Organotin-Mediated Exchange Diffusion of Anions in Human Red Cells

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ABSTRACT Organotin cations (RSn+) form electrically neutral ion pairs with monovalent anions. It is demonstrated that the tin derivatives induce exchange diffusion of chloride in red cells and resealed ghosts, without any detectable increase of membrane permeability to net movements of chloride ions. The obligatory anion exchange is believed to be due to the permeation of electroneutral ion pairs, whereas the organic cation (RSn+) has an extremely low membrane permeability. Exchange fluxes of chloride increased with the lipophilicity of the substituting group (Rs). At the same molar concentration of organotin, the relative potencies of the tin derivatives as anion carriers (with trimethyltin as a reference) were: methyl 1, ethyl 30, propyl = phenyl 1,000, and butyl 10,000. Tributyltin-mediated anion exchange was studied in detail. The organotin-induced anion transport increased through the sequence: F− << Cl− < Br− < I− = SCN− << OH−. Partitioning of tributyltin into red cell membranes was greater in iodide than in chloride media (partition coefficients 6.6 and 1.7 × 10−3 cm, respectively). Bicarbonate, fluoride, nitrate, phosphate, and sulphate did not exchange with chloride in the presence of tributyltin. Chloride exchange fluxes increased linearly with tributyltin concentrations up to 10−5 M, and with chloride concentrations up to at least 0.9 M. The apparent turnover number for tributyltin-mediated chloride exchange increased from 15 to 1,350 s−1 between 0 and 38°C. These figures are minimum turnover numbers, because it is not known what fraction of the organotin in the membrane exists as chloride ion pairs.

INTRODUCTION

This work deals with exchange diffusion of anions mediated by lipophilic organotin compounds. Many biological membranes possess transport systems which can perform an obligatory "self-exchange" of isotopes of one chemical ion species or a "heteroexchange" of ions of different chemical species. The phenomenon was named by Ussing (1948), who wrote: "in its ideal form such a mechanism will always take up one ion, when it gives off another, so that no net change in the ion concentration on either side of the membrane need take place. Such a diffusion we call exchange diffusion."

The above-mentioned criterion of ideality implies that the membrane permeability measured by isotope exchange is out of proportion to the low membrane conductance of the same ion, because the obligatory exchange mechanism cannot mediate a net flow of current through the membrane. This leads to the
paradox that an extremely large permeability to a tracer ion may be accompa-
nied by a very low electrical conductance. The anion transport system in
erthrocytes is a remarkable example. Measured as chloride self-exchange, the
permeability to $^{36}\text{Cl}^-$ efflux at 38°C is $4 \times 10^{-4} \text{ cm} \cdot \text{s}^{-1}$ (Brahm 1977), whereas
the permeability to a net transfer of chloride ions through the same membrane
is four orders of magnitude lower (Hunter 1971, 1977; Knauf et al., 1977).
Electrically silent anion exchange has also been described in artificial mem-
branes. Shean and Sollner (1966) demonstrated that certain lipophilic secondary
amines can perform an obligatory exchange of anions through thick hydropho-
bic membranes, and it was later shown by Gutknecht et al. (1978) that the
secondary amines are also capable of mediating exchange diffusion of bromide
in bimolecular lecithin membranes, which maintained a high electrical resis-
tance.

The purpose of the present work is to examine the exchange diffusion
induced in red cell membranes by organotin compounds. Selwyn et al. (1970)
showed that organotin derivatives can mediate chloride-hydroxyl exchange
across the membranes of mitochondria, erythrocytes, and liposomes. These
results were confirmed and extended in red blood cells by Aubert and Motais
(1975). Stimulated by these observations we decided to examine if the organotin
compounds mediate electrically silent exchange diffusion of anions in red cells
and in lipid membranes. We have found that the trialkyltin derivatives can
induce an anion exchange permeability, which is several orders of magnitude
higher than the permeability to net anion movements deduced from indirect
and direct measurements of membrane conductance in erythrocyte and artificial
membranes. Brief reports of our work have been published previously (Toste-
son et al., 1977; Wieth and Tosteson, 1977). During the preparation of this
article, a report has confirmed that organotin compounds can mediate a self-
exchange of chloride in red cells (Motais et al., 1977), but it has not been
considered previously to which extent the membrane conductance of red cells is
affected by the organometallic cations.

**METHODS**

*Organotin Compounds*

Trimethyltin chloride, triethyltin chloride, and triphenyltin chloride were obtained from
Merck-Schuchardt (Darmstadt, West Germany), tripropyltin chloride and tributyltin
chloride from BDH Chemical Ltd., Poole, England. The organotin salts were dissolved
in ethanol (99% vol/vol) to give the desired concentration in the cell suspension, when
diluted 700–1,000-fold with electrolyte medium. The ethanol concentration in the media
was thus maximally 0.15% (vol/vol). The organotin was dissolved in the medium before
the cells were injected. A constant rate of anion exchange was found to be established
within the first few seconds of the experiments.

*Preparation of Red Cells and Ghosts*

Freshly drawn heparinized human blood was centrifuged, plasma and buffy coat were
removed, and the cells were resuspended and washed in a 165 mM KCl solution. The
preparation and labelling of resealed ghosts with isotopes followed the protocol given by
Funder and Wieth (1976). The pH of the ghost preparations was adjusted before packing
the ghosts into nylon centrifuge tubes. Red cells were titrated to acid pH values with 
CO₂. When the desired pH value had been reached, the bicarbonate formed by the 
titration was removed by repeated washings of the cells in the bicarbonate-free electrolyte 
medium used for the subsequent experiment.

Inactivation of the Normal Anion Transport System

In most experiments anion transport mediated by the organotin compounds was studied 
in red cell membranes, whose natural anion transport mechanism had been inhibited 
irreversibly with the amino-group reagent 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid 
(DIDS), a potent inhibitor of anion transport in red cells (Cabantchik and Rothstein, 
1974). Chloride transport is inhibited >99% when binding is 1.1 × 10⁻⁶ DIDS molecules 
per cell (Lepke et al., 1976; Ship et al., 1977), and excess binding of DIDS causes no 
further reduction of anion transport (Funder et al., 1978). The DIDS treatment was 
carried out by incubating red cells or resealed ghosts for 45 min at 38°C in a medium 
containing 5-10 × 10⁻⁶ molecules per cell. The methods employed for preparation and 
purification of DIDS were recently reported by Funder et al., 1978.

Labelling with Isotopes

In order to label red cells or ghosts with radioactive anions, tracer was added to the cell 
suspension briefly before the addition of DIDS. Among the anions studied iodide has 
the slowest rate of exchange through the natural exchange system, but inasmuch as the 
T₁/₂ of exchange is 2 s at 38°C (Dalmark and Wieth, 1972), complete equilibration was 
obtained in <15 s after addition of tracer, whereafter DIDS was added. The cells were 
separated from the radioactive medium by centrifuging the suspension in nylon tubes 
(i.d. 3 mm) for 10 min at 50,000 g. The amount of extracellular fluid trapped was 2% (wt/ 
wt) in a column of packed erythrocytes, and 8-12% (wt/wt) between packed resealed 
ghosts (Funder and Wieth, 1976). The packed cells or ghosts were isolated from the 
radioactive medium by cutting the tube 1 mm below the interface between cells and 
medium. 

All radioactive anions except °SF⁻ were obtained and used in concentrations as 
described by Dalmark and Wieth (1972), where information about sampling and counting 
techniques is also stated. The radioactive fluoride (°SF⁻) was a gift from the Niels Bohr 
Institute of Theoretical Physics, University of Copenhagen. The isotope has a half-life of 
1.8 h, so the amount of radioactivity added to the cell suspensions was increased to 10 
μCi per milliliter.

Electrolyte Media

Media containing monovalent anions were 165 mM solutions of the appropriate potas-
sium salts, buffered with phosphate (0.5 mM), which was added as KH₂PO₄ and titrated 
to the desired pH with KOH. Minor modifications of these basic media are specified in 
the text. Control experiments showed that both natural and organotin-mediated ex-
change diffusion of chloride takes place at identical rates in KCl, NaCl, and LiCl media. 
The isotonic phosphate and sulphate media used in the heteroexchange experiments 
(Table IV) were 100 mM K₂SO₄ + 0.5 mM KH₂PO₄, and 130 mM KH₂PO₄. Both media 
were titrated to pH 6.8 (0°C) before being used for flux experiments.

Anion Exchange Experiments

The efflux of radioactive anions from labelled cells or ghosts was initiated by injecting a 
packed cell sample (usually 200 mg) into 40 ml of the well-stirred, thermostated, 
electrolyte medium. Serial samples of cell-free medium were obtained by the Millipore-
Swinnex technique (Millipore Corp., Bedford, Mass.; Dalmark and Wieth, 1972) at
appropriate time intervals, thereby following the accumulation of radioactivity as a function of time. The rate coefficient for anion efflux \( (k, \text{s}^{-1}) \) was calculated from the slope of a graph of \( \ln \left(1 - \frac{a_t}{a_u}\right) \) vs. time \( (t) \), determined by linear regression analysis, \( a_t \) being the activity in the extracellular compartment at time \( (t) \), and \( a_u \) the activity at isotopic equilibrium. The unidirectional anion flux was determined from the relation:

\[
J_{\text{exch}} = k \left( \frac{V}{A} \right) C_A \text{ mol} (\text{cm}^2 \cdot \text{s})^{-1}.
\]  

\( V \) is the solvent volume in red cells or ghosts determined from the mean cell volume with correction for the solids content assuming a density of 1.3 for hemoglobin; \( A \) is the mean surface area of erythrocytes and ghosts \( (1.42 \cdot 10^{-6} \text{ cm}^2 \text{ (cell)}^{-1}) \), cf. Funder and Wieth (1976); and \( C_A \) is the intracellular concentration of the anion \( \text{mmol} (\text{cm}^3 \text{ cell water})^{-1} \). The term \( k \left( \frac{V}{A} \right) \text{ cm} (\text{s})^{-1} \) equals the apparent permeability coefficient to anion exchange \( P_{\text{cl}}^{\text{exch}} \).

In steady-state self-exchange experiments, we followed the isotope exchange to 80-90% completion. In the heteroexchange experiments, the initial efflux was calculated from the initial slope of isotope release.

Preparation of \( ^{42}\text{K} \)-Loaded Cells

10 ml of freshly drawn heparinized blood was titrated to pH 6.6 (38°C), washed in the 150 mM KCl medium, and resuspended to 15 ml. A small volume (10--15 μl) of a 150 mM KCl solution containing 15 μCi \( ^{42}\text{K} \) (AEK, RISO, Denmark) was added, followed by 15 μl of a solution of valinomycin (grade A, Calbiochem Behring Corp., San Diego, Calif.) in ethanol (1 μmol/ml). When the cells were treated with DIDS, this compound was added dissolved in 150 mM KCl to give a resultant amount of 10^9 molecules per cell. The suspension was now incubated for 45 min at 38°C, and the cells were isolated for the subsequent flux experiments. Control analyses showed that the specific activity of \( ^{42}\text{K} \) after loading of the cells was between 90 and 100% of the specific activity in the extracellular phase.

\( ^{42}\text{K} \) Efflux Experiments

Determination of the rate coefficients of \( ^{42}\text{K} \) efflux from the valinomycin-treated cells was performed by injecting ~200 mg of cells into 40 ml well-stirred thermostated medium containing valinomycin 10^-6 M (ethanol 0.1%). The techniques of sampling and of calculating the rate coefficients were identical to those used in the \( ^{36}\text{Cl}^- \) efflux experiments. For each cell preparation, duplicate experiments were carried out at extracellular potassium concentrations of 0.15, 1.5, 15, 75, and 150 mM, in media containing 27 mM sucrose and (NaCl + KCl) equal to 150 mM. It was found that organotin-mediated self-exchange of chloride took place at the same rate in KCI and NaCl media in the presence and absence of valinomycin.

Membrane Permeability to Electrodiffusion of Chloride

It was essential to determine if tributyltin has any effect on the chloride conductance of the red cell membrane. Direct measurement of the conductance of human red cells is not feasible, but several quantitative estimates have been made by indirect methods, based on the qualitative observation of Chappel and Crofts (1966) that net transfer of anions becomes rate-limiting to the efflux of KCl from cells, if the normal low cation permeability of the membrane is raised a few orders of magnitude by ionophores that increase the potassium permeability. We have used the valinomycin method of Hunter (1971, 1977) to determine the effect of tributyltin on \( P_{\text{cl}}^{\text{exch}} \) of normal and of DIDS-treated human red cells. We determined the rate of \( ^{42}\text{K}^+ \) efflux into the above-mentioned media at 38°C, pH 6.6. Stability of extracellular pH of the unbuffered media during the efflux
of $^{42}\text{K}^+$ showed that there was no significant transport of hydroxyl or hydrogen ions, suggesting that it is justifiable to consider chloride and potassium as the only permeating ion species, as it is implied in the constant field treatment employed by Hunter (1977).

The rate coefficient of $^{42}\text{K}^+$ efflux ($k$, s$^{-1}$) was determined by linear regression analysis of the data in experiments, where $K_o$ was above 15 mM. At lower external potassium concentrations, where the cells shrink due to a net loss of KCl, we determined the rate coefficient from the initial rate of isotope release, i.e., from the samples taken before 20% of cellular KCl had been lost.

Under the present experimental conditions $K_i = Cl_i = Cl_o = 150$ mM at 38°C, pH 6.6. Therefore, the constant field equation takes the following simple form:

$$V_m = \psi_t - \psi_o = \frac{-RT/F \ln \frac{150 + P_k/P_{\text{eff}} \times 150}{150 + P_k/P_{\text{eff}} \times K_o}}{150 + P_k/P_{\text{eff}} \times K_o} = -RT/F \ln B,$$

where $K_o$ is the variable extracellular potassium concentration. The flux of $^{42}\text{K}^+$, which is determined experimentally, is affected by the membrane potential:

$$J_k = P_k K_i = \frac{\ln B}{B - 1}. \quad (3)$$

A sensitive way of determining $P_{\text{Cl}, \text{net}}$ is to plot $\ln B/(B - 1)$ vs. $K_o$ as shown in Fig. 11. It should be noted that the value of $\ln B/(B - 1)$ is equal to the ratio $k_{s}/k_{150}$, where $k_{150}$ is the rate coefficient of $^{42}\text{K}^+$ efflux in the 150 mM KCl medium, and $k_s$ is the rate found at a lower KCl concentration. The potassium permeability ($P_k$) is equal to $k_{150}(V/A)$, $V$ being the solvent volume and $A$ the membrane area of the cells.

**Partitioning of Organotin between Cells and Medium**

As shown in Results, red cell membranes take up considerable amounts of the lipophilic organotin derivatives ($T$). The partitioning was analyzed by a biological method, previously used by Hunter (1974) to determine the uptake of valinomycin into red cell membranes.

The method is based on determinations of rates of transport in suspensions containing a fixed amount of organotin and a variable concentration of cells. Inasmuch as the organotin-mediated anion transport at a fixed hematocrit varied linearly with the amount of $T$ in the medium, it is safe to assume that the adsorption of $T$ to cell membranes follows Henry's law within wide limits (cf. Fig. 2):

$$T_t = T_m + T_c = T_m + \alpha h T_m, \quad (4)$$

where $T_t$ is the total amount of organotin in the suspension, $T_m$ is the amount of organotin in the aqueous medium, and $T_c$ is the amount of organotin taken up by the cells. The linear adsorption coefficient, $\alpha$, is the ratio: (mole $T$ per liter cells/mole $T$ per liter medium), and $h$ is the fractional cell volume of the suspension. Anion self-exchange is a linear function of the amount of organotin in the cell membrane, so keeping $T_t$ constant:

$$k_h = k_0[1/(1 + \alpha h)], \quad (5)$$

where $k_0$ is the rate coefficient of anion self-exchange as the hematocrit tends to zero, and $k_h$ is the rate of self-exchange when the fractional cell volume of the suspension is $h$. The equation can be rearranged for a linear relation:

$$\frac{1}{k_h} = 1/k_0 + \alpha h/k_0. \quad (5 a)$$

Both $k_0$ and $\alpha$ can be found by linear regression analysis of a plot of $1/k_h$ vs. $h$. An
example of the determination of \( \alpha \) is shown in Fig. 6. Experimental evidence showed that tributyltin is only adsorbed to the cell membranes, and not to any measurable degree to hemoglobin or to other cellular constituents. From the value of \( \alpha \), it is therefore possible to estimate the surface concentration of organotin molecules, assuming that they are located at the two membrane interfaces. The partition coefficient is redefined as the ratio between the organotin at the membrane surface \( (T_d \text{ mol cm}^{-2}) \) and the concentration in the medium \( (T_m \text{ mol cm}^{-2}) \),

\[
\beta = \frac{T_d}{T_m} = \frac{\alpha}{A_{RBC}},
\]

where \( A_{RBC} \) is the total surface area (external + internal) of 1-ml red cells with a mean cell volume of \( 87 \times 10^{-12} \text{ ml} \) and a cell surface area of \( 1.42 \times 10^{-7} \text{ cm}^2 \). The number of organotin molecules per square centimeter \( (N) \) will now be given by:

\[
N = \beta T_m N_A, \tag{6a}
\]

where \( N_A \) is Avogadro's number \( (6.023 \times 10^{23} \text{ molecules/mole}) \).

**RESULTS**

*Chloride Self-Exchange*

The effect of tributyltin on the rate of \(^{36}\text{Cl}^-\) efflux from intact and from DIDS-treated human red cells at \( 0^\circ \) is illustrated in Fig. 1. In the DIDS-treated cells, where the natural anion exchange mechanism is inhibited, addition of tributyltin (5.2 \( \mu \text{mol/liter cell suspension} \)) increased the exchange flux from 1.4 to 85 pmol

![Figure 1](image-url)
(cm² × s)⁻¹. A similar increase of the exchange flux was found in intact cells (Fig. 1), demonstrating that the artificial ionophore creates a transport pathway for chloride in parallel with the natural anion transport system.

Table I shows that the effect of tributyltin on chloride exchange was the same in red cells and in resealed ghosts, indicating that the presence of hemoglobin does not affect the tin-mediated transport of anions. This was further substantiated by the observation that the addition of a membrane-free red cell lysate to the flux medium did not change the rate of chloride exchange. Table I also demonstrates that phloretin, which is a potent inhibitor of the natural transport system, only has a moderate effect on the tributyltin-mediated chloride transport.

At a hematocrit of 0.5% the chloride self-exchange was found to be a linear function of the amount of tributyltin added to the suspension up to 10⁻⁵ mol/liter (Fig. 2). The flux leveled off, when more organotin was added, and an

| Tributyltin, 5.2 µmol/liter cell suspension; hematocrit 0.5%, pH 6.8, 0°C. Values are means (SD) from series of 10 experiments in each category. |

| TABLE I | CHLORIDE SELF-EXCHANGE IN DIDS-TREATED RED CELLS AND RESEALED GHOSTS MEDIATED BY TRIBUTYL Tin |
|---------|-----------------------------------------------|
| Phloretin(mM) | DIDS-treated red cells | DIDS-treated ghosts |
| 0 | 100.1 (8.4) | 98.9 (11.9) |
| 0.25 | 42.6 (1.7) | 40.3 (6.5) |

**Figure 2.** Chloride self-exchange flux (J) in DIDS-treated red blood cells as a function of the amount of tributyltin added per liter cell suspension (0°C, pH 6.8, hematocrit 0.5%).
increase from 30 to 50 μmol/liter suspension caused almost no increase of the self-exchange flux.

Organotin-induced chloride transport increased linearly with chloride concentration. Fig. 3 shows the result of an experiment, where chloride concentrations in cells and media were increased from 165 to 600 mM by the addition of ammonium chloride. Similar results were obtained by varying the concentration of KCl between 165 and 900 mM in resealed ghosts, demonstrating that the results shown in Fig. 3 were not caused by the presence of ammonium ions.

The pH dependence of the tin-mediated chloride exchange was examined in ghosts to avoid difficulties imposed by the variable hydroxyl ion concentration across the erythrocyte membrane, when pH is varied. The results shown in Fig. 4 demonstrate that tributyltin-mediated chloride exchange decreased steeply with increasing pH. The inhibition of chloride exchange by OH\(^{-}\) was well described by assuming an apparent dissociation constant for R\(_3\)SnOH of 10\(^{-8}\) M, meaning that the flux was half-maximal at pH 6.9 at 0°C, where the ionization constant for water is 10\(^{-14}\). At pH 6.8 where most of the chloride self-exchange determinations were carried out, the flux would accordingly be 56% of the maximum tributyltin-mediated transport.

The red cell membrane has a very low permeability to hydrogen and hydroxyl ions, when the natural anion exchange is inhibited (Motais et al., 1977; Jennings, 1978). This was confirmed in the experiment shown in Fig. 5, which demonstrates that the DIDS-treated red cells were virtually impermeable to H\(^{+}\) and OH\(^{-}\) at 0°C, and that an imposed pH gradient vanished rapidly when tributyltin was added. The rapid relaxation of extracellular pH after addition of tributyltin

![Figure 3. Chloride self-exchange flux as a function of chloride concentration in medium and cell water. The chloride concentrations were increased by the addition of NH\(_4\)Cl to the media used for preequilibration and experiments (0°C, pH 6.8, hematocrit 0.5%, tributyltin 5.2 × 10\(^{-6}\) mol/liter suspension).]
confirms that this compound can perform a rapid Cl⁻/OH⁻-exchange as first demonstrated by Selwyn et al. (1970).

In order to determine the partitioning of tributyltin between red cell membranes and medium, the rate of anion transport was determined in a series of experiments, where varied numbers of cells were exposed to a fixed amount of tributyltin. Fig. 6 shows that the time constant of anion exchange (τ) increased linearly with the cell concentration of the suspension. The mean value of the cellular partition coefficient (α) was 55.2 (SD 4.3, n = 5). Assuming that the tributyltin molecules were all located in the membrane-surfaces, we calculated a membrane partition coefficient (β) of $1.65 \times 10^{-3}$ cm (cf. Methods). Similar values were determined at 25°C, so it can be assumed that the adsorption of tributyltin depends little on temperature. This is important for the interpretation of the temperature dependence of tributyltin-mediated chloride exchange, which could be described by an apparent activation energy of 20 kcal/mol, as shown in the Arrhenius diagram of Fig. 7.

**Self-Exchange of Other Inorganic Anions**

Measurements of the rate of self-exchange of inorganic anions other than chloride showed that the relative transport rates were: SCN = I > Br > Cl >> F (Table II). The ability of tributyltin to transport fluoride was poor. The

![Figure 4. pH dependence of tributyltin-mediated chloride exchange in DIDS-treated resealed ghosts (0°C, cytocrit 0.5%, tributyltin 5.2 × 10⁻⁷ mol/liter suspension). The fully drawn line describes the relation: \( J = \frac{150}{1 + \frac{10^{-14.9 - \text{pH}}}{10^{-8}}} \) mol (cm² x s)^⁻¹.

\( 10^{-14.9} \) is the ionization constant for water at 0°C, \( 10^{-(14.9 - \text{pH})} \) is the hydroxyl ion concentration, and the apparent dissociation constant for membrane-bound tributyltin hydroxide is \( 10^{-8} \) M at a chloride concentration of 165 mM.
exchange flux of iodide was about five times larger than the chloride exchange
determined in media containing the same amounts of cells and of the organotin
compound. In 165 mM KI medium it was found, in experiments similar to that
shown in Fig. 6, that the partition coefficient of tributyltin was about four times
larger than the value found in chloride media ($\alpha_{KI} = 200$). This result suggests
that the differing tributyltin uptake induced by the anions determines the rates
of chloride and iodide transport.

![Graph](image)

**Figure 5.** The low permeability of DIDS-treated cells to H$^+$ and OH$^-$ at 0°C. (A) The pH response of a suspension of DIDS-treated cells to additions of acid and base (0°C, hematocrit 1%) before and after the addition of tributyltin. (B) The pH changes in tributyltin-treated cells. 40 ml of an unbuffered 165 mM KCl solution were used in both experiments. The additions of HCl and KOH were 100 µl of a 0.1 M solution (10 x 10$^{-4}$ mol H$^+$ or OH$^-$ corresponding to 2.5 x 10$^{-4}$ mol/liter suspension. The amount of tributyltin (TBT) added in ethanolic solution was 1.5 x 10$^{-7}$ mol (3.8 x 10$^{-8}$ mol/liter cell suspension). Note the large pH changes in the cell suspension before the addition of organotin. The pH changes and the response time of the glass electrode were similar to those found in a cell-free medium indicating that the cells were practically impermeable to H$^+$ and OH$^-$ at 0°C. When organotin had been added, the suspension responded to pulses of acid or base with much smaller pH excursions which relaxed to a new equilibrium value in the course of few minutes.

In the absence of tributyltin the rate of iodide self-exchange is extremely slow
in DIDS-treated cells (the half-time of exchange being ~4 h at 0°C). The rate of
exchange was doubled after the addition of $5 \times 10^{-9}$ mol tributyltin per liter of
cell suspension, and Table III shows that a further 1,000-fold increase of the
tributyltin concentration to $5 \times 10^{-4}$ mol/liter suspension caused a 1,000-fold
increase of the self-exchange flux from 0.7 to 700 picomol $\times \text{cm}^{-2} \times \text{s}^{-1}$. The
Arrhenius activation energy of the tributyltin-mediated iodide transport be-
tween 0 and 38°C was 15.0 kcal/mol (SD 0.5).
**Heteroexchange of Anions**

So far we have only described tracer experiments in which there were no net movements of anions across the red cell membrane. The ability of tributyltin to exchange anions was also measured in heteroexchange experiments, where DIDS-treated chloride- or iodide-loaded cells were suspended in isotonic media of various potassium salts as shown in Tables IV and V. Because the cation permeability of the red cell is extremely low, intracellular anions can only leave the cells in exchange for the foreign anions of the extracellular phase. Therefore, one can measure the relative ease with which chloride or iodide can exchange with other anions. The results confirmed that bromide, and especially iodide and thiocyanate are transported more readily than chloride itself. In addition the results showed that the rates of fluoride, nitrate, sulphate, and phosphate transfer were extremely slow.

Fig. 8 shows that the slow rate of chloride efflux into a nitrate or fluoride medium was completely unaffected by the addition of 2 mM bicarbonate to the medium, in contrast to the dramatic effect of bicarbonate on chloride heteroexchange through the natural anion transport system (Fig. 5 in Wieth, 1972), supporting the conclusion of Motais et al. (1977) that bicarbonate is not transported by organotin derivatives. One might argue that heteroexchange of chloride with extracellular bicarbonate is impeded by the shift of pH from 6.8 to 7.1 after addition of bicarbonate. However, it may be noted that a similar pH change only reduced the rate of chloride self-exchange by 30% (cf. legend of Fig. 4).
Self-Exchange of Chloride or Iodide in the Presence of High Concentrations of Other Anions

The rates of chloride and iodide exchange were also determined in cells which had been preequilibrated in media containing 2 mM chloride or iodide plus 165 mM the anion in question. The results are shown in Tables VI and VII. It was not possible to demonstrate any kind of competition between chloride and the more rapidly transported anions such as bromide, iodide, or thiocyanate. On the contrary, the rate coefficients varied much in the same way as in the heteroexchange experiments, suggesting that ions favoring the partitioning of tributyltin into the membrane increase the rate of $^{38}$Cl$^-$ efflux. The rates of chloride and of iodide self-exchange decreased by 50-70% in the nitrate medium. Nitrate has a very low affinity for organotin and the concentration of nitrate ion pairs in the membrane must accordingly be low. The decreased rates of halide exchange in the presence of nitrate fits with the concept that the total amount of ion pairs in the membrane plays an important role for the rate of halide transport, a point that is further dealt with in the Discussion. Halide exchange was strongly inhibited by fluoride, which is known to form water-soluble, long-chained complexes with organotin molecules, (Cotton and Wilkinson, 1966).

Anion Exchange by Other Organotin Compounds

Other trialkyl- and triaryltin derivatives than tributyltin can mediate exchange diffusion of anions in red cells as shown in Table VIII, which shows the chloride

\[
\ln k = -9.9(10^3/T) + 28.8 \quad (r = 0.996).
\]
exchange flux, induced by varying concentrations of trimethyl-, triethyl-, tripropyl-, and triphenyltin. The dose relationship for tributyltin-mediated chloride exchange was shown in Fig. 2. The relative effects of the tin compounds were compared to that of trimethyltin by dividing the organotin anion exchange by the concentration of organotin in the suspension. The normalized exchange transport induced by trimethyltin was thus: 

\[
(1.4 \times 10^{-12}/0.7 \times 10^{-3}) = 2 \times 10^{-9} \text{ mol x cm}^{-2} x \text{s}^{-1}
\]

at a hypothetical trimethyltin concentration of 1 mol per liter.

### TABLE II

**SELF-EXCHANGE OF MONOVALENT INORGANIC ANIONS THROUGH DIDS-TREATED RED CELL MEMBRANES IN THE ABSENCE AND PRESENCE OF TRIBUTYLtin**

| Radioactive anion | Tributyltin absent | Tributyltin |
|-------------------|-------------------|-------------|
|                   | \( \text{Rate coefficient} \) | \( T_{1/2} \) | \( \text{Rate coefficient} \) | \( T_{1/2} \) |
| \(^{18}\text{F}^-\) | \(1.4 \times 10^{-2}\) | 48.6 | 0.04 | 17.9 |
| \(^{36}\text{Cl}^-\) | \(9.81 \times 10^{-3}\) | 70.7 | 0.84 | 0.83 |
| \(^{82}\text{Br}^-\) | \(2.35 \times 10^{-3}\) | 294.5 | 1.77 | 0.39 |
| \(^{125}\text{I}^-\) | \(2.92 \times 10^{-3}\) | 257.4 | 4.33 | 0.16 |
| \([^{14}\text{C}]\text{SCN}^-\) | \(3.55 \times 10^{-2}\) | 19.5 | 3.85 | 0.18 |

Tributyltin, \(5.2 \times 10^{-4}\) mol/liter cell suspension; hematocrit 0.5\%, pH 6.8, 0°C.

### TABLE III

**IODIDE SELF-EXCHANGE IN DIDS-TREATED RED CELLS AS A FUNCTION OF TRIBUTYLtin CONCENTRATION**

| Tributyltin | Iodide self-exchange flux \(10^{10}(\text{mol/cm}^{-2}x \text{s})\) |
|-------------|-------------------------------------------------------------|
| 0 | 0.006 |
| \(5.2 \times 10^{-9}\) | 0.682 |
| \(5.2 \times 10^{-8}\) | 6.48 |
| \(1.04 \times 10^{-7}\) | 13.38 |
| \(5.2 \times 10^{-7}\) | 97.3 |
| \(1.04 \times 10^{-6}\) | 147.7 |
| \(5.2 \times 10^{-6}\) | 711.5 |

Hematocrit 0.5\%, pH 6.8, 0°C. The self-exchange flux was a linear function of the amount of tributyltin added to the suspension between concentrations of \(5 \times 10^{-9}\) and \(5 \times 10^{-6}\) mol/liter cell suspension.

**It is likely that the lipophilicity of the organotin compound determines its ability to induce anion transport. We have attempted to determine the partitioning of triethyltin between medium and cells by the method used for determining partitioning of tributyltin (Fig. 6). The rate of chloride exchange was found to be independent of variations of the hematocrit between 0.5 and 5\%, as would be expected if—as judged from the relative potencies—the uptake of triethyltin is a 100-fold smaller than the uptake of tributyltin. We have been able to determine the cellular partition coefficient for tripropyltin. The value \((\alpha = 5)\) was ten times lower than that found for tributyltin. once more suggesting a simple relation**
between the amount of organotin taken up by the membranes and the relative rates of transport.

**Exchange Diffusion of Chloride without Increase of Chloride Conductance**

In order to evaluate the mechanism by which organotin compounds induce anion transport in red cell membranes, it was essential to determine the effect of tributyltin not only on exchange movements of anions but also on the ability of chloride ions to carry electrical current through the membrane.

$P_{\text{cl}^{-}}$ was determined in the presence and absence of tributyltin by the method of Hunter (1977), according to which the rate of $^{42}$K efflux from valinomycin-treated cells is measured as a function of extracellular potassium concentration.

### Table IV

| Extracellular Anion | Rate Coefficient of $^{36}$Cl$^{-}$ Efflux ($10^{4} \text{ s}^{-1}$) | Initial Chloride Efflux ($10^{4} \text{ mol cm}^{-2} \text{ s}^{-1}$) |
|---------------------|-------------------------|--------------------------|
| Chloride            | 15                      | 112.5                    |
| Bromide             | 21                      | 157.5                    |
| Iodide              | 36                      | 270.0                    |
| Thiocyanate         | 35                      | 262.5                    |
| Fluoride            | 0.9                     | 6.8                      |
| Nitrate             | 0.9                     | 7.0                      |
| Sulfate             | 1.1                     | 8.2                      |
| Phosphate           | 0.6                     | 4.6                      |

Tributyltin, $5.2 \times 10^{-4}$ mol/liter cell suspension; hematocrit 0.5%, pH 6.8, 0°C. The cells contained 172 mmol Cl$^{-}$ per kilogram cell water, and the intracellular chloride was labelled with $^{36}$Cl$^{-}$. The initial rate of tracer efflux was determined, and the initial chloride efflux was calculated from the initial rate of isotope release. It is a self-exchange flux in the presence of extracellular chloride, and a net flux (heteroexchange) in experiments where chloride exchanges with other anions. All experiments were performed with the same set of cells. The chloride self-exchange flux was $1.1 \times 10^{-10}$ mol cm$^{-2}$ s$^{-1}$ in the absence of tributyltin.

The rate of $^{42}$K efflux should not be affected by the external potassium concentration, if the chloride permeability were orders of magnitude higher than the valinomycin-induced potassium permeability, as would be the case if the large chloride permeability measured by tracer exchange ($P_{\text{cl}^{-}}^{\text{exch}}$) were a measure also of the permeability of the membrane to a net transfer of chloride ($P_{\text{cl}^{-}}^{\text{net}}$). Fig. 9 shows that the rate of $^{42}$K efflux was profoundly affected by changes in extracellular potassium concentration. The chloride permeabilities ($P_{\text{cl}^{-}}^{\text{net}}$) calculated from these experiments are summarized in Table IX. It should be noted that $P_{\text{cl}^{-}}^{\text{net}}$ of intact red cells [$3.0 \times 10^{-8}$ cm(s)$^{-1}$] was $10^{4}$ times lower than the exchange permeability determined in intact red cells at 38°C (Brahm, 1977). DIDS treatment of the red cells reduced $P_{\text{cl}^{-}}^{\text{net}}$ by 65%. We could not demonstrate any effect of tributyltin on $P_{\text{cl}^{-}}^{\text{net}}$ at a tributyltin concentration of 5 μmol per liter.
cell suspension. The exchange permeability induced by this amount of tributyltin in DIDS-treated cells at 38°C is $5 \times 10^{-8} \text{ cm(s)}^{-1}$ (cf. Fig. 7). The conclusion is, therefore, that the chloride permeability induced by organotin compounds in red cell membranes is largely electrosilent, although it is clear that a small increase of $P_{Cl}$ cannot be detected with the indirect method employed.

### Table V

| Extracellular anion | Rate coefficient of $^{125}\text{I}^-$ efflux $\times 10^{4}$ (mol/cm²·s⁻¹) | Initial iodide efflux $\times 10^{5}$ (mol/cm²·s⁻¹) |
|---------------------|----------------------------------|----------------------------------|
| Iodide              | 17                               | 114.5                            |
| Chloride            | 2.8                              | 19.3                             |
| Bromide             | 4.7                              | 32.4                             |
| Thiocyanate         | 22.4                             | 154.5                            |
| Fluoride            | 0.28                             | 1.9                              |
| Nitrate             | 0.13                             | 0.9                              |

The experiments are analogous to the chloride heteroexchange experiments shown in Table IV, except that the tributyltin concentrations were 10 times lower ($5.2 \times 10^{-7}$ mol/liter cell suspension). The cells contained 180 mM I⁻ per kilogram cell water, and the intracellular iodide was labelled with $^{125}\text{I}^-$ (pH 6.8, 0°C, hematocrit 0.5%). All experiments were performed with the same set of cells. The iodide self-exchange flux was $6 \times 10^{-15}$ mol/cm²·s⁻¹ in the absence of tributyltin.

**Discussion**

The ability of organotin derivatives to exchange anions across biological membranes has previously been demonstrated by Selwyn et al. (1970) and by Motais et al. (1977). The present work represents a quantitative approach to the characterization of the exchange diffusion mechanism, and includes a study of...
the failure of the organometallic carriers to mediate a net flow of anions through the membranes. The latter property is responsible for the tight 1:1 coupling of the anion exchange, making the transport process a perfect example of exchange diffusion, as defined by Ussing (1948).

**The Mechanism of Organotin-Mediated Anion Exchange**

The monovalent organic tin compounds dissociate in aqueous solution according to the scheme (Sillén, 1971):

\[ \text{R}_3\text{SnCl} \rightleftharpoons \text{R}_3\text{Sn}^+ + \text{Cl}^- \]

Our experiments showed that a constant rate of anion exchange was established within the first few seconds after injecting red cells into a medium containing organotin. The equilibration of organotin between medium and cells must, therefore, occur rapidly. We assume that the exchange diffusion of anions occurs through the lipid phase of the membrane by the mechanism illustrated in Fig. 10. The electroneutral ion pair (\(\text{R}_3\text{SnCl}\)) can permeate the membrane much more easily than the cation (\(\text{R}_3\text{Sn}^+\)), which is largely excluded from the hydrophobic core of the membrane. The charges of the two ions in the ion pair are well shielded, because the ions display a perfect electron sharing in a low dielectric environment—comparable to that of a covalent bond (Clark and O'Brien, 1962). We have found no way of evaluating what fraction of the organotin in the surface membranes is present as ion pairs. In studies of artificial lipid membranes (Tosteson and Wieth, 1979) it was not possible to demonstrate any effect of tributyltin on surface potential, suggesting that a major fraction of the tributyltin in the membrane surface is electrically neutral, i.e., forms ion pairs. The ion pairs can shuttle between the two surface regions of the membrane by random walk and perform the exchange diffusion observed in self-exchange and in heteroexchange experiments. The linear increase of anion flux with both organotin concentration (Fig. 2 and Table III) and anion concentration (Fig. 3), shows that the organotin derivatives form 1:1 complexes with the transported anions.

| Electrolyte medium | Chloride self-exchange |
|--------------------|------------------------|
|                    | Rate coefficient \( \text{min}^{-1} \) | \(T_1\) |
| KBr                | 0.92                   | 0.84 |
| KI                 | 1.11                   | 0.62 |
| KSCN               | 1.68                   | 0.64 |
| KF                 | 0.07                   | 9.96 |
| KNO₃               | 0.58                   | 1.34 |

Media contained 2 mM KCl in addition to 165 mM of the potassium salts indicated in the table. The cell suspension contained \(5.2 \times 10^{-4}\) mol tributyltin per liter, hematocrit 0.5%, pH 6.8, 0°C. The rate coefficient of chloride exchange of DIDS-treated control cells equilibrated in a 165 mM KCl medium was 0.77 min\(^{-1}\). The results are mean values from duplicate experiments.
The Role of the Organic Substituting Groups

The potency of a homologous series of organotin derivatives varied with the length (and therefore with the hydrophobicity) of the substituting organic group (Table VIII). The sequence of relative potencies of the homologous series was

**TABLE VII**

**IODIDE SELF-EXCHANGE IN DIDS-TREATED RED CELLS LOADED WITH $^{125}$I**

| Electrolyte medium | Rate coefficient $\text{min}^{-1}$ | $T_1$ $\text{min}$ |
|--------------------|-----------------------------------|-------------------|
| KCl                | 0.34                              | 2.07              |
| KBr                | 0.46                              | 1.52              |
| KSCN               | 1.32                              | 0.53              |
| KF                 | 0.03                              | 24.4              |
| KNO$_3$            | 0.25                              | 2.80              |

Media contained 2 mM KI in addition to 165 mM of the potassium salts indicated in the table. The cell suspension contained 5.2 x 10$^{-7}$ mol tributyltin per liter, hematocrit 0.5%, pH 6.8, 0°C. The rate coefficient of DIDS-treated control cells equilibrated in a 165 mM KI medium was 0.93 min$^{-1}$. The results are mean values from duplicate experiments.

**TABLE VIII**

**DOSE-RESPONSE DATA OF ORGANOTIN-MEDIATED CHLORIDE EXCHANGE IN DIDS-TREATED HUMAN RED CELLS**

| Organotin compound | Concentration of cell suspension mol/liter | Organotin-induced chloride self-exchange $10^{12}$ (mol/cm$^2$·s$^{-1}$) |
|--------------------|------------------------------------------|--------------------------------------------------|
| Trimethyl          | 0.7 x 10$^{-3}$                          | 1.4                                              |
| Triethyl           | 8.3 x 10$^{-4}$                          | 1.7 x 10$^{-4}$                                  |
| Tripropyl          | 6.0 x 10$^{-4}$                          | 1.2 x 10$^{-4}$                                  |
| Triphenyl          | 6.0 x 10$^{-4}$                          | 6.0 x 10$^{-4}$                                  |

pH 6.8, 0°C. The self-exchange flux in the absence of organotin [0.9 x 10$^{-18}$ mol/cm$^2$·s$^{-1}$] was subtracted from the flux found in the presence of organotin. The dose-response results for tributyltin are shown in Fig. 2.

the same as that reported by Selwyn et al. (1970) for the organotin-mediated Cl$^-$/OH$^-$ exchange in red cells. The results of Fig. 2 and of Table VIII show that the self-exchange of chloride increased by a factor of 10$^4$, when methyl groups were replaced by butyl groups.
The Role of the Anion Partner

The exchange flux of anions also depends on the nature of the anion partner of the ion pair (Table II). The equilibrium between ion pairs in electrolyte medium and membrane is established rapidly, and it appears likely that the concentration of tributyltin ion pairs in the membrane is proportional to the concentration of ion pairs in the aqueous phase, which increases through the series \( \text{Cl}^- < \text{Br}^- < \text{I}^- \). The association constants for ion pair formation between triphenyltin and chloride, bromide, or iodide are \( 10^3 \), \( 2 \times 10^3 \), and \( 5 \times 10^3 \) M\(^{-1}\), respectively.

\[
\begin{array}{c}
\text{Cl}^- \\
\text{Br}^- \\
\text{I}^-
\end{array}
\]

Figure 9. The rate of \( ^{42}\text{K}^+ \) efflux into media with varying potassium concentrations (NaCl + KCl = 150 mM). The potassium concentrations are indicated on the efflux curves. Experiments were performed under four sets of conditions in untreated red cells (left hand panels) and in DIDS-treated cells (right hand panels), in the absence (upper panels) and in the presence (lower panels) of tributyltin (5 x 10\(^{-6}\) mol per liter cell suspension, hematocrit 0.5%, 38°C). The pH of the unbuffered media was 6.6. It changed maximally 0.05 during the experiments, showing that there was no significant flux of hydrogen or of hydroxyl ions. The membrane permeabilities to electrodiffusion of chloride ions under the four experimental conditions were calculated from these results as described in Methods. The values are shown in Table IX.

(Sillén, 1971). The relative affinities of the anions for ion pair formation correspond to the observed relative rates of halide transport by tributyltin (Table II). It is, therefore, possible that the magnitude of halide transport simply is determined by the concentration of ion pairs in the membrane phase which, in turn, in the cases of chloride, bromide, and iodide, bears a simple relation to the concentration of ion pairs in the aqueous phase. This view is supported by our finding that both organotin partitioning and anion transport were found to be four to five times larger in iodide than in chloride media. This simple relationship does not hold for fluoride. Fluoride ions have a high affinity for organotin (Sillén, 1971), but fluoride was not transported through the membrane by tributyltin (Table II), and fluoride did not act as an exchange
partner in the heteroexchange experiments (Tables IV and V). Nevertheless, fluoride was a strong inhibitor of chloride and iodide exchange (Tables VI and VII). The data on fluoride transport do not fit with the idea that a high concentration of fluoride ion pairs in the aqueous phase should result in a high concentration of organotin in the membrane. A possible explanation for the apparent discrepancy is provided by Cotton and Wilkinson (1966, p. 477), who reported that fluoride ions form water-soluble long-chained polyionic complexes with organotin derivatives. We, therefore, suggest that partitioning of organotin into the membrane is decreased, because the polyionic fluoride complexes cannot be adsorbed into the membrane.

Hydroxyl ions have an extremely high affinity for organotin. The association constant for triphenylhydroxide is $10^{9.2}$ M$^{-1}$, six orders of magnitude higher
than the affinity for chloride, bromide, and iodide (Sillén, 1971). It is, therefore, not surprising that hydroxyl ions exert a strong inhibitory effect on organotin-mediated anion exchange. The self-exchange of chloride was inhibited, when pH increased above 6 (Fig. 4), and the results of Fig. 4 fitted well with an apparent dissociation constant for tributyltin hydroxide of $10^{-8}$ M at a chloride concentration of 165 mM. In other words, chloride exchange was inhibited 50\% at a hydroxyl ion concentration ($10^{-5}$ M at pH 6.9), which is about $10^7$ times smaller than the chloride concentration. Studies of the ability of organotin to equilibrate hydroxyl ions across the membrane have clearly demonstrated that OH$^-$ exchanges readily with chloride (Selwyn et al., 1970; Aubert and Motais, 1975; Fig. 5 of this article). The slow response time of the glass electrode does not permit a determination of the initial rate of hydroxyl transport in the experiment shown in Fig. 5 B. A very rough estimate of the apparent hydroxyl permeability induced by $3.8 \times 10^{-6}$ mol tributyltin per liter cell suspension is a value of $\sim 10^{-3}$ cm $\times$ s$^{-1}$, calculated by assuming that half of the total amount of hydroxyl ion transferred from medium to cells ($1.8 \times 10^{-9}$ mol/cm$^2$) is transferred during the 1st s after a sudden increase of the extracellular hydroxyl ion concentration by $2.5 \times 10^{-4}$ M.

No evidence of saturation of transport was found, when the chloride concentration was increased to 600 mM in red cells (Fig. 3) or to 900 mM in ghosts. We cannot present a quantitative explanation for this low apparent affinity. Although the association constant of tributyltin chloride in aqueous solution is not known, it is likely to be somewhat larger than the previously cited value of $10^3$ M$^{-1}$ for triphenyltin chloride, because the tendency of organotin derivatives to form ion pairs with halides increase with the lipophilicity of the organic groups (Sillén, 1971). Therefore, it was surprising that chloride fluxes did not show any tendency to saturate (Fig. 3). It must be noted, however, that...
several factors will tend to increase the concentration of tributyltin chloride in the membranes, when chloride concentration is increased. Increased formation of chloride ion pairs in the aqueous phase leads to an increased adsorption of ion pairs into the membranes. Moreover, the displacement of hydroxyl groups from tributyltin hydroxide at pH 6.8 will increase the amount of halide ion pairs by competition, contrasting the inhibitory effect of hydroxyl ions on chloride transport (Fig. 4). It is, furthermore, possible that the true association constant of tributyltin chloride of membrane-bound tributyltin differ from that found in the electrolyte solution, e.g., if the ion pair formation takes place in a microenvironment at the membrane surface that is characterized by a dielectric constant that is larger than that of the bulk solution. All these possible effects tend to lower the apparent affinity, as it can be evaluated by flux measurements.

We were not able to demonstrate clear evidence of competition between chloride and iodide for transport, when the self-exchange was studied at a concentration of 2 mM in the presence of 165 mM of the other anion (Tables VI and VII). The rate of iodide exchange decreased by a factor of three (from 0.9 to 0.3 min⁻¹), when most of the iodide was replaced with chloride (Table VII). This observation suggests that the decreased partitioning of tributyltin into the membrane in the presence of chloride is the cause of the decreased rate of iodide transfer. However, the simple relation between partitioning and transport did not hold for chloride: The rate of chloride exchange increased only by 40% (Table VI) in the presence of iodide, where the amount of tributyltin in the membrane is increased fourfold. In series of experiments where the effect of iodide substitution was studied over the whole concentration range between 0 and 165 mM, it was found that the small increase of the rate of chloride exchange shown in Table VI is significant. It is likely that the situation is complex, because the presence of iodide decreases the fractional amount of chloride ions forming ion pairs with organotin in the aqueous solution, while at the same time membrane transport of chloride is favored by the larger amount of tributyltin in the membrane, maybe because chloride transport through the membrane is facilitated by an exchange reaction of the type:

\[ \text{R}_3\text{SnI} + \text{Cl}^- \rightleftharpoons \text{R}_3\text{SnCl} + \text{I}^- . \]

**Adsorption of Tributyltin to the Membranes**

We found no difference between the chloride self-exchange induced by tributyltin in DIDS-treated red cells or ghosts (Table I), suggesting that organotin was not bound to other cellular constituents than the membranes. This conclusion was further substantiated by the finding that the rate of self-exchange was not affected by addition of a membrane-free lysate to the medium. At a constant hematocrit, anion self-exchange in DIDS-treated cells was a linear function of the amount of organotin present in the medium, when the concentrations were below \( \sim 5 \times 10^{-6} \) mol/liter suspension (Fig. 2, Table III), showing that the adsorption coefficient is constant within a wide concentration range. At a hematocrit of 0.5% the concentration of tributyltin in the medium (\( T_m \) of Eq. 4) is only 50-80% of the concentration in the suspension (\( T_r \) of Eq. 4) in iodide and chloride media, because a considerable fraction is taken up by the cells (\( \alpha_1 \sim \))
200, $\alpha_{Cl} \sim 50$). For cells suspended in a chloride medium, the number of tributyltin molecules in the membrane surfaces at a concentration ($T_m$) of $4 \times 10^{-9}$ M calculated by means of Eq. 6a was $4 \times 10^{12}$ tributyltin molecules per cm$^2$. This membrane density is equal to $1.1 \times 10^7$ organotin molecules per cell, approximately one organotin molecule per 20 phospholipid molecules (Bar et al., 1966). If evenly distributed in the membrane, the mean spacing distance between the tin molecules is $\sim 50$Å. The membrane becomes apparently saturated with organotin if the number of molecules is doubled (cf. Fig. 2). A density of $10^{13}$ per cm$^2$ corresponds to a spacing of about 30Å.

**The Turnover Number of Organotin-Mediated Anion Exchange**

It is only possible to relate the anion transport to the total amount of tributyltin present in the membrane ($T_a$). At a concentration in the medium ($T_m$) of $4 \times 10^{-6}$ M, the density of tin molecules in the membrane is $4 \times 10^{12}$ mol·cm$^{-2}$·s$^{-1}$. The organotin-facilitated chloride exchange was $10^{-10}$ mol·cm$^{-2}$·s$^{-1}$ at 0°C (Table I), and increased 22 and 90 times at 25 and 38°C, respectively (Fig. 6). The overall turnover numbers at 0, 25, and 38°C are, therefore, 15, 325, and 1350 s$^{-1}$. These rates are, of course, not measures of the transfer rates of ion pairs through the membrane, since the true turnover number cannot be determined as long as we do not know the degree of saturation of the membrane-bound carrier. According to the results of Fig. 4, chloride self-exchange was inhibited by $\sim 45\%$ at pH 6.8. Therefore, the true turnover number for chloride must be at least twice as big as the overall value, even if it is assumed that all tributyltin in the membrane is present as ion pairs: $R_3SnCl$ and $R_3SnOH$.

**Lack of Effect of Tributyltin on the $p_{cl}$ of Red Cells**

The organotin-mediated chloride transport was found to be electrically silent. $P_{cl}$ was determined by the valinomycin method both in normal and in DIDS-treated red cells (Fig. 9). An example of our experimental fit is shown in Fig. 11. The $P_{cl}$ found by us in intact red cells (Table IX) agrees with values reported by Hunter (1971, 1977) and by Knauf et al. (1977). Also, our observation that DIDS treatment of red cells (which reduces natural chloride exchange by 99.6%) caused a reduction of $P_{cl}$ of 70%, is in accordance with the observation of Knauf et al. (1977).

There was no flux of hydroxyl or hydrogen ions under the four sets of experimental conditions employed for the determination of $P_{cl}$ (Fig. 9). This was clear, because the pH of the unbuffered media did not change by more than 0.03-0.05 units even in the low potassium media, where the change of membrane potential causes a considerable disequilibrium of hydrogen and of hydroxyl ions across the membrane. The stability of extracellular pH was also observed in the presence of tributyltin. This shows that a chloride-hydroxyl exchange cannot be induced by changing the membrane potential, underlining the electroneutral nature of the organotin-mediated anion transfer.

The results of Table IX show that tributyltin did not cause any detectable increase of $P_{cl}$ in normal or in DIDS-treated red cells. However, the determination of $P_{cl}$ by the valinomycin method is indirect, making it possible that a
small increase of $P_{cl}^{0}$ might not have been detected. It is obvious from inspection of Fig. 11 that, although the differences found in the rate of potassium fluxes at low potassium concentrations in normal and in DIDS-treated cells are very reproducible (cf. Table IX), the indirect method could fail to detect a minor effect of tributyltin on the membrane permeability to electrodifussion of chloride ions. Therefore, we have also investigated the discrepancy between anion exchange and membrane conductance in artificial lipid membranes, where both conductance and tracer exchange can be determined directly with a high degree of precision. These studies, which confirmed that tributyltin mediates an electrically silent exchange diffusion of anions through thin lipid membranes, are reported in the following article (Tosteson and Wieth, 1979).

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