J. Membrane Biol. 49, 57–74 (1979)

Characteristics of Anion Transport in Cat and Dog Red Blood Cells

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Received 1 February 1979

Summary. Self-exchange of chloride and sulfate in dog and cat red cells has been measured under equilibrium conditions. The rates of efflux for these anions are approximately twofold higher in dog compared to cat red blood cells. Although the rates differ, the anion exchange systems of these two red cell types exhibit many common properties. The dependence of $^{35}SO_4$ efflux on the intracellular SO_4 concentration, the pH dependence and the inhibition of $^{35}SO_4$ efflux by Cl and SITS are almost identical in dog and cat red cells. Nystatin treatment was used to study the dependence of ^{36}Cl efflux on internal Cl. Chloride efflux exhibits saturation in both cell types with dog red cells possessing a higher V_{max} and $K_{1/2}$ than cat red cells. The number of anion transport sites was estimated by extrapolation to the number of molecules of dihydro DIDS (H_2DIDS, where DIDS is 4,4′-diisothiocyano-2,2′ stilbene-disulfonic acid) which were bound at 100% inhibition of transport. The results indicate that either the turnover numbers for anion transport differ in dog, cat, and human red cells or that there is heterogeneity in the function of the membrane components which bind H_2DIDS.

Much is now known about the transport of anions, such as chloride and sulfate, in red blood cells, particularly in those of man. Unidirectional movement of anions under steady-state conditions is rapid (Tosteson, 1959) under circumstances where the conductive permeability to anions is some 10^4 to 10^5 times slower (Scarpa, Cecchetto & Azzone, 1968; Hunter, 1971; Lassen, 1972). Demonstration of saturation kinetics, pH dependence, and competition among various anions indicates that in human red cells the rapid movement of anions is by a facilitated mechanism, lodged in the membrane, which mediates such self-exchange (Gunn *et al.*, 1973; Passow & Wood, 1974; Dalmark, 1975; Schnell, Gerhardt & Schoppe-Fredenburg, 1977).

While the rate of anion exchange has been measured in cat and

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0022-2631/79/0049-0057 $03.60

dog red cells (Deuticke & Gruber, 1970; Wieth *et al.*, 1974) as has the net Cl permeability of dog red cells (Parker, Castranova & Goldinger, 1977), an extensive study of the properties of the anion transport systems in these cell types has not been made. It is of interest to explore the kinetic properties of the anion transport systems in dog and cat red cells for two reasons. The first has to do with the fact that, compared to human and most other mammalian red cells (*see* Bernstein, 1954, and Parker, 1978), cat and dog red cells differ in their cation transport properties. These cells contain high Na and low K, but they control their composition and cell volume by some means which does not depend upon the operation of a cardiac glycoside-sensitive, adenosine triphosphate driven Na-K pump (Sorensen, Kirschner & Barker, 1962; Miles & Lee, 1972; Chan, Calabrese & Thiel, 1974). Therefore the membrane transport properties for cations are unique in dog and cat red cells. It seems of interest to determine if these cell types also possess anion transport systems which differ from human red cells. The second reason for studying anion transport in dog and cat red cells was to determine the relationship between the rate of anion self-exchange, the kinetic properties of the transport system, and the amount of membrane protein purported to be responsible (Cabantchik & Rothstein, 1972, 1974; Passow *et al.*, 1975; Lepke *et al.*, 1976; Ship *et al.*, 1977) for the facilitated translocation of anions. Given the marked differences in rates of anion self-exchange, there may be differences in the amount of transport protein found in these red cells or differences in the turnover number of each transport site in the various species. The results of this study indicate that the kinetic properties of anion transport in dog, cat, and human red cells are very similar. There seems to be no correlation between the number of anion transport sites identifiable with H_2DIDS and the rate of anion self-exchange in these cell types. Therefore either the turnover rates differ in these cell types or the means of estimating the amount of membrane transport protein involved is misleading. Preliminary accounts of portions of this work have been previously reported (Castranova, Weise & Hoffman, 1976, 1978).

Materials and Methods[1]

Sulfate Efflux

A measure of SO_4 transport was obtained by determining the rate constant of efflux for $^{35}SO_4$ self-exchange under equilibrium conditions at 37 °C. The method is a modification of that described in detail by Gardos, Hoffman and Passow (1969).

[1] *Abbreviation:* H_2DIDS is 4,4′-diisothiocyano-dihydrostilbene-2,2′-disulfonic acid. Tritiated H_2DIDS is represented by [^{3}H]H_2DIDS.

Venous blood was drawn into a heparinized container. The blood was centrifuged and the plasma and buffy coat were removed. The red cells were then washed 3 times (each with ten volumes wash solution) by alternate resuspension and centrifugation (at 2 °C and 12,100·*g*) in SO_4-flux medium (mM: 126 NaCl, 5 KCl, 10 Na_2SO_4, 5 NaHEPES (N-2-hydroxyethyl piperazine-N′-2-ethane sulfonic acid), 5 glucose, and 50 sucrose (pH = 7.4)). The red cells were incubated at a 10% hematocrit for 3 hr at 37 °C to load cells with $^{35}SO_4$ (New England Nuclear, NEX-04) which was added to the cell suspension to give 10 μCi/ml. After loading, the cells were removed from the radioactive medium and again washed 3 times but with iced (0 °C) SO_4-flux medium. To measure rates of $^{35}SO_4$ efflux, these cells were then added to flasks containing SO_4-flux medium at 37 °C (0.5% hematocrit) and incubated.

Chloride Efflux

Red cells were washed 3 times in Cl-flux medium (mM: 139 NaCl, 5 KCl, 5 NaHEPES, 5 glucose, and 50 sucrose (pH = 7.4)). The cells were incubated in this medium containing 1 μCi/ml ^{36}Cl (ICN Pharmaceutical, #63005) at 10% hematocrit for 3 hr at 37 °C and then for an additional 20 min at 0 °C. The suspension was then centrifuged using a Sorvall HB-4 rotor at 2 °C and 27,000·*g* for 5 min, and the radioactive supernatant removed.

These unwashed, packed cells were added to 0 °C Cl-flux medium, magnetically stirred, to give a hematocrit of 1%. Supernatant samples were taken after mixing at intervals of a few sec by means of a rapid filtering technique (Wieth *et al.*, 1973).

Calculation of Rate Constants

Efflux of tracer anions is a first-order process described by the equation

$$\ln\left(1-\frac{P_t}{P_\infty}\right)=-kt$$

where P_t and P_∞ are the amounts of radioactivity in the medium at times t and infinity, respectively; k is the rate constant for efflux, and t is time.

The amount of $^{35}SO_4$ or ^{36}Cl transported out of cells at any time (P_t) was determined by analysis of supernatant samples taken at various times after addition of the labeled cells to isotope-free medium. These samples were acidified with trichloroacetic acid (TCA), centrifuged, and aliquots added to ACS fluor (Amersham/Searle) for liquid scintillation counting. Five different time samples for each experiment were routinely taken, and each experiment was carried out at least in duplicate. The radioactivity in the medium at equilibrium (P_∞) was obtained from a sample of the cell suspension. These samples were treated with TCA, centrifuged to precipitate protein, and aliquots added to ACS fluor for liquid scintillation counting. The rate constants (time^{-1}) for SO_4, ${}^0k_{SO_4}$, and Cl, ${}^0k_{Cl}$, efflux were taken as the slopes of least squares lines fit to plots of ln $(1-P_t/P_\infty)$ *vs.* time. Correlation coefficients were generally 0.98 or greater.

Nystatin Treatment

The ionic content of cat and dog red cells was altered using nystatin as described by Cass and Dalmark (1973). Thus, red cells were washed 3 times (at 2 °C with 10 volumes solution for each wash, centrifuged at 12,000·*g*) in pretreatment-medium (mM: 150 NaCl, 10 NaHEPES, 5 KCl, and 30 sucrose (pH = 7.2, 25 °C)) and then resuspended at 20%

hematocrit in this medium. Nystatin (5 mg/ml in methanol) was added to give 50 μg/ml. After 30 min at 0 °C, the suspension was centrifuged and the packed cells resuspended at 20% hematocrit in a medium of different NaCl concentration. This suspension contained 35 μg/ml nystatin and was equilibrated for 20 min at 0 °C. The centrifuging (12,100·*g* at 2 °C), resuspension, and 20 min equilibrium were repeated, varying the NaCl concentration by 20 mM at each cycle, until the cells had been equilibrated in the medium of desired NaCl concentration. The cells were then washed, as before, eight times at 25 °C in the respective equilibration media, removing the nystatin from the cells, and restoring the membrane barrier to cation movement. Cells were washed a ninth time in the appropriate Cl-flux media (mM: 20–200 NaCl, 5 KCl, 10 NaHEPES, 5 glucose (pH 7.4)) and then resuspended (10% hematocrit) in that medium. One mCi/ml ^{36}Cl was then added to the suspensions to load cells with isotope (1 hr, 0 °C) in preparation for measuring rates of efflux. Those measurements were made as described above for labeled cells introduced into the respective media at 0 °C.

H_2DIDS Binding

Venous blood was drawn into a heparinized container. The blood was centrifuged, and the plasma and buffy coat were removed. The red cells were washed, as described before, 3 times in an iced (0 °C) incubation medium (mM: 135 NaCl, 5 KCl, 10 Na_2SO_4, 5 NaHEPES, and 5 glucose (pH = 7.4)). The red cells were resuspended at 0.75% hematocrit in incubation medium. The cell suspension was divided into aliquots for SO_4 efflux, H_2 = DIDS binding, and electrophoresis studies. Those samples used for SO_4 efflux received 10 μCi/ml $^{35}SO_4$. All the three aliquot groups were incubated for 3 hr at 37 °C. After this time, various concentrations of [^{3}H]H_2DIDS (the kind gift of P.A. Knauf, S. Ship, and W. Breuer) were added to all three groups of suspensions and the incubation was continued for 30 min at 37 °C. The cells were then centrifuged, the radioactive medium was removed by suction, and the cells in each group were washed again 3 times in iced-incubation medium containing 0.5% albumin to remove any noncovalently bound inhibitor, i.e., [^{3}H]H_2DIDS, as well as supernatant $^{34}SO_4$ where present.

The cell samples which were loaded with $^{35}SO_4$ were used to determine the effect of various concentrations of H_2DIDS on sulfate transport. The rate constant for SO_4 efflux was determined as described above. Estimates of $^{35}SO_4$ were unaffected by prior exposure of the cells in this group to different concentrations of [^{3}H]H_2DIDS.

Those cell samples used to determine the number of [^{3}H]H_2DIDS molecules bound per cell at various concentrations of the inhibitor were suspended at a 50% hematocrit in incubation medium. Aliquots were taken from each cell suspension and used for cell counting with a Coulter Counter (Model Z_{BI}). Then 10 μl aliquots of each cell suspension were added to 1 ml of protosol (New England Nuclear). The cells were digested for 2 hr at 25 °C. After digestion 10 ml of ACS fluor (Amersham/Searle) were added and the samples counted for ^{3}H using a liquid scintillation counter. The number of molecules of H_2DIDS per cell was calculated from the cpm/aliquot, the counting efficiency (23%), the specific activity (79 mCi/mmol as determined by Knauf, Ship and Breuer, *see* acknowledgment), the number of cells per aliquot, and Avogadro's number.

Cells which had been treated with 0.3 μM [^{3}H]H_2DIDS were added to 10 volumes of hemolyzing medium (0.1 mM Na_2EDTA brought to pH 7.5 (at 23 °C) with Tris), mixed, and placed at 0 °C for 10 min to allow for 100% hemolysis to occur. After centrifugation of the hemolysis mixture at 0 °C and 48,000·*g* for 10 min, the supernatant was removed and the ghosts were washed 4 times by alternate centrifugation at 0 °C and 48,000·*g* for 5 min and resuspension in washing medium (mM: 15.3 NaCl, 1.7 Tris, and 0.1 Na_2EDTA (pH 7.5)). The ghosts were diluted in 4 volumes of washing medium and this mixture

was added to 4 volumes of solubilizer solution (2% sodium dodecylsulfate (SDS), 20% sucrose, 20 mM Tris, 2 mM Na_2EDTA, 40 mM dithiothreitol (DTT), and 40 μg/ml pyronin Y, (pH 9)) and incubated at 100 °C for 10 min. After electrophoresis, staining with Coomassie Brillant Blue, and destaining, gel scans were run (Fairbanks, Steck & Wallach, 1971). Then the gels were sliced, and each slice was digested in 500 μl of 35% hydrogen peroxide at 80 °C to which 10 ml of ACS fluor (Amersham/Searle) was added before counting for ^3H in a liquid scintillation counter.

Results

The efflux rate constants for sulfate ($^0k_{SO_4}$) and chloride ($^0k_{Cl}$) self-exchange in cat and dog red blood cells are given in Table 1. It should be noted that $^0k_{SO_4}$ was measured at 37 °C and $^0k_{Cl}$ was determined at 0 °C. The movement of both SO_4 and Cl in dog red cells is about twice as fast as that measured in cat red cells. The observed differences in rates are due neither to variations in surface area to volume ratios (they are almost the same for both cell types (*see* legend, Table 2)) nor to differences in electrochemical gradients since both cat and dog cells are found to have the same Donnan distribution for anions under our experimental conditions (Castranova, Weise & Hoffman, 1979).

Lepke and Passow (1971) have reported that Cl inhibits the transport of SO_4 in human red cells. This is also true in cat and dog red cells as shown in Fig. 1. In both cat and dog red cells the $^0k_{SO_4}$ decreases as the concentration of Cl in the medium is raised. Although the values of $^0k_{SO_4}$ in dog red cells are greater than that for cat cells at any given Cl concentration (Fig. 1*A*), the Cl inhibition curve is qualitatively the same for both types of red cells (Fig. 1*B*).

Figure 2*B* shows the variation in SO_4 efflux ($^0M_{SO_4}$) as sulfate replaces Cl in the medium. Although $^0M_{SO_4}$ at any given sulfate concentration (Fig. 2*A*) is always greater in dog compared to cat cells, the same concentration dependence is exhibited by both cell types (Fig. 2*B*). The

Table 1. Anion transport in cat and dog red cells

Red cell type	$^0k_{SO}$ at 37 °C (hr^{-1})	$^0k_{Cl}$ at 0 °C (min^{-1})
Cat	0.98 ± 0.04	4.3 ± 0.1
(*n*)	(6)	(3)
Dog	1.86 ± 0.08	8.2 ± 0.1
(*n*)	(5)	(3)

The number of experiments are given as *n*. Values are means of *n* determinations ± SEM.

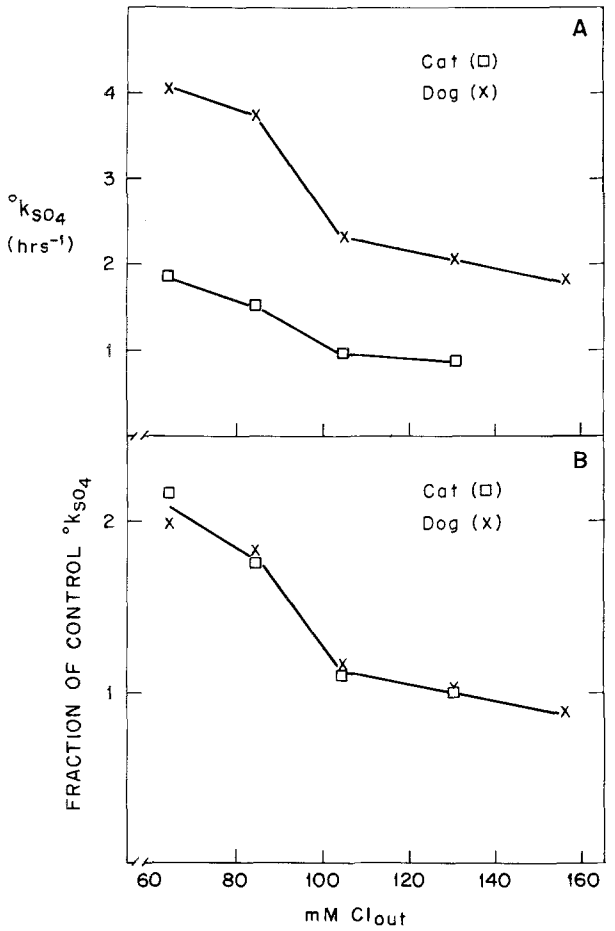

Fig. 1. (*A*): Rate of SO_4 efflux from cat and dog red blood cells in media of different Cl concentration (mM: 5 NaHEPES, 10 Na_2SO_4, 5 KCl, 60–132 NaCl, 158–10 sucrose, 5 glucose (pH 7.4)). NaCl and sucrose concentrations were varied so that media were all approximately 325 mosmol. Cells were incubated in above media at 10% hematocrit for 3 hr before rate constants for SO_4 efflux were determined as described in *Materials and Methods*. Note that since these cells have been equilibrated in media of different anion concentration, the internal anion content of these cells will also differ. (*B*): The same data plotted as the fraction of the control $^{0}k_{SO_4}$ measured in a medium containing 130 mM NaCl

supra-linear dependence of $^{0}M_{SO_4}$ on $[SO_4]_i$ in cat and dog red cells is similar to that reported for human red cells (Passow & Wood, 1974; Schnell *et al.*, 1977). No saturation of $^{0}M_{SO_4}$ is observed presumably because $^{0}M_{SO_4}$ is increased both by increasing sulfate concentration and by decreasing Cl inhibition. In work with human cells, Schnell *et al.* (1977) have found that $^{0}M_{SO_4}$ does exhibit saturation kinetics if the SO_4 concentration is varied at constant Cl.

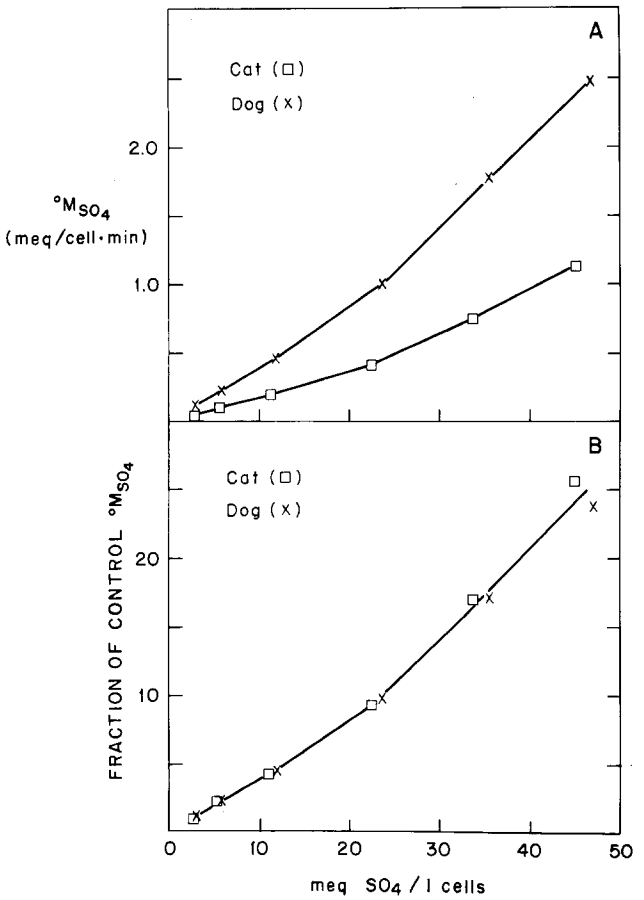

Fig. 2. (*A*): Sulfate efflux as a function of cellular SO_4 in cat and dog red cells. Cells were incubated for 3 hr at 10% hematocrit and 37°C in flux media (mM: 5 KCl, 5 glucose, 5 HEPES, 50 sucrose, 5–80 Na_2SO_4, 132–38 NaCl (pH 7.4)). 1 mM Na_2SO_4 replaced 1.26 mM NaCl so that all media were approximately 325 mosmol. Rate constants for SO_4 efflux were determined as in *Materials and Methods*; cellular concentrations of sulfate, $[SO_4]$, were calculated using a Donnan ratio of 0.7, and $^{0}M_{SO_4}$ was calculated from $^{0}k_{SO_4} \times [SO_4]$. (*B*): The same data plotted as the fraction of control $^{0}M_{SO_4}$ taken at 5 mM Na_2SO_4. Data are means of two determinations

SITS (4-acetamido-4′-isothiocyano-stilbene-2,2′-disulfonic acid) has been shown to be a potent inhibitor of anion transport in human red blood cells (Knauf & Rothstein, 1971; Cabantchik & Rothstein, 1972). Figure 3 shows that this is also true for dog and cat red cells, both of which show the same response to increasing amounts of SITS until almost all (96–97%) SO_4 efflux is inhibited. This is greater than the 85% maximal inhibition reported in human red cells and may reflect

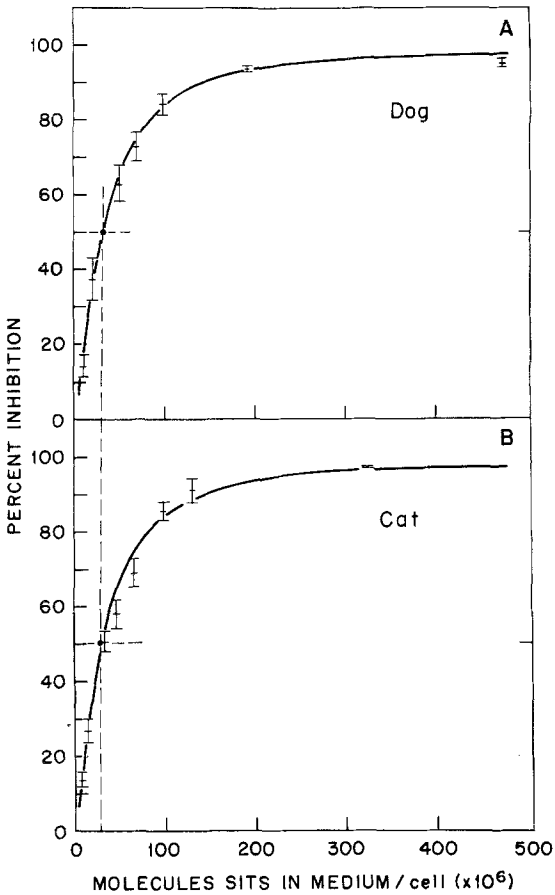

Fig. 3. Percent inhibition of the rate constant for sulfate efflux in dog (*A*) and cat (*B*) red blood cells as a function of SITS concentration. Rate constants measured as described in *Materials and Methods*. SITS was present in the media during the efflux measurements. For comparative purposes the concentration of SITS which gives 50% inhibition is approximately 2.5 μM for both dog and cat red cells. Data are means ± SEM of three experiments

the fact that the human red cells were washed after SITS treatment (Cabantchik & Rothstein, 1972). Indeed, washing cat and dog red cells 3 times in flux medium containing 5 mM glycine reverses approximately 50% of the inhibition caused by SITS.

The $^{0}k_{SO_4}$ in dog and cat red blood cells is dependent on external pH (Fig. 4). Although $^{0}k_{SO4}$ in dog red cells remains greater than that of cat red cells at all pH values (Fig. 4*A*), both cell types exhibit a similar pH dependence with a maximum rate at pH 6.3 (Fig. 4*B*). The pH maximum of SO_4 movement in human red cells has been reported as 6.2 by Schnell *et al.* (1977) and 6.0 by Passow and Wood (1974).

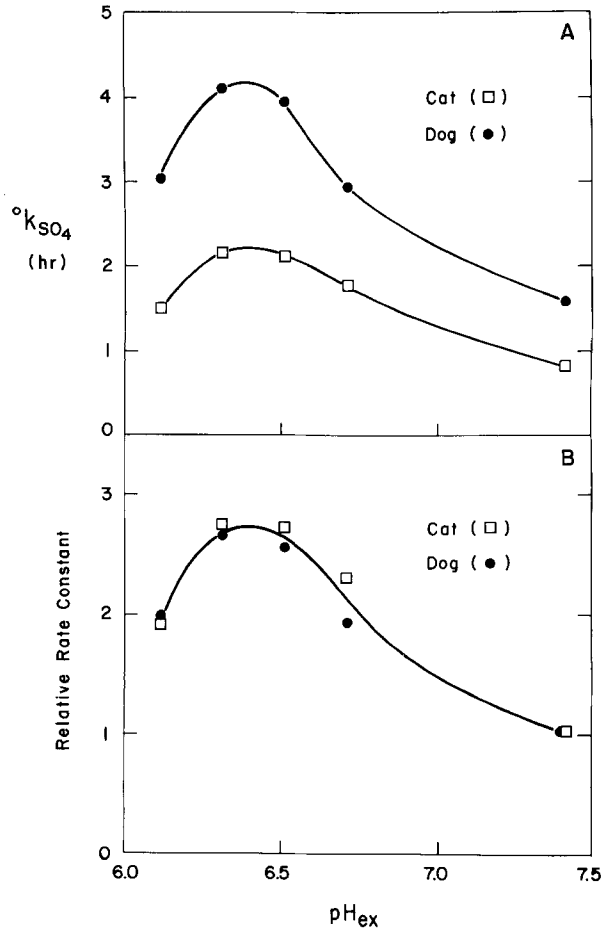

Fig. 4. (*A*): The rate constant for sulfate efflux as a function of external pH in cat and dog red blood cells. Red cells were washed and incubated for 3 hr at 37 °C in media of the appropriate pH (mM: 145 NaCl, 5 KCl, 10 Na_2SO_4, 5 glucose, and 10 buffer (NaPIPES for pH 6.1–6.7 and NaHEPES for pH 7.4)). Efflux was then measured in the appropriate adjusted media as described in *Materials and Methods*. (*B*): The same data plotted as fraction of control $^{o}k_{SO_4}$ measured at pH 7.4. Data are means of two determinations

Using a nystatin treatment method like that described by Cass and Dalmark (1973), we have studied the concentration dependence of Cl efflux in dog and cat red blood cells. A maximum Cl efflux of 466 mM/liter cell $H_2O \cdot$ min was observed at an intracellular Cl of 117 mM Cl/liter cell H_2O in dog red cells. This efflux is 43% greater than that in cat red cells (325 mM/liter cell $H_2O \cdot$ min at 58 mMCl/liter cell H_2O). At high Cl concentrations both cell types exhibit Cl self-inhibition (Fig. 5*A*) similar to that described by Dalmark (1975) for Cl transport in human

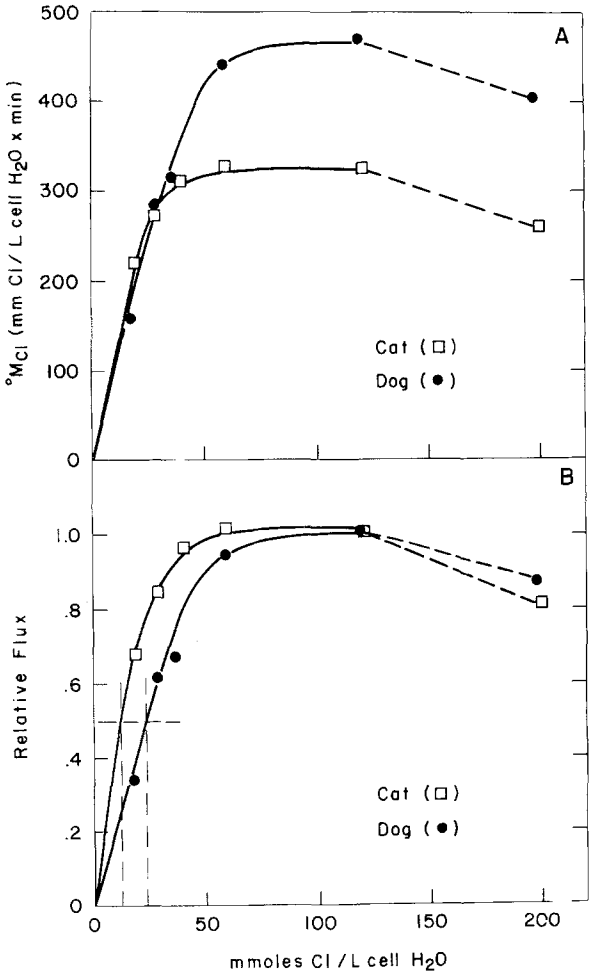

Fig. 5. (*A*): Chloride efflux as a function of internal Cl concentration in nystatin treated dog and cat red blood cells. (*B*): The same data plotted as the fraction of maximum efflux

erythrocytes. The apparent $K_{1/2}$'s for activation of Cl self-exchange, taken from the data in Fig. 5*B*, are approximately 12.5 and 22.5 mM/liter cell H_2O for cat and dog red cells, respectively. In human red blood cells values for $K_{1/2}$ range from 26–44 mM Cl/liter cell H_2O (Gunn *et al.*, 1973; Dalmark, 1975).

In order to study the number and types of membrane components involved in anion translocation in cat and dog red cells, we studied the binding of [^{3}H]H_2DIDS (tritiated dihydro analog of DIDS where DIDS is 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid) to these cells.

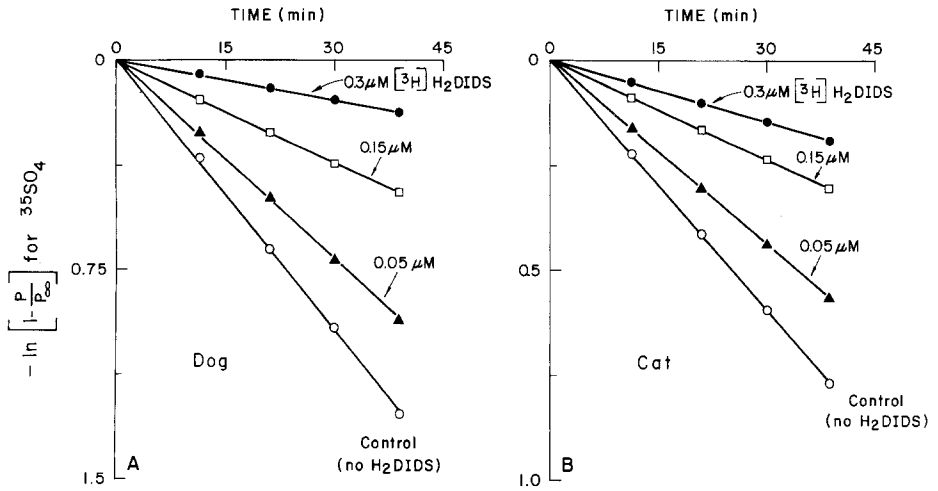

Fig. 6. The effect of various concentrations of [^{3}H]H_2DIDS on the time-course of sulfate efflux in dog (*A*) and cat (*B*) red blood cells. Cells were loaded with $^{35}SO_4$ by incubation at a 0.75% hematocrit for 3 hr at 37 °C in flux medium (mM: 135 NaCl, 5 KCl, 10 Na_2SO_4, 5 NaHEPES, and 5 glucose (pH=7.4)). After this time various concentrations of [^{3}H]H_2DIDS were added to the cell suspensions and the incubation was continued for 30 min at 37 °C. The cells were than washed 3 times in ice-cold flux medium containing 0.5% albumin. Sulfate efflux was measured at a 0.5% hematocrit at 37 °C in flux medium

This approach is based on previous work where it was shown that H_2DIDS could be bound covalently (irreversibly) to band III protein of human red blood cells, in parallel with its inhibition of SO_4 transport, thereby implicating this protein in anion transport (Cabantchik & Rothstein, 1972, 1974; Passow *et al.*, 1975; Lepke *et al.*, 1976; Ship *et al.*, 1977).

Figure 6 shows the effect of three different concentrations of [^{3}H]H_2DIDS on $^{35}SO_4$ efflux in dog and cat red cells. These data are summarized in Fig. 7 which shows that ${}^0k_{SO_4}$ is reduced by [^{3}H]H_2DIDS to the same extent in dog and cat red blood cells. These data also show that [^{3}H]H_2DIDS is a more potent inhibitor of SO_4 efflux than SITS (*compare* Fig. 3). In addition, the concentration of [^{3}H]H_2DIDS which gives half-maximal inhibition of sulfate efflux as found in the present experiments (0.12 μM [^{3}H]H_2DIDS) is more than an order of magnitude lower than that reported for [^{3}H]H_2DIDS by Ship *et al.* (1977) for human red cells. This difference is probably due to the fact that they performed binding studies at a 25% hematocrit. Use of such a high hematocrit may explain their observation of a linear relationship between inhibition of SO_4 efflux and inhibitor concentration rather than

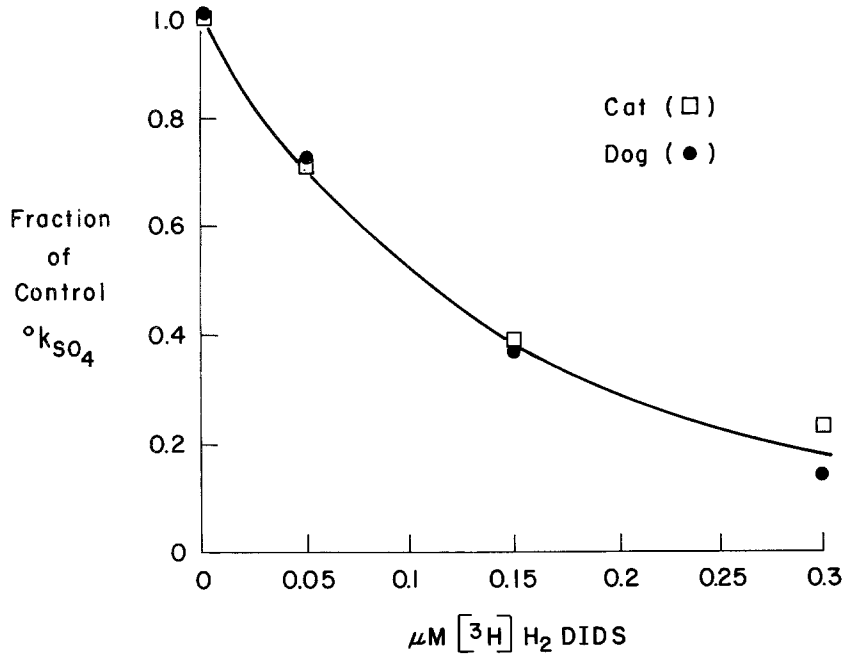

Fig. 7. A dose-response curve showing inhibition of $^{o}k_{SO_4}$ by [^{3}H]H_2DIDS. This figure summarizes data presented in Fig. 6. Maximum inhibition of 97–98% is obtained at 0.75 μM [^{3}H]H_2DIDS (data not shown). Data are means of two determinations

the dose-response curve obtained here. On the other hand, Shami, Rothstein and Knauf (1978) found that the concentration of H_2DIDS which gave half-maximal inhibition of Cl efflux, at low hematocrit values comparable to those used here, was 0.3 μM.

The relationship between the degree of inhibition of $^{34}SO_4$ efflux by [^{3}H]H_2DIDS and the extent of irreversible binding of this inhibitor is shown in Fig. 8. Note that the relationship is linear in both dog and cat red cells. By extrapolation, 1.2×10^6 molecules of [^{3}H]H_2DIDS are bound per cell at 100% inhibition in cat red cells. This number of binding sites is similar to that reported by Lepke *et al.* (1976) and Ship *et al.* (1977) for the binding of this substance to human red cells. Dog red cells possess fewer [^{3}H]H_2DIDS binding sites per cell (0.81×10^6) than either cat or human red cells. Therefore, the higher rate of anion transport in dog red cells compared to cat erythrocytes is not due to a greater number of transport sites but seems to be due to a higher turnover number (Table 2). These differences between cat and dog red cells are magnified if the results are expressed as binding sites per unit membrane area (*see* legend, Table 2).

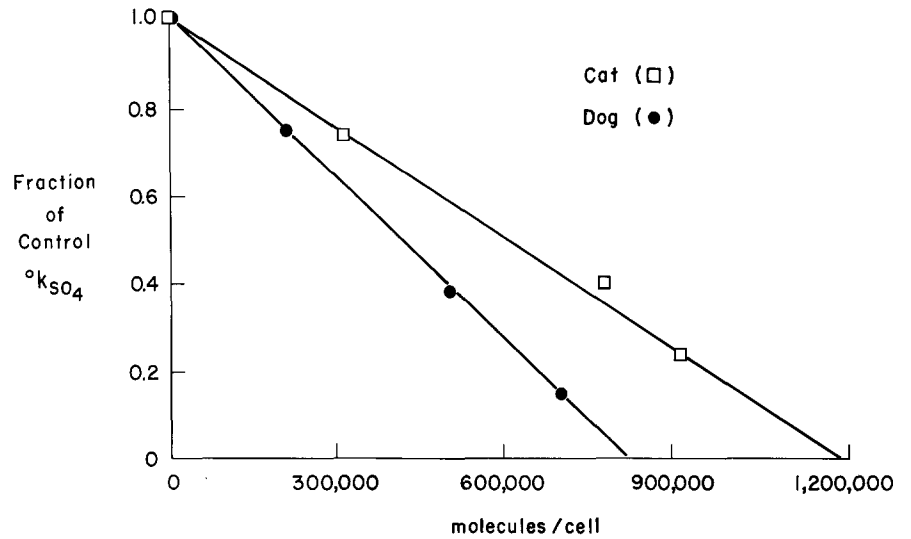

Fig. 8. The relationship between the number of $[^3H]H_2DIDS$ molecules bound per cell and inhibition of sulfate efflux. Cells were incubated at a 0.75% hematocrit for 3 hr at 37 °C with $^{35}SO_4$ in flux medium (mM: 135 NaCl, 5 KCl, 10 Na_2SO_4, 5 NaHEPES, and 5 glucose (pH 7.4)). After this time, $[^3H]H_2DIDS$ (0.05–0.3 μM) was added to the cell suspensions and incubation was continued for 30 min at 37 °C. The cells were washed 3 times in ice-cold flux medium containing 0.5% albumin. Aliquots of red cells were used to measure $^{0}k_{SO_4}$ at 0.5% and 37 °C. Other aliquots of cells were used for cell counting and for detection of ^3H. The number of molecules of H_2DIDS per cell was calculated from the cpm/aliquot, the counting efficiency (23%), the specific activity, the number of cells per aliquot, and Avogadro's number. Data are means of two determinations

Table 2.

Red cell type	Number H_2DIDS binding sites per cell	Cl turnover number (ions per site per sec)
Dog	0.81×10^6	4.27×10^2
Cat	1.19×10^6	1.13×10^2
Human	1.15×10^6	1.79×10^2

The number of anion transport sites was calculated from the number of $[^3H]H_2DIDS$ bound per cell at 100% inhibition of sulfate transport as shown in Fig. 8 for dog and cat red cells. Data for human red cells are taken from Ship *et al.* (1977) and Lepke *et al.* (1976). Turnover numbers were calculated using $^{0}k_{Cl}$ values obtained at 0 °C. Data for $^{0}k_{Cl}$ in dog and cat red cells are shown in Table 1 while that for human red cells are unpublished data from our laboratory, i.e., 3.75 min^{-1}. Values of Cl content of cells for dog, cat, and human were calculated from the Donnan ratio for Cl and was approximately 101 mM/liter cell water in each case. The water content of dog and cat cells is 72 and 71% (vol/vol), respectively. Mean cell volume and surface area for dog red cells is 69 μm^3 and 120.8 μm^2 (Emmons, 1927) or 68 μm^3 and 106 μm^2 (Castle & Daland, 1937), giving an average surface/volume ratio of 1.68. Mean cell volume and surface area for cat red cells is 43 μm^3 and 81.7 μm^2 (Emmons, 1927) or 57 μm^3 (Albritton, 1952) and 73 μm^2 (Rich *et al.*, 1967), giving an average surface/volume ratio of 1.59.

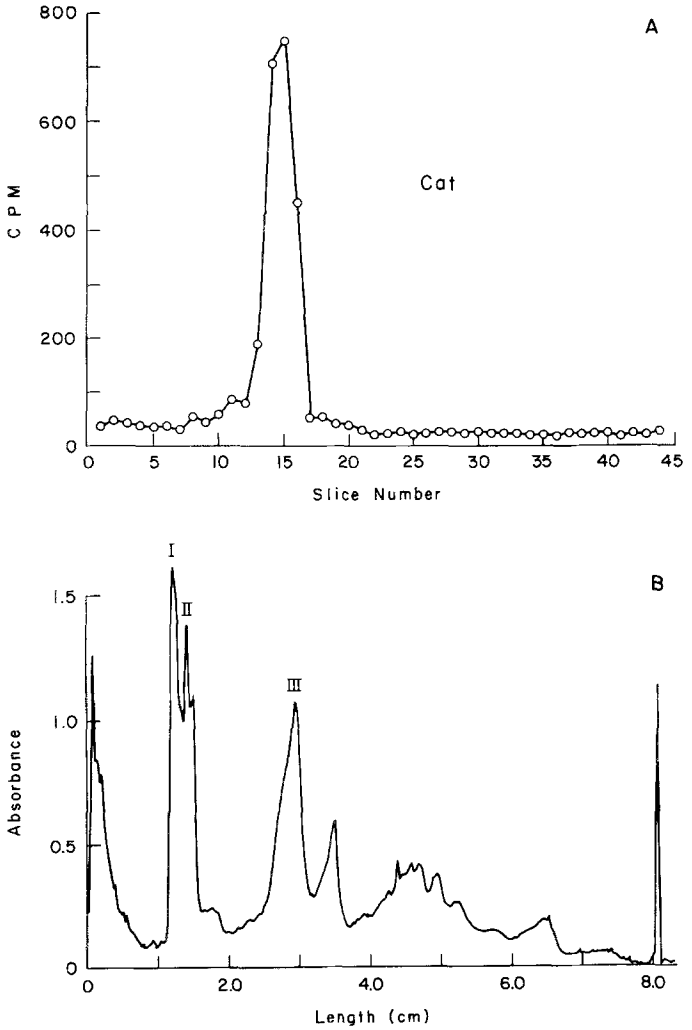

Fig. 9. (*A*): Radioactivity profile of membrane proteins from cat red cells separated by SDS polyacrylamide gel electrophoresis. Red cells were treated at 0.75% hematocrit with 0.3 μM [^{3}H]H_2DIDS for 30 min at 37 °C before lysis. Background of 33 cpm has not been subtracted from the data. (*B*): Gel scans showing membrane proteins stained with Coomassie Brilliant Blue. Band III is identified according to the nomenclature used by Fairbanks *et al.* (1971)

In order to identify which membrane proteins might be associated with [^{3}]H_2DIDS, ghosts were prepared from labeled dog and cat red cells. The membrane was solubilized and the proteins separated by SDS polyacrylamide gel electrophoresis. Figure 9*A* shows the radioactivity profile of membrane proteins obtained from cat red cells. Figure 9*B* shows, in a paired gel, the staining pattern of membrane proteins obtained

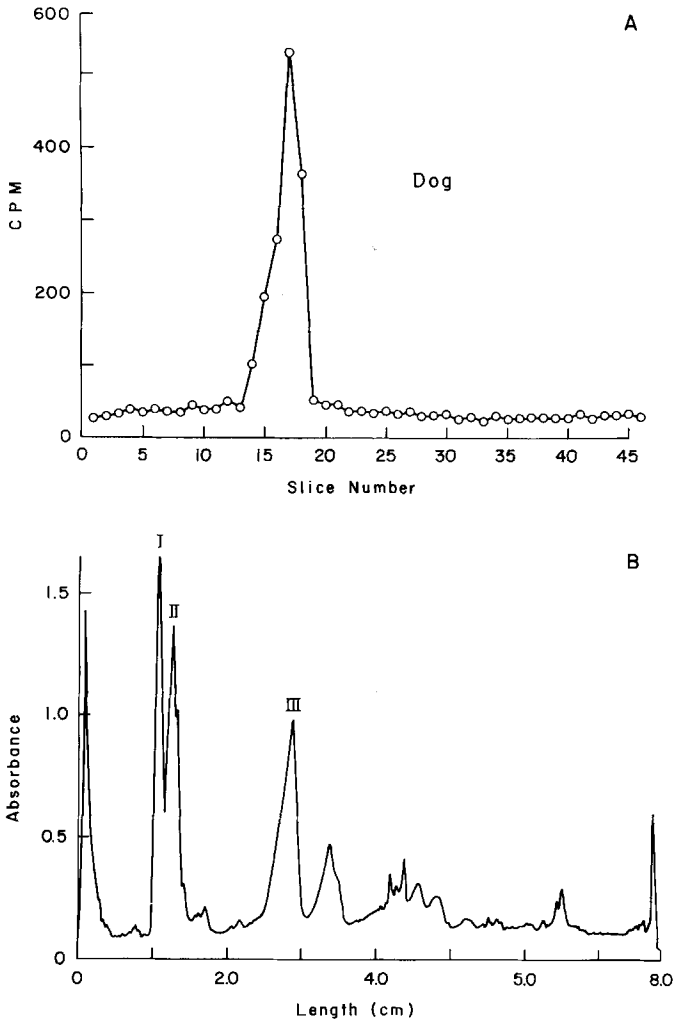

Fig. 10. (*A*): Radioactivity profile of membrane proteins from dog red cells separated by SDS polyacrylamide gel electrophoresis. Red cells were treated at 0.75% hematocrit with 0.3 μM [^{3}H]H_2DIDS for 30 min at 37 °C before lysis. Background of 33 cpm has not been subtracted from the data. (*B*): Gel scans showing membrane proteins from dog red cells stained with Coomassie Brilliant Blue. Band III is identified according to the nomenclature used by Fairbanks *et al.* (1971)

from cat red cells as part of the same experiment. Note that almost all (98%) of the [^{3}H]H_2DIDS label is found in five slices corresponding to band III. Figure 10A shows the radioactivity profile and Fig. 10B, the staining pattern of membrane proteins in a comparable experiment carried out using dog red cells. Here again about 98% of the [^{3}H]H_2DIDS label is found in five slices corresponding to band III. As in Fig. 8,

more [^{3}H]H_2DIDS appears on gels of cat red cell membranes than those of the dog when comparable amounts of membrane protein are put on the gels, indicating proportionate recovery of [^{3}H]H_2DIDS. The labeling patterns of both dog and cat red cell membranes are very similar to that for [^{3}H]H_2DIDS labeling of human red cell membranes (Ship *et al.*, 1977).

Discussion

Results presented in this study indicate that the rates of both Cl and SO_4 efflux are twice as fast in dog red cells as those measured in cat erythrocytes. Although the rates differ, the anion exchange systems in dog and cat red cells are very similar in many respects. The dependence of ${}^0M_{SO_4}$ on intracellular SO_4 concentration, the inhibition of SO_4 efflux by Cl and SITS, and the pH dependence of ${}^0k_{SO_4}$ are almost identical in dog and cat red cells. The only differences found for Cl self-exchange between the two red cell types were a lower $K_{1/2}$ and V_{max} in cat compared to dog red cells.

Since anion transport in cat and dog red cells appears to be saturated at physiological Cl concentrations, changes in $K_{1/2}$ are not as important as changes in V_{max} in explaining the differences in transport rates between cat and dog cells. The observed changes could result from differences in the numbers of ions transported per site per sec in cat and dog cells or from differences in the density of transport sites on cat and dog cell membranes.[2] Results from H_2DIDS binding studies indicate that the greater rate of anion transport in dog red cells is not due to a greater number of transport sites. In fact, there are more H_2DIDS binding sites on cat than on dog red cells. This suggests that if H_2DIDS binding is an accurate measure of the number of transport sites, the turnover number for dog red cells should be very high. Table 2 compares values for the number of anion transport sites per cell and the turnover number of these sites in human, cat, and dog red cells. These parameters are similar in human and cat red cells as are the rate constants for

[2] It is also of interest that the transport rates can be affected by membrane phospholipid composition. Deuticke and Gruber (1970) have reported a correlation between red cell membrane phospholipid composition and the rates of anion transport in red cells from various animals. In their study they found that anion flux tended to increase with increasing phosphatidylcholine and decreasing sphingomyelin contents. Since dog red cell membranes have more phosphatidylcholine and less sphingomyelin than cat red cell membranes, differences may exist regarding physical properties of these membranes that may underlie differences in transport rate or turnover number.

Cl and SO_4 efflux, while dog red cells possess a lower number of transport sites and a much larger turnover number than either human or cat red cells. On the other hand, if the turnover rate for anion transport sites in human, dog, and cat red cells were the same, then it would indicate that H_2DIDS binds to nonspecific sites as well as to authentic anion transport sites. This type of heterogeneity in band III protein cannot be eliminated on the basis of the data presented in this paper.

We wish to thank Dr. Jack Kaplan for his advice in the case of nystatin treatment of red cells and Drs. P.A. Knauf, S. Ship, and W. Breuer for supplying both nonradioactive H_2DIDS and radioactive [^{3}H]H_2DIDS. We also thank Dr. B. Forbush for his advice and help with the SDS-gel electrophoresis analysis.

This investigation was supported by U.S. Public Health Service Grants HL-09906 and AM-17433 and Fellowship HL-00400 and HL-01799.

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