Anion/Anion Exchange  
in Human Neutrophils

LOUIS SIMCHOWITZ, RONALD RATZLAFF, and  
PAUL DE WEER

From the Department of Medicine, the John Cochran Veterans Administration Medical Center, and  
the Departments of Medicine and Cell Biology and Physiology, Washington University School of  
Medicine, St. Louis, Missouri 63125

ABSTRACT  Of the total one-way chloride fluxes (~1.4 meq/liter cell water·  
min) in steady state human polymorphonuclear leukocytes bathed in 148 mM  
Cl media, ~70% behaves as self-exchange mediated by a nonselective anion  
carrier that is not inhibited by stilbene disulfonates. Five properties of this  
carrier-mediated exchange were investigated: (a) substrate saturation is seen  
with respect to 36Cl influx as a function of the external Cl concentration [for  
normal-Cl cells, the apparent K_m(Cl) is ~22 mM when Cl replaces para-amino-  
hippurate (PAH) and ~5 mM when Cl replaces glucuronate], and with respect  
to 36Cl efflux as a function of the concentration of internal Cl replacing PAH  
(apparent K_m(Cl) = 35 mM for cells bathed in 148 mM Cl]; (b) there is trans  
stimulation of 36Cl influx by internal Cl (replacing PAH) with an apparent  
K_m(Cl) = 35 mM, and of 36Cl efflux by external Cl with an apparent K_m(Cl) =  
22 mM (Cl replacing PAH) or ~5 mM (Cl replacing glucuronate); (c) there is  
substrate competition between Cl and PAH, but the carrier appears devoid of  
affinity for glucuronate; (d) influxes and effluxes mediated by the carrier are  
subject to competitive inhibition by extracellular α-cyano-4-hydroxycinnamate  
(CHC), with an apparent K_i = 9 mM in Cl medium or ~1 mM in PAH medium  
(transport of the inhibitor itself is very slow); and (e) internal Cl and external  
Cl or PAH undergo 1:1 countertransport, which is CHC sensitive. A simple  
equilibrium-competition model is proposed that accounts for all the extracel-  
lar ligand interactions presented for normal-Cl cells. Least-squares values of  
the carrier's true Michaelis constants for extracellular Cl, PAH, and CHC are  
5.03 ± 0.83, 50.3 ± 14.9, and 0.29 ± 0.09 mM, respectively.

INTRODUCTION

From the findings of the preceding article (Simchowitz and De Weer, 1986),  
it appears that the bulk of one-way Cl fluxes across the membrane of steady  
state human polymorphonuclear leukocytes represents carrier-mediated self-  
exchange. Such exchange should exhibit saturation, trans effects, specific inhi-  
bition, and possibly substrate competition and countertransport. We now present  

Address reprint requests to Dr. Louis Simchowitz, John Cochran V.A. Medical Center (151/JC),  
915 N. Grand Ave., St. Louis, MO 63125.

J. GEN. PHYSIOL. © The Rockefeller University Press - 0022-1295/86/08/0195/23$1.00  
195  
Volume 88  August 1986  195–217
evidence for all five properties, and propose a simple equilibrium model that accounts for the kinetics of the carrier with respect to extracellular ligands.

**METHODS**

The incubation media, cell isolation procedures, reagents, assay techniques, and flux measurements were as described in the preceding article (Simchowitz and De Weer, 1986). Nigercin was purchased from Calbiochem-Behring, La Jolla, CA, Na glucuronate and glucuronic acid from Sigma Chemical Co., St. Louis, MO, and $^3H$PAH (2.4 Ci/mmol) from New England Nuclear, Boston, MA. A 3-mM stock solution of nigercin in dimethylsulfoxide was made and diluted as needed into the appropriate medium. For the experiments of Fig. 2, $^{36}$Cl-loaded cells were needed with [Cl] between 0 and 80 meq/liter cell water. To obtain a sufficiently high intracellular specific activity, these cells were prepared as follows: to achieve [Cl] < 40 meq/liter cell water, neutrophils were incubated for 2–6 h in PAH medium to which 0.3 μCi/ml $^{36}$Cl ([Cl]° = 0.6 mM) was added; to achieve 40 < [Cl] < 80 meq/liter cell water, cells were labeled with $^{36}$Cl in the usual manner in 148 mM Cl medium and then placed in unlabeled PAH medium for 0, 0.5, or 1 h. The intracellular Cl concentrations were estimated by coulometry (see Fig. 1A of Simchowitz and De Weer, 1986).

**Least-Squares Fitting**

The straight lines through the origin of Fig. 8 were obtained by conventional least-squares techniques. Of the remaining theoretical curves, 11 (Figs. 1, 4–7, and 9–11) are governed by a number of parameters, three of which (the true Michaelis constants for Cl, PAH, and CHC) appear, in various combinations, in one or more of the 11 relevant equations. The least-squares values for all the parameters of these 11 curves were obtained, in a single operation, from a nonlinear least-squares program designed to fit 11 equations, governed by common parameters, to the total data set. The principle of this method is best explained by comparison with a conventional nonlinear least-squares fit, using two simple examples. In the conventional formulation (see, for example, De Weer and Lowe, 1973), all the data follow an equation or a set of equations that contain all the adjustable parameters. The “best” (least-squares) values for the parameters are found by solving

$$\text{minimum} = \sum g_i \left( v_i^{\text{obs}} - v_i^{\text{cal}} - \sum a_j E_j \right)^2,$$

where $n$ is the total number of observations; $p$ is the number of adjustable parameters; $g_i$ is the weighting factor for the $i$th observation; $v_i^{\text{obs}}$ is the $i$th observed value; $v_i^{\text{cal}}$ is the value of the dependent variable(s) calculated at the corresponding value of the independent variable(s) using provisional estimates for all $p$ parameters; $E_j$ is the difference between the “best” (least-squares) and the provisional estimate of the $j$th parameter; and $a_j$ is the derivative of the function with respect to the $j$th parameter, evaluated at the value of the independent variable(s) in the $i$th observation and for the provisional values of all $p$ parameters. For example, in a fit of the Michaelis-Menten equation (with parameters $K_m$ and $V_{max}$) to reaction velocity $v$ as a function of the substrate concentration, the best values for $K_m$ and $V_{max}$ are obtained from

$$\text{minimum} = \sum g_i \left( v_i^{\text{obs}} - v_i^{\text{cal}} - \frac{\delta v}{\delta K_m} E_{K_m} - \frac{\delta v}{\delta V_{max}} E_{V_{max}} \right)^2.$$

The analysis we face in this article, however, is somewhat different. The 11 data sets...
(types of experiments) mentioned above do not all follow a single equation. Instead, the various data sets are fitted by individual equations governed by parameters, some of which govern more than one of these equations. The least-squares criterion in this case becomes

$$\text{minimum} = \sum_{i} \sum_{k} g_{ik} \left( v_{i}^{ob} - v_{i}^{a} - \sum_{j} a_{ik} E_{j} \right)^{2},$$

where \( m \) is the number of types of experiments; \( n_k \) is the number of observations in the \( k^{th} \) type of experiment; \( p \) is the number of adjustable parameters; \( g_{ik} \) is the weighting factor for the \( i^{th} \) observation in the \( k^{th} \) type of experiment (i.e., whose behavior is described by the \( k^{th} \) function); \( v_{i}^{ob} \) is the \( i^{th} \) observed value of the dependent variable in the \( k^{th} \) type of experiment; \( v_{i}^{a} \) is the corresponding value calculated from the \( k^{th} \) function, using provisional estimates of the relevant parameters; \( E_{j} \) is the difference between the provisional estimate of the \( j^{th} \) adjustable parameter and its "best" (least-squares) estimate; and \( a_{ik}^{p} \) is the derivative of the \( k^{th} \) function with respect to the \( j^{th} \) parameter, evaluated for the value(s) of the independent variable(s) at the \( i^{th} \) observation in the \( k^{th} \) type of experiment, and for the provisional values of the parameters to be refined. Whenever a given function is independent of the \( j^{th} \) parameter, \( a_{ik}^{p} \) is zero. A simple example of this kind might be the following three types of experiments: (a) activation of \( ^{36}\text{Cl} \) influx by external \( \text{Cl} \), where \( v_{i} \) is governed by the parameters \( K_{m}(\text{Cl}) \) and \( V_{max} \); (b) activation of [\( ^{3}\text{H} \)] PAH influx by external PAH, where \( v_{i} \) is governed by the parameters \( K_{m}(\text{PAH}) \) and \( V_{max} \); and (c) activation of \( ^{36}\text{Cl} \) efflux by external \( \text{Cl} \) (replacing PAH, where \([\text{Cl}]_{o} + [\text{PAH}]_{o} = \) constant), where \( v_{i} \) is governed by \( K_{m}(\text{Cl}) \), \( K_{m}(\text{PAH}) \), and \( V_{max} \). That is, Eq. 1 contains \( K_{m}(\text{Cl}) \), Eq. 2 contains \( K_{m}(\text{PAH}) \), and Eq. 3 contains both. (That the three equations in this example happen to be of the same form is immaterial.) The least-squares parameter values are found by solving

$$\text{minimum} = \sum_{i} g_{1i} \left( v_{i}^{ob} - v_{i}^{1} - \left[ \frac{\delta v_{1}}{\delta K_{m}(\text{Cl})} \right]^{0} E_{K_{m}(\text{Cl})} - \left[ \frac{\delta v_{1}}{\delta V_{max}} \right]^{0} E_{V_{max}} \right)^{2}$$

$$+ \sum_{i} g_{2i} \left( v_{i}^{ob} - v_{i}^{2} - \left[ \frac{\delta v_{2}}{\delta K_{m}(\text{PAH})} \right]^{0} E_{K_{m}(\text{PAH})} - \left[ \frac{\delta v_{2}}{\delta V_{max}} \right]^{0} E_{V_{max}} \right)^{2}$$

$$+ \sum_{i} g_{3i} \left( v_{i}^{ob} - v_{i}^{3} - \left[ \frac{\delta v_{3}}{\delta K_{m}(\text{Cl})} \right]^{0} E_{K_{m}(\text{Cl})} - \left[ \frac{\delta v_{3}}{\delta V_{max}} \right]^{0} E_{V_{max}} \right)^{2}.$$
remainder, presumably Cl/Cl exchange, displaying saturation kinetics with an apparent $K_m(Cl) = 22$ mM and a $V_{max} = 1.20$ meq/liter-min.

Fig. 2 shows saturation of $^{36}Cl$ efflux as a function of $[Cl]_i$. Batches of cells were depleted of Cl by incubation in PAH medium for various lengths of time;
this procedure replaces some of their intracellular Cl with PAH (see below). After subtraction of a linear leak component taken from Simchowitz and De Weer (1986), a saturable, presumably exchange, component remains that is well described by a Michaelis-Menten curve with an apparent $K_m(\text{Cl}) = 35 \text{ mM}$ and a

![Graph showing the dependence of $^{36}\text{Cl}$ efflux from human neutrophils into 148 mM Cl medium on intracellular Cl concentration. The curve labeled "total" drawn through the data ($n = 3$) is the sum of the other two. The line labeled "leak" obeys the equation: efflux rate (in meq/liter·min) = 0.005 [Cl] (in mM) and is taken from Fig. 3 of Simchowitz and De Weer (1986). The curve labeled "exchange" is a Michaelis-Menten equation fit to the data from which the "leak" component had been subtracted; the least-squares values of its parameters are: apparent $K_m(\text{Cl}) = 35 \pm 12 \text{ mM}$ and $V_{\text{max}} = 1.40 \pm 0.62 \text{ meq/liter·min}$. The $K_m$ value was obtained simultaneously for the present data and those of Fig. 3; see Methods.

$V_{\text{max}} = 1.40 \text{ meq/liter·min}$. The difference in the apparent $K_m(\text{Cl})$ values between the outside and inside of the membrane (22 vs. 35 mM; in both cases, Cl replaced PAH) could be due either to genuine asymmetry of the putative carrier or to the presence of an unidentified competing ligand inside the cell; we did not pursue this question.
Evidence for trans Effects

trans stimulation of Cl influx is shown in Fig. 3, where the initial rate of $^{36}$Cl influx (other than active uptake or electrodiffusive leak) from a 148 mM Cl medium is plotted against the intracellular Cl concentration. In the nominal absence of intracellular Cl, Cl influx should consist of three components: (a) ~0.1 meq/liter·min of electrodiffusive leak (Table I of Simchowitz and De Weer, 1986). Uptake of $^{36}$Cl from a 148 mM Cl, 1 mM 2-DOG medium was then measured for 10 min ($n = 3$) and the initial rate (after subtraction of a 0.1 meq/liter·min electrodiffusive component, taken from Simchowitz and De Weer, 1986) plotted against [Cl]. The data were fit to the sum of a constant (the intercept) presumably representing exchange of external Cl for internal PAH at [Cl] = 0, and a Michaelis-Menten equation representing the transition between internal PAH/external Cl exchange and internal Cl/external Cl exchange. Least-squares values were: intercept = 0.11 meq/liter·min and $V_{\text{max}}$ = 1.70 meq/liter·min (variances not calculated); apparent $K_m(\text{Cl}) = 35 \pm 12$ mM. The latter was obtained simultaneously for the present data and those of Fig. 2; see Methods.

![Dependence of exchange carrier-mediated $^{36}$Cl influx rate on intracellular Cl concentration.](image-url)
1986); (b) \( \sim 0.25 \text{ meq/liter-min} \) of active (2-DOG-sensitive) uptake; and (c) a small amount of exchange for intracellular PAH, since Cl-depleted cells contain PAH, for which the carrier has affinity (see below). To estimate this third component more accurately, the influx experiments of Fig. 3 were carried out on cells treated with 2-DOG to block active uptake; a small (0.1 meq/liter-min) electrodiffusive component was also subtracted from the data. The influx rate depended markedly on the intracellular concentration, as expected for an exchange carrier. The data were very well fitted by a Michaelis-Menten curve \( [K_m(\text{Cl}) = 35 \text{ mM}; V_{\max} = 1.7 \text{ meq/liter-min}] \) superimposed on a [Cl]-independent influx of 0.11 meq/liter-min. This intercept presumably represents the exchange of external Cl for internal PAH. In separate experiments, we also measured \(^{3}H\)PAH efflux from markedly Cl-depleted (hence PAH-loaded) cells into Cl medium. The carrier-mediated (i.e., CHC-sensitive) efflux rate amounted to 0.13 \( \pm 0.04 \text{ meq/liter-min} \), which is in agreement with the intercept of Fig. 3.

trans stimulation of \(^{36}\text{Cl}\) efflux from normal-Cl cells is illustrated in Fig. 4. (The data also document substrate competition, which will be described shortly.) In the two curves labeled "PAH medium," Cl gradually replaced PAH. Efflux of \(^{36}\text{Cl}\) into a Cl-free, 5 mM K, 148 mM PAH medium (0.90 \( \pm 0.05 \text{ meq/liter-min} \); first data point on the curve with open squares) is similar to the Cl loss into the same medium found in the preceding article (Simchowitz and De Weer, 1986). Raising [Cl], stimulates \(^{36}\text{Cl}\) efflux with Michaelis-Menten kinetics [apparent \( K_m(\text{Cl}) = 22 \text{ mM} \)] to the level appropriate for normal-Cl cells in Cl media (see Simchowitz and De Weer, 1986, and Fig. 2 above). The curve with solid squares, also labeled "PAH medium," represents experiments that were similar but were carried out in the presence of 2 \( \mu \text{M} \) nigericin and 85 mM K (i.e., at \( V_m = -10 \text{ mV} \)), the rationale for which will be explained below. Here, too, external Cl trans-stimulated \(^{36}\text{Cl}\) efflux with an apparent \( K_m(\text{Cl}) = 22 \text{ mM} \). However, when external Cl replaced glucuronate (lower curve of Fig. 4 [open circles], again in the presence of nigericin and 85 mM K), it trans-stimulated \(^{36}\text{Cl}\) efflux much more effectively [apparent \( K_m(\text{Cl}) = 5.0 \text{ mM} \)].

Extracellular Substrate Competition: Stimulation of Cl Efflux

PAH, which is very impermeant in erythrocytes (Schanker et al., 1964; Lassen, 1972; Aubert and Motais, 1975), is not an inert anion for the carrier under investigation here. We will adduce further evidence (carrier-mediated \(^{3}H\)PAH influx and internally consistent competition kinetics between Cl, PAH, and CHC) to support this conclusion, but some has already been presented (Simchowitz and De Weer, 1986): the anion-carrier inhibitor CHC reduced net Cl efflux into PAH medium by about half, which suggests that internal Cl/external PAH exchange was taking place (i.e., that the exchanger is not perfectly selective for Cl). Two conditions must be met for our conclusions regarding the carrier's kinetics with respect to extracellular Cl, PAH, and CHC to be valid: (a) the affinity of the carrier for the "inert" anion substitute must be negligible, and (b) the concentration of any competing high-affinity substrate must be low. Of several anions tested (unpublished), only glucuronate had negligible affinity for the carrier; bicarbonate, however, had an affinity as high as or higher than that
of Cl, and a similar $V_{\text{max}}$. The following evidence is pertinent to the present article. Cl efflux into Cl-free (85 mM K) glucuronate medium (0.38 ± 0.04 meq/liter-min; first data point on the glucuronate curve in Fig. 4) significantly

![Graph showing efflux of Cl from normal-Cl human neutrophils by external Cl](image)

**FIGURE 4.** Stimulation of $^{36}$Cl efflux from normal-Cl human neutrophils by external Cl. Fluxes were calculated from the rate coefficients and the assumption that $[\text{Cl}] = 80$ meq/liter cell water. In the curves labeled "PAH medium" (□, ▴), Cl replaced PAH; in the curve labeled "glucuronate medium" (○), Cl replaced glucuronate. The curve with the uppermost origin (□) is a rectangular hyperbola: efflux (in meq/liter-min) = $0.91 + 0.76 [\text{Cl}]_o/[([\text{Cl}]_o + 22.1)]$ where $[\text{Cl}]_o$ is in millimolar. The data for the middle curve (◇), also in PAH medium, and for the lower curve (○) labeled "glucuronate medium," were obtained on "pH-clamped" cells, i.e., cells bathed in 85 mM K and 2 μM nigericin, pH 7.40. The curves are again rectangular hyperbolae. Middle (PAH) curve: efflux = $0.70 + 0.92 [\text{Cl}]_o/[([\text{Cl}]_o + 22.1)]$. Lower (glucuronate) curve: efflux = $0.39 + 1.28 [\text{Cl}]_o/[([\text{Cl}]_o + 5.03)]$. The single point on the ordinate (●), labeled "CHC," represents efflux, 0.26 ± 0.04 meq/liter-min, from cells exposed to 40 mM CHC in Cl-free (glucuronate) medium. Each data point represents four experiments ± SEM. The technique by which the curves were fitted, and their quantitative relationship with the curves shown in the other figures, are described in the Methods and Discussion.
exceeded that predicted (~0.20 meq/liter·min; Simchowitz and De Weer, 1986) for electrodiffusive efflux at this potential. 40 mM CHC brought this efflux down to 0.26 ± 0.04 meq/liter·min (solid circle, Fig. 4), which is close to the predicted electrodiffusion level. The 0.12 meq/liter·min CHC-sensitive difference, if indeed it is anion/anion exchange, could represent the exchange of internal Cl for external glucuronate, or for a contaminating anion, or both. When we took extreme precautions to eliminate bicarbonate from the Cl-free glucuronate medium (using a carbonate-free base to neutralize glucuronic acid and prolonged gassing with nitrogen), the CHC-sensitive $^{36}$Cl efflux into glucuronate media was insignificant (0.02 ± 0.03 meq/liter·min). We conclude (a) that glucuronate itself has negligible affinity for the carrier (this is supported by the observation that 148 mM Na glucuronate, replacing isotonic mannitol, had no effect on $^{36}$Cl uptake from 5 mM Cl medium), and (b) that the small amount of CHC-sensitive $^{36}$Cl efflux into glucuronate media probably represents HCO$_3$/$^36$Cl exchange.

Since it is laborious to rid solutions completely of bicarbonate, we now examine whether the persistence of a small HCO$_3$/Cl exchange component in nominally “inert” medium materially affects our conclusions. If 0.12 meq/liter·min of $^{36}$Cl efflux into Cl-free glucuronate medium (Fig. 4) is in fact HCO$_3$/Cl exchange (and assuming that, as mentioned above, the $V_{max}$ values for Cl and HCO$_3$ transport are comparable), HCO$_3$ must be present at a concentration ~1/10 the value of its $K_m$. Since bicarbonate presumably was present in all our experiments, it follows from simple kinetic considerations that the $K_m$ values we report may be systematically overestimated by ~10%, well within experimental accuracy. We therefore chose not to go to extreme lengths to rid solutions routinely of bicarbonate. There was a further complication, however. The small HCO$_3$/Cl exchange taking place in glucuronate medium produces intracellular alkalization. The carrier is dramatically stimulated by the elevation of intracellular pH (unpublished observations), and this thwarts controlled kinetic experiments. To circumvent this problem, we found it necessary to “pH-clamp” the cells at their normal intracellular pH of ~7.25 (Simchowitz and Roos, 1985) by bathing them in a medium that had a pH of 7.40 as before, but contained 85 mM K and 2 μM nigericin, a known K/H-exchanging ionophore (Pressman, 1969). Under these conditions, [H$^+$/][H$^+$/] = [K$^+$]/[K$^+$]. A penalty for this maneuver is that the cells are necessarily depolarized, but as already shown (Simchowitz and De Weer, 1986), membrane potential affects carrier-mediated flux little, if at all. This lack of effect of membrane potential on carrier-mediated exchange was confirmed here: repetition of the trans experiment described above ($^{36}$Cl efflux stimulation by external Cl replacing PAH), in the presence of 85 mM K and 2 μM nigericin (Fig. 4, middle curve), gave an indistinguishable curve except for a downward displacement entirely accounted for by the lower membrane potential (approximately ~10 mV in 85 mM K medium vs. approximately ~53 mV in 5 mM K medium). The difference between the rates of $^{36}$Cl efflux (0.91 ± 0.16 compared with 0.70 ± 0.15 meq/liter·min) agrees well with the approximately twofold (~0.2 meq/liter·min) difference in electrodiffusive $^{36}$Cl efflux predicted for the corresponding membrane potentials (Simchowitz and De Weer, 1986).

The 0.44 meq/liter·min difference between $^{36}$Cl efflux into 85 mM K PAH
medium and that into 85 mM K glucuronate, 40 mM CHC medium presumably reflects external PAH/internal Cl exchange. As [Cl]o is raised, replacing glucuronate (Fig. 4), 36Cl efflux tends toward a maximum similar to that shown for the PAH/Cl substitution experiment, which is further proof that 85 mM K and 2 µM nigericin did not materially affect our observations. Finally, the apparent $K_m$(Cl) for stimulation of 36Cl efflux into glucuronate medium is ~5 mM, much lower than that for efflux into PAH medium (~22 mM), as indeed expected if PAH were a much better competitor for the carrier than glucuronate.

**Substrate Competition for Carrier-mediated Influx**

If extracellular PAH competes with Cl in stimulating 36Cl efflux, then Cl influx as a function of [Cl]o should, in the absence of PAH, display a $K_m$(Cl) much lower than that found in Fig. 1 (~22 mM), where Cl replaced PAH. This is borne out in Fig. 5, where the rate of 36Cl influx from media containing 85 mM K and 2 µM nigericin is plotted against the concentration of external Cl replacing glucuronate. As in Fig. 1, the curve fitted to the data is the sum of a linear leak (now somewhat larger because it is appropriate for ~10 mV), a saturable active component (both taken from Simchowitz and De Weer, 1986), and a remainder, presumably exchange, displaying saturation kinetics with $K_m$(Cl) = 5.0 mM and $V_{max}$ = 1.42 meq/liter·min.

The quantitative relationships between the various "true" and apparent constants will be considered in the Discussion. For now, the data fit the notions that (a) external Cl stimulates both Cl influx and Cl efflux in Michaelis-Menten fashion with an apparent $K_m$(Cl) whose value depends on the nature of the replacement anion; (b) internal Cl similarly stimulates both Cl efflux and Cl influx in Michaelis-Menten fashion; (c) PAH partially sustains carrier-mediated exchange with Cl (and competes with it); and (d) glucuronate has negligible affinity for the carrier.

**Competitive Inhibition by CHC**

In addition to substrate saturation, substrate competition, and trans effects, we found the exchange carrier to be subject to competitive inhibition by CHC, a known inhibitor of anion transport (Halestrap, 1976). In Fig. 6, the influx of 36Cl into normal-Cl cells as the concentration of CHC (replacing Cl) is raised from 0 to 40 mM is plotted. We have shown previously (Simchowitz and De Weer, 1986) that CHC inhibits the "active" uptake component as well, with $K_i$ = 1.7 mM; consequently, a correction for active transport inhibition was applied to the data. The remainder was very well fitted by a combination Michaelis-Menten inhibition/electrodiffusive leak curve. The apparent $K_i$(CHC) was 9.4 mM; we will show (see Discussion) that the "true" $K_i$(CHC) is ~0.3 mM. (For comparison, $K_i$'s of 0.006, 0.06, and 0.13 mM have been reported for inhibition of rat liver mitochondrial monocarboxylate, human red cell monocarboxylate, and human red cell inorganic anion transport, respectively [Halestrap and Denton, 1975; Halestrap, 1976].)

Inhibition and competition are also seen in Fig. 7, where the rate of 36Cl efflux from normal-Cl cells into Cl or PAH medium is plotted as a function of CHC
concentration (0-40 mM). The control efflux rates in the absence of CHC, 1.62 ± 0.11 and 0.94 ± 0.04 meq/liter-min for Cl and PAH medium, respectively, are similar to the rightmost and leftmost points on the curve describing the transition between PAH medium and Cl medium in Fig. 4 (open squares). The data of Fig. 7 suggest that Cl efflux consists of two fractions: a CHC-resistant (presumably electrodiffusive) fraction, and one whose magnitude and CHC sensitivity depend on the nature of the extracellular anion. Inhibition by CHC is

![Diagram](https://via.placeholder.com/150)
well fitted with Michaelis-Menten kinetics, apparent $K_i$ values being high (9.4 mM) in Cl medium and low (1.2 mM) in PAH medium, as expected for competition with a high- and low-affinity substrate, respectively. (Quantitative

**FIGURE 6.** Inhibition by CHC of $^{36}$Cl influx into normal-Cl neutrophils ($n = 6$). External [Cl] varied between 148 and 108 mM as [CHC] was raised to 40 mM. The curve drawn through the data is the sum of the other two. The curve labeled "active" represents the expected magnitude of the active uptake component (0.24 meq/liter-min) and its inhibition by CHC ($K_i = 1.7$ mM), taken from Simchowitz and De Weer (1986). The curve labeled "exchange + leak" was obtained by least-squares fitting to the experimental data, from which the "active" component had been subtracted, a Michaelis-Menten inhibition equation (see Discussion, Eq. 2):

$$\text{flux (in meq/liter-min)} = \frac{13.2}{9.36 + [\text{CHC}]} - 0.12$$

where [CHC] is in millimolar. The procedure used for fitting this and other curves is described in the Methods and Discussion.

relationships will be discussed later.) At 40 mM CHC, $^{36}$Cl efflux into Cl or PAH medium is reduced to levels (0.45 ± 0.06 and 0.38 ± 0.03 meq/liter.min, respectively) not appreciably different from net Cl or $^{36}$Cl efflux into CHC-containing media (Simchowitz and De Weer, 1986). We also tested whether
CHC itself is carried into the cell from a medium containing 40 mM CHC and 108 mM Cl. Taking advantage of the inhibitor's strong absorption at 340 nm in alkaline media (Halestrap and Denton, 1975), we found an uptake rate not exceeding 0.04 meq CHC/liter cell water·min.

**FIGURE 7.** Inhibition by CHC of $^{36}$Cl efflux from normal-Cl neutrophils into Cl or PAH medium. The major external anion concentration varied between 148 and 108 mM as [CHC] was raised to 40 mM. The curves (n = 6 for each) are Michaelis-Menten inhibition equations, with the following constants (fluxes are in meq/liter·min): for Cl medium, flux = $14.7/(9.36 + [CHC]) + 0.06$; and for PAH medium, flux = $0.61/(1.15 + [CHC]) + 0.41$. The technique by which these curves were fitted is described in the Methods and Discussion.

**Carrier-mediated PAH Influx**

The finding that half of the net Cl efflux (Simchowitz and De Weer, 1986), or half of the $^{36}$Cl efflux (Fig. 7) into PAH medium, is CHC sensitive suggests that the exchange carrier may lack absolute specificity and may transport PAH. To check this, we measured $[^3]$H]PAH uptake by normal-Cl cells from 148 mM PAH medium in the presence and absence of CHC, as shown in Fig. 8. The influx rate (linear for at least 45 min) was $0.50 \pm 0.02$ meq/liter cell water·min, of which $90 \pm 5\%$ was CHC sensitive. When the $[^3]$H]PAH influx rate is plotted
(Fig. 9) against the extracellular concentration of PAH (replacing glucuronate), saturation is evident. After correction for CHC-insensitive influx, assumed to be a linear leak component (we will show next that 40 mM CHC fully inhibits carrier-mediated flux), the difference yields a Michaelis-Menten curve with $K_m$ (PAH) = 50 mM (presumably the "true" $K_m$, since the carrier appears to lack affinity for the replacement anion glucuronate). Inhibition of $[^3H]$PAH influx by CHC is shown in Fig. 10. The data are well fitted by a Michaelis-Menten inhibition curve with an apparent $K_i$ (CHC) = 1.2 mM. This low value (expected for an inhibitor competing with a low-affinity substrate) is identical to the apparent $K_i$ (CHC) for inhibition of $^{36}$Cl efflux into PAH medium (Fig. 7). (See Discussion for quantitative relationships.)

The question arises whether PAH influx into normal-Cl cells is indeed accompanied by a counter-efflux of Cl. To test this, we measured $^{36}$Cl efflux as a function of the concentration of external PAH (replacing glucuronate). Fig. 11 shows that PAH, replacing glucuronate, stimulates $^{36}$Cl efflux along a Michaelis-Menten curve whose $K_m$ (50 mM) is identical to that which fits the CHC-sensitive
PAH influx data of Fig. 9. In spite of the unavoidable scatter in the data (fluxes are calculated from differences between relatively small rate coefficients of intracellular $^{36}$Cl loss), the origin and endpoint of the curve in Fig. 11 are comparable to the corresponding glucuronate medium and PAH medium points on the ordinate of Fig. 4.

**FIGURE 9.** Rate of $[^{3}H]$PAH influx into normal-Cl neutrophils as a function of the concentration of extracellular PAH replacing glucuronate. The media contained 85 mM K and 2 μM nigericin. Cells were exposed to solutions containing various concentrations of labeled PAH (5 μCi/ml), and $[^{3}H]$PAH influx was measured as in Fig. 8 for 20–40 min. The influx rates were calculated from the linear slopes ($n = 5$). A Michaelis-Menten equation fit by least-squares to these influx rates (not shown) yielded $K_m(\text{PAH}) = 52 \pm 11$ mM and $V_{\text{max}} = 0.65 \pm 0.06$ meq/liter·min. The actual curve drawn through the data ("total") is the sum of the other two. The line labeled "leak" is the CHC-resistant, presumably passive and linear component taken from Fig. 8; its equation is: influx (in meq/liter·min) = $0.00033 [\text{PAH}]_o$ (in mM). The curve labeled "exchange" is a Michaelis-Menten equation fitted to the data from which the leak component had been subtracted, with $K_m(\text{PAH}) = 50.3 \pm 14.9$ mM and $V_{\text{max}} = 0.60 \pm 0.24$ meq/liter·min. The procedure by which this curve was fit is described in the Methods and the Discussion.
Exchange Stoichiometry

The data presented in this and the preceding article suggest that a CHC-sensitive carrier mediates electroneutral anion/anion exchange in human neutrophils. To check whether the stoichiometry is indeed 1:1 as expected, we have summarized in Fig. 12 all the relevant data from Figs. 1 and 4–11, as well as from the preceding article. Individual values of 2-DOG–resistant, CHC-sensitive $^{36}\text{Cl}^-$ or $[^3\text{H}]\text{PAH}$ influx are plotted against corresponding values of carrier-mediated $^{36}\text{Cl}^-$ efflux obtained or computed for particular external concentrations of Cl, PAH, or CHC. The data strongly support 1:1 stoichiometry for both Cl/Cl exchange (main figure) and PAH/Cl exchange (inset) throughout the experimental range. Note that at low external Cl concentrations in glucuronate (open circles) but not in PAH (solid circles) medium, $^{36}\text{Cl}^-$ efflux exceeds $^{36}\text{Cl}^-$ influx, a

![Graph](image_url)

**Figure 10.** Inhibition by CHC of $[^3\text{H}]\text{PAH}$ influx into normal-Cl human neutrophils. Cells were exposed to PAH media labeled with $[^3\text{H}]\text{PAH}$ ($5 \mu\text{Cl/ml}$). External [PAH] varied between 148 and 116 mM as [CHC] was raised to 32 mM. Influx was measured as in Fig. 8, and calculated from the linear slopes ($n = 4$). The equation of the Michaelis-Menten inhibition curve is: flux (in meq/liter·min) = $0.49/(1.15 + [\text{CHC}]) + 0.011$. The procedure by which this curve was fit is described in the Methods and the Discussion.
disparity that disappears with increasing $[\text{Cl}]_o$. For example, at $[\text{Cl}]_o = 0$, there is obviously no influx, but $^{36}\text{Cl}$ efflux was $0.12 \pm 0.03$ meq/liter·min (the open circle on the abscissa). As discussed above, this small efflux probably represents the exchange of internal Cl for another extracellular substrate, presumably HCO$_3$. A similar reasoning applies to the inset.

\[ \text{FIGURE 11. Stimulation of } ^{36}\text{Cl} \text{ efflux from normal-Cl human neutrophils by extracellular PAH, replacing glucuronate. The cells were "pH-clamped," i.e., bathed in 85 mM K and 2 \mu M nigericin, pH 7.40. Fluxes (n = 5) were calculated as in Fig. 4. The curve fitted to the data is a rectangular hyperbola: efflux (in meq/liter·min) = 0.34 + 0.60[PAH]/([PAH] + 50.3). The single point (●) labeled "CHC" is taken from Fig. 4 and represents }^{36}\text{Cl} \text{ efflux into 108 mM glucuronate medium containing 40 mM CHC. The technique by which the curve was fitted is described in the Methods and the Discussion.} \]

**DISCUSSION**

**Properties of the Exchange Carrier**

Steady state human neutrophils exchange $^{36}\text{Cl}$ with their 148 mM Cl bathing medium at a rate of $\sim 1.4$ meq/liter cell water·min, but lose Cl at a rate of only $\sim 0.8$ meq/liter·min when placed in PAH medium or $\sim 0.4$ meq/liter·min when bathed in CHC. Similarly, the net rate of Cl gain by low-Cl cells placed in Cl medium is very much slower ($\sim 0.2$ meq/liter·min) than the one-way $^{36}\text{Cl}$ influx measured in steady state normal-Cl cells ($\sim 1.4$ meq/liter·min). These facts, combined with evidence of saturation and trans stimulation of one-way fluxes, strongly suggest that the bulk of $^{36}\text{Cl}$ movements across the membrane of steady state neutrophils represents electrically silent, carrier-mediated self-exchange. When carrier-mediated $^{36}\text{Cl}$ influx was plotted against carrier-mediated $^{36}\text{Cl}$
efflux measured under similar conditions, near-identity was obtained over the whole experimental range (Fig. 12).

Of the various compounds tested (Simchowitz and De Weer, 1986), only CHC,
a known monocarboxylate anion transport blocker (Halestrap, 1976; Deuticke, 1982), inhibited the putative carrier-mediated transport; it did this by immobilizing the carrier rather than by competing for its transport function. Disulfonic stilbenes were ineffective. Thus, the carrier described here clearly differs from the anion exchanger ("band 3") of red blood cells. Also, the rate of Cl/Cl exchange in neutrophils is roughly four orders of magnitude slower than in the erythrocytes (for reviews, see Sachs et al., 1975; Knauf, 1979; Lowe and Lambert, 1983). Since Korchak et al. (1980, 1982) found that SITS inhibited sulfate fluxes in isolated human neutrophils, SO₄ and Cl must enter these cells by separate pathways. Our findings also suggest that, if neutrophil membranes indeed harbor polypeptides immunologically related to band 3 (Kay et al., 1983), they do not exhibit the carrier-kinetic and pharmacologic characteristics of erythrocyte band 3 protein.

The Cl/Cl exchange is stimulated by internal Cl with an apparent $K_m$ of ~35 mM (Fig. 3) and by external Cl with an apparent $K_m$ of ~22 mM (Fig. 1) when Cl replaces PAH. However, PAH is not nearly as inert as it is in erythrocytes. For example, the initial rate of net Cl loss into PAH medium (0.81 meq/liter-min) exceeds by 0.43–0.51 meq/liter-min that into CHC-containing media (Fig. 1A of the preceding article), and the $^{36}$Cl efflux rate into PAH medium (0.90–0.94 meq/liter-min) is further reduced by ~0.5 meq/liter-min in the presence of CHC (Fig. 7). This suggests that ~0.5 meq/liter-min of carrier-mediated Cl/PAH countertransport is taking place; indeed, Fig. 8 shows ~0.45 meq/liter-min of CHC-sensitive $[^3H]$PAH influx under similar conditions. Thus, PAH is a weak substitute for Cl on the carrier, and the rate at which cells lose Cl into a PAH medium, other than by electrodiffusion, is determined by the rate of carrier-mediated PAH entry. Carrier-mediated $[^3H]$PAH influx and $^{36}$Cl efflux are both stimulated by PAH with the same low affinity ($K_m$ ≈ 50 mM) and to the same extent (Figs. 9 and 11). When carrier-mediated $[^3H]$PAH influx was plotted against PAH-stimulated $^{36}$Cl efflux measured under similar conditions, near-identity was obtained over the whole experimental range (Fig. 12, inset), which confirms 1:1 stoichiometry for PAH/Cl exchange.

A corollary of the nonselectivity of this carrier is that its apparent $K_m$ for Cl should depend on the nature of the substituted anion, and this is seen in Figs. 1, 4, and 5: in the presence of glucuronate, which arguably lacks affinity for the carrier, the apparent $K_m$(Cl) is much lower (5 mM) than in the presence of PAH (22 mM). Also, the apparent $K_i$ for carrier inhibition by CHC is much lower in PAH medium (1.2 mM; Figs. 7 and 10) than in Cl medium (9.4 mM; Figs. 6 and 7), as expected if PAH were a substrate with lower affinity.

**Kinetic Model**

The external substrate and inhibitor interactions in normal-Cl cells can be interpreted quantitatively with a simple equilibrium carrier model that exhibits competition kinetics. All 11 theoretical curves drawn in Figs. 1, 4–7, and 9–11 were based on a single model, a description of which follows. Let $K_m$(Cl), $K_m$(PAH), and $K_i$(CHC) be dissociation constants for the following equilibria between the carrier's external binding site, E, and the respective external ligands:
THE JOURNAL OF GENERAL PHYSIOLOGY

E + Cl ⇔ E·Cl ... K_m(Cl)
E + PAH ⇔ E·PAH ... K_m(PAH)
E + CHC ⇔ E·CHC ... K_i(CHC)

(The fact that the extracellular ligand interactions for normal-Cl cells are well accounted for by this simple equilibrium model obviously does not prove that the carrier-ligand interactions are indeed at equilibrium, i.e., that the computed "true" K_m's represent thermodynamic dissociation constants.) The model assumes that Cl efflux is stimulated (not necessarily to the same extent) by the binding of Cl or PAH, that an equivalent influx of Cl or PAH accompanies Cl efflux, and that binding of the competitive inhibitor CHC blocks the carrier's function. For the reasons enumerated in the Results, glucuronate appears to be devoid of affinity for the carrier. Consequently, the Michaelis constant of the curves describing activation of Cl influx and efflux by external Cl replacing glucuronate (Figs. 4 and 5) is a proper estimate of K_m(Cl). Second, the Michaelis constant of the curves describing activation of PAH influx (Fig. 9) or ^36Cl efflux (Fig. 11) by external PAH replacing glucuronate is a proper estimate of K_m(PAH). Three of the seven remaining curves are functions of both K_m(Cl) and K_m(PAH). For example, activation of Cl influx by external Cl, replacing PAH, with [Cl]_o + [PAH]_o = 148 mM (Fig. 1, "exchange" curve), follows a Michaelis-Menten equation where

\[
K_{m}^{app}(Cl) = K_{m}(Cl) \cdot \frac{K_{m}(PAH) + 148}{K_{m}(PAH) - K_{m}(Cl)}
\]

(1)

Similarly, it can be shown that the increment in carrier-mediated Cl efflux produced by the transition between an all-PAH medium and an all-Cl medium, sum = 148 mM (Fig. 4, the two curves labeled "PAH medium"), follows a Michaelis-Menten curve of the form given below, with K_{m}^{app}(Cl) as in Eq. 1:

\[
\Delta \text{flux} = \frac{\Delta \text{flux}_{\text{max}} \cdot [Cl]_o}{[Cl]_o + K_{m}^{app}(Cl)},
\]

where

\[
\Delta \text{flux}_{\text{max}} = \left[ V_{\text{max}}(Cl) - V_{\text{max}}(PAH) \cdot \frac{148 + K_{m}(Cl)}{148 + K_{m}(PAH)} \right] \cdot \frac{K_{m}(PAH)}{K_{m}(PAH) - K_{m}(Cl)},
\]

and V_{\text{max}}(Cl) and V_{\text{max}}(PAH) represent flux velocities at saturating Cl or PAH concentrations, respectively. With regard to the four equations describing the action of CHC, competitive inhibition of either ^36Cl influx (Fig. 6) or ^36Cl efflux (Fig. 7) by the inhibitor replacing Cl follows an inhibition equation of the form:

\[
\text{flux} = V_{\text{min}} + \frac{(V_{\text{max}} - V_{\text{min}}) \cdot K_{i}^{app}}{[CHC] + K_{i}^{app}},
\]

(2)

where

\[
K_{i}^{app} = K_i \cdot \frac{K_{m}(Cl) + 148}{K_{m}(Cl) - K_i}.
\]

(3)
Finally, competitive inhibition of $^{36}\text{Cl}$ efflux (Fig. 7) or [$^3\text{H}$]PAH influx (Fig. 10) by CHC replacing PAH also follows an equation as in Eq. 2, but where

$$K_{\text{app}}^\text{eff} = \frac{K_m(\text{PAH}) + 148}{K_m(\text{PAH}) - K_i}.$$  \hspace{1cm} (4)

Although all these equations have a familiar form, some of the constants have a physical meaning that is less than straightforward. For example, Eq. 2, applied to influx (as in Figs. 6 and 10), should go through 0 at [CHC] = 148 mM, where the substrate vanishes, and $V_{\text{min}}$ must be negative. This behavior simply follows from the design of our experiments, in which we varied the concentrations of two ligands reciprocally.

Since the Michaelis-Menten constants for all 11 kinetic curves should be functions of only three true constants in various algebraic combinations, we designed a program (see Methods) to least-squares fit a unique set of constants to the combined data of all these figures (494 observations) simultaneously. Table I lists the refined values for “true” $K_m$(Cl), $K_m$(PAH), and $K_i$(CHC). Obviously, the accuracy of the low-affinity binding constant for PAH is limited by the physiological range available for experimentation, but reasonable accuracy can be claimed for $K_m$(Cl) and $K_i$(CHC). From these three numbers, all other apparent $K_m$ and $K_i$ values used in this article [except $K_m$(Cl) for internal Cl; Figs. 2 and 3] can be derived as described; they are also listed in Table I. As the figures show, these kinetic parameters fit most of the data very well. (We realize that our fitting procedure ignores certain second-order effects, e.g., changing baselines in Fig. 4: as [Cl]$_o$ is raised from 0 to 148 mM, electrodiffusive $^{36}\text{Cl}$ efflux must drop somewhat because of the single-filing behavior described in the preceding article. The magnitude of the baseline shift is probably small, and its kinetics are unknown. If the inhibition of electrodiffusive $^{36}\text{Cl}$ efflux is approximately linear between 0 and 148 mM external Cl, it can be shown that the apparent $K_m$’s for the carrier would be unaffected. Similarly, we applied no electrodiffusion corrections to the $^{36}\text{Cl}$ influx data of Fig. 6 or the [$^3\text{H}$]PAH influx data of Fig. 10, because their magnitudes must have varied very little over the experimental ranges.)

The exact mechanism of the exchange carrier remains unresolved, although a few models can already be reasonably eliminated. Electroneutral exchange can, in principle, be achieved through a variety of mechanisms. “Pure” anion/anion exchange requires a mechanism in which either (a) the anion-loaded carrier can cross the membrane but the empty one cannot, or (b) the carrier requires loading from both sides of the membrane before it can proceed (Knauf, 1979; Gunn and Fröhlich, 1979). Even an apparently nonspecific anion carrier, as described here, may in fact have a specific requirement for an anion that is always present in the medium. Thus, what appears to be Cl/Cl or Cl/PAH exchange may in fact be (c) parallel Cl/OH and OH/Cl or OH/PAH exchanges or (d) parallel Cl/HCO$_3$ and HCO$_3$/PAH exchanges (no extraordinary precautions were taken in our study to eliminate HCO$_3$ completely). Still other mechanisms may masquerade as electroneutral anion/anion exchange, such as (e) parallel operation of Na + Cl and Na + PAH cotransport in opposite directions (the lack of effect of the
removal of Na [Simchowitz and De Weer, 1986] pleads against this hypothesis here) or (f) parallel K + Na + 2 Cl and K + Na + 2 PAH cotransport (also unlikely since furosemide and large variations in [K]o were without effect [Simchowitz and De Weer, 1986]), or (g) parallel H + Cl or H + PAH cotransport. (The last three models would not predict 1:1 exchange stoichiometries under all conditions, as found here.) More complicated parallel mechanisms such as (h) Na + Cl cotransport combined with Na/H exchange and H + Cl or H + PAH cotransport are unlikely, since our data would require that all carriers involved have the same inhibitor specificity.

We conclude that isolated human neutrophils possess a relatively nonselective anion exchanger that accounts for the major part of the steady state 36Cl fluxes across the cell membrane. As Cl/HCO3 exchanger, the carrier appears to play a role in intracellular pH recovery from alkaline loads (Simchowitz and Roos, 1985). What other physiologic roles this carrier may play in neutrophil function remain to be elucidated.

We acknowledge the expert technical assistance of Ms. Arabella R. Kizzart and Mr. William H. Miller, and the secretarial skills of Janice Jones and Cheryl Krah. We also thank Drs. Robert Putnam, R. F. Rakowski, Luis Reuss, and Albert Roos for their comments on the manuscript. This work was supported by the Veterans Administration, by an Arthritis Foundation Clinical Research Center grant, and by National Institutes of Health grant NS 11223.

Original version received 20 August 1984 and accepted version received 7 April 1986.

### TABLE I

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

| Ligand | Condition | Text equation | Value |
|--------|-----------|---------------|-------|
| Cl     | Replacing PAH | 1 | 22.1† |
| PAH    | —         | —             | —     |
| CHC    | Replacing Cl  | 3 | 9.4** |
|        | Replacing PAH | 4 | 1.2# |

True and apparent kinetic constants were derived by least-squares fitting the equilibrium-competition carrier model described in the text to all the data of Figs. 1, 4–7, and 9–11 simultaneously (494 observations on normal-Cl cells). The model assumes that glucuronate has negligible affinity for the carrier, as strongly suggested by the data (see Results).

* From simultaneous least-squares fit to all data in Figs. 1, 4–7, and 9–11.
† Variances were not calculated.
‡ Used to calculate "glucuronate" curve in Fig. 4 and "exchange" curve in Fig. 5.
§ Used to calculate "exchange" curve in Fig. 1 and "PAH" curves in Fig. 4.
¶ Used to calculate "exchange" curve in Fig. 9 and curve in Fig. 11.
** Used to calculate "exchange + leak" curve in Fig. 6 and "chloride" curve in Fig. 7.
†† Used to calculate "PAH" curve in Fig. 7 and curve in Fig. 10.
REFERENCES

Aubert, L., and R. Motais. 1975. Molecular features of organic anion permeability in ox red blood cell. *Journal of Physiology.* 246:159–179.

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

De Weer, P., and A. G. Lowe. 1973. Myokinase equilibrium: an enzymatic method for the determination of stability constants of magnesium complexes with adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate in media of high ionic strength. *Journal of Biological Chemistry.* 248:2829–2835.

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.

Kay, M. M. B., C. M. Tracey, J. R. Goodman, J. C. Cone, and P. S. Bassel. 1983. Polypeptides immunologically related to band 3 are present in nucleated somatic cells. *Proceedings of the National Academy of Sciences.* 80:6882–6886.

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

Korchak, H. M., B. A. Eisenstat, S. T. Hoffstein, P. B. Dunham, and G. Weissmann. 1980. Anion channel blockers inhibit lysosomal enzyme secretion from human neutrophils without affecting generation of superoxide anion. *Proceedings of the National Academy of Sciences.* 77:2721–2725.

Korchak, H. M., B. A. Eisenstat, J. E. Smolen, L. E. Rutherford, P. B. Dunham, and G. Weissmann. 1982. Stimulus-response coupling in the human neutrophil. The role of anion fluxes in degranulation. *Journal of Biological Chemistry.* 257:6916–6922.

Lassen, U. 1972. Membrane potential and membrane resistance of red cells. In *Oxygen Affinity of Hemoglobin and Red Cell Acid Base Status.* P. Astrup and M. Rørth, editors. Munksgaard, Copenhagen. 291–304.

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

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. 2:613–703.

Schanker, L. S., J. M. Johnson, and J. J. Jeffrey. 1964. Rapid passage of organic anions into human red cells. *American Journal of Physiology.* 207:503–508.

Simchowitz, L., and P. De Weer. 1985. Chloride movements in human neutrophils. Diffusion, exchange, and active transport. *Journal of General Physiology.* 88:167–194.

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