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Movement of Thallous Ion Across the Ascites Cell Membrane

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Summary. The movement of thallous ion (Tl^+) across the ascites cell membrane has been characterized. Analogous to previous findings for $^{86}Rb^+$ (used as a tracer for K^+), $^{204}Tl^+$-influx could be resolved into three components: a ouabain-inhibitable "pump" flux, a passive flux, and a furosemide- or NO_3^--sensitive "exchange" flux. Although Tl^+ moved approximately nine times faster across the membrane than K^+, the pump/leak ratio was equal for the two ions. This suggests that the pump- and leak-pathways share a common rate-limiting step. The exchange mechanism was shown to provide close coupling between the Tl^+- and K^+-gradients.

Thallous ion (Tl^+) has a crystal radius similar to that of K^+, and has been shown to substitute for K^+ in a number of enzymes (Williams, 1970). In membrane-bound systems the behavior of Tl^+ as compared to that of K^+ is determined by the relative rates with which the two ions participate in pump- and leak-processes. Extensive evidence for several cell types indicates that Tl^+ substitutes for K^+ both at the level of the Na^+/K^+ pump and at that of passive leak processes (e.g., red blood cells, Skulskii, Manninen & Järnefelt, 1973; squid axons, Landowne, 1975; frog nerve, Hille, 1973): On the one hand, Tl^+ generally appears to have a higher affinity than K^+ for the Na^+/K^+ ATPase; on the other hand, Tl^+ is accepted either more or less readily than K^+ by the various leak pathways, depending upon the conditions (influx or efflux; resting *vs.* stimulated ion flux).

In certain bacteria the passage of Tl^+ across the plasma membrane is determined largely by its lipid solubility, making it possible to estimate the membrane potential from the distribution of $^{204}Tl^+$ (Bakker, 1978). We set out to characterize Tl^+ movements in ascites cells in the hope that this ion would prove to be a useful probe of the membrane potential

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in those cells. However, in the course of our experiments it became evident that Tl^+ possessed not only a rapid passive transmembrane flux compared to K^+, but also substituted for K^+ with a comparably higher affinity at the level of the Na^+/K^+ pump and the K^+ exchange diffusion system.

Materials and Methods

Ascites cells (a nonspecific strain kindly donated by Dr. J. Molnar from the University of Illinois) were obtained from cultures propagated in the abdominal cavities of Swiss Webster mice for 6 to 12 days. The cells were washed three times with HEPES-buffered mammalian Ringer. Depending upon the experiment, the solution contained as main anion either Cl^- (this will be referred to as "Cl^--saline") or NO_3^- ("NO_3^--saline"). The cell concentration was determined by spinning duplicate samples for 10 min in capillary tubes at 1,000 rpm. Extracellular (^{14}C-sucrose-accessible) space under these conditions was 20%; dry weight, obtained after dehydration at 100 °C overnight, was 16%. Results are expressed per ml cell water; to this end, intracellular H_2O in all cases has been taken as (100 − 20 − 16 =) 64% of the original total pellet volume, without considering possible volume changes under certain conditions (addition of ouabain; incubation at 0 °C).

Washed cells were suspended in the appropriate incubation solution at a final concentration of $1^1/_2$–2%. Experiments were performed in a shaking water bath at 37 °C. In some cases DNAse (final concentration, 20–50 μg/ml) was added to prevent cell aggregation. This had no effect on the steady-state ion content. $^{204}Tl^+$ (sulfate salt, Amersham) and $^{86}Rb^+$ (chloride, New England Nuclear) were added when indicated, at final activities of 5–20 nCi/ml ($^{86}Rb^+$ acts as a tracer for K^+ in ascites cells: Mills & Tupper, 1975).

Uptake was determined at the indicated times by spinning down duplicate 3-ml samples in 5-ml disposable pyrex tubes for 1 min at 1,500 rpm. The supernatant was decanted and saved, and the tubes were left inverted on filter paper for some minutes and carefully swabbed to remove any remaining supernatant. Pellets were resuspended in 0.5 ml H_2O, and 0.5 ml 10% TCA was added. After centrifugation, 0.4 ml aliquots of the supernatant were taken for the determination of cell radioactivity, and, where indicated, K^+ content. For determination of radioactivity, the aliquots were counted in 4 ml Budget Solve (R.P.I.) in a Picker Liquimat scintillation counter. For K^+ content, the samples were diluted so that the final solution contained 5% TCA, 10% isopropanol, and 15 mM LiCl, and measured on a Beckman Model B flame photometer. Radioactivity and K^+ content were determined in an identical way in suitably-diluted samples of the supernatant.

In the experiments of Fig. 3 and Table 1, data were corrected for the contribution of extracellular isotope content by subtracting the value obtained by extrapolation to zero time. For the other experiments, no such correction was made, i.e., all radioactivity or K^+ associated with the cells was considered to be intracellular. From the zero time extrapolation mentioned above this was calculated to lead to maximal errors of 10%.

Points shown in the figures are average of duplicates; duplicates were equal within 5%.

Experimental Setup

It should be emphasized that some experiments were designed to measure unidirectional-, and others to measure net fluxes. For the experiments of Fig. 3 and Table 1, cells were pre-equilibrated with 10 μM cold Tl-acetate and 4 mM K^+. Unidirectional fluxes of Tl^+ or K^+ were determined as tracer fluxes of $^{204}Tl^+$ or $^{86}Rb^+$, respectively, and expressed

as changes in activity ratio with time; the activity ratio is given by the respective radioactivity per ml cell water over that per ml supernatant.

In the other experiments, cells were pre-equilibrated with $^{204}Tl^+$ plus 10 μM cold Tl-acetate. Net ion movements were determined from the tracer flux of $^{204}Tl^+$ or the change in analytical K^+ content, respectively, and expressed as changes in concentration ratios; the concentration ratio is given by the respective ion content per ml cell water over that per ml supernatant. In the figures, the concentration ratios of Tl^+ and K^+ are denoted as R_{Tl} and R_K, respectively.

Solutions and Chemicals

Cl^--saline (pH 7.4–7.6) contained (in mM): NaCl, 80; KCl, 4 or as indicated; $NaHCO_3$, 25; Na_2HPO_4, 0.6; NaH_2PO_4, 1; $CaCl_2$, 2; $MgCl_2$, 1; HEPES, 50 neutralized with NaOH, 25; and glucose, 30. In NO_3^--saline, NaCl was replaced by 80 mM $NaNO_3$, and KCl by 5 mM KOH-HEPES. In the experiments of Fig. 3 and Table 1, simplified Ringer solutions were used, containing (in mM): NaCl or $NaNO_3$, 100; HEPES, 50; neutralized with NaOH, 20, and KOH, 5; $CaCl_2$, 1; $MgSO_4$, 1; and glucose, 20.

Ouabain, DNAse (deoxyribonuclease I) and valinomycin were purchased from Sigma. Crystalline furosemide was kindly supplied by Dr. Dettelbach from Hoechst. Valinomycin was dissolved in absolute ethanol (stock solution: 10 mg/ml); where applicable, an identical volume of ethanol ($\leqq$0.4% vol/vol) was added to controls.

Results

Tl^+ at a concentration of 10 μM equilibrated rapidly in ascites cells, reaching a stable distribution within 30 min (Fig. 1). The concentration ratio after 30 min had a value of 37 ± 3 (SD) in three different experiments. This ratio was independent (within SD) of the Tl^+-concentration from 1 to 100 μM but decreased to approximately 25 at 1 mM (not shown).

To test the assumption that Tl^+ might distribute passively according to the membrane potential, we measured the Tl^+ accumulation as a function of the K^+ concentration gradient; the latter was varied by changing the external K^+ concentration. Burckhardt (1977), by use of the fluorescent dye DiO-C_6-(3), has shown that the membrane potential in ascites cells increased with the logarithm of the K^+ concentration ratio, but levelled off at higher ratios (lower external K^+ concentrations); this presumably is due to the depolarizing contribution of Na^+. In the presence of the specific K^+ ionophore valinomycin, the membrane potential was elevated to the K^+ equilibrium potential even at higher K^+ concentration ratios. If Tl^+ would distribute passively according to the membrane potential, a double-logarithmic plot of the Tl^+ concentration ratio against the K^+ concentration ratio would yield a bending-off curve in the absence and a straight line with slope one in the presence of valinomycin (*compare* Burckhardt, 1977: Figs. 6 and 7). Actually, we

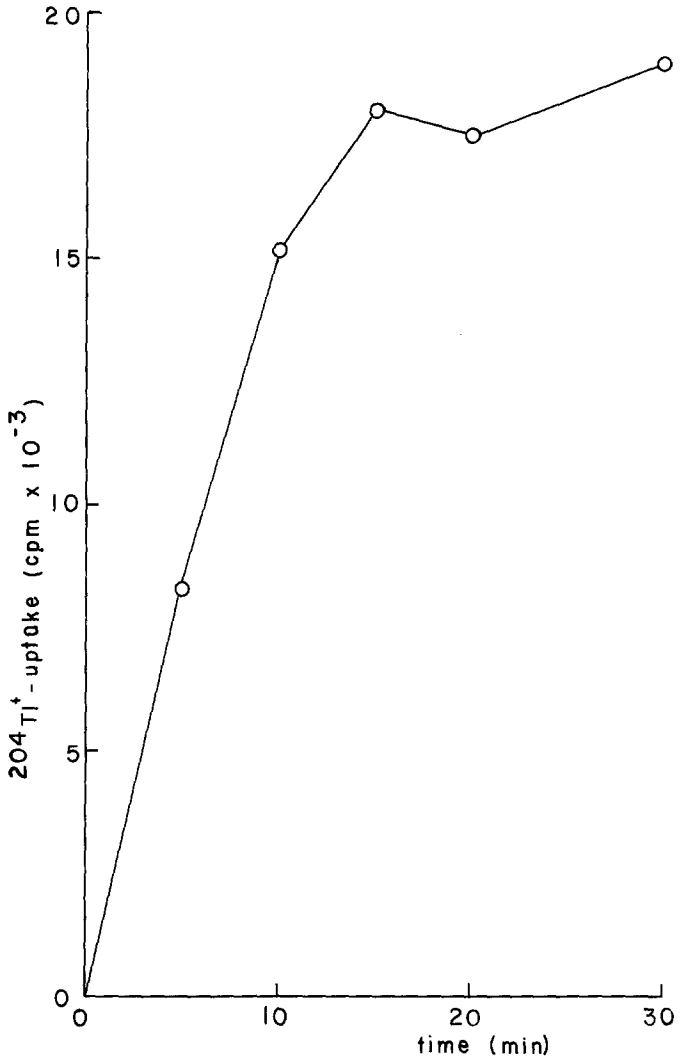

Fig. 1. Uptake of Tl^+ in ascites tumor cells. Cells were preincubated at 37 °C for $1^1/_2$ hr in Cl^--saline containing 5 mM K^+. At zero time $^{204}Tl^+$ plus 10 μM cold Tl-acetate was added, and the increase in cellular radioactivity with time was followed as described in *Methods*. Points are average of duplicate determinations; duplicates were equal within 5%

obtained (Fig. 2) a straight-line relationship with a slope of one both in the absence (open circles) and presence (closed squares) of valinomycin. Also when furosemide was added to block K^+ self-exchange, the same relationship was found (open triangles). The ordinate intercept in all cases corresponded to a Tl^+ concentration ratio of 1.6. The fact that the Tl^+ concentration ratio did not bend off in the absence of valinomycin

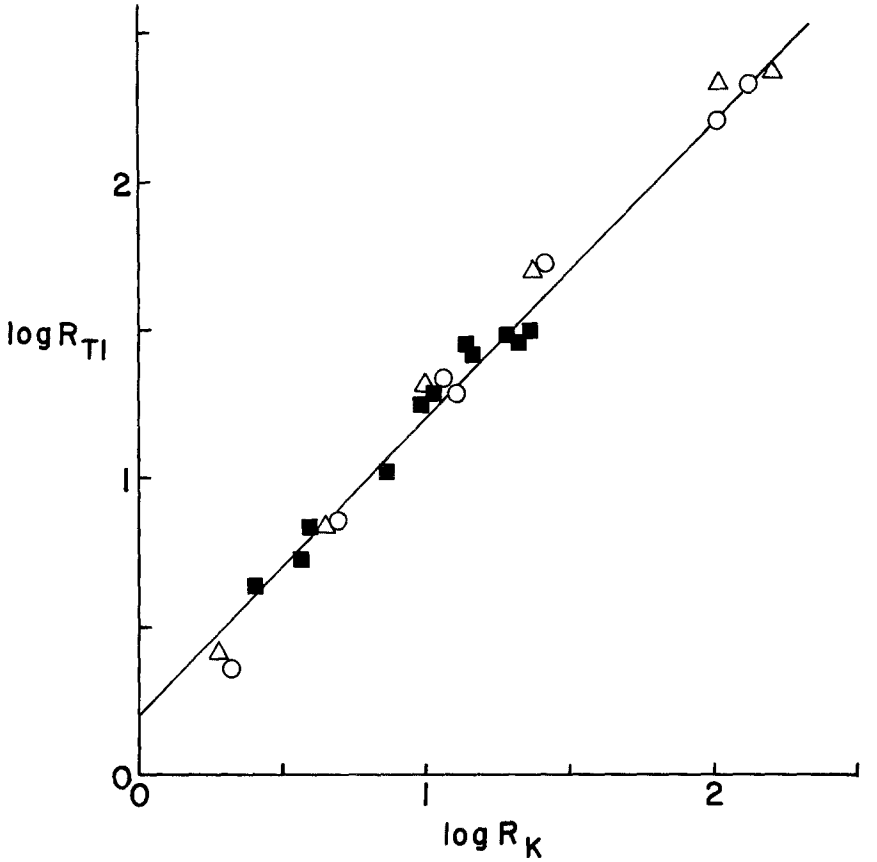

Fig. 2. Tl^+ concentration ratio as a function of K^+ concentration ratio in the absence or presence of inhibitors. Cells were washed in K^+-free Cl^--saline and incubated for 1 hr with different K^+-concentrations (0.4–85 mM). Results of several experiments were pooled. $^{204}Tl^+$ plus 10 μM cold Tl^- acetate and inhibitors were added at zero time. Duplicate samples for determination of cellular radioactivity and K^+ content were taken 20 and 30 min thereafter, and the values averaged; duplicates and 20 and 30 min values were equal within 10%. Cellular K^+ concentrations ranged from 80–190 mM. ○, no additions; △, furosemide, 1 mM; ■, ouabain, 1.2 mM, plus valinomycin, 20 μg/ml

(Fig. 2) suggested that the distribution of Tl^+ was not purely passive, but rather followed that of K^+.

To determine what pathways in addition to passive leakage contributed to Tl^+ entry, we analyzed unidirectional Tl^+-influx more closely (Fig. 3). In Cl^--saline, ouabain suppressed the initial rate of influx of the control by 27%, furosemide by 47%, and the two inhibitors together by 93%. In NO_3^--saline, irrespective of the absence or presence of furosemide, rates were equal to the corresponding rates in Cl^--saline plus furosemide. These results were qualitatively similar to those obtained previ-

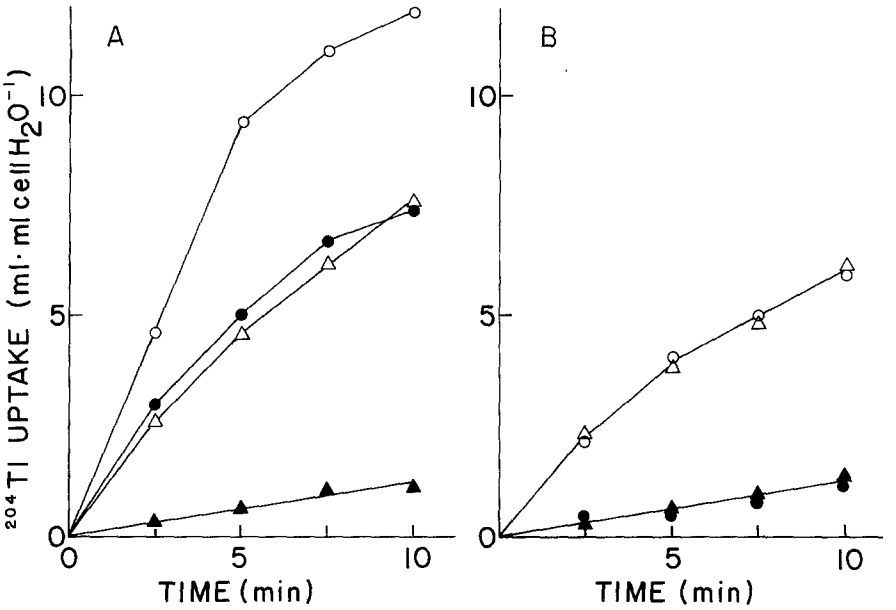

Fig. 3. Effect of inhibitors and NO_3^- on $^{204}Tl^+$ influx. Cells were preincubated at 37 °C for $1^1/_2$ hr in the respective salines with 4 mM K^+ and 10 μM cold Tl^+. At zero time the inhibitors and $^{204}Tl^+$ were added and influx was determined and expressed as outlined in *Methods*. Points are average of duplicates; duplicates were equal within 5%. ○, no additions; △, furosemide, 1 mM; ●, ouabain, 1.2 mM; ▲, furosemide, 1 mM, plus ouabain, 1.2 mM. (*A*): Cl^--saline; (*B*): NO_3^--saline

Table 1. Effect of inhibitors and NO_3^- on the rate constants of $^{204}Tl^+$- and $^{86}Rb^+$-influx

Major anion	$^{204}Tl^+$-influx k_i (min^{-1})			$^{86}Rb^+$-influx k_i (min^{-1})		
	Cl^-	NO_3^-	Furosemide- or NO_3^--sensitive component	Cl^-	NO_3^-	Furosemide- or NO_3^--sensitive component
Control	1.9	0.9	0.9	0.21	0.10	0.10
+furosemide (1 mM)	1.0	0.9		0.11	0.10	
+ouabain (1.2 mM)	1.2	0.14	1.1	0.20	0.016	0.18
+furosemide (1 mM) and ouabain (1.2 mM)	0.14	0.14		0.016	0.016	

Influx rate constants (k_i) were calculated from the initial rate of uptake of $^{204}Tl^+$ and $^{86}Rb^+$. The initial rate was estimated from the extrapolated ordinate intercept and the first time point of the uptake curves in Fig. 3 and similar ones for $^{86}Rb^+$-uptake. Results are for one representative experiment; in two other experiments quantitatively comparable results were obtained (maximal scattering, 20%).

ously for $^{86}Rb^+$-influx (Tupper, 1975; Bakker-Grunwald, 1978). In these previous experiments the results were interpreted to mean that K^+ enters ascites cells by three pathways: a ouabain-inhibitable pump, furosemide- or NO_3^--sensitive exchange diffusion, and a passive leak.

The relative rates of $^{204}Tl^+$- and $^{86}Rb^+$-influx in both Cl^-- and NO_3^--saline are compared in Table 1. In view of the fact that Tl^+ and K^+ were present at very disproportionate concentrations (10 μM *vs.* 5 mM), results are expressed as increases in activity ratio with time, i.e., in a unit formally corresponding to an influx rate constant rather than as absolute rates. The rate constants for $^{204}Tl^+$-influx were all approximately 9 times higher than those for $^{86}Rb^+$-influx, except for that in Cl^--saline in the presence of ouabain, which was relatively somewhat smaller (6 times that of $^{86}Rb^+$).

The results shown in Fig. 3 and Table 1 indicated that, like $^{86}Rb^+$-influx, $^{204}Tl^+$-influx consisted of three components: a ouabain-inhibitable pump flux (difference in influx in the presence and absence of ouabain in either Cl^--saline plus furosemide or NO_3^--saline), a passive flux (ouabain-insensitive flux in either Cl^--saline plus furosemide or NO_3^--saline), and an exchange flux (difference between influx in Cl^--saline and that in either Cl^--saline plus furosemide or NO_3^--saline). Thus, like K^+, Tl^+ appeared to be accepted by both the Na^+/K^+ pump and a furosemide- or NO_3^--sensitive exchange mechanism. The next point to establish was whether Tl^+ and K^+ shared the same exchange mechanism. If so, this would account for a furosemide- or NO_3^--sensitive coupling between the Tl^+- and K^+-gradients. However, under steady-state conditions a close coupling would be expected even in the absence of Tl^+/K^+ exchange because of the similarity in pump/leak ratio for the two ions (Table 1). In the following experiments we therefore attempted to uncouple the gradients on a transient base.

For the experiment shown in Fig. 4, cells were preincubated in Cl^--saline to obtain high Tl^+- and K^+-concentration ratios. Then ouabain was added, and the decay of the Tl^+- and K^+-concentration ratios followed as a function of time both in the presence and absence of furosemide. It should be emphasized that this experimental setup allowed for the measurement of *net* rather than unidirectional ion fluxes (*see also Methods*). The K^+ content of the cells decreased both in the presence and absence of furosemide from 115 to 82 mM in 1 hr, indicating that furosemide did not influence the passive leak rate of K^+. In the absence of furosemide, the ratio of R_{Tl} over R_K was maintained at 1.2; in the presence of furosemide the Tl^+ concentration ratio decayed significantly

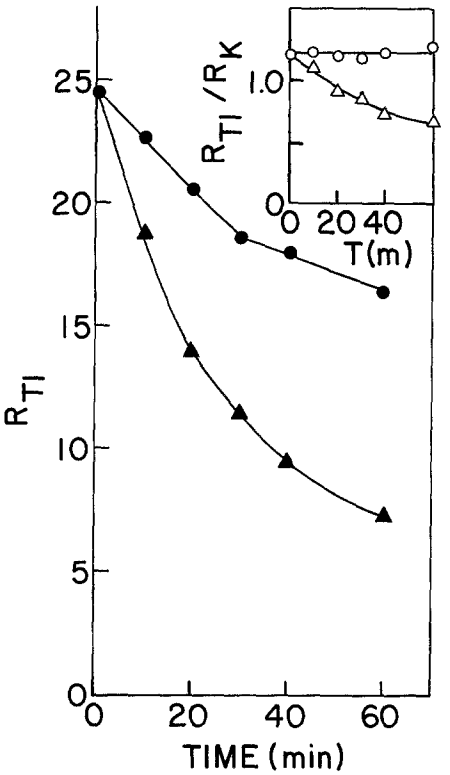

Fig. 4. Ouabain-induced decay of the Tl^+ concentration ratio in the absence or presence of furosemide; comparison with decay of K^+ concentration ratio. Cells were preincubated at 37 °C for $1^1/_2$ hr in Cl^--saline containing 4 mM K^+. During the last 20 min, $^{204}Tl^+$ plus 10 μM cold Tl^+ were present. Ouabain (1.2 mM) with or without furosemide (1 mM) was added at zero time, and cellular Tl^+- and K^+-content were followed in time and expressed as described in *Methods*. K^+ content decreased in 1 hr, independent of the absence or presence of furosemide, from 115 to 82 mM. Determinations were performed in duplicates; all duplicates were equal within 5%. Insert: ratio of Tl^+- and K^+ concentration ratios as a function of time after addition of ouabain. ○, ●: no furosemide; △, ▲: furosemide present

faster, and the ratio of R_{Tl} over R_K dropped to 0.7 in 1 hr (Fig. 4, insert). Similar results were obtained in an experiment in which Cl^- plus furosemide was replaced by NO_3^--saline (not shown; absolute values of R_{Tl} and R_K were equal within 20%, and their ratio within 5%, to those shown in Fig. 4). Thus, the close coupling between the two ion gradients in Cl^--saline apparently was dependent upon the action of a common furosemide- or NO_3^--sensitive exchange mechanism.

In a different experiment (Fig. 5) cells were suspended in either Cl^--saline or NO_3^--saline, and incubated at 0 °C for 90 min in order to

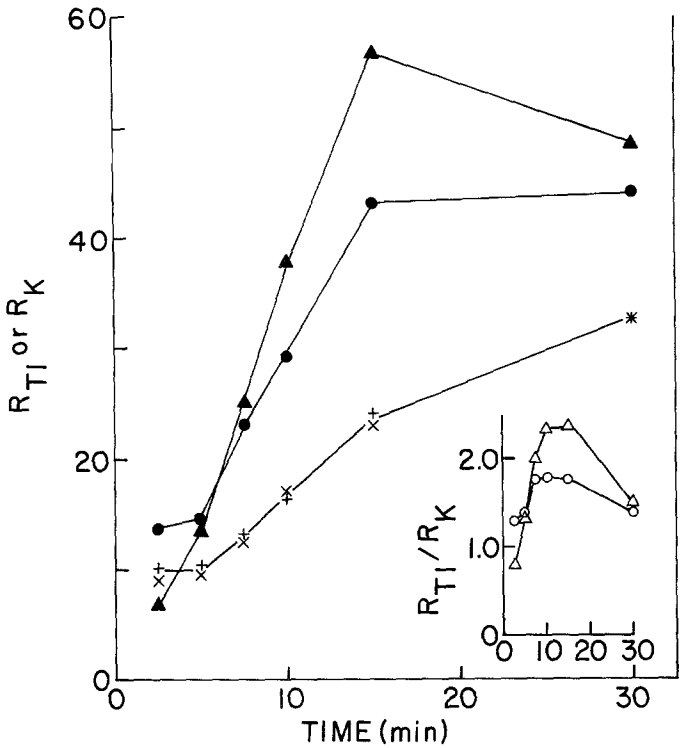

Fig. 5. Accumulation of Tl^+ and K^+ in NO_3^--saline and Cl^--saline after cold treatment of cells. Cells were incubated at 0 °C in K^+-free Cl^-- or NO_3^--saline for $1^1/_2$ hr to deplete them of internal K^+. At zero time, 4 mM KOH neutralized with HEPES (pH 7.5) was added together with $^{204}Tl^+$ plus 10 μM cold Tl^+, and the cells were transferred to 37 °C. During the course of the experiment intracellular K^+ increased in both saline systems from 36 to 111 mM; extracellular K^+ decreased from 4.0 to 3.4 mM (average of duplicates, equal within 5%). ○, ●, +: Cl^--saline; △, ▲, ×: NO_3^--saline; ●, ▲: Tl^+ concentration ratio; +, ×: K^+ concentration ratio

deplete them of their internal K^+. At zero time they were transferred to a 37 °C water bath and Tl^+ was added. The time course of K^+- and Tl^+ uptake was subsequently followed in both saline systems. That of K^+ uptake was identical in Cl^-- and NO_3^--saline: this shows that NO_3^- did not interfere with active K^+ pumping. Tl^+ uptake in Cl^--saline was slower than in NO_3^--saline. The insert in Fig. 5 shows that the ratio of R_{Tl} over R_K reached a maximum of 2.4 in NO_3^--saline, but was kept closer to the final steady-state value (approximately 1.6) in Cl^--saline. Similar results were obtained when NO_3^--saline was replaced by Cl^--saline plus 1 mM furosemide (not shown; absolute values of R_{Tl} and R_K were equal within 15%, and their ratio within 5%, to those of Fig. 5). Again, these results point to a coupling between the Tl^+-

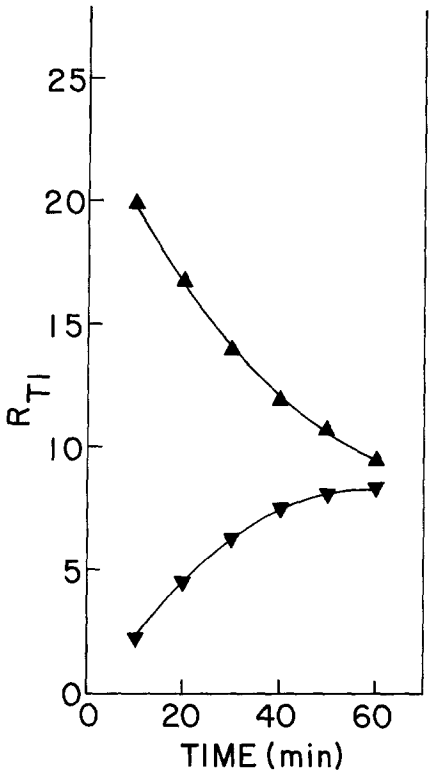

Fig. 6. Uptake and release of Tl^+ in NO_3^--saline after addition of ouabain. Cells were preincubated for $1^1/_2$ hr. $^{204}Tl^+$ plus 10 μM cold Tl^+ was either present during preincubation or added at zero time, together with ouabain, 1.2 mM. The Tl^+ concentration ratio was followed in time as described in *Methods*. ▲, Tl^+ present during preincubation; ▼, Tl^+ added at zero time

and K^+-gradients in Cl^--saline, which must be ascribed to a common exchange mechanism. The "overshoot" of R_{Tl} in NO_3^--saline, corresponding to a maximal Tl^+ concentration ratio of 57, indicates that during the initial uptake phase the pump/leak ratio for Tl^+ was elevated considerably above the steady-state value. This presumably is due both to enhanced activity of the Na^+/K^+ pump and to increased passive influx caused by an elevated membrane potential.

At this point it seemed obvious that Tl^+ in ascites cells would only distribute passively according to the membrane potential if both pump- and exhange pathways were blocked. To obtain an estimate of how long it would take the Tl^+ ion to equilibrate under those conditions we incubated cells in NO_3^--saline, and added $^{204}Tl^+$ (plus 10 μM cold Tl^+) either beforehand or at zero time together with ouabain. The two curves did not quite converge after 1 hr (Fig. 6).

Discussion

In our search for a convenient probe for the membrane potential in ascites cells we investigated the possibility that in those cells, like in certain bacteria (Bakker, 1978), Tl^+ might move across the plasma membrane predominantly by passive leak; in that case it would be possible to calculate the membrane potential directly from the distribution of $^{204}Tl^+$. However, this was excluded by the experiment of Fig. 2: under conditions (at high K^+ concentration ratios in the absence of valinomycin) where the membrane potential was shown to be significantly lower than the K^+ equilibrium potential (Burckhardt, 1977), the distribution of Tl^+ still followed that of K^+. Based on the evidence presented in this paper, this can now be explained as follows: (i) although Tl^+ moves across the membrane considerably faster than K^+, the steady-state pump/leak ratios for the two ions are similar; (ii) the K^+ self-exchange system accepts Tl^+. Thus, even outside the steady state, when the Na^+/K^+ pump is either blocked by ouabain or stimulated by preincubation at 0 °C, K^+/Tl^+ exchange will tend to equilibrate the two ion gradients.

In conclusion, our data suggest that the only way to allow for a passive equilibration of Tl^+ is by blocking both pump- and exchange activities. But as can be seen from Fig. 6, this equilibration is too slow to make it a useful probe for the potential in ascites cells.

Nevertheless, two interesting points need some comment. First, the rate constant for both the pump- and leak-influx were nine times higher for Tl^+ than for K^+; this suggests that pump- and leak-pathway share a common rate-limiting step. A similar conclusion was drawn by Landowne (1975) based on his data on Tl^+-fluxes in squid axon. A close connection between the two pathways has also been inferred by Tosteson and Hoffman (1960) based on a comparison of ion fluxes in HK and LK red blood cells. The exchange rate for $^{204}Tl^+$ was also 6 to 9 times that for $^{86}Rb^+$. Other data, such as the complementarity between pump- and exchange activities found under certain conditions (Mills & Tupper, 1976) also appear to indicate that the pump- and exchange pathways share common features.

Second, to our knowledge the evidence presented here is the first suggesting that Tl^+ is accepted by the K^+ exchange system. This evidence consists of the observation (Figs. 4 and 5) that under transient conditions the exchange system provided for a furosemide- or NO_3^--sensitive coupling between the Tl^+- and K^+-gradients. In the experiment of Fig. 4, the common exchange mechanism effectively acted as a secondary K^+-

driven Tl^+-pump-, in that of Fig. 5, as a Tl^+-driven K^+-pump of the antiport type (Mitchell, 1968). In neither case were effects on the Tl^+-gradient reflected in the K^+-gradient because of the 400-fold concentration difference between the two ions.

From the ordinate intercept in Fig. 2, the distributions of Tl^+ and K^+ correlated within a factor 1.6. A similar ratio was found in the experiments of Figs. 4 and 5. It probably arises from reversible partitioning of Tl^+ in, or reversible binding at, some subcellular compartment. Energy-dependent uptake by the mitochondria can be excluded, since valinomycin at a concentration that completely uncouples the mitochondria (Burckhardt, 1977) had no effect on the ordinate intercept in Fig. 2.

In this paper, $^{204}Tl^+$ was used as a tracer for both unidirectional and net fluxes of Tl^+. In view of the limited solubility of TlCl, the initital extracellular concentration of Tl^+ usually was kept at 10 μM. Ions at low concentrations tend to engage in irreversible binding and chemical reactions. That these processes were negligible under our conditions follows from the fact that the Tl^+ concentration ratio was independent of the Tl^+ concentration in the range from 1 to 100 μM. Another point of consideration is the difference in the degree of dissociation of various Tl^+-salts. From the dissociation constants given by Sillén and Martell (1964) it can be estimated that at a total Tl^+ concentration of 10 μM about 70% of the Tl^+ is present as the free ion in Cl^--saline *vs.* 80% in NO_3^--saline. Moreover, results obtained in Cl^--saline plus furosemide were always quantitatively comparable to those obtained in NO_3^--saline. Thus, differences in dissociation constant cannot account for the difference in Tl^+ uptake pattern in Cl^-- and NO_3^--saline. In conclusion, we feel that under all conditions tested here $^{204}Tl^+$ behaved as a probe for the movement of free Tl^+ ion.

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Note added in Proof. After this work had been completed, a paper (Skulskii *et al.*, 1978) came to our attention comparing $^{204}Tl^+$- and $^{86}Rb^+$ pump- and leak fluxes in human and cat erythrocytes. Also for those cells the authors concluded that Tl^+ and alkali metal cations appeared to overcome a common membrane barrier.

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