. To investigate this suspensions of erythrocytes in unbuffered isotonic NaCl were lysed by addition of Triton X-100 (1% v/v) and the lysates

were then titrated with 1 M-NaOH. The starting pH of lysates of cells which had been previously titrated to an extracellular pH of 7·35 (20 °C) was 7·08 + 0·05 (S.D., n = 8) at 10 °C. Increases in the pH of the lysates caused by additions of NaOH equivalent to 10 and 40% of cell chloride were 0·17 ± 0·01 pH (S.D., n = 8) and 0·80 ± 0·02 pH (S.D., n = 8) respectively. The rate of chloride/chloride exchange has been found to be almost independent of pH in the pH range 6·5–7·5 (Gunn *et al.* 1973) and therefore, if as seems likely, chloride/bicarbonate and chloride/chloride exchanges use the same membrane transport system, the above alterations in pH are unlikely to affect the rate of chloride/bicarbonate exchange during the period when the initial rate of change of extracellular pH is measured.

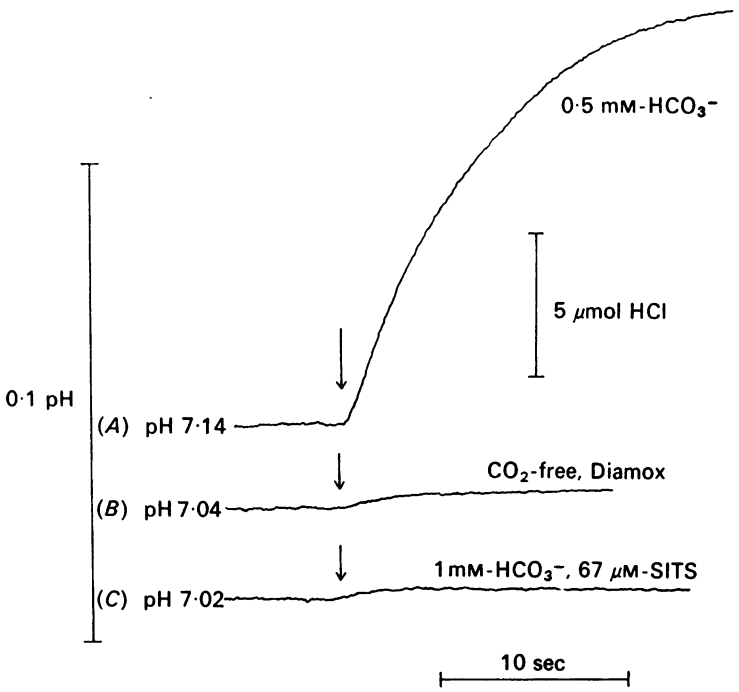


Fig. 1. pH changes associated with the addition of erythrocytes to media of different compositions. The upper trace, *A*, describes the change in pH of 15 ml gluconate–imidazole medium (pH 7·14, 10 °C) containing 0·5 mM-$NaHCO_3$ before and after addition of 0·25 ml packed erythrocytes at the time indicated by the arrow. The upward curve of the trace indicates acidification of the medium. The lower traces show that only small changes in pH occurred after additions of 0·25 ml packed erythrocytes to a similar medium containing either (*B*), no bicarbonate, CO_2 or carbonic anhydrase and 0·067 mM-acetazolamide (Diamox), or (*C*), 1 mM-$NaHCO_3$ and 0·067 mM-SITS.

Dependence of chloride/bicarbonate exchange on bicarbonate concentration

Fig. 2 illustrates the dependence of the initial rate of chloride-bicarbonate exchange (measured by monitoring changes in extracellular pH) on the extracellular concentration of bicarbonate in a Na gluconate medium buffered/with imidazole–malate (pH 7·0). The result is consistent with the existence of a saturable anion carrier with classical Michaelis-Menten kinetics. As indicated in Table 1, values for V_{max} and K_m (bicarbonate) for bicarbonate/chloride exchange were fairly similar in imidazole-malate and BES-Na buffered gluconate, but higher values for both these parameters were found in the sucrose media. The fact that the K_m for bicarbonate is lower in the gluconate than in the sucrose media suggests that gluconate does not

inhibit bicarbonate/chloride exchange by completing for the anion transport site and so it seems probable that the different V_{max} found in gluconate and sucrose are attributable to the different ionic strengths of the media. In subsequent experiments gluconate was preferred to sucrose in order to provide physiological ionic strength, and imidazole-malate was used as buffer because malate has been reported by Aubert & Motais (1975) to have no effect on erythrocyte anion transport.

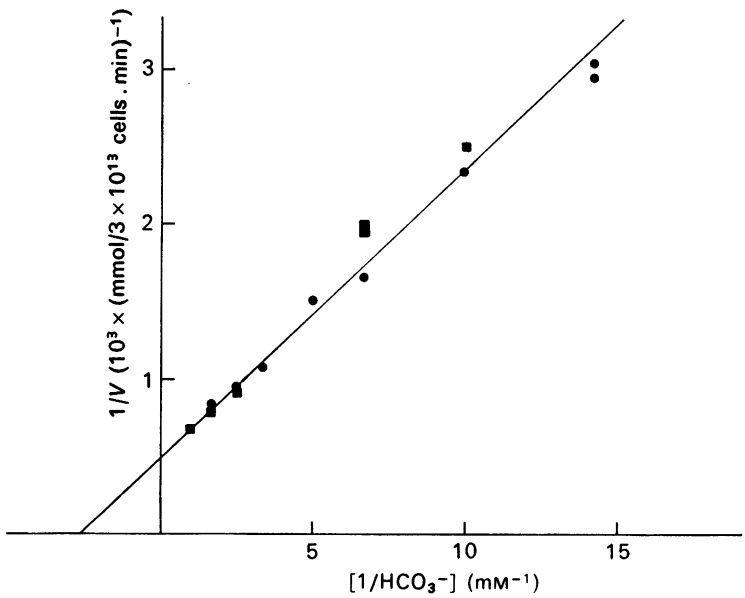


Fig. 2. Lineweaver-Burk plot illustrating the dependence of the rate of chloride/bicarbonate exchange on the extracellular bicarbonate concentration. Rates of chloride/bicarbonate exchange were calculated from the rates of acidification of the medium (15 ml gluconate–imidazole medium, pH 7·02–7·07, 10 °C) at varying concentrations of added bicarbonate following addition of 0·25 ml packed erythrocytes. The symbols, ■ and ●, represent the results of two similar experiments carried out with different batches of erythrocytes.

TABLE 1. Kinetic parameters for chloride/bicarbonate exchange across the erythrocyte membrane in different media. V_{max} and K_m, and their standard errors, were calculated from rates of acidification of media (15 ml initial pH 7·00–7·14) containing varying concentrations of bicarbonate following addition of packed erythrocytes (0·25 ml). Parameters were computed using the method of Wilkinson (1961).

Medium	V_{max} (mmol/3 × 10^{13} cells.min)	K_m for bicarbonate (mM) (± S.E.)
Gluconate–imidazole	2033 ± 80	0·39 ± 0·03
Gluconate–BES	1659 ± 98	0·30 ± 0·05
Sucrose–imidazole	2485 ± 328	0·44 ± 0·12
Sucrose–BES	3562 ± 402	0·73 ± 0·15

Inhibition of chloride/bicarbonate exchange by halide anions

The presence of halide anions in the extracellular medium was found to reduce the rate of chloride/bicarbonate exchange. As shown in Fig. 3*A–C*, Dixon plots for data from the inhibitory effects of chloride and bromide were linear and indicated

competitive inhibition in both cases with K_i of 4 mM for chloride and 7 mM for bromide. Lineweaver-Burk plots of the dependence of the rate of chloride/bicarbonate exchange on bicarbonate concentration with and without extracellular chloride at 5 mM also indicated competitive inhibition with a K_i for chloride of 4 mM. Measurements of inhibition by fluoride were complicated by the fact that the final extracellular pH

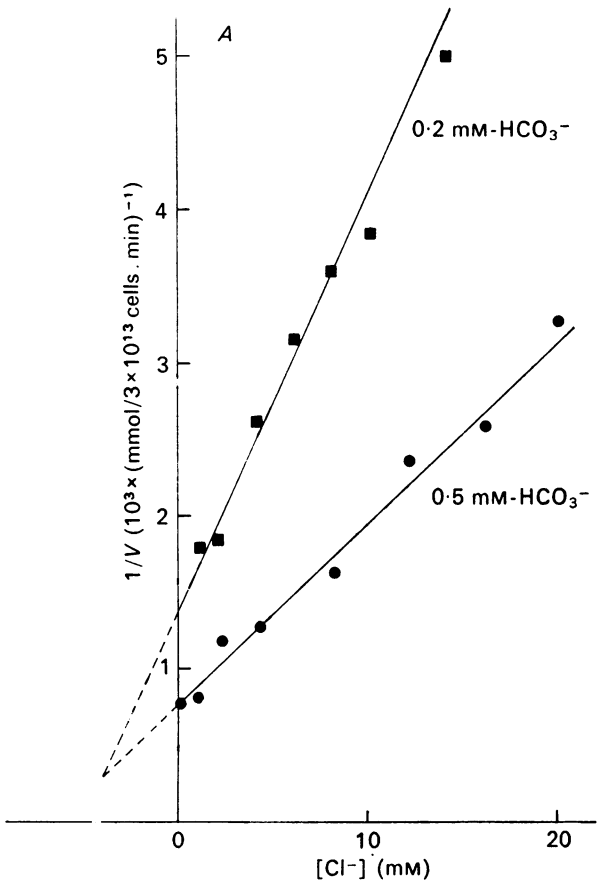
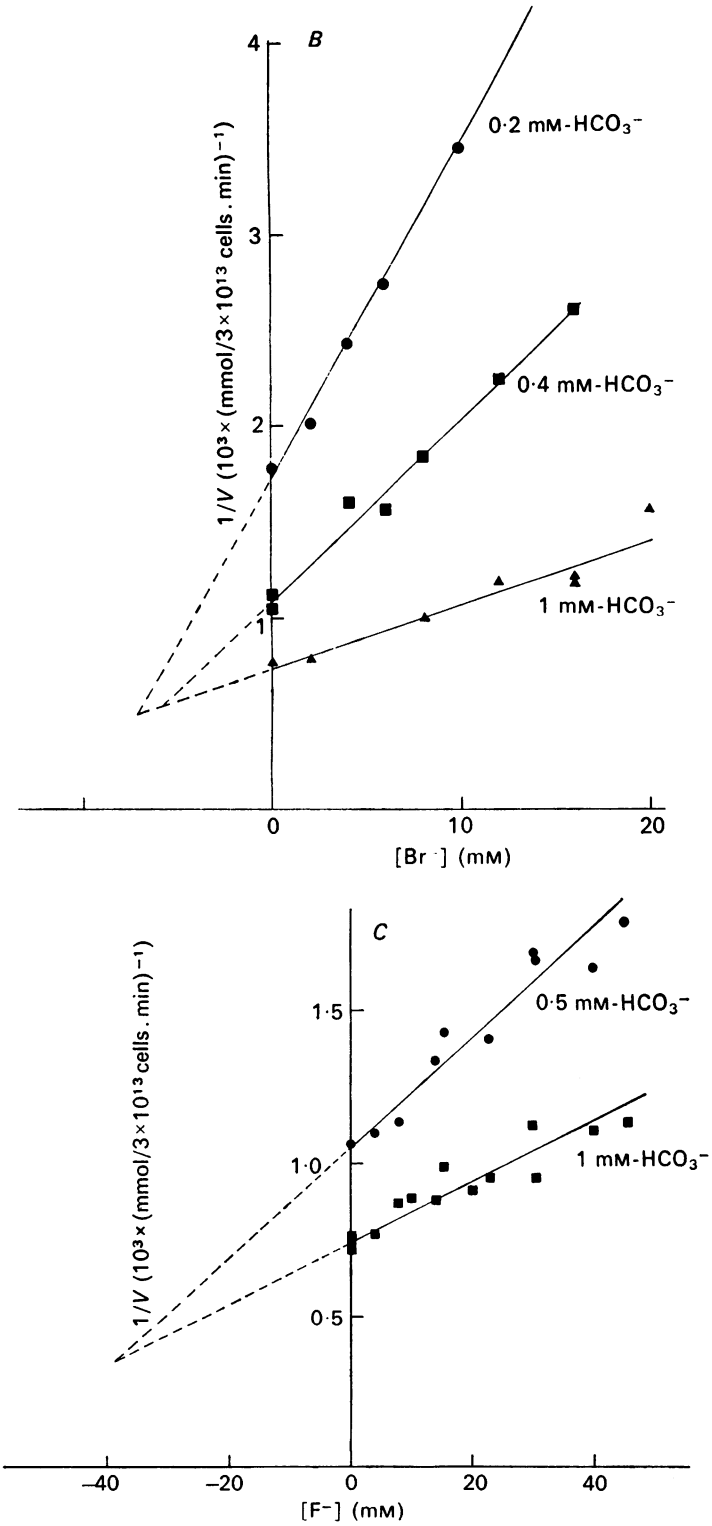


Fig. 3. Dixon plots of the inhibitory effects of extracellular chloride, bromide and fluoride on chloride/bicarbonate exchange. Rates of chloride/bicarbonate exchange between erythrocytes (0·25 ml) and gluconate–imidazole medium (pH 7·00–7·07, 10 °C) were measured: *A*, in the presence of varying concentrations of chloride and ■ 0·2 mM-HCO_3^-, ● 0·5 mM-HCO_3^-; *B*, in the presence of varying concentrations of bromide and ● 0·2 mM-HCO_3^-, ■ 0·4 mM-HCO_3^-, ▲ 1 mM-HCO_3^-; *C*, in the presence of varying concentrations of fluoride and ● 0·5 mM-HCO_3^-, ■ 1 mM-HCO_3^-.

after completion of bicarbonate/chloride exchange was not stable in the presence of fluoride (Fig. 4). This effect was observed even in the presence of 4 mM-fluoride and presumably indicate that fluoride increases the effective permeability of the erythrocyte membrane to protons and/or hydroxyl ions. Despite this difficulty it is clear that fluoride is much less effective than chloride or bromide in inhibiting chloride/bicarbonate exchange and the results in Fig. 3*C* suggest a K_i for fluoride of about 38 mM. Inhibitory effects of iodide on chloride/bicarbonate exchange were less



Figs. 3*B* and *C*. For legend see facing page.

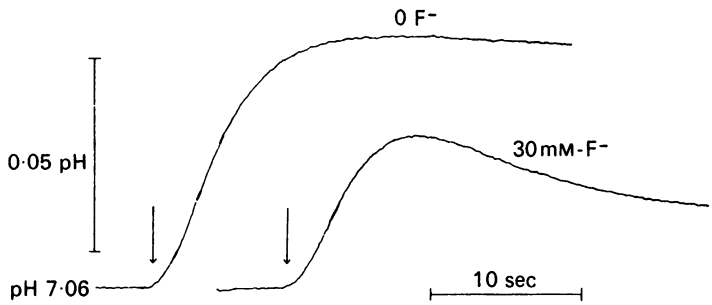


Fig. 4. The effect of fluoride on the stability of the pH gradient set up after exchange of intracellular chloride for extracellular bicarbonate. Changes in extracellular pH are shown following addition of erythrocytes (0·25 ml) at the arrow to 15 ml gluconate–imidazole medium (pH 7·06, 10 °C) containing 0·5 mM-$NaHCO_3$ with or without replacement of 30 mM-Na gluconate with 30 mM-NaF. Deflexions of the traces in the upwards direction indicate acidification of the media associated with the exchange of chloride for bicarbonate. The later downward deflexion of the trace for the experiment with 30 mM-NaF indicates a decay of the substantial pH gradient set up as a result of the exchange.

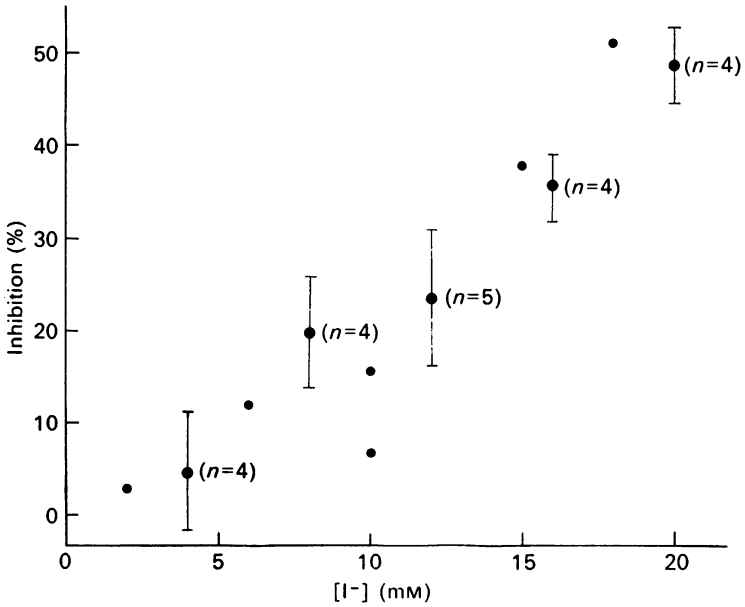


Fig. 5. The inhibition of chloride/bicarbonate exchange by iodide. Rates of chloride/bicarbonate exchange were measured in gluconate–imidazole medium (pH 7·04–7·07, 10 °C) containing 0·5 mM-$NaHCO_3$. Results are given as % inhibition of the rate of extracellular acidification relative to the rate found in the absence of iodide and points represent either single measurements or the mean of several experiments (number indicated) with error bars covering twice the S.E. of mean.

reproducible than those of the other halides and Fig. 5 combines the results of four experiments carried out with different batches of cells. In agreement with the results of Dalmark (1976) a Dixon plot of the data for iodide inhibition appeared to curve upwards indicating a complex mechanism of inhibition, while results with 0·2 mM- and 1 mM-bicarbonate were consistent with a partial competitive inhibition by iodide.

Temperature

The temperature-dependence of the rate of bicarbonate/chloride exchange was studied between 2 and 16 °C in the presence of both 0·5 mM and 1 mM extracellular bicarbonate. As shown in Fig. 6 the Arrhenius plot was linear in the presence of 0·5 mM-bicarbonate and the activation energy was calculated as 13·9 ± 0·7 kcal.mol^{-1}. This value cannot, however, be accepted as the true activation energy since any

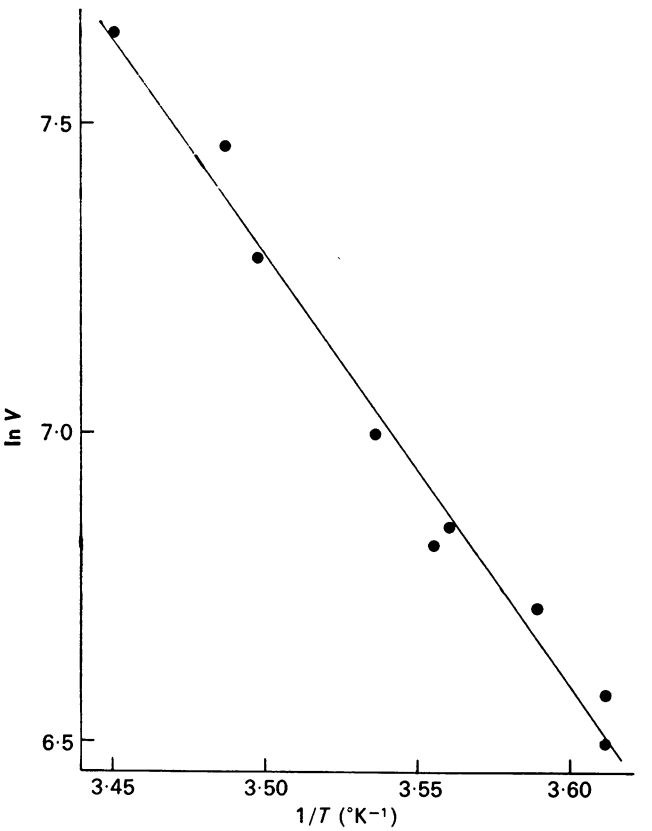


Fig. 6. Arrhenius plot of the temperature dependence of the initial rate of chloride/bicarbonate exchange (V) measured after the addition of 0·25 ml packed erythrocytes to 15 ml gluconate–imidazole medium (pH 7·04) containing 0·5 mM-$NaHCO_3$.

strong temperature dependence of the K_m for bicarbonate would change the degree of saturation of the anion carrier system by bicarbonate at different temperatures. To investigate this the bicarbonate concentration dependence of chloride/bicarbonate exchange was studied at both 2·3 and 10 °C using the same batch of cells. The results showed that temperature had a relatively small effect on the K_m for bicarbonate (measured as 0·55 (± 0·10, S.E. of mean) mM at 2·3 °C and 0·42 (± 0·08, S.E. of mean) mM at 10 °C while V_{max} increased from 707 (± 59, S.E. of mean) m-moles/10^{13} cells.min at 2·3 °C to 1854 (± 163, S.E. of mean) m-moles/10^{13} cells.min at 10 °C., indicating an activation energy of 19·4 kcal.mol^{-1}.

DISCUSSION

The results described in this paper are consistent with a 1:1 stoicheiometric exchange of intracellular chloride for extracellular bicarbonate when erythrocytes containing chloride but little bicarbonate are added to a chloride-free medium. The use of a pH electrode to monitor this exchange in a lightly buffered medium provides a more rapid and flexible method of following the exchange of anions than the measurement of the rate of efflux of ^{36}Cl from cells described by Dalmark and Wieth (1972). The latter method involves separation of the cells from the medium and, because of the rapidity of the anion movements, even the rapid filtration method employed restricts most useful measurements to temperatures of about 0 °C. Isotopic chloride exchange studies have also been carried out under equilibrium conditions with similar concentrations of anions on both sides of the cell membrane and, whilst this has the advantage that the intracellular pH and the membrane potential are kept constant throughout experiments, the interpretation of inhibition studies is complicated by the presence of inhibitor anions on both sides of the membrane and the exchange of inhibitor anions for chloride. The present work avoids the latter difficulties while minimizing possible effects of alterations in pH and membrane potential by measuring initial rates of chloride/bicarbonate exchange.

TABLE 2. Kinetic parameters of the anion transport system of the erythrocyte membrane

Authors	System measured	Temperature (°C)	V_{max} (mmol/ 3×10^{13} cells. min)	K_m or K_i for Cl^- (mM)	K_m or K_i for HCO_3 (mM)	Competitive K_i mM		
						F^-	Br^-	I^-
Gunn *et al.* (1973)	Cl^-/Cl^-	0	900	26	6–11	33–36	12–14	2–4
Dalmark (1975, 1976)	exchange	10	7200*	—	—	—	—	—
Chow *et al.* (1976)	Cl^-/HCO_3^-	2	62–123†	—	> 20	—	—	—
	exchange	10	587†	—	> 20	—	—	—
This paper	Cl^-/HCO_3^-	2·3	707	—	0·55	—	—	—
	exchange	10	2033	4	0·39	38	7	20‡

* Calculated from V_{max} at 0 °C assuming a Q_{10} of 8.

† Measured rates converted from units given in Chow *et al.* (1976).

‡ Iodide concentration causing 50% inhibition of Cl^-/HCO_3^- exchange in the presence of 0·5 mM-HCO_3^-.

Table 2 compares the results obtained in the present studies with those of Dalmark (1976) and of Chow *et al.* (1976). Substantial differences both in the rates of anion exchange found and in the apparent affinities of the anion system for chloride and bicarbonate are evident. It is possible, however, to reconcile at least some of these differences by consideration of the experimental conditions under which the various measurements were made. The studies of chloride self-exchange employed erythrocytes which had been treated with nystatin in order to make possible loading of the cells with high concentrations of chloride. It seems reasonable to suggest that nystatin may have modified both the affinity of the anion carrier for chloride and rate of movement of the carrier through its interaction with the membrane com-

ponents, while the high ionic strengths used may also have had similar effects. The fact that we found higher values for both V_{max} and K_m (for bicarbonate) when measuring bicarbonate/chloride exchange in sucrose, rather than a gluconate medium, lends support to the latter possibility. It is also worth noting that the measured K_m for chloride is (assuming a saturable carrier system analogous to an enzyme) a function of all the rate constants involved in the transport process (Dixon and Webb, 1964) and is not therefore a reliable measure for the dissociation constant of the carrier-chloride complex, whereas K_i for a competitive inhibitor is a true measure of the carrier-inhibitor dissociation constant. It would therefore seem that our value of 4 mM for the K_i for chloride inhibition of chloride/bicarbonate exchange is likely to be a better estimate of the affinity of the transport system for chloride than the K_m for chloride found from studies of chloride self-exchange. The relatively high affinity for chloride found in this work helps to explain the relatively low rates of exchange of extracellular chloride for intracellular bicarbonate observed by Chow *et al.* (1976). The erythrocytes used in the latter experiments contained about 150 mM-chloride and since this is thirty times greater than the K_i for chloride, the effective apparent K_m for bicarbonate would also be expected to be thirty times greater than the K_m of 0·4 mM found under the nominally chloride-free conditions employed in our studies. Since the experiments of Chow *et al.* were usually carried out with intracellular bicarbonate concentrations of about 5–9 mM and with a transmembrane bicarbonate gradient of about 6 mM, their measured rates of chloride/bicarbonate exchange would be expected to be lower than V_{max} by a factor of about three (1 + (apparent K_m)/(bicarbonate)). Multiplying the rates observed by Chow *et al.* by this factor would bring their rates closer to those observed in our experiments. An effective K_m of 12 mM for bicarbonate would also help to explain the fact that Chow *et al.*(1976) did not observe saturation kinetics for the bicarbonate concentration-dependence of chloride/bicarbonate exchange because few measurements were made at bicarbonate concentrations greater than 20 mM.

The relatively low temperature dependence of chloride/bicarbonate exchange reported in this paper is difficult to reconcile with the activation energy of about 30 kcal.mole^{-1} found by Dalmark (1972, 1975) for chloride self-exchange and chloride/bicarbonate exchange 0 and 10 °C, and the high Q_{10} found by Chow *et al.* (1976) for chloride/bicarbonate exchange at low temperatures. A strong temperature dependence of the affinity of the anion carrier for bicarbonate and/or chloride could, in theory, explain the differences but our measured value for the K_m for bicarbonate at 2·3 °C is not very different from that found at 10 °C. Furthermore, it would be necessary to postulate that the affinity of the anion carrier for chloride decreases with temperature to account for our low temperature dependence, whereas comparison of Dalmark's K_m for chloride (26 mM at 0 °C) and our K_i for chloride (4 mM at 10 °C) implies that affinity for chloride increases with temperature. Further work will be needed to resolve these discrepancies.

Our inhibition studies indicate that the apparent affinities of the transport system for anions decrease in order $HCO_3^- > Cl^- > Br^- > F^-$ (with the position of I^- unclear) and both the absolute magnitude and the order of these affinities differ substantially from those found by Dalmark (1976) for anion inhibition of chloride self-exchange ($I^- > HCO_3^- > Br^- > Cl^- > F^-$). It seems unlikely that the differences

in temperature and pH used in the two studies can account for the different order of affinities of the carrier system for the anions and the inhibitor concentrations used in our studies are too low to affect seriously the secondary regulatory sites on the anion carrier system proposed by Dalmark (1976). Alternative explanations must therefore be considered. One possibility is that the transport system is asymmetrical, since in the experiments of Dalmark inhibitors of chloride self exchange are present on both sides of the red cell membrane, whereas in our experiments inhibitors are present (initially) only in the extracellular medium. Evidence for an asymmetric arrangement of the anion carrier system(s) in the erythrocyte membrane has, in fact, been obtained from studies of the inhibitory effects of phlorizin (Schnell, Gerhardt, Lepke & Passow, 1973) on chloride/iodide exchange and of trypsin (Lepke & Passow, 1976) and 4,4'-diacetamido-2,2'-disulphonic acid (Kaplan, Scorah, Fasold & Passow, 1976) on sulphate equilibrium exchange. Furthermore, energy-dependent chloride/bicarbonate exchange has been found to be involved in the regulation of intracellular pH in the squid giant axon (Boron & De Weer (1976), Russell & Boron (1976)) and it may well turn out that chloride/bicarbonate exchange is also important for intracellular pH regulation in other types of cell.

REFERENCES

AUBERT, L. & MOTAIS, R. (1975). Molecular features of organic anion permeability in ox red blood cells. *J. Physiol.* **246**, 159–179.

BORON, W. F. & DE WEER, P. (1976). Active proton transport stimulated by CO_2/HCO_3^-, blocked by cyanide. *Nature, Lond.* **259**, 240–241.

CABANTCHIK, Z. I. & ROTHSTEIN, A. (1972). The nature of the membrane sites controlling anion permeability of human red blood cells as determined by studies with disulfonic stilbene derivatives. *J. Membrane Biol.* **15**, 311–330.

CHOW, E. I., CRANDALL, E. D. & FORSTER, R. E. (1976). Kinetics of bicarbonate–chloride exchange across the human red blood cell membrane. *J. gen. Physiol.* **68**, 633–652.

COUSIN, J. L. & MOTAIS, R. (1976). The role of carbonic anhydrase inhibitors on anion permeability into ox red blood cells. *J. Physiol.* **256**, 61–80.

COUSIN, J. L., MOTAIS, R. & SOLA, F. (1975). Transmembrane exchange of chloride with bicarbonate ion in mammalian red blood cells: evidence for a sulphonamide-sensitive carrier *J. Physiol.* **253**, 385–399.

DALMARK, M. (1972). The effect of temperature, bicarbonate, carbon dioxide, and pH on the chloride transport across the human erythrocyte membrane. In *Oxygen Affinity of Hemoglobin and Red Cell Acid–Base Status*. Alfred Benzon Symposium IV, pp. 320–331. Copenhagen: Munksgaard.

DALMARK, M. (1975). Chloride transport in human red cells. *J. Physiol.* **250**, 39–64.

DALMARK, M. (1976). Effects of halides and bicarbonate on chloride transport in human red blood cells. *J. gen. Physiol.* **67**, 223–234.

DALMARK, M. & WIETH, J. O. (1972). Temperature dependence of chloride, bromide, iodide, thiocyanate and salicylate transport in human red cells. *J. Physiol.* **224**, 583–610.

DE DUVE, C. (1948). A spectrophotometric method for simultaneous determination of myoglobin and hemoglobin in extracts of human muscle. *Acta chem. scand.* **2**, 264–289.

DIRKEN, M. N. J. & MOOK, H. W. (1931). The rate of gas exchange between blood cells and serum. *J. Physiol.* **73**, 349–360.

DIXON, M. & WEBB, E. C. (1964). *The Enzymes*, 2nd edition, chap. IV. London: Longmans, Green & Co.

FUNDER, J. & WIETH, J. O. (1967). Trapping of sodium, potassium, sucrose, and albumin in mammalian red blood cells. *Acta physiol. scand.* **71**, 105–112.

GROS, G. & MOLL, W. (1971). The diffusion of carbon dioxide in erythrocytes and hemoglobin solutions. *Pflügers Arch.* **324**, 249–266.

GUNN, R. B., DALMARK, M., TOSTESON, D. C. & WIETH, J. O. (1973). Characteristics of chloride transport in human red blood cells. *J. gen. Physiol.* **61**, 185–206.

KAPLAN, J. H., SCORAH, K., FASOLD, H. & PASSOW, H. (1976). Sideness of the inhibitory action of disulphonic acids on chloride equilibrium exchange and net transport across the human erythrocyte membrane. *FEBS Letters* **62**, 182–185.

LEPKE, S. & PASSOW, H. (1976). Effects of incorporated trypsin on anion exchange and membrane proteins in human red blood cells. *Biochim. biophys. Acta* **298**, 529–533.

LUCKNER, H. (1939). Über die Geschwindigkeit das Austausche der Atemgase im Blut. *Pflügers Arch. ges. Physiol.*, **250**, 301–311.

PIIPER, J. (1964). Geschwindigkeit des CO_2-Austasches zwischen Erythrocyten und Plasma. *Pflügers Arch. ges. Physiol.* **279**, 500–512.

RUSSELL, J. M. & BORON, W. F. (1976). Role of chloride transport in regulation of intracellular pH. *Nature, Lond.* **264**, 73–74.

SCHNELL, K. F., GERHARDT, S., LEPKE, S. & PASSOW, H. (1973). Asymmetric inhibition by phlorizin of halide movements across the red blood cell membrane. *Biochim. biophys. Acta* **318**, 474–477.

TOSTESON, D. C. (1959). Halide transport in red cells. *Acta physiol. scand.* **46**, 19–41.

WILKINSON, G. N. (1961). Statistical estimations in enzyme kinetics. *Biochem. J.* **80**, 324–332.