Interactions of Bromide, Iodide, and Fluoride with the Pathways of Chloride Transport and Diffusion in Human Neutrophils

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ABSTRACT Isolated human neutrophils possess three distinct pathways by which Cl⁻ crosses the plasma membrane of steady state cells: anion exchange, active transport, and electrodiffusion. The purpose of the present work was to investigate the selectivity of each of these separate processes with respect to other external halide ions. (a) The bulk of total anion movements represents transport through an electrically silent anion-exchange mechanism that is insensitive to disulfonic stilbenes, but which can be competitively inhibited by α-cyano-4-hydroxycinnamate (CHC; Ki ~ 0.3 mM). The affinity of the external translocation site of the carrier for each of the different anions was determined (i) from substrate competition between Cl⁻ and either Br⁻, F⁻, or I⁻, (ii) from stimulation of 36Cl⁻ efflux as a function of the external concentrations of these anions, (iii) from changes in the apparent Ki for CHC depending on the nature of the replacement anion in the bathing medium, and (iv) from activation of 82Br⁻ and 125I⁻ influxes by their respective ions. Each was bound and transported at roughly similar rates (Vmax values all 1.0–1.4 meq/liter cell water · min); the order of decreasing affinities is Cl⁻ > Br⁻ > F⁻ > I⁻ (true Km values of 5, 9, 23, and 44 mM, respectively). These anions undergo 1:1 countertransport for internal Cl⁻. (b) There is a minor component of total Cl⁻ influx that constitutes an active inward transport system for the intracellular accumulation of Cl⁻ ([Cl⁻]i ~ 80 meq/liter cell water), fourfold higher than expected for passive distribution. This uptake is sensitive to intracellular ATP depletion by 2-deoxy-D-glucose and can be inhibited by furosemide, ethacrynic acid, and CHC, which also blocks anion exchange. This active Cl⁻ uptake process binds and transports other members of the halide series in the sequence Cl⁻ > Br⁻ > I⁻ > F⁻ (Km values of 5, 8, 15, and 41 mM, respectively). (c) Electrodiffusive fluxes are small. CHC-resistant 82Br⁻ and 125I⁻ influxes behave as passive leak fluxes through low-conductance ion channels: they are nonsaturable and strongly voltage dependent. These anions permeate the putative Cl⁻ channel in the sequence I⁻ > Br⁻ > Cl⁻ with relative permeability ratios of 2.2:1.4:1, respectively, where PCl⁻ ~ 5 × 10⁻⁹ cm/s.

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INTRODUCTION

Neutrophils and other phagocytic cells play vital roles in the normal host defense against invading microbes and, in certain pathological conditions, they contribute to tissue injury as well. The studies presented here are part of a long-range research plan aimed at understanding the physiology of human neutrophil function. These investigations represent a continuing effort toward a systematic examination of the membrane properties of resting cells in order to provide the proper framework for evaluating the changes in ion fluxes that occur in cells activated by chemotactic factors and other stimulatory agents. The present work arises as a direct outgrowth of our recent reports on Cl\(^{-}\) movements in resting neutrophils (Simchowitz and De Weer, 1986; Simchowitz et al., 1986).

Steady state human polymorphonuclear leukocytes exchange Cl\(^{-}\) with their 148 mM Cl\(^{-}\) bathing medium at a rate of ~1.4 meq/liter cell water \(\cdot\) min. In previous reports (Simchowitz and De Weer, 1986; Simchowitz et al., 1986), we have presented evidence for three distinct pathways to account for all \(^{36}\)Cl\(^{-}\) movements across the neutrophil plasma membrane. (a) Approximately 30% of Cl\(^{-}\) efflux and ~8% of Cl\(^{-}\) influx behave as electrodiffusion through a low-permeability pathway \(\left(\begin{array}{c}
P_a \\
K_a \\
P_{Cl} \\
P_{K} \\
PK:Pa \
\end{array}\right) \approx 4-5 \times 10^{-9} \text{ cm/s}, P_K: P_{Cl} \approx 10\); these fluxes are nonsaturating and strongly voltage sensitive. (b) Approximately 20% of Cl\(^{-}\) influx behaves as active transport that requires metabolic energy and could be inhibited by 2-deoxy-D-glucose, \(\alpha\)-cyano-4-hydroxy-cinnamate (CHC), furosemide, and ethacrynic acid; this active process is probably responsible for the intracellular accumulation of Cl\(^{-}\) \(\left([\text{Cl}^-]\right) \approx 80 \text{ meq/liter cell water}\), fourfold above expected passive distribution for the cell's resting membrane voltage of approximately -53 mV (Seligmann and Gallin, 1980; Simchowitz et al., 1982; but see Simchowitz and De Weer, 1986). (The underlying mechanism has not been clarified, nor has the nature of the relationship to intracellular ATP depletion. In view of this, we chose the general term "active" to describe this process, which could conceivably represent a form of either primary or secondary active transport. While this Cl\(^{-}\) uptake system exhibits some of the properties that one might associate with a primary ATP-driven Cl\(^{-}\) pump, it should be emphasized that the existence of such a pump has not as yet been demonstrated in these cells.) (c) The major portion (~70%) of one-way Cl\(^{-}\) influx and efflux represents electrically silent Cl\(^{-}\)/Cl\(^{-}\) self-exchange, which is mediated by a nonselective anion carrier insensitive to disulfonic stilbenes. The properties of this anion exchange, which we have shown to function physiologically as a Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange mechanism in the regulation of intracellular pH after imposed alkalinization (Simchowitz and Roos, 1985), were characterized in some detail in our earlier article (Simchowitz et al., 1986), in which we demonstrated substrate saturation, "trans" effects, specific inhibition, substrate competition, and countertransport. The carrier is not specific for Cl\(^{-}\): although it binds Cl\(^{-}\) with relatively high affinity \(K_m \approx 5 \text{ mM}\), \(\beta\)-aminohippurate (PAH) is also transported, but with comparatively low affinity \(K_m \approx 50 \text{ mM}\). These substrate interactions are competitively inhibited by CHC, a known monocarboxylate anion inhibitor in mitochondria and red blood cells (Halestrap and Denton, 1975; Halestrap, 1976; Deuticke, 1982), with \(K_i \approx 0.3 \text{ mM}\). A simple equilibrium carrier model satisfactorily accounts for the kinetics of the carrier with respect to the various extracellular ligands, Cl\(^{-}\), PAH, and CHC.
The present work was undertaken to characterize the selectivity of these three pathways of Cl⁻ movement with respect to other halide ions. We find that these anions permeate the putative Cl⁻ channel in the sequence I⁻ > Br⁻ > Cl⁻, with relative permeabilities of 2.2:1.4:1. On the other hand, the ATP-dependent system for active Cl⁻ uptake binds anions in the sequence Cl⁻ > Br⁻ > I⁻ > F⁻, with $K_m$ values of 4.8, 7.8, 14.6, and 40.9 mM, respectively. The present study also confirms the relative nonselectivity of the anion-exchange carrier with respect to other halides. All were bound and transported, in order of decreasing affinity: Cl⁻ > Br⁻ > F⁻ > I⁻. Affinities were determined from substrate competition between Br⁻, F⁻, or I⁻, and either Cl⁻ or PAH, from trans activation of $^{36}$Cl⁻ efflux by external halides replacing glucuronate (an inert replacement anion), from changes in the apparent $K_i$ for CHC inhibition depending on the nature of the replacement anion, and from influx measurements of labeled Br⁻ and I⁻ as a function of the external concentrations of these ions. The true $K_m$ values for Cl⁻, Br⁻, F⁻, and I⁻ are 5.0, 9.4, 23.2, and 44.2 mM, respectively. The results also show that these anions undergo 1:1 countertransport with Cl⁻.

**METHODS**

*Incubation Media*

The standard medium used throughout this study had the following composition (millimolar): 140 NaCl, 5 KCl, 1 CaCl₂, 0.5 MgCl₂, 5.6 glucose, 5 HEPES buffer (pH 7.40), and 1 mg/ml crystalline bovine serum albumin. Where required, N-methyl-D-glucamine or K⁺ replaced Na⁺ and either Br⁻, F⁻, I⁻, PAH, or glucuronate replaced Cl⁻. When F⁻ was used as the substitute for Cl⁻, Ca²⁺ and Mg²⁺ were omitted. In preliminary studies, it was ascertained that the lack of external Ca²⁺ and Mg²⁺ had no effect on unidirectional $^{36}$Cl⁻ fluxes measured in 148 mM Cl⁻ medium. All solutions were routinely gassed with N₂ immediately before experimentation in order to deplete the amount of HCO₃⁻ in these nominally CO₂/HCO₃⁻-free media. When the effect of 1 mM 2-deoxy-D-glucose (2-DOG) was tested, the cells were first pretreated with the drug for 10–15 min in glucose-free medium and then resuspended in the experimental solutions. Thus, the flux measurements were also conducted with 2-DOG in the absence of glucose. The lack of glucose in the bathing media per se had no effect on any of the assays.

*Neutrophils*

Normal human peripheral blood neutrophils were isolated by sequential dextran sedimentation and Ficoll-Hypaque (Pharmacia Fine Chemicals, Inc., Piscataway, NJ) gradient centrifugation (Böyum, 1968). Contaminating erythrocytes were removed by lysis in distilled water for 30 s. The neutrophils were washed twice and then counted. Purity averaged 97%, as judged by Wright's stain. Viability, assessed by eosin Y exclusion, averaged 99% and was not affected by any of the agents tested in this study. The cells were prepared at 37°C, spun in unrefrigerated centrifuges, and kept at 37°C for at least 60 min before and during all assays. (Storage for up to 3 h at that temperature in 5 mM K⁺, 148 mM Cl⁻ medium had no effect on intracellular K⁺, Na⁺ [Simchowitz et al., 1982], or Cl⁻ content [Simchowitz and De Weer, 1986], or on the subsequent behavior of the neutrophils.) Thus, the cells, which had an internal Cl⁻ concentration of ~80 meq/liter cell water, will henceforth be termed "normal Cl⁻." For some studies, a batch of Cl⁻-depleted cells ([Cl⁻]ₐ ~ 2 meq/liter cell water) was prepared by incubating cells in 148 mM PAH medium for ~5 h at 37°C.
Reagents

We obtained crystalline bovine serum albumin, PAH, sodium PAH, 2-DOG, glucuronic acid, sodium glucuronate, N-methyl-D-glucamine, and HEPES from Sigma Chemical Co., St. Louis, MO; CHC from Aldrich Chemical Co., Milwaukee, WI; nigericin from Calbiochem-Behring Corp., La Jolla, CA. A 3-mM stock solution of nigericin in dimethylsulfoxide was made and diluted as needed into the appropriate medium. All inorganic salts were purchased from Fisher Scientific, St. Louis, MO. Isotopes were purchased from New England Nuclear, Boston, MA: ^{36}Cl^− (specific activity, 13.8 mCi/g Cl), ^{82}Br^− (specific activity, 3.2 mCi/mg Br), and ^{125}I^− (carrier free). For experiments with ^{82}Br^−, supplied as NH₄[^{82}Br] in 0.1 N NH₄OH, the NH₄OH was removed by evaporation under a stream of N₂ and the remaining salt was then dissolved in medium. For the ^{125}I^− studies, the small amount of free I⁻ was removed by incubating a small aliquot of the Na[^{125}I] solution with 5 mM Na₂S₂O₃, evaporating with N₂, and then diluting the residue in medium. Furosemide was a generous gift of Hoechst Laboratories, Somerville, NJ; the potentiometric dye diS-C₅(5) (3,3'-dipropylthiadicarbocyanine iodide) was a gift from Dr. Alan Waggoner of Carnegie-Mellon University, Pittsburgh, PA.

^{36}Cl^−, ^{82}Br^−, and ^{125}I^− Flux Measurements

The technique described by Naccache et al. (1977) was employed. Neutrophils (7–10 x 10⁶ cells/ml) were incubated at 37°C in capped plastic tubes (Falcon Plastics, Oxnard, CA). Influx experiments were performed in the presence of ^{36}Cl^− (1.5 μCi/ml) or ^{82}Br^− (2.0 μCi/ml) or ^{125}I^− (3.0 μCi/ml). At stated intervals, triplicate 0.5-ml aliquots were layered on 0.7 ml silicone oil (Versilube F-50, General Electric, Waterford, NY) and spun at 8,000 g for 1 min; cell separation occurred in <5 s. The pellets were excised and either counted directly (^{82}Br^− or ^{125}I^−) in a gamma counter (model 5500, Beckman Instruments, Palo Alto, CA) or dispersed (^{36}Cl^−) in 10 ml of Aquasol 2 (New England Nuclear) and counted in a liquid scintillation counter (LS 7000, Beckman Instruments). Influxes, corrected for zero-time “uptake” (which represents label trapped in the extracellular space), followed single-exponential equations of the form:

\[ C_t = C_\infty [1 - \exp(-kt)], \]

where \( C_t \) is the cell label at time t, \( C_\infty \) is the cell label at steady state, and k is the rate coefficient. Eq. 1 was fitted to the data by a nonlinear least-squares program, and the initial influx rate was computed from the product \( kC_\infty \). In some cases, influx was so slow as to appear linear over the period of study; the rate was then simply the slope of the straight line.

For the efflux studies, neutrophil suspensions (2–3 x 10⁷/ml) were incubated with ^{36}Cl^− (2.5 μCi/ml) for 1–2 h at 37°C. The cells were then washed twice and resuspended in unlabeled medium. Triplicate samples were taken at stated intervals and spun and counted as above. In order to correct for the daily variation in the extent of cell loading, the data for each experiment were normalized to 1.0 at zero time by dividing the counts per minute at any time t by the number of counts per minute in the cell pellet at zero time. Efflux kinetics were first order, and the rate coefficients were determined by least-squares fitting of single exponentials to the time course of radioactivity remaining in the cells. All figure symbols are averages ± SEM.

Fluorescence Measurements

A 1-mM stock solution of diS-C₅(5) in ethanol was kept in the dark at 4°C. Experiments were performed in a total volume of 3 ml containing 4 x 10⁶ neutrophils, 1 μM dye, and 0.1% ethanol. After equilibration for 5–10 min at 37°C, the fluorescence of the dye-cell suspensions was measured at 37°C in a spectrofluorometer (model 430, Turner Associates, Palo Alto, CA). Excitation was at 622 nm; emission was at 670 nm. At the concentrations employed, and
for the duration and conditions of the assays, neither ethanol nor the dye affected neutrophil viability as assessed by eosin Y exclusion, or functional integrity as measured (Simchowitz, 1985) by the superoxide generation response elicited by a 5-min exposure to the synthetic chemotactic factor N-formyl-methionyl-leucyl-phenylalanine (0.1 μM). The intracellular ion concentrations or active and passive fluxes of K⁺ and Na⁺ were similarly unaffected (Simchowitz et al., 1982). In the absence of cells, changes in the medium's anion composition did not alter dye fluorescence, except in the case of iodide, which quenched the dye.

RESULTS

Halide Ion Permeabilities

We determined the permeability of the neutrophil plasma membrane to Br⁻ and I⁻, relative to that for Cl⁻, from the passive influxes of the labeled anions. The rates of

![Figure 1. Rates of CHC-resistant ⁸²Br⁻ and ¹²⁵I⁻ influx into Cl⁻-depleted cells ([Cl⁻]₀ ~ 2 mM). The bathing solutions contained 3–100 mM of either Br⁻ or I⁻ (balance, PAH) and 40 mM CHC. Experiments were performed over the course of 1 h, during which influxes were linear with time. Influx rates (n = 4 for each), which were computed from the slopes of the tracer uptake and plotted against the external anion concentration, are linearly proportional to [Br⁻]₀ and [I⁻]₀. The slopes of the lines are 0.79 ± 0.04 (middle line) and 1.31 ± 0.08 (upper line). The slope of the Cl⁻ line (lower line), taken from Simchowitz and De Weer (1986), is 0.56 ± 0.02.

influx of ⁸²Br⁻ and ¹²⁵I⁻ into Cl⁻-depleted cells were measured in the presence of 40 mM CHC, an inhibitor of both anion exchange and active transport (Simchowitz and De Weer, 1986; Simchowitz et al., 1986). As previously shown with ³⁵Cl⁻ (Simchowitz and De Weer, 1986), under these conditions the residual influxes and effluxes behave as electrodiffusive fluxes through ion channels: they do not saturate, they are voltage sensitive, and they exhibit flux ratios appropriate to constant-field behavior. Fig. 1 shows the rates of CHC-insensitive ⁸²Br⁻ and ¹²⁵I⁻ influxes plotted against the external anion concentration. The ³⁵Cl⁻ influx data of Simchowitz and De Weer (1986) are also shown for comparison. For both ⁸²Br⁻ and ¹²⁵I⁻, CHC-resistant influxes were linear functions of [Br⁻]₀ and [I⁻]₀ between 3 and 100 mM, with no evidence of saturation, which suggests that these fluxes, as already documented for Cl⁻, are also electrodiffusive in nature. Assuming membrane voltage (Vₘ) to be roughly constant
and largely independent of the nature or concentration of the major anion in the bathing medium (see below), the slopes of the lines imply a relative I:Br:Cl permeability ratio of 2.3:1.4:1. Since \( P_{\text{Cl}} \) (4–5 \( \times \) 10\(^{-9} \) cm/s) is \( \sim \)1/10 of \( P_{\text{K}} \) (Simchowitz and De Weer, 1986), one would expect \([\text{Br}^-]\) and \([\text{I}^-]\) to have little effect on membrane potential on the basis of these results. Our tests with the voltage-sensitive dye diS-C\(_3\)(5) support this: as with Cl\(^-\) (Simchowitz and De Weer, 1986), variations of 

![Figure 2](image-url)

**Figure 2.** Effect of membrane potential on \(^{82}\text{Br}^-\) and \(^{125}\text{I}^-\) influxes into Cl\(^-\)-depleted ([Cl\(^-\)], \( \sim \) 2 mM) cells. The media contained 98 mM PAH, 40 mM CHC, 10 mM of either NaBr or NaI, and K\(^+\) at concentrations ranging from 0.2 to 124 mM (balance, N-methyl-D-glucamine). (A) \(^{82}\text{Br}^-\) influx at various \([\text{K}^+]_o\)'s, following the method of Fig. 1. The rates were linear, and the values of the slopes were (milliequivalents per liter times minutes): 0.00180 \( \pm \) 0.00125 at 0.2 mM K\(^+\), 0.00423 \( \pm \) 0.00201 at 3.1 mM K\(^+\), 0.0108 \( \pm \) 0.0013 at 12 mM K\(^+\), 0.0196 \( \pm \) 0.0028 at 40 mM K\(^+\), 0.0279 \( \pm \) 0.0023 at 85 mM K\(^+\) (containing 2 \( \mu \)M nigericin), and 0.0311 \( \pm \) 0.0013 at 124 mM K\(^+\) (n \( = \) 3 for each set). (B) \(^{125}\text{I}^-\) influx at various \([\text{K}^+]_o\)'s. The rates were linear, and the values of the slopes were (milliequivalents per liter times minutes): 0.00190 \( \pm \) 0.00140 at 0.2 mM K\(^+\), 0.00648 \( \pm \) 0.00073 at 3.1 mM K\(^+\), 0.0147 \( \pm \) 0.0010 at 12 mM K\(^+\), 0.0324 \( \pm \) 0.0021 at 40 mM K\(^+\), 0.0412 \( \pm \) 0.0020 at 85 mM K\(^+\) (containing 2 \( \mu \)M nigericin), and 0.0478 \( \pm \) 0.0029 at 124 mM K\(^+\) (n \( = \) 3 for each set).

external Br\(^-\) between 0 and 148 mM (replacing PAH or glucuronate) had little effect on resting \( V_m \), which remained constant at approximately \(-50 \) mV. Unfortunately, a technical problem (quenching of dye fluorescence in 148 mM I\(^-\) medium) prevented our assessment of the effect of external I\(^-\) concentrations on \( V_m \).

Passive fluxes should also be voltage sensitive. The resting membrane potential of these cells is about \(-53 \) mV (Seligmann and Gallin, 1980; Simchowitz et al., 1982; Simchowitz and De Weer, 1986). The K:Na:Cl permeability ratio is 10:1:1 (Simcho-
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witz and De Weer, 1986). We manipulated the cells' membrane potential by varying $[K^+]_o$ between 0.2 and 124 mM in 10 mM Na$^+$ media (equivalent replacement with N-methyl-D-glucamine). The media also contained 40 mM CHC, 98 mM PAH, and 10 mM of either Br$^-$ or I$^-$. Furthermore, we assumed the following intracellular ion concentrations: 120 mM $[K^+]_i$, 25 mM $[Na^+]_i$ (Simchowitz et al., 1982), and ~2 mM $[Cl^-]_i$ (Simchowitz and De Weer, 1986). Under these conditions, and given (as will be shown later) that $P_{Br}$ and $P_{I}$ are, respectively, ~1.4 and ~2.3 times $P_{Cl}$ and that the products $P_{Br}[Br^-]_i$ and $P_{I}[I^-]_i$ are negligibly small during the course of the experiments, the constant-field membrane potential equation (Goldman, 1943; Hodgkin and Katz, 1949):

$$V_m = \frac{RT}{F} \ln \left( \frac{P_{K}[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i + P_{X}[X^-]_i}{P_{K}[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o + P_{X}[X^-]_o} \right)$$

(2)

(where X stands for Br$^-$ or I$^-$, P represents permeability, and the other symbols have their usual meaning) reduces to ($[K^+]_o$ in millimolar):

$$V_m = \frac{RT}{F} \ln \left( \frac{[K^+]_o + 1.2}{124} \right).$$

(3)

Fig. 2 shows the time courses of $^{85}$Br$^-$ (A) and $^{125}$I$^-$ (B) influxes at $[K^+]_o$ = 0.2, 3.1, 12, 40, 85 (plus 2 µM nigericin), and 124 mM, or, from Eq. 3, $V_m$ = −120, −90, −60, −30, −10, and 0 mV, respectively. As expected for electrodiffusive fluxes, depolarization caused the CHC-insensitive $^{85}$Br$^-$ and $^{125}$I$^-$ uptake rates to increase dramatically as $[K^+]_o$ was raised from 0.2 to 124 mM. At about the normal resting potential ($V_m$ approximately −60 mV), influxes of 0.0108 ± 0.0013 and 0.0147 ± 0.0010 meq/liter·min from 10 mM Br$^-$ and 10 mM I$^-$ media, respectively, are found, similar to the influx rates from 10 mM Br$^-$ and 1$^-$ media (0.0085 ± 0.0009 and 0.0143 ± 0.0024 meq/liter·min, respectively), at a $V_m$ of approximately −53 mV (Fig. 1). Also shown in the figures is the apparent lack of effect of 2 µM nigericin on these CHC-resistant influxes, the data at 85 mM K$^+$ having been performed in the presence of this drug.

The complete absence of saturation and the striking voltage dependence of the CHC-resistant fluxes described here support the notion that these fluxes are indeed channel-mediated. In Fig. 3, the unidirectional $^{85}$Br$^-$ and $^{125}$I$^-$ influx rates taken from Fig. 2, A and B, are plotted, after conversion to picomoles per square centimeter times seconds, against $V_m$ computed from Eq. 3. If constant-field behavior (Goldman, 1943) prevails, electrodiffusive influxes should show the following dependence on $V_m$:

$$M^X_m = P_X \cdot \frac{V_m F}{RT} \cdot \frac{[X^-]_o}{1 - \exp \left( - \frac{V_m F}{RT} \right)}$$

(4)

where $P$ is the permeability coefficient and $X^-$ stands for Br$^-$ or I$^-$. As can be seen in the figure, both the $^{85}$Br$^-$ and $^{125}$I$^-$ influx data (i.e., from 10 mM Br$^-$ or I$^-$ media into Cl$^-$-depleted cells) fit the constant-field prediction well, with least-squares permeability coefficients for Br$^-$ and I$^-$ of 6.7 ± 1.5 × 10$^{-5}$ and 1.0 ± 0.2 × 10$^{-8}$ cm/s, respectively. These values can be compared with that of 5.1 ± 0.2 × 10$^{-9}$ cm/s for $P_{Cl}$ (Simchowitz and De Weer, 1986), which was obtained under similar conditions, giving a relative I:Br:Cl permeability ratio of 2.0:1.3:1.
Halide Selectivity of Anion Exchange

Fig. 4 shows the time course of $^{36}$Cl$^-$ efflux into media where all extracellular Cl$^-$ (148 mM) was replaced with either Br$^-$, F$^-$, I$^-$, or glucuronate. The media contained 85 mM K$^+$ and 2 μM nigericin (see below for explanation). All effluxes were first order; the rates were calculated on the assumption of an initial [Cl$^-$]$_i$ of 80 meq/liter cell water. While the rates of efflux into Cl$^-$ and Br$^-$ media were similar (1.56 ± 0.14 and 1.50 ± 0.05 meq/liter·min), those into F$^-$ and I$^-$ media were somewhat lower (0.98 ± 0.08 and 1.20 ± 0.03 meq/liter·min). By comparison, the efflux of $^{36}$Cl$^-$ into 148 mM glucuronate medium (nominally substrate free) was much smaller (0.26 ± 0.03 meq/liter·min): only slightly greater than that into 108 mM glucuronate medium containing 40 mM CHC (rate, 0.22 ± 0.02 meq/liter·min), where anion exchange is maximally suppressed. This efflux closely approximates the small residual CHC-resistant flux that can be attributed to the passive leak of Cl$^-$ through ion channels at a $V_m$ of approximately −10 mV (0.2 meq/liter·min; Simchowitz et al., 1986).

In preliminary studies, we determined that during exposure to 148 mM Cl$^-$, Br$^-$, or I$^-$ in 5 mM K$^+$ medium, intracellular pH remained at its normal (Simchowitz and Roos, 1985) value of ~7.25 (pH$_i$ 7.40). However, high external concentrations of F$^-$ caused a gradual intracellular acidification of 0.2–0.3 units over 10–20 min. This pH$_i$
transient is probably related to the generation of protons that accompanies the well-known respiratory burst in response to $F^-$ and follows a similar time course (Curnutte and Babior, 1975). (Since hydrofluoric acid exhibits a $pK'$ of ~3 in aqueous media, the rapid influx of HF via non-ionic diffusion could in theory contribute to the intracellular acidification that is seen in cells bathed in high-[$F^-$] media. In practice, this mechanism is unlikely in that it is incompatible with the kinetics of the observed pH transient: the acidification can only first be detected at ~7 min. This time course does not conform to expectations for entry of undissociated HF, which should be gradual, beginning immediately upon exposure to $F^-$. Rather, the course of the intracellular acidification coincides precisely with the delayed onset of an $F^-$-induced respiratory burst in which protons are generated de novo along with reduced oxygen species such as superoxide radicals.) In contrast, resuspension of cells in 148 mM glucuronate in 5 mM K$^+$ medium led to an intracellular alkalinization through the following mechanism. As noted above, the exchange carrier appears to be devoid of affinity for glucuronate (Simchowitz et al., 1986), which can thus be considered an inert replacement anion. On the other hand, the affinity for HCO$_3^-$ is high (see below). For the purpose of this study (characterization of halide ion interactions with the anion-exchange carrier), the presence of small quantities of HCO$_3^-$, which are normally found in the media, represents a nuisance and is highly undesirable for the reasons outlined below. In fact, we were able to reduce the amount of HCO$_3^-$ in the media to a considerable extent by routinely gassing the solutions with N$_2$ just

![Figure 4](image-url). Time course of $^{36}$Cl$^-$ loss from normal-Cl$^-$ cells into media containing 148 mM of various anions. The lines represent least-squares fits to single exponentials; the labels describe the conditions under which the efflux took place. Fluxes were calculated from the rate coefficients and the assumption that [Cl$^-$]i = 80 meq/liter cell water at zero time. Results are from three to four experiments for each condition. (2) Efflux into 148 mM Cl$^-$ medium containing 85 mM K$^+$ and 2 $\mu$M nigericin; rate, 1.56 ± 0.14 meq/liter-min. (o, o) Efflux into 148 mM Br$^-$ medium containing either 5 mM K$^+$ (o) or 85 mM K$^+$ and 2 $\mu$M nigericin (o); rate for the combined data, 1.50 ± 0.05 meq/liter-min. (A) Efflux into 148 mM I$^-$ medium containing 85 mM K$^+$ and 2 $\mu$M nigericin; rate, 1.20 ± 0.03 meq/liter-min. (2) Efflux into 148 mM F$^-$ medium containing 85 mM K$^+$ and 2 $\mu$M nigericin; rate, 0.98 ± 0.08 meq/liter-min. (6) Efflux into 148 mM glucuronate medium containing 85 mM K$^+$ and 2 $\mu$M nigericin; rate, 0.26 ± 0.03 meq/liter-min. (o) Efflux into 40 mM CHC plus 108 mM glucuronate medium containing 85 mM K$^+$ and 2 $\mu$M nigericin; rate, 0.22 ± 0.02 meq/liter-min.
before experimentation. However, since it is technically very difficult to rid solutions completely of HCO₃⁻, attempts to measure ³⁶Cl⁻ efflux into a nominally "inert" medium such as glucuronate resulted in internal Cl⁻/external HCO₃⁻ exchange and consequent intracellular alkalinization. It also happens that the activity of the anion exchanger is very sensitive to pH in this range: transport rates are dramatically enhanced by raising the pH and reduced by lowering it. Thus, these pH changes and their secondary effects on the fluxes introduced other variables and frustrated our attempts to control the experimental conditions. So, in order to avoid these complications from even trace amounts of HCO₃⁻ (derived perhaps from cell metabolism), we took the added precaution of "pH-clamping" the neutrophils at their normal pHₐ of 7.25 using a high-K⁺/nigericin technique as previously described (Simchowitz and Roos, 1985; Simchowitz et al., 1986): the cells were bathed in medium of pHₐ 7.40 as before, but now containing 85 mM K⁺ and 2 μM nigericin, a known K⁺/H⁺-exchanging ionophore (Pressman, 1969). Under these conditions, [H⁺]_/[H⁺]₀ = [K⁺]_/[K⁺]₀.

In earlier studies (Simchowitz and De Weer, 1986; Simchowitz et al., 1986), we showed that the presence of 85 mM K⁺ (with consequent depolarization) and 2 μM nigericin in the bathing media did not materially affect our observations since membrane potential has little if any effect on the flux through the anion exchanger. These findings are confirmed in Fig. 4, which shows that the rate of ³⁶Cl⁻ efflux into 148 mM Br⁻ was indistinguishable under the two conditions, i.e., at 5 mM K⁺ or at 85 mM K⁺ plus 2 μM nigericin. The slight reduction in the efflux rate into 85 mM K⁺ (from 1.55 to 1.43 meq/liter·min) may be due in part to an approximately twofold reduction in the minor passive ³⁶Cl⁻ efflux component caused by the depolarization from approximately −53 mV to approximately −10 mV as [K⁺]₀ was raised from 5 to 85 mM.

In Fig. 5 A, the initial rate of ³⁶Cl⁻ efflux has been plotted as a function of the concentration of external Br⁻, F⁻, or I⁻ replacing glucuronate. For each anion, substrate saturation was clearly evident. Increasing concentrations of Br⁻, I⁻, or F⁻ stimulated the rate of ³⁶Cl⁻ efflux along Michaelis-Menten activation curves, although the affinities differed considerably: the Kₘ values for Br⁻, F⁻, and I⁻ were 8.6 ± 1.8, 22.9 ± 2.7, and 47.0 ± 7.3 mM, respectively. Since these studies were performed in glucuronate, an inert anion substitute, these kinetic constants can be taken as estimates of the true Michaelis constants for Br⁻, F⁻, and I⁻.

Substrate competition is shown in Fig. 5 B. Since PAH also serves as a substrate for the exchange carrier (Kₘ ~ 50 mM; Simchowitz et al., 1986), it should compete with Br⁻, F⁻, and I⁻ for the same external transport sites and cause a decrease in apparent affinity for each of the halide ions. This expectation is verified by the data of Fig. 5 B, where the initial rate of ³⁶Cl⁻ efflux is plotted as a function of the external concentrations of Br⁻ or F⁻ replacing PAH. There is substrate saturation as in Fig. 5 A, and the endpoints at 140 mM Br⁻ and F⁻ in B are similar to those in A. However, the apparent Kₘ's for Br⁻ and F⁻ are considerably higher in PAH medium (37.2 ± 19.8 and 98.5 ± 58.6 mM, respectively) than in glucuronate medium (8.6 ± 1.8 and 22.9 ± 2.7). These results parallel earlier observations (Simchowitz et al., 1986) that Cl⁻ acts with affinities of 22.1 vs. 5.0 mM, respectively, in PAH vs. glucuronate media. Since I⁻ and PAH have comparable true Kₘ's (~40–50 mM), it is impossible to measure an apparent Kₘ(I⁻) in PAH medium.
Competitive inhibition by CHC, a known inhibitor of anion exchange (Halestrap and Denton, 1975; Halestrap, 1976), is illustrated in Fig. 6. Our group (Simchowitz et al., 1986) has previously reported a "true" $K_i$ for CHC of $\sim 0.29$ mM. In the presence of constant concentrations of extracellular substrate (100 mM; balance, glucuronate), CHC reduced the rate of $^{36}\text{Cl}^-$ efflux along Michaelis-Menten inhibition curves, with apparent $K_i$ values of $2.60 \pm 0.44$, $1.57 \pm 0.42$, and $1.16 \pm 0.41$ mM in $\text{Br}^-$, $\text{F}^-$, and $\text{I}^-$ media, respectively, as expected from the order of decreasing affinities: $\text{Br}^- > \text{F}^- > \text{I}^-$ (Fig. 5 A).

Substrate competition for the external carrier site is further shown in Fig. 7 A, where the ability of the other halide ions to compete with $^{36}\text{Cl}^-$ was tested. The experiments were conducted in glucuronate medium and the figure displays the
effect of increasing concentrations (0–140 mM) of Br\(^-\), F\(^-\), or I\(^-\) on the initial rate of \(^{36}\)Cl\(^-\) influx from a 5 mM Cl\(^-\) medium. Inhibition of \(^{36}\)Cl\(^-\) influx followed Michael-Menten kinetics, with apparent \(K_i\) values of 23.0 ± 4.6, 49.9 ± 6.8, and 94.8 ± 21.2 mM for Br\(^-\), F\(^-\), and I\(^-\), respectively. In the presence of two competing substrates, \(S_1\) and \(S_2\) (instead, one may be a competitive inhibitor), the apparent affinity constant is related to the true kinetic constant as given by the expression:

\[
K_{m}^{app}(S_i) = K_{m}^{true}(S_i) \cdot \left[ 1 + \frac{[S_2]}{K_{m}^{true}(S_2)} \right].
\]

Since, for the experiments presented in Fig. 7 A, external Cl\(^-\) was present at a concentration equal to its true \(K_m\) (5.0 mM; Simchowitz et al., 1986), the apparent constants for Br\(^-\), F\(^-\), and I\(^-\) should be about twice the true \(K_m\) values found in Fig. 5 A. This was indeed the case.

### Figure 6

Inhibition by CHC of \(^{36}\)Cl\(^-\) efflux from normal-Cl\(^-\) neutrophils into Br\(^-\), F\(^-\), or I\(^-\) medium. The major external anion concentration was kept constant at 100 mM as [CHC]\(_o\) was raised from 0 to 40 mM (balance, glucuronate). The media contained 85 mM K\(^+\) and 2 \(\mu\)M nigericin. The curves (\(n = 3\) or 4) are Michaelis-Menten inhibition equations superimposed on a constant background flux of 0.22 meq/liter·min, representing electrodiffusive \(^{36}\)Cl\(^-\) efflux at -10 mV. The equations yielded the following constants (fluxes are in milliequivalents per liter times minutes): for Br\(^-\) medium, flux = 3.56/(2.60 + [CHC]\(_o\)) + 0.22; for F\(^-\) medium, flux = 1.32/(1.57 + [CHC]\(_o\)) + 0.22; for I\(^-\) medium, flux = 1.13/(1.16 + [CHC]\(_o\)) + 0.22. The apparent \(K_i\) values for CHC were 2.60 ± 0.44, 1.57 ± 0.42, and 1.16 ± 0.41 mM in 100 mM Br\(^-\), F\(^-\), and I\(^-\) medium, respectively.

The ability of non-halide anions to compete with Cl\(^-\) for binding to the exchanger was also investigated (Fig. 7 B). HCO\(_3^-\), NO\(_3^-\), and SCN\(^-\) inhibited the initial rate of \(^{36}\)Cl\(^-\) influx from 5 mM Cl\(^-\) media with apparent \(K_i\)'s of 8.3 ± 1.8, 16.8 ± 2.5, and 111 ± 36 mM (or, from Eq. 5, true \(K_m\)'s of 4.1, 8.4, and 55.6 mM), respectively. We also found (data not shown) the initial rate of \(^{36}\)Cl\(^-\) efflux into 20 mM HCO\(_3^-\) and 140 mM NO\(_3^-\) (balance, glucuronate) to be 1.39 ± 0.09 and 1.48 ± 0.12 meq/liter·min (\(n = 3\)), similar to that into 140 mM Cl\(^-\) medium. The \(^{36}\)Cl\(^-\) efflux rate into 140 mM SCN\(^-\) was slightly lower: 1.24 ± 0.11 meq/liter·min (\(n = 3\)). These results imply
that external HCO₃⁻, NO₃⁻, and SCN⁻ are also transported inward via the carrier in exchange for internal Cl⁻. On the contrary, the divalent anion SO₄²⁻ at external concentrations of 0–95 mM had no effect at all on the rate of ³⁶Cl⁻ influx from 5 mM Cl⁻ media (Fig. 7 B). A similar lack of effect was observed with oxalate (data not shown). These data strongly suggest that the anion-exchange system of human neutrophils possesses little if any affinity for SO₄²⁻, unlike the situation in human erythrocytes, where Cl⁻ and SO₄²⁻ bind to the inorganic anion exchanger with closely similar Kᵢ's (Schnell et al., 1977; Gunn, 1978; Barzilay and Cabantchik, 1979; Milanick and Gunn, 1984).

![Diagram of anion influx](image)

**Figure 7.** Effect of different anions on the initial rate of ³⁶Cl⁻ influx into normal-Cl⁻ neutrophils from 5 mM Cl⁻ medium. The media contained 85 mM K⁺ and 2 μM nigericin and 1 mM 2-DOG to block active transport; the concentrations of the other seven anions tested (Br⁻, I⁻, F⁻, HCO₃⁻, NO₃⁻, SCN⁻, and SO₄²⁻) were varied between 0 and 140 mM by replacement with glucuronate. The influx of ³⁶Cl⁻ was measured at 5 and 10 min and the initial influx rates were calculated from Eq. 1. The curves follow Michaelis-Menten inhibition equations superimposed on a constant background of 0.0073 meq/liter-min, representing passive ³⁶Cl⁻ influx at 5 mM Cl⁻. (A) Data for Br⁻, I⁻, and F⁻ (n = 4 for each). The apparent Kᵢ's for Br⁻, F⁻, and I⁻ are 23.0 ± 4.6, 49.9 ± 6.8, and 94.8 ± 21.2 mM, respectively. (B) Data for HCO₃⁻, NO₃⁻, SCN⁻, and SO₄²⁻ (n = 3 or 4). The apparent Kᵢ's for HCO₃⁻, NO₃⁻, and SCN⁻ are 8.3 ± 1.8, 16.8 ± 2.5 and 111 ± 36 mM, respectively. The data points for SO₄²⁻ were fitted to a straight line with slope 0.00024 ± 0.00046.

The foregoing results indicate that (a) Br⁻, F⁻, and I⁻ bind to an external site on the anion exchanger and are presumably carried inward, (b) these halide ions behave as substrates that compete with Cl⁻ and PAH for binding to the carrier, and (c) CHC competitively inhibits these interactions. The next series of experiments using ⁸²Br⁻ and ¹²⁵I⁻ demonstrate directly that these ions are indeed transported inward via a 1:1 countertransport for internal Cl⁻.

Fig. 8, A and B, displays the time courses of ⁸²Br⁻ and ¹²⁵I⁻ influx into normal-Cl⁻
cells from 148 mM Br\(^{-}\) and I\(^{-}\) media. Both influxes could be fitted to single exponentials, with influx rates of 1.45 ± 0.10 and 1.33 ± 0.07 meq/liter·min, respectively, comparable to the rates of \(^{36}\)Cl\(^{-}\) efflux into 148 mM Br\(^{-}\) or I\(^{-}\) (1.50 and 1.20 meq/liter·min; Fig. 4). These influxes were markedly inhibited by 40 mM CHC, which blocks both anion exchange and active transport (Simchowitz and De Weer, 1986; Simchowitz et al., 1986). The influxes of \(^{82}\)Br\(^{-}\) and \(^{125}\)I\(^{-}\) measured in the presence of 40 mM CHC (0.22 ± 0.02 and 0.24 ± 0.02 meq/liter·min) were slightly larger than the CHC-insensitive influxes (0.12 and 0.19 meq/liter·min) into Cl\(^{-}\)-depleted cells (from Fig. 1, but adjusted to an external anion concentration of 148 mM), which were nonsaturable and probably represent passive electrodiffusive influx as discussed above. These small differences can be satisfactorily attributed to residual carrier-

![Graph showing time course of \(^{82}\)Br\(^{-}\) influx](image1)

**Figure 8.** (A) Time course of \(^{82}\)Br\(^{-}\) influx into normal-CI\(^{-}\) neutrophils from 148 mM Br\(^{-}\) medium. Upper curve: influx measurements carried out using the same procedure as for CI\(^{-}\). The data were fitted to a single exponential (initial rate, 1.45 ± 0.10 meq/liter·min) constrained to a final uptake of 80.0 meq/liter cell water, the normal internal Cl\(^{-}\) level (when maximal uptake was treated as a least-squares adjustable parameter, a value of 66.7 ± 21.8 meq/liter cell water was obtained). Middle curve: \(^{82}\)Br\(^{-}\) influx from 148 mM Br\(^{-}\) medium in the presence of 1 mM of either 2-DOG or furosemide. The curve is a single-exponential fit to the combined data, with an initial rate of 1.22 ± 0.04 meq/liter·min. Lower curve: influx of \(^{82}\)Br\(^{-}\) from a 108 mM Br\(^{-}\) medium, in the presence of 40 mM CHC. The slope of the straight line (corrected to 148 mM Br\(^{-}\) to compensate for dilution by CHC) was 0.22 ± 0.02 meq/liter·min. Results are from three to five experiments. (B) Time course of \(^{125}\)I\(^{-}\) influx from I\(^{-}\) medium. Upper curve: influx from 148 mM I\(^{-}\) medium. The data were fitted to a single exponential as in A (initial rate, 1.33 ± 0.07 meq/liter·min). Middle curve: influx in the presence of 1 mM 2-DOG. Single exponential with initial rate 1.15 ± 0.08 meq/liter·min. Lower curve: see A. The slope of the straight line (corrected to 148 mM I\(^{-}\)) was 0.24 ± 0.02 meq/liter·min. Results are from three to four experiments.
mediated anion exchange in normal-Cl\(^-\) cells at 40 mM CHC. In the presence of 1 mM of either 2-DOG or furosemide, inhibitors of active Cl\(^-\) transport (Simchowitz and De Weer, 1986), the initial rate of \(^{82}\)Br\(^-\) influx decreased by \(\sim 15\%\) to 1.22 \(\pm 0.04\) meq/liter·min (Fig. 8 A). The reduction of \(^{82}\)Br\(^-\) influx by the two drugs, 0.23 meq/liter·min, is similar to the reported magnitude (\(\sim 0.25\) meq/liter·min) of the active transport component of \(^{36}\)Cl\(^-\) influx (Simchowitz and De Weer, 1986). 2-DOG also lowered the initial rate of \(^{125}\)I\(^-\) influx by 0.18 meq/liter·min (Fig. 8 B).

**Figure 9.** Rate of \(^{82}\)Br\(^-\) influx into normal-Cl\(^-\) neutrophils as a function of the extracellular Br\(^-\) concentration, where Br\(^-\) replaced either glucuronate (A) or PAH (B). After a 10-min pretreatment with 1 mM 2-DOG (to block active transport), the cells were exposed to solutions containing 1 mM 2-DOG and various concentrations of labeled Br\(^-\) (2 μCi/ml) and influxes were measured as in Fig. 8. Results are from four to six experiments. (A) Media containing 85 mM K\(^+\) and 2 μM nigericin, where Br\(^-\) replaced glucuronate. The curve drawn through the data ("total") is the sum of the other two curves shown. The line labeled "leak" is the CHC-resistant, presumably passive, linear component taken from Fig. 1 and corrected to \(-10\) mV; its equation is: influx \(= 0.0021\) mM [Br\(^-\)]\(_o\). The curve labeled "exchange" is a Michaelis-Menten equation fitted to the data from which the leak component had been subtracted, with \(K_m(Br^-) = 6.2 \pm 0.6\) mM and \(V_{max} = 1.19 \pm 0.10\) meq/liter·min. (B) Media containing 85 mM K\(^+\) and 2 μM nigericin, where Br\(^-\) replaced PAH. See above. The line labeled "leak" is as in A. The parameters of the Michaelis-Menten equation describing the "exchange" curve are: apparent \(K_m(Br^-) = 31.4 \pm 8.2\) mM and \(V_{max} = 1.34 \pm 0.13\) meq/liter·min.

In Figs. 9 and 10, the initial \(^{82}\)Br\(^-\) and \(^{125}\)I\(^-\) influx rates are plotted against the external concentrations of these ions replacing either glucuronate or PAH. The experiments were performed in the presence of 1 mM 2-DOG to block active transport. All three panels show substrate saturation. Br\(^-\) stimulates its own influx with a \(K_m\) of 6.2 \(\pm 0.6\) mM when replacing glucuronate (Fig. 9 A), and with an apparent \(K_m\) of 31.4 \(\pm 8.2\) mM when replacing PAH (Fig. 9 B). This is as expected since, in the
latter case, external PAH and Br\(^-\) compete for the same sites on the anion exchanger. These constants are similar to the true and apparent dissociation constants for Br\(^-\) activation of \(^{36}\)Cl\(^-\) efflux into glucuronate (8.6 ± 1.8 mM; Fig. 5 A) and PAH media (37.2 ± 19.8 mM; Fig. 5 B). Uptake of \(^{125}\)I\(^-\) also follows a Michaelis-Menten curve, with a \(K_m\) of 55.4 ± 16.8 mM in glucuronate, similar to the \(K_m\) value (47.0 ± 7.3 mM) for \(\text{I}^-\) stimulation of \(^{36}\)Cl\(^-\) efflux into glucuronate medium (Fig. 5 A). As mentioned earlier, an apparent \(K_m(\text{I}^-)\) cannot be measured in PAH medium since \(K_m(\text{PAH})\) is similar (∼50 mM) to that for \(\text{I}^-\).

**FIGURE 10.** Rate of \(^{125}\)I\(^-\) influx into normal-Cl\(^-\) neutrophils as a function of the extracellular \(\text{I}^-\) concentration. See legend to Fig. 9 A. The cells had been pretreated with 1 mM 2-DOG and experiments were performed in media containing 1 mM 2-DOG, 85 mM K\(^+\), and 2 \(\mu\)M nigericin, where \(\text{I}^-\) replaced glucuronate. The curve drawn through the data ("total") is the sum of the other two curves shown. The line labeled "leak" has been taken from Fig. 1 and corrected to −10 mV; its equation is: influx = 0.0034 mM [\(\text{I}^-\)]\(_o\). The parameters of the Michaelis-Menten equation describing the "exchange" curve are \(K_m(\text{I}^-) = 55.4 \pm 16.8 \text{ mM and } V_{\text{max}} = 1.26 \pm 0.17 \text{ meq/liter-min (}n = 4\).

**FIGURE 11.** Effect of external Cl\(^-\) on the initial rate of \(^{85}\)Br\(^-\) influx into normal-Cl\(^-\) neutrophils from 20 mM Br\(^-\) medium. The media contained 85 mM K\(^+\) and 2 \(\mu\)M nigericin and 1 mM 2-DOG to block active transport, Cl\(^-\) replaced glucuronate. The influx rates (\(n = 4\)) were calculated as in Fig. 8. The curve is a Michaelis-Menten inhibition equation (apparent \(K_i\), 13.5 ± 4.6 mM) superimposed on a background influx rate of 0.042 meq/liter-min, representing passive \(^{85}\)Br\(^-\) influx at a \(V_m\) of approximately −10 mV.
It was shown above (Fig. 7 A) that \( ^{36}\text{Cl}^- \) influx is competitively inhibited by external \( \text{Br}^- \). The converse also holds true (Fig. 11): influx of \( ^{85}\text{Br}^- \) from 20 mM \( \text{Br}^- \) medium (balance, glucuronate) is inhibited by external \( \text{Cl}^- \) with an apparent \( K_i \) of 13.5 \( \pm \) 4.6 mM, as expected from Eq. 5, if \( K_{\text{m,ex}}(\text{Cl}^-) = 5.0 \) mM (Simchowitz et al., 1986) and \( K_{\text{m,ex}}(\text{Br}^-) = 8.6 \) mM (Fig. 5 A).

The influxes of \( ^{85}\text{Br}^- \) and \( ^{125}\text{I}^- \), which presumably reflect transport via the anion exchanger, should be inhibited by CHC. This point has already been documented for 40 mM CHC (Fig. 8, A and B). The dose dependence of CHC inhibition of \( ^{85}\text{Br}^- \) and \( ^{125}\text{I}^- \) influx (from 100 mM media) is plotted in Fig. 12. Taking into account the CHC-insensitive background influxes of \( ^{85}\text{Br}^- \) and \( ^{125}\text{I}^- \) (0.21 and 0.34 meq/liter-min, respectively; Figs. 1 and 3), the graphs show Michaelis-Menten inhibition curves with apparent \( K_i \)'s for CHC of 3.43 \( \pm \) 0.82 and 0.94 \( \pm \) 0.27 mM in \( \text{Br}^- \) and \( \text{I}^- \) media, respectively, comparable to those for inhibition of \( ^{36}\text{Cl}^- \) efflux into 100 mM \( \text{Br}^- \) or \( \text{I}^- \) (2.60 \( \pm \) 0.44 and 1.16 \( \pm \) 0.41 mM, respectively; Fig 6).

We have previously concluded that when normal-\( \text{Cl}^- \) cells are placed in 148 mM \( \text{Cl}^- \) or \( \text{PAH}^- \) medium, the anion exchanger engages in 1:1 \( \text{Cl}^-/\text{Cl}^- \) or \( \text{Cl}^-/\text{PAH}^- \) countertransport: the ratio of CHC-sensitive \( ^{36}\text{Cl}^- \) or \( [\text{H}]\text{PAH} \) influx to \( ^{36}\text{Cl}^- \) efflux was close to unity (Simchowitz et al., 1986). Fig. 13 illustrates the same relationship between \( ^{85}\text{Br}^- \) or \( ^{125}\text{I}^- \) influx on the one hand and \( ^{36}\text{Cl}^- \) efflux on the other. The data were taken from Figs. 5, 6, 9, 10, and 12 after appropriate subtractions of the passive leak fluxes. The line of identity is shown for comparison. The slopes of the lines (not...
shown), representing the average carrier-mediated $^{85}$Br$^-$ or $^{125}$I$^-$ influx/$^{36}$Cl$^-$ efflux ratios, are $0.97 \pm 0.03$ and $0.92 \pm 0.03$ for the Br$^-$ and I$^-$ data, respectively.

Selectivity of Active Transport

Fig. 8 suggests that 1 mM 2-DOG or furosemide reduces the initial rate of $^{85}$Br$^-$ and $^{125}$I$^-$ influx from 148 mM media by 0.23 and 0.18 meq/liter·min, respectively. These flux magnitudes are similar to the value of $-0.25$ (range, 0.20–0.27) meq/liter·min for the component of $^{36}$Cl$^-$ influx (~20% of total) that behaves as active transport (Simchowitz and De Weer, 1986). Thus, it would appear that this active transport system also possesses affinity for Br$^-$ and I$^-$. This question is addressed in Fig. 14, which shows the ability of the other halide anions to inhibit 2-DOG-sensitive

![Figure 13](image-url)

**Figure 13.** Plot of 2-DOG-resistant, CHC-sensitive $^{85}$Br$^-$ or $^{125}$I$^-$ influx against anion-stimulated $^{36}$Cl$^-$ efflux, at various extracellular Br$^-$, I$^-$, or CHC concentrations. The data for $^{85}$Br$^-$ influx and $^{36}$Cl$^-$ efflux in glucuronate (○, □) or PAH (■) media were taken from Figs. 5, 6, 9, and 12; those for $^{125}$I$^-$ influx and $^{36}$Cl$^-$ efflux in glucuronate media (▲, △) were taken from Figs. 5 A, 6, 10, and 12. In order to derive information for only the exchange fluxes, all data were adjusted by subtracting the appropriate small passive leak fluxes. In addition, for the data on $^{36}$Cl$^-$ efflux as a function of external Br$^-$ replacing PAH (Fig. 5 B), corrections were also applied for the amount of Cl$^-$/PAH$^+$ exchange. The filled symbols refer to results on the external Br$^-$ or I$^-$ dependence of $^{85}$Br$^-$ or $^{125}$I$^-$ influx or of $^{36}$Cl$^-$ efflux. The open symbols denote experiments performed in the presence of varying concentrations of CHC. The line of identity, where the average influx/efflux ratio = 1.0, is shown for comparison. The least-squares slopes of the proportionality lines (not shown) fitted through the points were $0.97 \pm 0.03$ for the Br$^-$ data and $0.92 \pm 0.05$ for the I$^-$ data.

$^{36}$Cl$^-$ influx, which we have operationally defined as the active transport component (Simchowitz and De Weer, 1986). For these experiments, the external Cl$^-$ concentration was 5 mM, and the balance was PAH. The initial rate of 2-DOG-sensitive $^{36}$Cl$^-$ influx was inhibited with Michaelis-Menten kinetics: the apparent $K_i$s for Br$^-$, I$^-$, and F$^-$ were $14.2 \pm 2.9$, $36.7 \pm 9.6$, and $83.6 \pm 20.1$ mM, respectively. Since these studies were conducted at a [Cl$^-$] of 5 mM, roughly equal to its $K_m$ ($4.8 \pm 1.2$ mM; Simchowitz and De Weer, 1986), these values correspond to true $K_m$'s (Eq. 5) of 7.0, 18.0, and 40.9 mM, respectively, assuming that this active transport system lacks affinity for PAH.

We also directly determined the [Br$^-$] and [I$^-$] dependence of this active uptake component. In Fig. 15, the differences in the initial influx rates of $^{85}$Br$^-$ or $^{125}$I$^-$ into Cl$^-$-depleted cells, in the presence and absence of 1 mM 2-DOG, have been
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Figure 14. Active (2-DOG-sensitive) $^{36}$Cl$^-$ influx from 5 mM Cl$^-$ into Cl$^-$-depleted cells: effect of other halide anions. The concentrations of Br$^-$, I$^-$, and F$^-$ were varied between 0 and 140 mM by replacement with PAH. Data represent the differences in influx rates, at each concentration of Br$^-$, I$^-$, or F$^-$, between cells treated with and without 1 mM 2-DOG ($n = 3$ for each). Least-squares fits of the data to Michaelis-Menten inhibition equations yielded apparent $K_i$'s of $14.2 \pm 2.9$, $36.7 \pm 9.6$, and $83.6 \pm 20.1$ mM for Br$^-$, I$^-$, and F$^-$, respectively.

Plotted against the external concentrations of Br$^-$ or I$^-$ (0.3–80 mM; balance, PAH). Least-squares fits yield Michaelis-Menten activation curves with the following parameters: for Br$^-$, $K_m = 8.2 \pm 3.2$ mM and $V_{max} = 0.21 \pm 0.03$ meq/liter·min; for I$^-$, $K_m = 13.2 \pm 5.2$ mM and $V_{max} = 0.17 \pm 0.02$ meq/liter·min.

Figure 15. 2-DOG-sensitive $^{85}$Br$^-$ or $^{125}$I$^-$ influx into Cl$^-$-depleted cells as a function of [Br$^-$]o or [I$^-$]o. The cells were depleted of internal Cl$^-$ by a 5-h exposure to PAH medium. They were then incubated in media with various labeled Br$^-$ or I$^-$ concentrations (0.3–80 mM; balance, PAH) in the presence or absence of 1 mM 2-DOG. The influx rates ($n = 6$ for each) of $^{85}$Br$^-$ and $^{125}$I$^-$ were measured as in Fig. 8. The data points represent paired differences between the $^{85}$Br$^-$ or $^{125}$I$^-$ influxes measured with and without 2-DOG in the medium. The least-squares parameters of the Michaelis-Menten curves are: for Br$^-$, $K_m = 8.2 \pm 3.2$ mM and $V_{max} = 0.205 \pm 0.030$ meq/liter·min; for I$^-$, $K_m = 13.2 \pm 5.2$ mM and $V_{max} = 0.168 \pm 0.022$ meq/liter·min.
DISCUSSION

Selectivity of Anion Exchange

We investigated the extent to which halides other than Cl\(^-\) are handled by the anion-exchange carrier of human neutrophils. This involved several methods, as follows: (a) Substrate competition: in the presence of another substrate such as Cl\(^-\) or PAH, the apparent \( K_m \) for Br\(^-\), F\(^-\), or I\(^-\) depends on the nature of the substituted anion (i.e., the apparent \( K_m \) rises in the presence of competing substrates of increasing affinity); (b) Specific inhibition: the apparent \( K_i \) for the competitive inhibitor CHC similarly depends on the nature of the replacement anion; (c) Trans effects: Br\(^-\), F\(^-\), and I\(^-\) stimulate the counterefflux of Cl\(^-\); and (d) The uptake rate of labeled substrate as a function of its external concentration.

These substrate and inhibitor interactions in normal-Cl\(^-\) cells can be accounted for by a single-site equilibrium carrier model. This model, based on competition kinetics, has been described in detail in one of our previous reports on anion exchange (Simchowitz et al., 1986). Thus, only a brief quantitative description of its application to the present studies is provided below. Since the Michaelis-Menten constants for the 17 kinetic curves in Figs. 5, 6, 7A, and 9-12 should all be functions of six true constants \([K_m(\text{Cl}^-), K_m(\text{Br}^-), K_m(\text{I}^-), K_m(\text{F}^-), K_m(\text{PAH}), \text{and } K_i(\text{CHC})]\) in various algebraic combinations, we modified the program (designed and kindly provided by Dr. Paul De Weer) used in our original report (Simchowitz et al., 1986) to obtain separate programs for Br\(^-\), I\(^-\), and F\(^-\) in order to least-squares fit a unique \( K_m \) value to all the data derived with that anion simultaneously. In these analyses, \( K_m(\text{Cl}^-), K_m(\text{PAH}), \) and \( K_i(\text{CHC}) \) were taken as 5.0, 50.3, and 0.29 mM, respectively, the values previously computed using a more complicated version of this program. Table I lists the refined values for "true" \( K_m(\text{Br}^-), K_m(\text{F}^-), \) and \( K_m(\text{I}^-) \), as well as the appropriate derived (apparent) constants. Considering the external concentration ranges available for experimentation and the frequency with which each parameter appeared in the various equations, reasonable accuracy can be claimed for all three values.

From these numbers, all other apparent \( K_m \) and \( K_i \) values with respect to the anion exchanger in this article can be derived as described above. In addition, for convenience, the true \( K_m \) values for the other lyotropic anions HCO\(_3^-\), NO\(_2^-\), and SCN\(^-\) are also given in Table I. These values were calculated from Eq. 5 on the assumption that true \( K_m(\text{Cl}^-) = 5.0 \, \text{mM} \) (Simchowitz et al., 1986).

Several points should be made clear concerning the physical meaning of the constants listed in Table I. At present, there is insufficient information available to even attempt to formulate a descriptive model of anion exchange in neutrophils. However, on the not-unreasonable assumption that the mechanism follows ping-pong kinetics with obligatory exchange, as in red cells (for reviews, see Gunn and Fröhlich, 1979; Fröhlich and Gunn, 1986), it is important to emphasize some of the implications of such a scheme and the theoretical limitations imposed on the analysis of these measured parameters.

Following the mathematical analysis of Fröhlich and Gunn (1986), it appears that Eq. 5 is a good approximation for competition among substrates, but it is not exact except for the case where \( S_1 \) (i.e., anions other the Cl\(^-\)) is not transported. Where \( S_1 \) is transported at a rate similar to that for \( S_2 \) (i.e., Cl\(^-\)), as in the present studies, then \( S_1 \) contributes to shifts in the steady state distribution of the various conformational...
hyperbolic function of the \textit{trans} concentration of the substrate) independently of the transport rate of \( S_1 \). In the experiments reported here, because the carrier is nearly (~80%) saturated by internal \( \mathrm{Cl}^- \) at the resting \([\mathrm{Cl}^-]_i\) of 80 meq/liter cell water, Eq. 5 probably constitutes a good approximation and the quantitative comparisons are therefore valid.

As alluded to above and addressed more fully by Fröhlich and Gunn (1986), even the derived "true" \( K_m \) values, the half-saturation constants for activation of anion

| Ligand | True \( \text{mM} \) | Apparent \( \text{mM} \) | \( s \) |
|--------|-----------------|-----------------|-----|
| CI\(^-\) | 5.0 \( \pm 0.8 \) | Replacing PAH | 18.8 \( \pm 2.0 \) |
| Br\(^-\) | 9.4 \( \pm 1.0^*\) | 5 CI\(^-\) | 5.0 CI\(^-\) |
| F\(^-\) | 23.2 \( \pm 2.9^t\) | Replacing PAH | 171 \( \pm 40^t\) |
| I\(^-\) | 44.2 \( \pm 7.5^t\) | 5 CI\(^-\) | 88.4 \( \pm 15.1^t\) |
| HCO\(_3\)\(^-\) | 4.1 \( \pm 0.9^{**}\) | 5 CI\(^-\) | 8.3 \( \pm 1.8^{**}\) |
| NO\(_3\)\(^-\) | 8.4 \( \pm 1.3^{**}\) | 5 CI\(^-\) | 16.8 \( \pm 2.5^{**}\) |
| SCN\(^-\) | 55.6 \( \pm 18.2^{**}\) | 5 CI\(^-\) | 111 \( \pm 36^{**}\) |
| PAH | 50.3 \( \pm 14.9 \) | 100 Br\(^-\) | 5.4 \( \pm 0.3^{R} \) |
| CHC | 0.29 \( \pm 0.09 \) | 100 F\(^-\) | 1.5 \( \pm 0.2^{R} \) |

True and apparent kinetic constants derived by least-squares fitting the single-site competition kinetics carrier model described in the text to all the data derived with each halide ion in Figs. 5, 6, 7 A, 9, 10, 11, and 12 simultaneously. For convenience, comparable data for other lyotropic anions that were obtained from Fig. 7 B have also been listed. The model is based on the fact that glucuronate has negligible affinity for the anion-exchange carrier. The true Michaelis constants for CI\(^-\), PAH, and CHC were taken as those values previously reported (Simchowitz et al., 1986): 5.0, 50.3, and 0.29 mM, respectively.

\(^*\)Fig. 5, 6, 7 A, 9, 11, and 12.
\(^t\)Figs. 5 B and 9 B.
\(^R\)Fig. 7 A.
\(^1\)Figs. 5, 6, and 7 A.
\(^2\)Figs. 5 A, 6, 7 A, 10, and 12.
\(^{**}\)Fig. 7 B.
\(^{**}\)Figs. 6 and 12.

...
transport, do not necessarily represent equilibrium dissociation constants for binding of these substrates to the external translocation site of the carrier. In reality, the Michaelis constants for flux activation depend on a number of additional factors, including the trans concentration of substrates, binding and/or translocation asymmetries, and whether the ion-binding step or translocation is rate-limiting. Thus, the Michaelis constants obtained experimentally are not real constants, but rather complex functions of other kinetic parameters.

The topic of anion selectivity in physical and biological systems has been the subject of several extensive reviews (Diamond and Wright, 1969; Wright and Diamond, 1977). In 1961, Eisenman put forth a unifying theory to explain the occurrence of only 11 of the 120 possible sequences of alkali metal selectivity (Eisenman, 1961). On the basis of biophysical principles, he postulated that differences in anionic field strength in the membrane determine which sequence is preferred in a given system. Since the original report, the selectivities for the halide series (Diamond and Wright, 1969; Wright and Diamond, 1977) have also been calculated according to this theory and related to the sequences observed in nature (7 out of a possible 24).

The human neutrophil anion exchanger, which functions physiologically as a Cl-/HCO₃⁻ exchanger in the regulation of intracellular pH from alkaline loads (Simchowitz and Roos, 1985), binds anions in a series of decreasing affinities HCO₃⁻ > Cl⁻ > Br⁻ > NO₂⁻ > F⁻ > I⁻ > SCN⁻, which corresponds to sequence 5 for the halides. This is reminiscent of the sequence (NO₂⁻ > Cl⁻ > Br⁻ > SCN⁻ > I⁻ > F⁻) reported by Aicken and Brading (1985) for the Cl⁻/HCO₃⁻ exchanger of smooth muscle cells of guinea pig vas deferens.

In the most well-studied system, the ability of the inorganic anion-exchange (Cl⁻/HCO₃⁻) mechanism of red blood cells to handle other anions has come under intense scrutiny (for reviews, see Sachs et al., 1975; Gunn, 1979; Knauf, 1979; Lowe and Lambert, 1983). Several points comparing the human erythrocyte system to that of human neutrophils bear emphasis since important distinctions between the two are evident. In regard to affinities, Dalmark (1976), measuring self-exchange fluxes, reported that affinities decreased in the order I⁻ > HCO₃⁻ > Br⁻ > Cl⁻ > F⁻ (sequence 1), whereas Lambert and Lowe (1978), measuring changes in extracellular pH resulting from CI⁻/HCO₃⁻ exchange, observed the order HCO₃⁻ > Cl⁻ > Br⁻ > F⁻ (sequence 4 or 5; the exact position of I⁻ was uncertain, although its affinity was less than those of Cl⁻ or Br⁻). In addition, Gunn and Fröhlich (1979) have reported comparable affinities for Cl⁻ and Br⁻. The relative affinities for Cl⁻ and HCO₃⁻ have been studied by a number of investigators (for review, see Lowe and Lambert, 1983): the $K_m$ values derived for Cl⁻ range from 3 to 65 mM and those for HCO₃⁻ from 0.5 to 43 mM, with most reports finding a higher affinity for HCO₃⁻ over Cl⁻ by a factor of 2–3. Most of the apparent disparities in absolute values can be readily explained by taking into account the different experimental conditions of temperature, pH, ionic strength, use of ionophores, and presence or absence of competing anions on one or both sides of the membrane given the known asymmetry of the system (Gunn and Fröhlich, 1979).

It is of note that the anion-exchange mechanism of human neutrophils appears to be devoid of affinity for divalent anions such as SO₄²⁻ and oxalate. In decided contrast, the red cell anion exchanger both binds and transports these anions. While Cl⁻ and SO₄²⁻ possess comparable affinities (Schnell et al., 1977; Barzilay and Cabant-
chick, 1979; Milanick and Gunn, 1982), the transport rate for Cl\(^-\) is 1,000-fold greater than that for SO\(_4^{2-}\).

It should also be pointed out that for human neutrophils the maximal translocation rates for Cl\(^-\), Br\(^-\), I\(^-\), and F\(^-\) (and apparently also for HCO\(_3^-\), NO\(_3^-\), and SCN\(^-\)) are all of roughly similar magnitude (1.0–1.4 meq/liter·min). This is strikingly different from the situation in human erythrocytes, where the transport rates are very dependent on the nature of the transported anion. The transport rates of other anions have been studied in detail by a number of investigators, who found exchange rates to decrease in the order: Cl\(^-\) > Br\(^-\) > F\(^-\) > I\(^-\) (Tosteson, 1959); Cl\(^-\) = Br\(^-\) > I\(^-\) > NO\(_3^-\) > SCN\(^-\) (Wieth, 1970); Cl\(^-\) > Br\(^-\) > HCO\(_3^-\) > NO\(_3^-\) > I\(^-\) > SCN\(^-\) (Dalmark and Wieth, 1972); Cl\(^-\) > Br\(^-\) > I\(^-\) (Dalmark, 1976); Cl\(^-\) > NO\(_3^-\) > F\(^-\) > Br\(^-\) > I\(^-\) (Wieth, 1979); and Cl\(^-\) > Br\(^-\) > F\(^-\) > I\(^-\) > NO\(_3^-\) > SCN\(^-\) (Obaid et al., 1980). These results generally fall within sequence 4 or 5. The rates for Cl\(^-\) and I\(^-\), generally the fastest and slowest, respectively, among the halides differ by a factor of 100. As pointed out by Milanick and Gunn (1984), in red blood cells, the transport rates of the halide ions

### Table II

**Kinetic Constants for the Active Cl\(^-\) Transport System in Human Neutrophils**

| Ligand | True | Apparent |
|--------|------|----------|
|        | Value| Condition | Value |
| Cl\(^-\) | 4.8 ± 1.2 | 5 Cl\(^-\) | 15.9 ± 4.3* |
| Br\(^-\) | 7.8 ± 2.1* | 5 Cl\(^-\) | 29.8 ± 7.6* |
| I\(^-\) | 14.6 ± 3.7* | 5 Cl\(^-\) | 83.6 ± 20.1* |
| F\(^-\) | 40.9 ± 9.8* | 5 Cl\(^-\) | 83.6 ± 20.1* |

See Table I for details. The data have been taken from Figs. 14 and 15. The least-squares programs were based on the assumptions that the carrier has no affinity for PAH and that the true \(K_m\) for Cl\(^-\) is 4.8 mM (Simchowitz and De Weer, 1986).

*Figs. 14 and 15.

Selectivity of Active Transport

Human neutrophils possess an active inward transport system for the intracellular accumulation of chloride ([Cl\(^-\)]\(_i\) ~ 80 meq/liter of cell water; Simchowitz and De Weer, 1986), fourfold higher than expected for passive distribution at the cell's normal resting potential of approximately −53 mV (Seligmann and Gallin, 1980; Simchowitz et al., 1982). Unlike anion exchange, this uptake is dependent on intracellular ATP and is sensitive to furosemide and ethacrynic acid, although CHC inhibits both carrier-mediated processes (Simchowitz and De Weer, 1986). As with results concerning anion exchange as discussed above, the data pertaining to the true and
apparent $K_m$'s for binding of the various halide ions to the external translocation site of the active transport system were analyzed along similar lines and are compiled in Table II. As explained more fully in the preceding section, the data of Fig. 15 (stimulation of 2-DOG-sensitive $^{85}$Br⁻ or $^{125}$I⁻ influx by external Br⁻ or I⁻) can be represented by a standard Michaelis-Menten activation equation, while those of Fig. 14 showing inhibition of $^{36}$Cl⁻ influx by external Br⁻, I⁻, or F⁻ follow equations of the form given by Eq. 5. The data obtained with Br⁻ and I⁻ were each fitted separately to an abbreviated version of the program outlined in the foregoing section, based on the assumptions that the true $K_m$ for Cl⁻ is 4.8 mM (Simchowitz and De Weer, 1986) and that the carrier has no affinity for PAH. The least-squares values for "true" $K_m$(Br⁻) and "true" $K_m$(I⁻) came to 7.8 ± 2.1 and 14.6 ± 3.7 mM, respectively. The "true" $K_m$ for F⁻, derived solely from the set of data showing substrate competition between external F⁻ and Cl⁻ (Fig. 14), is 40.9 ± 9.8 mM. The order of affinities, Cl⁻ > Br⁻ > I⁻ > F⁻, corresponds to sequence 4.

Ion Permeabilities

Our estimate of $P_{Cl}$ in human neutrophils, $5.1 \times 10^{-9}$ cm/s (Simchowitz and De Weer, 1986), is of the same order of magnitude as that for human erythrocytes, $\sim 2 \times 10^{-8}$ cm/s (Hunter, 1977; Knauf et al., 1977). With respect to the anion selectivity of ion channels in neutrophils, permeabilities were observed to decrease in the order I⁻ > Br⁻ > Cl⁻, with permeability ratios of 2.2:1.4:1.0 (averages of data from Figs. 1 and 3). This is similar to the findings in human red cells, where the relative permeability ratio is 5:1.5:1 for I⁻, Br⁻, and Cl⁻, respectively (Hunter, 1977). These ratios correspond to sequence 1, reflecting interactions with a cationic site of low field strength (Diamond and Wright, 1969; Wright and Diamond, 1977).

In summary, the anion-exchange system of human neutrophils possesses affinity for all halide ions and for several other monovalent anions, but seems to lack affinity for divalent anions. The order of decreasing affinities is $\text{HCO}_3^- \sim \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{F}^- > \text{I}^- \sim \text{PAH}^- \sim \text{SCN}^-$ (sequence 5). However, the neutrophil's active inward transport system for the intracellular accumulation of Cl⁻ binds halides in the sequence Cl⁻ > Br⁻ > I⁻ > F⁻, corresponding to sequence 4. The sequences of both carriers differ markedly from that of halide selectivity in the Cl⁻ channel, where permeabilities decrease in the order I⁻ > Br⁻ > Cl⁻ (sequence 1).

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