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

LOUIS SIMCHOWITZ

From the Department of Medicine, the John Cochran Veterans Administration Medical Center, and the Departments of Medicine and of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110

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; K_i ~ 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 *trans* stimulation of $^{36}Cl^-$ efflux as a function of the external concentrations of these anions, (*iii*) from changes in the apparent K_i for CHC depending on the nature of the replacement anion in the bathing medium, and (*iv*) from activation of $^{82}Br^-$ and $^{125}I^-$ influxes by their respective ions. Each was bound and transported at roughly similar rates (V_{max} values all 1.0–1.4 meq/liter cell water · min); the order of decreasing affinities is $Cl^- > Br^- > F^- > I^-$ (true K_m 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 $Cl^- > Br^- > I^- > F^-$ (K_m values of 5, 8, 15, and 41 mM, respectively). (*c*) Electrodiffusive fluxes are small. CHC-resistant $^{82}Br^-$ and $^{125}I^-$ 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 $P_{Cl} \sim 5 \times 10^{-9}$ cm/s.

Address reprint requests to Dr. Louis Simchowitz, John Cochran V.A. Medical Center, 915 N. Grand Ave., St. Louis, MO 63125.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/88/06/0835/26 $2.00
Volume 91 June 1988 835–860

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·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 ($P_{Cl} \simeq 4$–5×10^{-9} cm/s, P_K:$P_{Cl} \sim 10$); 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, α-cyano-4-hydroxycinnamate (CHC), furosemide, and ethacrynic acid; this active process is probably responsible for the intracellular accumulation of Cl^- ($[Cl^-]_i \simeq 80$ 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 ($K_m \sim 5$ mM), *p*-aminohippurate (PAH) is also transported, but with comparatively low affinity ($K_m \sim 50$ 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 $K_i \sim 0.3$ 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 squence $Cl^- > Br^- > I^- > F^-$, 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 $CaCl_2$, 0.5 $MgCl_2$, 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^-, Ca^{2+} and Mg^{2+} were omitted. In preliminary studies, it was ascertained that the lack of external Ca^{2+} and Mg^{2+} had no effect on unidirectional $^{36}Cl^-$ fluxes measured in 148 mM Cl^- medium. All solutions were routinely gassed with N_2 immediately before experimentation in order to deplete the amount of HCO_3^- in these nominally CO_2/HCO_3^--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.) These 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 ($[Cl^-]_i \sim 2$ 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 $NH_4[^{82}Br]$ in 0.1 N NH_4OH, the NH_4OH was removed by evaporation under a stream of N_2 and the remaining salt was then dissolved in medium. For the $^{125}I^-$ studies, the small amount of free I_2 was removed by incubating a small aliquot of the $Na[^{125}I]$ solution with 5 mM $Na_2S_2O_3$, evaporating with N_2, and then diluting the residue in medium. Furosemide was a generous gift of Hoechst Laboratories, Somerville, NJ; the potentiometric dye diS-C_3(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 × 10^6 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:

$$C_t = C_\infty [1 - \exp(-kt)], \tag{1}$$

where C_t is the cell label at time t, C_∞ 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_∞. 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 × 10^7/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-C_3(5) in ethanol was kept in the dark at 4°C. Experiments were performed in a total volume of 3 ml containing 4 × 10^6 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

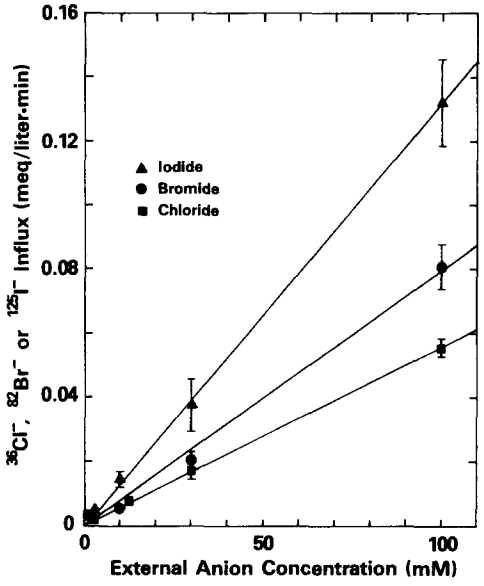

FIGURE 1. Rates of CHC-resistant $^{82}Br^-$ and $^{125}I^-$ influx into Cl^--depleted cells ($[Cl^-]_i \sim 2$ mM). The bathing solutions contained 3–100 mM of either Br^- or I^- (balance, PAH) and 40 mM CHC. Experiments were performed over the course of 1 h, during which influxes were linear with time. 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 $[Br^-]_o$ and $[I^-]_o$. 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 $^{82}Br^-$ and $^{125}I^-$ 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 $^{36}Cl^-$ (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 $^{82}Br^-$ and $^{125}I^-$ influxes plotted against the external anion concentration. The $^{36}Cl^-$ influx data of Simchowitz and De Weer (1986) are also shown for comparison. For both $^{82}Br^-$ and $^{125}I^-$, CHC-resistant influxes were linear functions of $[Br^-]_o$ and $[I^-]_o$ 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 (V_m) 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_{Cl} (4–5 × 10^{-9} cm/s) is ~1/10 of P_K (Simchowitz and De Weer, 1986), one would expect [Br^-] and [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

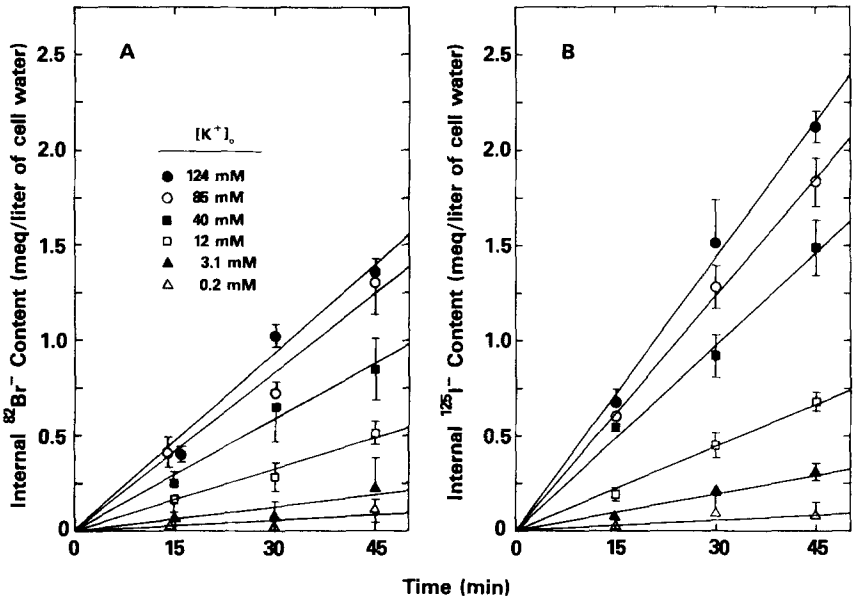

FIGURE 2. Effect of membrane potential on $^{82}Br^-$ and $^{125}I^-$ influxes into Cl^--depleted ($[Cl^-]_i$ ~ 2 mM) cells. The media contained 98 mM PAH, 40 mM CHC, 10 mM of either NaBr or NaI, and K^+ at concentrations ranging from 0.2 to 124 mM (balance, *N*-methyl-D-glucamine). (*A*) $^{82}Br^-$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

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):

$$V_m = \frac{RT}{F} \ln \left(\frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i + P_X[X^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o + P_X[X^-]_o} \right) \quad (2)$$

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

$$V_m = \frac{RT}{F} \ln \left(\frac{[K^+]_o + 1.2}{124} \right). \quad (3)$$

Fig. 2 shows the time courses of $^{82}Br^-$ (*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 $^{82}Br^-$ 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 I^- 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 $^{82}Br^-$ 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:

$$M_{in}^X = P_X \cdot \frac{V_m F}{RT} \cdot \frac{[X^-]_o}{1 - \exp(-V_m F/RT)}, \quad (4)$$

where *P* is the permeability coefficient and X^- stands for Br^- or I^-. As can be seen in the figure, both the $^{82}Br^-$ 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^{-9} 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

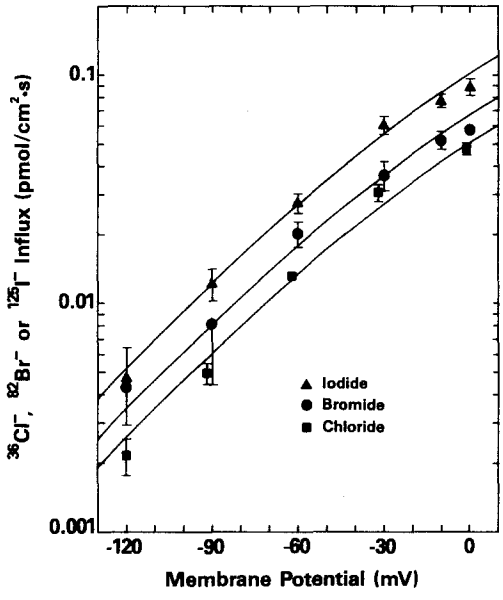

FIGURE 3. Replot of the slopes from Fig. 2 against the corresponding membrane potentials computed from Eq. 3. Upper curve: slopes (least-squares value ± SEM) from Fig. 2 *B* after conversion to picomoles per square centimeter times seconds, representing rates of $^{125}I^-$ influx from a 10 mM I^- medium into Cl^--depleted cells kept at the indicated membrane potential. The curve is a least-squares fit of a constant-field equation (Eq. 4) to the variance-weighted data, with P_I as the sole adjustable parameter. The best value was $P_I = 1.0 \pm 0.2 \times 10^{-8}$ cm/s. Middle curve: as above, with slopes (least-squares value ± SEM) from Fig. 2 *A*, representing rates of $^{82}Br^-$ influx from a 10 mM Br^- medium into Cl^--depleted cells as a function of membrane potential. The best value was $P_{Br} = 6.7 \pm 1.5 \times 10^{-9}$ cm/s. Lower curve: $^{36}Cl^-$ influx data taken from Simchowitz and De Weer (1986) adjusted to 10 mM Cl^- in the medium. The best value for P_{Cl} was $5.1 \pm 0.2 \times 10^{-9}$ cm/s.

(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^+ media, intracellular pH remained at its normal (Simchowitz and Roos, 1985) value of ~7.25 (pH_o 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-$[F^-]_o$ media. In practice, this mechanism is unlikely in that it is incompatible with the kinetics of the observed pH_i 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

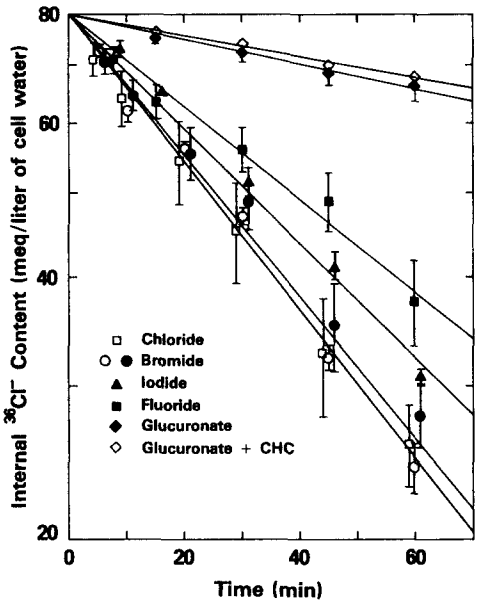

FIGURE 4. Time course of $^{36}Cl^-$ loss from normal-Cl^- cells into media containing 148 mM of various anions. The lines represent least-squares fits to single exponentials; the labels describe the conditions under which the efflux took place. Fluxes were calculated from the rate coefficients and the assumption that $[Cl^-]_i$ = 80 meq/liter cell water at zero time. Results are from three to four experiments for each condition. (□) Efflux into 148 mM Cl^- medium containing 85 mM K^+ and 2 μM nigericin; rate, 1.56 ± 0.14 meq/liter·min. (●, ○) Efflux into 148 mM Br^- medium containing either 5 mM K^+ (○) or 85 mM K^+ and 2 μM nigericin (●); rate for the combined data, 1.50 ± 0.05 meq/liter·min. (▲) Efflux into 148 mM I^- medium containing 85 mM K^+ and 2 μM nigericin; rate, 1.20 ± 0.03 meq/liter·min. (■) Efflux into 148 mM F^- medium containing 85 mM K^+ and 2 μM nigericin; rate, 0.98 ± 0.08 meq/liter·min. (◆) Efflux into 148 mM glucuronate medium containing 85 mM K^+ and 2 μM nigericin; rate, 0.26 ± 0.03 meq/liter·min. (◇) Efflux into 40 mM CHC plus 108 mM glucuronate medium containing 85 mM K^+ and 2 μM nigericin; rate, 0.22 ± 0.02 meq/liter·min.

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

before experimentation. However, since it is technically very difficult to rid solutions completely of HCO_3^-, attempts to measure $^{36}Cl^-$ efflux into a nominally "inert" medium such as glucuronate resulted in internal Cl^-/external HCO_3^- 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_i 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 HCO_3^- (derived perhaps from cell metabolism), we took the added precaution of "pH-clamping" the neutrophils at their normal pH_i 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 pH_o 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, $[H^+]_i/[H^+]_o \simeq [K^+]_i/[K^+]_o$.

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 $^{36}Cl^-$ 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 $^{36}Cl^-$ efflux component caused by the depolarization from approximately -53 mV to approximately -10 mV as $[K^+]_o$ was raised from 5 to 85 mM.

In Fig. 5 *A*, the initial rate of $^{36}Cl^-$ 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 $^{36}Cl^-$ efflux along Michaelis-Menten activation curves, although the affinities differed considerably: the K_m 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 ($K_m \sim 50$ 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 $^{36}Cl^-$ 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 K_m'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 K_m's (~40–50 mM), it is impossible to measure an apparent $K_m(I^-)$ 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 ~0.29 mM. In the presence of constant concentrations of extracellular substrate (100 mM; balance, glucuronate), CHC reduced the rate of $^{36}Cl^-$ efflux along Michaelis-Menten inhi-

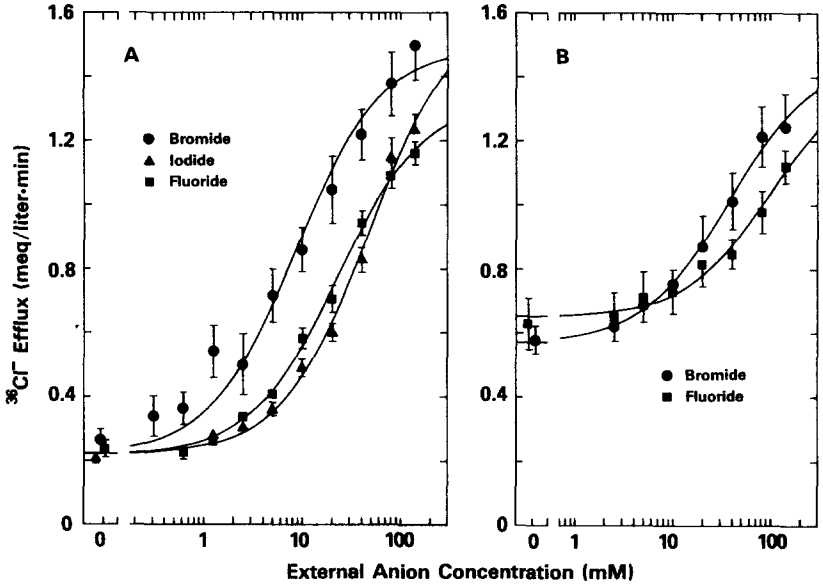

FIGURE 5. Stimulation of $^{36}Cl^-$ efflux from normal-Cl^- human neutrophils as a function of the extracellular concentrations of either Br^-, F^-, or I^-, replacing glucuronate or PAH. The bathing media contained 85 mM K^+ and 2 μM nigericin, pH 7.40. Fluxes (n = 3 or 4) were calculated as in Fig. 4. (*A*) Media in which Br^-, F^-, or I^- replaced glucuronate. The curves fitted to the data represent Michaelis-Menten activation equations superimposed on a constant background flux of 0.22 meq/liter·min, representing electrodiffusive $^{36}Cl^-$ efflux at −10 mV. The parameters of the Michaelis-Menten equations are as follows: for Br^- medium, K_m = 8.6 ± 1.8 mM and V_{max} = 1.28 ± 0.07 meq/liter·min; for I^- medium, K_m = 47.0 ± 7.3 mM and V_{max} = 1.39 ± 0.09 meq/liter·min; for F^- medium, K_m = 22.9 ± 2.7 mM and V_{max} = 1.11 ± 0.04 meq/liter·min. (*B*) Media in which Br^- or F^- replaced PAH. The curves fitted to the data represent Michaelis-Menten activation equations superimposed on background fluxes into 148 mM PAH medium of 0.57 ± 0.05 and 0.65 ± 0.03 meq/liter·min (Br^- and F^- curves, respectively). The equations describing the curves are as follows: for Br^- medium, flux = 0.89 $[Br^-]_o$/(37.2 + $[Br^-]_o$) + 0.57; for F^- medium, flux = 0.78 $[F^-]_o$/(98.5 + $[F^-]_o$) + 0.65. The Michaelis-Menten parameters are as follows: apparent $K_m(Br^-)$ = 37.2 ± 19.8 mM and apparent $K_m(F^-)$ = 98.5 ± 58.6 mM.

bition curves, with apparent K_i values of 2.60 ± 0.44, 1.57 ± 0.42, and 1.16 ± 0.41 mM in Br^-, F^-, and I^- media, respectively, as expected from the order of decreasing affinities: $Br^- > F^- > I^-$ (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 $^{36}Cl^-$ 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:

$$K_m^{app}(S_1) = K_m^{true}(S_1) \cdot \left[1 + \frac{[S_2]_o}{K_m^{true}(S_2)}\right]. \quad (5)$$

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.

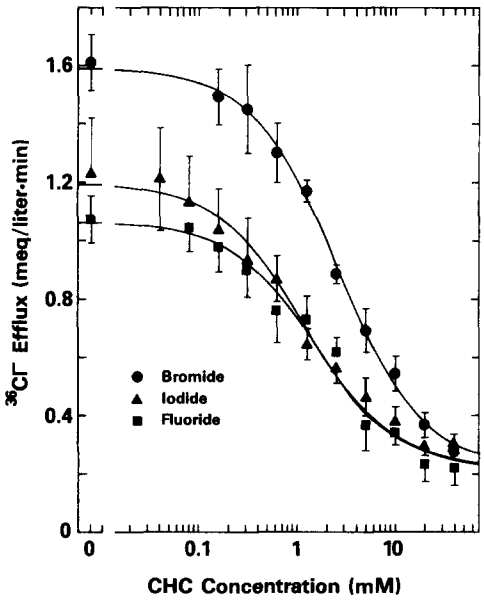

FIGURE 6. Inhibition by CHC of $^{36}Cl^-$ efflux from normal-Cl^- neutrophils into Br^-, F^-, or I^- medium. The major external anion concentration was kept constant at 100 mM as $[CHC]_o$ was raised from 0 to 40 mM (balance, glucuronate). The media contained 85 mM K^+ and 2 μM nigericin. The curves (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 HCO_3^-, NO_3^-, and SCN^- are also transported inward via the carrier in exchange for internal Cl^-. On the contrary, the divalent anion SO_4^{2-} at external concentrations of 0–95 mM had no effect at all on the rate of $^{36}Cl^-$ 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 SO_4^{2-}, unlike the situation in human erythrocytes, where Cl^- and SO_4^{2-} bind to the inorganic anion exchanger with closely similar K_m's (Schnell et al., 1977; Gunn, 1978; Barzilay and Cabantchik, 1979; Milanick and Gunn, 1984).

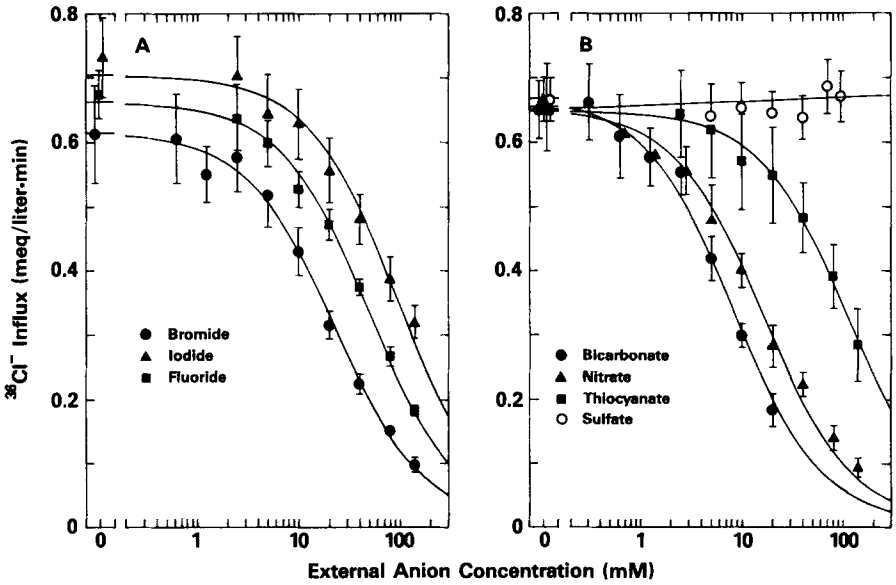

FIGURE 7. Effect of different anions on the initial rate of $^{36}Cl^-$ influx into normal-Cl^- neutrophils from 5 mM Cl^- medium. The media contained 85 mM K^+ and 2 μM nigericin and 1 mM 2-DOG to block active transport; the concentrations of the other seven anions tested (Br^-, I^-, F^-, HCO_3^-, NO_3^-, SCN^-, and SO_4^{2-}) were varied between 0 and 140 mM by replacement with glucuronate. The influx of $^{36}Cl^-$ 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 $^{36}Cl^-$ influx at 5 mM Cl^-. (*A*) Data for Br^-, I^-, and F^- ($n = 4$ for each). The apparent K_i'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 HCO_3^-, NO_3^-, SCN^-, and SO_4^{2-} ($n = 3$ or 4). The apparent K_i's for HCO_3^-, NO_3^-, and SCN^- are 8.3 ± 1.8, 16.8 ± 2.5 and 111 ± 36 mM, respectively. The data points for SO_4^{2-} 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 $^{82}Br^-$ and $^{125}I^-$ 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 $^{82}Br^-$ and $^{125}I^-$ 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;

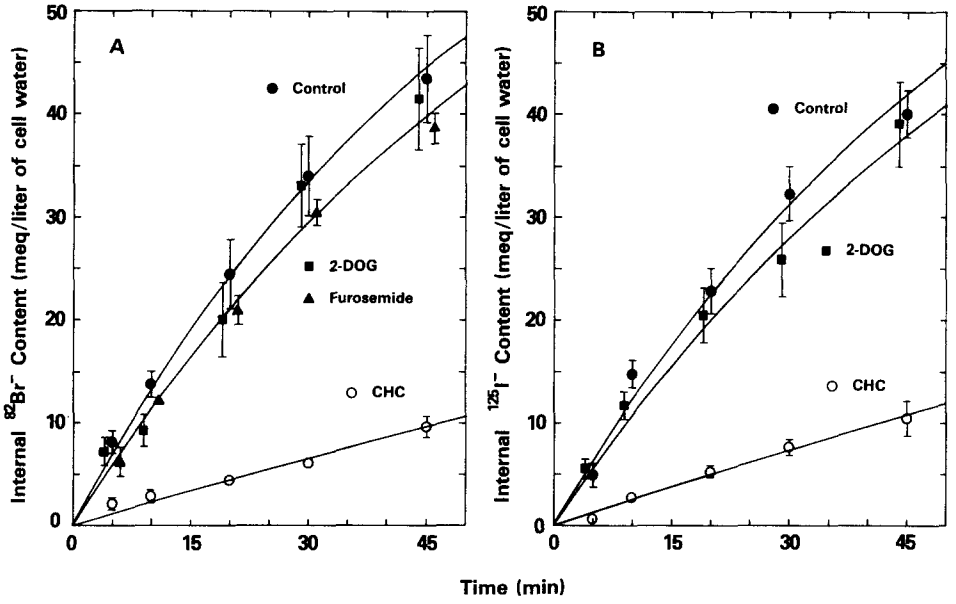

FIGURE 8. (*A*) Time course of $^{82}Br^-$ influx into normal-Cl^- neutrophils from 148 mM Br^- medium. Upper curve: influx measurements carried out using the same procedure as for Cl^-. The data were fitted to a single exponential (initial rate, 1.45 ± 0.10 meq/liter·min) constrained to a final uptake 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.

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-

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 ~15% to 1.22 ± 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 (~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*).

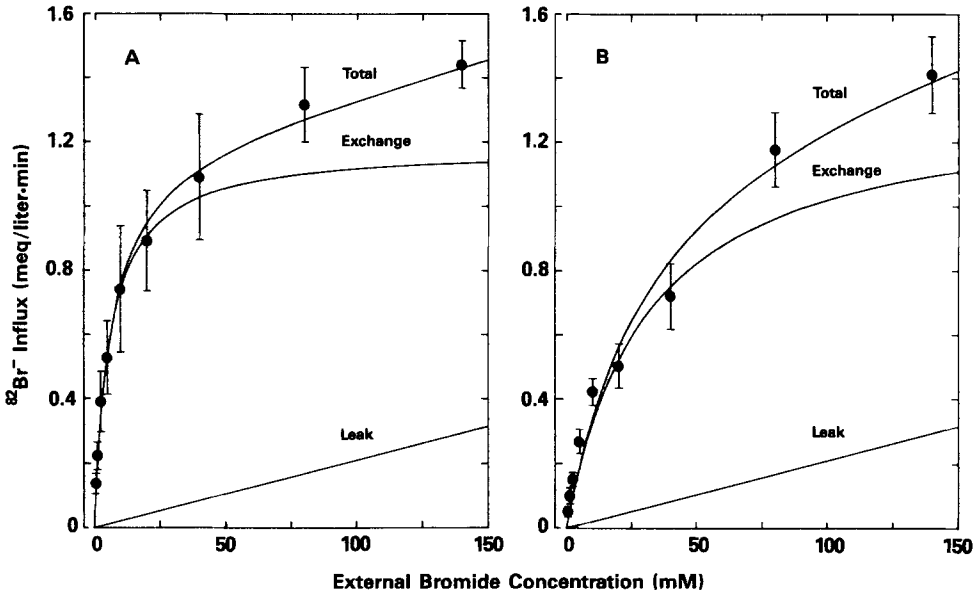

FIGURE 9. Rate of $^{82}Br^-$ influx into normal-Cl^- neutrophils as a function of the extracellular Br^- concentration, where Br^- replaced either glucuronate (*A*) or PAH (*B*). After a 10-min pretreatment with 1 mM 2-DOG (to block active transport), the cells were exposed to solutions containing 1 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 ± 0.6 mM and V_{max} = 1.19 ± 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 ± 8.2 mM and V_{max} = 1.34 ± 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 ± 0.6 mM when replacing glucuronate (Fig. 9 *A*), and with an apparent K_m of 31.4 ± 8.2 mM when replacing PAH (Fig. 9 *B*). This is as expected since, in the

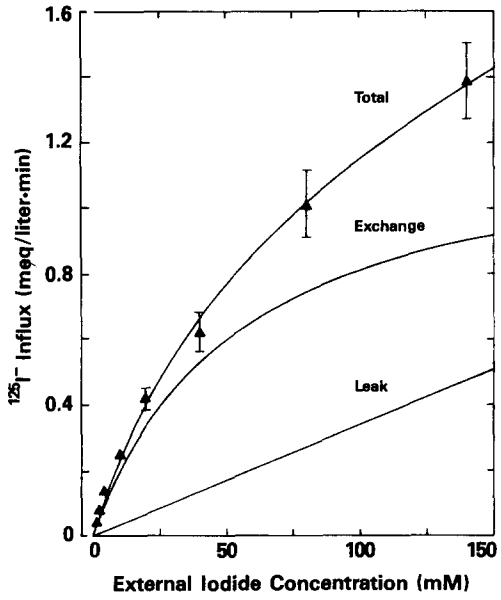

FIGURE 10. Rate of $^{125}I^-$ influx into normal-Cl^- neutrophils as a function of the extracellular I^- concentration. See legend to Fig. 9 *A*. The cells had been pretreated with 1 mM 2-DOG and experiments were performed in media containing 1 mM 2-DOG, 85 mM K^+, and 2 μM nigericin, where I^- replaced 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 $[I^-]_o$. The parameters of the Michaelis-Menten equation describing the "exchange" curve are $K_m(I^-)$ = 55.4 ± 16.8 mM and V_{max} = 1.26 ± 0.17 meq/liter·min (n = 4).

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 I^- stimulation of $^{36}Cl^-$ efflux into glucuronate medium (Fig. 5 *A*). As mentioned earlier, an apparent $K_m(I^-)$ cannot be measured in PAH medium since K_m(PAH) is similar (~50 mM) to that for I^-.

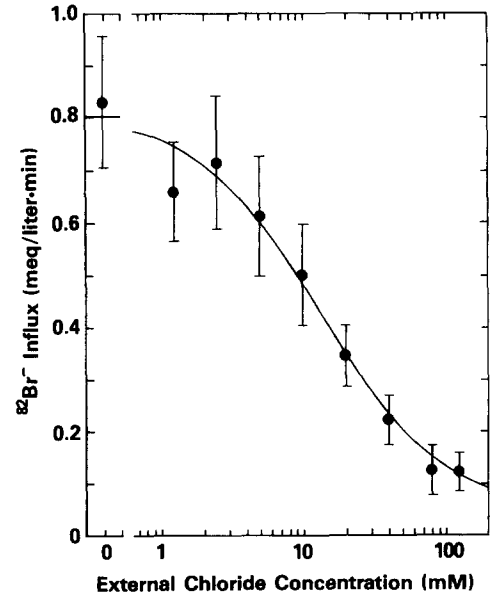

FIGURE 11. Effect of external Cl^- on the initial rate of $^{82}Br^-$ influx into normal-Cl^- neutrophils from 20 mM Br^- medium. The media contained 85 mM K^+ and 2 μM nigericin and 1 mM 2-DOG to block active transport. Cl^- replaced glucuronate. The influx rates (n = 4) were calculated as in Fig. 8. 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 $^{82}Br^-$ influx at a V_m of approximately -10 mV.

It was shown above (Fig. 7 *A*) that $^{36}Cl^-$ influx is competitively inhibited by external Br^-. The converse also holds true (Fig. 11): influx of $^{82}Br^-$ from 20 mM Br^- medium (balance, glucuronate) is inhibited by external Cl^- with an apparent K_i of 13.5 ± 4.6 mM, as expected from Eq. 5, if $K_m^{true}(Cl^-)$ = 5.0 mM (Simchowitz et al., 1986) and $K_m^{true}(Br^-)$ = 8.6 mM (Fig. 5 *A*).

The influxes of $^{82}Br^-$ and $^{125}I^-$, 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 $^{82}Br^-$ and $^{125}I^-$ influx (from 100 mM media) is plotted in Fig. 12. Taking into account the CHC-insensitive background influxes of $^{82}Br^-$ and $^{125}I^-$ (0.21 and 0.34

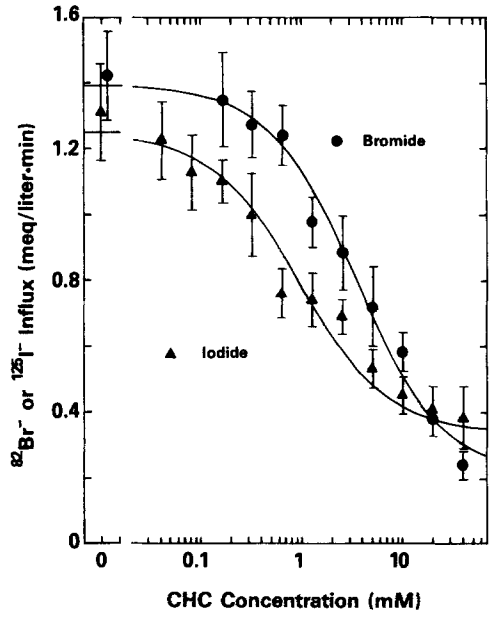

FIGURE 12. Inhibition by CHC of $^{82}Br^-$ and $^{125}I^-$ influx into normal-Cl^- human neutrophils. Experiments were performed in the presence of 1 mM 2-DOG to block active transport. Cells were exposed to 100 mM Br^- or I^- media labeled with $^{82}Br^-$ (2 μCi/ml) or $^{125}I^-$ (3 μCi/ml). The concentration of CHC was varied between 0 and 40 mM (balance, glucuronate) in the presence of 85 mM K^+ and 2 μM nigericin. Influxes (n = 4 for each) were measured as in Fig. 8 and calculated from Eq. 1. The curves are least-squares fits to Michaelis-Menten inhibition equations superimposed on a constant, CHC-insensitive background representing the leak, presumably passive, component taken from Fig. 1 after adjustment to −10 mV. These leak fluxes, at an anion concentration of 100 mM, were 0.21 and 0.34 meq/liter·min for Br^- and I^-, respectively. The equations describing the curves are: for Br^- medium, flux (in milliequivalents per liter times minutes) = $4.05/(3.43 + [CHC]_o) + 0.21$; for I^- medium, flux = $0.85/(0.94 + [CHC]_o) + 0.34$. The apparent K_i values for CHC were 3.43 ± 0.82 and 0.94 ± 0.27 mM in 100 mM Br^- and I^- medium, respectively.

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 ± 0.82 and 0.94 ± 0.27 mM in Br^- and I^- media, respectively, comparable to those for inhibition of $^{36}Cl^-$ efflux into 100 mM Br^- or I^- (2.60 ± 0.44 and 1.16 ± 0.41 mM, respectively; Fig 6).

We have previously concluded that when normal-Cl^- cells are placed in 148 mM Cl^- or PAH^- medium, the anion exchanger engages in 1:1 Cl^-/Cl^- or Cl^-/PAH^- countertransport: the ratio of CHC-sensitive $^{36}Cl^-$ or [^{3}H]PAH influx to $^{36}Cl^-$ efflux was close to unity (Simchowitz et al., 1986). Fig. 13 illustrates the same relationship between $^{82}Br^-$ or $^{125}I^-$ influx on the one hand and $^{36}Cl^-$ 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 $^{82}Br^-$ or $^{125}I^-$ influx/$^{36}Cl^-$ efflux ratios, are 0.97 ± 0.03 and 0.92 ± 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 $^{82}Br^-$ 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

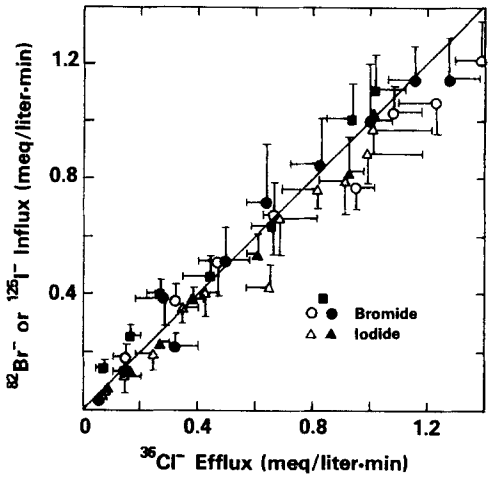

FIGURE 13. Plot of 2-DOG–resistant, CHC-sensitive $^{82}Br^-$ or $^{125}I^-$ influx against anion-stimulated $^{36}Cl^-$ efflux, at various extracellular Br^-, I^-, or CHC concentrations. The data for $^{82}Br^-$ influx and $^{36}Cl^-$ efflux in glucuronate (●, ○) or PAH (■) media were taken from Figs. 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 $^{82}Br^-$ 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 ± 0.03 for the Br^- data and 0.92 ± 0.03 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 ± 2.9, 36.7 ± 9.6, and 83.6 ± 20.1 mM, respectively. Since these studies were conducted at a $[Cl^-]_o$ of 5 mM, roughly equal to its K_m (4.8 ± 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 $[Br^-]_o$ and $[I^-]_o$ dependence of this active uptake component. In Fig. 15, the differences in the initial influx rates of $^{82}Br^-$ or $^{125}I^-$ into Cl^--depleted cells, in the presence and absence of 1 mM 2-DOG, have been

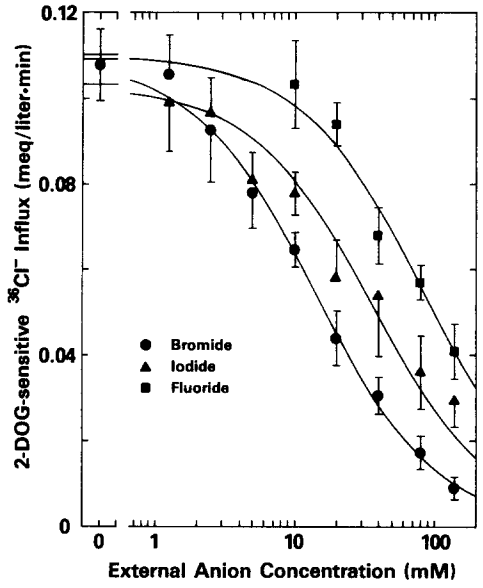

FIGURE 14. Active (2-DOG–sensitive) $^{36}Cl^-$ influx from 5 mM Cl^- into Cl^--depleted cells: effect of other halide anions. The concentrations of Br^-, I^-, and F^- were varied between 0 and 140 mM by replacement with PAH. Data represent the differences in influx rates, at each concentration of Br^-, 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 ± 2.9, 36.7 ± 9.6, and 83.6 ± 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 ± 3.2 mM and V_{max} = 0.21 ± 0.03 meq/liter·min; for I^-, K_m = 13.2 ± 5.2 mM and V_{max} = 0.17 ± 0.02 meq/liter·min.

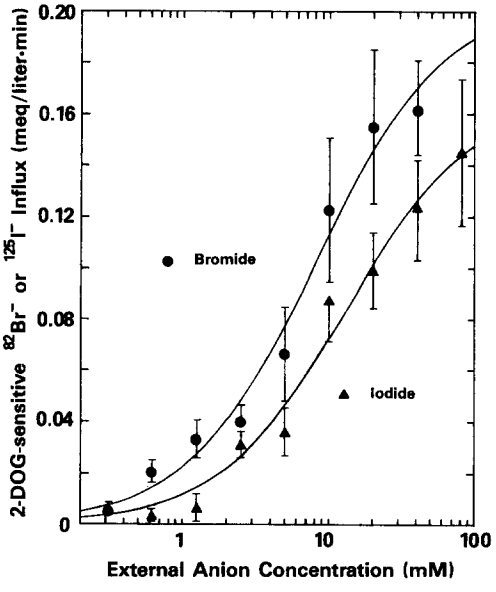

FIGURE 15. 2-DOG–sensitive $^{82}Br^-$ or $^{125}I^-$ influx into Cl^--depleted cells as a function of $[Br^-]_o$ or $[I^-]_o$. The cells were depleted of internal Cl^- by a 5-h exposure to PAH medium. They were then incubated in media with various labeled Br^- or I^- concentrations (0.3–80 mM; balance, PAH) in the presence or absence of 1 mM 2-DOG. The influx rates (n = 6 for each) of $^{82}Br^-$ and $^{125}I^-$ were measured as in Fig. 8. The data points represent paired differences between the $^{82}Br^-$ 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 ± 3.2 mM and V_{max} = 0.205 ± 0.030 meq/liter·min; for I^-, K_m = 13.2 ± 5.2 mM and V_{max} = 0.168 ± 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, 7 *A*, and 9–12 should all be functions of six true constants [$K_m(Cl^-)$, $K_m(Br^-)$, $K_m(I^-)$, $K_m(F^-)$, K_m(PAH), and K_i(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(Cl), K_m(PAH), and K_i(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" $K_m(Br^-)$, $K_m(F^-)$, and $K_m(I^-)$, 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_3^-, and SCN^- are also given in Table I. These values were calculated from Eq. 5 on the assumption that true $K_m(Cl^-)$ = 5.0 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

states of the transporter (e.g., recruitment between inward- and outward-facing states), which in turn affects the different K_m values, including the true $K_m(S_2)$. In essence, S_1 influences the apparent $K_m(S_2)$ in a way that depends on the *trans* concentration of S_2 (i.e., $[Cl^-]_i$), the effect of S_1 being greatest at low $[Cl^-]_i$. Fortunately, these considerations have relatively little effect on this study since the apparent $K_m(S_2)$ increases to the same saturating value (i.e., the Michaelis constant, the concentration of the anion on the *cis* side of the membrane that causes half the maximal flux, is a

TABLE I

Kinetic Constants for the External Binding Sites of the Anion-Exchange Carrier of Human Neutrophils

Ligand	Michaelis constants		
	True	Apparent	
		Condition	Value
	mM	*mM*	*mM*
Cl^-	5.0 ± 0.8		
Br^-	9.4 ± 1.0*	Replacing PAH	45.7 ± 5.9‡
		5 Cl^-	18.8 ± 2.0§
F^-	23.2 ± 2.9‖	Replacing PAH	171 ± 40‡
		5 Cl^-	46.4 ± 5.8§
I^-	44.2 ± 7.5¶	5 Cl^-	88.4 ± 15.1§
HCO_3^-	4.1 ± 0.9**	5 Cl^-	8.3 ± 1.8**
NO_3^-	8.4 ± 1.3**	5 Cl^-	16.8 ± 2.5**
SCN^-	55.6 ± 18.2**	5 Cl^-	111 ± 36**
PAH	50.3 ± 14.9		
CHC	0.29 ± 0.09	100 Br^-	3.4 ± 0.3‡‡
		100 F^-	1.5 ± 0.2‡‡
		100 I^-	1.0 ± 0.1‡‡

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 Cl^-, 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.

‡Figs. 5 *B* and 9 *B*.

§Fig. 7 *A*.

‖Figs. 5, 6, and 7 *A*.

¶Figs. 5 *A*, 6, 7 *A*, 10, and 12.

**Fig. 7 *B*.

‡‡Figs. 6 and 12.

hyperbolic function of the *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 Cl^- at the resting $[Cl^-]_i$ 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

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^-/HCO_3^- exchanger in the regulation of intracellular pH from alkaline loads (Simchowitz and Roos, 1985), binds anions in a series of decreasing affinities $HCO_3^- \sim Cl^- > Br^- \sim NO_3^- > F^- > I^- \sim SCN^-$, which corresponds to sequence 5 for the halides. This is reminiscent of the sequence ($NO_3^- > Cl^- \sim Br^- > SCN^- \sim I^- > F^-$) reported by Aicken and Brading (1985) for the Cl^-/HCO_3^- exchanger of smooth muscle cells of guinea pig vas deferens.

In the most well-studied system, the ability of the inorganic anion-exchange (Cl^-/HCO_3^-) 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 $I^- > HCO_3^- > Br^- > Cl^- > F^-$ (sequence 1), whereas Lambert and Lowe (1978), measuring changes in extracellular pH resulting from Cl^-/HCO_3^- exchange, observed the order $HCO_3^- > Cl^- > Br^- > F^-$ (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 HCO_3^- 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 HCO_3^- from 0.5 to 43 mM, with most reports finding a higher affinity for HCO_3^- 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 SO_4^{2-} and oxalate. In decided contrast, the red cell anion exchanger both binds and transports these anions. While Cl^- and SO_4^{2-} 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	Michaelis constants		
	True	Apparent	
		Condition	Value
	mM	*mM*	*mM*
Cl^-	4.8 ± 1.2		
Br^-	7.8 ± 2.1*	5 Cl^-	15.9 ± 4.3‡
I^-	14.6 ± 3.7*	5 Cl^-	29.8 ± 7.6‡
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.

‡Fig. 14.

(except F^-) and other monovalent anions such as HCO_3^-, NO_3^-, and SCN^- seem to fit the relationship in which the transport rate is inversely proportional to the unhydrated radius of the anion. These findings, together with the relative lack of sensitivity of Cl^-/Cl^- exchange in human neutrophils to disulfonic stilbenes (Simchowitz and De Weer, 1986), emphasize that major differences exist between the anion exchanger of leukocytes and that of red blood cells.

Selectivity of Active Transport

Human neutrophils possess an active inward transport system for the intracellular accumulation of chloride ($[Cl^-]_i \sim 80$ 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 $^{82}Br^-$ 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, 5.1×10^{-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 $HCO_3^- \sim Cl^- > Br^- \sim NO_3^- > F^- > I^- \sim PAH^- \sim SCN^-$ (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).

I would like to acknowledge the expert technical assistance of Arabella R. Kizzart, Jacquelyn T. Engle, Ajuah O. Davis, and William H. Miller, and the secretarial skills of Sue Eads and Janice Wuelling. I am especially grateful to Dr. Paul De Weer for his helpful discussions while the work was in progress, for supplying the least-squares programs, and for his comments on an early version of this manuscript. I also wish to thank Ronald W. Ratzlaff for his diligent efforts in connection with the computer files.

This work was supported by the Veterans Administration and by U.S. Public Health Service grant GM-38094.

Original version received 31 August 1987 and accepted version received 21 December 1987.

REFERENCES

Aickin, C. C., and A. F. Brading. 1985. The effects of bicarbonate and foreign anions on chloride transport in smooth muscle of the guinea-pig vas deferens. *Journal of Physiology.* 366:267–280.

Barzilay, M., and Z. I. Cabantchik. 1979. Anion transport in red blood cells. II. Kinetics of reversible inhibition by nitroaromatic sulfonic acids. *Membrane Biochemistry.* 2:255–281.

Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scandinavian Journal of Clinical and Laboratory Investigation.* 21(Suppl. 97):77–89.

Curnutte, J. R., and B. M. Babior. 1975. Effects of anaerobiosis and inhibitors on O_2^- production by human granulocytes. *Blood.* 45:851–861.

Dalmark, M. 1976. Effects of halides and bicarbonate on chloride transport in human red blood cells. *Journal of General Physiology.* 67:223–234.

Dalmark, M., and J. O. Wieth. 1972. Temperature dependence of chloride, bromide, iodide, thiocyanate and salicylate transport in human red cells. *Journal of Physiology.* 224:583–610.

Deuticke, B. 1982. Monocarboxylate transport in erythrocytes. *Journal of Membrane Biology.* 70:89–103.

Diamond, J. M., and E. M. Wright. 1969. Biological membranes: the physical basis of ion and nonelectrolyte selectivity. *Annual Review of Physiology.* 31:581–646.

Eisenman, G. 1961. On the elementary atomic origin of equilibrium ionic specificity. *In* Symposium on Membrane Transport and Metabolism. A. Kleinzeller and A. Kotyk, editors. Academic Press, Inc., New York, NY. 163–179.

Fröhlich, O., and R. B. Gunn. 1986. Erythrocyte anion transport: the kinetics of a single-site obligatory exchange system. *Biochimica et Biophysica Acta.* 864:169–194.

Goldman, D. E. 1943. Potential, impedance and rectification in membranes. *Journal of General Physiology.* 27:37–60.

Gunn, R. B. 1978. Considerations of the titratable carrier model for sulfate transport in human red blood cells. *In* Membrane Transport Processes. J. F. Hoffman, editor. Raven Press, New York, NY. 1:61–77.

Gunn, R. B. 1979. Transport of anions across red cell membranes. *In* Membrane Transport in Biology. Vol. II: Transport across Single Biological Membranes. G. Giebisch, D. C. Tosteson, and H. H. Ussing, editors. Springer-Verlag, Berlin. 59–80.

Gunn, R. B., and O. Fröhlich. 1979. Asymmetry in the mechanism for anion exchange in human red cell membranes. Evidence for reciprocating sites that react with one transported anion at a time. *Journal of General Physiology.* 74:351–374.

Halestrap, A. P. 1976. Transport of pyruvate and lactate into human erythrocytes. Evidence for the involvement of the chloride carrier and a chloride-independent carrier. *Biochemical Journal.* 156:193–207.

Halestrap, A. P., and R. M. Denton. 1975. The specificity and metabolic implications of the inhibition of pyruvate transport in isolated mitochondria and intact tissue preparations by α-cyano-4-hydroxycinnamate and related compounds. *Biochemical Journal.* 148:97–106.

Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *Journal of Physiology.* 108:37–77.

Hunter, M. J. 1977. Human erythrocyte anion permeabilities measured under conditions of net charge transfer. *Journal of Physiology.* 268:35–49.

Knauf, P. A. 1979. Erythrocyte anion exchange and the band 3 protein: transport kinetics and molecular structure. *Current Topics in Membranes and Transport.* 12:249–363.

Knauf, P. A., G. F. Fuhrmann, S. Rothstein, and A. Rothstein. 1977. The relationship between anion exchange and net anion flow across the human red blood cell membrane. *Journal of General Physiology.* 69:363–386.

Lambert, A., and A. G. Lowe. 1978. Chloride/bicarbonate exchange in human erythrocytes. *Journal of Physiology.* 275:51–63.

Lowe, A. G., and A. Lambert. 1983. Chloride-bicarbonate exchange and related transport processes. *Biochimica et Biophysica Acta.* 694:353–374.

Milanick, M. A., and R. B. Gunn. 1982. Proton-sulfate co-transport mechanism of H^+ and sulfate addition to the chloride transporter of human red blood cells. *Journal of General Physiology.* 79:87–113.

Milanick, M. A., and R. B. Gunn. 1984. Proton-sulfate cotransport: external proton activation of sulfate influx into human red blood cells. *American Journal of Physiology.* 247:C247–C259.

Naccache, P. H., H. J. Showell, E. L. Becker, and R. I. Sha'afi. 1977. Transport of sodium, potassium, and calcium across rabbit polymorphonuclear leukocyte membranes. *Journal of Cell Biology.* 73:428–444.

Obaid, A. L., T. F. Leininger, and E. D. Crandall. 1980. Exchange of HCO_3^- for monovalent anions across the human erythrocyte membrane. *Journal of Membrane Biology.* 52:173–179.

Pressman, B. C. 1969. Mechanism of action of transport-mediating antibiotics. *Annals of the New York Academy of Sciences.* 147:829–841.

Sachs, J. R., P. A. Knauf, and P. B. Dunham. 1975. Transport through red cell membranes. *In* The Red Blood Cell. 2nd edition. D. M. Surgenor, editor. Academic Press, Inc., New York, NY 2:613–703.

Schnell, K. F., S. Gerhardt, and A. Schöppe-Fredenburg. 1977. Kinetic characteristics of the sulfate self-exchange in human red blood cells and red blood cell ghosts. *Journal of Membrane Biology.* 30:319–350.

Seligmann, B. E., and J. I. Gallin. 1980. Use of lipophilic probes of membrane potential to assess human neutrophil activation. Abnormality in chronic granulomatous disease. *Journal of Clinical Investigation.* 66:493–503.

Simchowitz, L. 1985. Intracellular pH modulates the generation of superoxide radicals by human neutrophils. *Journal of Clinical Investigation.* 76:1079–1089.

Simchowitz, L., and P. De Weer. 1986. Chloride movements in human neutrophils: diffusion, exchange, and active transport. *Journal of General Physiology.* 88:167–194.

Simchowitz, L., R. Ratzlaff, and P. De Weer. 1986. Anion/anion exchange in human neutrophils. *Journal of General Physiology.* 88:195–217.

Simchowitz, L., and A. Roos. 1985. Regulation of intracellular pH of human neutrophils. *Journal of General Physiology.* 85:443–470.

Simchowitz, L., I. Spilberg, and P. De Weer. 1982. Sodium and potassium fluxes and membrane potential of human neutrophils. Evidence for an electrogenic sodium pump. *Journal of General Physiology.* 79:453–479.

Tosteson, D. C. 1959. Halide transport in red cells. *Acta Physiologica Scandinavica.* 46:19–41.

Wieth, J. O. 1970. Effect of some monovalent anions on chloride and sulfate permeability of human red cells. *Journal of Physiology.* 207:581–609.

Wieth, J. O. 1979. Bicarbonate exchange through the human red cell membrane determined with [^{14}C]bicarbonate. *Journal of Physiology.* 294:521–539.

Wright, E. M., and J. M. Diamond. 1977. Anion selectivity in biological systems. *Physiological Reviews.* 57:109–156.